KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

FACULTY OF BIOSCIENCES

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

PRODUCT DEVELOPMENT OF SPORTS DRINK USING COCONUT WATER AND PINEAPPLE JUICE

THIS DISSERTATION IS PRESENTED TO THE DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE MSC. FOOD SCIENCE AND TECHNOLOGY

DAVID ASANTE- DONYINAH

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DECLARATION

I declare that I have undertaken the study reported herein under the supervision of Prof. J.H. Oldham and that except portions that are duly cited this dissertation is the outcome of my research work.

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DATE David Asante-Donyinah

…… ……

DATE Prof. J. H. Oldham

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ABSTRACT

In this project a sports drink product was developed from coconut water and pineapple juice. In the formulations three different pineapples varieties grown locally were used in different combination with the coconut water. The pineapple varieties were smooth cayenne, MD2 and sugar loaf variety. Proximate, chemical and sensory properties of the product were analysed. From the sensory analysis there were no significant differences in all the formulations that were made so the sample that contained a high energy value, brix and a percentage carbohydrate that allows for easy gastric emptying was selected. With these criteria the formulation with the pineapple variety known as MD2 (55% coconut water: 45% pineapple juice) which is available throughout the year was selected. The final selected sports drink product contained a brix of 6.8, a percentage carbohydrate of 5.61%, and vitamin C content of 161.67 mg/100 ml. Statsgraphics software was used to model and predict the shelf life of the final product. The shelf life of the sports drink was 12 months with a pH of 3.80. The electrolytes contained in the sports drink was similar to that lost through sweating and these were potassium (837.67 mg/L), sodium (273.94 mg/L), phosphate (186.66 mg/L), magnesium (55.51 mg/L), bicarbonate (46.33 mg/L) chlorine (45 mg/L), calcium (28.00 mg/L) and carbonate (6.00 mg/L).

CHAPTER ONE

1.0 INTRODUCTION

Since the earliest days of sporting endeavor, man has always sought means of achieving success over his fellow competitors. It is said that Charmis of Sparta attributed his success in foot races in the ancient Olympics to consumption of a diet rich in dried figs, an early example of convenience food rich in carbohydrate (Harris, 1996). The pursuit of success has continued to the present day but science has played an increasingly important part in understanding the physiological achievements of these successes. In particular, this knowledge has facilitated the development of suitably formulated sports drinks which have enabled active individuals to capitalize on their talents.

It is now clear that what we eat and drink has profound effect upon sports performance and this fact was reinforced by a recent publication by Maughan *et al*., (1993) of consensus statement agreed by a group of leading academics in sport science which included the following statement: loss of fluid and reduction of the body's carbohydrate stores are the two major causes of fatigue in prolonged exercise and the evidence clearly indicates that sports drinks which contain an energy source in the form of carbohydrate together with electrolytes particularly sodium are more effective than plain water in improving performance in sports. Despite this acceptance and the large numbers of scientific publications which acknowledge the substantial benefits that correctly formulated sports drink can confer upon those engaged in sporting activities, it must be recognized that the advantages that these sports drink offer are less crucial to the armchair athlete, for whom flavour and overall acceptability remain an important issue.

In the 1960's a number of pioneering studies emanating from Scandinavia demonstrated the relationship between diet, muscle glycogen storage and exercise potential (Bergstrom and Hultman, 1966; Bergstrom *et al.*, 1967). In the 1960's, much attention was directed to the augmentation of glycogen reserves prior to competition and this led to the development of a small number of specialized sports products, including a sports drink 'Dynamo'containing a high concentration of carbohydrate in addition to a mixture of electrolytes intended to aid the replacement of those lost in sweat. The product contained polymers providing 1170 KJ of carbohydrate energy in a palatable formulation (Bergstrom and Hultman, 1966; Bergstrom *et al.*, 1967). At this time the emphasis was on carbohydrate provision. Subsequently, the performance of water as nutrient in preventing deterioration in physical performance was perceived, and this realization led to the development of isotonic sports beverages (Below *et al*., 1995).

A wide range of flavours are employed by the manufacturers of sports drinks ranging from fruit punch, exotic fruits or grape flavoured for the United States market compared to the more traditional citrus flavours popular in Europe. Orange remains the principal flavour followed closely by lemon and mixed citrus on the sports market (Berning *et al*., 1998).

Recently milk- base drinks have entered the market with R'Activ being particularly successful in the German market. It should be noted that while orange remains a popular flavour with the general public it is not a universally popular flavour when consumed after strenuous exercise (Berning *et al*., 1998).

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1.1 STATEMENT OF PROBLEM AND JUSTIFICATION

Coconut is among the most important nut crops around the world. It has so many uses but in this study the young endosperm inside a young coconut known as coconut water is the point of focus. Coconut water is low in calories. It has sodium, potassium, phosphorus, chloride and magnesium. These are the main electrolytes found in coconut water with vitamin C and glucose also being present (Magda, 1992; Campos *et al*., 1996).

Coconut water has a therapeutic effect by acting as an anticarcinogenic agent (Sylianco *et al*., 1992). Despite the numerous benefits that coconut water offers, there is a problem of marketing, packaging, and sales of wholesome coconut water. The street vendors who sell the coconut water do not practice hygiene in the sale of the coconut water. The street vendors sell the coconut water close to open sewage drainages and they use unsanitized knives to open the coconut and pour the coconut water into unsterilized polyethylene bags. These bags acts as packaging materials and could contaminate the coconut water and cause diseases thereby defeating the benefits derived from taking the coconut water. Coconut water is said to be a natural sports drink because of its electrolyte and carbohydrate content which are similar to that lost by athletes through sweat during strenuous exercise. For this reason developing a sports drink product from coconut water could solve the problem. The electrolyte content of pineapple juice and the enzyme bromelain which is used in muscle repair makes a combination of coconut water and pineapple juice good raw materials for formulating a sports drink, with the final product having a good flavour.

1.2 MAIN OBJECTIVE

Adding value to coconut water through the product development of sports drink using coconut water as the base material for functional properties and pineapple juice for sensory and some functional properties.

1.3 SPECIFIC OBJECTIVES

1. Formulation of coconut based sports drink for athletes as well as non-athletes.
2. Determining the appropriate method of preservation of the coconut water sports drink.
3. Using sensory analysis and physicochemical analysis to choose the best formulation.
4. Determination of shelf life of the sports drink.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 THE IMPORTANCE OF WATER TO THE BODY

Water is the medium in which the biochemistry of the body takes place. Water is continually being lost in urine (in the process of excreting waste products) and sweat (exercise and sporting activities); a constant intake of water is required to maintain fluid balance. This is controlled principally by the thirst mechanism. When total body water drops, hormonal messages are sent to the brain to create thirst. Excessive water intake on the other hand stimulates an increase in urine production (www.mcb.berkeley.edu).

Apart from providing the perfect chemical environment for our bodies, water accounts for around 70% of the human body weight. The loss of even a tiny fraction of this water can significantly reduce the performance of the human body. For this reason it is vital for all serious athletes to maintain good hydration (www.mcb.berkeley.edu).

Water has an extraordinary property that is the ability to stop our bodies from over heating by evaporating via the skin in the form of sweat. This is particularly important during exercise, when heat output rises (Sawka *et al*., 2005).

Glycogen (stored muscle carbohydrate) is the body's principal fuel for high intensity activities, and replenishing glycogen stores with dietary carbohydrate is vital to continuing high performance water is very important in this process (Puckett and Wiley, 1932).

2.2 WATER BALANCE IN THE BODY

Body water balance represents the net difference between fluid intake and loss. Normal body water turn over in sedentary adults is from 1 to 3 L/day, the range primarily is accountable to difference in insensible water loss, or evaporation of moisture from the skin (Sawka *et al.,* 2005). Large variations in fluid intake are controlled by the kidneys, which can produce more or less urine, depending on changes in body fluid volumes. Water loss in air exhaled from the lungs is often ignored with respect to water balance because it is usually offset by water production occurring during aerobic metabolism (Sawka *et al.,* 2005). Over the course of a day, humans usually regulate daily body water balance remarkably well as a result of thirst and hunger drives. This is accomplished by physiological responses to changes in body water volume and to changes in concentration of dissolved substances in body fluids and by non- regulatory social-behavioral factors, such as drinking fluids at meetings and parties (Sawka *et al*., 2005).

Although minor perturbation in daily body water balance are easily restored to normal, the imposition of exercise and environmental stress onto daily activity can seriously threaten fluid balance homeostasis, performance, and health (Casa *et al.,*2000). Abating these consequences is the underlying and unifying basis for developing guidelines for fluid intake before, during and after exercise (Casa *et al*., 2000; Convertino *et al*., 1996), but hydration assessment remains a key component for ensuring full rehydration in athletes performing frequent and intense exercise in hot weather. The selection of an appropriate hydration assessment method is a controversial aspect of fluid balance science (Oppliger and Bartok, 2002). All hydration assessment techniques vary greatly in their applicability due to methodological limitations such as the necessary circumstances for measurement

(reliability), ease and cost of application (simplicity), sensitivity for detecting small, but meaningful changes in hydration status (accuracy), and the type of dehydration anticipated (Oppliger and Bartok, 2002; Sawka *et al*., 2005).

Most circumstances involving strenuous physical exercise require the formation and vapourization of sweat as the principal means of heat removal. When sweat losses produce a body water deficit, the reduced volume of body fluids contains a greater than normal concentration of dissolved substance such as sodium and potassium.

2.3 IMPORTANCE OF HYDRATION TO THE BODY DURING SPORTING ACTIVITY

A comprehensive hydration strategy involves ensuring good hydration before training/competition, maintaining it during exercise and then replacing any shortfall as soon as possible afterwards. Although the composition of sweat varies from person to person (partly as a function of acclimitisation) a litre of sweat typically contains the following Calcium-0.02 g, Magnesium-0.05 g, Sodium-1.15 g, Potassium-0.23 g, Chloride-1.48 g (Lentner, 1981). For this reason it is important to rehydrate with a properly formulated isotonic electrolyte to replace what is lost through sweat.

2.4 ADVANTAGES OF AN ELECTROLYTE MINERAL-CONTAINING DRINK OVER DRINKING PURE WATER ALONE

Although the amount of mineral lost in sweat is generally small in proportion to total body stores of electrolyte prolonged heavy sweating can lead to significant mineral losses particularly of sodium. Drinking pure water effectively dilutes the concentration of electrolyte minerals in the blood, which can impair a number of

normal physiological processes. An extreme example of such impairment is 'hyponatraemia', when low plasma sodium levels can be literally life threatening (www.diagnose-me.com).

Drinks containing electrolytes (minerals) particularly sodium are known to promote thirst, thereby stimulating a great voluntary intake of fluid (Wilk and Bar-Or, 1996). There is also evidence that drinks containing sodium enhance the rate and completeness of rehydration after a bout of exercise (Wemple *et al.*, 1997).

When the electrolyte minerals particularly sodium are present in appropriate concentration, the rate of fluid absorption from the small intestine into the rest of the body appears to be enhanced, especially with small amounts of glucose (Lambert *et al.*, 1996). This is particularly important when rapid uptake of fluid is required, such as during strenuous exercise in the heat.

2.5 EFFECT OF SODIUM ON RESTORATION OF HYDRATION

Research evidence suggests that fluid containing significant amounts of electrolytes (especially sodium) have a slightly greater impact in restoring hydration than fluids with little or no electrolyte/sodium (Sheriffs *et al.*, 1996).

However, the amount of sodium in the drink is critical as it was shown by research which compared rehydration efficiently using each of the following:

- A 6% carbohydrate solution with no added sodium.
- A 6% carbohydrate solution with (0.58 g) of sodium per litre.
- A 6% carbohydrate solution with (1.16 g) of sodium per litre.

The subjects dehydrated by 3% of body weight during 90 minutes of exercise and drank as much as they wanted of one of the above beverages during a three-hour

recovery period. The researchers found that the beverage with 0.58 g of sodium per liter stimulated the greatest fluid intake, while the high sodium drink either suppressed thirst or diminished the palatability of the fluid (Wemple *et al*., 1997).

2.6 FLUID ABSORPTION IN THE BODY

The rate of fluid absorption in the body is determined by a two-stage process:

The first is Gastric emptying, (this is how quick ingested fluid leaves the stomach). In a more dilute solution, this is often the key step that determines the overall rate of fluid absorption.

The second is Intestinal absorption. This indicates the rate of absorption across the intestinal wall (Ryan *et al.,* 1998).

2.6.1 GASTRIC EMPTYING

Optimal fluid absorption requires rapid gastric emptying and efficient uptake in the intestine.

Fluid absorption tends to take place in the small intestine rather than the stomach. Studies have shown that the larger the volume of fluid in stomach, the more rapid the emptying into the small intestine, which means that maintaining a larger fluid volume in the stomach by repeated drinking will maximize the rate of fluid (and nutrient) delivery to the small intestine (Noakes *et al.*, 1991; Mitchel and Voss, 1990).

Gastric emptying rate is also influenced by fluid composition. Early studies showed that, regardless of their electrolyte or glucose content, solution with lower overall concentration (or osmolality) than body fluids were emptied as rapidly as plain water (Hunt and Pathak, 1960; Hunt and Stubbs, 1975). With glucose solution, for example,

this would allow for concentration of up to 2.5% (2.5 g per litre of water). At that level it seemed that the concentration above this threshold would slow gastric emptying. But more recent work has established that drinks containing glucose concentrations of up to around 4-5% are emptied as rapidly as water. Beyond a concentration of 5%, glucose solution are emptied more slowly from the stomach, but they can nevertheless result in a faster delivery of glucose overall (Hunt *et al.*, 1985). This is because the increase in glucose per unit volume delivered by these more concentrated drinks more than makes up for the reduced volume absorbed. Where fluid replacement is of lower importance than energy replacement, more concentrated drinks may be preferable (Hunt *et al.*, 1985).

2.6.2 INTESTINAL ABSORPTION

After gastric emptying, ingested fluids are absorbed in the small intestine. Pure water, or very dilute solution, diffuses readily across the intestine. However, research has shown that dilute glucose/ electrolyte solutions with a concentration that is slightly less than that of plasma maximize the rate of water absorption (Lifshitz and Wapnir, 1985). The researchers found that optimum hydration from the intestine was obtained with a solution containing 1.38 g of sodium and 20.0 g glucose per liter of water.

Where energy (i.e. glucose) replacement is the main goal, studies have shown that uptake from the small intestine into the body rises as the concentration of glucose rises in the intestine. This is simply because there is more glucose available per unit volume for absorption.

However, very concentrated solutions of glucose (more than 6%) can have an adverse effect on fluid balance. This is due to the process known as osmosis, where water

separated by permeable membrane (in this case the intestinal wall) passes from a more dilute to a more concentrated solution (Lifshitz and Wapnir, 1985).

When a drink with a very high concentration of glucose is ingested, the fluid in the bloodstream (on the other side of the intestinal wall) will be relatively dilute and by comparison the osmotic pressure exerted by the very concentrated glucose solution will actually draw out of the blood stream and into the intestine. This results in a loss of available body water, effectively increasing dehydration (Ryan *et al.,* 1998).

Although it has a chemical structure similar to glucose, the fruit sugar fructose diffuses passively across the intestinal wall. Studies have shown that fructose is absorbed more slowly than glucose and that it promotes less water uptake (Fordtran, 1975). Moreover, fructose is known to exert a greater osmotic pressure, which means that, for a given concentration, it is more likely to draw water into the intestine, which can cause abdominal distress. These properties make fructose much less desirable as an energy component in sports drinks than glucose (Murray *et al.*, 1989).

A study on cyclists compared the effect of glucose and fructose in a 6% solution during a 1hr 45min bout of cycling (Murray *et al.*, 1989). By comparison with glucose, fructose was associated with gastrointestinal distress, greater loss of plasma volume, higher levels of stress hormone and substantially poorer exercise performance.

Properly formulated carbohydrate/ electrolyte drinks can and do increase hydration and as a bonus, supply extra carbohydrate to working muscles (Murray *et al.*, 1989).

2.7 HYPONATRAEMIA

Hyponatraemia is a disorder in fluid-electrolyte balance that results in an abnormally low plasma sodium concentration less than 135 mmol per litre compared with a normal range of 138-142 mmol/L. Sustained decrease in plasma sodium concentration disrupts the dynamics of water exchange (osmotic balance) across the blood brain barrier, resulting in a rapid influx of water into the brain. This can cause swelling in the brain, leading to a series of increasingly severe neurological responses, such as confusion, seizure, coma and even death (www.diagnose-me.com).

The lower the blood sodium and the faster it falls, the greater the risk of life-threatening consequences. A drop in plasma sodium concentration to 125-135 mmol/L often results in little more than gastrointestinal symptoms, such as bloating and nausea. Below 125 mmol/L, the symptoms become more severe and can include confusion, throbbing headache, wheezy breathing, swollen hands and feet, unusual fatigue and reduced coordination. Below 120 mmol/, the risk of seizure, coma and death is increased (Nelson *et al.,* 1989).

Hyponatraemia in athletes is often, although not always, caused by excessive drinking of water. During exercise, urine production is decreased, reducing the body’s ability to excrete excess water, while at the same time sodium losses are increased through sweating. The combined effect makes it much more likely that the body's sodium content will be significantly diluted (Nelson *et al.,* 1989).

2.8 SPORTS DRINKS

A sport drink is a beverage designed to help athletes rehydrate, as well as replenish electrolytes, sugars and other nutrients that are lost or depleted after strenuous exercise, training or competition (Berning *et al.*, 1998).

Companies marketing these products point out the fact that plain water, despite its association with good health, can be toxic if consumed in large amount (water intoxication). This can happen because over consumption of water reduces levels of electrolytes such as sodium and potassium in the body by dilution, interfering with the nervous system (Berning *et al.*, 1998). An example of a sport drink on the Ghanaian market is 'Lucozade Sports'. Sports drinks are popular among athletes because they provide the necessary electrolytes to support extended exercise. They help keep the body balanced and contain the right amount of electrolyte in the fluids (Berning *et al.*, 1998). However, not all drinks advertised as sports drink are suitable for this purpose, and professional advice should be sought for potentially risky situations such as those described in this chapter. Currently the largest growing segment of the sports drinks manufacturers are to specially target and develop products aimed at non-athletes, such as low sodium or low calorie sports drinks. Non-athletes who use sports drink should also be aware that sports drinks for athletes typically contain high levels of carbohydrates which will result in weight gain if consumed without a corresponding increase in exercise activity. Sports drinks are not to be confused with energy drinks (e.g. Full Throttle, Lucozade, Mountain Dew AMP, or Monster Energy). Sports drinks are intended to replenish electrolytes, sugar, water and other nutrients, and are usually isotonic (containing the same proportions as found in the human body). Energy drinks, on the other hand, simply provide lots of sugars and caffeine (Bonci, 2002).

2.8.1 ENERGY DRINK VERSUS SPORTS DRINKS

A number of energy drinks are now marketed as ‘sports energy drinks’. Although this term suggests that energy drinks are beneficial for exercise or sports performance, their composition is diverse and at the current time none of those sold meet international guide lines for sports (electrolyte) drinks (Canadian Broad Casting Cooperation, 2005). An ideal sports drink has three important criteria- carbohydrate level, electrolyte level and no carbonation. Using these criteria energy drinks are judged below.

2.8.1.1 CARBOHYDRATES LEVELS IN SPORTS DRINKS COMPARED TO ENERGY DRINKS

The amount of carbohydrate present in most energy drinks (e.g. 10-12%) is similar to soft drinks and is much higher than sports drinks (usually 6-8%). Australian international guidelines for optimal fluid replacement recommend beverages have an absolute maximum of 8% carbohydrate as levels greater than this delay stomach emptying and increase the risk of dehydration and gastrointestinal upset during exercise. ‘Gatorade’ has an optimum level of 6% (Berning *et al.*, 1998).

2.8.1.2 ELECTROLYTES LEVELS IN SPORTS DRINKS COMPARED TO ENERGY DRINKS

Sodium is an important electrolyte in sports as it helps to optimize hydration and maintain thirst drive. Sodium also has a role in preventing low levels of sodium in the blood (hyponatraemia) which may occur during prolonged exercise in the heat and is potentially life threatening. Most energy drinks have either too little sodium or too much. Where as sports drinks are formulated with the right mix of carbohydrates and sodium for optimal hydration and taste (Berning *et al.*, 1998).

2.8.1.3 CARBONATION OF ENERGY DRINKS COMPARED TO SPORTS DRINKS

Most energy drinks are carbonated while sports drinks are not carbonated. Studies have shown a trend that carbonation increases the risk for gastrointestinal discomfort during exercise (Berning *et al.*, 1998). Also carbonation can cause burning sensation in the throat and form carbon dioxide in the stomach which causes nausea, vomiting and stomach ache (Berning *et al.*, 1998).

2.8.2 TYPES OF SPORTS DRINKS

Sports drinks are generally classified into three major groups or types and this classification is based on the percentage of carbohydrate in the sports drinks. These are isotonic sports drinks, hypotonic sports drinks and hypertonic sports drinks.

2.8.2.1 ISOTONIC SPORTS DRINKS

These contains electrolytes and 6-8% carbohydrates which when taken in quickly replaces lost electrolytes as a result of sweating and supplies a boost of carbohydrate. It is a good choice for athletes engaged in middle and long distance running or team sports (www.mamasheath.com).

2.8.2.2 HYPOTONIC SPORTS DRINKS

These sports drinks contain electrolytes and a low level of carbohydrates which quickly replace fluids lost during sweating (www.mamasheath.com). It is suitable for athletes who need fluid without the boost of carbohydrate such as jockeys and gymnast (www.mamasheath.com).

2.8.2.3 HYPERTONIC SPORTS DRINKS

Hypertonic sports drinks contain a high level of carbohydrate and they are used to supplement daily carbohydrate intake normally after exercise to top up muscle glycogen stores. They are suitable for ultra distance events (cycling, marathon and cross country) where high levels of energy are required and hypertonic drinks can be taken during exercise to meet the energy requirements. If used during exercises, hypertonic drinks need to be used in conjunction with isotonic drinks to replace fluids (www.mamasheath.com).

2.8.3 RATE OF ABSORPTION OF SPORTS DRINKS

There are two main factors that affect the speed at which fluid from a drink gets into the body. These are carbohydrate level and electrolytes such as sodium and potassium.

The higher the carbohydrate levels in a drink the lower the rate of stomach emptying. Isotonic drinks with a carbohydrate level of between 6% and 8% are emptied from the stomach at a rate similar to water (Berning *et al.,* 1998). Sodium and potassium in drink will reduce urine output. Reduced urine output enables the fluid to empty quickly from the stomach, promotes absorption from the intestine and encourages fluid retention (Nose *et al.,* 1988).

2.8.4 REASONS FOR THE DEVELOPMENT OF SPORTS DRINK

The restoration of body fluid balance following dehydration induced by exercise occurs through regulatory responses which stimulate ingestion of water and sodium. Heavy sweating during exercise can cause the loss of 1 litre of body fluids (Costill, 1977). If proper hydration is achieved during the course of the exercise there will not be any need for a post exercise rehydration (Costill, 1977). In reality this cannot be achieved so therefore there is the need for a post exercise rehydration especially in the case whereby the athlete is expected to compete again. If there is sufficient time to rehydrate before the next event fluid ingestion and solid food are the choice, however if the time before the next event is short (2 hours) an athlete should replace fluids to achieve rehydration (Galloway, 1999). The restoration of body fluid losses is

necessary for optimal cardiovascular function and thermoregulation during subsequent exercise (Costill, 1977). Replacement of water and electrolytes during rehydration may be limited by gastric emptying and intestinal reabsorption as well as the body's ability to retain ingested fluid that is to prevent diuresis (Gisolf *et al.*, 1990).

A sport drink is a beverage that contains carbohydrates and other supplement to help replenish fluids and nutrients used during vigorous exercise and sporting events (www.mamasheath.com). Sports drinks are available in a variety of flavours. Some are premade liquids packaged in bottles and cans while others are mix- it- yourself- powders (www.mamasheath.com).

2.8.5 IMPORTANCE OF SPORTS DRINK FORMULATION

Formulation of sports drink is very important because high concentration of carbohydrates in sport drinks slow mineral absorption (Ryan *et al.*, 1998). For this reason a blend of simple carbohydrates (i.e., sucrose, glucose and fructose), at an overall concentration that is between 6 % - 8 %, can help speed fluid absorption (Shi *et al.*, 1995).The amount of sodium content is important in the sports drink because sodium will keep the thirst mechanism active for better drinking and will result in a more complete rehydration (Wemple *et al.*, 1997). Taste and Flavour contributes to overall acceptance of the sports drink (Passe *et al.*, 1999). According to research, exercisers who drink sports drinks during activity can work out harder (Below *et al.*, 1995; Davis *et al.*, 2000) and are less likely to over heat following a workout (Ballard *et al.*, 2007). Research also shows that consuming carbohydrates during exercise makes activity feel easier.

2.9 IMPORTANCE OF ELECTROLYTES IN THE HUMAN BODY

Many electrolytes are essential minerals and they control osmosis of water between body compartments. Apart from this, electrolytes help maintain the acid-base required for normal cellular activities. The sweat that evaporates from the skin contains a variety of electrolytes. The electrolyte composition can vary from person to person but is generally Sodium, Potassium, Calcium, Magnesium, Chlorine, Bicarbonate, Phosphate, Sulphate (www.mamasheath.com).

2.10 CAFFEINE AND PERFORMANCE

In the past, researchers and nutrition experts cautioned against the ingestion of caffeine-containing beverages when engaging in physical activity because it was believed that the caffeine would have a diuretic or laxative effect. This would keep individuals from maintaining an adequate hydration status during bouts of physical activity and could lead to dehydration (Grandjean and Campbell, 2004).

More recent research, however, suggests that this may not be true for all individuals since some individuals seem to develop a tolerance for caffeine (Grandjean and Campbell, 2004). For these individuals who tolerate caffeine without problems drinking caffeine containing drinks may not be a serious concern. However caffeine is regulated as a sports enhancing agent. Caffeine seems to act as diuretic or laxative, causing you to have to go to the bathroom more often or causing gastrointestinal discomfort, you may want to avoid its use before or during exercise. Caffeinated beverages are often carbonated and carbonation can cause stomach discomfort during exercise in some individuals (Grandjean and Campbell, 2004).

Caffeinated beverages are also poor choices for post –exercise hydration because they are not designed with optimal ingredients to rehydrate and balance electrolytes. They have high levels of carbohydrate, which can slow down fluid absorption. Caffeine has been noted as being ergonic (i.e. performance enhancing) compound because it appears to perk up the central nervous system. Recent work reported that 3-9 mg caffeine per kilogram of body weight consumed one hour prior to exercise increased endurance running and cycling performance in laboratory tests (Grandjean and Campbell, 2004). Large amounts of caffeine have been banned by Olympic Athleletic Officials and other Sports Regulatory Bodies. Thus it is important to note that while caffeine can have performance enhancing qualities its controlled use is better (Grandjean and Campbell, 2004). For average active teenager or adult who is exercising with goals of enjoyment and self improvement, using caffeine defeats these purposes. Proper training and nutritional habits are sensible and productive approaches (Grandjean and Campbell, 2004). Some sports drinks on the market today include 'Gatorade' by Pepsi Cola, Inc., 'All Sports' by Monarch Beverage Co., 'PowerAde' by Coca-Cola, 'Accelerade' by Pacific Health Laboratories, 'Lucozade Sport' by Glaxo Smith Kline, 'Cera Sport' by Cera Products.

2.11 COCONUT

The Coconut palm (*Cocos nucifera*) is a very important cash crop along the coastal belt of Ghana. It is estimated that Ghana produces a total of 224,000 tons of coconut annually from 43,000 hectares of land (Wadhwa & Kumar, 1992; Ofori & Nkansah-Poku, 1995). An important characteristic of coconut cultivation in Ghana is its ability to spread wealth and generate employment in rural areas where few other employment opportunities exist (Adams *et al*., 1996)

2.11.1 COCONUT WATER

Coconut water is the liquid endosperm of *Cocos nucifera*. The coconut fruit is unique in that it accumulates large amounts of this liquid over periods of a year or more in its life cycle. The greatest amount of coconut water is found in young, green coconuts and provides nourishment for the growth of the solid endosperm (coconut meat) inside the hard shell of the fruit. When the fruit matures, both the solid endosperm and the remaining coconut water serve as nutrients for the developing embryo and seedling. Thus coconut water serves as a natural reservoir of nutrients to promote tissue growth (Rursegloove, 1992). Coconut water has long been a popular drink in the tropics, especially in Tropical Asia and Trinidad and Tobago, where it is available fresh or bottled. It is naturally fat-free, and low in food energy (16.7 calories or 70 kJ per 100 g). Due to its sterility, pH, mineral, and sugar content, coconut water had been successfully used as liquid in intravenous therapy in emergency situations (Campbell-Falck *et al.*, 2000). Coconut water can also be found in regular cans or tetra paks (and often has coconut pulp or coconut jelly added) and is also marketed as a sports drink because of its high potassium and mineral content which helps the body recover from rigorous exercise.

2.11.2.1 COMPOSITION OF COCONUT WATER

According to research, coconut water accounts for 25% of the weight of the fruit, and its basic composition is 95.5% water, 4% carbohydrates, 0.1% fat, 0.02% calcium, 0.01% phosphorous, 0.005% iron, in addition to amino acids, vitamin C, B complex

vitamins and mineral salts (Shah, 1956). The nutritional composition of coconut water obtained from fruits at different stages of maturity has been determined. The medium is rich in proteins, amino acid, sugars, vitamins, minerals and growth hormones essential to the promotion of tissue growth (Shah, 1956). In addition, shikimic acids and quinic acids have been detected in samples of coconut water from fruits at different stages of maturity, with the alicyclic acids in aromatic biosynthesis, indicates their importance in developing coconut flavour (Shah, 1956). They may also play a significant role in the nutrition of plant and tissue cultures (Shah, 1956). The coconut water contains high mineral content and is shown in Table 1.

TABLE 1 MINERAL CONTENT OF COCONUT WATER

Mineral	**Mg/100g**
Potassium	312.0
Chloride	183.0
Sodium	105.0
Phosphorous	37.0
Magnesium	30.0
Sulfur	24.0
Copper	0.04
Iron	0.10

http://www.freepatentsonline.com

2.11.2.2 CHANGES IN CHEMICAL COMPOSITION OF COCONUT WATER DURING MATURATION OF FRUIT

Flavour varies depending on the stage of maturation of the fruit. Coconut water is the juice of the endosperm found within the cavity of the coconuts, which begin to form around 2 month after the natural opening of the inflorescence. Changes in chemical composition of coconut (*Cocos nucifera*) water, including total and soluble solids, titrable acidity (as citric acid), turbidity, ash, lipids and sugars were investigated in four varieties of coconuts at four stages of fruit. The most significant change was observed in the volume of nut water, which increased during development from 233 ml to 504 ml, with the greatest quantity found at 9 months. Fat, protein, soluble solids, acidity and turbidity also increased steadily with maturity, while pH and ash showed variation throughout maturation. The interaction of variety and stage of maturity of the fruit appeared to have a significant effect on the chemical composition of the coconut water (Jackson *et al.*, 2004)

2.11.2.3 USE OF COCONUT WATER FOR ORAL REHYDRATION

In some countries coconut water is used as a solution for oral hydration, as part of the daily diet and as protein supplement when nutritional deficits are intense (Kuberski *et al.*, 1979).

2.11.2.4 INTRAVENOUS USE OF COCONUT WATER

Some studies have suggested that coconut water can be used for intravenous rehydration (Campbell-Falck *et al.*, 2000). Other studies suggest that coconut water can be used for electrolyte replacement in a wide range of situations.

2.11.2.5 PRESERVATION OF COCONUT WATER

After harvesting the coconut from the tree the husk must be peeled with a sanitized stainless steel knife and the coconut water is cooled at a temperature of 4°C and then transferred to a well sterilized bottle and stored at 4°C (Campos *et al.*,1996). Polyphenol oxidase and peroxidase are inactivated by heating at 90°C for 550 seconds but this usually affects the taste and also addition of ascorbic acid can also preserve it. A combination of heat treatment with potassium metabisulfite or ascorbic acid or both do not affect the taste quality of the coconut water (Campos *et al.*, 1996).

FAO-funded research indicates that coconut water of good drinking quality is clear and colourless, with a pH of 4.5 to 5.5 and a Brix^{o} level (a measurement of sugar concentration) of 5 to 6.5 per 100 ml, it should have a total microbiological count of less than 5,000, less than 10 coliform bacteria and zero faecal coliform. For small-scale processors without access to a laboratory for microbiological testing, FAO recommends some simple measures, such as checking the product for traces of fermentation or foreign objects , giving it a “nose test”. A rancid odour, for example, indicates that the small quantity of fats in the liquid have oxidized (FAO, 2007).

Coconut water is considered a low acid food because its pH falls between 4.5-5.5. According to US FDA such foods require a temperature of approximately 115-125°C to render it commercially sterile, but it also significantly degrades the sensory

attributes, such as taste and aroma of the coconut water. For example, the low-acid heat process produces a foul, overcooked aroma and taste in the coconut water. In view of this a method has been developed which involves the step of adding a pH lowering component to coconut water until the pH of the resulting composition is below 4.5. In preferred embodiment, a food grade acid, preferably phosphoric acid is added to lower the pH (Haynes *et al*., 2004). In a further embodiment, the coconut water and acid mixture, having a pH below 4.5 are subjected to a pasteurization process to achieve commercial sterilization of the resulting coconut water or coconut water beverage. The pasteurization process is performed at time and temperature conditions which are sufficient for high-acid products and which are lower or less harsh than those required for low-acid products (Haynes *et al.*, 2004). Multiple fruit juice sources can be included in the coconut water to lower the pH and add flavour. Other additives can also be added to coconut water (Haynes *et al.*, 2004).

For proper storage a temperature of 12^{o}C is needed using polythene as a packaging material. This can last for a period of four weeks (Maciel *et al.*, 1992).

2.12 PINEAPPLE

The pineapple (Ananas *comosus*) is native to southern Brazil and Paraguay where wild relatives occur. It was spread by the Indians up through South and Central America to West Indies before Columbus arrived. In 1643 Columbus found the fruit on the island of Gaudaloupe and carried it back to Spain and it was spread around the world on sailing ships that carried it for protection against scurvy. The Spanish introduced it into the Philippines and may have taken it to Hawaii and Guam early in

16th Century. The pineapple reached England in 1660 and began to be grown in green houses for its fruit around 1720 (Maxwell and Maxwell, 1984).

Ghana's pineapple industry has grown significantly over the past twenty years. Commercially produced pineapples are grown within a 50km radius of the capital, Accra. From here they are shipped to export markets such as Germany, Holland and Italy by sea or air. From 1994 to 1999, the total value of Ghana's pineapple exports increased nearly threefold, from US $5.3 million to US $15.5 million. Pineapple processing has also increased, with a number of companies involved in manufacturing single-strength juice and juice concentrate. In addition, the company Blue Skies recently established a plant for processing pineapple into slices and mixed fruit salads (www.freshplaza.com/news_detail.asp?id=704).

2.12.1 CULTIVARS OF PINEAPPLE

There are about six different cultivars of pineapples grown around the world. These are kona sugarloaf, hilo, natal green, pernambuco, red Spanish and smooth cayenne (Maxwell and Maxwell, 1984).

Kona sugarloaf has an average of 5-6 lbs white flesh with no woodiness in the center. It is cylindrical in shape and has high sugar content but no acid. This makes it an incredibly delicious fruit (Maxwell and Maxwell, 1984).

Smooth Cayenne has pale yellow to yellow flesh with a cylindrical shape. It contains a high sugar and acid content. Leaves are without spines and the fruit are well adapted to canning and processing (Maxwell and Maxwell, 1984).

Natal Queen has a golden yellow flesh with crisp and delicate mild flavour. It is well adapted to fresh consumption and keeps well after ripening. The leaves are very spiny (Maxwell and Maxwell, 1984).

Pernambuco has pale yellow to white flesh, sweet, melting and excellent for eating fresh with spiny leaves. It is poorly adapted for shipping (Maxwell and Maxwell, 1984).

Red Spanish has pale yellow flesh with pleasant aroma and it is squarish in shape with spiney leaves. It is well adapted for shipping as fresh fruits to distant markets (Maxwell and Maxwell, 1984). In Ghana there are three varieties of pineapple that are grown by farmers these are kona sugarloaf, smooth cayenne and a hybrid called MD2.

2.12.2 STORAGE OF PINEAPPLE

Cold storage at a temperature of 4.4°C and lower causes chilling injury and breakdown in pineapples. At 7-8°C and above, 80-90% relative humidity and adequate air circulation, normal ripening progresses during and after storage. At best, pineapples may be stored for no more than 4-6 weeks. There is a possibility the storage life might be prolonged by dipping the fruits in a wax emulsion containing a suitable fungicide. Irradiation extends the shelf life of half ripe pineapples by about one week (Donald *et al.*, 1971).

2.12.4 FOOD USES OF PINEAPPLE

Field ripe fruits are best for eating fresh, and it is only necessary to remove the crown, rind, eyes and core. In Panama, very small pineapples are cut from the plant with a few inches of stem to serve as a handle, the rind is removed except at the base, and the flesh is eaten out-of-hand like corn on the cob (Donald *et al.*, 1971).

The flesh of larger fruits is cut up in various ways and eaten fresh, as dessert, in salads, compotes and otherwise, or cooked in pies, cakes, puddings, or as a garnish on ham, or made into sauce or preserves (Donald *et al.*, 1971).

Canned pineapple is consumed throughout the world. The highest grade is the skinned, cored fruit sliced crosswise and packed in syrup. Undersize or overripe fruits are cut into “spears”, chunks or cubes. Surplus pineapple juice used to be discarded after extraction of bromelain but today there is a growing demand for it is a beverage. Crushed pineapple juice, nectar, concentrate, marmalade and other preserves are commercially prepared from the flesh remaining attached to the skin after the cutting and trimming of the central cylinder. All residual parts cores, skins and fruit ends are crushed and given a first pressing for juice to be canned as such or prepared as syrup used to fill the cans of fruits, or is utilized in confectionery and beverages, or converted into powder pineapple extract which has various roles in the food industry. Chlorophyll from the skin and ends imparts a greenish hue that must be eliminated and the juice must be used within 20 hours as it deteriorates quickly. A second pressing yields “skin juice” which can be made into vinegar or mixed with molasses for fermentation and distillation of alcohol (Donald *et al.*, 1971)

2.12.5 HEALTH BENEFITS OF PINEAPPLE JUICE

Pineapple is loaded with vitamins and minerals. The obvious benefits of pineapple are all the vitamins and minerals the fruit has which include calcium, potassium, fibre, and vitamin C. In addition it is low in fat and cholesterol (www.mamasheath.com). The benefits of pineapple can be achieved through eating fresh, canned, or frozen pineapple and or by drinking its juice. One of the benefits of pineapple is that it helps to build healthy bones. Pineapples are rich in manganese, a trace mineral that is

needed for the body to build bone and connective tissues. Just one cup of pineapple provides 73% of the daily recommended amount of manganese. The benefits of pineapple can affect the growth of bones in young people and the strengthening of bones in older people (www.mamasheath.com).

While many people often take extra vitamin C or drink extra orange juices when they have a cold, few consider eating pineapple. The benefits of pineapple when you have a cold or cough are the same as the benefits of orange juice, but there is an additional benefit of pineapple. Bromelain, which is found in pineapples, has been found to help suppress cough and loosen mucus (www.mamasheath.com).

2.13 BROMELAIN

The proteolytic enzyme, bromelain, or bromelin, was formerly derived from pineapple juice but now it is gained from the mature plant stems salvaged when fields are being cleared. The yield of bromelain from 167 kg of stem juice is 3.6 kg of bromelain. The enzyme is used like papain from papaya for tenderizing meat. In modern therapy, it is employed as a digestive and anti-inflammatory action after surgery, and to reduce swellings in cases of physical injuries (Donald *et al*, 1971).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SOURCE OF RAW MATERIALS

The coconuts and the sugarloaf pineapples used were obtained from Central Region. The MD2 and smooth cayenne pineapples were purchased from the eastern region precisely.

3.2 EXTRACTION OF COCONUT WATER FROM THE COCONUTS AND EXTRACTION OF PINEAPPLE JUICE

The coconuts and pineapples were washed thoroughly with water to remove the sand and dirt on them after that they were washed with chlorine water to reduce the microbes that might be on the raw materials.

The coconut water was extracted by cracking the coconut shell and the coconut water obtained was stored in a refrigerator at temperature of $4^{o}C$ in sterilized plastic container.

The skin of the pineapple was carefully peeled after which it was diced into pieces to increase the surface area for maximum extraction of the juice. The diced pineapple was carefully loaded into an aseptically hygienic blender and blended. The juice was extracted by filtering the blended pineapple mass through a cheese cloth. The residue fibre in the cheese cloth was reblended and strained for juice. The fresh juice was poured into containers and immediately stored in the refrigerator at 5 ^{o}C to prevent fermentation.

3.3 FORMULATION OF THE SPORTS DRINK

The extracted coconut water and pineapple juice from the three different varieties of pineapple (smooth cayenne, kona sugarloaf, and MD2) were blended into twelve different products. Each variety of pineapple was blended with the coconut water to form 4 different products with different concentrations (Table 2). The formulations are shown in Table 2.

TABLE 2

FORMULATION OF SPORTS DRINK FROM COCONUT WATER AND PINEAPPLE JUICE

PINEAPPLE VARIETY	PIEAPPLE JUICE COMPOSITION (%)	COCONUT WATER COMPOSITION (%)	SAMPLE CODE
Smooth cayenne	30	70	(SC1)
Smooth cayenne	35	65	(SC2)
Smooth cayenne	40	60	(SC3)
Smooth cayenne	45	55	(SC4)
Sugarloaf	30	70	(SL1)
Sugarloaf	35	65	(SL2)
Sugarloaf	40	60	(SL3)
Sugarloaf	45	55	(SL4)
MD2	30	70	(MD1)
MD2	35	65	(MD2)
MD2	40	60	(MD3)
MD2	45	55	(MD4)

3.4 PRESERVATION OF THE FORMULATED SPORTS DRINKS

The formulated sports drinks were preserved by pasteurizing the drinks at a temperature of 70 degrees Celsius for 30 minutes and adding 0.01% food grade sodium benzoate and 0.02% orthophosphoric acid to lower the pH to 3.8. The pasteurized formulations were then filled in glass bottles which had tightly fitted lid with gasket and stored in a refrigerator at a temperature of 5^{o}C.

3.5 PROXIMATE ANALYSIS OF THE FORMULATED SPORTS DRINKS

Percentage moisture, protein, fat, ash, fibre and carbohydrate were determined according to AOAC (1999) procedures**.**

3.6 DETERMINATION OF VITAMIN C IN THE FORMULATED SPORTS DRINKS

A set of 50 ml flasks were labeled in triplicate for blank, ascorbic acid standard and sports drink sample. About 5 ml of acid stabilization solution (made from 40 g of HPO_3 dissolved in 200 ml of glacial acetic acid) was added to each flask. About 2 ml of distilled water was added to the flask labeled as blank and 2 ml of sports drink sample and ascorbic acid samples were added to two other flasks designated for the sports drink samples and ascorbic acid samples respectively. The samples were then titrated against the dye solution prepared from 0.042 g of $NaHCO_3$ and 0.05 g of sodium 2, 6-dichloroindolphenol until a light but distinct rose pink colour persists for at least 5 seconds (AOAC, 1999).

3.7 DETERMINATION OF TOTAL SOLIDS IN THE FORMULATED SPORTS DRINKS

For the determination of total solids, 10 ml of the samples were measured and weighed into a 50 mm diameter flat bottomed petri dish, the samples were then evaporated on a boiling water bath until it solidified and was dried for two and a half hours in an oven at a temperature of 100^{o} C. It was then cooled in a dessicator and weighed. The difference in weight between the initial and final weight was recorded as total solids (AOAC, 1999).

3.8 DETERMINATION OF SOLUBLE SOLIDS IN THE FORMULATED SPORTS DRINKS

The total soluble solids of the samples were measured with a refractometer at temperature of 20^{o} C and the refractive index obtained was used to find the degree brix from a chart which has a correlation of refractive index with the degree brix (AOAC, 1999).

3.9 DETERMINATION OF MINERALS (ELECTROLYTES) IN THE FORMULATED SPORTS DRINKS

About 15 ml of the samples were ashed and 5 ml of concentrated HCl was added to digest the ashed samples. The mixtures were then poured into 50 ml flasks and distilled water was added to make up to the 50 ml mark. After the dilution each electrolyte was determined using different methods.

3.9.1 DETERMINATION OF SODIUM ION AND POTASSIUM ION IN THE FORMULATED SPORTS DRINKS

Sodium ion and potassium ion measurements were determined using the flame photometer by preparing six serial standards of 2, 5, 10, 20, 30, 50 mg/l from the stock standard solution of 100 mg/l sodium ion and potassium ion. The serial standards were then aspirated and the reading was noted. The sports drink samples were then aspirated and the readings were also recorded. Standard curves were then plotted and the concentration of the electrolytes calculated (Morin, 1960).

3.9.2 DETERMINATION OF MAGNESIUM ION IN THE FORMULATED SPORTS DRINKS

Six serial standards were prepared from pure $MgCl_2$ salt. About 2 ml of calmagilte solution was pipetted into labeled test tubes. About 20 µl of standard /sample were added to the tubes and incubated at room temperature for 5 minutes. The reagent (calmagilte) was used as a blank and the absorbances were measured at 520 nm on a spectrometer. Standard curves were then plotted and the concentration of the magnesium electrolytes calculated (Morin, 1960).

3.9.3 DETERMINATION OF PHOSPHORUS AS PHOSPHATE ION IN THE FORMULATED SPORTS DRINKS

About 0.50 ml of six serial dilutions were prepared from the standard solution, the samples and 2.5 ml of colour developing reagent (50 ml H_2SO_4+5 ml Potassium Antimonyl Tatarate+30 ml of Ammonium Molybdate and 15 ml of Ammonium

Molybdate). The mixtures were then incubated for 20 minutes. The absorbances were then read at 770 nm from the spectrophotometer. The standard values obtained were then used to plot standard curve and the concentrations of Phosphorus from the samples were then calculated (Tietz, 1995).

3.9.4 DETERMINATION OF CALCIUM ION IN THE FORMULATED SPORTS DRINKS

About 0.5 ml of each serial dilution prepared from the standard and the samples were taken and 3.5 ml of working O-Cresolphthalein Complexone reagent were added. They were then incubated at room temperature for 15 minutes. The absorbances of each were read at 570 nm on spectrophotometer. Standard curves were plotted from the standard values. From the equation of the curve the concentrations of calcium from the individual samples were then calculated (Farell and Kaplan, 1984).

3.10 DETERMINATION OF pH OF THE FORMULATED SPORTS DRINKS

The pH of the formulated sports drinks were determined using a pH meter (model: 526WTW) which was standardized at pH 7.0 with BDH buffers. The pHs of the formulated sports drinks were read at room temperature.

3.11 DETERMINATION OF PERCENTAGE CARBOHYDRATE IN THE FORMULATED SPORTS DRINKS

The percentage carbohydrate was determined by summing all the proximate values and the sum subtracted from 100 percent (AOAC, 1999).

3.12 DETERMINATION OF ENERGY VALUE OF THE FORMULATED SPORTS DRINKS

The energy value was calculated by using the following factors.

(9xFat) + (4x Protein) + (4xCarbohydrate) = Energy/calorie

1 calorie=4.182KJ

(AOAC, 1999).

3.13 SENSORY ANALYSIS OF THE FORMULATED SPORTS DRINKS

The samples prepared were analysed by a well trained sensory panelist (20 people) who were trained for one week with raw and pasteurized pineapple juice, raw and pasteurized coconut water, and the final sports drink samples that were formulated. Lucozade sports drink was used as the standard. Flavour, aroma, mouth feel, aftertaste, colour and overall acceptance were the main parameters evaluated. The evaluation was carried out using the acceptance test (9 point hedonic scale). The results of overall evaluation were analysed using one way anova (Lawless and Heymann, 1998).

3.14 SHELF LIFE DETERMINATION OF THE SPORTS DRINKS FORMULATED

The shelf life of the final product was determined by checking the pH weekly, observation of gas production, change of colour and odours for a period of six month. The pH was used because when fermentation starts to take place there is the formation of alcohol which shifts the pH towards the basic region because of the ROH functional group (Labuza and Labuza, 2004). Statsgraphics software was used to predict the shelf life of the products.

3.15 MICROBIAL ANALYSIS OF THE FINAL SPORTS DRINK PRODUCED

Microbial analysis was done on the final sports drink produced to check for bacteria and faecal coliforms and whether the pasteurization was adequate. Some of the samples were kept on the shelf (room temperature) and some in a fridge of an average temperature of 8^{o}C. For every week the growth of microorganism in the samples were determined for a period of 6 months.

3.16 DETERMINATION OF THE PRESENCE OF BROMELAIN IN THE FINAL SPORTS DRINK PRODUCT

About 7 g of gelatin was dissolved in 15 ml of hot water stirred and allowed to cool. The gelatin solution was left over night to solidify. About 0.1 g of solidified gelatin was put in 7 different beakers. The pHs of the sports drink samples which were pasteurized at a temperature of 70^{0} C for 30 minutes were recorded. About 5 ml of distilled water were added to each of the beakers. About 2 ml of distilled water was

added to beaker 1 which was the control. Two ml of the sports drink was added to each of the 6 remaining beakers. The activity of bromelain was measured at 450 nm at one hour interval (Hagar and Blackwell, 2006).

3.17 STATISTICAL ANALYSIS

One way analysis of variance (ANOVA) was used as the research design to study the effect of variation in the means of the physicochemical parameters determined. This is as a result of varying the percentage of coconut water and pineapple juice. The one way ANOVA was also used to compare the physicochemical parameters of the formulated sports drink to a standard sports drink (Lucozade sports drink).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

The results of the proximate analysis and energy value of the study are presented in tabular (Appendix 1) and graphical forms.

4.1 MOISTURE CONTENT OF THE FORMULATED SPORTS DRINKS

The mean percentage moisture content of the twelve formulated sports drinks are shown in Figure 1. The standard sports drink (Lucozade sports drink) had the highest mean percentage moisture of 95.01 % this could be due to the fact that the ingredients used in the Lucozade sports drink formulation contained water and had no fibre. Sample SC1 (30 % smooth cayenne: 70 % coconut water) had the highest moisture content of 93.49 % and sample SL3 (40 % sugar loaf: 60 % coconut water) had the lowest moisture content of 92.58 %. There was no significant difference ($P< 0.05$) between the mean percentage moisture content of the twelve formulated sports drinks. This result shows that statistically the mean percentage moisture content of the twelve formulated sports drinks were the same at 95 % confidence level. When means of the percentage moisture content of the sports drinks were compared to the mean moisture content of Lucozade sports there was no significant difference($P<0.05$).

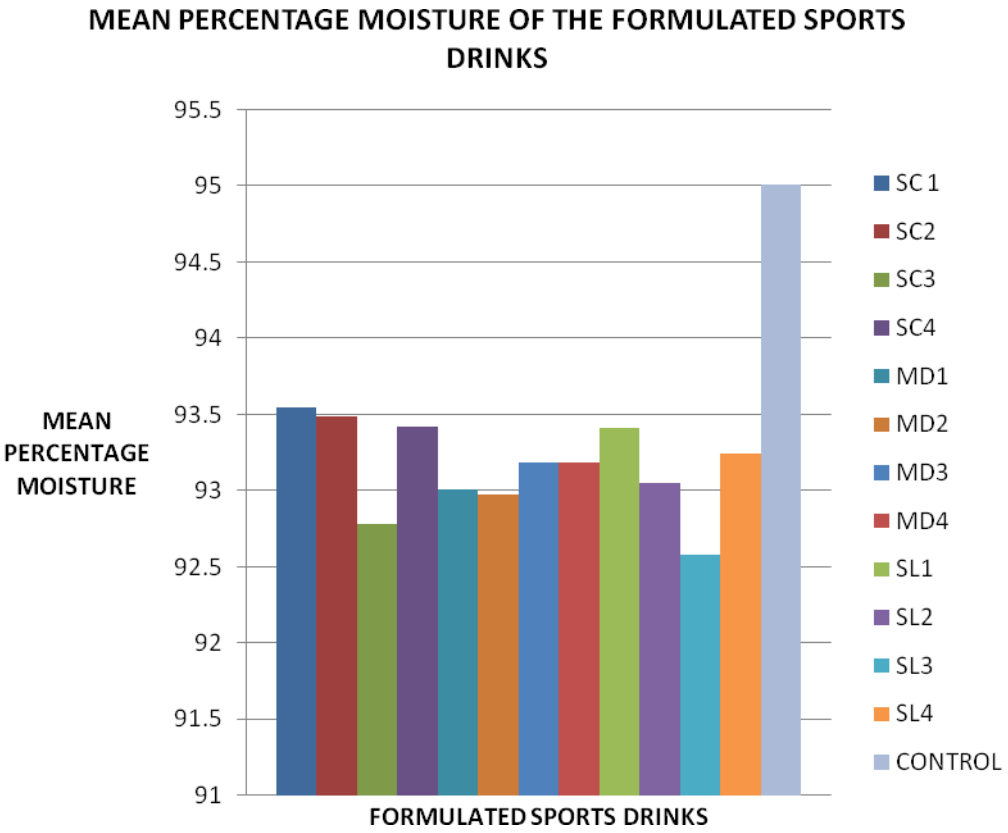

FIGURE 1 MEAN PERCENTAGE MOISTURE CONTENT OF THE FORMULATED SPORTS DRINKS

4.2 CRUDE FAT OF THE FORMULATED SPORTS DRINKS

The percentage crude fat of the sports drinks are shown in Figure 2**.** The crude fat in the sports drink formulated might have come from both the coconut water used which contains some essential fatty acid which the heart needs and the pineapple variety used. SC2 (35% smooth cayenne: 65 % coconut water) had the lowest mean percentage crude fat. The formulated sports drink with the highest crude fat was MD4 (45 % MD2: 55 % coconut water). There were significant differences ($P<0.05$) between the twelve formulated sports drink. The significant differences between the mean percentage crude fat values came from the variety of the pineapple used. Sports drink formulated using smooth cayenne variety had lower crude fat value compared

with the sports drinks formulated using MD2 and sugarloaf variety. The presence of crude fat gives an idea that essential fatty acids could be present in the sports drink formulated (Sylianco *et al*., 1992). This gives the formulated sports drinks an added value.

A high level of crude fat also gives an idea that the energy value of the formulated sports drinks will be high.

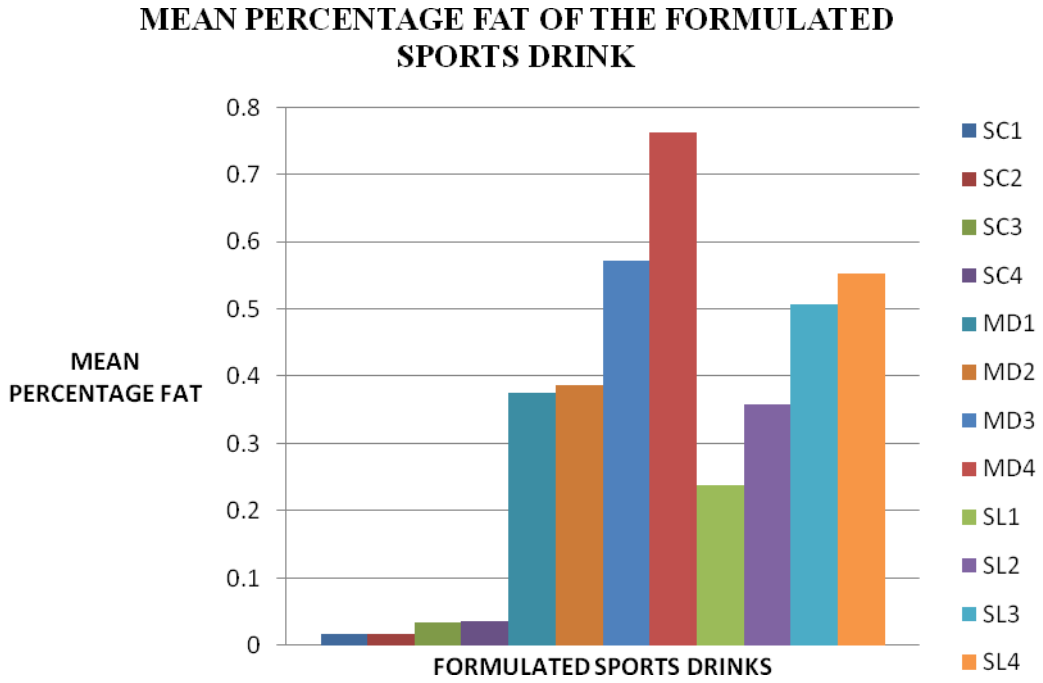

FIGURE 2 MEAN PERCENTAGE FAT OF THE FORMULATED SPORTS DRINKS

4.3 CRUDE PROTEIN OF THE FORMULATED SPORTS DRINKS

Protein is also one of the determined parameters that are used to calculate for the energy value of the sports drink. The results for crude protein are shown in Figure 3. The standard sports drink (Lucozade sports drink) did not contain any protein. SL4 (45 % sugarloaf: 55 % coconut water) contained the highest mean percentage crude

protein of 0.156 %. The formulated sports drink with the lowest mean percentage crude protein was SC1 (30 % smooth cayenne: 70 % coconut water). There was no significant difference ($P<0.05$) between the mean percentage crude protein of the formulated sports drinks. However the mean crude protein measured could be due to the proteolytic enzyme bromelain found in pineapples since enzymes are proteins (Donald *et al.*, 1971).

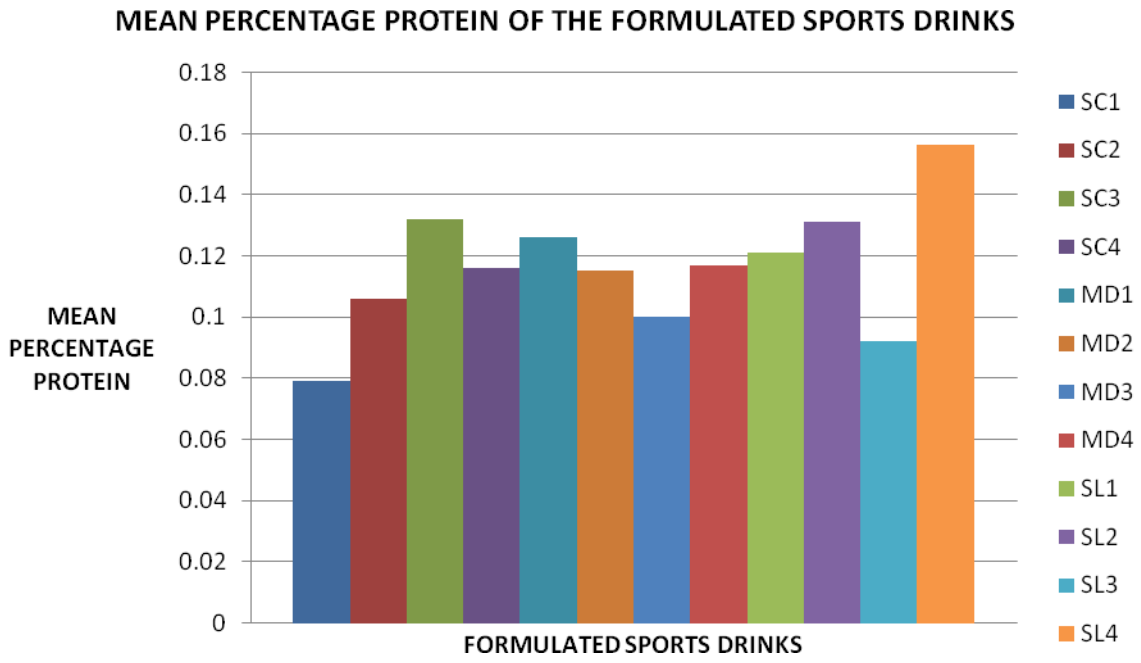

FIGURE 3 MEAN PERCENTAGE PROTEIN OF THE FORMULATED SPORTS DRINKS

4.4 CRUDE ASH OF THE FORMULATED SPORTS DRINKS

The results of the mean percentage ash content of the formulated sports drink samples are shown in Figure 4.

The standard sports drink used (Lucozade sports drink) recorded a lower ash content (0.008 %) compared to the twelve formulated sports drink. The quantity of electrolyte in Lucozade sports might have contributed to the low ash content. The formulated

sports drink sample labeled MD2 (35 % MD2: 65 % coconut water) contained the highest mean percentage ash (0.040 %) and SL1 (30 % sugarloaf: 70% coconut water) contained the lowest mean percentage ash (0.013 %).

The mean percentage ash obtained from the formulated drinks are significantly different at $P<0.05$. This could be due to the amount of coconut water and pineapple juice in the formulated sports drinks. Since coconut water contains high levels of electrolytes varying the quantity of electrolyte could affect the amount of electrolyte in the sports drinks. The soil types on which the pineapples are grown could also affect the amount of ash in the formulated sports drinks (Donald *et al*., 1971).

Ash gives an indication of the amount of minerals in a sample. Sports drinks are used to replace electrolytes that are lost through sweat so a high ash value gives a good indication of the amount of electrolytes the samples contain.

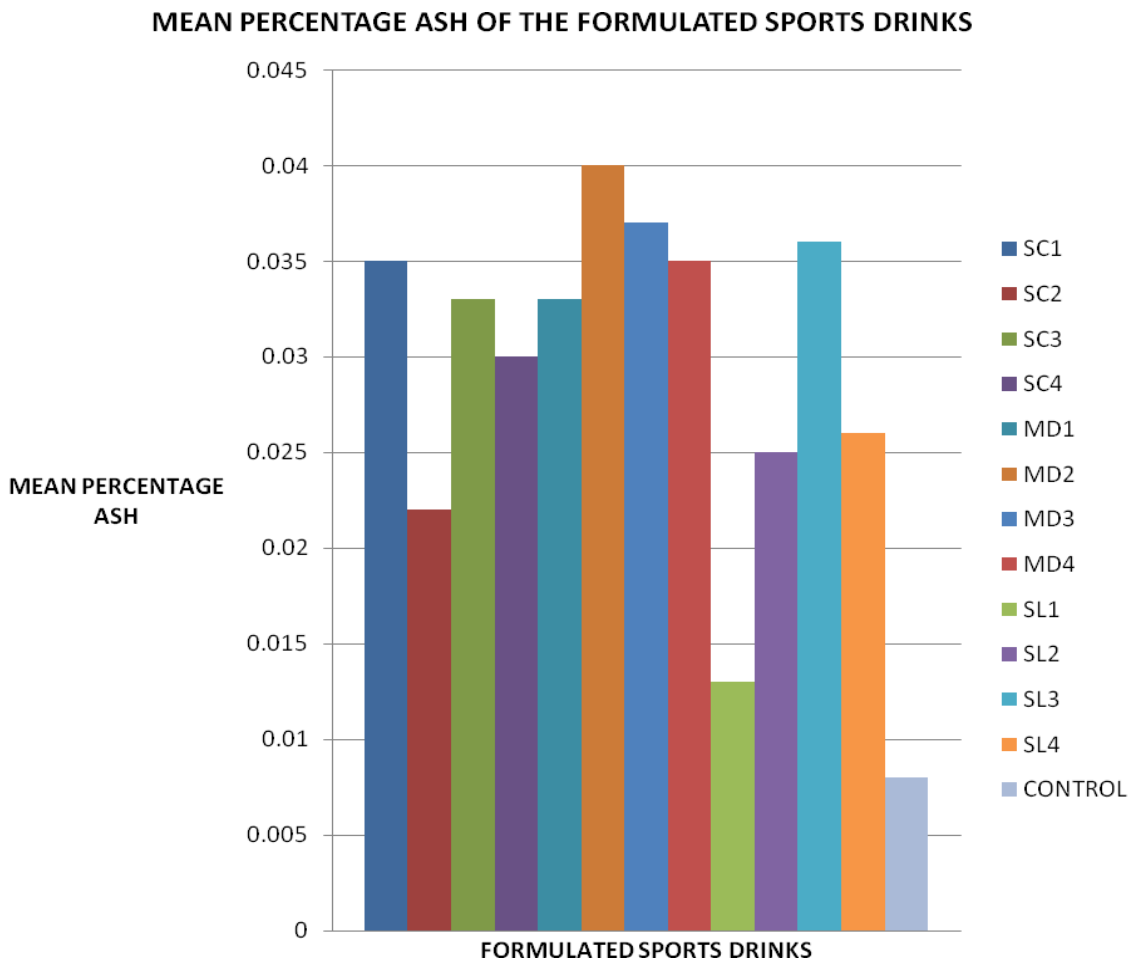

FIGURE 4 MEAN PERCENTAGE ASH OF THE FORMULATED SPORTS DRINKS

4.5 CRUDE FIBRE OF THE FORMULATED SPORTS DRINKS

The crude fibre content of the formulated sports drinks are shown in Figure 5.

The formulated sports drink SC4 had the highest percentage crude fibre of 1.090 %, followed by SL4 which contained 1.020 % crude fibre. The sample labeled MD1 contained the least percentage fibre of 0.032 %. The standard sports drink (Lucozade sports drink) did not contain any fibre. There was a significant difference ($P<0.05$) in the mean percentage crude fibre of the formulated sports drinks. The significant

difference could be attributed to the variety of pineapple used and also settling of the fibre content during the analysis.

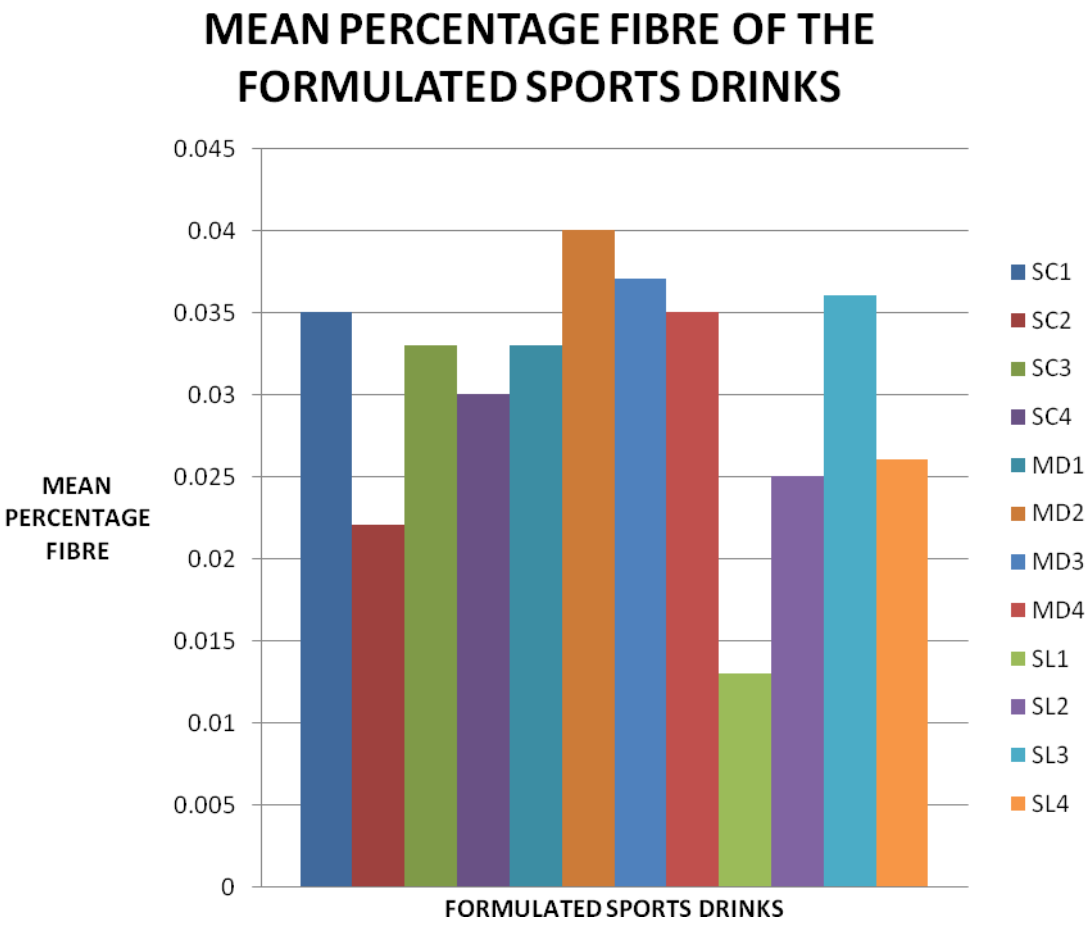

FIGURE 5 MEAN PERCENTAGE FIBRE OF THE FORMULATED SPORTS DRINKS

4.6 CARBOHYDRATE OF THE FORMULATED SPORTS DRINKS

The results for the percentage carbohydrate are shown in Figure 6. The mean percentage carbohydrate of the standard sports drink (Lucozade sports drink) was 6.40 %. The mean percentage carbohydrate were not significantly different ($P>0.05$) and also there was no significant difference between the mean percentage carbohydrate of the Lucozade sports drink and the formulated sports drinks. Sample MD1 (6.42%) recorded a mean percentage carbohydrate close to Lucozade sports drink (6.40 %).

The mean percentage carbohydrate values obtained makes all the formulated sports drink isotonic (Shi *et al*., 1995).

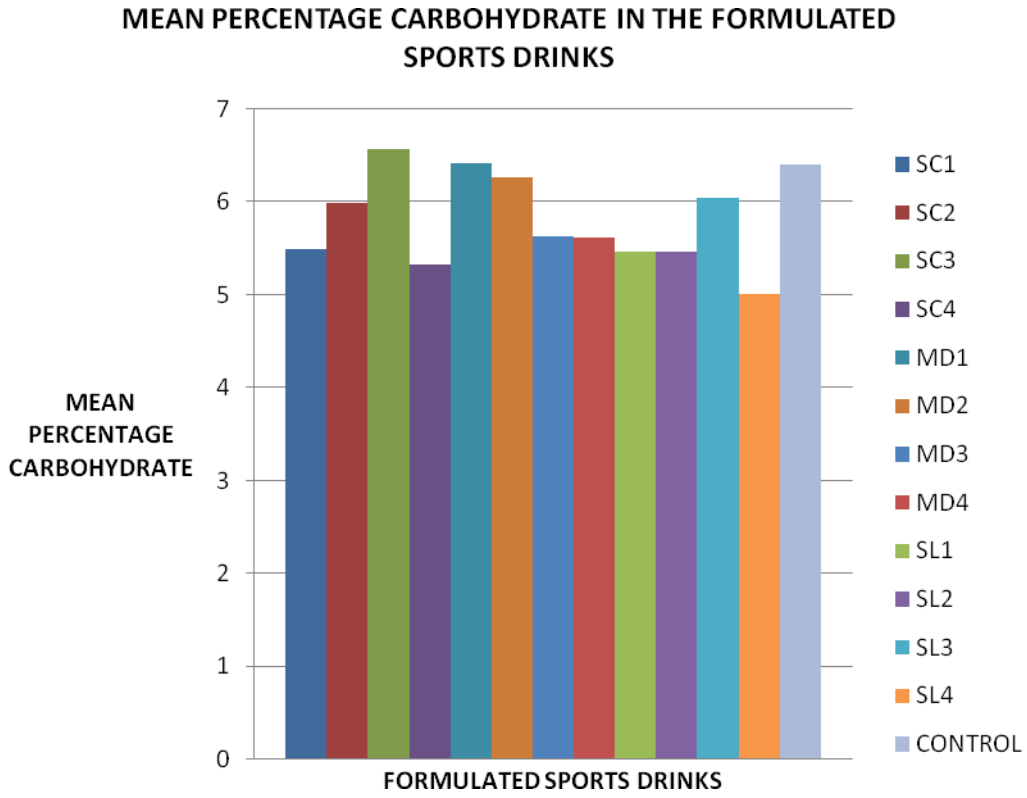

FIGURE 6 MEAN PERCENTAGE CARBOHYDRATE OF THE FORMULATED SPORTS DRINKS

4.7 ENERGY LEVELS OF THE FOMULATED SPORTS DRINKS

The energy value of the formulated sports drinks are shown in Figure 7. The energy value of the standard sports drink (Lucozade sports) was 117 KJ/100 ml. This was higher than some of the sports drinks formulated. However samples MD1, MD2, SL2 and SL3 had higher energy values than Lucozade sports drink. There was a significant difference ($P<0.05$) in the energy value of the formulated sports drinks and this could have been due to the proportions of coconut water and pineapple juice used in the various formulations. The variety of the pineapple used also had an effect on the

energy value of the formulated sports drinks. When the energy values of the sports drinks formulated using the smooth cayenne variety were compared with the sports drink formulated from MD2 and sugarloaf, the energy values obtained from samples labeled SC were the lowest. This shows that the variety of the pineapple used also had an effect on the energy values due to different levels of sugars present in the pineapple varieties.

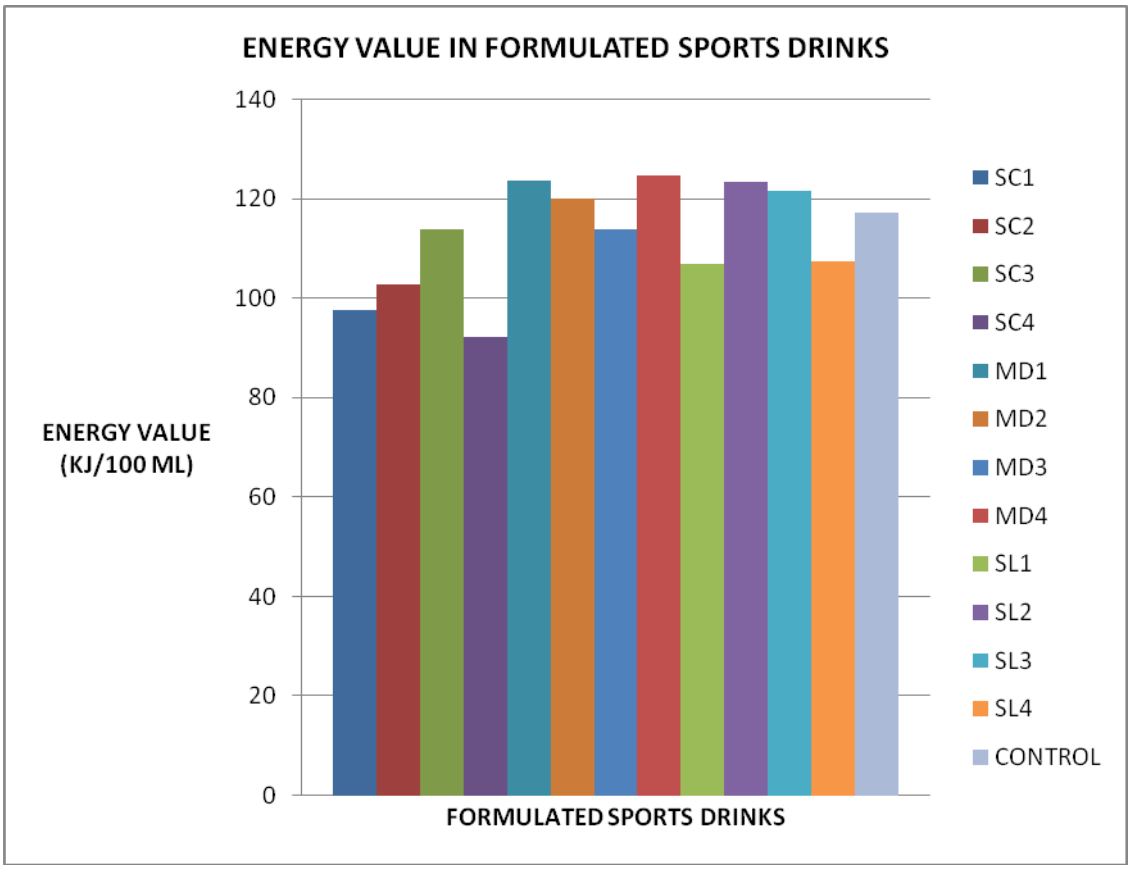

FIGURE 7 ENERGY VALUES OF THE FORMULATED SPORTS DRINKS

The results of Vitamin C, Total Solids, Total Soluble Solids Contents and pH of the Formulated Sports Drinks are presented in tabular (Appendix 2) and graphical forms.

4.8 VITAMIN C LEVELS OF THE FORMULATED SPORTS DRINKS

The results for the mean vitamin C of the formulated sports drinks are shown in Figure 8.

The formulated sports drinks contained vitamin C because of the pineapple juice used in the formulation. However the standard sports drink (Lucozade sports drink) did not contain any vitamin C because of the ingredients that were used in the formulation. Sample SL4 recorded the highest mean vitamin C content (180.67 mg/100 ml), followed by SL2 which had a mean vitamin C of 170.07 mg/100 ml and SC3 recorded the least mean vitamin C content. The presence of vitamin C in the sports drinks was due to the pineapple juice in the sports drinks. There were significant differences ($P<0.05$) in vitamin C content of the formulated sports drinks. The difference might be due to the variety of pineapple used. The results showed that sports drink samples formulated from smooth cayenne had lowest vitamin C content whiles sports drinks formulated from MD2 and sugar loaf varieties had high vitamin C content. This shows that the variety of pineapple used contributed to the vitamin C levels.

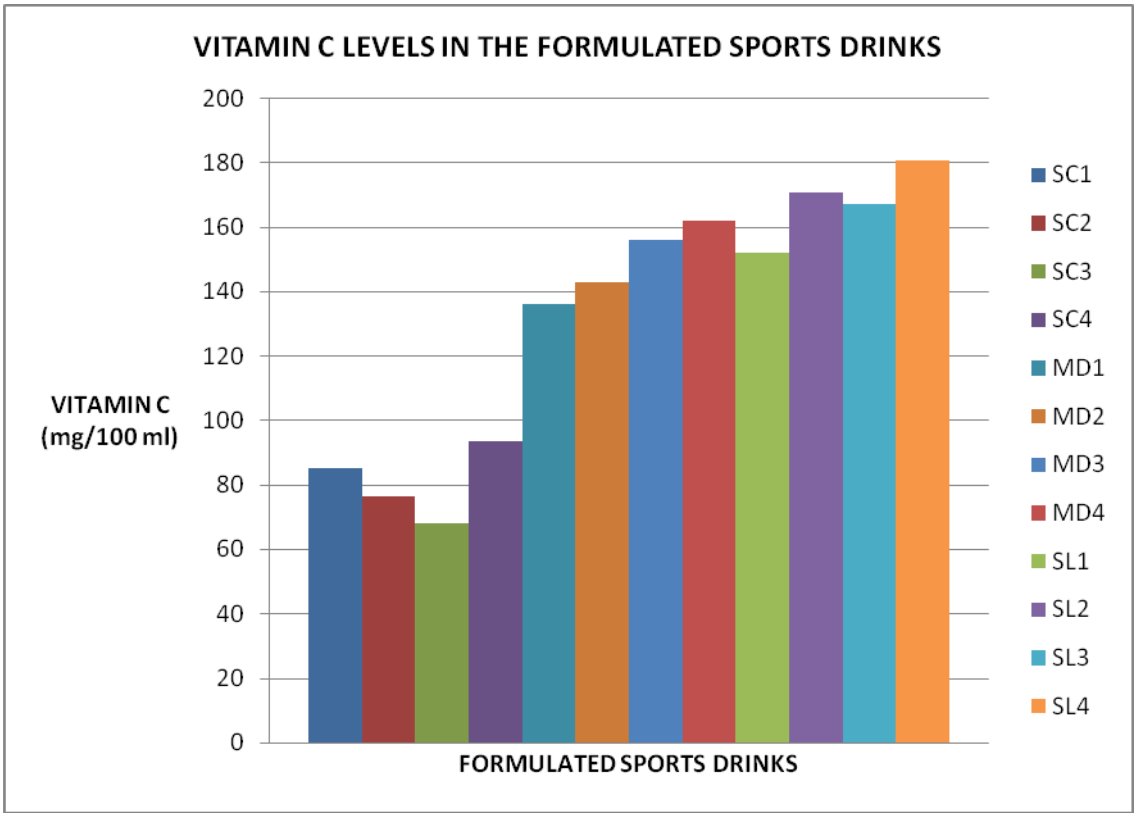

FIGURE 8 MEAN VITAMIN C LEVELS IN SPORTS DRINKS

4.9 PERCENTAGE TOTAL SOLIDS OF THE FORMULATED SPORTS DRINKS

The results of the formulated sports drinks are shown in Figure 9. The percentage total solids of the standard sports drink (Lucozade sports) was 4.64 %. This was lower than the formulated sports drinks and might be due to the ingredients that were used in the formulation. The total solids recorded in the Lucozade sports drink might have been the gum and glucose syrup that was used in the formulation. The difference in the percentage total solids were significant at $P<0.05$. The difference could be attributed to the variety of pineapple used. The sports drinks formulated from smooth cayenne had higher percentage total solids than that of formulated from MD2 and sugarloaf. Sample SC2 had the highest percentage total solids of 9.93 %. The lowest recorded total solids was 5.79 % (MD1). The sugars from the coconut water and pineapple as well as the fibre from the pineapple might have contributed to the total solids.

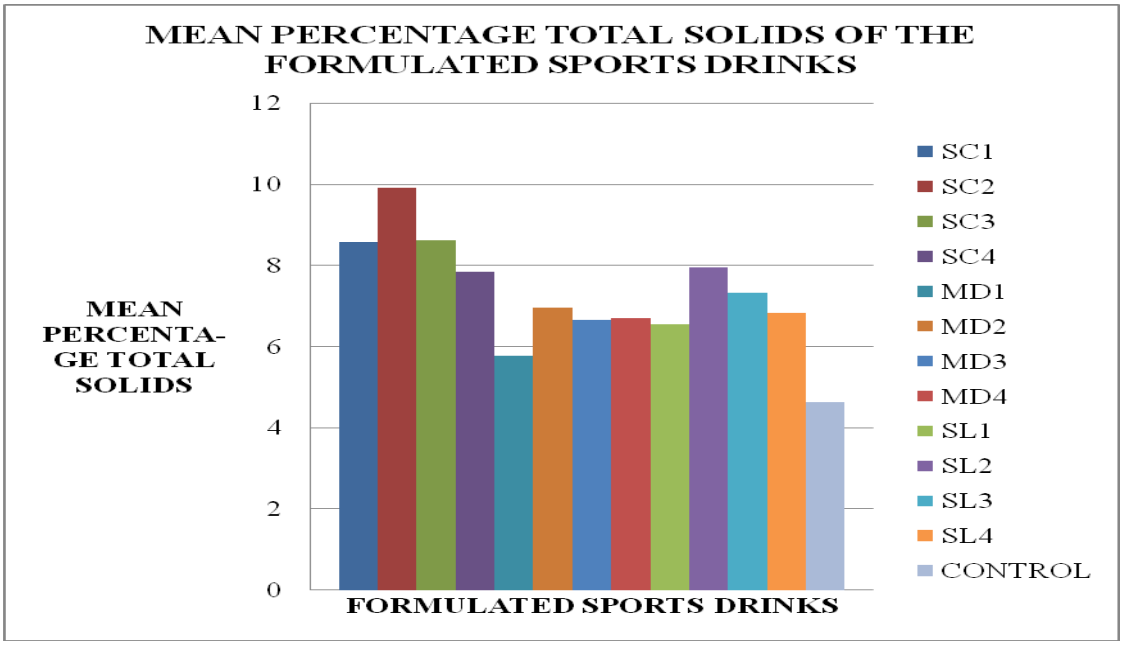

FIGURE 9 MEAN PERCENTAGE TOTAL SOLIDS OF THE FORMULATED SPORTS DRINKS

4.10 TOTAL SOLUBLE SOLIDS (BRIX^{o}) OF THE FORMULATED SPORTS DRINKS

The recorded brix of formulated sports drink are shown in Figure 10. The standard sports drink sample (Lucozade sports drink) recorded a brix of 5.00 degree brix. This was lower than that recorded by the formulated sports drinks. The formulated sports drink from sugarloaf pineapple variety had the highest recorded brix. This is because sugarloaf is a variety of pineapple that is very sweet compared to MD2 and smooth cayenne. Sports drinks samples formulated from MD2 pineapple variety had the lowest ^{o}brix. The differences in the brix recorded were statistically significant at $P<0.05$. These differences in the brix could be attributed to the variety of pineapple used.

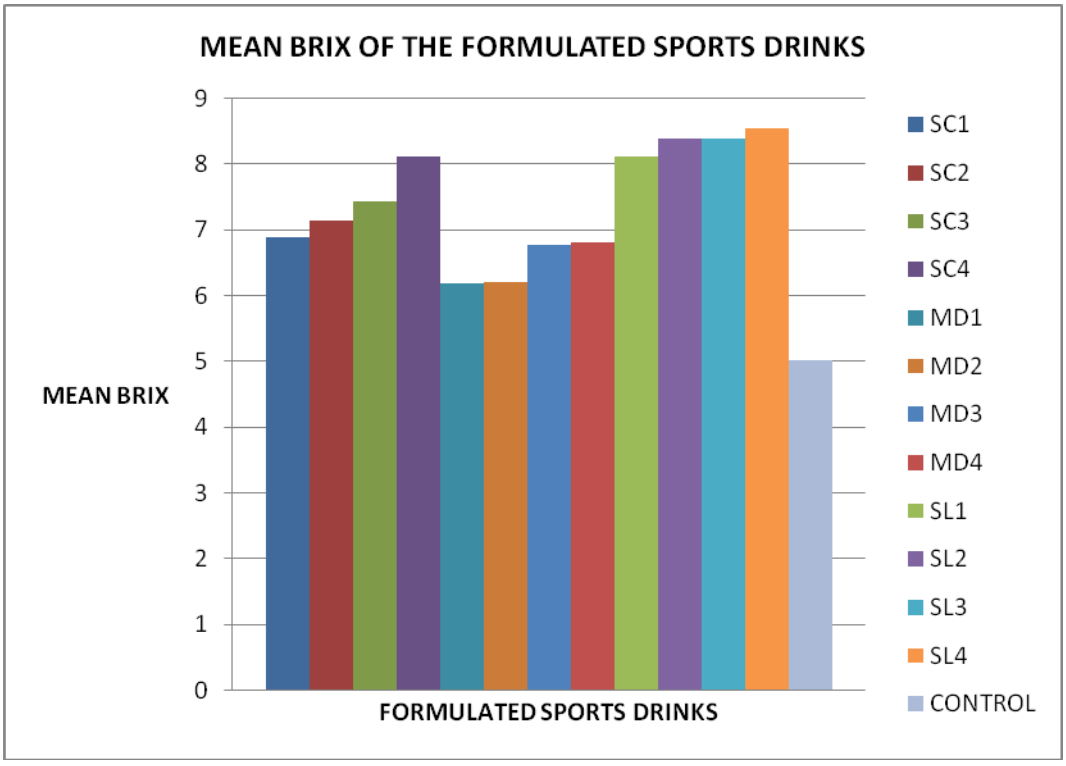

FIGURE 10 MEAN BRIX OF THE FORMULATED SPORTS DRINKS

4.11 pH OF THE SPORTS DRINKS FORMULATED

The pHs recorded from the sports drinks formulated are shown in Figure 11. The standard sports drink had a pH of 3.66. This was lower than the formulated sports drinks which had pHs ranging between 4.40-4.72. The high pH range of the formulated sports drinks was due to the proportion of coconut water. The coconut water is a low acid food and because the formulated sports drink has a high proportion of coconut water than pineapple juice which is a high acid food, the coconut water could influence the pH more than the pineapple juice. The variety of pineapple used also contributed to the pHs recorded because sports drinks sample formulated from MD2 pineapple variety recorded higher pH compared to sports drinks formulated from smooth cayenne and sugarloaf. The difference in the pH recorded was significant at $P<0.05$.

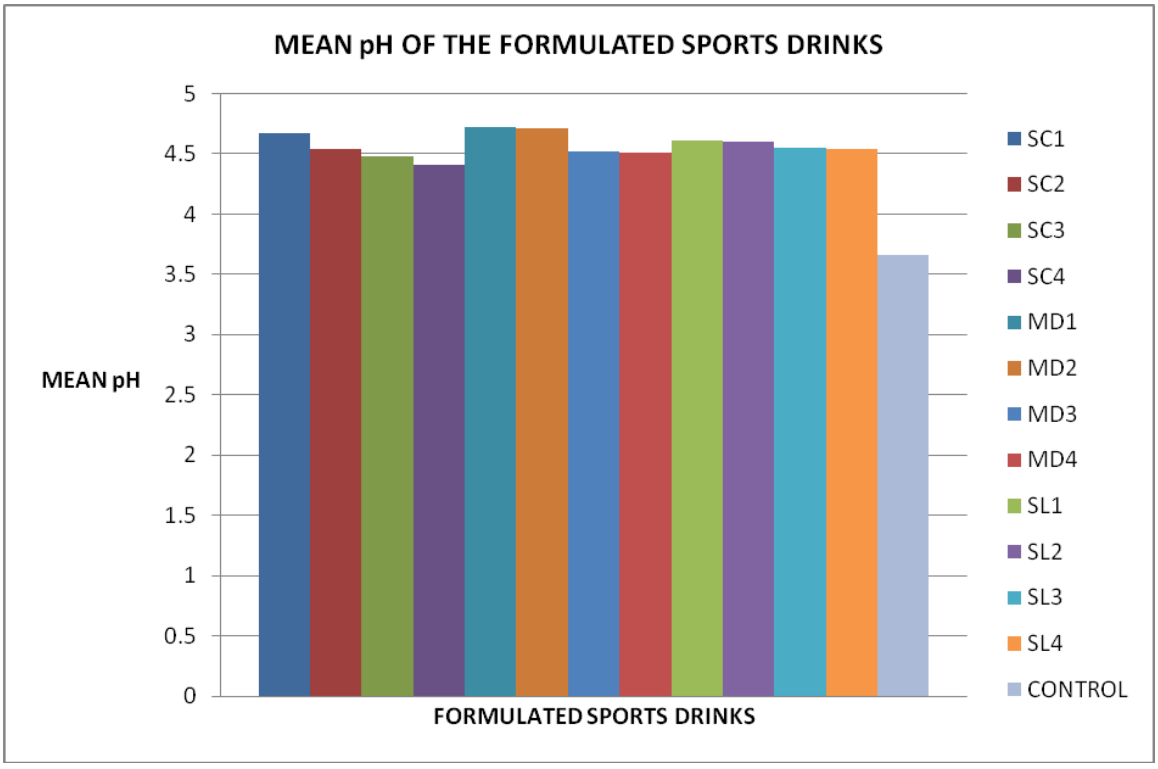

FIGURE 11 MEAN pH OF THE FORMULATED DRINKS

4.12 SENSORY ANALYSIS OF THE FORMULATED SPORTS DRINKS

Table 3 shows the responses of the sensory panelists.

Table 3 SENSORY ANALYSIS OF THE FORMULATED SPORTS DRINKS

Sports Drinks	Taste	Aroma	Flavour	Colour	Aftertaste	Overall Acceptability
SC1	4.65	4.58	4.09	4.27	4.03	4.59
SC2	4.25	4.06	4.24	4.09	4.27	4.03
SC3	4.68	4.73	4.06	4.06	4.70	4.44
SC4	4.98	4.72	4.73	4.73	4.75	4.25
MD1	4.15	4.25	4.72	4.72	4.87	4.44
MD2	4.70	4.70	4.25	4.70	4.80	4.64
MD3	4.27	4.69	4.70	4.23	4.71	4.15
MD4	4.59	4.23	4.34	4.22	4.50	4.99
SL1	4.68	4.22	4.09	4.44	4.56	4.44
SL2	4.64	4.44	4.24	4.12	4.53	4.66
SL3	4.18	4.24	4.09	4.27	4.03	4.59
SL4	4.93	4.28	4.70	4.69	4.19	4.25
Lucozade	4.03	4.59	4.95	4.80	4.53	4.79

SL4 scored the highest with taste, this could be due to the high brix (8.53 ^{O}Brix) of the formulated sports drink. Samples labeled MD2 scored highest with aftertaste, this could be due to the low acid: high sugar balance. The standard sports drink (Lucozade sports drink) scored highest with colour and flavour, this could be due to the beta carotene coloration and carbonation of the sports drink respectively. When it came to

overall acceptability MD4 scored the highest. Since the formulated sports drinks had almost the same sensory attributes other parameters like high energy value and percentage carbohydrate must be used to select the final product. There were no significant differences in the responses of the sensory panelist when their responses were analysed statistically at $P<0.05$. This showed that the products had almost the same sensory attributes.

Using these parameters sample MD4 (45% MD2:55 % coconut water) was selected from the formulations because of its high energy value of 124.47 KJ and its percentage carbohydrate of 5.62%.

4.13 ELECTROLYTES LEVELS OF THE SPORTS DRINKS

The measurement of electrolytes in the sports drink (sample MD4) showed that it contained a high level of potassium (837.67 mg/ L) , sodium (273.94 mg/L), phosphate (186.67 mg/L), magnesium (55.53 mg/L), bicarbonate (46.33 mg/L), and low levels of chlorine (45 mg/L), calcium (28.00 mg/ L) and carbonate (6.00 mg/L). These are shown in **Figure 12.** Lucozade sports drink contains 9.0 mg/100 ml of potassium and 50 mg/100 ml of sodium.

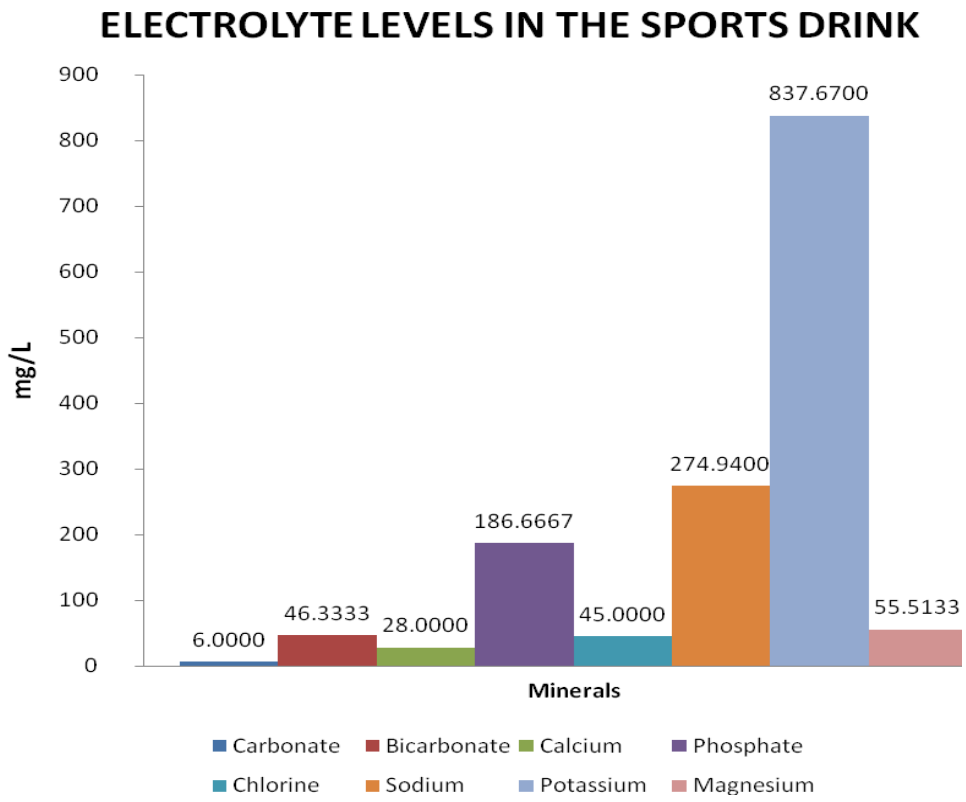

FIGURE 12 ELECTROLYTES LEVELS IN THE SELECTED SPORTS DRINK (SAMPLE MD4)

The high levels of sodium and potassium makes the sports drink a good sports drink. The sodium in the sports drink will contribute to palatability and also encourage drinking as well replacing that lost through sweat. Sodium also is a key element in the maintenance of fluid and acid-base balance. It transmits nerve impulse and helps to control in the muscle contraction as well as regulating permeability of the cell membrane. Potassium affects heartbeat and aids muscle contraction. Calcium combines with other minerals within a protein framework to give structure and strength to bones and teeth, also calcium assists in blood clotting so whenever there is an open cut it helps prevent loss of blood (Haris, 1996). These nutritional compositions makes the sports drink an excellent choice for sports men.

4.14 DETERMINATION OF THE PRESENCE OF BROMELAIN IN THE FINAL SPORTS DRINK PRODUCT

The absorbance for the first ten minutes was 0.7. The absorbance rose to 0.87 after 50 minutes and dropped after 60 minutes. This shows that bromelain was present in the sports drink and was able to digest the gelatin because of the changes in the absorbance curve (Figure 13). The presence of bromelain gives the sports drink an added advantage over other sports drinks. Bromelain in the sports drink will help in muscle repair and heal inflammation (Donald *et al*., 1971). These are the most common problems that sports men encounter during training sessions and competition.

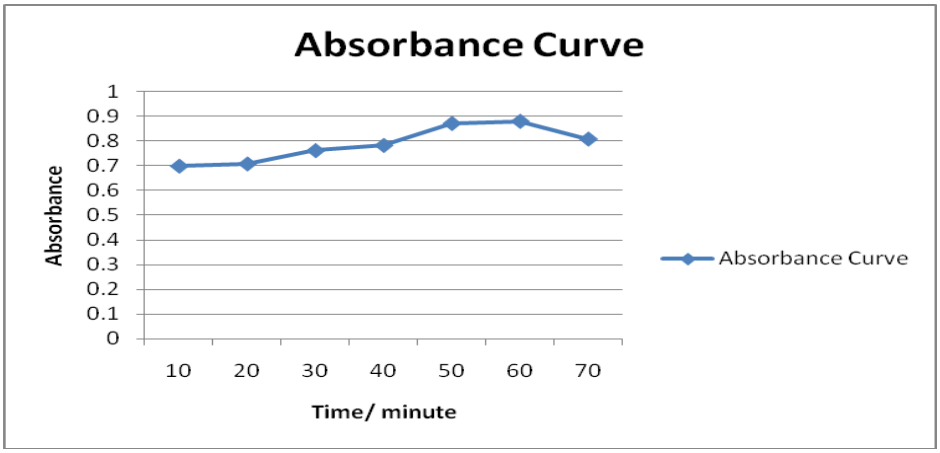

FIGURE 13 ABSORBANCE CURVE OF THE SPORTS DRINKS FORMULATED READ AT 450 nm

4.15 MICROBIOLOGICAL ANALYSIS OF THE FINAL SPORTS DRINK OBTAINED

From the microbiological results (Figure 14) for samples kept in the fridge at temperature of 8^{o}C and on the shelf at a temperature of 25^{o} for six month, in the initial reading there was no growth (Nil). There was also no growth (Nil) during the first month for the samples kept in the fridge. The shelf sample had a growth of 2 counts but during the second month there were growth in both the samples kept in the fridge and the shelf. The count for the sample in the fridge was 1 and the shelf sample had 5 counts. The counts increased to 3 for fridge sample and 6 for shelf sample for the third month. The counts for the fourth month were 4 and 7 for the fridge sample and shelf sample respectively.

For the fifth and sixth month the counts were the same 5 and 8 for both the fridge sample and the shelf sample. The growth of these microorganisms were due to the fact that the sports drink samples were natural fruit juices and that during pasteurization at temperature of 70^{o}C for 30 minutes there could have been some spores that were not destroyed by the temperature used. The pH of the formulated sports drinks might have increased and this could affect the effect that the sodium benzoate was having on the sports drinks with time and microorganisms could proliferate.

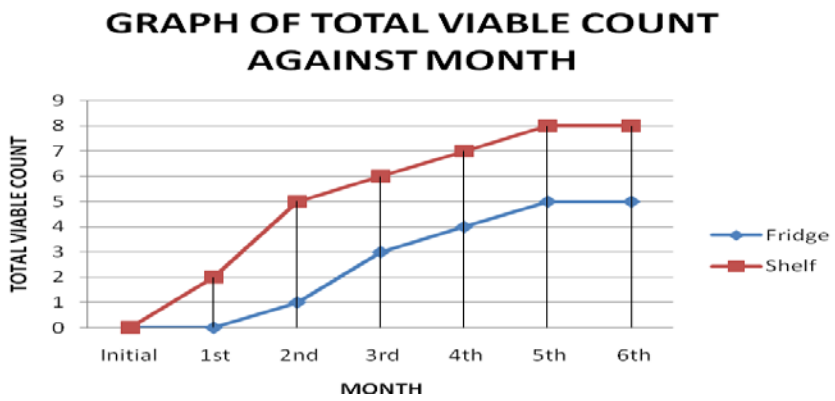

Figure 14 TOTAL VIABLE COUNT OF THE FORMULATED SPORTS DRINK

4.16 SHELF LIFE DETERMINATION OF THE SPORTS DRINK OBTAINED

Statgraphics was used to determine the shelf life using pH readings for six months. The pH was used as a parameter for determining shelf life because coconut water is a low acid food and hence the pH must be lowered to make it a high acid food to enhance and extend the shelf life because microorganisms proliferate very well in low acid foods. An increase in pH after it was lowered gives a sign of growth of microorganisms. This software generated an equation that could be used to determine the shelf life by creating a model.

The StatAdvisor

From **Appendix 13** and **14** the output showed the results of fitting the squared-Y square root-X model to describe the relationship between pH Fridge and Months (where pH fridge means the sample that was kept in the fridge). The equation of the fitted model was

pH Fridge = sqrt(14.5458 + 0.365508*sqrt(Months))

Since the P-value in the ANOVA table was less than 0.05, there was a statistically significant relationship between pH Fridge and Months at the 95.0% confidence level.

The R-Squared statistic indicated that the model as fitted explains 91.9625% of the variability in pH Fridge after transforming to a logarithmic scale to linearize the model. The correlation coefficient equals 0.958971, indicating a relatively strong relationship between the variables. The standard error of the estimate showed the standard deviation of the residuals to be 0.0994924. This value was used to construct prediction limits for new observations by selecting the Forecasts option from the text menu.

The mean absolute error (MAE) of 0.0729759 is the average value of the residuals. The Durbin-Watson (DW) statistic tests the residuals to determine if there is any significant correlation based on the order in which they occur in the data file. Since the P-value was less than 0.05, there is an indication of possible serial correlation at the 95.0% confidence level. The residuals were plotted versus row order to see if there was any pattern that could be seen. This is shown in **Appendix 22 and 23**.

From **Appendix 24** the lack of fit test was designed to determine whether the selected model was adequate to describe the observed data, or whether a more complicated model should be used. The test was performed by comparing the variability of the current model residuals to the variability between observations at replicate values of the independent variable X.

From **Appendix 16** the results of fitting several curvilinear models to the data. Of the models fitted, the squared-Y square root-X model yields the highest R-Squared value with 91.9625%. This was the currently selected model.

From **Appendix 18** the output shows the results of fitting a squared-Y square root-X model to describe the relationship between Shelf pH and Months where shelf pH is the sample that was kept on the shelf for shelf life studies. The equation of the fitted model is

Shelf pH = sqrt(14.5 + 0.509954*sqrt(Months))

Since the P-value in the ANOVA table was less than 0.05, there was a statistically significant relationship between Shelf pH and Months at the 95.0% confidence level.

The R-Squared statistic indicates that the model as fitted explains 97.674% of the variability in Shelf pH after transforming to a logarithmic scale to linearize the model. The correlation coefficient equals 0.988302, indicating a relatively strong relationship between the variables. The standard error of the estimate shows the standard deviation of the residuals to be 0.0713636. This value was used to construct prediction limits for new observations by selecting the Forecasts option from the text menu.

The mean absolute error (MAE) of 0.0419464 is the average value of the residuals. The Durbin-Watson (DW) statistic tests the residuals to determine if there is any significant correlation based on the order in which they occur in the data file. Since the P-value is greater than 0.05, there is no indication of serial autocorrelation in the residuals at the 95.0% confidence level.

Appendix 19 shows predicted values for Shelf pH using the fitted model. In addition to the best predictions, the table shows:

(1) 95.0% prediction intervals for new observations

(2) 95.0% confidence intervals for the mean of many observations

The prediction and confidence intervals correspond to the inner and outer bounds on the graph of the fitted model.

Appendix 21 shows the results of fitting several curvilinear models to the data. Of the models fitted, the squared-Y square root-X model yields the highest R-Squared value with 97.674%. This was the selected model.

From the equation generated with the statsgraphics software

Shelf pH = sqrt(14.5 + 0.509954*sqrt(Months))

pH Fridge = sqrt(14.5458 + 0.365508*sqrt(Months))

From literature the average pH of the coconut water is 4.0-5.5. Fixing a pH of 3.97 into the equation generated the month taken for the pH of the preserved samples to increase to 3.97 for both the samples stored on the shelf and in the fridge as 12.79 month which is approximately 12 months. This correlates with the results obtained from the microbial studies of the samples.

The final sports drink obtained is shown in **Picture 1**

PICTURE 1 THE FINAL SPORTS DRINK PRODUCT OBTAINED

CHAPTER FIVE

5.1 CONCLUSION

From the results obtained a sports drink can be prepared from coconut water and pineapple juice in a ratio of 55 percent of coconut water to 45 percent of pineapple juice. Food grade phosphoric acid and sodium benzoate can be used as preservative and a pasteurizing temperature of 70^{o}C. The MD2 pineapple variety was chosen as the best variety for formulation with coconut water. MD2 juice formulation with coconut water has an average brix of $6.80\ ^{0}$ brix, Vitamin C content of 161.67 mg/100 ml, and percentage carbohydrate of 5.61 % making it easy for gastric emptying, energy of 124.47 KJ per 100 ml. The formulated sports drink also contains bromelain a proteolytic enzyme which aids in muscle repair. These parameters make the formulation a good sports drink and with the availability of the raw materials throughout the year this product could be economically viable.

The shelf life of the sports drink was approximately twelve months using statsgraphics to model and predict the shelf life.

5.2 RECOMMENDATIONS

Other fruits such as pawpaw, water melon should be studied to see if they can be used for a sports drink formulation. Also the amount of bromelain should be isolated and quantified.

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APPENDIX

APPENDIX 1 **Proximate Composition And Energy Value Of The Sports Drinks**

Sports Drinks	**Moisture (%)**	**Crude Fat (%)**	**Crude Protein (%)**	**Crude Ash (%)**	**Crude Fibre (%)**	**Carbo-hydrate (%)**	**Energy Value KJ/100ml**
SC1	93.55 (0.08)	0.018 (0.016)[a]	0.079 (0.03)	0.035 (0.001)[a]	0.942 (0.018)[a]	5.49 (0.02)	97.55 (0.02)[a]
SC2	93.49 (0.08)	0.017 (0.008)[a]	0.106 (0.03)	0.022 (0.017)[a]	0.324 (0.017)[a]	5.99 (0.03)	102.64 (0.01)[a]
SC3	92.78 (0.30)	0.035 (0.010)[a]	0.132 (0.04)	0.033 (0.009)[a]	0.427 (0.018)[a]	6.57 (0.01)	11.67 (0.03)[a]
SC4	93.42 (0.13)	0.036 (0.003)[a]	0.116 (0.03)	0.030 (0.006)[a]	1.090 (0.001)[a]	5.32 (0.03)	92.20 (0.01)[a]
MD1	93.01 (0.52)	0.376 (0.005)[b]	0.126 (0.03)	0.033 (0.002)[b]	0.032 (0.000)[b]	6.42 (0.01)	123.53 (0.02)[b]
MD2	92.98 (0.16)	0.386 (0.000)[b]	0.115 (0.04)	0.040 (0.003)[b]	0.099 (0.000)[b]	6.27 (0.23)	119.87 (0.20)[b]
MD3	93.19 (0.03)	0.571 (0.002)[c]	0.100 (0.04)	0.037 (0.003)[b]	0.383 (0.000)[b]	5.63 (0.12)	113.70 (0.12)[b]
MD4	93.19 (0.31)	0.761 (0.000)[d]	0.117 (0.02)	0.035 (0.003)[b]	0.304 (0.000)[b]	5.61 (0.24)	124.47 (0.03)[b]
SL1	93.41 (0.57)	0.238 (0.067)[e]	0.121 (0.05)	0.013 (0.006)[c]	0.488 (0.061)[c]	5.47 (0.21)	106.81 (0.04)[c]
SL2	93.05 (0.46)	0.359 (0.030)[f]	0.131 (0.03)	0.025 (0.003)[c]	0.922 (0.020)[c]	5.47 (0.02)	123.35 (0.04)[c]
SL3	92.58 (0.45)	0.507 (0.011)[f]	0.092 (0.02)	0.036 (0.008)[c]	0.754 (0.000)[c]	6.04 (0.03)	123.37 (0.05)[c]
SL4	93.25 (0.23)	0.552 (0.002)[f]	0.156 (0.02)	0.026 (0.003)[c]	1.020 (0.041)[c]	5.01 (0.01)	121.43 (0.07)[c]
Lucozade	95.01 (0.01)	Nil	Nil	0.008 (0.003)[d]	Nil	6.40 (0.03)	117.00 (0.02)[d]

Mean values along the same column with the same superscript are not significantly different. ($p<0.05$)

APPENDIX 2

Vitamin C, Total Solids, Total Soluble Solids Contents and pH Of the Formulated Sports Drinks

Sports Drinks	Vitamin C mg/100 ml	Total Solids (%)	Total Soluble Solids (^{o}Brix)	pH
SC1	85.00 (0.02)^{a}	8.58 (0.12)^{a}	6.87 (0.05)^{a}	4.67 (0.00)$^{\mathbf{a}}$
SC2	76.43 (0.03)^{a}	9.93 (0.13)^{a}	7.13 (0.11)^{a}	4.54 (0.00)$^{\mathbf{a}}$
SC3	68.03 (0.02)^{a}	8.64 (0.24)^{a}	7.43 (0.10)^{a}	4.47 (0.00)$^{\mathbf{a}}$
SC4	93.27 (0.01)^{a}	7.85 (0.14)^{a}	8.10 (0.05)^{a}	4.40 (0.00)$^{\mathbf{a}}$
MD1	136.07 (0.02)^{b}	5.79 (0.20)^{b}	6.17 (0.00)^{b}	4.72 (0.00)$^{\mathbf{b}}$
MD2	142.60 (0.01)^{b}	6.98 (0.28)^{b}	6.20 (0.02)^{b}	4.71 (0.00)$^{\mathbf{b}}$
MD3	155.73 (0.05)^{b}	6.67 (0.20)^{b}	6.76 (0.06)^{b}	4.52 (0.00)$^{\mathbf{b}}$
MD4	161.67 (0.02)^{b}	6.71 (0.26)^{b}	6.80 (0.02)^{b}	4.51 (0.00)$^{\mathbf{b}}$
SL1	152.00 (0.01)^{c}	6.57 (0.21)^{c}	6.10 (0.01)^{c}	4.61 (0.00)$^{\mathbf{c}}$
SL2	170.67 (0.05)^{c}	7.97 (0.34)^{c}	6.37 (0.11)^{c}	4.60 (0.00)$^{\mathbf{c}}$
SL3	167.13 (0.02)^{c}	7.33 (0.18)^{c}	8.37 (0.10)^{c}	4.55 (0.00)$^{\mathbf{c}}$
SL4	180.67 (0.01)^{c}	6.85 (0.32)^{c}	8.53 (0.06)^{c}	4.54 (0.00)$^{\mathbf{c}}$
Lucozade	Nil	4.64 (0.12)^{d}	5.00 (0.01)^{d}	3.66 (0.00)$^{\mathbf{d}}$

Mean values along the same column with the same superscript are not significantly different. ($p<0.05$)

APPENDIX 3

ANOVA TABLE OF MEAN PERCENTAGE MOISTURE CONTENT OF THE FORMULATED SPORTS DRINKS AND LUCOZADE SPORTS DRINK

Moisture					P<0.05
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.237	12	.118	.590	.560
Within Groups	6.415	32	.200		
Total	6.651	44			

APPENDIX 4

ANOVA TABLE OF MEAN PERCENTAGE FAT CONTENT OF THE FORMULATED SPORTS DRINKS AND LUCOZADE SPORTS DRINK

Fat					P<0.05
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.554	12	.777	49.705	.000
Within Groups	.500	32	.016		
Total	2.054	44			

APPENDIX 5

ANOVA TABLE OF MEAN PERCENTAGE ASH OF THE FORMULATED SPORTS DRINKS AND LUCOZADE SPORTS DRINK

Ash					P<0.05
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	12	.000	5.765	.007
Within Groups	.002	33	.000		
Total	.003	45			

APPENDIX 6

ANOVA TABLE OF MEAN PERCENTAGE PROTEIN OF THE FORMULATED SPORTS DRINKS

Protein					P<0.05
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	2	.001	.537	.590
Within Groups	.035	32	.001		
Total	.036	34			

APPENDIX 7

ANOVA TABLE OF MEAN PERCENTAGE FIBRE OF THE FORMULATED SPORTS DRINKS

Fibre					P<0.05
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.406	2	1.203	19.550	.000
Within Groups	2.031	33	.062		
Total	4.436	35			

APPENDIX 8

ANOVA TABLE OF MEAN VITAMIN C AMOUNT IN THE FORMULATED SPORTS DRINKS

Vitamin C					P<0.05
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	50164.776	2	25082.388	204.380	.000
Within Groups	4049.900	33	122.724		
Total	54214.676	35			

APPENDIX 9

ANOVA TABLE OF MEAN PERCENTAGE TOTAL SOLIDS IN THE FORMULATED SPORTS DRINKS AND LUCOZADE SPORTS DRINK

TOTAL SOLIDS					P<0.05
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	30.938	2	15.469	18.156	.000
Within Groups	28.116	33	.852		
Total	59.054	35			

APPENDIX 10

ANOVA TABLE OF MEAN BRIX IN THE FORMULATED SPORTS DRINKS AND LUCOZADE SPORTS DRINK

$Brix^{O}$					P<0.05
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20.727	2	10.364	85.022	.000
Within Groups	4.022	33	.122		
Total	24.750	35			

APPENDIX 11

ANOVA TABLE OF MEAN PERCENTAGE CARBOHYDRATE IN THE FORMULATED SPORTS DRINKS AND LUCOZADE SPORTS DRINKS

CARBOHYDR-ATE					P<0.05
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20.727	2	10.364	52.484	.000
Within Groups	4.022	33	.122		
Total	24.750	35			

APPENDIX 12

ANOVA TABLE OF MEAN ENERGY IN THE FORMULATED SPORTS DRINKSAND LUCOZADE SPORTS DRINK

ENERGY					P<0.05
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20.727	2	10.364	85.022	.000
Within Groups	4.022	33	.122		
Total	24.750	35			

APPENDIX 13 ANOVA TABLE OF SENSORY ANALYSIS

ANOVA TABLE OF SENSORY ANALYSIS						P<0.05
		Sum of Squares	df	Mean Square	F	Sig.
Taste	Between Groups	.959	8	.120	.062	1.000
	Within Groups	313.684	162	1.936		
	Total	314.643	170			
Aroma	Between Groups	6.737	8	.842	.551	.817
	Within Groups	247.789	162	1.530		
	Total	254.526	170			
Flavour	Between Groups	3.556	8	.444	.286	.970
	Within Groups	251.789	162	1.554		
	Total	255.345	170			
Colour	Between Groups	2.012	8	.251	.206	.989
	Within Groups	197.368	162	1.218		
	Total	199.380	170			
Aftertaste	Between Groups	1.766	8	.221	.184	.993
	Within Groups	194.421	162	1.200		
	Total	196.187	170			
Overall	Between Groups	7.801	8	.975	.957	.472
	Within Groups	165.053	162	1.019		
	Total	172.854	170			

SHELF LIFE MODELING USING STATSGRAPHICS

Simple Regression - pHFridge vs. Months

Dependent variable: pHFridge

Independent variable: Months

Squared-Y square root-X: Y = sqrt(a + b*sqrt(X))

APPENDIX 14 Coefficients OF Regression

	Least Squares	Standard	T	
Parameter	Estimate	Error	Statistic	P-Value
Intercept	14.5458	0.0837006	173.783	0.0000
Slope	0.365508	0.0483246	7.56362	0.0006

APPENDIX 15

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	0.566289	1	0.566289	57.21	0.0006
Residual	0.0494936	5	0.00989873		
Total (Corr.)	0.615783	6			

DETERMINATION OF CORRELATION COEFFICIENT

Correlation Coefficient = 0.958971

R-squared = 91.9625 percent

R-squared (adjusted for d.f.) = 90.355 percent

Standard Error of Est. = 0.0994924

Mean absolute error = 0.0729759

Durbin-Watson statistic = 1.15944 (P=0.0287)

Lag 1 residual autocorrelation = 0.250929

APPENDIX 16

Analysis of Variance with Lack-of-Fit

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	0.566289	1	0.566289	57.21	0.0006
Residual	0.0494936	5	0.00989873		
Lack-of-Fit	0.0494936	5	0.00989873		
Pure Error	0.0	0			
Total (Corr.)	0.615783	6			

APPENDIX 17

Comparison of Alternative Models

Model	Correlation	R-Squared
Squared-Y square root-X	0.9590	91.96%
Square root-X	0.9581	91.80%
Double square root	0.9577	91.72%
Logarithmic-Y square root-X	0.9573	91.64%
Reciprocal-Y square root-X	-0.9564	91.47%
Squared-Y	0.8380	70.23%
Linear	0.8362	69.92%
Square root-Y	0.8353	69.77%
Exponential	0.8344	69.62%
Reciprocal-Y	-0.8326	69.32%
Double squared	0.6778	45.94%
Squared-X	0.6755	45.62%
Square root-Y squared-X	0.6743	45.47%
Logarithmic-Y squared-X	0.6731	45.31%
Reciprocal-Y squared-X	-0.6708	45.00%
Logarithmic-X	<no fit>	
Square root-Y logarithmic-X	<no fit>	
Multiplicative	<no fit>	
Reciprocal-Y logarithmic-X	<no fit>	
Squared-Y logarithmic-X	<no fit>	
Reciprocal-X	<no fit>	
Square root-Y reciprocal-X	<no fit>	
Double reciprocal	<no fit>	
Squared-Y reciprocal-X	<no fit>	
Logistic	<no fit>	
Log probit	<no fit>	

Simple Regression – Shelf pH vs. Months

Dependent variable: Shelf pH

Independent variable: Months

Squared-Y square root-X: Y = sqrt(a + b*sqrt(X))

APPENDIX 18

Coefficients

	Least Squares	Standard	T	
Parameter	Estimate	Error	Statistic	P-Value
Intercept	14.5	0.0622139	233.067	0.0000
Slope	0.509954	0.0393475	12.9603	0.0002

APPENDIX 19

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	0.855424	1	0.855424	167.97	0.0002
Residual	0.020371	4	0.00509276		
Total (Corr.)	0.875795	5			

Correlation Coefficient = 0.988302
R-squared = 97.674 percent
R-squared (adjusted for d.f.) = 97.0925 percent
Standard Error of Est. = 0.0713636
Mean absolute error = 0.0419464
Durbin-Watson statistic = 2.6081 (P=0.6140)
Lag 1 residual autocorrelation = -0.427464

APPENDIX 20

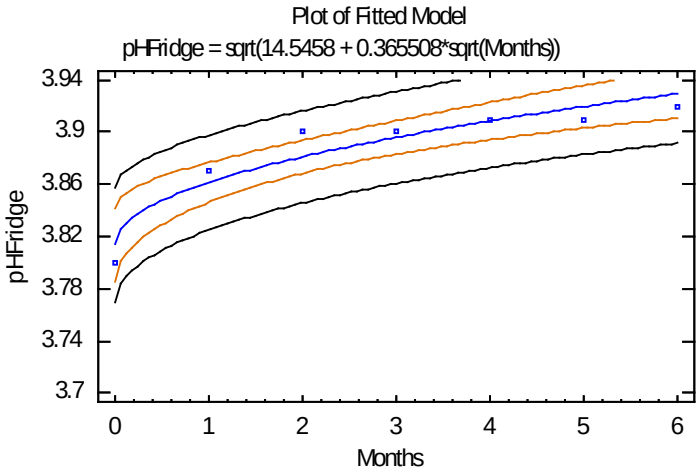
Predicted Values

		95.00%		95.00%	
	Predicted	Prediction	Limits	Confidence	Limits
X	Y	Lower	Upper	Lower	Upper
0.0	3.80789	3.77321	3.84225	3.78514	3.8305
6.0	3.96852	3.93779	3.99901	3.95077	3.98619

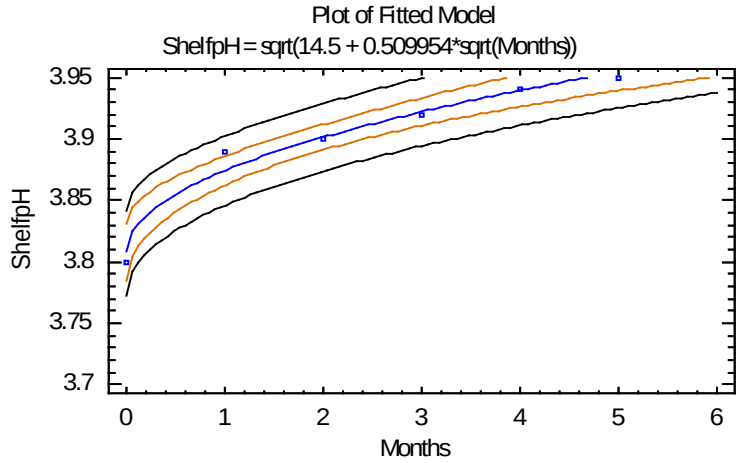
APPENDIX 21

Comparison of Alternative Models

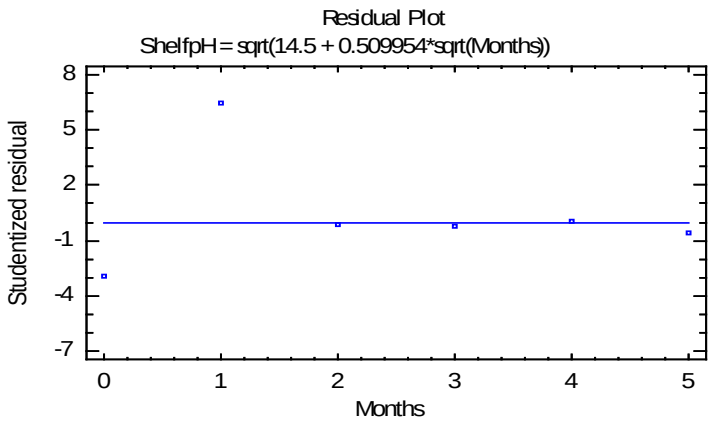
Model	Correlation	R-Squared
Squared-Y square root-X	0.9883	97.67%
Square root-X	0.9877	97.55%
Double square root	0.9873	97.48%
Logarithmic-Y square root-X	0.9870	97.42%
Reciprocal-Y square root-X	-0.9863	97.28%
Squared-Y	0.9123	83.24%
Linear	0.9100	82.82%
Square root-Y	0.9089	82.61%
Exponential	0.9077	82.40%
Reciprocal-Y	-0.9054	81.97%
Double squared	0.7859	61.76%
Squared-X	0.7824	61.21%
Square root-Y squared-X	0.7806	60.94%
Logarithmic-Y squared-X	0.7789	60.67%
Reciprocal-Y squared-X	-0.7754	60.12%
Logarithmic-X	<no fit>	
Square root-Y logarithmic-X	<no fit>	
Multiplicative	<no fit>	
Reciprocal-Y logarithmic-X	<no fit>	
Squared-Y logarithmic-X	<no fit>	
Reciprocal-X	<no fit>	
Square root-Y reciprocal-X	<no fit>	
S-curve model	<no fit>	
Double reciprocal	<no fit>	
Squared-Y reciprocal-X	<no fit>	
Logistic	<no fit>	
Log probit	<no fit>	



APPENDIX 22 **Plot Of Fitted Model**



APPENDIX 23 **Plot of Fitted Model**



APPENDIX 24 **Residual plot**

APPENDIX 25

SAMPLE CALCULATIONS

MOISTURE

Weight of crucible =

Weight of crucible + weight of wet sample =

Weight of crucible + weight of dried sample =

$$\text{Percentage Moisture} = \frac{\text{weight of sample} - \text{weight of dried sample} \times 100}{\text{Weight of wet sample} - \text{weight of crucible}}$$

FAT

Weight of flask =

Weight of flask + Sample =

Weight of flask + Fat =

$$\text{Percentage Fat} = \frac{\text{Weight of flask + Fat} - \text{Weight of Flask}}{\text{Weight of flask + Sample} - \text{Weight of crucible}}$$

PROTEIN

$$\text{Percentage Protein} = \frac{100(V_A - V_B) \times 0.01401 \times 100}{\text{Weight of Sample} \times 10}$$

ASH

Weight of crucible + weight of wet sample =

Weight of crucible + weight of ashed sample =

$$\%\ \text{Ash} = \frac{\text{weight of sample} - \text{weight of ashed sample} \times 100}{\text{Weight of wet sample + weight of crucible} - \text{weight of crucible}}$$

FIBRE

$$\%\ \text{Crude Fibre} = \frac{\text{Dry Residue Weight- Ignited Residue Weight} - \text{Blank Weight Loss}}{\text{Sample Weight}}$$

VITAMIN C

$$\text{Ascobic Acid (mg/100 ml)}$$

$$= \frac{(\text{Net ml Titrant for Sample})\,(\text{Ascorbic Acid Equivalent})}{(\text{Sample Volume})}$$

$$= \frac{(\text{Net ml Titrant for Sample})\,\dfrac{(\text{mg Ascorbic Acid in Stardand})}{(\text{Net ml Titrant for Standard})}}{(\text{ml Sample})} \times 100$$

$$= \frac{(\text{Net ml Titrant for Sample})\,\dfrac{(\text{ml Standard})(\text{mg/ml Standard})}{(\text{Net ml Titrant for Standard})}}{(\text{ml Sample})} \times 100$$

$$= \frac{(\text{Net ml Titrant for Sample})(\text{ml Standard})(\text{mg/ml Standard})}{(\text{Net ml Titrant for Standard})(\text{ml Sample})} \times 100$$

where

(Net ml Titrant for Sample) = (ml Titrant for Sample) – (ml Titrant for Blank)

(Net ml Titrant for Standard) = (ml Titrant for Standard) – (ml Titrant for Blank)

TOTAL SOLIDS

Weight of dish =

Weight of dish + sample =

Weight of dish +dried Sample =

$$\% \text{ Total Solids} = \frac{\text{Weight of dish + wet sample – weight of dish+ dried sample X 100}}{\text{Weight of dish}}$$

CARBOHYDRATE

Percentage carbohydrate = 100- (% protein+ % fat +% moisture + % fibre +% ash)

APPENDIX 26

SENSORY QUESTIONNAIRE

INSTRUCTION No:

You are provided with 9 samples. Please indicate your preference for each sample using the hedonic scale provided

Sample Code	Taste	Aroma	Flavour	Colour	After taste	Overall acceptance
01						
02						
03						
04						
05						
06						
07						
08						
09						

Like extremely (1) Like very much (2) like moderately (3)

Like slightly (4) Neither like nor dislike (5) Dislike slightly (6)

Dislike moderately (7) Dislike very much (8) Dislike extremely (9)

CONTENTS

LIST OF TABLES

LIST OF FIGURES