UNIVERSITY OF CAPE COAST

OPTIMIZATION OF MICROWAVE PASTEURISATION AND STORAGE ON THE PHYSICOCHEMICAL PROPERTIES OF JUICE FROM THREE PINEAPPLE VARIETIES

OFORIWAAH KUKUWAA SMITH

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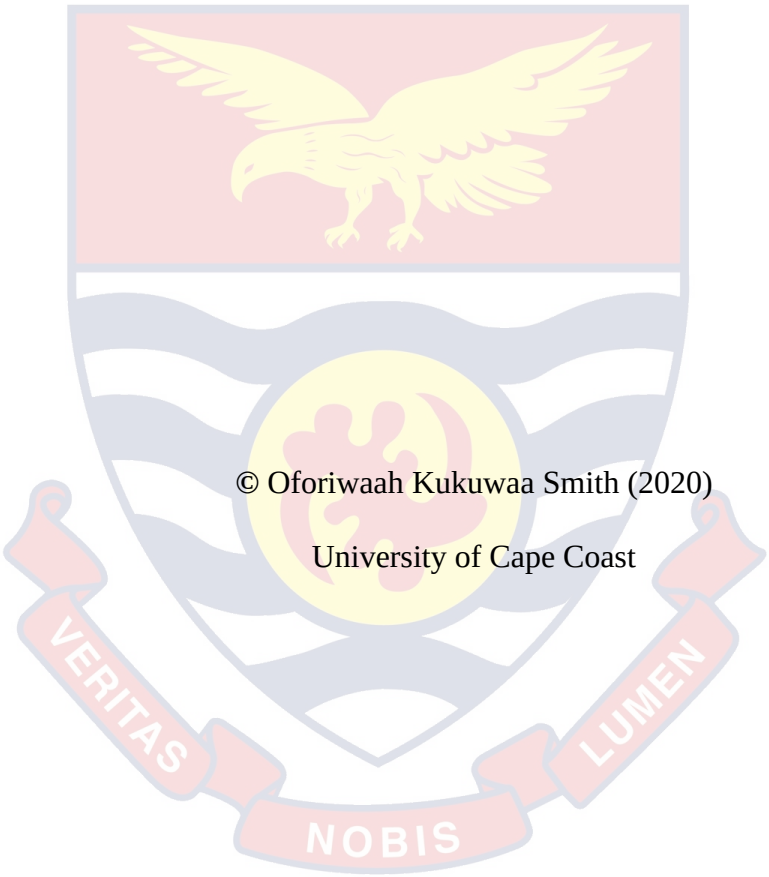

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OPTIMIZATION OF MICROWAVE PASTEURISATION AND STORAGE ON THE PHYSICOCHEMICAL PROPERTIES OF JUICE FROM THREE PINEAPPLE VARIETIES

BY

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Thesis submitted to the Department of Agricultural Engineering of School of Agriculture of the College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Master of Philosophy degree in Food and Postharvest Technology

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JULY 2020

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of the author research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: ..... Date: .....

Oforiwaah Kukuwaa Smith

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised by the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature: ..... Date: .....

ABSTRACT

The objective of the present study is to investigate the effect of pineapple variety and size on the yield and optimization on the physicochemical properties of the juice pasteurised by microwave and conventional method employing Response Surface Method (RSM). The pineapple variety significantly ($p<0.001$) influenced the total soluble solids, titratable acidity and ascorbic acid. Large size smooth cayenne variety had the highest (559.256 ml/kg) juice content. The sugarloaf variety recorded the highest brix content while the MD2 variety had the highest titrable acidity(TA) and acsobic acid(AA). Data obtained were compared with conventional pasteurisation (80-90^{o}C for 60-180 secs). It was shown that microwave pasteurised pineapple juice had a significant positive influence on total soluble solids, pH, total antioxidant content, ascorbic acid and total phenol compared to conventional pasteurisation treatment. The optimised microwave and conventional pasteurised juice were obtained at 900W/180secs and 90^{o}C/60 secs, respectively. Optimised microwave pasteurised pineapple juice was prepared and stored under room temperature, supermarket and refrigeration conditions and quality changes studied every (7) day for 21 days. The shelf life of refrigerated microwave pasteurised pineapple juice was 21 days compared to only 14 days for supermarket and room temperature conventional pasteurised pineapple juice. No significant changes were detected in total antioxidant values after storage of both pasteurised pineapple juice. However, the differences in pH, total soluble solids (TSS), titratable acidity (TA) total phenolic (TP) content, ascorbic acid content, and total phenols (TP) throughout the 21days storage period was observed to decrease in a fluctuating order under room and supermarket condition. The degradation of these nutrients was rapid in the conventional pasteurised juice during storage, as compared to refrigerated microwave pasteurised pineapple juice. Therefore, it can be concluded that microwave treatment is a potential alternative for fruit juice pasteurisation, especially for small scale industrial application.

KEY WORDS

Pineapple

Storage

Microwave pasteurisation

Juice

Physicochemical properties

Optimization

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DEDICATION

I dedicate this work first and froemost to God, my parents Samuel Smith and Florence Smith and my Siblings: Naana Conuaba Smith, Robert Dennis Smith, John Smith and my best friend Gilbert Charles Buckman for the support given me during the period.

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LIST OF ABBREVIATIONS

TSS- Total Soluble Solids

MD2- Del Monte

MHz- MegaHertz

TA- Titrable Acidity

TP- Total Phenols

TAC- Total Antioxidant Capacity

RSM- Response Surface Method

TCD- Total Colour Difference

SC- Smooth Cayenne

USDA- United States Department of Agriculture

CFU- Colony Forming Unit

pH- Potential Hydrogen

ANOVA- Analysis of Variance

APC- Aerobic Plate Count

TC- Total Coliforms

YM- Yeast and Mould

LDL- Low Density Lipoprotein

VLDL- Very Low Density Lipoprotein

OA- Overall Acceptability

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Fruit juice consumption has become a popular snack among people of all ages and is highly known as a healthy beverage (Dennison, Rockwell, & Baker, 1997). Fruit juice intake is very easy compared to that of the fruit itself. Young children, the aged and unwell people might encounter a bit of a problem in taking food, not to mention taking off fruits peels. Juice consumption is an essential, nutrients substitute. Juice from fruits contains high nutrients and extra health advantages from phytochemicals. Study from global food market submits that all kinds and varieties of juice play a key role in nutrition sustenance and satisfaction (Bates, Morris, & Crandall, 2001). The sugar composition of pineapple ranges from 12-15% which comprises two out of three of sucrose and the remaining are fructose and glucose. Pineapple pH is 3.71 making it acidic and the percentage of the acidity is 53.5 % (Sairi, Law, & Sarmidi, 2004). There are quite a few healthy-living elements in fruits, comprising fiber and elevated levels of phenolic acids, flavonoids, vitamins and minerals. Phenolic acids and flavonoids could offer long-term guard against most long-lasting infections (Mullen, Marks, & Crozier, 2007).

According to Joy and Abraham (2013), drinking fresh fruit juice maintains and stabilises the amount of moisture in the human body in a tremendous way. Pineapple is a well-known fruit and is enjoyed by many people regardless of their age group. It contains an enzyme known as vitamin C and bromelain that performs a key curative function in the body. With countless

health benefits, bromelain is a natural anti-inflammatory enzyme. They are very minimal in terms of saturated fat, cholesterol and sodium in pineapple fruit. For the immune and digestive systems, it is a helpful source of dietary fiber that is needed. Some anti-inflammatory effects are also present in the fruit.

Pineapple fruit juice is an alcohol-free beverage which is one of the several popular fruit juice products and requires continues to rise primarily owing to an increase in understanding of its health advantages among consumers. The juice contains around 83.7% moisture, 16% total soluble solids, 0.4% fibre and a rich source of Vitamin C, particularly sucrose and glucose, and fructose. (Nwachukwu & Ezejiaku, 2014).

Pineapple (*Ananas comosus*) is a fruit that is found mostly in the tropics and could be eaten entirely fresh, liquid or canned. Nowadays, the fruit is of great financial significance. They are being shipped in greater amounts to non-pineapple growing areas of the globe (Ackom & Tano-Debrah, 2012). The widespread supply of exporting pineapple fresh fruit is primarily as a result of the minimal impact of conveyance on their quality compared to other tropical fruits like papaya and mango (Essia Ngang et al., 2014). Pineapple is having a global production of about eighteen million tons. It is noted as Ghana's greatest significant sole contemporary export profits earner. The estimate in 2001 for worth of pineapple distribution from Ghana to European Union states was roughly 30 million Euros. The main trades in countries for pineapples are Switzerland, Italy, Belgium, France, Luxembourg, Netherlands and United Kingdom. Market overseas has mostly been for the Europe marketplaces because of the geographical supply shift from the Mediterranean to West Africa after the 1980s (Baafi, Osei, Agyeman, & Afriyie, 2015). Pineapple (*Ananas*

cosmosus) is widely cultivated in several nations comprising Malaysia, and for long it has been part of the major widespread "non-citrus" subtropical and tropical fruitlet due to its appealing taste and revitalising sugar-acid equilibrium (Adzahan et al., 2011).

The juice obtained from pineapple fruit contains a very high amount of antioxidants and phenolic compound (Paniagua-Martínez, Mulet, García-Alvarado, & Benedito, 2018). When it comes to the horticulture area in Ghana, the pineapple industry is highly developed and can mostly be found in the Volta, Central, Eastern, Western and Greater Accra regions of Ghana. In the year 2004, the exportation of pineapples was said to have contributed over sixty percent of Ghana' non-traditional trades producing over twenty thousand jobs and a projected income of GH¢6 million to 2,500 homes in the rural societies (Kuwornu, Nafeo, & Osei-Asare, 2013).

In terms of perishability, pineapple fruits are highly perishable and it requires different processing methods to make it have a longer shelf life. When fruits are being harvested, they have to be preserved within four to forty-eight hours period because as time passes the fruit spoilage also intensifies (Joy & Minu, 2013). If pineapple juice is not kept well in a refrigerator the shelf life is very short. There is exposure of the juice to microbial contamination by several means due to the traditional method of preparation (Nwachukwu & Ezejiaku, 2014).

Due to yeast fermentation, pineapple could literally ruin the black rot (Chalara paradox - Thielaviopsis paradox), black spot(Penicillium funiculosum; Fusarium subglutenanas) and fruit red (*Saccharomys spp.; Saccaromyces cerevisae; Hanseniaspora valbyensis; Candida intermedia)*

(Aspergillus flavus; Rhizopus oryzae; Hendersonula toruloidea)(Quyen, Joomwong, & Rachtanapun, 2013). Pineapple in its fresh-cut or juices form is also highly vulnerable to bacterial spoilage, high respiration rate and production of ethylene, leading to surface degeneration, quick microbial growth, enzymatic browning, weight loss and unpleasant volatile production, which tends to greatly lessen the product shelf life (Corbo et al., 2009).

Nevertheless, the small life of fruits has made food scientists to come out with different technologies that will increase the quantity plus quality of freshly-cut produce and juices with the key aim of growing their production without affecting quality and the surroundings. Moreover, preserving and processing fresh products like pineapple is not that easy or direct as it was done in the past. Several new methods of preservation are developed to fulfil present economic preservation demands and satisfying the consumer in terms of nutrition and sensory aspects, absence of chemical preservatives, and safety of the environment.

1.2 Problem Statement

Earlier studies conducted on postharvest losses of fruits and vegetables in Ghana by Abano, Ma, and Qu (2014) reveals that over 50% of the farm produce go to waste during peak harvest due to high perishability of fruits and vegetables and lack of farmer's knowledge regarding processing, packaging and storage channels. Concerning the varieties of pineapple, it is suggested that more than 100 varieties of pineapple are available, however, when it comes to the commercial cultivation of them, only six to eight are well known (Medina & García, 2005)

In Ghana, we have three main varieties of pineapple that are mostly known and could be found on the local markets and groceries which include smooth cayenne, del monte (MD-2), and sugarloaf variety. Most of the local market women and local pineapple juice processors lack knowledge on the varieties that are available on the market and the ones that can be used for producing high-quality juice. Many local processors purchase any variety that they find available as far as it is pineapple fruit they see and they use for juice which sometimes makes local processors make losses.

A research conducted by Wardy, Saalia, Steiner-Asiedu and Budu (2009) made us know that every variety of pineapple has its own amount of juice that it produces but it was based on a specific geographical location. The location geography also has influence on the juice yield and quality as stated by Sairi et al (2004) that juice obtained from pineapple fruit varies with respect to the topography, culture and seasons of harvesting and processing. Although using different varieties brings variability in the pineapple juice produced but sometimes could result in lesser juice production as a result of the particular variety used by the local juice processor. Ghanaians will prefer to consume freshly made-in Ghana fruit juice and there is an increase in demand for them but the form in which the produce comes and the package limits its acceptability by modern day consumers.

Skills on how to process and preserve fresh food and fruits are always being developed to fit in to the present-day consumer requests for healthy plus safe foods. High earnings, development, demographic changes, enhanced transport system, and perceptions of consumers concerning food safety and quality are causing a change in global food intake. The modern day consumer wants

delicious, healthier, natural and fresh food and juices, made in an environmentally pleasant way with sustaining approaches and minor carbon footprints (Shah, Shamsudin, Rahman, & Adzahan, 2016). Processing fresh fruits to juice without additives and preservatives, packaging and storage of them to enhance its shelf life still remains a global challenge.

1.3 Justification of the Study

Processing of fruit like pineapple by crushing and blending seems to be some of the ideal techniques to refine the nutrient attribute of fruit juice. Thought of innovative product improvement using size reduction method like blending and juicing produces a fresh healthy natural juice for consumption (Jan & Masih, 2012).

The use of a clean technology (such as UV radiation) to kill microorganisms has been interesting in recent times, due to the higher concernes raised to keep the environment away from chemicals. Various experiments for different methods to stabilise fruit juice microbial loads that can lessen the damage to the sensory characteristics are considered as an alternative to conventional pasteurisatation (Cañumir, Celis, De Bruijn, & Vidal, 2002). The aim of thermal pasteurisation (microwave and hot water treatment) is to enhance the durability of the handled juice and reduce warming time by technology.

It is intended to rekindle made-in-Ghana food products with respect to fresh fruit juice and to reduce their importation by processing, packaging and storing of available tropical fruits like pineapple. The study also tends to draw local processors of pineapple fruit juice attention to the particular variety that is being used to produce the juice into the market and best for their businesses. The thermally pasteurised juice from the different varieties will be characterised

using the physicochemical and sensory evaluation methods to know the one that is best preferred by consumers. The research sought to also know the best means of enhancing the shelf life of various pineapple variety juices using plastic and glass containers as packaging methods.

1.4 General Objective

This research was designed to optimize the effects of developing a pineapple juice, and to study the yield potential, sensory properties, physiochemical, and microbial quality of microwave and conventional pasteurised pineapple juice during room temperature, open air supermarket and refrigeration storage.

1.5 Specific Objectives

The specific objectives of the study seeks to determine;

1. The influence of pineapple variety and fruit size on juice yield, physicochemical (pH, TA, TSS, colour, ascorbic acid, phenolic and flavonoids) and sensory properties of pineapple juice.
2. The influence of pasteurisation (microwave and conventional) treatment on physicochemical (colour, pH, TSS, TA, TP, AA, TAC and TP) and microbial quality.
3. The effect of independent variables (storage condition and time) on physicochemical properties and microbial quality of optimised pasteurised pineapple juice.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background of Pineapple Production

Ananas comosus (pineapple) is an extremely sweet and nutritious fruit found in the tropics. In Australia, Mexico, Kenya, the Philippines, Hawaii, the Caribbean region, Malaysia, Taiwan, Thailand and South Africa, pineapple is mainly grown (Patil, Jadhav, & Deshmukh, 2013). It has been one of the most popular non-citrus tropical and subtropical organic products for a few times, owing to its enticing taste and invigorating acid-sugar balance, to an excellent degree (Bartolomé, Rupérez, & Fúster, 1995). In recent times, they are made accessible in fresh, canned and in juice form (Arthey & Ashurst, 1995). Pineapples are known to originate locally from southern part of Paraguay and Brazil. Indians at that era outspread the pineapple through Southern and Central America to West Indies before the arrival of Columbus. In those early centuries, Columbus discovered the fruit on the desert island of Gaudaloupe and took it to Spain. During that era, sailing ships that conveyed pineapples for safety against scurvy spread them worldwide. Previously, people who lived in Spain brought pineapples to the Philippines and probably to Hawaii and Guam. (Arthey & Ashurst, 1995). Pineapple got to England in 1660 and around 1720, the fruits were nurtured and cultivated in greenhouses for their fruits (Maxwell & Maxwell, 1984). Pineapple production in Ghana is widespread and is mainly cultivated in Ghana's Central and Western regions.

Most tropical fruit grown in Ghana during peak harvest are either consumed fresh, sold at a relatively low price or wasted because processing

facilities are insufficient (Yeboah & Kunze, 2004). A large portion of the fresh pineapple fruit goes towards the juice and juice industry. The fresh fruit market also exports approximately 5 million tons annually (Smart & Simmonds, 1995). In the past two decades, there has been a significant growth in the export of pineapples from Ghana's pineapples industry. These commercially processed pineapples are produced more often than not within a 50 km radius of the capital, Accra, from where they are exported by sea or air to export markets such as Germany, Holland and Italy. Whitfield (2010) reported the subsequent increase in the total sea and air loads Ghana pineapple industry exported estimating 56,000 metric tonnes in 2003 to a peak of about 71,000 metric tonnes in 2004 before it reduced steadily to about 60 percent over five years falling to 31,000 metric tonnes in 2009 (GEPA, 2010). Also, with a variety of companies' pineapple processing has increased (e.g. Blue Skies) involved in producing slices and mixed fruit salads, single-strength juice and juice concentrates.

2.2 General Description of the Pineapple Plant

The *Ananas Comosus* is part of the Bromeliaceae family. A pineapple plant is commonly grown in the form of a 1-2 m high and wide spinning top. The stem, separate fruits, the crown, root and the shoots are notable morphological features. Slender, tapering and pointed, it leaves in a spiral rosette. The margins of the leaves are most commonly spike, and maybe green or otherwise strewn in the middle or along its margin with colour red, yellow or ivory. The leaves envelop the tin with a diameter of two thirds, according to d'Eeckenbrugge, Leal, and Bartholomew, (2003).

The fruit of the pineapple has a core, fruitlets, collective flesh and the shell of the fruit. Using the crowns, suckers and stem shoots of good viable

plants, pineapples are replicated by vegetative propagation means. To ensure crop uniformity, these products must be picked correctly by size (Duke, 1983). Nonetheless, under supervised cultivation, sexual reproduction is possible. The plant develops stem shoots, axial sprout suckers, which are capable of producing new axils of growth and thus new fruits. Significant commercial cultivation areas are located between latitudes 30 ° North and South, with some areas considered marginal for different reasons (Bartholomew, Paul, & Rohrbach, 2003). Minor plantations extend the production of pineapples to subtropical areas with mild climates beyond 30 ° North and South latitudes and even in protective shelters.

2.3 Pineapple Propagation

Crowns, slips, suckers, and ratoons were all usually used for pineapple multiplication using vegetative means. Sowing is needed only in breeding programs and is traditionally the product of hand pollination. The seeds germinate slowly and hard. Seeds are treated with sulfuric acid to germinate within 10 days, but higher germination rates (75-90 percent) and faster seedling growth are the results of intermittent mist planting of untreated seeds. Pineapple seedlings will be planted at the age of 15 to18 months, and start producing fruit 16 to 30 months after. Fruit propagate from vegetative plants in 15 to 22 months (Morton, 1987).

2.4 Pineapple varieties

When it comes to foreign trade, the different cultivars of pineapple are classified into four major classes: 'Smooth Cayenne,' 'Red Spanish,' 'Queen,' and

'Abacaxi,' regardless of the variety in each class (Morton, 1987). A new variety called 'Del monte Gold' or MD2 'has been produced lately.

2.4.1 Smooth Cayenne Variety

Smooth cayenne was chosen and cultivated by Indians in Venezuela, and introduced from Cayenne (French Guyana) in 1820. It was also known as "Sarawak' or "Kew" in India, Sri Lanka, Malaysia and Thailand. From there, it was added and dispersed to Queensland, Australia, and Jamaica in the Royal Botanical Gardens, Kew, England. Since the plant, with the exception of the needle on the end and scala (4 to 10 lbs) is close to spine independence, it is essential globally, although it is prone to disease and has little acidity, even though it has an orange rind, yellow flesh, low in fibres, juiciness and mildly acidic savour. It is valued mainly for cannery, with ample fibre for firmer bits and cubes, and while disease-prone and not shipped well, it is of growing worldwide importance. It is particularly prized for canning with a sufficient amount of fibre and exceptional aroma for firm cuts and cubes (Morton, 1987). Since the cultivar was introduced from Hawaii to the Philippines in 1912, the industry in Philippines boosted from the semi-wild, mostly seedy, casual growing kind. There are several ' Smooth Cayenne ' clones in Hawaii that were chosen to resist mealybug wilt. It is Taiwan's premier cultivar. The Queensland Department of Primary Industries published in 1975 a dual-purpose cultivar named 'Queensland Cayenne' after 20 years of breeding and research. After two decades of selection and evaluating clones 'Smooth Cayenne,' the Pineapple

Research Station in South Africa, East London, has selected 4 as the superior for the canning industry.

Plate 0-1: Smooth cayenne pineapple fruit

2.4.2 Sugar Loaf Variety

'Sugarloaf' has a clear connection to 'Abacaxi' in Central as well as South America, Puerto Rico, Cuba and the Philippines. The plant and crown leaves grow easily, while the unreliable hypothesis has arisen that pineapple ripeness is implied as the leaves become loose. The pan normally weighs 0.68-1.36 kg and often appears conical, circular, not bright in colour. The fruit flesh is white to yellow in appearance, juicy as well as very sugary. This variety is highly susceptible to shipment tender (Morton, 1987).

Plate 0-2: Sugar loaf pineapple fruit

2.4.3 MD 2 Variety

This form of cultivar is a hybrid originating from the non-operational Pineapple Research Institute's breeding programme in Hawaii, which carried out a research on behalf of Maui Land and Del Monte Pineapple Company. The fruit pulp is gold in colour, appears barrel-like and has an extra sweet taste. It is internally known as MD 2 (Crane & Balerdi, 2009). The MD 2 has less acid content and additional nutritional benefits; thus, it has become very popular in the fresh-cut market and the processing industry. To resolve issues of inequality in the shape of pineapple fruits and at the same time to heighten sugar contents, MD 2 was developed (Sauls, 1998). The MD2 pineapple variety had earned more from the time when it was introduced to the European market in 1996 compared to Smooth Cayenne, Sugar Loaf and other pineapple varieties (Wardy et al., 2009). MD2 is defined as super-sweet, self-matured and with an extended shelf life (Achuonjei et al., 2003).

Plate 0-3: MD2 pineapple fruit

2.5 Pineapple Juice

Many countries have started processing tropical fruits over the last two decades because of the sudden rise in demand for tropical fruit and fruit products on international markets. Amongst products made from tropical fruits, the most popular ones are fruit juices, nectars and drinks. Approximately 10 to 25 per cent of pineapple juices obtained from canning industries are not suitable for or concentrate juice processing as a result of the high acidity (Adhikary et al., 1987). Numerous attempts have been made to minimise sweetening by adding sweeteners, quick alkali neutralisation or partly eliminating it by ion exchange methods (Vibhakar, Prabhakar, & Bhatnagar, 1966). Fruit juice with the original fresh fruit characteristics and free of artificial additives are increasingly in demand. This contributes to the quest for innovations that can enhance the sensory, nutritional and microbiological fruit juice quality (Carneiro et al., 2002).

2.5.1 Juice Yield of Pineapple Fruits

More often than not, rippened or over maturing pineapple fruits produce viscous juices and wetter wastes which make further downstream processing very difficult (Zalita et al., 2009). Since most consumers prefer to buy more juice than flesh, the volume of juice that can be extracted from a given variety per fruit becomes essential. Because of this, inferences could be established signifying which of the varieties may be most lucrative in the manufacture of profit-making pineapple juice. Wardy et al. (2009) described that Sugarloaf gives the maximum juice volume of 205.72ml per fruit, with MD2 following (143.12-191.43 ml per fruit) and smooth cayenne giving the minimum juice capacity between 91.7-108.65 ml per fruit. Sugarloaf variety is thought to contain much juice, few fibres and much sugar from the report, offering the greatest likelihood and benefits in this enterprise. Nonetheless, sugarloaf with a substantial volume of juice can be more vulnerable to cracking and other post-harvest mechanical bruises compared to smooth cayenne and MD2 (Wardy et al., 2009).

2.5.2 Pineapple Juice Colour

Visual colour, the priority of the perceptions of consumers, is an important characteristic that affects a product's consumer preference and often represents 40 percent of the acceptance criteria (Kolawole et al., 2007). Furthermore, it is a measure of pigment concentration that can be determined instantly for on-line quality control using tristimulus colorimeters. Numerous results showed that many reactions through heat application influenced the colour. Carotenoids are the most common, followed by phytic acid, proteins,

lipids, mycotoxins, amino acids, oxidative modification, peroxidations, crosslinking and auto-oxidation reactions.

Hunter colour parameters; lightness values (L *), redness values (a *), and yellowing values (b *), and total colour difference values (TCD) have been commonly used in food production. For the consistency of the pineapple concentrate production, the colour ratios must be correct. The key parameters for pineapple juice consistency determination are lightness and yellowness. In TCD, which is the most important index of colour changes of pineapple juice, a colour difference can be demonstrated in the same study. Scientists have applied a zero-order and first-order kinetic model to explain food colour shift and non-enzymatic browning (Cohen, 1998). MD2 is golden brown in colour, while Smooth Cayenne has a light-yellow colour. When mixed, MD2's golden-brown colour complements the yellow colour of Smooth Cayenne to give their blends a deeper rich yellow colour (Wardy et al., 2009).

2.5.3 Nutritional Properties of Pineapple

Pineapple is cultivated mostly for its fruits. It is consumed fresh, bottled, frozen, and in juice forms. Nearly 70 percent of pineapple is eaten in the fresh state, according to Francis (1982). Vitamins, essential amino acids and minerals, such as calcium, iron, magnesium, phosphorus, potassium, zinc, copper, manganese and selenium, are considered to be rich in pineapples. They contain high potassium and vitamin C and have good antioxidant activity in the body. Generally, pineapple acidity varies from 0.6-1.2 per cent, 87 per cent of it being citric acid and 13 per cent is malic acid. Table 2.1 displays the nutrient content of three pineapple varieties with liquid content and total sugars of sugarloaf much higher as compared to the MD-2 and smooth cayenne.

Table 0.1: Nutrient content of three pineapple varieties. (Value per 100grams of edible portion of raw fruit).

Nutrient	MD-2	Sugarloaf	Smooth cayenne
Water (ml)	86	92	87
Energy (KJ)	215	214	190
Protein(g)	0.53	0.52	0.55
Total lipid (fat) (g)	0.11	0.12	0.13
Carbohydrate (g)	13.50	13.10	11.82
Iron (mg)	0.25	0.26	0.28
Calcium (g)	13	13	13
Fructose (g)	2.15	2.31	1.94
Fiber (total dietary) (g)	13.50	13.10	-
Glucose (dextrose) (g)	1.70	1.82	1.76
Total sugars(g)	10.32	13.56	8.29
Sucrose (g)	6.47	8.3	4.59
Potassium (mg)	108	112	125
Vitamin K	0.7	0.7	0.7
Vitamin E(mcg)	0.02	0.02	-
Vitamin A (IU)	57	55	52
Choline (mg)	5.4	5.2	5.6
Vitamin B3 (mg)	0.507	0.502	0.47
Vitamin B6 (mg)	0.114	0.114	0.106
Folate (mcg)	19	19	11
Vitamin C (mg)	56.4	42.5	16.9
Vitamin B1 (mg)	0.080	0.0790	0.078
Vitamin B2 (mg)	0.033	0.031	0.029
Sodium (mg)	1	1	1
Magnesium (mg)	12	12	12
Manganese (mg)	0.818	0.825	1.593

Source: (USDA, 2009)

2.6 Medicinal Uses of Pineapple

Pineapple has an outstanding medicinal property, known in folk medicine for decades. It contains the bromelain medicinal ingredient, which is a group of enzymes that hydrolyse proteins, thus helping in the process of digestion (Walker, Bundy, Hicks, & Middleton, 2002). Clinical tests have established that pineapples contain a great amount of the bromelain, a natural anti-inflammatory enzyme which has several health benefits and facilitates rapid wound curing. Bromelain is very effective at reducing swelling and pain in the management of fractures and sprains. Such powerful anti-inflammatory effect can also help to alleviate rheumatoid arthritis symptoms and decrease postoperative swelling. However, by the presence of heat, the enzyme present in the petals and fruit flesh is inactivated.

2.7 Physicochemical Properties of Pineapple

2.7.1 Total Titratable Acidity (TTA) of Pineapple Juice

Increases in fruit acidity are associated with its maturity (Singleton & Gortner, 1965); however, the titratable acidity of MD2 is slightly effected at maturity stages M1 and M3 (Kotey, 2006). The Smooth Cayenne's higher acidity may mean a longer shelf life and a potentially higher astringency index. The hydrolysis of sucrose and pectin methoxyl groups, gelling properties can be impaired under high temperatures when acidity is high. Citric acid and malic acid are two main organic acids in pineapple. (Saradhuldhat & Paull, 2007).

2.7.2 Potential Hydrogen (pH) of Pineapple Juice

The pH value provides a measure of a product's acidity or alkalinity, while TA provides an amount of the acid volume present (Dadzie & Orchard,

1997). Assessment of pH and titratable pineapple acidity is mainly used to measure consumption quality. They could be known as fruit maturity indicators or ripeness indicators. It also affects the fruit's taste (sweet or sour) and defines the fruit's marketable quality to a large extent. Sugarloaf, its pH being high might not be ideal for producing jams than the SC, and the MD2 as its glycoside associations at low acidity will remain relatively less secure. The pH of pineapple will vary from 3.20-4.00, according to the America Center for Food Safety and Applied Nutrition of the Food and Drug Administration (2000). Malic acid is the primary acid present in pineapple.

The pH values obtained represent the microbial stability of the various varieties to a considerable extent. The MD2 and Smooth Cayenne are expected to keep more than the Sugarloaf with lower pH levels. Acidifiers may be required to improve the Sugarloaf products ' holding properties. Increasing the sugar level of fruits improves senescence.

2.7.3 Total Soluble Solids (TSS) of Pineapple Juice

Harrill (1998) mentioned TSS also being known as Brix. The OECD, (1999) also suggested that a refractometer tests TSS as Brix in graduations of 0.1 percent. Brix is a calculation of the percentage of total sugars in known vegetable juice weight. It is typically a total of the pounds of fructose, sucrose, vitamins, hormones, amino acids, proteins, minerals, and other solids in any particular plant juice (Harril, 1998).

In many plants, nutrients are stored as starch during the growth of a fruit's flesh, which is converted into sugars during the maturing process. Progressive ripening leads to higher sugar levels (OECD, 1999). Soluble solids relative to total sugars are also found to affect the sweetness index

(Bartholomew, Paul, & Rohrbach, 2003). As an indicator of fruit maturity and quality, the amount of total sugars is often used (Paull, 1993) and ranges from 10.8-17.5 percent (Dull, 1971) for pineapples, and less variation among varieties. The marginally greater values, however, correspond to the sweetness rankings. Soluble Solid content and Dry Matter content are important features used to measure the maturity of certain vegetables and fruits (Kays, 1991), and may affect fresh produce's flavour quality, nutritional status, and post-harvest storage potential.

2.7.4 Ascorbic Acid in Pineapple Juice

Pineapple is high in ascorbic acid (vitamin C), and thus protects the body from free radicals that cause atherosclerosis and bronchitis. This has a positive effect on proper functions (Coveca, 2002). Based on the cultivar, developmental stage, storage conditions and the part of the fruit, the ascorbic acid content is highly variable. Vitamin C content for MD-2 and sugarloaf is 56.4 mg / 100 ml (or 58.2 mg / 100 ml) which is sufficient to meet the vitamin C dietary requirement (or RDA) for adults (> 19 years) as the daily recommended intake or average daily intake is 75 mg / day for women and 90 mg/day for men. There has been a proposal on the basis of clinical and epidemiological studies that a 100 mg/day dietary intake of ascorbic acid is connected with a decreased rate of mortality from heart disease, stroke and cancer (Naidu, 2003). Vitamin C aids in iron absorption in human gut, and it also promotes wound healing in skin and tissues (Janine, 2010).

2.7.5 Antioxidant Activity of Pineapple Juice

Tropical and subtropical fruit antioxidant activity is usually ascribed to elevated levels of L-ascorbic acid. Nonetheless, several experiments have also shown that key function, in terms of antioxidant capacity, lies in the synergism of many active compounds, indicating that other substances, other than L-ascorbic acid, can act as antioxidants themselves, as well as substances that do not have any antioxidant capacity but may increase L-ascorbic acid's antioxidant capacity (Leong & Shui, 2002). The water-ethanol extracts from various pineapple parts affect the levels of cholesterol, triglycerides, LDL and VLDL particles, and the serum levels of aspartate transaminase and alanine transaminase. (Fleming, 2004).

The pineapple crust methanol extract has shown modulatory effect of lipid peroxidation in the liver caused by alcohol, including loss of hepatic biomarkers in rat plasma. The capacity of antioxidant is achieved by various mechanisms: free radical scavenging, inhibition of oxidative enzymes, increased catalytic activity, and chelation of metals (Erukainure, Ajiboye, Adejobi, Okafor, & Adenekan, 2011).

2.8 Microbial Load of Pineapple

Fruits may have wide and diverse bacterial communities. Numerous work on fruit bacteria has been centred on limited number of pathogenic bacteria and, as a result, we know much less about the general existence and distribution of certain bacterial communities found in products and how the composition of these communities differs between types of products. In contrast, there is a lack of a thorough understanding of the possible influences

of different farming activities on the bacterial species to which buyers are exposed.

The causative organisms of microbial spoilage of fruit and fruit juices may be pathogens such as yeasts and moulds. Because of the low pH of most fruits, the principal spoilage agents may be detected. Many bacteria, like the *Campylobacter spp*, may cause fruit and fruits juice spoilage (Walker & Phillips, 2008). In microorganisms, yeasts and moulds are having a comparative benefit over bacteria, which could also penetrate bruised tissues of several fruits due to its ability to grow at a lesser pH range of 2.2 to 5.0.

2.9 Sensory evaluation of pineapple juices

Institute of Food Technology describes sensory assessment as a scientific approach that includes the sight, smell, touch, taste and hearing senses to induce, measure, evaluate and classify responses to products as supposed. This scientific system is composed of two parts: measures that are analytical and affective. Analytical measurements are used to define product variations or to classify the object (descriptive analysis). Small panels that have undergone some training typically perform empirical sensory assessments. Affective measurements specify which samples are favoured over others and normally involve a large number of panellists. The involvement of human perception in sensory testing implies that quality attributes should always be evidently clear in terms that are important to consumer satisfactoriness. The only way to ascertain what consumers like and what they dislike is by means of effective consumer testing (Barrett, Beaulieu, & Shewfelt, 2010).

Kemp, Hollowood, and Hort (2011) conveyed that the sensory properties for many food products depreciate ahead of microbial quality. For

this reason, sensory testing can be used in tandem with microbial tests to determine product shelf life and inconsistencies through the supply chain. Sensory attributes for pineapple juice include appearance, sweetness, sourness, odour, texture, firmness and its overall acceptability. Sensory analysis is a better tool in providing rapid assessment of the pineapple juice quality (Masniza et al., 2010).

2.10 Pasteurisation Processing on Pineapple Juice Quality

So many products can be formed as a result of processing fresh pineapple; this includes, canned slices, concentrated juice, fresh juice, fruit chips, and pasteurised juice. The most common pineapple products is pasteurised juice. Consequently, the processing can influence the organoleptic and nutritional values of the refined pineapple.

2.10.1 Effect of Pasteurisation on Vitamin C

Vitamin C is a delicate material that could quickly vanish under several circumstances, including exposure to light, high temperatures and access to oxygen. It is recorded that the vitamin C content of fresh pineapple juice varies from 9.2 to 93.8 mg/100 mL. This indicates that pineapple fruit has a wide difference in vitamin C. Research has shown that approximately 84.2 ± 9.6 mg/100 mL of Vit C is found in fresh pineapple juice, while the concentrate value of commercial pineapple juice ranges between 8.5 ± 1.4 and 58 ± 4.9 mg/100 mL, respectively. Another study found that 94 percent of the ascorbic acid content was lost by the pasteurisation of the juice at 99 ^{o}C for 17 minutes. This drop in vitamin C may be due to its oxidation during heat treatment into

dehydroascorbic acid. Drop in the ascorbic acid concentration from 33.5 ± 1.9 to 12.8 ± 1.1 mg/100 mL was caused by pasteurisation at 90 ^{o}C for 3 min.

2.10.2 Effect of Pasteurisation on Soluble Solids Content

Sucrose and glucose are dominant sugars present in pineapple products. Recent research on brix content of pineapple varieties; MD2, Smooth Cayenne, and Sugarloaf has been published by (Wardy et al., 2009). The sugar content for the variety MD2 was found to be the highest, Sugarloaf and SC followed. Nevertheless, Sugar loaf had the maximum index of brix / acid ratio, 15.4, with MD2 (12.7) and SC (6.98) following respectively. This suggests Sugarloaf's potential for the production of juice.

Even in these modern times, not many studies have been conducted on that variety; it is important to classify fresh and pasteurised Sugarloaf juice. Dietary energy is provided by sugars in pineapple juice. Juices play a significant role on consumer's satisfaction.

2.10.3 Effect of Pasteurisation on Pineapple Juice's Microbiological Quality

In stores, supermarkets, restaurants, and cafes, most fruit juices and beverages available are pasteurised. Juice spoilage is mainly due to aerobic acid-tolerant bacterial contamination and also yeasts and moulds. It has been confirmed that fresh pineapple juice had 1.9×10^3 organisms/mL of coliforms and 3.2×10^4 organisms/mL of YM. Badge & Tumane (2011) noticed that bacterial counts are contained between 1×10^5 and 15×10^5 CFU/mL in unpasteurised pineapple juice. In all non-pasteurised pineapple juices, *Staphylococcus aureus* and *Escherichia coli* were present. This presence of both

pathogenic and non-pathogenic microorganisms in fresh juice was due to the contamination of infected fruit caused by insects. The organoleptic variations in the pasteurised pineapple juice are as a result of these microorganisms, rendering them unsuitable for consumption.

Bacteria, yeast and moulds are pineapple juice spoilage microorganisms since they are acid-resistant. As a result, the regular thermal pasteurisation applied for juices certainly destroys them. Conventional heat treatments could be used using hot-filled pasteurization methods. In citrus peeling, juices are heated up to 92 and 105 ^{o} C for 15 to 30 seconds in the hot-fill process. Yeasts can grow in the presence of acidic environments. They can grow easily in fresh pineapple juice because the pH of the juice is lower than 4. Yeasts, by the nature of their genetic makeup, are sensitive to heat. They can be easily killed by excessively high temperatures. Heat treatments used were, for example, 74^{o} C/16s or 85^{o} C/1s in the past. More attention must be given to this matter because of this recently discovered issue. On the other hand, juice obtained from pineapple has a low pH (< 4.6) and even under such environments, hardly any *Clostridium botulinum* bacteria can develop or even become harmful. Pineapple juice can also be put through relatively mild heat treatment. This would preserve the juiciness of the fruit and keep the fruit in a state of complete ripeness.

2.10.4 Effect of Pasteurisation on Pineapple Juice Organoleptic Quality

There are some researches on the organoleptic properties of pineapple juice. Among them are the organoleptic consistency of three forms of pineapple juice in Ghana, specifically MD2, SC and Sugarloaf. Pasteurisation results have not been studied in terms of the general sugarloaf juice quality and the sensory properties of pasteurised juice to be precise. The consistency of fruit juice may

be compromised by heating methods, resulting in dissatisfaction on the part of the customer. The key causes of these problems have been found to be non-enzymatic browning reactions and pigment damage and not to cause browning enzymes as they are prone to heat at temperatures > 50 ^{o} C. Heat processing effect on Hunter colour variables (L, a, b, and ΔE) between 55 and 95 ^{o} C was studied by Rattanathanalerk et al. (2009) It was noted that pineapple juice became darker with an increase in temperature and time associated with a decrease in L value.

After 12-month, the pineapple juice in cans was examined by Ewaidah in a range of temperatures and the variety was not mentioned. There was a statistically significant difference ($P < 0.05$) between the juice kept at 5 and 24 ^{o} C and the juice kept at 33 and 42 ^{o} C. The best colour for pineapple juice was obtained at 5 ^{o} C, but the worst colour for pineapple juice was obtained at 42 ^{o} C. When it came to the taste of the pineapple juices, the difference in the two refrigerated juices differed significantly ($p <0.05$). Nevertheless, no significant difference was seen in juice storage at the two temperatures. The highest scores were given to pineapple juice kept at 5 ^{o} C during refrigeration, while juice kept at 33 and 42 ^{o} C during refrigeration received lower scores of 5.0, 2.3, respectively. Therefore maintaining all the pineapple juice's organoleptic qualities was essential.

CHAPTER THREE

EFFECT OF PINEAPPLE VARIETY AND FRUIT SIZE ON JUICE YIELD AND PHYSICOCHEMICAL PROPERTIES OF PINEAPPLE JUICE

3.1 Introduction

In Ghana, many people are unaware of the actual constituents of the variety of pineapple of which they purchase their juice when they need them; they buy any pineapple fruit or juice based on their accessibility. As known, pineapple is one of the lowest caloric fruits and a valuable source of ascorbic acid. Ripe pineapple flesh contains approximately 85% water, 0.7% citric acid, and saccharides at about 14% Brix, with a pH of 3.4. Sucrose, glucose and fructose are predominant sugars in plant beverages (2.2 percent). In addition, it is a rich source of numerous inorganic and organic acids, such as malic, oxalic and phosphoric acid. Fresh pineapple juice has fructose at 17.2-47.5 g/l; glucose at 12.1-45.2 g/l; and citric acid as 4.6-12.1 g/l. The pineapple fruit or juice is acidic and has a pH of 3.71 and acidity percentage of 53.5%.

Pineapple juice is an excellent source of vitamins., particularly vitamin C, and contains different amounts depending on the type of pineapple fruit (Mahdavi, Nikniaz, Rafraf, & Jouyban, 2010). Even within the same species, its content can vary (Po & Po, 2012). There are about ninety pineapple varieties grown and know world-wide. However, only three varieties of pineapple are mostly grown in Ghana. The cultivated varieties are Smooth cayenne, Sugarloaf and MD2. Sugarloaf pineapple variety has a conical shape, with very sweetened taste. Also, smooth cayenne has a sweet taste and juicy but lacks the bright

yellow colour, which is used as an indicator for a ripened pineapple by most consumers. The MD2 pineapple variety is a super-sweet, self-ripening and has long shelf-life with a higher value as compared to smooth cayenne pineapple variety (Achuonjei et al., 2003).

In addition, as customers are searching for a better quality of fruit juice with unique features, new varieties are emerging to fulfil this requirement. It is for this reason that the study is carried out, assessing pineapple variety quality in regards to their physicochemical and sensory properties. A study by Wardy et al. (2009), in assessing the physicochemical and sensory characteristics of Smooth cayenne, Sugarloaf and MD2 revealed that; Sugarloaf had juice yield of 205.72 ml/kg, which was significantly ($p < 0.05$) different from juice yield obtained from MD2 pineapple variety (134.191 ml/kg) and SC pineapple variety (134.191 ml/kg). While there was no significant difference ($p > 0.05$) in the pineapple fruit flavour among the pineapple varieties, there were significant differences in the overall acceptability for the fresh pineapple juice. The differences in pineapple fruit juice physiochemical and sensory properties may not be only dependent on the variety, but also the size of pineapple fruit used for the processing of the juice. With this revealing information, the study intends to assess the influence of pineapple variety and fruit size on the quality of the juice. The specific objectives were to;

i. determine the influence of pineapple variety and fruit size on juice yield
ii. evalute the influence of pineapple variety and fruit size on physicochemical properties (TSS, TA, Ascorbic acid and pH).

iii. examine the effect of pineapple variety and fruit size on juice organoleptic characteristics (colour, aroma, taste, aftertaste and overall preference).

3.2 Materials and Methods

3.2.1 Raw Materials Collection and Preparation

Pineapple fruit varieties; SC, Sugarloaf and MD2, were obtained from Greenfields Limited, at Ekumfi in the Central region of Ghana, which is an accredited farm of the Ministry of Food and Agriculture, Ghana. Pineapple fruits were harvested in the early hours of the morning at full ripening stage. Harvested fruits were sorted into various sizes (small, medium and big) and cleaned to ensure that they were no bruised ones. Fruits were stored at room temperature three days before juicing.

The obtained raw materials were processed following Hazzard Analysis Critical Control Points (HACCP) guidelines. Briefly, according to the guidelines, all raw fruits and vegetables selected were of high-quality class, harvested at the right physiologically mature stage, cleaned before processing and rinsed thoroughly with tap water of drinking quality standard.

3.2.2 Pineapple Fruit Size (Weight) Determination (g)

In order to get the fruit sizes of the pineapple fruits and categorising them into small, medium and large, the fruits were weighed using a toploader balance, the weight of the samples was determined, and then the fruits were grouped into various sizes (large, medium, small).

3.2.3 Pineapple Juice Extraction

The cleaned pineapples varieties were peeled by hand using stainless steel knives and sliced. Pineapple juice was extracted by use of a juice machine after blending with commercial type juice blender (Dessini). The obtained juice was pasteurised using the thermal pasteurisation (microwave and conventional) method and was then stored at different storage conditions (room temperature, supermarket condition and refrigeration condition) for further investigations.

3.3 Experimental Design

A Response Surface Method in an I-optimal design was deployed to the study the influence of pineapple variety and fruit size-independent factors on pineapple juice sensory, yield and physicochemical properties. The coding was done from -1 to +1 through 0 for minimum, maximum, and centre point, respectively. The actual levels of the independent variables are presented in Table 3.1. The complete design were made up of 27 treatment combinations, as presented in Table 3.2

Table 0.2: Independent Variables and their Level Used to Design the Experiment

Independent Variables	Levels		
	-1	0	+1
Variety (X_1)	Smooth cayenne	Sugarloaf	MD2
Size (X_2)	Big	Medium	Small

Table 0.3: Complete Design Showing Independent Variables and their Levels

	Independent variables	
Run	X_1:Variety	X_2:Size
1	Sugarloaf (0)	Small (1)
2	MD2 (1)	Big (-1)
3	MD2 (1)	Small (1)
4	MD2 (1)	Big (-1)
5	Sugarloaf (0)	Small (1)
6	Sugarloaf (0)	Big (-1)
7	Smooth cayenne (1)	Medium (0)
8	MD2 (1)	Medium (0)
9	Sugarloaf (0)	Medium (0)
10	Smooth cayenne (1)	Big (-1)
11	Smooth cayenne (1)	Medium (0)
12	Sugarloaf (0)	Small (1)
13	MD2 (1)	Big (-1)
14	MD2 (1)	Small (1)
15	Sugarloaf (0)	Medium (0)
16	MD2 (1)	Medium (0)
17	Smooth cayenne (1)	Small (1)
18	Sugarloaf (0)	Medium (0)
19	Sugarloaf (0)	Big (-1)
20	Sugarloaf (0)	Big (-1)
21	Smooth cayenne (1)	Small (1)
22	Smooth cayenne (1)	Small (1)
23	Smooth cayenne (1)	Big (-1)
24	Smooth cayenne (1)	Medium (0)
25	Smooth cayenne (1)	Big (-1)
26	MD2 (1)	Small (1)
27	MD2 (1)	Medium (0)

The effects of each factor were assessed on the responses according to the linear, quadratic and interactive model presented in equation (3.1):

$$Y = b_0 + \sum_{i=1}^{j} b_i x_j + \sum_{i=1}^{j} b_{ii} x_i^{\,2} + \sum\sum b_{ij} x_i x_j \qquad (3.1)$$

Where Y = dependent variable (response), bo = constant, bi = linear coefficient, bii the quadratic coefficient and bij the interaction coefficients xi and xj are independent variable levels. By holding the control constant and varying the two other variables, a three-dimensional plot was created. The independent variables were investigated for their effects on the dependent variables (responses). The responses measured were pH, TSS, beta-carotene, and the organoleptic characteristics (colour, aroma, taste, consistency, and overall preference) of the beverage.

3.4 Optimisation of the Juice Processing Variables

The optimisation of pineapple juice processing was achieved using Design-Expert Software version 11.1, by adopting the multivariate response method called Desirability index, DI, as described by Myers, Montgomery, Vining, Borror, and Kowalski (2004) using Equation (3.2).

$$DI = \left[\prod_{i=1}^{n^*} di\,(Y_i)\right]^{\frac{1}{n^*}} \qquad (3.2)$$

Where n is the number of responses, di represents the desirability index for each response variable, and n is the number of response variables. The desirability index ranges between 0 to 1, with 0 being the least desirable and 1 the most desirable. The goal of optimisation studies is to maximise the desirability index. The optimisation process incorporates goals and criteria for the independent and dependent variables. For this study, the goals for the

independent variables and responses were one of these five criteria: minimise, target, maximise, in range, and none.

3.5 Physicochemical Properties Determination

3.5.1 Determination of TSS (Brix)

A refractometer measures the TSS or sugar as ^{o}brix in 0.1% graduations. The total sugars of the juice samples was determined by the use of a handheld refractometer (ATAGO N1), it was calibrated with distilled water. A juice drop was placed on the refractometer prism, and the cover plate lowered to take the readings against the direction of light. The procedure was followed for all the samples, by ensuring the refractometer prism was rinsed with distilled water and dried with a soft, lint-free tissue each time measurement was made.

3.5.2 Determination of Titratable Acidity (TA)

The TA was determined as in the process of Rekha et al. (2012)using the titration method up to pH 8.1. A 10 ml fruit juice sample was diluted to 100 ml using distilled water and homogenised by shaking. The resulting solution was filtered through Whatman filter paper to get a clear filtrate. A 10 ml of the filtrate was pipetted into a 200 ml conical flask and titrated against standardised 0.1N NaOH solution from the burette using phenolphthalein as an indicator. The endpoint of the titration will be reached when the filtrate changed to a permanent pink colour. The volume of NaOH solution required for titration was noted. Titrable acidity (TA) of the juice sample was calculated according to equation (3.3).

$$\text{TA}\left(\% \frac{\text{w}}{\text{w}}\right) = \frac{\text{Net ml of titrant x Normality of titrant x 6.4}}{\text{sample weight}} \quad (3.3)$$

3.5.3 Ascorbic Acid (AA) Determination

The AA content of the pineapple varieties were determined each by the spectrophotometric method according to the protocol reported by Harris & Ray (1935)and with the slight modifications proposed by Abano, Ma, and Qu (2014). 5ml of pineapple juice was poured into a 25ml volumetric flask and diluted to the mark with 4% oxalic acid. 10ml aliquot was reassigned into a conical flask, and bromine water was added dropwise to fill it till it turns orange-yellow colour due to the excess bromine. The excess bromine was blown off. 0.5ml of the brominated extract was transferred into test tubes in triplicate, respectively. The volume was made up to 3ml with 4% oxalic acid. 1ml of DNPH reagent was added, followed by 1ml 10% of thiourea. A blank preparation was similarly made, but water in place of sample extract was used. The content was mixed thoroughly and incubated at 37^{o}C for three hours. The orange-red crystals formed (osazone) was dissolved with 4ml of 80% sulphuric acid. The absorbance of each solution was taken at 540 nm using a visible spectrophotometer.

A standard ascorbic acid was prepared similarly by pipetting 100, 200, 300, 400 and 500 µL of 10mg/ml standard ascorbic acid solution into a series of test tubes. All the reagents were similarly added as above, and absorbances were taken at 540nm. A standard calibration plot ascorbic acid concentration versus absorbance was prepared, and the AA content was calculated from the mathematical standard plot.

$$Y = 0.0024x+0.0866 \quad (4)$$

Where;

Y = absorbance and x = concentration (µg/ml).

3.5.4 Determination of Potential Hydrogen (pH)

Pineapple juice pH samples were determined using a pH meter (JENWAY) after calibration with buffer solutions of pH 4.0 and 7.0, respectively. The juice sample was put in a 100 ml beaker and stirred thoroughly. The pH electrode was immersed in the beaker and the reading of pH probe is taken after the reading stabilised. Samples were assessed when their temperature was 25 ^{o}C.

3.6 Statistical Analysis

To investigate and optimise the influence of pineapple variety fruit size on the juice yield, physicochemical, and organoleptic properties of the pineapple juice. Regression equations were obtained using Design-Expert Software (version 11.11), by fitting a quadratic polynomial model to the mean values of the experimental results (Stat-Ease, Inc., Minneapolis, USA). ANOVA was performed to determine the statistical significance of the model terms at a 5 percent probability. The accuracy of the model to describe the response variables was diagnosed based on the determination coefficient (R^2) values.

3.7 Results and Discussion

The effects of pineapple variety and fruit size on pineapple juice quality are described and explained in terms of juice yield of the pineapple juice. Also, the changes in TSS, TA, Ascorbic acid and pH, and as well as the organoleptic characteristics expressed by colour, flavour, taste, aftertaste, and overall acceptance were studied in response to the applied treatments. Pineapple varieties (Smooth Cayenne, Sugarloaf and MD2) and fruit size were the factors

used to prepare pineapple juice (big, medium and small). The relevance of the changes recorded in the studied samples in response to independent factors was expressed, focusing on the data obtained through statistical analysis.

Table 3.3 displays the results of the 27 experiments conducted according to the RSM. Regression analysis was used, as shown in Table 3.4, to analyse the statistical significance of the variables. It displays the quadratic models' approximate regression coefficients for the response variables, along with the corresponding decision coefficients (R^2).

Table 0.4: Effects of Pineapple Variety and Fruit Size on Juice yield, TSS, pH, TA and Sensory Attributes of Pineapple Juice

Run No.	Coded values		Experimental values of the experiment									
	X_1: Variety	X_2: Size	Juice Yield (ml/kg)	TSS (%brix)	pH	TA (%)	AA (mg/L)	Colour	Aroma	Taste	Aftertaste	OA
1	1	3	406.421	13.0	4.00	0.6272	43.0833	6.44	6.26	6.48	6.28	6.52
2	3	1	391.932	11.0	4.02	0.7398	131.8333	6.60	6.28	5.48	5.44	5.96
3	3	3	382.471	11.8	4.00	0.7962	177.6667	6.66	6.20	5.34	5.18	5.82
4	3	1	380.270	11.2	4.03	0.7552	129.3333	6.38	5.78	5.54	5.28	5.84
5	1	3	483.244	12.9	4.00	0.6144	46.0000	6.52	6.28	6.48	6.40	6.60
6	1	1	299.862	12.9	4.11	0.6093	46.4167	6.94	6.90	7.06	6.78	7.06
7	2	2	430.346	13.9	4.03	0.6016	71.0000	4.98	6.14	6.46	5.90	6.16
8	2	2	361.268	10.9	4.01	0.7475	113.0833	6.60	6.24	5.64	5.52	5.82
9	3	2	340.210	13.9	4.05	0.6067	43.5000	6.50	6.52	6.86	6.76	6.92
10	1	1	516.262	12.9	4.02	0.5862	114.3333	5.14	5.84	6.04	5.66	5.88
11	2	2	484.142	13.0	4.04	0.6195	63.9167	5.30	5.82	6.28	6.06	6.30
12	1	3	427.957	13.0	4.00	0.6067	47.6667	6.26	6.18	6.24	6.42	6.64
13	3	1	384.979	11.0	4.03	0.7450	127.2500	6.22	6.08	5.42	5.58	5.76

Table 3.3.Con't

14	3	3	351.018	11.9	4.00	0.7936	178.9167	6.32	6.08	5.60	5.32	5.72
15	1	2	342.928	13.9	4.03	0.5837	36.4167	6.46	6.32	6.56	6.46	6.72
16	3	2	296.004	11.0	4.02	0.7501	118.9167	6.50	6.02	5.88	5.66	6.08
17	2	3	413.103	13.5	4.00	0.7347	106.0000	4.14	5.30	5.46	5.26	5.44
18	1	2	341.693	14.0	4.04	0.5888	39.7500	6.46	6.34	6.68	6.60	6.82
19	1	1	326.114	13.5	4.12	0.5862	46.0000	6.94	6.62	6.96	6.30	6.96
20	1	1	320.387	13.6	4.12	0.5939	45.5833	6.92	6.48	6.98	6.90	7.30
21	2	3	425.346	13.0	4.01	0.7424	109.7500	4.12	5.10	5.28	5.24	5.42
22	2	3	391.859	12.0	4.02	0.7296	106.0000	4.34	5.02	5.40	5.12	5.26
23	2	1	559.256	12.8	4.04	0.5914	116.4167	5.62	7.12	5.98	5.78	6.04
24	2	2	469.832	13.7	4.05	0.6118	102.2500	5.18	5.94	6.24	5.94	6.36
25	2	1	510.579	14.0	4.04	0.5990	102.6667	5.68	5.74	6.04	6.08	6.12
26	3	3	335.810	11.7	4.00	0.7910	184.7500	6.48	6.26	5.72	5.70	5.74
27	3	2	363.480	11.0	4.03	0.7552	113.9167	6.00	6.16	5.68	5.42	5.76

Values are means of triplicate determination

Pineapple variety: Smooth cayenne (1), Sugarloaf (2), MD2 (3)

Fruit size: Big (1), Medium (2), Small (3)

Table 0.5: Regression Coefficients and R^2 values for the Model Terms in the Quadratic RSM for the Various Responses of Pineapple Juice

	b_o	X_1	X_2	X_1X_2	X_1^2	X_2^2	R^2
Juice Yield	429.274	-12.0874	-9.5207	-26.7556	-84.2945**	38.3578	0.4595
p-values		0.3813	0.4889	0.1067	0.0018	0.1201	
TSS	13.0695	-0.9128**	0.0467	0.3109	-0.2826	-0.3945	0.5626
p-values		0.0001	0.8093	0.1808	0.4082	0.2520	
pH	4.03278	-0.0124*	-0.0260**	0.0170*	0.0035	-0.0058	0.6674
p-values		0.0290	< 0.0001	0.0122	0.7110	0.5369	
TA	0.638865	0.0750**	0.0316**	0.0068	0.0080	0.0392*	0.7555
p-values		< 0.0001	0.0067	0.5869	0.6678	0.0454	
AA	79.7536	43.6319**	5.6259	16.6309*	-11.7329	33.5350**	0.7775
p-values		< 0.0001	0.3308	0.0205	0.2492	0.0028	
Colour	5.26334	-0.0233	-0.2100	0.0305	1.3267**	-0.2400	0.6249
p-values		0.8668	0.1411	0.8517	< 0.0001	0.3296	
Aroma	5.94262	-0.0964	-0.1979	0.0795	0.4226*	-0.1731	0.3986
p-values		0.3287	0.0528	0.4903	0.0207	0.3170	
Taste	6.1135	-0.4514**	-0.1443	0.1099	0.3420*	-0.4194**	0.6899
p-values		< 0.0001	0.0954	0.2702	0.0278	0.0086	
Aftertaste	5.86518	-0.4278**	-0.1145	-0.0004	0.3922*	-0.3544*	0.6210
p-values		< 0.0001	0.2118	0.9969	0.0199	0.0334	
OA	6.0976	-0.3929**	-0.1582	0.0293	0.4909**	-0.3650*	0.6668
p-values		0.0001	0.0709	0.7673	0.0029	0.0220	

*significant at $p<0.05$, **significant at $p<0.01$, b_o is the intercept of the model

3.7.1 Effect of Pineapple Variety and Fruit Size on Juice Yield

Pineapple is more preferred for its juice than fresh, and thus the volume of juice yield is of significant importance. From the experimental values of the effect of pineapple variety and fruit size, the juice yield of the three pineapple varieties ranged from 296.004 ml/kg – 559.256 ml/kg in the range of the independent variables (Table 3.3). The processing of large size Sugarloaf variety yielded the highest (559.256 ml/kg) juice content. The study showed that large size Smooth cayenne pineapple variety had the second highest (516.262 ml/kg) juice yield content. The lowest juice yield (296.004 ml/kg) was recorded from medium size MD2 pineapple variety. From the results, on average, Smooth Cayenne is seen to have more juice giving the best prospects and advantages in this enterprise. The difference in juice yield among the varieties may be due to the varietal differences of the pineapples, climatic conditions, soil characteristics and other environmental factors.

The quadratic effect of pineapple variety (X_1) on juice yield was very profound and showed statistical significance ($p<0.01$). On the other hand, the linear and quadratic effect of fruit size (X_2) also showed a p-value higher than 0.05. Furthermore, the interaction between X_1 (pineapple variety) and X_2 (fruit size) gave p-value higher than 0.05, which is considered insignificant. The R^2 value of the model for juice yield was 0.4595 (Table 3.4). It implies it is less sufficient for a good model and has more deviation from the graphical fit. The 3D response surface plot showing the effect of the relationship between pineapple variety and fruit size on pineapple juice yield is presented in Fig. 3.1.

$$Y = 429.27 - 12.09X_1 - 9.52X_2 - 26.76X_1X_2 - 84.29X_1^2 + 38.36X_2^2$$

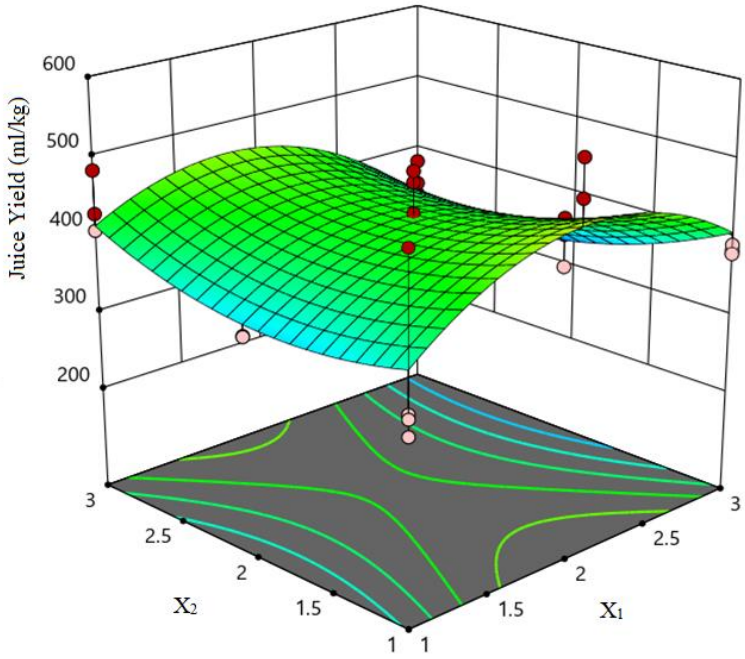

Figure 0.1: 3D RSM plot for the effect of pineapple variety (X_1) and fruit size (X_2) on the Juice Yield of pineapple juice

3.7.2 Effect of Pineapple Variety and Fruit Size on Physiochemical Properties

3.7.3 Effect of Pineapple Variety and Fruit Size on Total Soluble Solids (TSS)

According to McAllister (1980), the Total Soluble Solid is an essential parameter in fruit juice and is often used for grading its quality (Bartholomew et al., 2003). The total sugars content for pineapples ranges between 10.8 - 17.5% (Dull, 1971) with minimal differences between varieties. From the study, TSS of the pineapple juice ranged from 10.9 to 14.0 % in the range of the independent variables (Table 3.3). The data for pineapple varieties for the study are within these limits. Among all the experimental units in processing the pineapple juice, medium-sized Sugarloaf pineapple variety had the highest

(14.0 %) brix content. The lowest TSS content (10.9 %) in pineapple juice was recorded for medium size, Smooth cayenne pineapple variety. In general, the Sugarloaf pineapple fruit indicated to have averagely higher TSS content, compared to Smooth cayenne and MD2 pineapple varieties. It can therefore be said that the TSS of pineapple juice varied mainly as a function of the pineapple variety used in the juice processing. As shown in Table 3.4, although the effect of the interaction between pineapple variety and fruit size (X_1X_2) on total soluble solids was insignificant, the linear effect of pineapple variety (X_1) was seen to have a profound significant ($p<0.01$) effect on the TSS value of the pineapple juice. Also, the quadratic effect of the pineapple variety (X_1^2) and fruit size (X_2^2) showed no statistically significant ($p<0.05$) effect on the TSS level of the pineapple juice. The R^2 of the models for TSS (%brix) was 0.5626. This value is sufficient for a good model, with moderate deviation from the graphical fit. Figure 3.2 shows a graphically represented 3D response surface generated by the model. It depicts the relationship between pineapple variety and fruit size on TSS content estimations

$$Y = 13.07 - 0.913X_1 + 0.047X_2 + 0.311X_1X_2 - 0.283X_1^2 - 0.395X_2^2$$

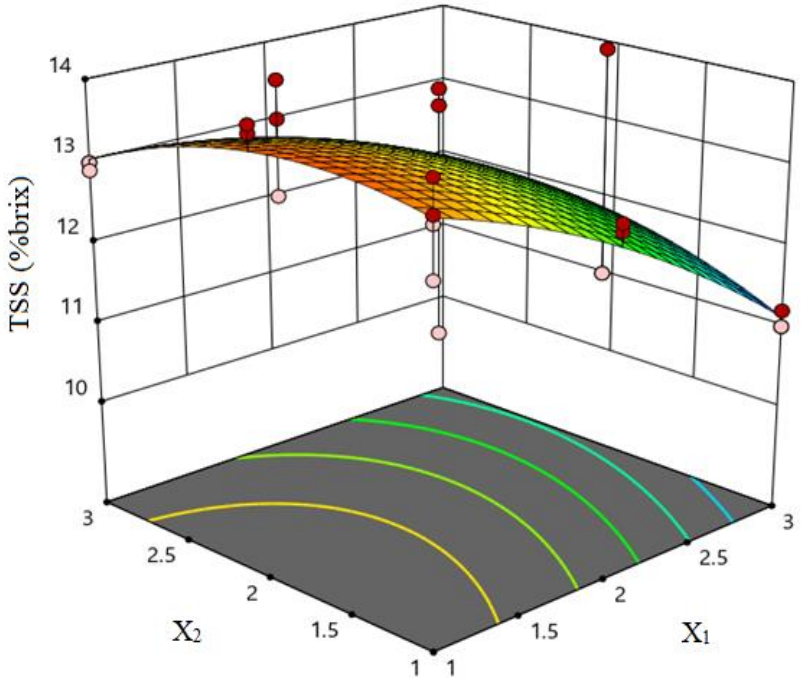

Figure 0.2: 3D RSM plot for the effect of pineapple variety (X_1) and fruit size (X_2) on the TSS of pineapple juice

3.7.4 Effect of Pineapple Variety and Fruit Size on Potential Hydrogen (pH)

The pH of the pineapple juice collected ranged from 4.00 to 4.055 from the experimental values of the pineapple variety effect and fruit size (Table 3.3). This corresponds to the pH range of 4 to 4.5 in pineapple fruits, as recorded by Kim, Park, Cho, and Park (2001). As can be seen from Table 3.4 and Figure 3.3, the pH spectrum of the pineapple juice suggests that the pineapple juice is greatly influenced by both the pineapple variety and the size of the fruit. A significant ($p<0.05$) low pH of pineapple juice was reported irrespective of the pineapple variety and fruit. Furthermore, the interaction effects between pineapple variety and fruit size (X_1X_2), was seen to have a positive highly significant effect on the pH of the pineapple juice obtained. The quadratic

effects of the independent variables were, however, non-significant ($p>0.05$) on pineapple juice pH (Table 3.4). The R^2 of the models for pH was 0.6674. This value is adequately sufficient for a good model, with less deviation from the graphical fit.

$$Y = 4.03 - 0.012X_1 - 0.026X_2 + 0.017X_1X_2 + 0.004X_1^2 - 0.006X_2^2$$

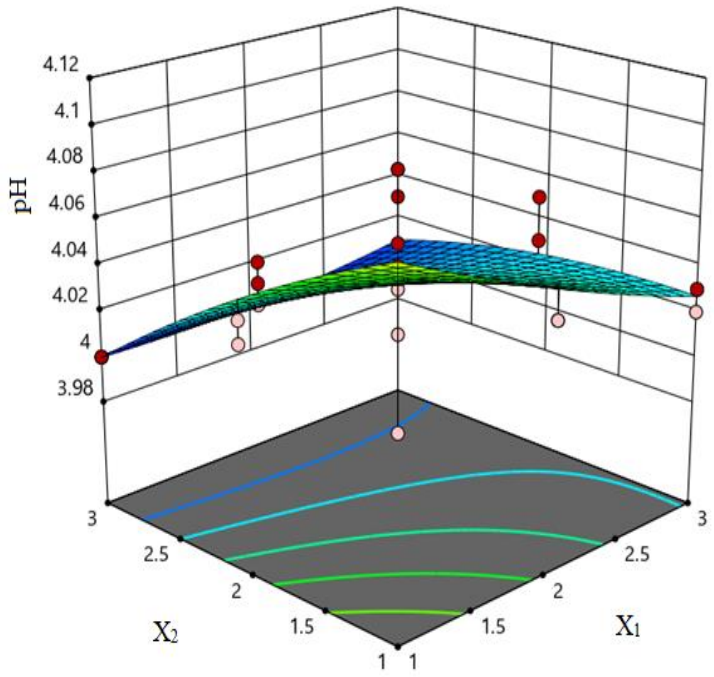

Figure 0.3: 3D RSM plot for the effect of pineapple variety (X_1) and fruit size (X_2) on the Potential Hydrogen (pH) of pineapple juice

3.7.5 Effect of Pineapple Variety and Fruit Size on Titratable Acidity (TA)

The titratable acidity content of the three pineapple varieties ranged from 0.5837 to 0.7962% (Table 3.3). The highest (0.7962%) titratable acidity content of pineapple juice was obtained from small size, MD2 pineapple variety, while the lowest (0.5837%) was obtained from a medium-size, Smooth cayenne pineapple variety. The higher acidity of the MD2 pineapple variety may imply a more extended storage life (Wardy, Saalia, Steiner-Asiedu, Budu, & Sefa-

Dedeh, 2009). This may be very useful for juice processors as longer shelf life allows sufficient time for handling, processing and selling.

The variation in titratable acidity content of the pineapple juice as affected by the independent variables is shown in Table 3.4, and the 3D response surface plots in Figure 3.4. The main effect of pineapple variety (X_1) and fruit size (X_2) significantly ($p<0.01$) affected the pineapple juice titratable acidity content positively. Furthermore, the interaction effects between pineapple variety and fruit size (X_1X_2) significantly ($p<0.01$) affected the titratable acidity content of the pineapple juice positively. This was not the same for the quadratic term, as the effect of the pineapple variety (X_1^2) and fruit size (X_2^2) showed no statistical significance ($p<0.05$) on titratable acidity of pineapple juice. The R^2 of the models for titratable acidity was 0.7555. This value is adequately sufficient for a good model, with less deviation from the graphical fit.

$$Y = 0.639 + 0.075X_1 + 0.032X_2 + 0.007X_1X_2 + 0.008X_1^2 - 0.039X_2^2$$

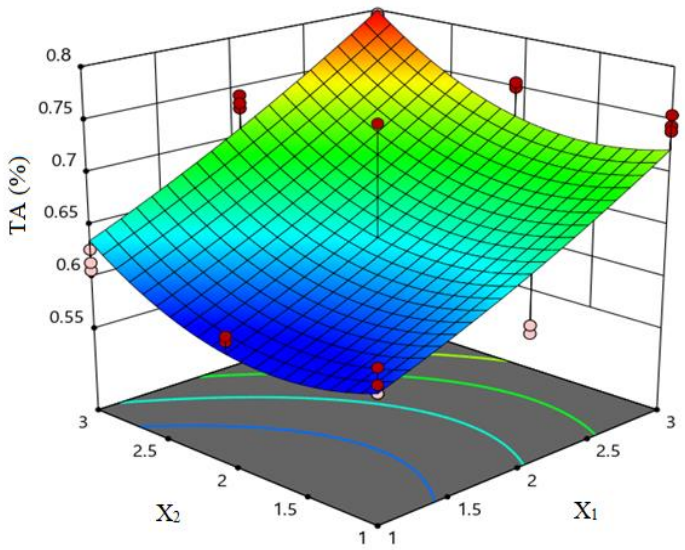

Figure 0.4: 3D RSM plot for the effect of pineapple variety (X_1) and fruit size (X_2) on the Titratable Acidity (TA) of pineapple juice

3.7.6 Effect of Pineapple Variety and Fruit Size on Ascorbic Acid (AA)

In general, the ascorbic acid content of fruit is an essential quality index due to its health significance as a vitamin and cell antioxidant. In addition, ascorbic acid acts as an antioxidant in food production by being preferentially oxidized (Bartholomew et al., 2003). Pineapple juice's ascorbic acid content varies depending on the cultivar, the stage of maturity, the conditions of storage and the fruit component (Ihekoronye & Ngoddy, 1985). Among the pineapple varieties, the MD2 variety showed considerably higher ascorbic acid (184.750 mg/L) content, whiles the smooth cayenne variety had the lowest ascorbic acid content compared to sugarloaf pineapple variety (Table 3.3). The study by Lu, Sun, Wu, Liu, and Sun (2014) among pineapple types also showed that MD2 pineapple fruit contained the highest amount of vitamin C. As shown in Table 3.4, The concentration levels of AA in the pineapple juice was observed to be significantly influenced by the main effect of pineapple variety (X_1) at $p<0.01$, the interaction effect of pineapple variety and fruit size (X_1X_2) at $p<0.05$, and the quadratic effect of fruit size (X_2^2) at $p<0.0$. The R^2 of the models for ascorbic acid was 0.7775. This value is adequately sufficient for a good model, with a minor deviation from the graphical fit. Figure 3.5 shows a graphically represented 3D response surface generated by the model. It depicts the relationship between pineapple variety and fruit size on ascorbic acid content estimations

$$Y = 79.75 + 43.63X_1 + 5.63X_2 + 166.63X_1X_2 - 11.73X_1^2 + 33.53X_2^2$$

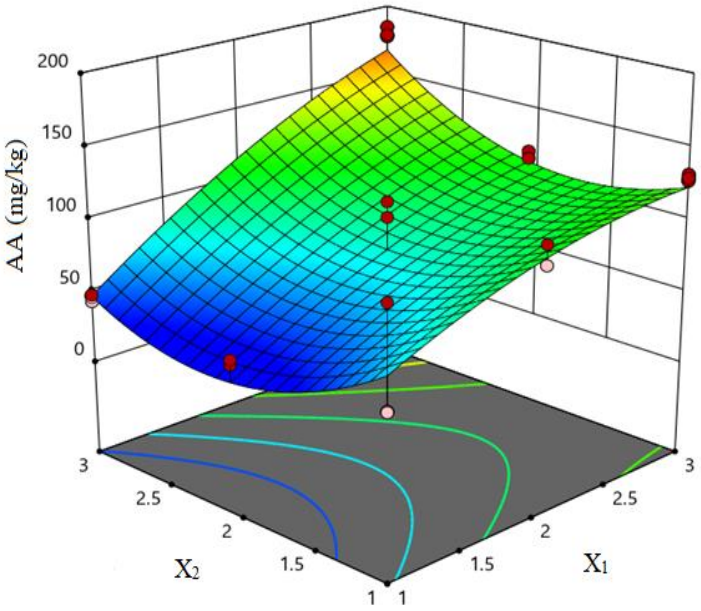

Figure 0.5: 3D RSM plot for the effect of pineapple variety (X_1) and fruit size (X_2) on the AA of pineapple juice

3.7.7 Effect of Pineapple Variety and Fruit Size on Juice Sensory Properties

3.7.8 Effect of Pineapple Variety and Fruit Size on Colour

The colour of food products is an important aspect that consumers take into consideration when making purchasing decisions. (Chen & Paull, 2001). The sensory score for colour ranged from an average value of 4.17 to 6.94 (Table 3.3). The colour (6.94) score of pineapple juice, obtained from the large size, Smooth cayenne pineapple variety, was the most preferred. This may probably be that the colour of the Smooth cayenne was more appealing than the other varieties studied, and since consumers will not appreciate pineapple juice that deviates from the natural pineapple juice colour. The Sugarloaf pineapple juice had the lowest colour (4.17) score ranking as compared to MD2 pineapple

variety. MD2 pineapple variety was the second most preferred colour of the pineapple juice.

As shown from Table 3.4 and Figure 3.6, the quadratic term of pineapple variety (X_1^2) was found to have a profound positive significant ($p<0.01$) effect on the colour of the pineapple juice. Colour of the pineapple juice insignificantly ($p<0.05$) decreased with a linear term of pineapple variety (X_1) and fruit size (X_2). Furthermore, the interaction effect between pineapple variety and fruit size (X_1X_2), and quadratic effect of the fruit size (X_2^2) was found to have no statistical ($p<0.05$) influence on pineapple juice colour. As was expected, this result shows that the sensory score for colour was affected only by the pineapple variety.

$$Y = 5.26 - 0.023X_1 - 0.210X_2 + 0.031X_1X_2 + 1.33X_1^2 - 0.24X_2^2$$

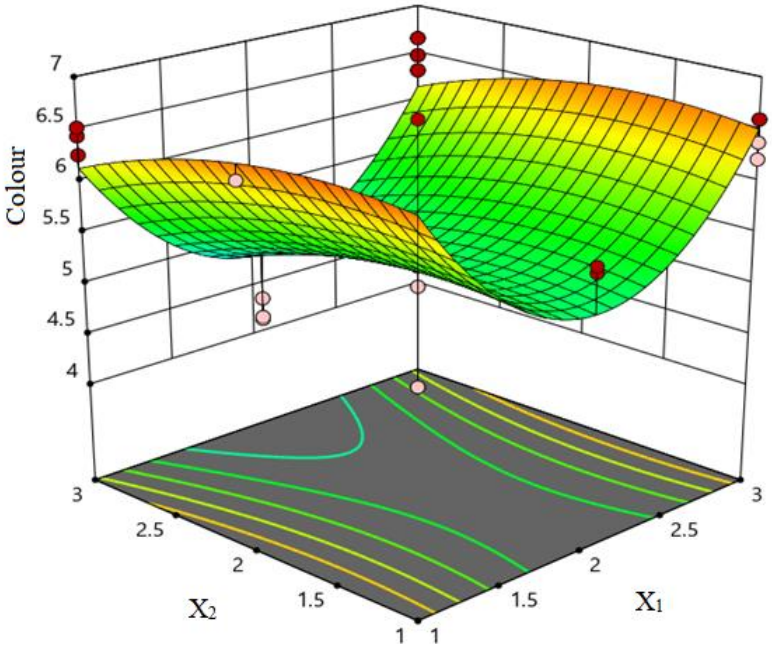

Figure 0.6: 3D RSM plot for the effect of pineapple variety (X_1) and fruit size (X_2) on the colour of pineapple juice

3.7.9 Effect of Pineapple Variety and Fruit Size on Aroma

The aroma score of the pineapple juice ranged from 5.02 to 7.12 in the range of the independent variables (Table 3.3). It implies that the consumers generally liked the aroma of the pineapple juice. Also, it is because the pineapple fruits used for the juice processing were all matured, and this improves on the aroma of the fruits (Bender, Brecht, Baldwin, & Malundo, 2000). Although, the study recorded that, the best aroma score (7.12) was obtained from the large size, Smooth cayenne pineapple variety, which was not significantly different from all the other treatments. As observed from Table 3.4 and Figure 3.7, a related trend from the colour response was observed for the aroma response. The quadratic term of pineapple variety (X_1^2) was found to have a positive significant ($p<0.05$) effect on the aroma of the pineapple juice. The aroma of the pineapple juice decreased insignificantly ($p<0.05$) according to the linear term of pineapple variety (X_1) and fruit size (X_2). Furthermore, the interaction effect between pineapple variety and fruit size (X_1X_2), and quadratic effect of the fruit size (X_2^2) was found to have no statistical ($p<0.05$) influence on pineapple juice aroma. As was expected, this result shows that the sensory score for aroma was affected only by the pineapple variety.

$$Y = 5.94 - 0.096X_1 - 0.198X_2 + 0.080X_1X_2 + 0.42X_1^2 - 0.173X_2^2$$

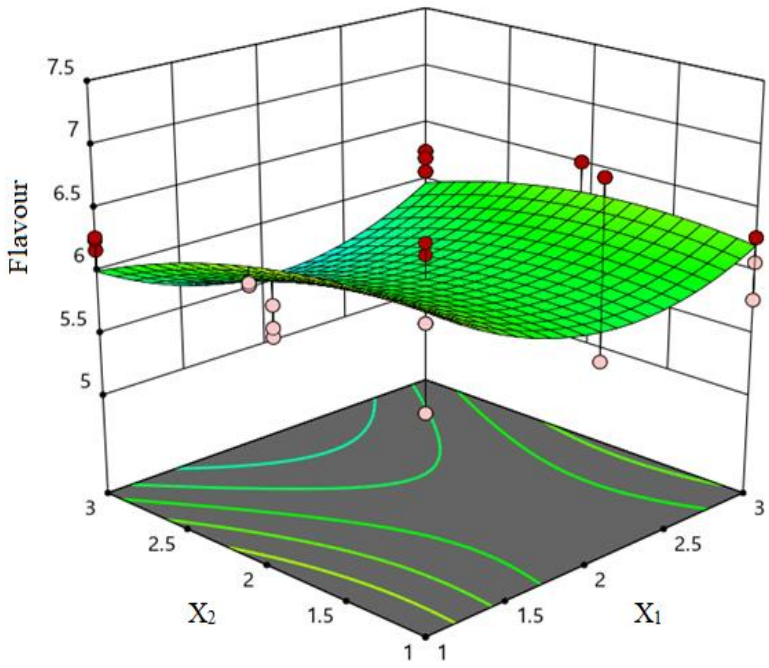

Figure 0.7: 3D RSM plot for the effect of pineapple variety (X_1) and fruit size (X_2) on the Flavour of pineapple juice

3.7.10 Effect of Pineapple Variety and Fruit Size on Taste

The average values for taste response ranged from 5.28 to 7.06, indicating that all products were acceptable in terms of taste (Table 3.3). Taste of juice produced from the large size, Sugarloaf pineapple fruit was most preferred (7.06) compared to the medium size, smooth cayenne (5.28). This may be due to the high TSS of the Sugarloaf variety compared to the MD2 and Smooth cayenne. The characteristic of taste is assesed by the content of sugar and organic acids (Kader, 2008).

The main effect of pineapple variety (X_1) negatively significantly ($p<0.01$) affected the taste of the pineapple juice as rated by the sensory panellists (Table 3.4). Furthermore, the quadratic term, as the effect of the pineapple variety (X_1^2) and fruit size (X_2^2) showed statistical significance

($p<0.05$) on the aroma of the pineapple juice. The R^2 of the models for titratable acidity was 0.6899. This value is adequately sufficient for a good model, with less deviation from the graphical fit. Figure 3.8 shows a graphically represented 3D response surface generated by the model. It depicts the relationship between pineapple variety and fruit size on the taste score of the pineapple juice

$$Y = 6.11 - 0.451X_1 - 0144X_2 + 0.109X_1X_2 + 0.342X_1^2 - 0.419X_2^2$$

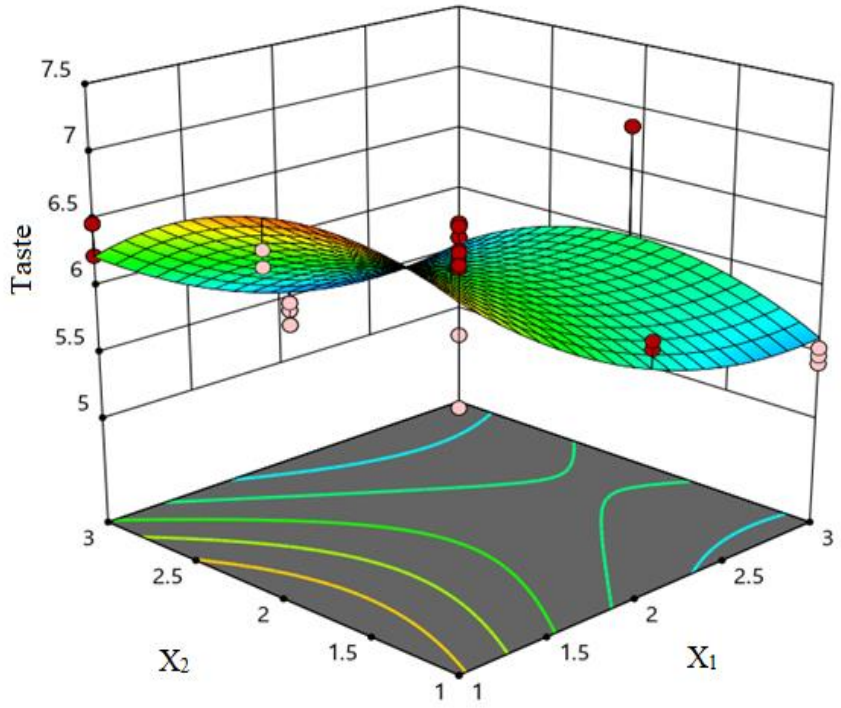

Figure 0.8: RSM plot for the effect of pineapple variety (X_1) and fruit size (X_2) on the Taste of pineapple juice

3.7.11 Effect of Pineapple Variety and Fruit Size on Aftertaste

Aftertaste is the lingering of the sense of taste of a product on taste buds. The sensory score for aftertaste ranged from an average value of 5.12 to 6.90 (Table 3.3). The least score for aftertaste was obtained for pineapple juice processed from small size, Sugarloaf variety (5.12), while the highest mean aftertaste score (6.90) was obtained for pineapple juice processed from the large

size, Smooth cayenne. This may be due to the sweetening nature of the pineapple variety.

As shown from Table 3.4 and Figure 3.6, the main effect of pineapple variety (X_1) negatively significantly ($p<0.01$) affected the aftertaste of the pineapple juice as rated by the sensory panellists (Table 3.3 and Figure 3.9). Furthermore, the quadratic term, as the effect of the pineapple variety (X_1^2) and fruit size (X_2^2) showed statistical significance ($p<0.05$) on the aftertaste of the pineapple juice. The R^2 of the models for titratable acidity was 0.6210. This value is adequately sufficient for a good model, with less deviation from the graphical fit.

$$Y = 5.87 - 0.43X_1 - 0.115X_2 - 0.0004X_1X_2 + 0.392X_1^2 - 0.354X_2^2$$

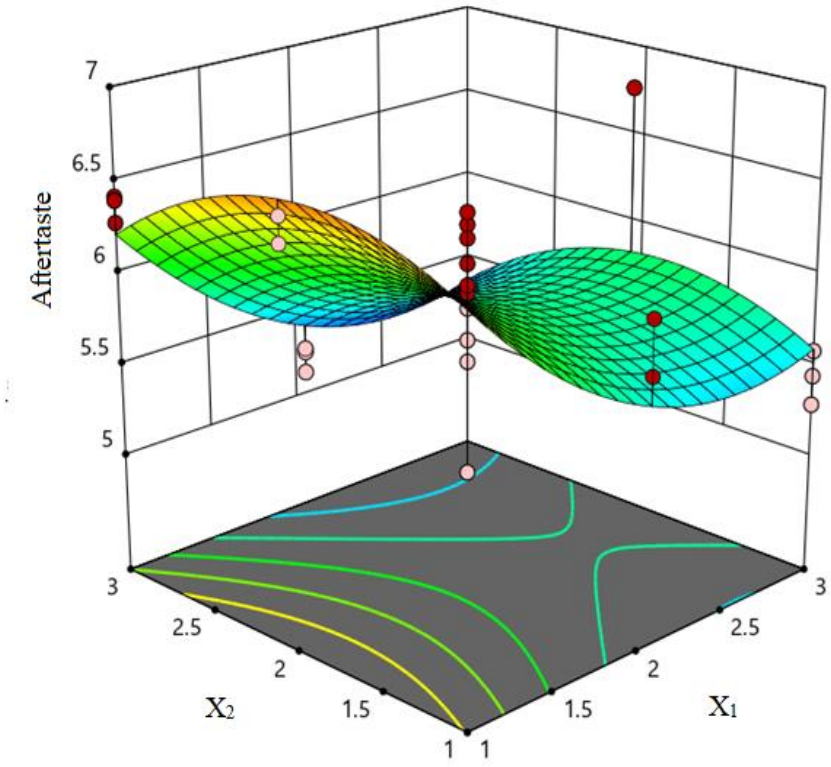

Figure 0.9: RSM plot for the effect of pineapple variety (X_1) and fruit size (X_2) on the Aftertaste of pineapple juice

3.7.12 Effect of Pineapple Variety and Fruit Size on Overall Acceptability

Average OA of the pineapple juice ranged from 5.26 to 7.30, indicating that all the products were slightly acceptable. Average overall acceptability was lowest from juice obtained from small size, Sugarloaf pineapple variety, and the highest score was obtained from the large size, Smooth cayenne pineapple variety (Table 3.3). The main effect of pineapple variety (X_1) negatively significantly ($p<0.01$) affected the OA of the pineapple juice as rated by the sensory panellists (Table 3.4 and Figure 3.10). Furthermore, the quadratic term, as the effect of the pineapple variety (X_1^2) and fruit size (X_2^2) showed statistical significance ($p<0.05$) on the overall acceptability of the pineapple juice. The R^2 of the models for OA was 0.6668. This value is adequately sufficient for a good model, with less deviation from the graphical fit.

$$Y = 6.10 - 0.393X_1 - 0.158X_2 + 0.029X_1X_2 + 0.491X_1^2 - 0.360X_2^2$$

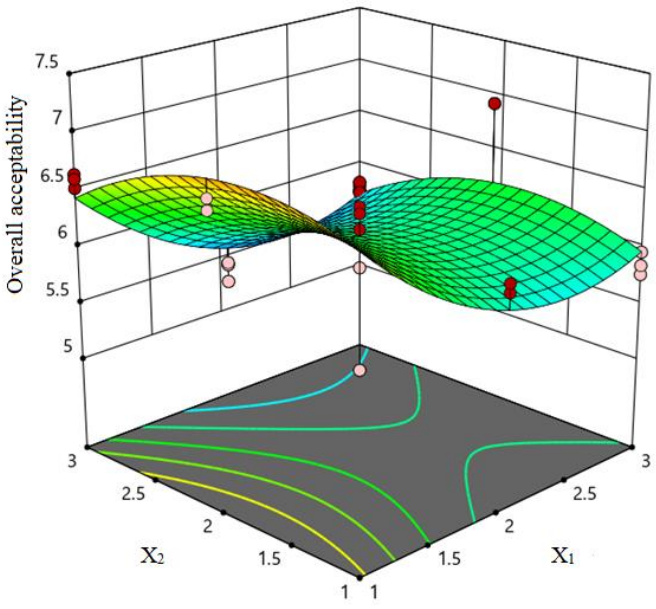

Figure 0.10: RSM plot for the effect of pineapple variety (X_1) and fruit size (X_2) on the Overall acceptability of pineapple juice

3.7.13 Optimization of the Variables and Responses

Using the Design-Expert 11.1 program (Stat-Ease, Inc., Minneapolis, USA), the levels of variables were optimised using the Desirability Index principle in equation 3.2. The criteria were to set targets for independent variables and response models to optimise the desirability index, explaining the highest amount of variation with the fewest terms chosen. From Table 4.7, all equations were moderately predictive for the model, except the equation for juice yield and aroma which were not good predictive (R^2 = 0.4595 and 0.3986) respectively. The desirability index of the response gave an overall desirability index of 0.620 (Figure 3.11). The result projected 95 percent confidence in the range of independent variables gave optimum pineapple variety (Smooth cayenne) and pineapple size (big). At this optimum condition, juice yield, Brix, pH, TA, AA, colour, aroma, taste, aftertaste and overall acceptability were found to be 376.180 ml/kg, 13.587%, 4.085, 0.584%, 66.271 mg/kg, 6.6, 6.6, 6.8, 6.5 respectively.

Table 0.6: Criteria Applied for Optimization of the Variables

Constraint	Goal	Lower limit	Upper limit
Pineapple variety	is in range	1	3
Pineapple size	is in range	1	3
Juice yield	none	296.004	559.256
Brix	maximize	10.9	14
pH	minimize	4	4.12
TA	minimize	0.58368	0.79616
AA	maximize	36.4176	184.75
Colour	maximize	4.12	6.94
Aroma	none	5.02	7.12
Taste	maximize	5.28	7.06
Aftertaste	maximize	5.12	6.9
Overall acceptability	maximize	5.26	7.3

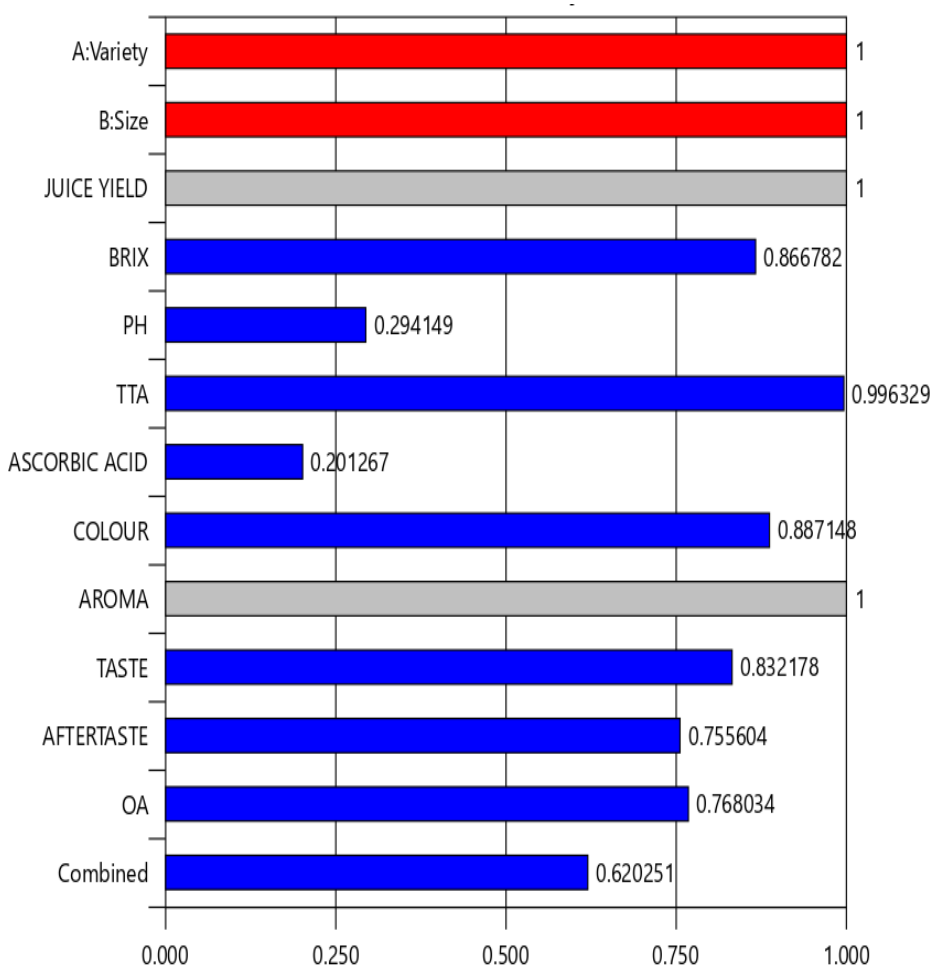

Figure 0.11: Desirability index graph for the variables after optimization

CHAPTER FOUR

INFLUENCE OF PASTEURISATION ON PINEAPPLE JUICE QUALITY

4.1 Introduction

There has been a growing interest of fruit juice in recent times as a result of the health benefits they provide and the ease of consumption they offer. Juice is an essential dietary health supplement. Pineapple is one of the most important fruits of Ghana. It can be bought fresh or processed for consumption. Pineapple juice is well-known for its sweet and sour taste as well as its benefits to the body. Its juice is considered to provide the antioxidant components, which include vitamin C, bromelain, carotenoids, phenolic compounds and flavonoids, among others (Larson, 1988).

Recent reports of foodborne occurrences related to the ingestion of untreated fruit juices (Aneja, Dhiman, Aggarwal, & Aneja, 2014; Vojdani, Beuchat, & Tauxe, 2008) are now available to consumers for high-quality, slightly processed and microbiologically safe fruit juice. Not all spoilage microbes can survive at a pH 3-4. The current spat in foodborne outbreaks associated with fruit juice has made most fruit juice production processors to formally integrate a pasteurisation method in order to ensure a safety free microbial and stability of fruit juice. The common processing method for microbe free fruit juice is the traditional heat treatments, also known as conventional pasteurisation, which remain of interest to preserve pineapple juice, as its aids in the inactivation of microbial growth, and enhancing fruit juice shelf life. Though, the higher amount of heat intensity could deteriorate

the nutritional qualities of fruit juices, by denaturing their physical and chemical properties (Aneja et al., 2014; Chen, Yu, & Rupasinghe, 2013). These limitations have inspired research and advancement of treatments with minimal effect on the physicochemical properties of pineapple juice. (Cortés, Esteve, & Frígola, 2008)

In order to produce a highly nutritious fruit juice, a potential alternative thermal pasteurisation was introduced, which is microwave pasteurisation. Microwave pasteurisation has been reported to be effective in maintaining the quality of fruit juices and enhancing the inactivation of microorganisms, which resulted in an extended shelf life (Demirdöven & Baysal, 2015). It also reduces a significant period during the process, thus lowers the risk of losing essential nutrition in the juice (Benlloch-Tinoco, Igual, Rodrigo, & Martínez-Navarrete, 2015).

In this study, the optimum conditions for microwave pasteurisation and conventional thermal treatment on pineapple juice will be determined. Influence of microwave and conventional heat treatment on physicochemical properties of pineapple juice will be evaluated. Smooth cayenne pineapple variety was used for juice production. This study would be beneficial for providing alternative juice pasteurisation for industrial application. This objective, therefore, seeks to research on the following specific objectives;

i. To evaluate the effect of conventional and microwave pasteurisation on physicochemical properties of pineapple juice
ii. To evaluate the effect of conventional and microwave pasteurisation on the microbial load of pineapple juice.

4.2 Materials and Methods

4.2.1 Pineapple Juice Extraction

Smooth cayenne pineapple variety of uniform pineapple size was manually peeled using stainless steel knife and chopped into pieces, and there was fruit extraction done using a juice extractor after blending with commercial type juice blender. The obtained juice was pasteurised using the thermal pasteurisation (microwave and conventional) methods. Pasteurised juice was stored at 4 ^{o}C refrigeration condition before juice quality determination.

4.2.2 Microwave Pasteurisation

The microwave pasteurisation of the pineapple juice was determined according to the protocol of González-Monroy, Rodríguez-Hernández, Ozuna, and Sosa-Morales (2018). A 245MHz microwave was used to pasteurise the juice. 250ml of pineapple juice was poured into a stainless-steel container. The microwave power ranged from 400W, 650W and 900W, and the time range was between 60-180sec. The microwave-assisted pasteurisation was carried out in batches. Microwaved juice was taken out immediately from the oven and cooled in an ice bath until room temperature. The cooled juice was bottled immediately.

4.2.3 Hot Water Pasteurisation (Conventional)

Pineapple fruit juice was pasteurised in batches with respect to their variety on a hot plate (Heidolph MR Hei-Standard). The temperatures that were used for the pasteurisation was 80, 85 and 90 ^{o}C, and the time ranges were 60 - 180 sec. The stainless-steel container was placed on the hot surface until the target temperature with the time reached using the digital thermometer and timer. The stainless-steel container was immediately taken off from the hot

surface into an ice bucket until room temperature and then packaged into their various packaging material.

4.3 Experimental Design

A Response Surface Method in an I-optimal design was deployed to study the effect of pasteurisation (microwave and conventional) on juice quality. The coding was done from -1 to +1 through 0 for minimum, maximum, and centre point, respectively. The actual levels of the independent variables for microwave pasteurisation and conventional pasteurisation are shown in Table 4.1.

Table 0.7: Independent Variables and their Level Used to Design the Experiment for Pasteurisation treatment

Pasteurisation	Independent Variables	Levels		
		-1	0	+1
Microwave	Microwave Power; X_1 (W)	400	650	900
	Microwave Time; X_2 (secs)	60	120	190
Conventional	Water Temperature; X_1 (^oC)	80	85	90
	Conventional Time; X_2 (secs)	60	120	180

The complete experimental design resulted in 27 treatment combinations each for microwave and conventional pasteurization treatment conditions (Table 4.2), The effects of each factor were assessed on the responses and partitioned into linear, quadratic and interactive components as represented in equation (4.1):

$$Y = b_0 + \sum_{i=1}^{j} b_i x_j + \sum_{i=1}^{j} b_{ii} x_i^{\,2} + \sum\sum b_{ij} x_i x_j \qquad (4.1)$$

Where Y= the dependent variable (response), bo=constant, bi=linear coefficient, bii the quadratic coefficient and bij the interaction coefficients, xi and xj are the independent variable levels, three-dimensional plots were generated by keeping one variable constant at the centre point and by varying the other two variables within the experimental range. Independent variables were investigated to find out their effects on dependent variables (responses). The measures were pH, total suspended solids, beta-carotene, and the organoleptic properties of the beverage.

Table 0.8: Complete Design Showing Independent Variables and their Levels

Run	Independent variables			
	Microwave		Conventional	
	X_1:Microwave power (W)	X_2:Microwave time (sec)	X_1:Conventional temperature (^oC)	X_2:Conventional time (sec)
1	400 (-1)	180 (1)	80 (-1)	180 (1)
2	600 (0)	60 (-1)	85 (0)	60 (-1)
3	400 (-1)	120 (0)	85 (0)	180 (1)
4	600 (0)	60 (-1)	90 (1)	180 (1)
5	900 (1)	120 (0)	85 (0)	120 (0)
6	400 (-1)	180 (1)	90 (1)	180 (1)
7	600 (0)	180 (1)	90 (1)	120 (0)
8	400 (-1)	60 (-1)	90 (1)	60 (-1)
9	600 (0)	120 (0)	80 (-1)	120 (0)
10	900 (1)	60 (-1)	80 (-1)	180 (1)
11	900 (1)	120 (0)	90 (1)	180 (1)
12	600 (0)	180 (1)	90 (1)	60 (-1)
13	600 (0)	60 (-1)	90 (1)	120 (0)
14	400 (-1)	120 (0)	85 (0)	180 (1)
15	400 (-1)	60 (-1)	90 (1)	60 (-1)
16	600 (0)	120 (0)	85 (0)	180 (1)
17	600 (0)	120 (0)	85 (0)	60 (-1)
18	400 (-1)	60 (-1)	80 (-1)	60 (-1)
19	900 (1)	60 (-1)	80 (-1)	60 (-1)
20	900 (1)	120 (0)	80 (-1)	180 (1)

Table 4.2.Con't D

21	400 (-1)	180 (1)	85 (0)	120 (0)
22	400 (-1)	120 (0)	80 (-1)	60 (-1)
23	900 (1)	60 (-1)	90 (1)	120 (0)
24	900 (1)	180 (1)	85 (0)	120 (0)
25	600 (0)	180 (1)	80 (-1)	120 (0)
26	900 (1)	180 (1)	85 (0)	60 (-1)
27	900 (1)	180 (1)	80 (-1)	120)

4.4 Optimization of the Juice Processing Variables

The optimization of the beverage production process was carried out using Design-Expert Software version 11.1 using a multivariate response method called Overall Desirability index, DI, as described by Myers and Montgomery (2002) using equation (4.2).

$$DI = \left[\prod_{i=1}^{n^*} di\,(Y_i)\right]^{\frac{1}{n^*}} \qquad (4.2)$$

Where n is the number of responses, di represents the desirability index for each response variable, and n is the number of response variables. The desirability index ranges between 0 to 1, with 0 being the least desirable and 1 the most desirable. The goal of optimization studies is to maximize the desirability index. The optimization process includes objectives and criteria for independent and dependent variables. For this study, the objectives for independent variables and responses were one of the five criteria: minimize, target, maximize, range, and none.

4.5 Physicochemical Properties Determination

4.5.1 Total Soluble Solids (Brix)

In graduations, a refractometer tests the % TSS or sugar in the solution. The TSS of the juice samples was determined using a handheld refractometer (ATAGO N1) that was calibrated with distilled water. A juice drop was placed on the refractometer prism, and the cover plate was lowered to take the readings against the direction of light. The procedure was followed for all samples, ensuring that the refractometer prism washed with distilled water and washed with soft, lint-free tissue each time the measurement was made.

4.5.2 Determination of Potential Hydrogen (pH)

The pH levels were determined by the use of a pH meter (JENWAY) and the samples were measured against a standardized pH solution. The juice sample was prepared by thoroughly stirring, placing the electrodes of a pH meter into the juice and taking a direct reading. Samples were measured at 25 ^{o}C when there was an average temperature.

4.5.3 Titrable Acidity (TA)

The TA was analysed by the Rekha et al. (2012) technique using the titration method up to pH 8.1. 10 ml of fruit sap sample was diluted using 100 ml of purified water and the mix was homogenized by shaking. The chemicals were then filtered through Whatman filter paper to produce a clear filtrate. Aliquot of the filtrate was pipetted into a 200 ml conical flask and titrated to a standardized 0.1N NaOH burette solution using phenolphthalein as an indicator. The endpoint of the titration was reached when the filtrate changed to a permanent pink colour. The amount of NaOH needed to titrate the sample

was noted. Titratable acidity (TA) of the juice sample will be calculated according to equation (4.3).

$$\text{TA}\left(\% \frac{\text{w}}{\text{w}}\right) = \frac{\text{Net ml of titrant x Normality of titrant x 6.4}}{\text{sample weight}}$$

(4.3)

4.5.4 Total Antioxidant Capacity (TAC) Determination

Pasteurised pineapple juice TAC was calculated using a spectrophotometer by the phosphomolybdenum assay method with minor modifications by Prieto et al. (1999) 0.5 mL of 1mg/mL of juice extract in distilled water was combined with 3 mL of phosphomolybdenum chemical agent (28 mM sodium phosphate and 74 mM ammonium molybdate in 0.6 M sulphuric acid) in covered test tubes. It was then incubated at 95 degree celsius for 90mins in a water bath. This was cooled at room temperature and the absorption of the solutions measured with the aid of the Ultra Violet-visible spectrophotometer (Varian Inc., Mulgrave, Australia) was 695 nm against the blank (1 mL water devoid of pineapple juice). Total antioxidant performance was expressed as an ascorbic acid equivalent.

4.5.5 Ascorbic Acid (AA) Determination

The vitamin C content of the pineapple varieties was determined by the spectrophotometer method described by Harris and Ray (1935), and the slight modification by Abano, Ma, and Qu (2014). Exactly 25mL of pineapple juice was diluted with 4% oxalic acid and the final solution used to determine the concentration of the oxalic acid. A 10 mL of the sample was conveyed into a flask and then bromine was slowly added dropwise in order to achieve a yellow-orange liquid. The excess bromine was blown off. 0.5 mL of the brominated

extract was transferred into test tubes in triplicate. The volume was made up to 3 mL with 4% oxalic acid. 1mL of DNPH reagent was added, followed by 1ml 10% of thiourea. A blank preparation was similarly made, but water in place of sample extract was used. The content was mixed thoroughly and incubated at 37^{o}C for three hours. The orange-red crystals formed (osazone) was dissolved with 4ml of 80% sulphuric acid. The absorbance of each solution was measured at 540 nm using a visible spectrophotometer.

A standard ascorbic acid was prepared similarly by pipetting 100, 200, 300, 400 and 500 µL of 10mg/ml standard ascorbic acid solution into a series of test tubes. All the reagents were similarly added as above, and absorbances were taken at 540nm. A standard calibration plot ascorbic acid concentration versus absorbance was prepared, and the ascorbic acid content was calculated from the mathematical standard plot.

$$Y = 0.0024x+0.0866 \quad (4.4)$$

Where;

Y = absorbance and x = concentration (µg/ml).

4.5.6 Total Phenolic Content (TPC) Determination

The total phenolic content of each pasteurised juice was measured using the Folin-Ciocalteu method (Singleton & Rossi, 1965). A standard curve was made by taking different concentrations of the phenol, and the results were expressed as mg phenolic equivalents per 100 mL. Approximately 0.5 ml of the sample was combined with 2 ml of the Folin–Ciocalteu chemical agent and 2 mL of an aqueous sodium carbonate solution. The mixture was incubated for thirty minutes under room temperature and then measured at 765nm. The

calibration curve was prepared using Sigma-Gallic Aldrich's acid and results were expressed in gallic acid equivalents. (mg·GAE/g) (Kasangana et al., 2015).

4.6 Microbial Analysis

Aerobic Plate Count, faecal coliforms, and *Salmonella sp*, Yeast and Moulds were determined using Standard plate count method (Chouhan, 2015). By ISO standards (ISO-4833-2, 2013), the samples were analysed for APC, TC, and *E. coli*, Yeast and Moulds all in CFU/ ml using the pour plate method. Culture media consisting of Plate Count agar (Oxoid, Hampshire, England), Peptone Water and Eosin Methylene Blue agar, Mannitol Salt Agar and Potato Dextrose agar were prepared in correspondence to the manufacturer's directions. Using Peptone Water as a diluent for recovery, 180 ml of peptone water was prepared in triplicate and sterilised by autoclaving all prepared media and Petri dishes at a temperature of 121°C, a pressure of 15 psi for 15 minutes. The sample was effectively homogenised, 20 mL of the sample test was aseptically measured into Peptone water and incubated in boiling water at 37 ^{o}C for 30 minutes. The samples were diluted with sterile water to 3-10. Triplicate dilutions of 0.1 mL and 1 mL of 10-2 dilution of the sample were placed on the agar plate and incubation was done for 48 hours at 37 °C. All colonies were counted and an average of duplicate samples was recorded as aerobic plate counts (CFU/ml) for the sample.

In addition, triplicate dilutions of 0.1 ml and 1 ml of 10 per sample were applied to Eosin Methylene Blue agar. For total coliform counts (CFU/ml) of the sample, one of the duplicated dilutions was kept in incubation for 48 hours at 37 °C. Triplicate dilutions of 0.1 ml and 1 ml of 10-1 per sample were applied

to Mannitol Salt Agar. For staphylococci counts (CFU/ml) for the sample, one of the duplicated dilutions was in incubation at 37°C for 48 hours.

For yeast and moulds, triplicate dilutions of 0.1 ml and 1 ml of 10^{-1} each sample were plated on Potato Dextrose agar supplemented with ampicillin. One each of the triplicate dilutions was incubated at room temperature for 7 days to observe for yeast and mould counts (CFU/ml) for the sample.

For E. coli presumptive test, colonies of at least 3 mm in diameter exhibiting a metallic sheen and a purple colour were identified as possible colonies of E. coli (Anjana et al., 2021; Leininger, Roberson, & Elvinger, 2001)(Oxoid.com, 2011). Isolated colonies were inoculated into the medium Oxoid SIM (Sulfite Indole Motility) stabbing the needle about two-thirds of the way to the depth. It was incubated at 37 °C for 24 hours or until growth was evident. Testing for the presence of indole, 5 drops of Kovác's reagent were added to the top of the deep. A positive indole test is indicated by the formation of red colour in the reagent layer at the top of the agar deep within seconds of the addition of the reagent. (MacWilliams, 2009).

4.7 Statistical Analysis

In this study, in order to examine and evaluate the influence of microwave and conventional pasteurization treatment on the physicochemical properties and microbial quality of pineapple juice, a quadratic polynomial model was fitted to the mean values of the experimental results in order to obtain the regression equations with Design-Expert Software version 111 (Stat-Ease, Inc., Minneapolis, USA). ANOVA tests were used to determine the statistical significance of the model terms' associations at a probability of 5%. The model

was assessed as being accurate to describe the response variables through the coefficients of determination (R^2) values.

4.8 Results and Discussions

The effect of pasteurisation on the quality of pineapple juice is discussed in terms of physicochemical properties such as pH, TSS, TA, vitamin C content, total antioxidant content (TAC) and total phenols (TP). The microbiological quality expressed by APC, coliform count, staphylococcus and yeast and mould (YM) was also investigated in response to the treatments used. For pineapple juice, the samples were subjected to microwave pasteurization at levels of 400 – 900 W for microwave time at levels of 60 – 180 seconds, and all these parameters were investigated.

The influence induced by microwave pasteurisation treatments on the analysed parameters was compared to the parameters of pineapple juice samples subjected to conventional pasteurization at levels of 80 – 90 ^{o}C for a conventional period of 60 – 180 seconds. Upon the data gotten through statistical processing, the significance of the changes noted in the analysed parameters in response to microwave and conventional pasteurised treatments has been reported.

The outcomes of the 27 experiments performed according to the Response Surface Method are shown in Table 4.3 and 4.4. Table 4.5 and 4.6 demonstrates the regression coefficients and coefficient of determination (R^2) values for quadratic models of the dependent variables of pineapple juice. When R^2 approaches unity, the better the model fits empirically in describing the effect of independent variables on responses.

Table 0.9: Effects of Microwave Power and Microwave Time on Physicochemical Properties and Microbial Load of Pineapple Juice

Run No.	Coded Values		Experimental values of the experiment									
	X_1: Microwave power (W)	X_2: Microwave time (secs)	ΔE	TSS (%Brix)	pH	TA (%)	TAC (mg/100g)	AA (mg/kg)	TP (mg/100g)	Aerobic plate count (CFU/ml)	Coliforms (CFU/ml)	Yeast and Mould (CFU/ml)
1	400	180	28.56211	14.2	4.69	1.2032	147.6416	33.4164	32.6541	2.34	2.16	2.15
2	650	60	23.7005	15	4.71	1.1904	145.2447	35.6812	36.4542	2.16	1.94	1.60
3	400	120	25.75339	14.2	4.69	1.088	184.8295	39.162	36.9062	2.30	1.00	2.00
4	650	60	22.52281	15	4.7	1.344	155.8403	36.4682	24.6265	2.20	1.94	1.68
5	900	120	23.27926	15.2	4.7	1.408	215.344	38.1639	38.8197	1.12	0	1.00
6	400	180	44.11692	14.4	4.69	1.1648	151.8757	33.4164	22.7137	2.32	2.22	2.16
7	650	180	23.6725	15.4	4.68	1.2416	214.7556	36.9333	33.7658	0	0.78	0
8	400	60	28.99434	14.4	4.69	1.2288	177.8452	41.3341	43.0828	2.33	1.20	2.32
9	650	120	24.714	14.6	4.69	1.152	198.2533	40.9771	32.3964	1.40	1.18	1.20
10	900	60	27.48855	14.6	4.7	1.2672	192.9877	37.8841	36.9497	1.82	1.85	1.48
11	900	120	22.78167	15	4.7	1.2544	203.0677	37.6647	43.6753	1.22	0	1.04
12	650	180	22.72946	15.4	4.7	1.3824	194.1074	38.0167	36.1089	0	0	0
13	650	60	23.2574	15	4.7	1.1392	153.2974	35.8883	37.5747	2.22	1.94	1.65
14	400	120	19.93067	14.2	4.69	1.28	202.6688	38.4571	37.0289	2.28	0.30	2.08
15	400	60	25.44609	14.8	4.69	1.28	143.1533	39.5883	37.7099	2.30	1.30	2.28
16	650	120	23.2254	14.2	4.71	1.2288	154.9418	38.3184	33.3826	1.30	1.30	1.28
17	650	120	26.09883	14.8	4.7	1.2544	192.1471	39.2739	32.7663	1.36	1.32	1.20
18	400	60	25.56081	14.6	4.69	1.2032	178.2682	39.2142	36.8353	2.36	1.18	2.36

Table 4.3.Con't D

19	900	60	22.81121	14.4	4.69	1.1776	208.0283	38.4654	38.1781	1.82	1.85	1.59
20	900	120	22.76759	15.2	4.71	1.2416	204.6199	37.4151	40.1892	1.22	0	1.04
21	400	180	56.31888	14	4.69	1.216	136.0685	33.4995	30.5417	2.32	2.19	2.12
22	400	120	19.43926	14	4.69	1.152	194.1059	38.8718	35.4338	2.34	1.08	2.28
23	900	60	23.86229	14.6	4.7	1.2416	211.008	39.3373	38.3009	1.82	1.85	1.60
24	900	180	23.57592	16	4.7	1.28	202.6323	47.0609	48.9359	0	0	0.00
25	650	180	22.05018	15.4	4.69	1.1904	223.5845	36.600	36.2322	0	0	0.30
26	900	180	25.23708	16.2	4.69	1.3056	212.1656	47.4739	58.3034	0.30	0	0
27	900	180	20.78264	16.2	4.7	1.2544	215.296	49.9934	47.1873	0.48	0	0

Values are means of triplicate determination

Table 0.10: Effects of Conventional Temperature and Conventional Time on Colour, Physicochemical Properties and Microbial Load of Pineapple Juice

Run No.	Coded values		Experimental values of the experiment									
	X_1: Conventional Temp (^oC)	X_2: Conventional time (secs)	ΔE	TSS (%Brix)	pH	TA (%)	TAC (mg/100g)	AA (mg/kg)	TP (mg/100g)	Aerobic plate count (CFU/ml)	Staph (CFU/ml)	Yeast and Mould (CFU/ml)
1	80	180	26.60778	15	4.8	0.5117	97.846	31.4706	47.6202	0	0	0
2	85	60	25.53483	16.1	4.9	0.5385	75.3034	46.3585	53.5892	2.06	1.11	1.3
3	85	180	28.1992	15.8	4.7	0.4649	66.0996	38.061	50.4889	2	0.9	1.38
4	90	180	26.23338	15	4.9	0.5123	69.8838	41.1035	52.4105	2.49	0	0.3
5	85	120	25.73132	15	4.8	0.5117	92.2583	29.0701	37.4678	2.05	0.3	1.49
6	90	180	27.43702	15.2	4.7	0.7205	61.5221	39.5481	48.9314	2.48	0	0
7	90	120	25.77978	15.6	4.8	0.5932	108.8714	39.8247	49.0362	2.12	0	1
8	90	60	24.12519	15.4	5	0.477	118.3636	49.736	54.6797	0.3	1	0.7
9	80	120	24.53148	15.1	4.8	0.5226	72.9802	30.1285	42.457	1.08	0	1.6
10	80	180	26.76692	15.1	4.7	0.6456	90.2029	32.3693	43.8785	0	0	0
11	90	180	27.31839	15	4.5	0.6724	50.1842	41.5128	49.0557	2.48	0	0
12	90	60	24.34803	15	4.9	0.701	106.9479	44.4554	56.0473	0	0	0.7
13	90	120	25.26564	15.8	4.8	0.5701	97.8714	23.5351	51.6445	2.22	0	1.04
14	85	180	28.0682	16	4.8	0.6332	79.4573	29.6949	48.4933	2.3	1	1.41
15	90	60	23.16746	15.6	4.6	0.7877	94.4166	30.099	55.6744	0	0	0.7
16	85	180	28.08733	15.9	4.6	0.6412	90.5652	22.231	50.7384	2.19	0.9	1.41
17	85	60	27.87554	15.4	4.7	0.7042	79.3023	42.7137	56.3195	2.12	1	1.41

Table 4.4.Con't D

18	80	60	27.22912	16.2	5	0.566	90.9363	36.1344	47.5338	2.36	1.46	1.56
19	80	60	27.01065	16.2	4.9	0.6174	97.4815	45.9481	42.9986	2.38	0	1.46
20	80	180	27.32873	15.1	4.7	0.6692	89.925	28.366	43.3796	0	0	0
21	85	120	26.95615	15.3	4.6	0.5117	102.8087	40.6942	49.7179	2.05	0.3	1.53
22	80	60	26.47118	16.2	4.8	0.5807	93.4974	40.3759	47.1661	2.32	1.3	1.56
23	90	120	25.80285	15.8	4.8	0.7164	92.4429	47.6951	54.8738	2.22	0	1.11
24	85	120	24.54776	15.2	4.6	0.5194	97.8186	28.8245	43.531	2.01	0.6	1.6
25	80	120	22.80307	15	4.7	0.6414	84.4423	22.9644	42.0865	0	0.78	1.36
26	85	60	27.16793	16	4.7	0.6794	94.3326	47.1869	57.6847	2.11	0.95	1.34
27	80	120	24.26455	15.1	4.7	0.6098	87.9368	27.9875	44.9269	0	0	1.6

Values are means of triplicate determination

Table 0.11: Regression Coefficients and R^2 values for the Model Terms in the Quadratic RSM for the Various Responses of Microwave Pasteurised Pineapple Juice

	b_o	X_1	X_2	X_1X_2	X_1^2	X_2^2	R^2
ΔE	21.2519	-1.97424*	0.823135	-2.15438*	2.53899	2.70609*	0.5083
p-values		0.0141	0.2783	0.0256	0.0718	0.0461	
Brix	14.8333	0.477778**	0.3**	0.5**	-0.296296**	0.377778**	0.9306
p-values		< 0.0001	< 0.0001	< 0.0001	0.0011	< 0.0001	
pH	4.70106	0.004444**	-0.00219298	0.000438596	-0.00439815	-0.00333333	0.3955
p-values		0.0085	0.1682*	0.8162	0.1288	0.2227	
TTA	1.23591	0.0341333	0.0106947	0.0217544	-0.00711111	0.0106667	0.2127
p-values		0.0555	0.5335	0.3003	0.8184	0.7182	
TAC	193.863	19.3718**	7.65992	4.5204	2.7502	-13.1198	0.4874
p-values		0.0010	0.1475	0.4724	0.7684	0.1506	
AA	37.7808	2.02772**	0.964622*	4.0115**	1.55119	0.203533	07415
p-values		0.0009	0.0819	< 0.0001	0.1195	0.8254	
T Phenols	33.8212	4.31295**	1.3261	5.94914**	4.7051*	0.831017	0.7480
p-values		0.0001	0.1674	< 0.0001	0.0107	0.6091	
Aerobic plate count	1.1169	-0.616111**	-0.648618**	-0.33761**	0.672917**	-0.127222	0.8658
p-values		< 0.0001	< 0.0001	0.0032	0.0002	0.3892	
Coliforms	0.707824	-0.393333**	-0.471338**	-0.653399**	-0.0696759	0.557778**	0.7407
p-values		0.0019	0.0004	< 0.0001	0.7327	0.0085	
Yeast and Mould	0.937963	-0.666667**	-0.567325**	-0.318202**	0.699074**	-0.163889	0.9419
p-values		< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0824	

*significant at p<0.05, **significant at p<0.01, b_o is the intercept of the

Table 0.12: Regression Coefficients and R^2 values for the Model Terms in the Quadratic RSM for the Various Responses of Conventional Pasteurised Pineapple Juice

	b_o	X_1	X_2	X_1X_2	X_1^2	X_2^2	R^2
ΔE	25.8849	-0.196431	0.728722**	0.779635*	-1.21363**	1.53398**	0.6638
p-values		0.4086	0.0051	0.0125	0.0067	0.0010	
Brix	15.4704	-0.0333333	-0.222222*	0.216667	-0.222222	0.244444	0.4127
p-values		0.7133	0.0215	0.0613	0.1664	0.1297	
pH	4.68519	-0.00555556	-0.061111*	0.00833333	0.0722222	0.0388889	0.2690
p-values		0.8446	0.0405	0.8103	0.1512	0.4315	
TTA	0.551196	0.0214556	-0.01005	-0.0102417	0.0392556	0.0405722	0.1601
p-values		0.3087	0.6301	0.6884	0.2829	0.2675	
TAC	91.2012	-0.263583	-8.60531**	-11.1831**	2.77002	-7.14408	0.5335
p-values		0.9236	0.0046	0.0029	0.5622	0.1437	
AA	31.4292	2.87583	-4.36948*	2.34388	-0.3564	6.99538*	0.4002
p-values		0.1328	0.0271	0.3098	0.9120	0.0394	
T Phenols	46.9958	3.90591**	-2.03871*	-1.09863	-1.20337	4.17814**	0.6751
p-values		< 0.0001	0.0162	0.2628	0.3829	0.0055	
Aerobic plate count	2.09556	0.342778**	0.0161111	1.18417**	-0.851667**	0.005	0.8581
p-values		0.0027	0.8747	< 0.0001	< 0.0001	0.9775	
Staph	0.574815	-0.141111	-0.223333*	0.146667	-0.532222**	0.314444	0.5648
p-values		0.1241	0.0193	0.1885	0.0022	0.0519	
Yeast and Mould	1.77926	-0.199444**	-0.346111**	0.231667*	-0.613889**	-0.523889*	0.8082
p-values		0.0082	< 0.0001	0.0115	< 0.0001	0.0002	

*significant at $p<0.05$, **significant at $p<0.01$, b_o is the intercept of the mode

4.8.1 Effect of Pasteurisation on Colour of Pineapple Juice

In order to quantify the colour changes in pineapple juices caused by the pasteurisation treatment, the total colour difference (ΔE) was evaluated from trichromatic CIE $L^*a^*b^*$ values. The obtained ΔE values of microwave and conventional pasteurisation of pineapple juices are presented in Table 4.3 and 4.4, respectively. The research on the influence of microwave pasteurisation with different microwave power and times on the colour difference of pineapple juice revealed that the pineapple juice treated with MP 400W/180 secs had higher colour difference (36.3189) value than pineapple juices treated with other microwave pasteurisation conditions. The pineapple juices treated at 400W for 120secs had lower colour difference (19.4393) value (Table 4.3). The power level of microwave treatment had a significant linear negative effect on the colour difference of the pineapple juice (Table 4.5). The relative yellow colour pigment decreased as time and temperature increased during the pasteurisation treatment methods as shown in Figure 4.1

In the case of the conventional pasteurisation, among the pasteurisation treatments, the colour difference in pineapple juice was generally high (28.1992) in samples held at 80 ^{o}C for 180 secs condition. A lower colour difference of pineapple juice was observed in samples held at 80 ^{o}C for 120secs. Although, generally, the average lowest colour difference was observed in pineapple juice treated at pasteurisation temperature of 90 ^{o}C (Table 4.4). The colour difference between the pineapple juice was insignificantly affected by the pasteurisation temperature (Table 4.5). Increase in the pasteurisation time (X2), the interaction of pasteurisation temperature and pasteurisation time (X1X2) and quadratic effect of pasteurisation time (X22) had a significantly

positive ($p<0.01$) effect on the colour difference of pineapple juice. Whiles, the quadratic effect of pasteurisation temperature (X_1^2) had a significantly negative ($p<0.01$) effect on the colour difference of pineapple juice.

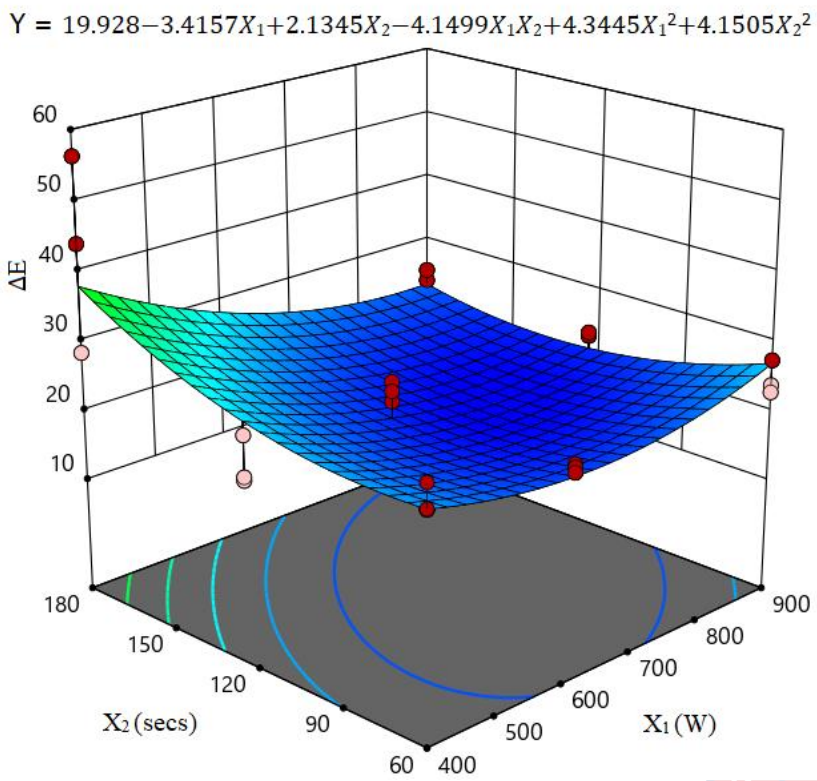

Figure 0.12: 3D RSM plot for the effect of microwave power (X_1) and microwave time (X_2) on the Colour (ΔE) of pineapple juice

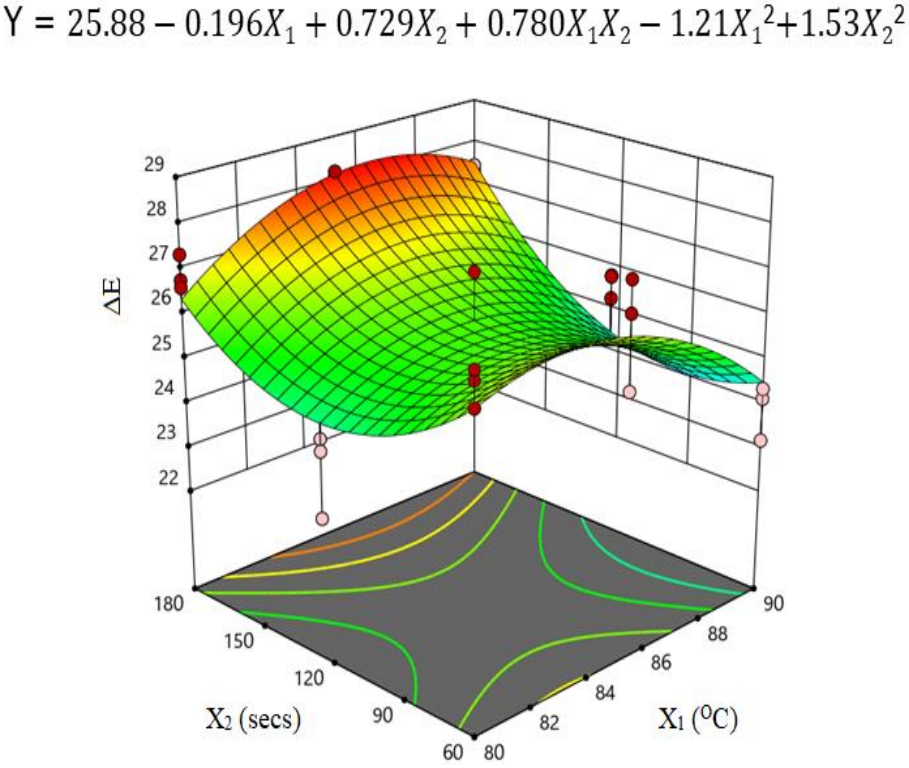

Figure 0.13: RSM plot for the effect of conventional temperature (X_1) and conventional time (X_2) on the colour (ΔE) of pineapple juice

4.8.2 Effect of Pasteurisation on Total Soluble Solids of Pineapple Juice

Total Soluble Solids (TSS) content of pineapple juice was studied in this study after pasteurisation. The total Soluble Solids (TSS) content of microwave and conventional pineapple juice pasteurisation is shown in Tables 4.3 and 4.4, respectively. Among microwave pasteurised pineapple juice, samples treated at 900W for 180 secs showed the highest total soluble solids (16.2 per cent), followed by samples treated at 900W/120 secs, 650W/180 secs and 900W/60 secs (Table 4.3). Among the treatments, the variances detected were noteworthy. The TSS content of pineapple juice was positively ($p<0.01$) influenced by the linear effect of microwave power (X1) and microwave time (X2), the interaction effect of microwave power and microwave time (X1X2) and the quadratic effect of microwave time (X2). As shown in Figure 4.4, when the microwave level and the microwave time increased, the total soluble solids in pineapple juice also increased. However, the quadratic effect of the microwave power did have a negative effect on the total soluble solids.

In comparison to the microwave pasteurisation, the statistical regression study of the effect of conventional pasteurisation on the total soluble solid content of the pineapple juice showed that it has no significant effect for conventional temperature ($p<0.05$). However, it has a significant effect on conventional time ($p<0.05$) (Table 4.6). Effectively, conventional pasteurisation decreased the total soluble solid content of the pineapple juice as compared to the microwave pasteurisation. At any conventional power, pasteurised pineapple juice presented lower total soluble solids than that found in microwave pasteurised pineapple juice. This explains reduced sugar loss at the temperature and time of treatment observed above. However, higher

temperature treatment over a more extended time disrupts the cell wall integrity making it less stable and highly porous for leaching to occur. The sugar content decreased compared to the pasteurised microwave, but this decrease did not have any impact on the nutritional content of pineapple juice as the sugar content of the fruit remained within the accepted range of 12 and 16 per cent brix for all treatment conditions.

Study by Saad (2017), reported that microwave pasteurisation of some mixture juice retained higher total soluble solids (TSS) as compared to conventional processing and power level had a significant effect on total soluble solids (TSS) after processing.

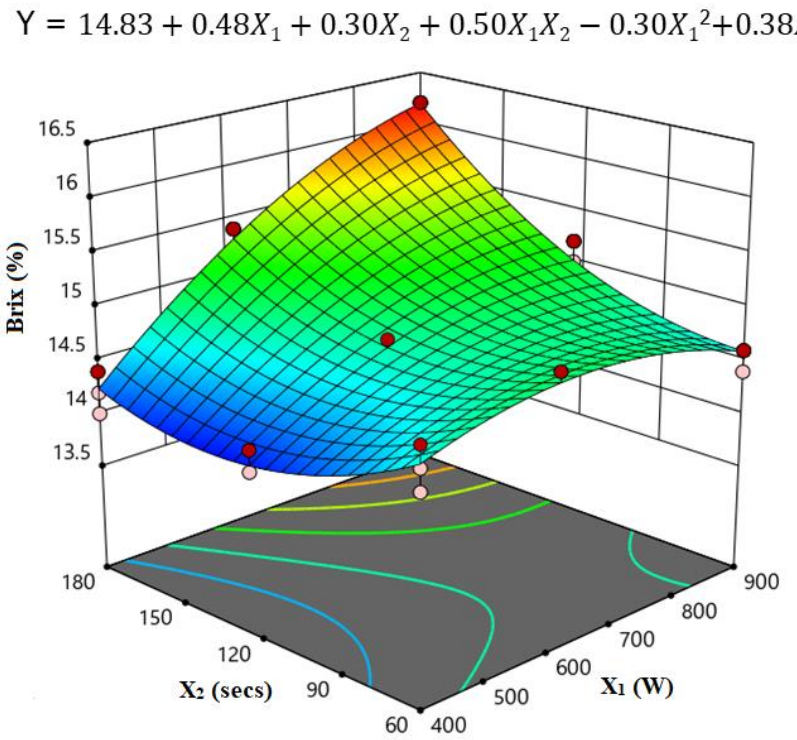

Figure 0.14: 3D RSM plot for the effect of microwave power (X_1) and microwave time (X_2) on the TSS of pineapple juice

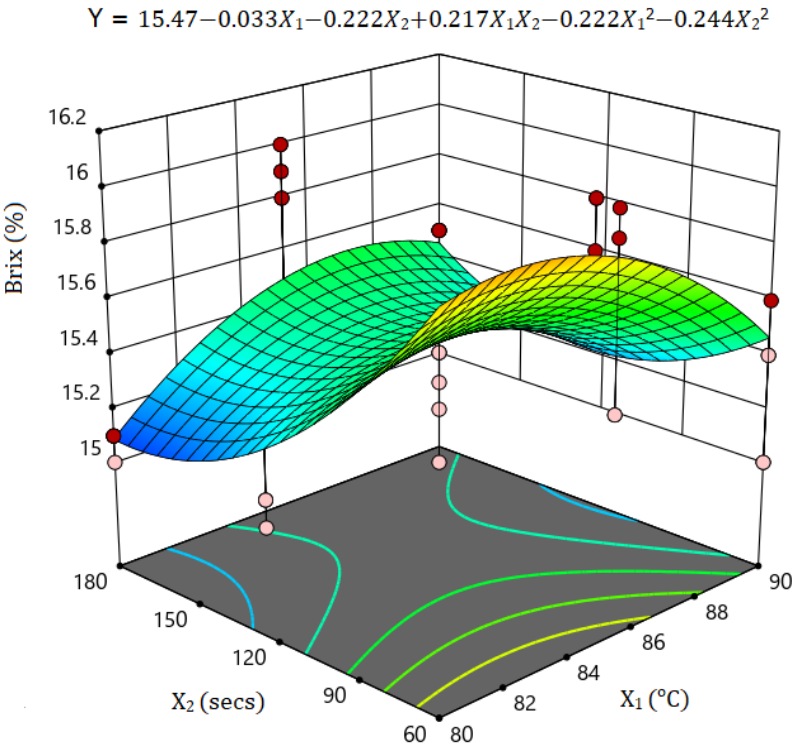

Figure 0.15: 3D RSM plot for the effect of conventional temperature (X_1) and conventional time (X_2) on the TSS of pineapple juice

4.8.3 Influence of Pasteurisation on Potential Hydrogen (pH) of Pineapple Juice

Results of pH of pineapple juices pasteurised by microwave on conventional pasteurisation is presented in Tables 4.3 and 4.4, respectively. From the experimental results, it can be observed that the pH of both microwave and conventional pasteurised pineapple juice samples were within the range of 4.5 to 5.0. Potential hydrogen of pineapple juice was observed to be significantly positively affected by microwave power at ($p<0.01$) and negatively affected by microwave time at ($p< 0.05$). However, conventional pasteurisation temperature treatment was observed to affect the pH of pineapple juice negatively. Thus, as the conventional pasteurisation temperature increases, the pH also decrease. Literature search revealed no documented studies indicating the effect of pasteurisation on pineapple juice. However, the decrease in pH in this study compares with studies by Rabie, Soliman, Diaconeasa, & Constantin (2015), where a pH reduction of 1.40 % was reported for Physalis juice.

$$Y = 4.70 + 0.04X_1 + 0.002X_2 + 0.0004X_1X_2 - 0.004X_1^2 + 0.003X_2^2$$

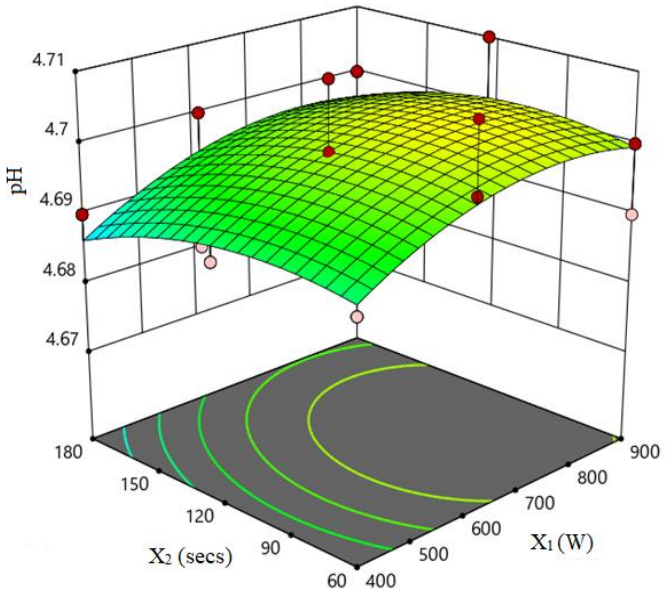

Figure 0.16: RSM plot for the effect of microwave power (W) and microwave time (secs) on the Potential Hydrogen (pH) of pineapple juice

$$Y = 4.69 - 0.006X_1 - 0.061X_2 + 0.008X_1X_2 + 0.07X_1^2 + 0.039X_2^2$$

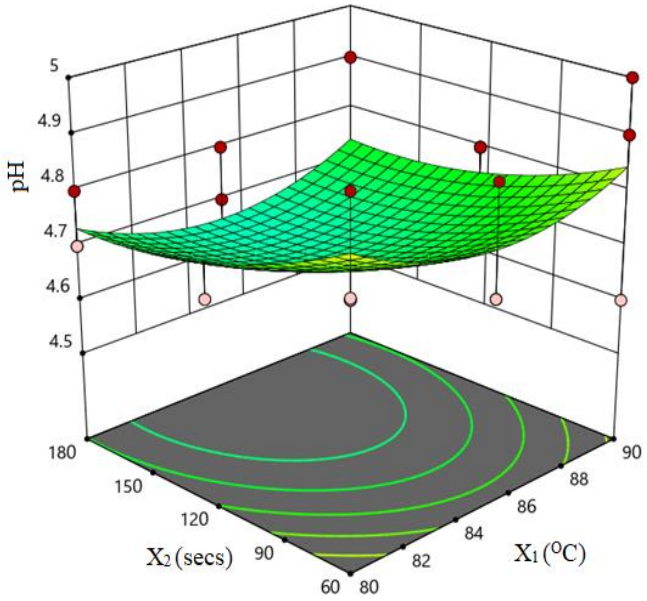

Figure 0.17: RSM plot for the effect of conventional temperature (°C) and conventional time (secs) on the Potential Hydrogen (pH) of pineapple juice

4.8.4 Effect of Pasteurisation on Titratable Acidity (TA) of Pineapple Juice

Acidity is an important factor that affects the taste of a number of fruits; therefore, the acidity content of all commodities must be analysed. The titrated acidity of microwave pasteurised pineapple juice was observed to be between 1.088 and 1.408 per cent (Table 4.3). Microwave power level 900W at microwave time 120secs recorded the highest titrable acidity content (1.408 per cent). As shown in Table 4.5 and Figure 4.7, the linear effect of microwave power, microwave time, the interaction influence of microwave power and time, the quadratic effect of microwave power and the quadratic effect of microwave time had no significant effect on the titrative acidity of pineapple juice. This study is similar to studies by Igual, García-Martínez, Camacho, and Martínez-Navarrete (2010), which showed that microwave heating did not have any effect on the citric acid content.

In the case of conventional pasteurisation of pineapple juice, the titrable acidity ranged from 0.4649 to 0.7877 per cent (Table 4.4). The highest titrable acidity was obtained from the 90^{o}C/60secs treated sample resulting in a titrable acidic content of 0.7877 per cent (Table 4.4). With regard to the effect of conventional pasteurisation on the titrable acidity content of pineapple juice after the process, the regression analysis showed that the effect of conventional time and the interaction effect of conventional temperature and conventional time had an insignificant negative effect on the titrable acidity content of pineapple juice, while the linear effect of conventional temperate had an insignificant negative influence on the titrable acidity content of pineapple juice (Table 4.6). Figure 4.8 exhibit the 3D response surface plot for the effect of

conventional temperature (X_1) and conventional time (X_2) on the titratable acidity. The graph depicts that increase in conventional temperature results in a marginal increase in titratable acidity, as the impact of the length of treatment time on titratable acidity of pineapple juice was negative.

Based on our findings, the acidity content of pasteurised pineapple juice by both microwave and conventional pasteurization was observed to have an increasing marginal trend, with microwave pasteurisation retaining high titratable acidity content. The increase in titratable acidity might be due to oxidation of reducing sugar, which can also be contributed to an increase in the acidity of fruit juice during pasteurization (Kumar, Khadka, Mishra, Kohli, & Upadhaya, 2017).

$$Y = 1.24 + 0.0.34X_1 + 0.011X_2 + 0.022X_1X_2 - 0.007X_1^2 + 0.011X_2^2$$

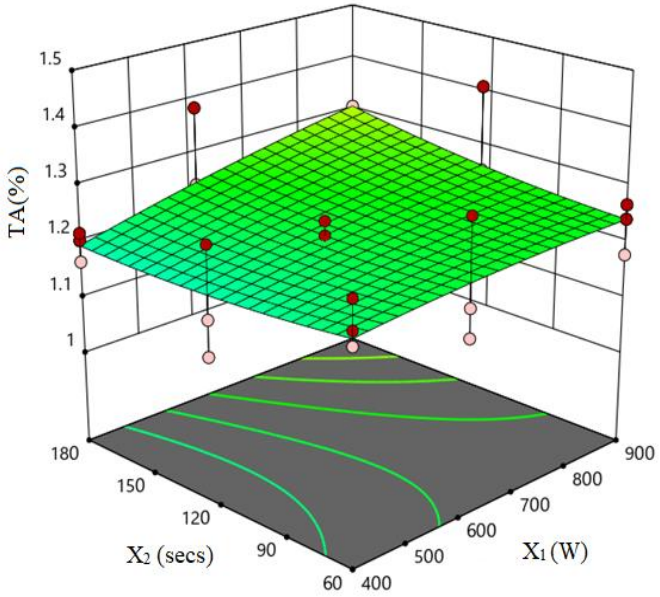

Figure 0.18: 3D RSM plot for the effect of microwave power (X_1) and microwave time (X_2) on the Titratable Acidity (TA) of pineapple juice

$$Y = 0.55 + 0.022X_1 - 0.010X_2 - 0.01X_1X_2 + 0.039X_1^2 + 0.041X_2^2$$

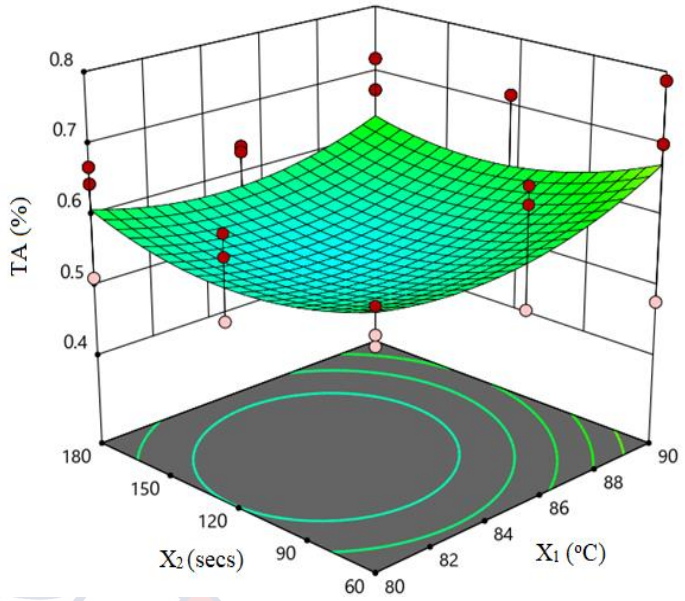

Figure 0.19: 3D RSM plot for the effect of conventional temperature (X_1) and conventional time (X_2) on the Titratable Acidity (TA) of pineapple juice

4.8.5 Effect of Pasteurisation on Total Antioxidant Content (TAC) of Pineapple Juice

The total antioxidant activities of the pineapple juice under microwave pasteurisation and conventional pasteurisation conditions are shown in Table 4.3 and 4.4, respectively. Generally, the two investigated pasteurisation treatment significantly affected the antioxidant capacity of the pineapple juice. Study on the effect of microwave pasteurisation with different microwave power and microwave times on the total antioxidant of pineapple juice revealed that the pineapple juice treated with MP 650W/180 secs, MP 900W/180 secs, MP 900W/120 secs, MP 650W/180 secs, and MP 900W/60 secs had higher total antioxidant than pineapple juices treated with other microwave pasteurisation conditions. The pineapple juices treated at 400W for 180secs had lowest total antioxidant (136.069 mg/100g) content (Table 4.3). The microwave power treatment had a significant linear positive effect on this antioxidant activity of the pineapple juice. This could be appreciated through the increase in TAC observed with the decrease of microwave heat (Table 4.5). Indeed, the increase in microwave power instead tended to reduce the negative impact of the impact of microwave time (Figure 4.9).

In the case of the conventional pasteurisation, the temperature treatment had an insignificant ($p<0.05$) linear negative effect on the total antioxidant content of pineapple juice (Table 4.4). Besides, a significant interaction negative effect on the antioxidant activity was also observed with pineapple juices. In fact, for the latter, the effect of conventional pasteurisation temperature and time on antioxidant activity was appreciated through the decrease observed with the increase with interaction effect of conventional pasteurisation temperature and

time (Table 4.6). A significant effect of time of treatment was noticed only with conventional temperature treatment, and this was quadratic. This could be seen through the rise of the antioxidant activity in samples treated for 60 and 120 seconds, an increase that was followed by a gradual reduction with time (pineapple juice treated for 180 seconds) (Table 4.4). As illustrated by Figure 4.10, a decrease in the total oxidant value of the pineapple juice was observed with a rise in pasteurisation temperature and time of treatment.

The Antioxidant activity of both the pasteurisation method of pineapple juice showed a decreasing trend to an increase in pasteurisation level and time level. However, our study showed that microwave pasteurisation treatments retained total antioxidant in the pineapple juices better than the conventional pasteurised juices regardless of the microwave pasteurisation conditions. Similar results were observed in studies by Saikia, Mahnot, & Mahanta, (2015) where microwaved pasteurised treatments had positive effects on the antioxidant activity of pineapple among other fruit juice.

$$Y = 193.863 + 19.3718X_1 + 7.6599X_2 + 4.5204X_1X_2 + 2.7502X_1^2 - 13.1198X_2^2$$

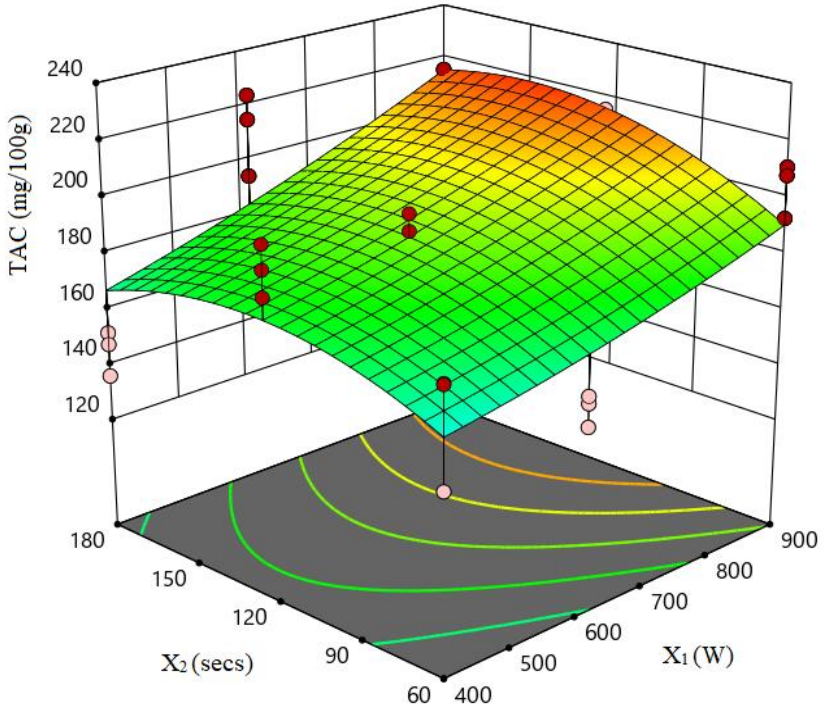

Figure 0.20: 3D RSM plot for the effect of microwave power (X_1) and microwave time (X_2) on the Total Antioxidant Content (TAC) of pineapple juice

$$Y = 91.20 - 0.264X_1 - 8.61X_2 - 11.18X_1X_2 + 2.77X_1^2 - 7.14X_2^2$$

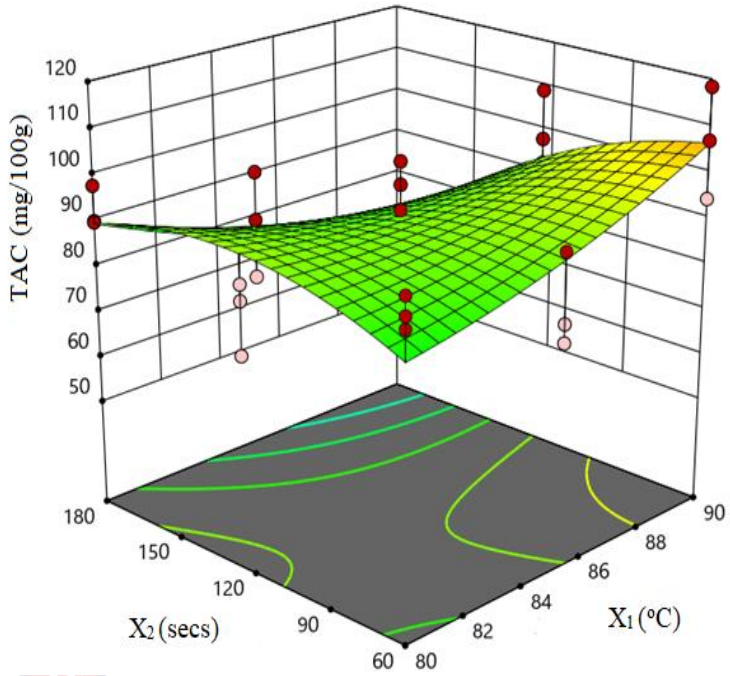

Figure 0.21: 3D RSM plot for the effect of conventional temperature (X_1) and conventional time (X_2) on the Total Antioxidant Content (TAC) of pineapple juice

4.8.6 Effect of Pasteurisation on Ascorbic Acid (AA) of Pineapple Juice

According to Zhang and Zhang (2014), ascorbic acid is a thermal sensitive polar molecule. It is therefore prone to degradation once a raw material is pasteurised. The effect of microwave power and microwave time on pineapple juice ascorbic acid is outlined in Table 4.3. The AA of microwave pasteurised pineapple juice was observed to be in the range of 33.4164 to 49.9934 mg/kg. Microwave power level 900W at microwave time 180secs was observed to record the highest ascorbic acid (49.9934 mg/kg) content (Table 4.3). As shown in Table 4.5 and Figure 4.11, the linear effect of microwave power, microwave time and quadratic effect of microwave power had a significantly ($p<0.01$) positive effect on AA of pineapple juice. This is study contrary to studies Paull and Chen (2000), as well as Lamikanra and Watson (2003), who reported that temperature treatment could increase protoplasmic viscosity as well as membrane impermeability. Since the loss of vitamin C is primarily through the membrane, retention observed could be attributed mostly to the impermeability caused by the heat treatment

On the other hand, conventional pasteurisation of pineapple juice was also observed to be in the range 22.231 to 49.736 mg/kg (Table 4.4). The highest AA was obtained from sample treated of 90^{o}C/60secs resulting in a having ascorbic content of 49.736 mg/kg (Table 4.4). About the influence of conventional pasteurisation on ascorbic acid content of pineapple juice after the process, the regression analysis showed that the effect of conventional time had a significant negative influence on the ascorbic acid content of pineapple juice. The statistical analysis also revealed an insignificant negative influence of the quadratic coefficient of conventional temperature-time on ascorbic acid of

pineapple juice (Table 4.6). As shown in Figure 4.12, the negative impact of the length of treatment time on pineapple juice was more important when the conventional temperature is above 80°C than below. Based on the results, lengthier conventional pasteurisation times decreased the ascorbic acid content, as a result of a likely increased duration of oxidative reactions and occurrence of thermolysis. This result is in accordance with the study by Padayatty et al. (2003), which reported that, ascorbic acid content decreased almost linearly with increase in period of treatment (10 to 30 min) for all treatment temperatures (60 to 80 °C). This may be due to the relatively high temperatures and the long period of treatment which are capable of depleting the levels of vitamin C. The tendency of vitamin C to leach into water-soluble media as reported by Davey et al. (2000) and Franke, Custer, Arakaki, and Murphy (2004), could also be a contributing factor to this loss.

$$Y = 37.78 + 2.03X_1 + 0.96X_2 + 4.01X_1X_2 + 1.55X_1^2 + 0.204X_2^2$$

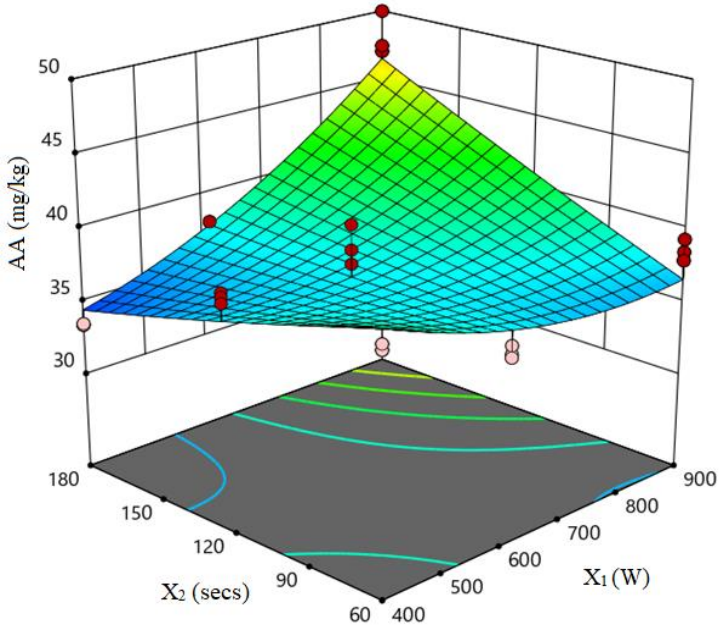

Figure 0.22: 3D RSM plot for the effect of microwave power (X_1) and microwave time (X_2) on the Ascorbic Acid (AA) content of pineapple juice

$$Y = 111.43 + 2.88X_1 - 4.37X_2 + 2.34X_1X_2 - 0.356X_1^2 + 7.00X_2^2$$

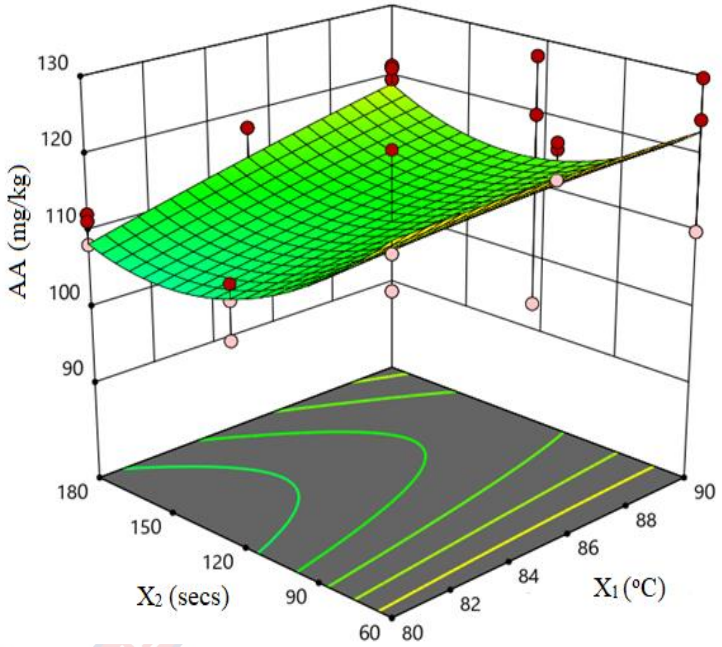

Figure 0.23: 3D RSM plot for the effect of conventional temperature (X_1) and conventional time (X_2) on the Ascorbic Acid (AA) content of pineapple juice

4.8.7 Effect of Pasteurisation on Total Phenols (TP) of Pineapple Juice

Phenolic compounds are one of the crucial quality parameters since they contribute to the fruit's colour and organoleptic characteristics (bitterness, flavour, and astringency) (Burin, Ferreira-Lima, Panceri, & Bordignon-Luiz, 2014). The effect of microwave pasteurisation and conventional pasteurisation on the total phenol content of pineapple juice is shown in Table 4.3 and 4.4, respectively. The total phenol content of microwave pasteurised pineapple juice samples varied from 22.7137 to 58.3034 mg/100g (Table 4.3). The highest total phenol content was yielded by the treatment of 900W/180secs, resulting in a having total phenol content of 58.3034 mg/100g (Table 4.3). From the regression coefficient table, the total phenol content of microwave pasteurised pineapple juice was shown to have a positive effect on the microwave power level ($p<0.01$) and not on the microwave time ($p<0.05$) (Table 4.5). Figure 4.13 shows changes in the total phenol content of microwave pasteurised pineapple juice in response to applied treatments. It can be noted that, in response to microwave pasteurisation, there were fewer losses in the total phenol content of the pineapple juice samples.

In the case of the conventional pasteurisation treatment, the total phenol content of pineapple juice samples varied from 22.7137 to 58.3034 mg/ (Table 4.4). The highest total phenol content was yielded by the treatment of 85^{o}C/60 secs resulting in a having total phenol content of 56.3195 mg/100g (Table 4.4). Regarding the effect of conventional pasteurisation on total phenol content of pineapple juice after the process, the regression analysis showed that the effects of coefficients of conventional temperature, conventional time, and quadratic coefficients of the conventional time were significant ($p<0.001$) in influencing

the total phenol content of pineapple juice (Table 4.6). As shown in Figure 4.14, with increasing the conventional temperature, the total phenol content of pineapple juice decreases. Given the variance pattern in both conventional pasteurisation and microwave pasteurisation of phenolic compounds, it was noted that the conventional temperature had a more negative effect on the total phenol content than the microwave power content. This is because the variations in conventional temperature were higher than that of microwave power.

Earlier works have revealed that the effect of thermal treatments on total phenolic concentrations is variable. Santhirasegaram, Razali, and Somasundram (2013), showed that, the total phenolic content of a mango juice treated at 90°C for 60 sec., was reduced by 38%. But no significant change in the total phenolic content was evident for peach pieces pasteurised at 90°C for 5 min, Oliveira, Pintado, and Almeida (2012) inconsistency approves the effect of thermal treatment hinges on the harshness of the heat process, the definite factors that are representative of the food matrix and the sensitivity of the different phytochemicals to temperature (Rawson et al., 2011).

$$Y = 33.82 + 4.31X_1 + 1.33X_2 + 5.95X_1X_2 - 4.71X_1^2 + 0.83X_2^2$$

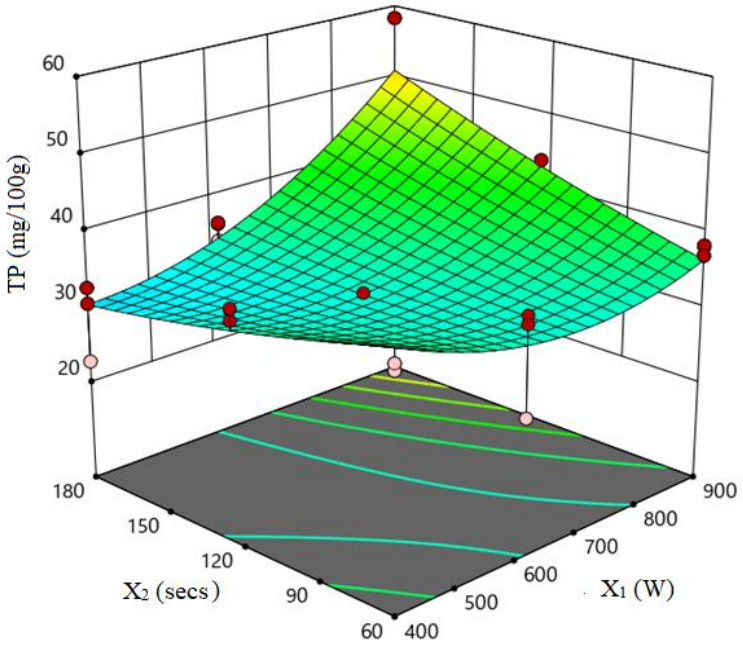

Figure 0.24: 3D RSM plot for the effect of microwave power (X_1) and microwave time (X_2) on the Total Phenols (TP) content of pineapple juice

$$Y = 47.00 + 3.91X_1 - 2.04X_2 - 1.10X_1X_2 - 1.20X_1^2 + 4.18X_2^2$$

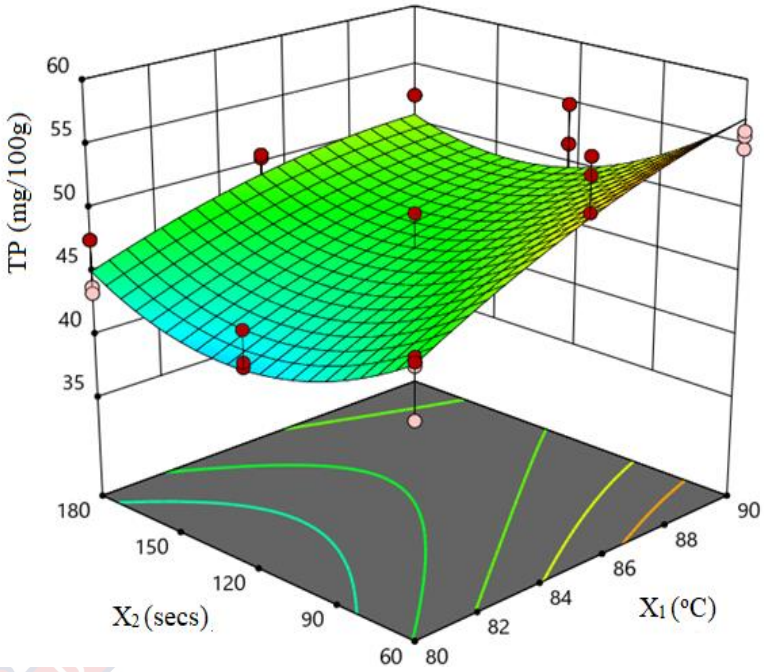

Figure 0.25: RSM plot for the effect of conventional temperature (X_1) and conventional time (X_2) on the Total Phenols (TP) of pineapple juice

4.8.8 Effect of Pasteurisation on Microbial Load Count of Pineapple Juice

Pasteurisation has been recognised as a possible breakthrough knowledge that can meet the requirements of the Food and Drug Administration (FDA) to achieve a 5-log decrease in microorganisms related with fruit juices (Salleh-Mack & Roberts, 2007). The microbial quality of the pineapple juice samples was evaluated based on four indicators, namely the APC, coliform count, staphylococcus and YM. In Table 4.3 and 4.4, a decrease in the microbiological load after microwave pasteurisation and conventional pasteurisation of pineapple juice can be observed. The microwave pasteurised pineapple juice samples showed values in the range of 0.0 to 2.34 log colony forming units (CFU)/mL for APC, 0.0 to 2.19 log CFU/mL for the coliform count and 0.0 to 2.36 log CFU/mL for YM. As shown in Table 4.2, APC, Coliform and YM recorded a decrease in microwave power from 400 to 900W, as well as an increase in microwave time from 60 to 180 sec. This shows that microbial spores may be resistant to microwave power treatment and that the destruction of spores occurs as microwave time increases.

Based on the regression coefficient results, the linear effect of microwave power, microwave time, and interaction effect of microwave power and microwave time on microbial count were significant ($p<0.01$) (Table 4.5) (Figure 4.15, 4.17 and 4.19). With increasing the microwave power, the effectiveness of microwave waves increased. This effect was pronounced at the 900W where the microbial count slope decreased with the increase of microwave time. Other researches have similarly indicated that the effect of microwave pasteurisation on the removal of microorganisms is significant (Canumir, Celis, de Bruijn, & Vidal, 2002).

From the regression coefficient results in Table 4.6, it was revealed that the quadratic effect of conventional temperature on aerobic plate count, staphylococcus and yeast and mould level was significantly ($p<0.01$) reduced, as the conventional temperature was increased (Table 4.6). This is possibly due to faster reaching to the higher temperatures and having an insufficient chance for microorganisms to adapt to the new conditions (Figure 4.16, 4.18 and 4.20). Also, the linear and quadratic effect of conventional time had a negative influence on yeast and mould level in pineapple juice. At lower conventional temperatures, increasing the conventional time did not affect the reduction of aerobic plate count. This shows that any increase in the independent variable would result in an immense negative value in the equation; as well as higher microbial removal.

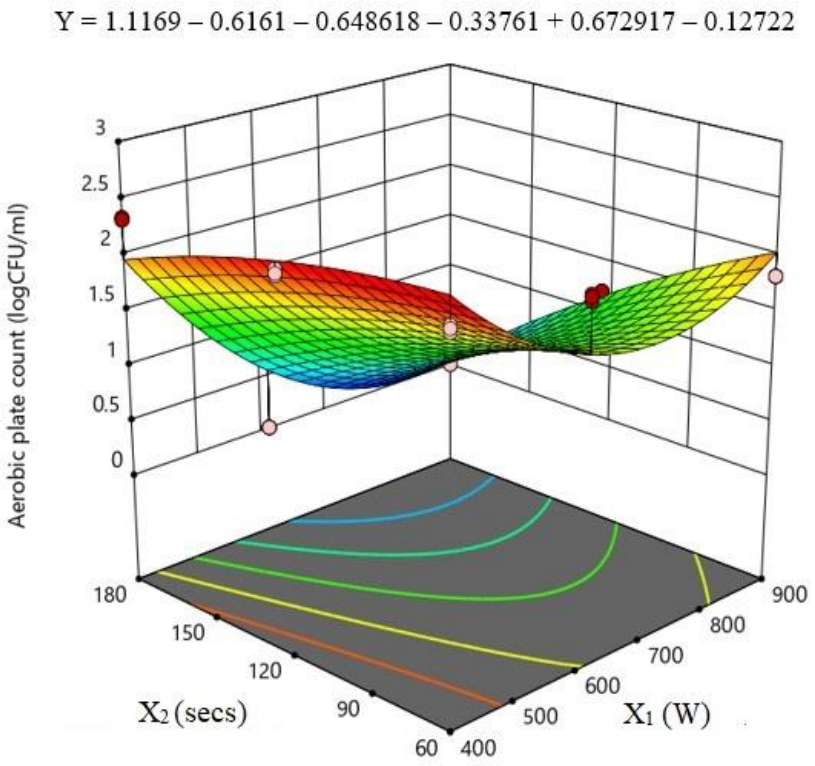

Figure 0.26: 3D RSM plot for the effect of microwave power (X_1) and microwave time (X_2) on the Aerobic Plate Count level of pineapple juice

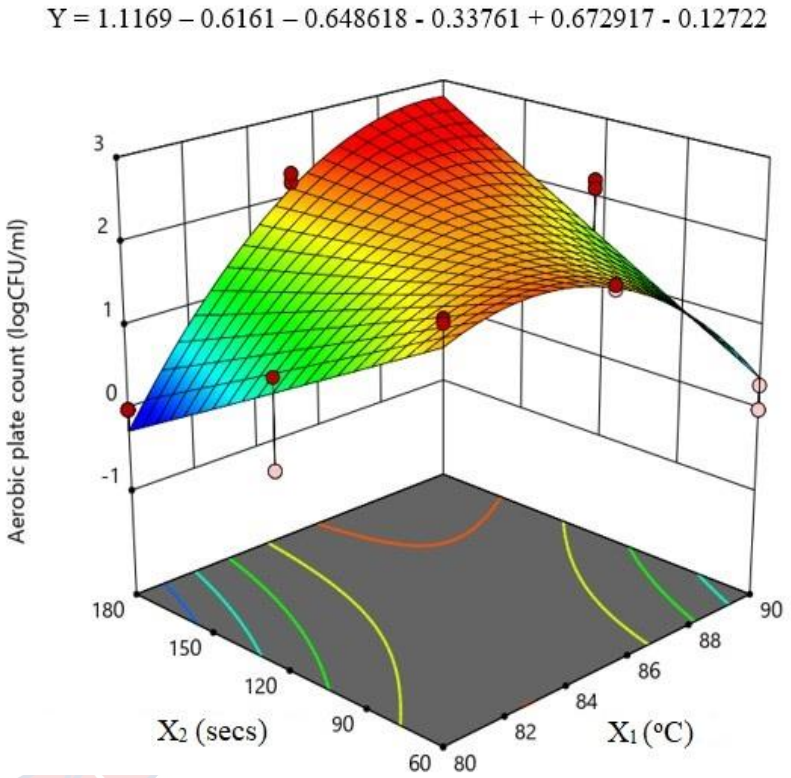

Figure 0.27: 3D RSM plot for the effect of conventional temperature (X_1) and conventional time (X_2) on Aerobic Plate Count level of pineapple juice

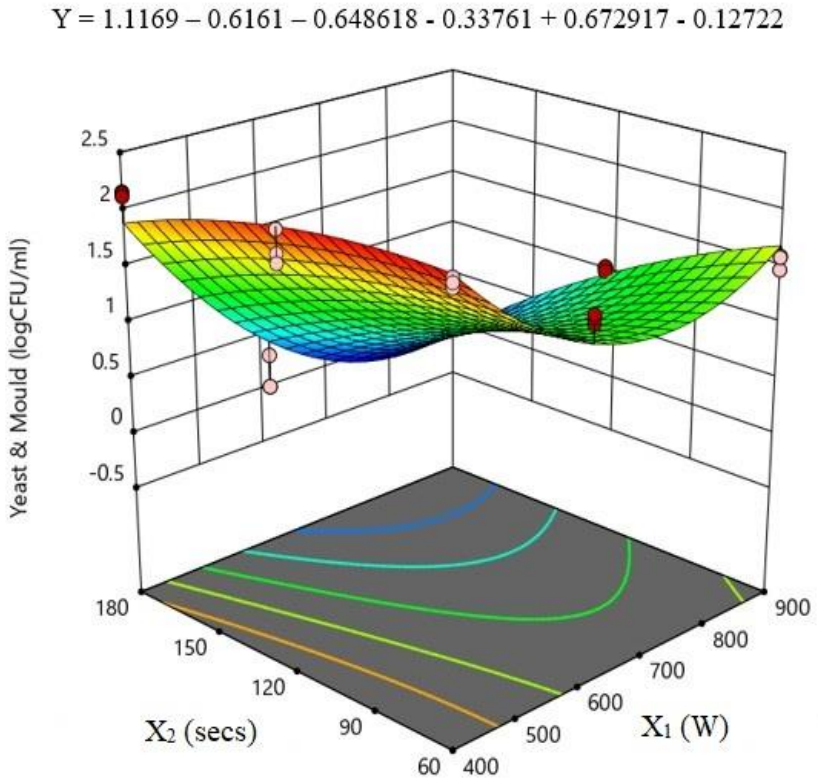

Figure 0.28: 3D RSM plot for the effect of microwave power (X_1) and microwave time (X_2) on the Yeast and Mould level of pineapple juice

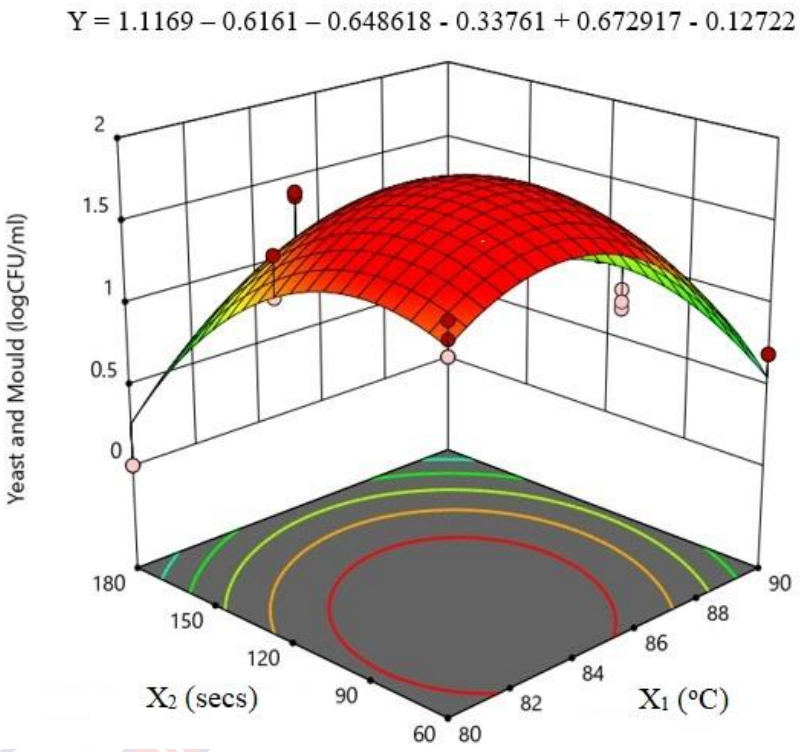

Figure 0.29: 3D RSM plot for the effect of conventional temperature (X_1) and conventional time (X_2) on the Yeast and Mould level of pineapple juice

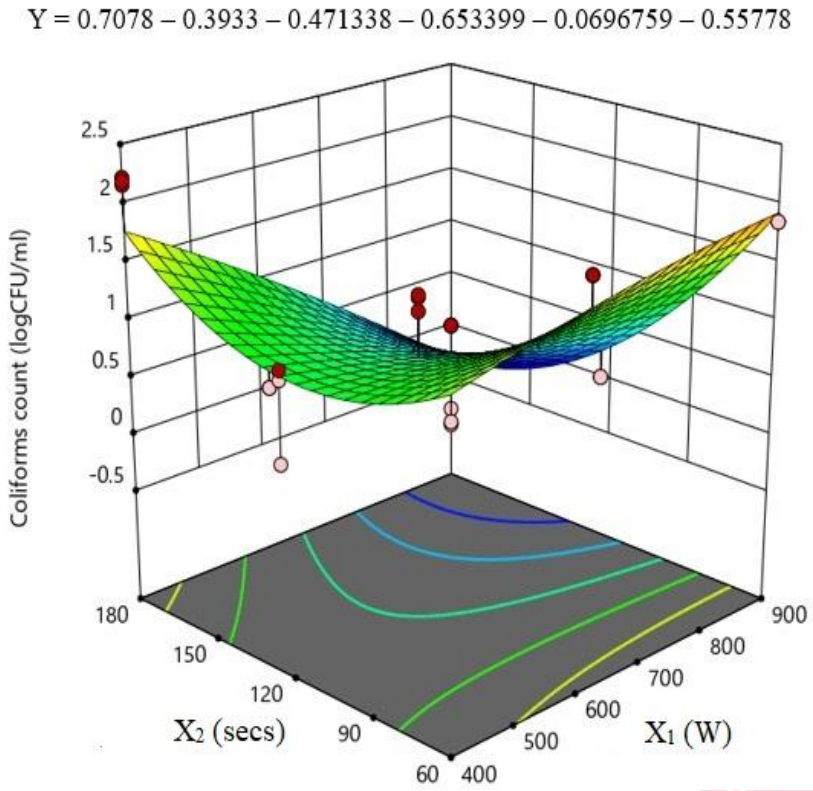

Figure 0.30: 3D RSM plot for the effect of microwave power (X_1) and microwave time (X_2) on the Coliform Count level of pineapple juice

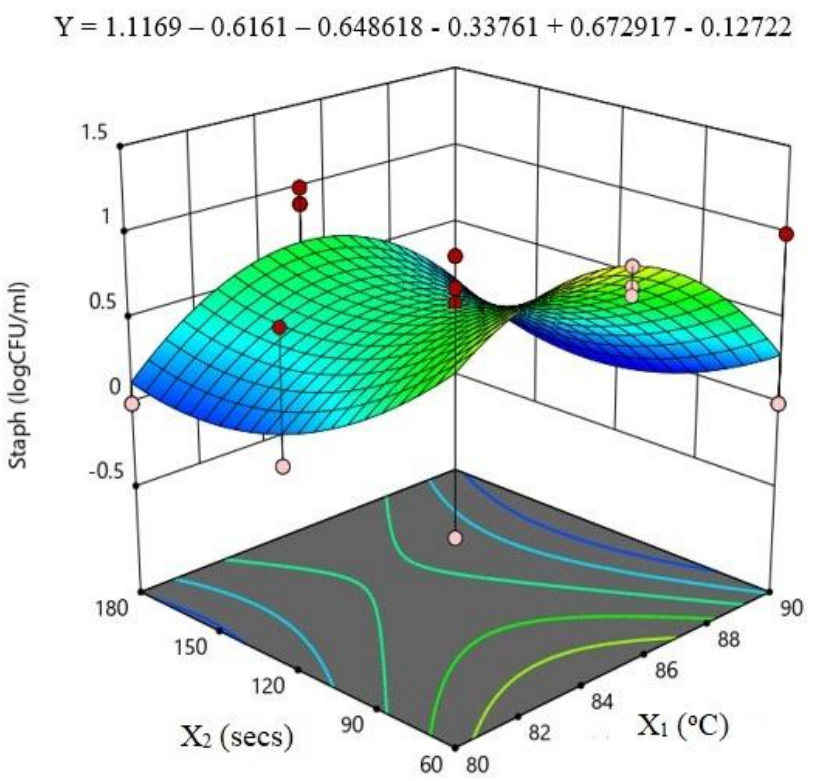

Figure 0.31: 3D RSM plot for the effect of conventional temperature (X_1) and conventional time (X_2) on the Staph level of pineapple juice

4.8.9 Optimization of the Variables and Responses

Using Design-Expert Software version 11.1 (Stat-Ease, Inc., Minneapolis, USA), the levels of variables were optimised using the Desirability Index principle in equation 2. The criterion for optimising the desirability index was to set goals for independent variables and responses. Models describing the greatest amount of variance in the least few terms were chosen. From table 4.5, the equations for brix, aerobic plate count and yeast and mould were highly predictive (R^2 = 0.9306, 0.9557 and 0.9513) respectively. Equations for ascorbic acid, total phenol and coliform count were moderately predictive (R^2 = 0.7415, 0.7480 and 0.6715) respectively. Whiles those for the total colour difference, pH, titratable acidity and total antioxidant capacity were not as good (R^2 = 0.4710, 0.3955, 0.2127 and 0.4874) respectively. The following criteria were applied to justify the choice of optimal conditions (Table 4.7). The desirability of the response gave an overall desirability of 0.921 (Figure 4.21). The result predicted with 95% confidence in the range of independent variables gave optimum microwave power 900W and microwave time 180 seconds. At this optimum condition, ΔE, Brix, pH, TA, TAC, AA, TP, Aerobic plate count, coliform and yeast and mould were found to be 22.989, 16.193%, 4.698, 1.306%, 215.046 mg/100g, 46.539 mg/kg, 50.946 mg/100g, 0.060 log CFU/ml, -0.322 log CFU/ml and -0.079 log CFU/ml respectively.

For the conventional pasteurisation treatment, the equations for aerobic plate count and yeast and mould were highly predictive (R^2 = 0.8581 and 0.8082) respectively. Equation for total colour difference, total antioxidant capacity, total phenol and staphylococcus were moderately predictive (R^2 = 0.6638, 0.5335, 0.6751 and 0.5648) respectively. Whiles those for brix, pH,

titratable acidity and ascorbic acid were not as good (R^2 = 0.4127, 0.2690, 0.1601 and 0.4002) respectively (Table 4.6). The following criteria were applied to justify the choice of optimal conditions (Table 4.8). The desirability of the response gave overall desirability of 0.692 (Figure 4.22). The result predicted with 95% confidence in the range of independence variables gave optimum conventional temperature 90 ^{o}C and conventional time 60 seconds. At this optimum condition, ΔE, Brix, pH, TA, TAC, AA, TP, Aerobic plate count, staph and yeast and mould were found to be 24.500, 15.465%, 4.844, 0.673%, 106.352 mg/100g, 42.970 mg/kg, 57.014 mg/100g, 0.391 log CFU/ml, 0.293 log CFU/ml and 0.556 log CFU/ml respectively.

Table 0.13: Criteria Applied for Optimization of the Variables from Microwave Pasteurised Treatment

Constraint	Goal	Lower limit	Upper limit
Microwave power (W)	is in range	400	900
Microwave time (sec)	is in range	60	180
ΔE	None	19.4393	56.3189
Brix	Maximize	14	16.2
pH	None	4.68	4.71
TA	None	1.088	1.408
TAC	None	136.069	223.584
AA	Maximize	33.4164	49.9934
TP	Maximize	22.7137	58.3034
Aerobic plate count	Minimize	0	2.36
Coliforms	Minimize	0	2.22
Yeast and Mould	Minimize	0	2.36

Table 0.14: Criteria Applied for Optimization of the Variables from Conventional Pasteurised Treatment

Constraint	Goal	Lower limit	Upper limit
Conventional temperature (^{o}C)	is in range	80	85
Conventional time (sec)	is in range	60	180
ΔE	Maximize	22.8031	28.1992
Brix	None	15	16.2
pH	None	4.5	5
TA	None	0.4649	0.7877
TAC	Maximize	50.1842	118.364
AA	None	13.5351	49.736
TP	Maximize	37.4678	57.6847
Aerobic plate count	Minimize	0	2.49
Stap	Minimize	0	0.146
Yeast and Mould	Minimize	0	1.6

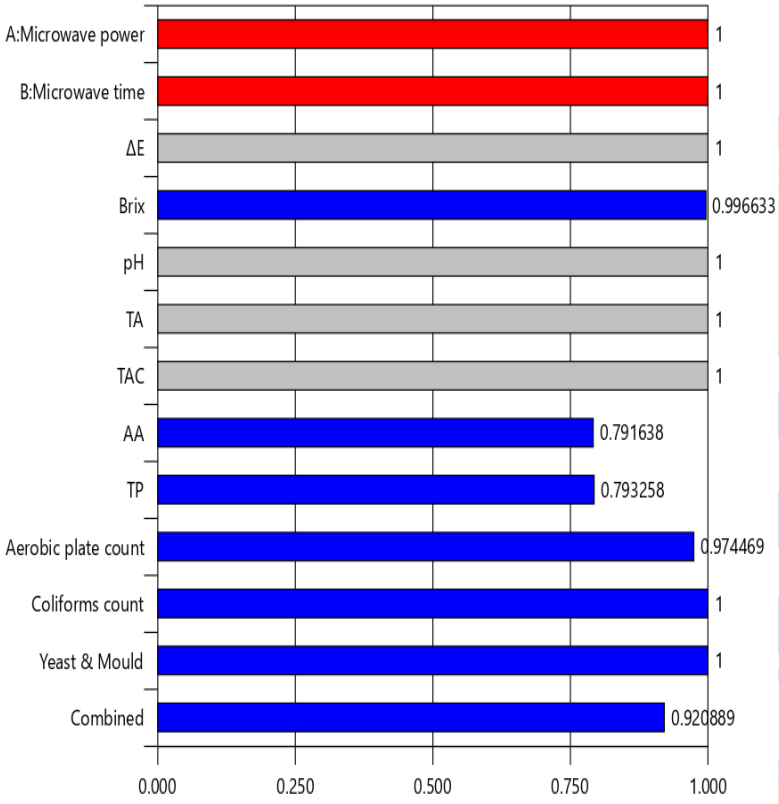

Figure 0.32: Effect of microwave power and microwave time on the graph of the desirability index for the optimal pineapple juice

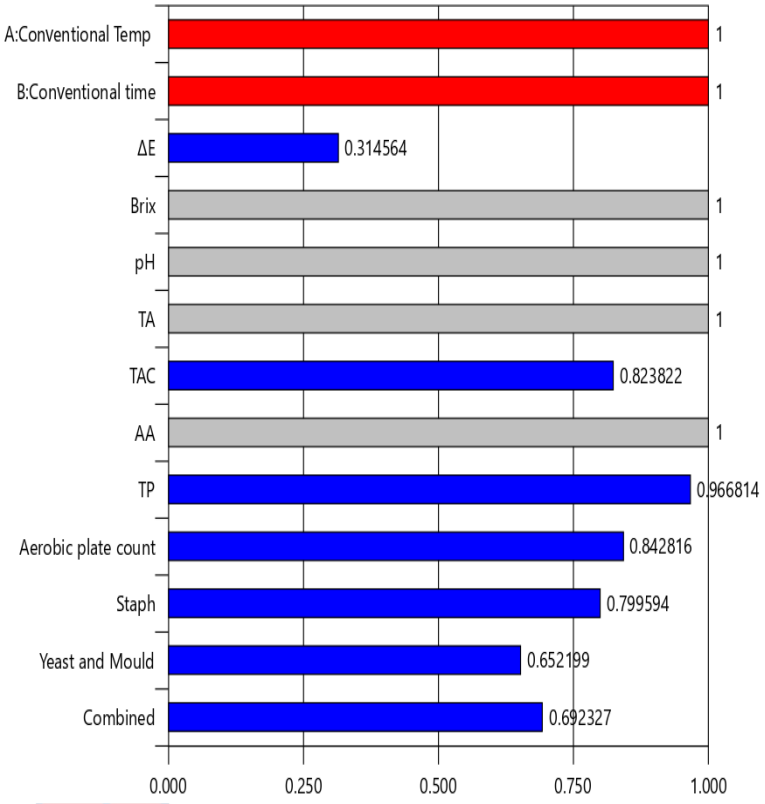

Figure 0.33: Effect of conventional temperature and conventional time on the graph of the desirability index for the optimal pineapple juice

CHAPTER FIVE

EFFECT OF STORAGE CONDITIONS ON OPTIMIZED PINEAPPLE JUICE SAFETY

5.1 Introduction

As known, pineapple juice has a unique flavour, and popularly consumed due to its fresh-like characteristics. It is complicated by many factors that contribute to its decomposition during storage, such as its pH, oxygen presence, temperature and enzyme activity (Bates et al., 2001). Inadequate storage is believed to cause deterioration and spoilage of fruit juice, as natural fruit juices are susceptible to spoilage due to their inherent properties. Also, fruit juice can spoil from microbial contamination, which makes it problematic to store. In the course of storage of pineapple juice, there is an inevitable decline in quality value; physicochemical properties are sensitive to some storage and environmental conditions (Olorunsogo & Adgidzi, 2013). This study employed the optimised conditions of the microwave, and conventional pasteurised treatment in objective two to study juice were obtained at 900 W/180 secs and 90 ^{o}C/60 secs, respectively. Optimised pasteurised pineapple juice was prepared and stored under room temperature, supermarket and refrigeration conditions and quality changes studied every (7) day for 21 days

Information on the physicochemical and microbial quality of conventional pasteurisation (90 ^{o}C at 60) and microwave pasteurisation (900 W at 180 secs) pineapple juice, stored at room temperature (28.5 ^{o}C), open-air supermarket (31.3 ^{o}C) and refrigeration (4.6 ^{o}C) conditions respectively are practically missing. In this context, few or no study may have been done

concerning the storage quality of pasteurised pineapple juice. The aim of this study was to optimize the process of inhibiting microbial growth using different pasteurization methods. The optimal condition obtained from this study could improve the process of inhibition of microbial growth during storage. The specific aims of the study were to

i. Determine the physicochemical properties of the pasteurised juice (room temperature, supermarket and refrigeration)
ii. Determine the microbial loads of the pasteurised pineapple juice under different storage conditions (room temperature, supermarket and refrigeration)

5.2 Materials and Methods

5.2.1 Juice Preparation, Bottling and Storage

Smooth cayenne pineapple fruit, large size at the same ripening stages was used for the experiment. This treatment condition was chosen because treated pineapple fruit juice had the most optimum qualities expected. The obtained juice was pasteurised using thermal pasteurisation (90^{o}C at 60 secs) and microwave pasteurisation (900 W at 180 secs). Bottling of pasteurised juice was carried out by using hot water sterilised bottles. Bottled juice was then cooled in a cold-water bath before it was sealed. Sealed bottle pasteurised pineapple juices were then stored at room temperature (28.5 ^{o}C), open-air supermarket (31.3 ^{o}C) and refrigeration (4.6 ^{o}C) conditions respectively for further study.

5.2.2 Pineapple Juice Extraction

Smooth cayenne pineapple varieties of large uniform size were peeled with a stainless steel knife and cut into pieces, and the juice extraction was done using an extractor machine. The obtained juice was pasteurised using the thermal pasteurisation (microwave and conventional) method, as stated below. Pasteurised juice was then stored at different storage conditions (room temperature, supermarket condition and refrigeration condition) for further investigations.

5.3 Experimental Design

The design used was a Completely Randomised Design (CRD). The treatments were storage conditions under room temperature, supermarket open-air and refrigeration. Each treatment was replicated three times.

5.4 Physicochemical Properties Determination

5.4.1 Determination of Total Soluble Solids (Brix)

A refractometer measures TSS or sugar as ^{o}brix in 0.1% graduations. The juice samples TSS was determined using a handheld refractometer (Atago Co. LTD., Tokyo, Japan), the calibration was done using distilled water. A drop of juice was placed on the refractometer prism, and the cover plate lowered to take the readings against the direction of light. The procedure was followed for all the samples, by ensuring the refractometer prism will be rinsed with distilled water and dried with a soft, lint-free tissue each time measurement was made.

5.4.2 Determination of Potential Hydrogen (pH)

The pH of the juice was obtained using a pH meter. Calibration was done with buffer solutions of pH 4 and 7. The juice sample was placed in a 100 ml

beaker, thoroughly mixed, and the pH meter was placed into its measurement device. Samples were taken at an average temperature of 25 ^{o}C.

5.4.3 Determination of Titratable Acidity (TA)

The acidity was measured using the method of (Rekha et al. 2012) up to pH 8.1. 10 ml fruit juice sample was diluted to 100 ml with distilled water and homogenised by shaking. The resulting solution was filtered using the Whatman filter paper to obtain a clear filtrate. 10 ml of filtrate was pipetted into a 200 ml conical flask and titrated to a standardised 0.1N sodium hydroxide burette solution using phenolphthalein as an indicator. The endpoint of the titration is reached when the filtrate is changed to a permanent pink colour. The volume of sodium hydroxide solution required for titration was noted. Titratable acidity (TA) of the juice sample was calculated according to Equation (5.1).

$$\text{TA}\left(\% \frac{\text{w}}{\text{w}}\right) = \frac{\text{Net ml of titrant x Normality of titrant x 6.4}}{\text{sample weight}} \quad (5.1)$$

5.4.4 Determination of Total Antioxidant Capacity (TAC)

The spectrophotometer was used to determine the TAC for pasteurised pineapple juice using the phosphomolybdenum assay method described by Prieto, Pineda and Aguilar (1999) with minor modifications. The 0.5 mL of 1 mg/mL sample was mixed with 3 mL of phosphomolybdate reagent (28 mM sodium phosphate and 74 mM ammonium molybdate in 0.6 M sulphuric acid). The incubation was then performed at 95 degrees Celsius for 90 minutes. This sample was run with the aid of a UV-visible spectrophotometer (Varian Inc., Mulgrave, Australia) at 695 nm, which was against the sample blank (1 mL

water without juice extract). TAC performance was expressed as a standardised, equivalent of AA(Kasangana, Haddad, & Stevanovic, 2015).

5.4.5 Ascorbic Acid Determination (AA)

The AA content of the pineapple varieties were determined each by the spectrophotometric technique according to the protocol reported by Harris and Ray (1935) and with the slight modifications proposed by Abano, Ma, & Qu (2014). 5ml of pineapple juice was transferred into a 25ml volumetric flask and diluted to the mark with 4% oxalic acid. 10ml aliquot was transferred into a conical flask, and bromine water was added dropwise to fill it till it turns orange-yellow colour due to the excess bromine. The excess bromine was blown off. 0.5ml of the brominated extract was transferred into test tubes in triplicate, respectively. The volume was made up to 3ml with 4% oxalic acid. 1ml of DNPH reagent was added, followed by 1ml 10% of thiourea. A blank preparation was similarly made, but water in place of sample extract was used. The content was thoroughly mixed and incubated at 37^{o}C for three hours. The orange-red crystals formed (osazone) was dissolved with 4ml of 80% sulphuric acid. The absorbance of each solution was measured at 540 nm using a visible spectrophotometer.

A standard ascorbic acid was prepared similarly by pipetting 100, 200, 300, 400 and 500 µL of 10mg/ml standard ascorbic acid solution into a series of test tubes. All the reagents were similarly added as above, and absorbances were taken at 540nm. A standard calibration plot ascorbic acid concentration versus absorbance was prepared, and the ascorbic acid content was calculated from the mathematical standard plot.

$$Y = 0.0024x+0.0866 \quad (5.2)$$

Where;

Y = absorbance and x = concentration (µg/ml).

5.4.6 Determination of Total Phenolic Content (TPC)

Each sample was tested for total phenols (Singleton & Rossi, 1965). The standard curve was plotted using different concentrations of Gallic acid (standard 0-200 µg/ml) and the results of the phenolic measurements were expressed as mg of Gallic acid equivalents (mg GAE) per 100 ml. The total phenol content of the pasteurised pineapple juice was measured spectrophotometrically using the Folin-Ciocalteu method. 5 ml (approx. 5g) were measured and diluted to 50 ml. Aliquots (0.5 mL of the sample) were mixed with 2 mL of Folin–Ciocalteu reagent and 2 mL of aqueous sodium carbonate (75 mg/mL) solution. The final mixture was incubated at room temperature for 30 minutes and the absorbance was read at 765 nm against the blank solution. Gallic acid was used to prepare the calibration curve and these results are expressed in gallic acid equivalents (mg5-007GAE/g) (Kasangana et al., 2015).

5.5 Microbial Analysis

Aerobic Plate Count, faecal coliforms, and *Salmonella sp*, Y&M were identified using Standard plate count technique (Chouhan, 2015). By ISO standards (ISO-4833-2, 2013) (Bender et al., 2000), the samples were analysed for APC, Total coliforms, and *E. coli*, Y&M all in CFU per ml using the pour plate method. Culture media consisting of Plate Count agar *(Oxoid, Hampshire,* England), Peptone Water (Oxoid) and Eosin Methylene Blue agar, Mannitol Salt Agar and Potato Dextrose agar were prepared according to the instructions

stated by the manufacturing company. Peptone water was used as a diluent for recovery, 180 ml of it was prepared in triplicate and sterilised by autoclaving with all prepared media and Petri dishes at a temperature of 121°C, a pressure of 15 psi for 15 minutes. The sample was well homogenised, 20 ml of the test sample was aseptically weighed into Peptone water and incubated in a water bath for 30minutes at 37°C. The sample was one after the other diluted to a 10^{-3} in sterile peptone water. Triplicate dilutions of 0.1 ml and 1 ml of 10^{-2} dilution of the sample were placed on the agar plate and kept warm at 37°C for 48 hours. All colonies were counted and an average of duplicate samples were recorded as aerobic plate counts (CFU/ml) for the sample.

In addition, triplicate dilutions of 0.1 ml and 1 ml of 10^{-1} per sample were plated on Eosin Methylene Blue agar. For total coliform counts (CFU/ml) of the sample, one of the duplicate dilutions was incubated at 37 °C for 48 hours. Triplicate dilutions of 0.1 ml and 1 ml of 10^{-1} per sample were plated on Mannitol Salt Agar. For staph counts (CFU/ml) for the sample, one of the duplicate dilutions was incubated at 37 °C for 48 hours.

For Yeast and Moulds, triplicate dilutions of 0.1 ml and 1 ml of 10^{-1} each sample were plated on Potato Dextrose agar supplemented with ampicillin. One each of the triplicate dilutions was incubated at room temperature for 7 days to observe for yeast and mould counts (CFU/ml) for the sample.

For the Escherichia coli presumptive test, isolated colonies of about 2-3mm diameter exhibiting a greenish metallic glow with reflected light and dark purple centres were identified as possible colonies of Escherichia coli (Anjana et al., 2021; Leininger, Roberson, & Elvinger, 2001). Isolated colonies were inoculated into the medium Oxoid SIM (Sulfite Indole Motility) stabbing the

needle about two-thirds of the way to the depth. It was in incubation at 37°C for 24 hours otherwise until growth was evident. Five drops of Kovác's reagent were added to the top of the deep to test for the presence of indole. A positive indole test is indicated by the formation of red colour in the reagent layer at the top of the agar deep within seconds of the addition of the reagent. (MacWilliams, 2009).

5.6 Statistical Analysis

In this study, a one-way ANOVA was performed using Minitab to determine the influence of storage conditions and storage time on specific chemical properties. Where there were significant differences, the means were separated using Tukey's pairwise comparison at a 5% significance level.

5.7 Results and Discussions

A completely randomised design was used to create the influence of independent variables (storage condition and storage days) on physical and chemical properties and microbial load of optimised pasteurised pineapple juice. The results of the experiment were performed according to the analysis of means model in ANOVA.

5.8 Influence of Storage Condition and Time on Potential Hydrogen (pH) of Pineapple Juice

Table 5.1 presents the effect of storage conditions and storage time on the microwave and conventional pasteurised pineapple juice. According to Sanchez-Vega et al. (2009), pH is one of the main characteristics that determine the stability of bioactive compounds in fruit juice. The results from pH readings showed significant changes throughout the duration of storage under different

conditions. During 21 days of storage, pH was observed to fluctuate in all treatments. This may be due to the initiation of fermentation and the presence of microorganism-reducing lactic acid. The pH of microwave pasteurised pineapple juice was higher than that of conventional pasteurised throughout the storage period. Microwave pasteurised pineapple juice, stored under room temperature and refrigeration showed a significant pH fluctuation ($p<0.05$) during three weeks of storage. In agreement with our studies, Cortés et al. (2008), similarly found out that the pH level of fresh, high-pressurised and pasteurised orange juice during seven weeks of storage at 2 ^{o}C and 10 ^{o}C increased significantly. The increase in pH level was considered to be caused by microorganisms, which causes the juice to spoil.

On the other hand, during storage under room temperature and refrigeration, there were no significant changes in the pH value of conventionally pasteurised pineapple juice. The above findings are consistent with that of Rivas, Rodrigo, Martinez, Barbosa-Cánovas and Rodrigo (2006), who disclosed no pH variation in thermally treated juice (blended orange and carrot juice) during refrigeration storage at 2^{o}C and 12 ^{o}C. Similarly, there was no significant changes detected in Yeom, Streaker, Zhang, and Min (2000) in heated orange juice stored at 4^{o}C and 22^{o}C.

Table 0.15: Influence of storage condition and storage days on potential hydrogen

Pasteurisation	Storage time (weeks)	Storage conditions		
		Room temp.	Supermarket	Refrigeration
Microwave	0	4.90 a	4.75 a	4.75 ab
	1	4.40 c	4.60 a	4.65 b
	2	4.65 b	4.00 a	4.80 ab
	3	ND	4.70 a	4.90 a
	Lsd	0.1757	0.1125	0.1299
Conventional	0	4.70 a	4.75 a	4.80 a
	1	4.55 a	4.30 b	4.65 a
	2	4.70 a	4.00 c	4.75 a
	3	ND	ND	4.75 a
	Lsd	0.5270	0.1757	0.3375

Values in each column with different letters indicate a significant difference between each other according to the least significant difference test

5.8.1 Influence of Storage Condition and Time on Titrable Acidity (TA) of Pineapple Juice

The titrable acidity for the pasteurised pineapple juice during storage was observed. The difference in titrable acidity due to storage conditions on pineapple juice is presented in Table 5.2. Similarly, the titratable acidity was observed to fluctuate in all the treatments during 21 days of storage as observed in pH determination. This could also be due to the activity of fermentation, resulting in the presence of lactic acid and the reduction of the microorganism.

It was observed that the titrated acidity of microwave pasteurised pineapple juice was higher than that of conventional pasteurised juice throughout the storage period. Both microwave and conventional pasteurised pineapple juice, stored at room temperature and in a supermarket condition, showed statistically significant titrated acidity fluctuations ($p<0.05$) during the three weeks of storage. Conversely, during storage under refrigeration, there was no significant change in the titrable acidity value in both microwave and conventionally pasteurised pineapple juice. The rate of decrease in refrigerated pasteurised pineapple juice was comparatively lower.

Table 0.16: Influence of storage condition and storage days on TA

Pasteurisation	Storage time (weeks)	Storage conditions		
		Room temp.	Supermarket	Refrigeration
Microwave	0	1.683 b	1.741 b	1.779 a
	1	2.272 a	2.566 a	2.157 a
	2	1.990 a	2.918 a	1.702 a
	3	ND	1.587 b	1.786 a
	Lsd	0.5846	0.5298	0.5243
Conventional	0	1.581 a	1.754 c	1.542 a
	1	1.786 a	2.144 b	1.549 a
	2	1.677 a	2.797 a	1.805 a
	3	ND	ND	1.574 a
	Lsd	0.7140	0.1921	0.2706

Values in each column with different letters indicate a significant difference between each other according to the least significant difference test

5.8.2 Influence of Storage Condition and Time on Total Phenols (TP) of Pineapple Juice

Table 5.3 shows the effect of storage conditions on total microwave phenolic and conventional pasteurised pineapple juice. According to Kaur and Kapoor (2001), phenolic compounds have health-enhancing properties and contribute to the flavour and colour attributes of fruits and vegetables. From the study, the total phenolic content of microwave pasteurised juice was maintained above that achieved in conventional pasteurised pineapple juice at the end of (21) days of storage. Changes in total phenol content of pasteurised juice have been observed to be negligible during the supermarket and refrigeration storage period. Phenolic compounds in juices have been maintained during storage because peroxidase, an enzyme that degrades phenolic compounds, has been inactivated. (Odriozola-Serrano, Soliva-Fortuny, Gimeno-Añó, & Martín-Belloso, 2008). Nevertheless, as shown in Table 5.3, the phenolic compounds in conventional pasteurised pineapple juice sharply increased during the first week and decreased slightly ($p>0.05$) during Week 2. Microwave pasteurised samples stored at room temperature and refrigeration were similar; total phenolic decreased sharply during Week 1, increased slightly ($p>0.05$) at Week 2, and decreased at Week 4 for the refrigerated sample. This observation is supported by the findings of Klimczak, Małecka, Szlachta and Gliszczyńska-ɯwigło (2007), which reported that total orange juice phenols decreased after four months of storage and increased significantly at the end of 6 months of storage. These findings reported that during storage, certain compounds could form and react with the Folin-Ciocalteau reagent to increase the phenolic content.

Table 0.17: Influence of storage condition and storage days on total phenols

Pasteurisation	Storage time (weeks)	Storage conditions		
		Room temp.	Supermarket	Refrigeration
Microwave	0	28.07 a	35.29 a	38.21 a
	1	25.75 a	29.94 a	34.00 a
	2	25.85 a	29.29 a	35.45 a
	3	ND	32.03 a	34.24 a
	Lsd	5.367	5.251	7.654
Conventional	0	26.62 ab	28.62 a	33.35 a
	1	28.40 a	26.74 a	32.26 a
	2	24.94 b	25.47 a	31.23 a
	3	ND	ND	28.13 a
	Lsd	1.843	11.11	9.21

Values in each column with different letters indicate a significant difference between each other according to the least significant difference test

5.8.3 Influence of Storage Condition and Time on Total Antioxidant (TAC) Content of Pineapple Juice

The effect of storage condition and time on the TAC of pasteurised pineapple juice is presented in Table 5.4. There was a general reduction in the total antioxidant capacity of pasteurised pineapple juice throughout the storage duration, irrespective of the storage condition. The total antioxidant content of the pasteurised pineapple juice was not statistically significantly different from each storage condition and time. Although, the retention of total antioxidant at the end of the storage duration was observed to be higher in the microwave

pasteurised refrigerated pineapple juice. The minimal decrease in TAC at refrigeration storage could be attributed to the minimal decreases in antioxidant compounds like phenolic content and vitamin C as compared to room and supermarket storage conditions, where some of these compounds decreased rapidly during storage. A similar result of a decrease in total antioxidant activity of fruit juices have been reported during storage of grapefruit juice at -18 and 4 ^{o}C (Igual et al., 2010) and commercial orange juices at 18, 28 and 38 ^{o}C (Klimczak et al., 2007). Furthermore, studies by Anaya-Esparza et al. (2017) and Mgaya-Kilima, Remberg, Chove, and Wicklund (2014) in other fruit juices, reported no changes in antioxidant capacity during storage.

Table 0.18: Influence of storage condition and storage days on TAC content

Pasteurisation	Storage time (weeks)	Storage conditions		
		Room temp.	Supermarket	Refrigeration
Microwave	0	122.1 a	124.7 a	137.6 a
	1	113.3 a	116.1 a	135.7 a
	2	105.1 a	112.8 a	129.3 a
	3	ND	110.0 a	119.0 a
	Lsd	34.93	15.44	19.23
Conventional	0	108.20 a	99.41 a	160.5 a
	1	97.50 a	102.30 a	125.3 a
	2	96.40 a	93.69 a	125.4 a
	3	ND	ND	113.6 a
	Lsd	19.35	54.09	84.26

Values in each column with different letters indicate a significant difference between each other according to the least significant difference test.

5.8.4 Influence of Storage Condition and Time on Ascorbic Acid (AA) of Pineapple Juice

The results on ascorbic content of pasteurised pineapple juice as affected by storage condition and storage time is presented in Table 5.5. It is clear from the data shown in Table 5.5 that the AA content of microwave pasteurised pineapple juice kept under supermarket storage condition was significantly ($p<0.05$) affected by the storage days. Thus, as the storage days increase, the ascorbic acid content decreases at an increasing rate. Significantly higher ascorbic acid (24.26 mg/kg) content was retained under refrigeration condition and the least ascorbic acid (11.73 mg/kg) under the supermarket storage condition. Ascorbic acid content has also been observed to decrease during and storage in studies by Attri, Krishna, Ahmed, and Kumar (2014) in Ginger squash, and Deka (2000) in Ready-To-Serve (RTS) drink prepared from mango: pineapple. Also, Plaza et al. (2006); Polydera, Stoforos, and Taoukis (2003); Tiwari, O'Donnell, Muthukumarappan, and Cullen (2009) observed a reduction in ascorbic acid in thermally pasteurised orange juice and Achinewhu and Hart (1994) observed a reduction in ascorbic acid in thermally pasteurised in pineapple juice during storage.

Table 0.19: Influence of storage condition and storage days on AA

Pasteurisation	Storage time (weeks)	Storage conditions		
		Room temp.	Supermarket	Refrigeration
Microwave	0	22.55 a	25.83 a	26.66 a
	1	20.89 a	22.56 ab	25.58 a
	2	18.02 a	18.20 bc	23.36 a
	3	ND	13.48 c	20.26 a
	Lsd	6.545	3.438	4.983
Conventional	0	19.78 a	19.15a	20.80 a
	1	16.92 a	22.29 a	16.68 a
	2	13.20 a	11.73 a	12.86 a
	3	ND	ND	11.87 a
	Lsd	6.673	31.34	7.845

Values in each column with different letters indicate a significant difference between each other according to the least significant difference test

5.8.5 Influence of Storage Condition and Time on Total Soluble Solids (TSS) of Pineapple Juice

Table 5.6 presents findings on the impact of storage conditions and time on the total soluble solids of pasteurised pineapple juices. Over the three weeks of storage, the total soluble solids of conventional pasteurised pineapple juice were lower than the microwave pasteurised juice. There was a significant difference between treatments. The maximum TSS was recorded in refrigerated pineapple juice for treatments on the 0th day of storage (16.60 ^{o}brix). The rate

of TSS decrease in pasteurised pineapple juice was relatively lower in refrigerated pineapple juice compared to pasteurised pineapple juice stored in the supermarket and room temperature. Total soluble solids (TSS) remained almost invariable and no significant changes were detected during storage under the conditions of the supermarket ($p<0.05$)

Reduction of TSS content in pineapple juice during storage time was due to the growth of yeasts and moulds where the microorganisms utilise the sugar for growth, thus, reducing the °Brix value (Shamsudin, Adzahan, Yee, & Mansor, 2014). All in all, the sugars of pasteurised pineapple juice during storage in different conditions was observed to decrease. TSS of refrigerated pineapple juice showed a substantial increase compared to room temperature and supermarket storage period from the one week. The total soluble solids of pineapple juice stored at room temperature and supermarket conditions showed a gradual increase from the two weeks of storage till the end. Our findings agree with the study by Bhupinder, Sharma, and Harinder (1991), as they observe a decrease in total soluble solids in sugarcane juice during storage. The changes in total soluble solids in fruit juice are as a result of the presence of microorganisms in the juice, according to Rivas et al. (2006), which causes the fermentation of sugar as the fruit juice deteriorates. Such phenomena could have occurred in the pasteurised pineapple juice, kept in room temperature during the third week of storage, as it was observed that the pineapple juice began to smell fermented as soon as the bottles were uncoated (Rosen & Gothard, 2010).

Table 0.20: Influence of storage condition and storage days on TSS

Pasteurisation	Storage time (weeks)	Storage conditions		
		Room temp.	Supermarket	Refrigeration
Microwave	0	15.55 a	15.75 a	16.60 a
	1	14.95 b	16.35 a	16.30 a
	2	15.00 b	15.80 a	15.80 b
	3	ND	15.95 a	15.65 b
	Lsd	0.1757	0.5626	0.2832
Conventional	0	15.00 a	16.25 a	16.00 a
	1	15.40 a	15.70 a	15.40 ab
	2	14.40 a	15.60 a	15.05 b
	3	ND	ND	15.10 b
	Lsd	0.3513	0.8783	16.00 a

Values in each column with different letters indicate a significant difference between each other according to the least significant difference test

5.8.6 Influence of Storage Condition and Days on Microbial Load Count of Pineapple Juice

Figures 5.1 and 5.2 demonstrate the effect of storage on the aerobic plate counts of microwave pasteurised and conventional pasteurised pineapple juice, respectively. It was observed that both conventional pasteurised pineapple juice stored under room temperature and open-air supermarket condition had gone bad, and not wholesome for consumption. Similarly, room temperature storage of microwave pasteurised pineapple juice was observed to have gone bad at

after 21 days of storage. The mean aerobic plate count (CFU/ml) among the storage conditions for microwave pasteurised pineapple juice was observed to range from ($\log_{10}$ 0.00 to $\log_{10}$ 2.81) CFU/ml, during the 21 days storage, whiles the mean aerobic plate count (APC) ranged from ($\log_{10}$ 0.00 to $\log_{10}$ 2.43) CFU/ml, for conventional pasteurised pineapple juice. Among the storage condition, conventional pasteurised pineapple juice stored under open-air supermarket condition showed the highest aerobic plate count ($\log_{10}$ 2.18) CFU/ml, with the least count ($\log_{10}$ 1.36) CFU/ml in refrigerated microwave pasteurised pineapple juice after 21 days of storage.

Figure 5.3 and 5.4 shows the effect of storage on the yeast and mould count of counts microwave pasteurised and conventional pasteurised pineapple juice, respectively. The mean yeast and mould count (CFU/ml) among the storage conditions for microwave pasteurised pineapple juice were observed to range from ($\log_{10}$ 0.00 to $\log_{10}$ 3.01) CFU/ml, during the 21 days storage, whiles the mean yeats and mould count (YM) ranged from ($\log_{10}$ 0.00 to $\log_{10}$ 3.12) CFU/ml, for conventional pasteurised pineapple juice. Among the storage condition, conventional pasteurised pineapple juice stored under room temperature showed the highest yeast and mould count ($\log_{10}$ 3.12) CFU/ml, with the least count ($\log_{10}$ 2.57) CFU/ml in refrigerated microwave pasteurised pineapple juice after 21 days of storage.

The low aerobic plate counts for refrigerated microwave pasteurised pineapple juicer could probably be due to reduced initial microbial loads of the pineapple juice, as well as reduced pH resulting from bacterial metabolic activities (Kaddumukasa, Imathiu, Mathara, & Nakavuma, 2017). In general, the microbial quality of the pineapple juice from all three pineapple varieties

was below the limit of microbial shelf life for juice, which is 6 log CFU/ml.

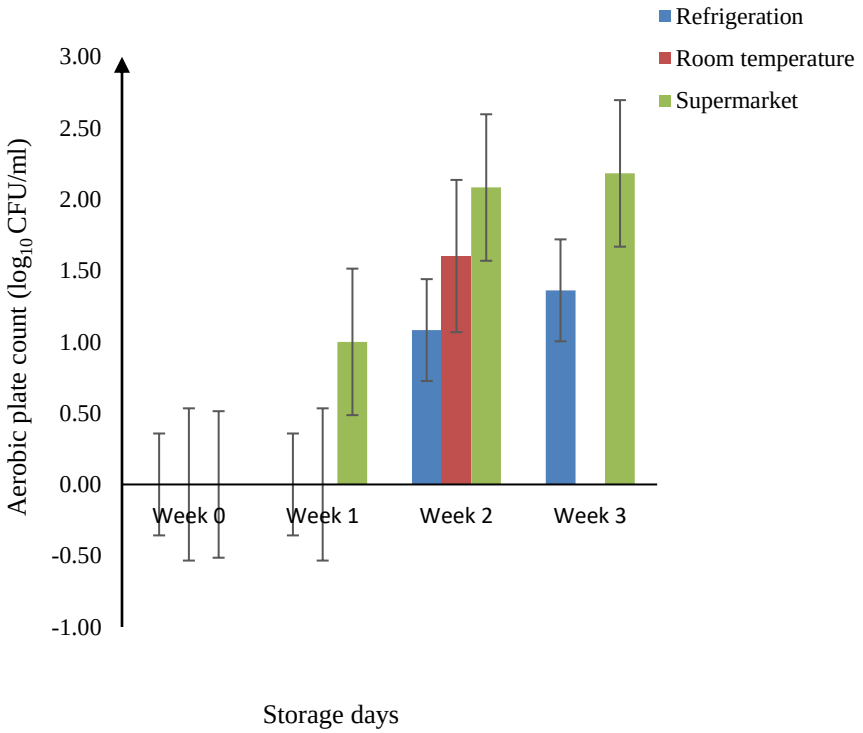

Figure 0.34: Influence of storage conditions and days on the aerobic plate count of microwave pasteurised pineapple juice

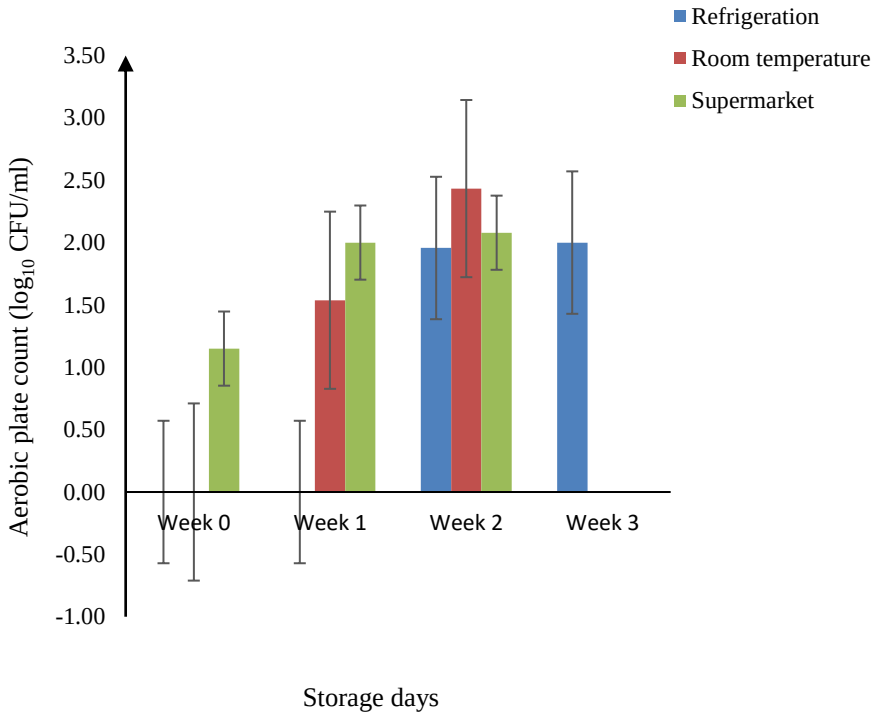

Figure 0.35: Influence of storage conditions and days on the aerobic plate count of conventional pasteurised pineapple juice

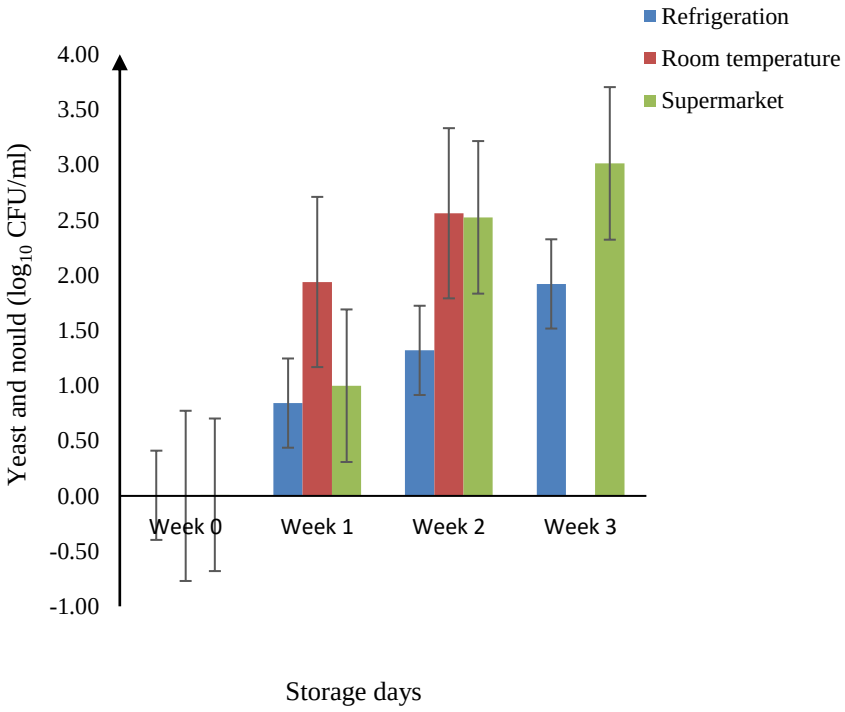

Figure 0.36: Effect of storage conditions and days on the yeast and mould of microwave pasteurised pineapple juice

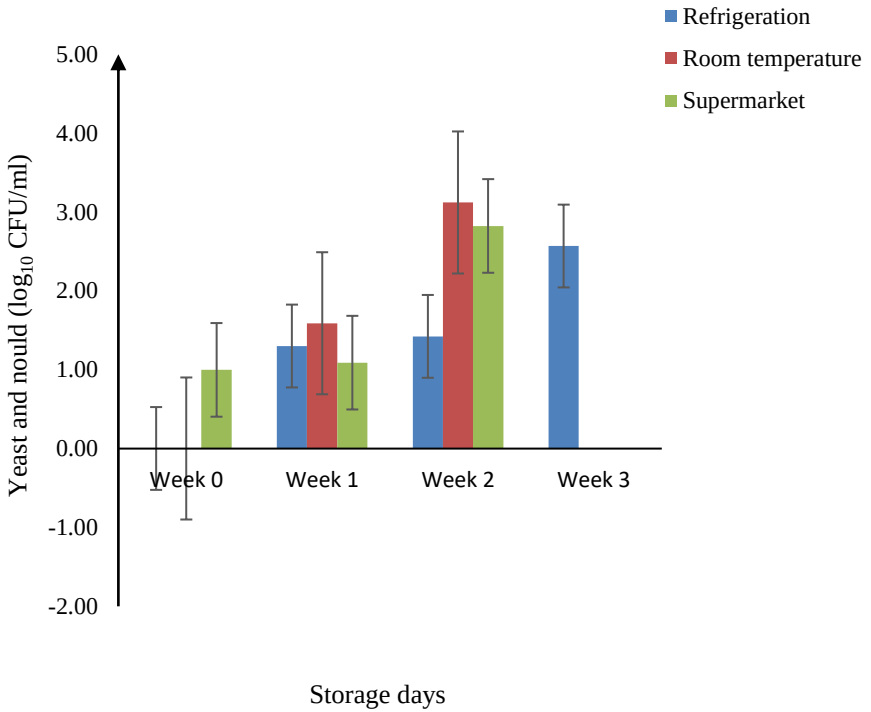

Figure 0.37: Effect of storage conditions and days on the yeast and mould of conventional pasteurised pineapple juice

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The effects of two independent variables (pineapple variety and pineapple size) on response variables juice yield, total soluble solids (TSS), titratable acidity (TA), ascorbic acid and pH, as well as the sensory attributes expressed by colour, flavour, taste, aftertaste, and overall acceptability were investigated. Within the range of the independent variables studied, the main effect of pineapple variety was observed to have positively significant ($p<0.001$) influence on total soluble solids, titratable acidity and ascorbic acid content. The highest TSS was obtained in sugarloaf variety; whiles MD2 pineapple variety recorded the highest titratable acidity and ascorbic acid content. The processing of large size smooth cayenne variety yielded the highest (559.256 ml/kg) juice content.

Also, the effects on independent variables microwave pasteurisation (400-900 W at 60-180 secs) and conventional pasteurisation (80-90^{o} at 60-180) on response variables (pH, total soluble solids, titratable acidity, total phenolic content, ascorbic acid content, total antioxidant content, total phenols, aerobic plate count, coliform count, staphylococcus and yeast and mould) were investigated. Increase in the levels of microwave power resulted in significant increased total soluble solids, pH, titratable acidity, ascorbic acid, and total phenol. The optimum level of microwave pasteurisation was obtained at microwave power 900W and microwave time 180 seconds. On the other hand, increase in conventional temperature resulted in an insignificant decrease in

total soluble solids, pH, titratable acidity, and ascorbic acid, but a significant increase in total phenol content, at conventional temperature 90^{o}C and conventional time 60 seconds. Microbial counts were significantly decreased, as microwave power and conventional temperature were increased.

For pasteurised optimised pineapple juice, stored under room temperature, open-air supermarket and refrigeration conditions, quality changes (pH, total soluble solids, titratable acidity, total phenolic content, ascorbic acid content, total antioxidant content, total phenols) and microbial safety were investigated. No significant changes were detected in total antioxidant values after storage of both pasteurised pineapple juice. However, the differences in pH, total soluble solids (TSS), titratable acidity (TA) total phenolic (TP) content, ascorbic acid content, and total phenols (TP) throughout the 21days storage period was observed to be decreased in a fluctuating order for room temperature and supermarket storage condition. The shelf life of refrigerated microwave pasteurised pineapple juice was 21 day compared to 14 days for supermarket and room temperature conventional pasteurised pineapple juice. The degradation of these nutrients was rapid in the conventional pasteurised sample during storage, as compared to refrigerated microwave pasteurised pineapple juice.

6.2 Conclusion

Statistical analysis using Response Surface Method in an I-optimal design appeared to be a valuable tool for studying the effects of independent variables, pineapple variety and fruit size, pasteurisation treatment (microwave power and microwave time, conventional temperature and conventional time, and storage conditions (room temperature, open-air supermarket and refrigeration) in

production of optimised pineapple juice. The response surface quadratic models and graphical representation of effects led to a better understanding of the effects of the different variables on the pineapple juice characteristics.

During storage of pasteurised pineapple juice, the microbial quality of the stored pasteurised pineapple juice was below the limit of microbial shelf life for juice. Microwave pasteurised pineapple juice was found to be the best since there was minimal ($\log_{10}$ 1.36) CFU/ml aerobic plate count and $\log_{10}$ 2.57) CFU/ml yeast and mould contamination after 3 weeks of storage.

6.3 Recommendation

In light of the results of this study, the following recommendations are made with some suggestions for further study.

1. To determine the effect of alternative packages on pasteurised pineapple juice physicochemical properties and shelf-life.
2. To determine the effect of microwave and conventional pasteurisation on the nutritional composition of pineapple juice.

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APPENDICES

APPENDIX B

ANOVA for conventional pasteurised pineapple juicie storage

ANOVA of the Effects of Storage Condition and Storage Duration of Conventional Pasteurised Pineapple Juice on pH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	1	0.005441	0.005441	0.61	
Storage condition	2	0.650286	0.325143	36.35	<.001
Storage duration	3	0.269115	0.089705	10.03	0.003
Storage condition*Storage duration	4	0.355557	0.088889	9.94	0.002
Residual	9	0.080500	0.008944		
Total	19	1.249500			

df =degree of freedom; SS = Sum of squares; MS = Mean square

ANOVA of the Effects of Storage Condition and Storage Duration of Conventional Pasteurised Pineapple Juice on Titratable Acidity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	1	0.012064	0.012064	1.34	
Storage condition	2	1.770898	0.885449	98.37	<.001
Storage duration	3	0.674972	0.224991	25.00	<.001
Storage condition*Storage duration	4	0.582938	0.145735	16.19	<.001
Residual	9	0.081009	0.009001		
Total	19	2.797873			

df =degree of freedom; SS = Sum of squares; MS = Mean square

ANOVA of the Effects of Storage Condition and Storage Duration of Conventional Pasteurised Pineapple Juice on Total Phenol

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	1	35.23	35.23	0.87	
Storage condition	2	28.82	14.41	0.36	0.710
Storage duration	3	3738.03	1246.01	30.81	<.001
Storage condition*Storage duration	4	391.60	97.90	2.42	0.125
Residual	9	364.01	40.45		
Total	19	3845.40			

df =degree of freedom; SS = Sum of squares; MS = Mean square

ANOVA of the Effects of Storage Condition and Storage Duration of Conventional Pasteurised Pineapple Juice Total Antioxidant Capacity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	1	32310.	32310.	2.76	
Storage condition	2	935871.	467936.	39.98	<.001
Storage duration	3	28748927.	9582976.	818.84	<.001
Storage condition*Storage duration	4	381434.	95359.	8.15	0.005
Residual	9	105329.	11703.		
Total	19	28262248.			

df =degree of freedom; SS = Sum of squares; MS = Mean square

ANOVA of the Effects of Storage Condition and Storage Duration of Conventional Pasteurised Pineapple Juice on Ascorbic Acid

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	1	2.91	2.91	0.17	
Storage condition	2	6.15	3.07	0.18	0.838
Storage duration	3	292.45	97.48	5.71	0.018
Storage condition*Storage duration	4	40.34	10.09	0.59	0.678
Residual	9	153.58	17.06		
Total	19	432.41			

df =degree of freedom; SS = Sum of squares; MS = Mean square

ANOVA of the Effects of Storage Condition and Storage Duration of Conventional Pasteurised Pineapple Juice on Total Soluble Solids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	1	0.00237	0.00237	0.09	
Storage condition	2	6.08441	3.04221	120.09	<.001
Storage duration	3	12.77661	4.25887	168.11	<.001
Storage condition*Storage duration	4	11.01023	2.75256	108.65	<.001
Residual	9	0.22800	0.02533		
Total	19	19.62800			

df =degree of freedom; SS = Sum of squares; MS = Mean square

ANOVA of the Effects of Storage Condition and Storage Duration of Conventional Pasteurised Pineapple Juice on Aerobic Plate Count

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	1	2.551E-05	2.551E-05	3.07	
Storage condition	2	4.836E+00	2.418E+00	2.914E+05	<.001
Storage duration	3	9.637E+00	3.212E+00	3.871E+05	<.001
Storage condition*Storage duration	4	4.507E+00	1.127E+00	1.358E+05	<.001
Residual	9	7.469E-05	8.299E-06		
Total	19	1.862E+01			

df =degree of freedom; SS = Sum of squares; MS = Mean square

ANOVA of the Effects of Storage Condition and Storage Duration of Conventional Pasteurised Pineapple Juice on Yeast Mould

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	1	0.000494	0.000494	0.27	
Storage condition	2	0.258954	0.129477	70.85	<.001
Storage duration	3	19.097146	6.365715	3483.09	<.001
Storage condition*Storage duration	4	1.702076	0.425519	232.83	<.001
Residual	9	0.016448	0.001828		
Total	19	21.008785			

df =degree of freedom; SS = Sum of squares; MS = Mean square

APPENDIX B

ANOVA for microwave pasteurised pineapple juice storage

ANOVA of the Effects of Storage Condition and Storage Duration of Microwave Pasteurised Pineapple Juice on pH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	1	0.001996	0.001996	1.10	
Storage condition	2	0.293773	0.146887	80.79	<.001
Storage duration	3	0.529757	0.176586	97.12	<.001
Storage condition*Storage duration	5	0.574097	0.114819	63.15	<.001
Residual	10	0.018182	0.001818		
Total	21	1.334545			

df =degree of freedom; SS = Sum of squares; MS = Mean square

ANOVA of the Effects of Storage Condition and Storage Duration of Microwave Pasteurised Pineapple Juice on Titratable Acidity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	1	0.00004	0.00004	0.00	
Storage condition	2	0.61195	0.30597	14.10	0.001
Storage duration	3	2.09573	0.69858	32.18	<.001
Storage condition*Storage duratic	5	1.25563	0.25113	11.57	<.001
Residual	10	0.21706	0.02171		
Total	21	3.77937			

df =degree of freedom; SS = Sum of squares; MS = Mean square

ANOVA of the Effects of Storage Condition and Storage Duration of Microwave Pasteurised Pineapple Juice on Total Phenol

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	1	70.43	70.43	1.81	
Storage condition	2	1285.78	642.89	16.48	<.001
Storage duration	3	1482.07	494.02	12.67	<.001
Storage condition*Storage duration	5	590.24	118.05	3.03	0.064
Residual	10	389.99	39.00		
Total	21	3798.06			

df =degree of freedom; SS = Sum of squares; MS = Mean square

ANOVA of the Effects of Storage Condition and Storage Duration of Microwave Pasteurised Pineapple Juice on pH Total Antioxidant Capacity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	1	1213.	1213.	0.11	
Storage condition	2	86365.	43182.	3.85	0.058
Storage duration	3	2389287.	796429.	70.92	<.001
Storage condition*Storage duration	5	431387.	86277.	7.68	0.003
Residual	10	112295.	11229.		
Total	21	2455476.			

df =degree of freedom; SS = Sum of squares; MS = Mean square

ANOVA of the Effects of Storage Condition and Storage Duration of Microwave Pasteurised Pineapple Juice on Ascorbic Acid

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	1	5.331	5.331	1.45	
Storage condition	2	117.311	58.655	15.92	<.001
Storage duration	3	290.524	96.841	26.28	<.001
Storage condition*Storage duration	5	20.625	4.125	1.12	0.409
Residual	10	36.848	3.685		
Total	21	355.290			

df =degree of freedom; SS = Sum of squares; MS = Mean square

ANOVA of the Effects of Storage Condition and Storage Duration of Microwave Pasteurised Pineapple Juice on Total Soluble Solids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	1	0.01796	0.01796	0.71	
Storage condition	2	2.98509	1.49255	58.85	<.001
Storage duration	3	0.88778	0.29593	11.67	0.001
Storage condition*Storage duration	5	1.20306	0.24061	9.49	0.001
Residual	10	0.25364	0.02536		
Total	21	4.75091			

df =degree of freedom; SS = Sum of squares; MS = Mean square

ANOVA of the Effects of Storage Condition and Storage Duration of Microwave Pasteurised Pineapple Juice on Aerobic Plate Count

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	1	1.723E-05	1.723E-05	2.29	
Storage condition	2	1.943E-02	9.717E-03	1289.65	<.001
Storage duration	3	9.650E+00	3.217E+00	4.269E+05	<.001
Storage condition*Storage duration	5	9.057E+00	1.811E+00	2.404E+05	<.001
Residual	10	7.534E-05	7.534E-06		
Total	21	1.673E+01			

df =degree of freedom; SS = Sum of squares; MS = Mean square

ANOVA of the Effects of Storage Condition and Storage Duration of Microwave Pasteurised Pineapple Juice on Yeast Mould

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	1	0.01214	0.01214	1.17	
Storage condition	2	2.30788	1.15394	111.25	<.001
Storage duration	3	23.24137	7.74712	746.91	<.001
Storage condition*Storage duration	5	4.63718	0.92744	89.42	<.001
Residual	10	0.10372	0.01037		
Total	21	30.10383			

df =degree of freedom; SS = Sum of squares; MS = Mean square

APPENDIX C