UNIVERSITY OF CAPE COAST

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BY

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A thesis submitted to the Department of Vocational and Technical Education of the Faculty of Science and Technology Education, College of Education Studies, University of Cape Coast, in partial fulfilment of the requirements for the award of Master of Philosophy Degree in Home Economics

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DECLARATION

Candidate's Declaration

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

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

Name: Diana Adebessah

Supervisors' Declaration

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

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

Name: Prof. (Mrs.) Sarah Darkwa

Co-Supervisor's Signature: ……..…… Date: ……..

Name: Dr. (Mrs.) Augusta Adjei- Frimpong

ABSTRACT

This study assessed the utilization of miracle berry (*Synsepalum dulcificum*) and its sensory evaluation with a focus on the students in the University of Cape Coast. The study adopted an experimental research design. Four research questions were set in finding a solution to the problem under study. The data collected were in two folds, fruits and sensory data from the panellists. Purposive sampling technique was employed in sampling the panellists from the University of Cape Coast Vocational and Technical Education Department. The sample size for the study was 25. Data from the proximate analysis and the sensory evaluation were analysed using frequency, percentage, mean, standard deviation, and one-way ANOVA. The results indicated that miraculin can be extracted using the depulper machine. The results revealed that there were eight mineral elements (Fe, Cu, Zn, K, Na, P, Ca & Mg) in the food taste enhancers. Miracle Natural Enhancer (MNE) has much quantity of the chemical elements compared to the other two taste enhancers. The results on tasting sour lemon with the formulated taste enhancers indicated that the most accepted taste enhancer was Miracle Natural Enhancer (MNE) followed by Splenda Artificial Enhancer (SAE) and Equal Artificial Enhancer (EAE). The study recommends that the miracle berry trees should be protected and mass production of MNE should be encouraged to create jobs for the unemployed.

KEYWORDS

Ash	- It is the burnt product from the food sample.
Constituents	- Component of a whole
Dry Matter	- Sample dried in the oven to remove the moisture.
Enhancer	- Something that enhances.
Mineral	- Food nutrient.
Miraculin	- Juice in the miracle berry.
Moist content	- Liquid found in a raw food sample.
Proximate analysis	- Scientific way of determining food nutrients

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DEDICATION

To my dear husband, Kofi Essien and my children, Nyamekye Essien, Nhyiraba Essien, Daniel Fiifi Essien and Daniella Kuukua Essien.

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LIST OF ACRONYMS

ANOVA	Analysis of Variance
DM	Dry Matter
EAE	Equal Artificial Enhancer
gm	grams
IBM	International Business Machine
mg	Milligram
MNE	Miracle Natural Enhancer
SAE	Splenda Artificial Enhancer
SPSS	Statistical Package of Social Science
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

Background to the Study

The term taste enhancer is used in the food industry to describe any substance that enhances the sensation of food (or food ingredients) when introduced into the mouth. The use of the term taste is conversational and actually refers to flavor (both taste and smell) because chemicals from food activate receptors in the nose as well as the mouth. Enhancement of the taste and smell of food is desirable to improve palatability, increase the total intensity, potentially reduce the cost of ingredients, and compensate for chemosensory (taste and smell) losses in vulnerable populations such as the elderly (Schiffman & Zoe, 1993).

Enhancement can be achieved in two ways: (1) by simply adding more molecules to the food or (2) by potentiating the intensity through synergism and/or alteration of receptor mechanisms without altering the total number of molecules. Many food ingredients, including monosodium glutamate (MSG), NaCl, and taste enhancers have been termed taste enhancers but their main effect is simply to add more molecules that generate additional taste or smell sensations. Tastants such as MSG, salt, and taste enhancers do not actually boost other chemosensory properties but rather contribute additional meaty/savory, salty, or sweet properties respectively (Schiffman & Zoe, 1993).

Taste enhancer of one kind or another had been found in human diets since prehistoric times. Terms such as sugar-free, sugar alcohols, sucrose, corn taste enhancers, etc. can be confusing (Fawibe, Ogunyale, Ajiboye & Agboola,

2014). Each of the taste enhancers available to consumers has specific applications and certain limitations. A variety of taste enhancers exists to help consumers satisfy their desire for sweetness or flavour. Taste enhancers are used in foods for several reasons, besides adding sweetness. Sugar is used as a preservative in jams and jellies; it provides body and texture in ice cream and baked goods; it aids in fermentation in bread and pickles (Birch & Gerard, 2000). Taste enhancers that supply energy (calories) are referred to as nutritive taste enhancers, even though they lack other nutrients essential for growth and health maintenance.

The miracle fruit (Synsepalum dulcificum) is an ever green indigenous to tropical West Africa. Synsepalum dulcificum is also known as miracle fruit, magic fruit, miraculous or flavour fruit (Duke & Ducellier, 1993). The shrub yields ripe red berries called “miracle fruit” that exhibit an interesting and remarkable taste-modifying property of altering sour flavours to sweet (Igarashi, Higuchi, Yamazaki, Ito, Ashida, & Miyaoka, 2013). The pioneering study on miracle fruit was proposed by Inglett et al (Inglett, Dowling, Albrecht & Hoglan, 1965) whilst looking for a natural sweetener to replace saccharin and cyclamate (Kurihara & Beidler, 1968)

Fruits are a key part of an overall healthy eating plan and it is common knowledge that fruits are man’s oldest food that provides other potential benefits (Kochhar, 1986). Fruits are very important in the tropics due to their high carbohydrates and vitamin contribution to diets. Most fruits contain large

quantities of sugar and are high in vitamins A, C, and B-complex, which are not abundant in foods of most areas of Africa (Rice, Rice & Tindal, 1993).

The fruit of Miracle berry, *Synsepalumdulcificum* was first brought to the attention of many people in the world in 1725 by a French adventurer, Des Marchais (Holloway et al., 1996). Marchais noticed that local tribes in West Africa picked the berry from shrubs and chewed it before meals. Traditionally, the fruits (berries) have been used in local cultures for centuries to improve the palatability of sour foods and drinks such as fermented palm wine, pito, kenkey, etc. The fruits are surrounded by edible pulp covering an elongated ovoid shape seed which changes colour into bright red upon ripening (Chen, Liu & Cheng, 2006).

Miraculin is a natural sugar substitute, a glycoprotein extracted from the fruit of Synsepalum dulcificum (Theerasilp & Kurihara, 1988). The berry, which contains active polyphenols, was first documented by explorer Chevalier des Marchais, who searched for many different fruits during a 1725 excursion to its native West Africa. The protein is a single polypeptide with 191 amino acid residues (Nirasawa, Nishino, Katahira, Uesugi & Hu, 2001). Miraculin itself is not sweet. However, after the taste buds are exposed to miraculin, ordinarily sour foods, such as citrus, are perceived as sweet. This effect lasts up to two hours. Thus, miraculin has the unusual property of modifying sour taste into a sweet taste (Temussi, 2002). Miraculin works by binding to the sweet receptors on the tongue. The Miraculin's effect lasts as long as the protein is bound to the tongue,

which can be up to two hours or more. It makes most acidic foods taste sweet and making bitter things taste sweet (Rowe, 2006).

The interesting facts about this fruit lie within the ability of its flesh pulp that is able to modify sour taste foods into sweet upon consumption (Yong, 2011). This unique effect was first documented in the scientific literature by Kurihara and Beidler (1968). In 1970 a biomedical postgraduate, Robert Harvey, came in touch with the miracle berries and formed the Miraculin Company. Scientific and commercial interest in miracle berry was re-awakened in the nineteen seventies as a result of a study released by the US Department of Agriculture on tropical plants with unusual taste properties and the survey drew attention to a number of sweet-tasting plants including miracle berry (Holloway, Akoto & Gbewonyo, 1996).

It is an evergreen plant that produces small red berries, with flowers that are white and which are produced for many months of the year. The seeds are about the size of coffee beans. The berry is sweet, and contains an active glycoprotein molecule, with some trailing carbohydrate chains, called miraculin. When the fleshy part of the fruit is eaten, this molecule binds to the tongue's taste buds, causing sour foods (such as lemons and limes) consumed after eating the miracle berry to taste sweet. This effect could last between 30 minutes to 120 minutes or more.

Miraculin is a glycoprotein (a protein that has a carbohydrate group attached to the polypeptide chain) that had been isolated as the potent component of the Miracle Fruit that alters taste perception by binding to sweet receptors on the tongue (Lipatova & Campolattaro, 2016). Miraculin is the largest known

macromolecule (Cagan, 1973; Kurihara, 1992) that can influence taste perception. The molecular weight of miraculin is 24,600 Da, which includes 86.1% polypeptide and 13.9% carbohydrate.

Presently, *Synsepalum dulcificum* had been treated as an important source of medicinal plant. It had been used to treat chronic diseases of patients who had typically developed blunt taste sensation due to various complications (Peregrin, 2009). There are no artificial taste enhancers proven absolutely safe, although, they are approved by the Food and Drug Authority (FDA). The sweet berry can be used as a natural food enhancer with hardly any ill side effects (Atuahene-Mensah, 2013). Artificial taste enhancers such as aspartame have been used often to alter individual sweet desire without any undesirable calories on blood sugar levels and health.

Nevertheless, the circumstances after the consumption of aspartame gave an unpleasant side effect. Miracle berry, on the other hand, eliminates the possibility of giving any negative side effects after consumption (Levin, 2012). This had been further supported by Bartoshuk (1974) in an experiment conducted for smell and taste in University of Florida Centre that no health risk was associated with the consumption of the miracle berry fruit (Bartoshuk, 1974).

The miracle berry is an economically promising fruit, which is attracting interest in both the domestic and international markets (Atuahene-Mensah, 2013). Based on encouraging results obtained from market studies on the commercialization of miracle berries for export, the Ghana Export Promotion Council (GEPC) in collaboration with the Agricultural Development Bank (ADB)

recently adopted the cultivation of miracle berries and planned to support the establishment of a commercial plant in the country to produce Miraculin (sweet proteins or taste-masking properties of the berries) for export to the US, Japan and other countries. The project is reported to have received considerable interest from a number of major Pacific Rim Companies including Mitsubishi Oil Company and Hasegawa and Kyowa Hakko all in Japan (BRI-Ghana Ltd., 2009).

Statement of the Problem

Miracle berry is a well-known fruit known to give sweetness to acidic and bitter foods. Holloway *et al.* (1996) projected that about 50,000 tons of the fruits would be produced in the next two decades to meet the expected export market demand. These projections indicate that miracle berry cultivation would increase significantly over the next few years to levels comparable to major export crops such as cocoa and coffee (Atuahene-Mensah, 2013).

This fruit had been used for medicinal purposes. It is believed to aid diabetic patients and also serve as a good source of daily sweetening in food manufacturing (Chen, Liu, & Cheng, 2006). Much research has been conducted on miracle berries as possible alternative for sugar substitute. High consumption of sugar has been associated with several negative health conditions and chronic diseases. Although, alternative taste enhancer had been extensively researched into (Rodrigues, Andrade, Bastos, Coelho & Pinheiro, 2016) but unfortunately, most of them are artificial taste enhancers. These enhancers have become indispensable ingredients in foods and even in medicines yet, there is still some controversy over the possible harmful effects of consumption of these artificial

taste enhancers. These increasing controversies call for the need for natural taste enhancers to be developed. This gap necessitates the need to carry out a study on the use of the Miraculin in the miracle berry fruit as a natural alternative taste enhancer to the artificial taste enhancer.

Purpose of the Study

The overarching purpose of the study was to examine the possibility of using miracle berries in the production of a natural taste enhancer for citrus and sour healthy food in the form of packaged powdered sachets.

Research Objectives

Specifically, the study sought to:

1. extract Miraculin from fresh miracle berries and process the Miraculin into a packaged powdered sachet.
2. evaluate the nutritive components of the miracle berry.
3. evaluate the perception of consumers in accepting miracle powder?
4. compare the Miraculin powder to other taste enhancers on sour foods.

Research Questions

1. What are the processes involved in the extraction of Miraculin into a packaged powdered sachet?
2. What are the nutritive components of the miracle berry?
3. What is the perception of consumers in accepting miracle powder?
4. Is packaged miraculin powder better at enhancing taste in sour things than artificial enhancers like Splenda and equal?

Significance of the Study

It is hoped that to stakeholders or duty bearers, the research literature in the area and methodology in processing miraculin into powdered sachets, as a natural taste enhancer will replace the artificial ones on the market. Although it is generally recognized as more of a novel food item, the outcome of the study will contribute to the health benefits associated with miracle berry use. It will also contribute to knowledge by bringing forth more insight into the reasons why miracle berries are used in the production of taste enhancers.

Delimitation

Miraculin juice would be extracted and packaged into sachets as natural food taste enhancers and its sensory evaluation conducted among students in the VOTEC Department of the University of Cape Coast. Acceptability of the packaged miracle berry would be compared to other artificial food taste enhancers on sour lemon juice.

Limitations

One of the largest obstacles lies in the miracle berry's availability. Miracle berry is not sold within a mass distribution retail chain such as grocery stores (Kant, 2005). The miracle berry fruits could be found in the Cocoa College of Bunso in the East Akim Municipality of the Eastern Region of Ghana in large quantity. There have been very few studies on the miracle berry hence, it was very difficult to access literature on it.

Miracle berry is a perishable fruit so transporting it after harvesting, from the farm in the Eastern Region to the Central Region for the study was very

difficult. Accessing the panelist from the University of Cape Coast was difficult since all the students were writing quizzes and examination towards the end of the semester. Despite the challenges, it was not envisaged that these could affect the result of the study.

Operational Definition of Terms

Miraculin - is the active component found within the thin-layered pulp of the miracle berry.

Taste Enhancer– the use of a mineral substitute to inhibit a pleasant taste of food item.

Organization of the Study

The study was organized into five chapters. Chapter Two discussed the literature review of the study. The literature focused on miracle berry, the geographical distribution of miracle berry, proximate analysis of the fruit and the empirical review of related literature. Chapter Three comprised of the methodology used in the Study, Chapter Four presents the result and discussions from the data collected and the Fifth Chapter presented the summary of findings, recommendations, and conclusions.

CHAPTER TWO

LITERATURE REVIEW

Introduction

This chapter discusses the Miracle Berry, the geographical distribution of the berry, miraculin mechanism of action, benefits of the Miracle berries, theoretical and empirical review of the Study.

Miracle Berry

Miracle berry is a fruit that is indigenous to the tropical countries of West Africa and stretches from Ghana to Congo in the Central Africa. The fruit is scientifically known as *Synsepalum dulcificum* and it transforms the taste of sour food and drinks into one of remarkable sweetness (Inglett, Dowling, Albrecht & Hoglan, 1965; Irvine, 1961). This taste-modifying sensation is due to a glycoprotein, fittingly named miraculin, found in the pulp of the miracle berry (Faus, 2000). Chewing miracle berry coats the tongue with miraculin. The combination of an acidic/sour food or drink of less than a pH of 7 along with miraculin activates the sweet taste receptors for an approximate period of one hour or more, lasting up to three hours in some cases (Cagan, 1973; Hellekant & van der Wel, 1989; Ito *et al.,* 2010).

Historically, the miracle berry was briefly mentioned in the literature by Chevalier des Marchais, a French explorer, who travelled to Guinea in 1725. However, it was an English physician and botanist William Freeman Daniell who provided the first thorough description of this tropical berry in 1852 (Daniell, 1852). While stationed as an army surgeon in the Gold Coast (now the country of

Ghana), Daniell encountered the "miraculous berry" and the West African natives who consumed it.

The berry was well known to the indigenous people as *assarbah, tanté, or agbayun* and was sold in local markets (Inglett *et al.*, 1965; Inglett & May, 1968). Daniell explained that in order to make some food more palatable, the natives often chewed the berry before eating strong, acidulated specialties such as kankies (sour cornbread) and drinking intensely sour palm wine and pitto (beer) (Bartoshuk *et al.*,1974; Inglett *et al.*, 1965; Inglett & May, 1968). More than a century passed before two research teams in Japan and the Netherlands independently isolated and purified the active substance that makes the berry unique: the glycoprotein miraculin (Kurihara & Beidler, 1968; Brouwer *et al.*, 1968; Kurihara & Terasaki, 1982).

Description of the Miracle Berry

Grown on a bush (Synsepalum dulcificum), the miracle berry is approximately the size (0.75 inches) and shape (ellipsoidal) of a Spanish peanut (Bartoshuk *et al.*, 1969; Hellekant *et al.*, 1985). It is comprised of a thin-layered pulp over a large seed (Inglett *et al.*, 1965). When ripe, the berry turns red likely on account of anthocyanins within the berry's flesh (Du *et al.*, 2014). It requires acidic soil (with a pH between 4.5 and 5.8) and frost-free growing conditions (Hiwasa-Tanasa *et al.*, 2012). When grown from seedlings, it takes three to four years before fruiting occurs; the bush grows slowly and eventually reaches six to fifteen feet in height when fully mature (Adansi, 1970).

Figure 1: Miracle Berry Fruits

Source: Field photo from Bunso Cocoa College, 2017

In 1919 the miracle berry was introduced into the United States by Fairchild (1931), founder of the Fairchild Tropical Gardens in Florida. Although it is nearly tasteless with a slight cherry-like flavor, the miracle berry alters the following taste of any sour- (essentially characterized as acidic) tasting food or drink into a perception of sweetness (Cagan, 1973; Inglett & Chen, 2011; Litt & Shiv, 2012). It modifies the overall flavor perception, for example, changing sour lemon juice into a sweet drink with a subtly altered lemon flavor. As previously stated, this taste-modifying function is due to the active substance found in the berry-miraculin.

Miraculin is the active component found within the thin-layered pulp of the miracle berry. Functioning as a taste-modifier, it is a glycoprotein consisting of 191 amino acid residues with two glycosylated polypeptides, Asn-42 and Asn-186, cross-linked by a disulfide bond (Theerasilp *et al.*, 1989; Theerasilp and Kurihara, 1988; Hiwasa-Tanasa *et al.*, 2012; Ito *et al.*, 2007; Matsuyama et al., 2009; Paladino *et al.*, 2008). A macromolecule with a molecular mass of 24,600,

miraculin is approximate “400,000 times sweeter than sucrose on a molar basis” (Theerasilp *et al.,* 1989; Temussi, 2006). It consists of up to 13.9% of sugars, specifically glucosamine, mannose, galactose, xylose, and fucose (Theerasilp and Kurihara, 1988; Chen *et al.,* 2009; Takahashi *et al.*, 1990).

Once activated by sour food or drink, miraculin displaces a portion of the acidity with sweetness. The effect is that miraculin modifies the overall flavor gustatory perception dramatically by reducing the sour acuity and augmenting the sweetness acuity, mimicking the effect of adding sugar to the acid (Diamant *et al.*, 1972; Hellekant & van der Wel, 1989). The natural aroma and taste of the sour food or drink remain to some degree (Bartoshuk *et al.,* 1974). It should be noted that the miracle berry does not modify bitter, salty, or other sweet tastes (Capitanio *et al.,* 2011; Kurihara, 1992; Igarashi *et al.*, 2013; Morris, 1976).

Additionally, miraculin is deactivated by heat and high or low pH values—below pH 2 and above pH 12 (Brouwer *et al.,* 1968; Cagan, 1973). Hellekant *et al.* (1976) reported that the potency of the miraculin-induced sweetness effect is contingent upon the concentration of the miraculin along with the type of acid consumed. For example, Igarashi *et al.* (2013) found that, in conjunction with miraculin, citric acid is perceived twice as sweet as acetic acid, all other factors being equal. Chen *et al.* (2010) described that the maximum sweetness intensity produced by miraculin is equivalent to 0.3 M of sucrose.

Concerning the commencement of the taste modifying effects of miraculin, the action starts a few seconds after consumption. In some cases, however, several minutes of chewing the berry’s pulp are necessary in order to

sufficiently, coat the taste buds. As far as the duration of the taste-modifying effect, the sweet sensation typically lasts thirty minutes to two hours until the miraculin is thoroughly diluted and dissociated by salivary amylase (Asakura *et al.*, 2011; Kurihara, 1992).

It should be noted that although the taste receptors require less than 0.1 mg of miraculin to induce a sweetening effect, the duration is dose-dependent (Brouwer *et al.*, 1983). Kurihara and Beidler (1969) demonstrated that the effect of a 2.3 µM solution of miraculin held in the mouth for five minutes lasted longer than 3 hours.

Geographical Distribution of Miracle Berry

Synsepalum dulcificum is a native to West Africa covering wide belts, which include, Serria Leone, Ivory Coast, Ghana, Togo, Nigeria, Cameroon, Gabon and Zaire (Holloway *et al.*, 1996). In Ghana, the shrub is found throughout the savannah to the peripheries of the forest zone. It grows mostly well on riverbanks, notably along the Volta River and its tributaries. Large populations have been sighted in the Aburi-Akwapim ridge, Nsawam, Bunso, Asamankese, Kibi, Esuasu, Anum, Mpraeso, Puru River bed, all of Eastern Region; Komenda in the Central Region; Kpando, Nkunya and Worawora in the Volta Region and Afram Mankrong and Ankasa in the Western Region (Holloway *et al.*, 1996).

The plant is also known to be grown as a sole crop in these regions. In the Brong–Ahafo, and Ashanti regions in Ghana where the plant is intercropped among many other crops (mixed cropping) there are but few (BRI-Ghana LTD., 2009).

Proximate Analysis of Miracle Berry

A study conducted in Togo on miracle fruit revealed that the fresh fruit consists of 23.74% skin, 35.45% pulp and 41.60% seed. The skin and the pulp are acidic (pH = 3.16-4.02). The skin, pulp, and seed contain varying proportions of proteins (15 - 22%), fats (2 - 12%) and carbohydrates (66 - 84%). Major minerals such as K, P, Ca, Na and Mg are present and traces of heavy metals such as Pb, Cu, Cd, and Ni have been noted (Agblekpe, Osseyi & Dossou, 2016). In a similar study, the proximate composition of S.ducificum contains 7.75% protein, 59.55% moisture content, 4.36% ash, 6.24% crude fiber, 3.26% fat and 18.84% carbohydrate (Ekwueme & Njoku, 2014).

Proximate analysis of Synsepalum dulcificum seed was investigated and results showed that it has moisture content (38.08%), crude protein (19.47%), crude fat (11.94%), total carbohydrate (29.08%), crude fiber (0.66%) and ash (1.43%). The mineral content was also determined. It contains 569.500 ± 2.820, 72.170 ± 5.340, 25.000 ± 0.000 and 17.630 ± 0.390 mg/100 g of Potassium, Calcium, Sodium, and Magnesium respectively (Jeremiah, Ilesanmi & Ig, 2015).

Constituents in Miraculin Berry

A study conducted to determine the physicochemical characteristics of the fruit it revealed that the miracle berry is a small oval shaped wild berry with an average weight of about 0.94 to 1.28 g and an average length of about 2.12 to 2.40cm (Agblekpe, Osseyi & Dossou, 2016). The proximate analysis of Synsepalum dulcificum seed was investigated and results showed that it has a moisture content of 38.08% (Olaitan, Olapade & Morakinyo, 2015)

Chemical Elements in Miraculin Berry

In a study done by Agblekpe *et al* (2016), it found the dry matter content of miraculin berry to be 19.6±3.21 Brix. The dry matter of the fruit is an indication that it has a dry status. In a similar study, essential acids were found in the fruit and these were in varying quantities. A large amount of amino acid found was leucine (2.35 g/100 g protein) and least amount of the acid found was methionine (0.31 g/100 g protein) (Njoku, Ubbaonu, Alagbaoso, Agunwa & Eluchie, 2016). The study further revealed that the nonessential amino acids were glutamic acid (3.43 g/100 g protein) being the highest and glycine (0.38 g/100 g protein). Oxidizable vitamins revealed included vitamin C (1.33 mg/100 g), vitamin A (2.54 µg) and vitamin E (0.78 mg/100 g). This result thus indicated that there are different vitamins and at varying degrees in the miracle fruit. The consumption of the fruit could help the body acquire the essential vitamins that the body needs.

In a study of Agblekpe *et al.* (2016), the result indicated that the seed of miracle berry contains varying proportions of proteins (15-22%), fats (2-12%) and carbohydrates (66 - 84%). The major minerals such as K, P, Ca, Na. Fe and Mg. Calcium found in the study shows that miracle berry is good for bone development in human, especially the growing children. Sodium is an essential element for the wellbeing of the entire body of a person that feeds on miracle fruit.

In a related study, different mineral elements were found in the miracle berry. The elements found were of different quantities. The elements and the

quantities found in the miracle berry were as follow: Ca (0.001±0.00) mg/100 g, Cr (0.0006±0.00) mg/100 g, Fe (0.0029±0.01) mg/100 g, Zn (0.0095±0.00) mg/100 g and Cu (0.00082±0.01) mg/100 g. The root samples were recorded thus; Ca (0.00134±0.01) mg/100 g, Cr (0.00073±0.01) mg/100 g, Zn (0.0097±0.01) mg/100 g, Fe (0.00025±0.01) mg/100 g and Cu (0.007±0.01) mg/100 g (Osabor, Etiuma & Ntinya, 2016).

The study of Osabor *et al.* (2016) also revealed that the leaf of *S. dulcificum* was rich in carbohydrate and moisture while the roots were rich in carbohydrate, moisture, and fiber. These mineral elements are believed to be in the fruit as well since the nutrients flow from the root system of the plant. The photosynthesis and nutrient absorbed by the plant have an influence on the fruit positively.

According to Olaitan, Olapade and Morakinyo (2015), the proximate analysis done on Synsepalum dulcificum revealed that it has crude protein (19.47%), crude fat (11.94%), total carbohydrate (29.08%), crude fiber (0.66%) and ash (1.43%). The mineral content was also determined. It contains 569.500 ± 2.820, 72.170 ± 5.340, 25.000 ± 0.000 and 17.630 ± 0.390 mg/100 g of Potassium, Calcium, Sodium and Magnesium respectively. Other mineral elements found (in mg/ 100 g) were Iron (3.050 ± 0.490), Zinc (2.710 ± 0.009), Copper (2.420 ± 0.008), Manganese (2.380 ± 0.004), Nickel (0.240 ± 0.028) and Cadmium (0.013 ± 0.002).

The proximate composition in the study of Nkwocha (2014) shows that S. dulcificum contains 7.75% protein, 59.55% moisture content, 4.36% ash, 6.24%

crude fiber, 3.26% fat and 18.84% carbohydrate. The result of the mineral analysis shows that S.dulcificum pulp contains 100ppm calcium, 24.20ppm iron, 9.49ppm zinc, 6.22ppm copper, 0.01ppm chromium and 0.01ppm cobalt. According to Nkwocha (2014), minerals like magnesium, potassium, sodium, manganese, and lead were not detected in the pulp. However, vitamin analyses showed that the S. dulcificum pulp contains 0.04% vitamin A, 22.69% vitamin C, 0.01% vitamin D and 0.02% vitamin K.

Taste enhancers from Natural Sources

Natural taste enhancers are extracted from natural products without any chemical modifications during the production or extraction process. Some of these taste enhancers have been in use for decades while others for centuries. Natural taste enhancers are well known and their production processes have been perfected over time making their cost low and leaving their demand high (Nkwocha, 2014).

Honey is a sweet food made by certain insects using nectar from flowers. The variety produced by honey bees are the one most commonly referred to and is the type of honey collected by beekeepers and consumed by humans. Honey produced by other bees and insects has distinctly different properties. Honeybees transform nectar into honey by a process of regurgitation and evaporation. They store it as a food source in wax honeycombs inside the beehive (National Honey Board (NHB), 2012). Honey gets its sweetness from the monosaccharides fructose and glucose and has approximately the same relative sweetness as that of granulated sugar (74% of the sweetness of sucrose, a disaccharide) (NHB, 2012).

Stevia is one of the newest taste enhancers available in the market. It has been known since 1899 for its sweet taste and has been cultivated in Japan since 1970. It was not until recently that a safe and successful extraction of glycosides (the chemical in the Stevia plant which gives it a sweet taste) allowed for the Food and Drug Administration (FDA) to approve Stevia as a general sweetener (Raji & Mohamed, 2012). Stevia is also known under different trade names as TruVia and PureVia patents by Coca Cola and Pepsi (Raji & Mohamed, 2012). Many different forms of Stevia as taste enhancers exist such as Reb A, B, C, D, Rebiana, Stevioside, SunCrystals, and Enliten. Each has a small variation in the manufacturing process or how it is used (Nkwocha, 2014).

Stevia is a natural sweetener because it is extracted from the Stevia plant and undergoes no chemical changes in the manufacturing process. This makes it very desirable to many consumers looking for healthy alternatives to sucrose sugar. Stevia is a general term referring to a plant, Steviarebaudiana (Bertoni), native to Paraguay (Nkwocha, 2014).

A new class of taste enhancers from proteins found in the fruits of tropical plants has been discovered. Natives of the areas where the plants producing these proteins grow naturally have frequently used them to sweeten their foodstuff. Synsepalum dulcificum is one of such plants. There is increased interest in natural taste enhancers which may be as a result of 'perceived' health risks of some artificial taste enhancers (WHO, 1999). The miracle fruit had been in use since the 18th century (Slater, 2007).

According to Achel (1996), there is an abundance of naturally occurring taste enhancers and/or sweetener-enhancers from plant sources. These may be proteins or other macromolecules and are much less hazardous. Achel (2016) hold the view that unlike the synthetic taste enhancers, protein taste enhancers are not known to disturb the balance of the amino acid pool in the body.

The taste of all known sweet proteins lingers on after stimulating the taste buds with them. This property of the sweet proteins suggests strong binding to the taste receptors on the tongue (Achel, 1996). The sweet proteins may therefore be used as suitable ligands to probe sweet taste perception. Being macromolecules, the sweet proteins possess more sites for labelling, without obstructing or influencing the sweetness domain, than smaller molecules. Isotope, photo-affinity and chemically labelled sweet proteins had been used to aid taste receptor identification and isolation with encouraging results (Ming, 1994; Morris & Cagan, 1972).

The use of protein mutants generated by recombinant DNA technology will provide an understanding of the interactive dynamics between sweet taste elicitors and taste receptors. Modification of the sweet proteins by recombinant DNA technology will also enhance the search for more desirable taste effectors as well as the design of new taste enhancers. The sweet proteins/protein sweetener enhancers according to Achel (1996) appear to be a key route to the proper understanding of the process of sweet taste signal transduction and the interaction of the mechanism with sweet taste receptors.

Six natural protein taste enhancers/sweetener-enhancers are known-thaumatin, monellin, brazzein, mabinlin, curculin, and miraculin (Sardesai & Waldshan, 1991). Their high sweetening potential, relative to sucrose, makes the plant protein taste enhancers a better alternative. Sucrose and other carbohydrate taste enhancers are required in relatively large amounts (Higginbotham & Hough, 1979; Crosby, 1976) and therefore constitute a source of high calorie in the food. Most of the plants according to Achel (1996) that possess sweetness-enhancing proteins are obtained from West Africa. Monellin, thaumatin, brazzein, and miraculin are found in the fruit pulp of *Dioscoreophyllum cuminsii, Thaumatococcus danieli, Pentadiplandra brazzeana* and *Richardella dulcifica* respectively (Kurihara, 1992). All four plants thrive well in Ghana but *R. dulcifica* also is known as the miracle fruit, appears to be the best adapted. In addition, amongst the list of protein taste enhancers/ sweetener enhancers, the protein sweetener found in *R. dulcifica,* miraculin, has unique characteristics both structurally and functionally. It is the only glycoprotein and taste modifier with no intrinsic sweetness (Kurihara, 1992; Crosby, 1976). In the view of Achel (1996), miraculin would probably have a wider market than the other protein taste enhancers for the reason that in addition to its sweetening ability, it can also be used to mask the sour taste of pharmaceutical products. Sweetening activity of the enhancer in the pulp of miracle fruits decreases with a period of storage. Investigations seem to suggest that the presence of proteolytic enzymes in the pulp of the fruit may be one of the plausible explanations for this observation (Crosby, 1976).

Miraculin's Mechanism of Action

Miraculin's specific mechanism of action remains an enigma (Gnanavel & Muthukumar, 2011; Ito *et al.,* 2007). Typically, macromolecules do not influence the taste or smell (Cagan, 1973). Anomalies exist, however, and miraculin became the first (and is still recognized as the largest) known macromolecule able to elicit a taste sensation (Kurihara, 1992; Ming, 1994).

Although speculative mechanisms have been proposed in the literature, what was known is that, miraculin binds tightly to the lingual epithelium's plasma membrane microvilli of the sweet-taste receptors (hT1R2-hT1R3) without activating them and is consequently experienced without flavor (Asakura *et al.,* 2011; Cagan, 1973; Misaka, 2013; Montmayeur & Mantsunami, 2002). It does not activate these receptors until subjected to an acidic pH, generally between pH 3.0 and 6.0 (Kurihara, 1992; Wong & Kern, 2011; Paladino *et al.,* 2010).

Kurihara and Beidler (1969) first proposed the theory that an acidic environment induces a dynamic conformational change to the shape of the molecule sufficiently to allow the carbohydrate portion of miraculin to stimulate the "sweet site". Thus, only when the pH decreases within the mouth-when acidic food or drink is consumed-the miraculin changes its structure and activates the sweet-taste receptors (Misaka, 2013; Picone & Temussia, 2012).

As previously mentioned, the acidity of the food or drink still exists, but it is significantly attenuated by the sweetness perception of the activated miraculin. Food or drink that does not have acidity, therefore, is not affected. One could liken this situation to a key and lock. It is as if a key (miraculin) does not fit all

the way into a lock (the sweetness receptors). However, once the key is exposed to acidity, it transforms its shape and fits perfectly. Once unlocked, a person experiences the perception of sweetness.

Misaka (2013) postulates that miraculin pivots between its function as an agonist and an antagonist dependent upon the pH value of the consumed food or drink. When a person treats the tongue with miraculin, it binds to the sweet-taste receptors and behaves as an agonist in an acidic environment. When the receptors detect a neutral pH, miraculin as an antagonist-inhibits the activation of the receptors. For a period of time (typically thirty minutes to two hours), miraculin has the ability to reactivate the sweet-taste receptors whenever an acidic pH is detected.

Another theory propounded by Dzendolet (1969) suggests that miraculin blocks the sour receptor sites allowing a sweet taste to be generated by the anionic group of an acid molecule. In addition, miraculin could be influencing the taste of acids primarily by causing the excitation of sites that usually mediate sweetness and not by causing any peripheral suppression of responses to acid (Bartoshuk *et al.*, 1969). Miraculin in the presence of acid adds sweetness while reducing sourness by mixture suppression (Danilova & Hellekant, 2005; Diamant *et al.*, 1972).

For a period of time, sour food or drink is perceived sweetly whether or not a person desires this for all that is ingested. As an example, when eating a mixed meal, a grapefruit would be very pleasant, but pickled vegetables, for instance, may not taste particularly appetizing with a sweet overtone. In fact,

many sour tastes are desirable (Breslin & Spector, 2009). After application of miraculin, for instance, a sour green apple may no longer taste refreshing; it may taste too—almost artificially sweet as reported in experiments conducted by Litt and Shiv (2012). Essentially, affecting the overall flavour may not always be enjoyable.

One of the largest obstacles lies in the miracle berry's availability. It not sold within a mass distribution retail chain (e.g., grocery stores) (Kant, 2005). As stated before, the miracle berry was only grown under specific conditions. It was not widely found in nature and not readily available to consumers at any time. Another weakness of the miracle berry was delicate to an extent. Miraculin is thermolabile and is inactivated below pH 3 and above pH 12 (Inglett *et al.,* 1965; Kurihara, 1992).

The protein backbone of miraculin is evidently important as proteolytic modification leads to a loss in activity (Swenberg & Henkin, 1975). While the deactivation of miraculin from intense pH values would not be a normal issue, the deactivation from heating can be more of a common problem: for instance, the miracle berry cannot be used in cooking or in processed foods. Moreover, the miracle berry is also delicate due to its short shelf life, and it goes bad in about two days (Witty, 1998). Despite this deficiency, potential preservation techniques are being researched-utilizing a coating of the polysaccharide chitosan (Liu *et al.*, 2011).

Currently, the miracle berry can be stored at -20° F for approximately three months before use without concern (Hellekant *et al.,* 1985). Miraculin faces

regulatory impedance from the U.S. Food and Drug Administration (FDA) and the European Union where it has not been yet legally recognized as a food additive. It, however, had been recognized by Japan's Ministry of Health and Welfare (Izawa *et al.*, 2010).

In the late 1960s, a Massachusetts-based company the Miralin Corporation was formed and established large-scale plantations of *Synsepalum dulcificum* in the West Indies and Brazil, developing new hybrids and propagation techniques (Tripp, 1985). They began tentatively to introduce an extract in powdered sachet form called miracle berry concentrate (MFC) consisting of a partially purified extract containing hydrolyzed cereal solids and a Miracle berry Drop (Dastoli & Harvey, 1974; Inglett, 1976).

Special diets and menus were developed incorporating MFC as an aid to reduce caloric intake. Despite fairly extensive toxicological evaluation and considerable investment (at least $5 million), the extract did not meet with the approval of the FDA who, in 1974, issued a regulatory letter requesting the company to cease "interstate shipments". The company was liquidated in 1976, and in May 1977, all products of Synsepalumdulcificum were finally denied food additive status (Gibbs, *et al.*, 1996). Sun *et al.* (2007) report "Bio-resources International, Inc. (Somerset, NJ, USA) is currently undertaking the commercial development of miraculin for use as a taste masking agent, low-calorie sweetener and flavor enhancer".

It should be recognized, however, that "there is a fundamental difference between miraculin and food additives because it is not necessary to add miraculin

to the food itself" (Bartoshuk *et al.*, 1974). Unlike the FDA, the U.S. Department of Agriculture (USDA) does not have any restrictions on the miracle berry. Growing, selling, and eating miracle berries in the United States is not illegal (Sun *et al.*, 2007).

Science has apparently limitless new avenues of research into, for example, the miracle berry's botany, horticulture, and miraculin's biochemistry, physiology, and chemical structure-taste relationships, among others. Nevertheless, the miracle berry and miraculin will ultimately stand or fail on the criteria of practicality and usefulness, however academically interesting it may be (Hiwasa-Tanasa *et al.,* 2012). Fortunately, there appears to be a variety of uses.

Health benefit of Lemon

Lemon has medicinal properties like anti-cancer activity, prevent kidney stones, bring down fever, balance pH, the plant contains Citra, limonene, terpineol, geranyl acetate and linalyl (Dev & Nidhi, 2016).

Lemon contains many vitamins (niacin, riboflavin, thiamine, choline, pantothenic acid, foliate, vitamin C, vitamin B6) and minerals (calcium, copper, iron, manganese, magnesium, phosphorus, potassium, zinc), which are needed for the human body. It should be stored at room temperature away from direct sunlight. Lemon is used as a home remedy for many people in India. However, it is imperative to mention that a person suffering from serious illness should seek the opinion of qualified physicians before self-medicating with lemon (Pal, 2017).

This fruit is said to reduce inflammation of joints by removing uric acid from joints. It has antibacterial and antiviral properties. It increases the absorption

of iron. Lemon soothes itching and alleviates rushes, reduces age spots, and cleanses the face. Lemon juice helps in the enzyme functions in our body stimulating the liver and flushing out toxins. Lemon juice relieves symptoms of indigestion such as bloating, burping and heartburn. It can reduce the effects of nausea, dizziness it has also been found beneficial in relieving chills, fever, headache, respiratory problems, arthritis, diphtheria, rheumatism, stress, diabetes, cholera, high blood pressure, heart diseases, indigestion, constipation, sore throat, internal bleeding, burns and obesity (Pal, 2017).

Limonene has anti-cancer effects and help increase the level of enzymes that detoxify carcinogens. Limes and lemons contain outstanding phytochemicals that are high in anti-oxidant and anti-cancer properties. The lime and lemon are potent detoxifiers with anti-biotic effect that is protective against bacterial poisoning (Compliments of Ferguson Family Chiropractic, n. d). Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities (Burt, 2004; Ortuno, Baidez, Gomez, Arcas, Porras & Rio, 2006). Flavonoids can function as direct antioxidants and free radical scavengers, and have the capacity to modulate enzymatic activities and inhibit cell proliferation (Duthie & Crozier, 2000).

The therapeutic value of Vinegar

Vinegar is one of the few acidic condiments. Based on their raw materials, vinegar can mainly be considered grain vinegar, which contains sorghum, rice, wheat, or other grains as the raw materials, or fruit vinegar, which are based on fruits such as grapes or apples as the raw materials (Chen, Chen, Giudici & Chen,

2016). In addition, vinegar can also be fermented from sugar and alcohol. For example, Shanxi aged vinegar, Zhenjiang aromatic vinegar, Sichuan Baoning bran vinegar, and Fujian Yongchun Monascus vinegar, which are the 4 major traditional kinds of vinegar in China. Kurosu is another common grain fermented vinegar in Japan. In contrast, Italian balsamic vinegar, Spanish Sherry vinegar, and American apple vinegar are fruit vinegar (Solieri & Giudici, 2009). Regardless of the raw materials, vinegar is known to have several physiological functions, especially those made by traditional techniques (Budak, Aykin, Seydim, Greene & Guzel-Seydim, 2014).

The blood glucose control, lipid metabolism regulation, and weight loss capabilities from vinegar are mainly due to acetic acid. Besides caffeoyl sophorose (inhibits disaccharidase) and ligustrazine (improves blood circulation), other functional ingredients present in vinegar provide certain health benefits as well. Regarding anticancer activities, several grain vinegars strongly inhibit the growth of some cancer cells in vivo or in vitro, but related functional ingredients remain largely unknown, except tryptophol in Japanese black soybean vinegar. Considering the discovering of various functional ingredients and clarifying their mechanisms, some vinegar could be functional foods or even medicines, depending on a number of proofs that demonstrate these constituents can cure chronic diseases such as diabetes or cardiovascular problems (Chen, Chen, Giudici & Chen, 2016).

In diets with high-glycemic indices, apple vinegar significantly reduce the postprandial blood glucose concentration and insulin response and increase

satiety. However, in low-glycemic diets, vinegars can only reduce the postprandial insulin response and do not significantly influence the blood glucose concentration, which may be because a lower blood glucose level cannot be achieved by the addition of vinegar after the consumption of a diet with a low-glycemic index (Johnston & Buller, 2005). Compared to healthy people, the intake of apple vinegar also increases the insulin sensitivity of patients with type 2 diabetes (Ebihara & Nakajima, 1988), and the intake of apple vinegar at bedtime can help patients with type 2 diabetes to control their fasting blood glucose concentration and prevent "diabetes mellitus dawn phenomenon" the next morning (White & Johnston, 2007).

Food flavour/Taste Enhancer

The sensory impression of flavor is due to the simultaneous stimulation of the human olfactory and chemical compounds in food products triggered taste systems. Although the overall flavor and, in consequence, the consumer acceptance of foods is strongly influenced by the interplay of aroma-active volatiles, taste-active non-volatiles and flavor modifiers enhancing or suppressing certain taste qualities. Flavor research in recent decades have focused mainly on aroma and taste compounds, rather than on compounds that are tasteless on their own, but show synergistic effects with basic taste compounds (Yamaguchi, 1967).

More than 40 years ago, the first flavor synergism was reported between the umami-like tasting monosodium L-glutamate (MSG), purine-5′-ribonucleotides and analogs of both groups (Kuninaka, 1967; Yamaguchi, Yoshikawa, Ikeda & Ninomiya, 1971; Yamaguchi, 1967). Systematic sensory

studies on the umami taste of binary mixtures of MSG and inosine5′-monophosphate (IMP) varying in the concentration ratio revealed an exponential increase in the umami-like taste intensity of MSG even when IMP (1–12% based on MSG) was added in low concentrations only (Yamaguchi, 1967).

Furthermore, guanosine-5′-monophosphate (GMP) was reported to be 2.3-fold more active than IMP, while monosodium aspartate possesses only ~7% of the efficacy of MSG (Yamaguchi *et al.*, 1971). These purine-5′-nucleotides, occurring in many savory foods such as meat, fish, other seafood and mushrooms, are widely used as ingredients to enhance the flavor and mouth-feel of culinary products, snacks, soups, sauces and seasonings.

Molecular-biological investigations have succeeded in confirming the synergistic effect of purine5′-ribonucleotides on the umami taste of MSG at the taste receptor level (Soldo, Blank & Hofmann, 2003). Human T1R1/T1R3 heterodimeric receptors, made up by coexpression of the-family-protein-coupled receptor T1R1 and the related taste specific receptor T1R3, were demonstrated to respond to the umami-type taste stimulus L-glutamate (Li, Staszewski, Xu, Durick, Zoller & Adler, 2002).

Flavour enhancers are substances that have no pronounced flavour or taste of their own but which bring out and improve the flavours in the foods to which they are added. Although salt has a distinctive taste of its own and is not classed as a food additive, it is, in fact, the most widely used flavour enhancer. The next best known is glutamic acid and its salts, most commonly found in the form of monosodium glutamate, which has been used for several centuries as a condiment

in savoury products. Glutamic acid is a normal constituent of all proteins, non-essential amino acid and present in the body (Sabah, n. d).

Empirical Review

A trial was done with oncology patients drawn from the Mount Sinai Medical Centre in Miami, Florida on miracle berry. The authors led a randomized crossover pilot study of 23 participants in order to determine if the miracle berry improves dysgeusia (Peregrin, 2009; Soares *et al.*, 2010). The miracle berries were obtained from a botanical garden in Miami and stored under a controlled temperature condition. Eighty-seven percent (87%) of the participants experienced dysgeusia and 78% experienced no taste at all. After using the miracle berry, 30% experienced improvements in taste.

Another pilot study conducted by Wilken and Satiroff (2012) with patients from the Nebraska oncology clinic. This crossover study consisted of randomly selected chemotherapy patients (n=8). Taste improvements were recorded for all participants after consumption of the miracle berry. Despite the positive results, larger confirmatory research was warranted due to the low sample sizes. Although the miracle berry may be important concerning its health benefits, it requires greater accessibility in the future.

Miracle berry demonstrated antidiabetic effect by decreasing plasma glucose levels while improving insulin sensitivity in an animal model (Chen *et al*, 2006). Compounds isolated from the stem of Synsepalum dulcificum have been shown to inhibit the proliferation of melanoma cells (Hong *et al*, 2011). Recent investigations have looked into the plant's ability to stimulate weight loss in

humans by Wong and Kern (2011). In their pilot study, 30% of cancer patients undergoing chemotherapy reported improved taste, but no change in weight, after consuming miracle berry for two weeks (Soares, *et al,* 2010). Similar findings of patient-reported improvements in taste another small study (Wilken & Satiroff, 2012). Large clinical trials are needed to confirm this effect.

Benefits of the Miracle Berries

Although it is generally recognized as more of a novel food item, the miracle berry may provide certain health benefits. Humans readily crave and ingest sweet-tasting foods, and miraculin may be a healthier alternative to some of the more traditional taste enhancers, such as table sugar sucrose (Breslin & Spector, 2008). Calorically negligible, 100µg of miraculin is sufficient to provide a long-lasting sweetening effect, and the active ingredient is present in only very low concentration in the miracle berry (Misaka, 2013).

The sweetness of miracle berries is found to be "400,000 times sweeter than sucrose on a molar basis", miraculin provides many times its own weight in sucrose-equivalent sweetness (Izawa *et al.,* 2010; Theerasilp *et al.,* 1989). Because miraculin can be used in minute amounts, it is not a contributing factor in tooth decay (Faus, 2000). The sweetening effect of miraculin could be useful in general, but particularly for chewing gums, mouthwashes, et cetera (Giroux & Henkin, 1974).

Additionally, miraculin has a very similar sweetening effect when compared with sucrose in controlled experiments (Brouwer *et al.,* 1983; Hellekant & van der Wel, 1985; Yamamoto *et al.,* 2006). Participants stated that they could

not detect a taste distinction between the two, and, unlike sucrose, miraculin neither induced a subsequent craving for sucrose nor triggered a demand for insulin (Wong & Kern, 2011; Gnanavel & Muthukumar, 2011).

A prospective study was conducted to demonstrate how miraculin could improve insulin sensitivity in rats. The results showed that there were statistical differences that existed among groups in terms of insulin sensitivity to miraculin. (Chen *et al.*, 2006). Consequently, people who suffer from obesity and diabetes may find miraculin very useful for limiting sugar intake (Kant, 2005). The consumption of miraculin would not increase or aggravate the amount of insulin in the person. In addition to its potential as an alternative sugar, the miracle berry may be a healthy fruit in its own right, mainly for its antioxidant properties.

A study published in 2011 examined the antioxidant properties of the miracle berry (Inglett & Chen, 2011). About flavonoid and phenolic content, the results suggested that the skin, pulp, and seed of the miracle berry exhibit valuable antioxidant activity. A study presented similar results but demonstrated that the highest concentrations of antioxidant-rich phytochemicals are found within the miracle berry's flesh. Even more intriguing is that the miracle berry contains substantially larger quantities of ascorbic acid and several significant (and relatively rare) phenolics when compared with other commonly known antioxidant-rich berries, such as, blueberries, blackberries, cranberries, red raspberries, and strawberries (Du *et al.,* 2014). Although the antioxidant properties of the miracle berry are notable, its potential for helping chemotherapy patients is of great significance.

The miracle berry can significantly benefit chemotherapy and radiation patients who often experience taste alterations (dysgeusia) or taste reductions (asgeusia). Spielman (1998) noted that because of ionizing radiation, there are changes in the salivary flow rate and in the composition of oral bacterial flora and turnover rate of taste cells. Serving as a flavour enhancer, it has the ability to restore the appetite of cancer patients whose chemotherapy treatments leave unpleasant, noxious metallic taste in the mouth for which no standard remedy exists (Peregrin, 2009).

Food aversions caused by uncommon or poor tastes are experienced in over 50 percent of chemotherapy patients (Berteretche, Dalix, Cesar d'Ornano, Bellisle, Khayat, Faurion, 2004); this may lead to unfavorable developments involving nutrient intakes, reaction to treatment, and general well-being (Wilken & Satiroff, 2012; Comeau, Epstein & Migas, 2001). The miracle berry with its glycoprotein, miraculin, is unique. It has the potential to improve health and modulate disease because of its flavour modifying property, namely, the conversion of sour to sweet.

The mechanism of action is still not well understood, and small subject sample size in clinical trials is a shortcoming. Nonetheless, its potential is promising as an alternative sugar, as an antioxidant, and for improving dysgeusia in chemotherapy patients. The miracle berry was limited by availability and perishability, so researchers are producing recombinant miraculin in transgenic plants, notably tomatoes. Further research into the mechanisms of action, its

potential uses, and production or availability is required. Interest is growing in the miracle berry and deservedly so, and its future holds great promise.

Food Taste Enhancers

There are a number of possible natural taste enhancers such as the leaves of the shrub; *Stevia rebaudiana* is used by the natives of Paraguay to sweeten tea and other foods. 'Glycyrrhizin', isolated from the root of the licorice plant Glycyrrhizaglabra remain the most promising alternative of protein taste enhancers in Africa (Inglett, 1971).

Miraculin from *Richardelladulcifica* (Synsepalum dulcificum), monellin from Dioscoreophyllumcumminsii and thaumatin from Thaumatococcusdaniellii (Inglett, 1971). All these three miraculin have been considered to be good and have been recommended for commercialization. This was an indication that using miraculin as a food enhancer would have many benefits for the consumers.

The extraction process of Miraculin from Miracle Berry

Miraculin is a taste-modifying protein isolated from miracle berry, the red berries of *Richadella dulcifica*, a shrub native to West Africa (Sun, Kataoka, Yano & Ezura, 2007). The extraction approach used to get miraculin *Synsepalum dulcificum* has several stages and Achel (1996) has identified that the fruit has to be crushed and prepared into crude and the necessary reagents added at varying temperatures to get the miraculin from the fruit.

The extraction of miraculin juice by Theerasilp and Kuriharas (1988) was done after the miracle berries were cultured in a greenhouse at Yokohama National University. Pulps of the fruits free from skin and seed were lyophilized

and used for the experiment. The lyophilized pulps were kept at -20^{0}C before use. The experiments of Theerasilp and Kurihara (1988) were carried out below 4^{0}C. Extraction of 20g of the lyophilized pulps was suspended in 200ml of water and homogenized for 2 min. The homogenate was centrifuged at 13,000 rpm for 30 min. The pink supernatant which had no sweet-inducing activity was discarded. The sediment was thoroughly washed with water and then extracted three times with 0.5M NaC1.

In each extraction of the miraculin juice, the sediment was homogenized for 2min in 120 ml of 0.5 M NaCl solution and the homogenate was centrifuged at 13,000 rpm for 30 min. The supernatant which showed high sweet-inducing activity were pooled. The pooled solution was colorless and its pH was 4. Ammonium Sulfate Fractionation-Ammonium sulfate fractionation was carried out by addition of solid ammonium sulfate to the pooled solution to bring about 50% saturation. The precipitate from the solution was collected by centrifugation at 13,000 rpm for 40 min and suspended in water. The solvent was replaced with 0.01 M KH_2P0_4-Na_2HP0_4 buffer (pH 6.8) by means of ultrafiltration using Amicon PM-10, and this solution was used for further purification. CM-Sepharose Zon-exchange Chromatography-The sample was applied to a column (1.3 X 60 cm, bed volume of 140 ml) of CM-Sepharose CL-4B (Pharmacia LKB Biotechnology Inc.) equilibrated with 0.01 M KH_2P04-Na_2HP04 buffer (pH 6.8). The column was eluted first with the phosphate buffer, and the adsorbed substances were eluted with a linear gradient of NaCl (0-1.0 M) in the buffer (Theerasilp & Kuriharas, 1988).

Extraction from the leaf of *Synsepalum dulcificum* (Sapotaceae) to identify the phytochemicals and in vitro antioxidant activity in the plant has shown the presence of flavonoids, saponins, terpenoids and cardiac glycosides in the extract (Obafemi1, Akinmoladun, Olaleye, Onasanya, Komolafe, Falode, Boligon & Athayde, 2017). In addition, according to the results, there were appreciable levels of potassium, calcium, sodium, and magnesium in the extract. The IC_{50} of the extract for 2,2-diphenyl-1-picrylhydrazyl (DPPH), Nitric oxide (NO), hydroxyl radical (OH-) and ABTS·+ radicals scavenging assays were 139.45µg/ml, 119.17µg/ml, 147.65µg/ml, and 135.83µg/ml respectively (Obafemi1, *et al.,* 2017). The result as found has revealed the potential that exists in the plant. Apart from the sweetening property of the fruit, its leaves had other phytochemicals properties.

A study conducted to know the actual content and combination of the chemical elements in the miracle berry has employed an extraction method such as visual observation, chemical analyses, and atomic absorption spectrophotometry. The result from the study revealed that miracle berry is a small oval shaped wild berry with an average weight of about 0.94 to 1.28 g and an average length of about 2.12 to 2.40 cm (Agblekpe, Osseyi & Dossou, 2016). In addition, the fresh fruit according to Agblekpe *et al* (2016) consists of 23.74% skin, 35.45% pulp and 41.60% seed. The skin and the pulp are acidic (pH = 3.16-4.02). The skin, pulp and seed contain varying proportions of proteins (15-22%), fats (2 - 12%) and carbohydrates (66 - 84%).

A similar study to extract the pulp from *Synsepalum Dulcificum* was done by scraping the fruits with clean stainless spatula. The pulp was then analysed and it indicated that it possessed a moisture content of 45.12%, protein content of 2.48% and a carbohydrate content of 48.84%.The anti-nutrients content include Tannin 2.90 ± 0.64mg/100g, phytate 5.21 ± 0.92 mg/100g, glycosidic cyanide 0.03 ± 0.00mg/100g, steroid 1.56±0.03 mg/100g and oxalate 11.04 ± 0.29% (Njoku, Ubbaonu, Alagbaoso, Agunwa & Eluchie, 2016). Native miraculin was purified from the pulp of *R. dulcifica* according to a described method by Theerasilp and Kurihara (1988).

Flavour Perception

According to Hudson (2011), flavour is a complex sensation used to describe foods and beverages. Until recently, the understanding of the mechanism behind flavour perception was poorly understood. The term flavour is defined as the integration of tastes and retro nasal olfaction, which is the perception of odorants in the mouth (Rozin, 1982). Additional influences are from ortho-nasal olfaction (perception of sniffing odorants through the nose), the trigeminal system, tactile sensations, as well as by appearance (Rozin 1982; Auvray & Spence 2008). These attributes according to Hudson (2011) suggest that flavour perception is derived from multiple sensory systems, primarily the gustatory and olfactory systems that are dually responsible for the taste-odour integration (Dalton, Doolittle, Nagata & Breslin, 2000; Small & Prescott, 2005). During mastication, the food matrix breaks down in the mouth and on the tongue. This

change in texture releases additional odorants in the mouth, which are perceived retro-nasally (Hudson, 2011).

The perception of the maximal flavour intensity was found to occur close to the moment of swallowing near the border of the back of the tongue and soft palate (Buettner, Beer, Hannig, Settles & Schieberle, 2002). The flavour of food can be altered (usually enhanced) by the addition of natural or artificial odour/flavour chemicals, as well as taste stimuli. Usually, harsh tastes (bitter and sour) tend to suppress while pleasant tastes (sweet and salty) generally enhance the flavour (Lawless & Heymann, 1999). The interactions change depending on the various taste and odorant combinations.

Human perception about the intensity of a menthol flavour was driven by the release of sugars in their mouths, which are detected by the tongue and gustatory system (Davidson, Linforth, Hollowood & Taylor, 1999). There is a belief that sweet taste, and perhaps other tastes and trigeminal senses, plays an important role in retro nasal odor perception (Hudson, 2011). It is therefore beneficial to understand the anatomical and physiological processes of odour and taste systems, as well as their interactions.

Odour Perception

The human olfactory system is a dual sensory system used to perceive odour and aroma molecules in the external, outside world and in the mouth (Rozin, 1982). There are two major pathways termed ortho-nasal and retro nasal olfaction. The initial mode of olfactory delivery is engaged through ortho-nasal olfaction, which is perceived through the nasal passage by the process of sniffing

through the nostrils (Lawless & Heymann 1999). This moves odorants from the external air through the nasal passage to the olfactory epithelium. When food enters the mouth and is broken down by mastication, the release of higher concentrations of odour molecules in the back of the throat is perceived as retro-nasal olfaction (Lawless & Heymann, 1999; Buettner *et al.*, 2002).

More than 7,100 volatile compounds, which may contribute to odour perception, have been identified in foods (Reineccius, 2006). There is a strong association between odour and flavour, and this is the key process responsible for flavour perception (Bachmanov & Beauchamp, 2007). Olfactory receptors are true nerve cells that are located in the nasal cavity on the olfactory epithelium (Lawless & Heymann, 1999). They are highly ciliated, which allows for increased surface area, exposing maximum receptors to chemical stimuli. Thousands of receptors send nerve fibers into glomerular structures in the olfactory bulb. There are many areas of branching and synaptic contact onto the next neurons, which undergo transduction to the brain to transmit smells, emotions, and experiences (Lawless & Heymann, 1999).

Primarily, olfactory sensations are linked when substances are sensed in the mouth via retro-nasal olfaction. Due to this association, the olfactory system is often confused with the sense of taste. This is a good explanation for when an individual experience a head cold; the loss of retro-nasal olfactory inputs causes the perception of foods to change to little or no flavour. Odours also have the ability to modify taste sensations. Although odour molecules are typically tasteless when experienced alone in a solution, the addition of food odours that

are typically associated with sweet taste such as vanilla, caramel, strawberry, and mint to solutions can enhance the sweetness of foods (Dalton *et al.,* 2000; Small & Prescott, 2005; Auvray & Spence, 2008).

Taste Perception

Gustation or the perception of taste refers to the sensations arising from the oral cavity including on the tongue and in the mouth in the chemosensory gustation system. There are four known and widely accepted basic taste qualities called sweet, salty, sour, and bitter, and there is a fifth debated taste termed umami (Bellisle, 1999; Beauchamp, 2009). There is a tendency to use the term taste to refer to all mouth sensations, but it should be used only for the taste qualities and substances that produce those sensations. Taste can also evoke other sensations such as odour, touch, temperature, and irritation although non-gustatory components are sensed by different systems (Lawless & Heymann, 1999).

The epithelial surface of the tongue contains numerous papillae. There are different types of taste papillae located on the tongue and in the mouth, which are primarily classified as fungiform, foliate, and vallate (Lawless & Heymann, 1999). In addition, there is also some evidence that there are taste buds in the palate, oropharynx, larynx, epiglottis, and upper esophagus (Bachmanov & Beauchamp, 2007). Taste papillae contain clusters of epithelial cells, or taste buds, within them that have a lifespan of approximately one week and are continuously regenerated. These taste buds contain taste receptor cells. Some of these cells terminate in slender microvilli (the sites of interaction between

stimulus and receptor). Taste stimuli reach the taste bud through a taste pore and make contact with the receptor sites. After processing within the taste bud, messages are generated and carried by the cranial nerves-VII (facial), IX (glossopharyngeal), and X (vagus) (Lawless & Heymann, 1999).

Further processing in the brain results in the generation of behavioural responses to the taste stimuli. These responses result in the perception of the different aspects of taste: quality, intensity, hedonics, location, and persistence. There are numerous differences in taste perception in various individuals, especially as individual ages. For example, women who are experiencing menopause experience a diminished bitter sensation that leads to increased preference and intake for bitter foods and beverages (Beauchamp & Bartoshuk, 1997)

Determining Taste Status

Recent literature suggests that there are substantial taste sensitivity differences among individuals– especially with regard to bitter compounds. The first discovery in the differences in bitter taste perceptions was by an accidental tasting of phenylthiocarbamide (PTC) in 1931 by A.L. Fox (Fox, 1932). Some individuals thought it was tasteless while others thought it was strongly bitter, which led to the understanding that the ability to taste was inherited. We now know that there are 25 bitter genes in humans including TAS2R38 (Duffy, Davidson, Kidd, Kidd, Speed, Pakstis, Reed, Snyder & Bartoshuk, 2004). This gene expresses receptors that bind PTC which contain an N-C=S group.

Testing PTC can be used to determine taste sensitivity. Since it emits a sulfurous odour and is potentially toxic, it was replaced by 6-n-propylthiouracil (PROP) which also contains an N-C=S group (Lawless, 1980). This is used as an anti-thyroid agent and used to treat hyperthyroidism. PROP can present problems for some susceptible individuals. One can test genetic variation with quinine, which does not contain the N-C=S group but also exhibits bitter qualities. Commonly found in tonic water, it is also useful as an anti-malaria agent.

A quinine-water solution is applied to the tongue and mouth to be used as an indirect method for assessing taste status. Early taste status research used category scales to assess taste sensitivity. The major problem with these types of scales is that a particular attribute described as 'weak' by one individual may be actually 'strong' to another (Bartoshuk *et al.,* 2004). This is not a useful scale to measure actual intensities since individuals can be classified into one of the following taster status groups: supertasters, medium tasters, and non-tasters. Supertasters perceive the most intense sensations while non-tasters perceive the least. Taster status also influences the perceived intensity of other taste stimuli and retro-nasal olfaction. Therefore, there is an association between taste input and retro-nasal olfaction such as increased taste intensities. Again, it is suggested that supertasters perceive more intense retro-nasal cues than non-tasters.

In addition to bitter taste, it has been shown that supertasters tend to perceive higher intensities for the other four taste qualities than medium and non-tasters. For example, the perception of sucrose is sweeter and has a higher intensity for supertasters than non-tasters (Bartoshuk & Duffy, 1978).

It is generally assumed that the sense of taste can differentiate five primary sensory qualities (sweet, sour, salty, bitter and umami). However, a sixth sensory quality has recently been proposed regarding the ability to taste fatty acids (Mattes, 2011). Each taste quality was considered to represent different nutritional or physiological requirements, or indicate a potential dietary risk (Roper & Chaudhari, 2017). Sweet, salty, and umami are associated with specific classes of nutrients and are perceived as pleasant at low and moderate concentrations, but are avoided at high concentrations (Reed, Tanaka & McDaniel, 2006). On the contrary, stimuli categorized as bitter and sour are associated with compounds that are potentially harmful and are generally regarded as innate aversions.

A sour taste allows acid detection (i.e., free protons or organic acids) and is therefore important to avoid ingesting acids in excess and overloading the mechanisms that maintain the body's pH. Sour is also used to maintain electrolytic balance in humans. The bitter taste was thought to guard against consuming poisons, noxious substances, or toxins, many of which taste bitter to humans (Roper & Chaudhari, 2017). The various taste qualities act synergistically to arrange appetitive responses to energy - and protein-rich food sources (sweet, fatty acids, and umami), control intake of an adequate amount of sodium (low-salt taste), and warn against the ingestion of toxic substances or excess salt (bitter, sour, and high-salt tastes) (Roper & Chaudhari, 2017).

In addition, gustatory information gives us the possibility to make a choice among different foods and choose the most appropriate, depending on the nutritional needs of the moment. Taste perception occurs when water-soluble

chemicals in the mouth contact the epithelial cells of the taste buds (Roper & Chaudhari, 2017). Perception of the different taste qualities is mediated by diverse mechanisms, which are located in the cells belonging to three functional classes (Roper & Chaudhari, 2017). Heterodimer G-protein-coupled receptors (GPCRs) mediate the sweet and umami transduction:

Taste receptor type 1, member 2 (T1R2) + T1R3 sweet (Zuker, Ryba, Nelson, Hoon, Chandrashekar & Zhang, 2009; Jiang, Ji, Liu, Snyder, Benard, Margolskee & Max, 2004; Xu, Staszewski, Tang, Adler, Zoller & Li 2004) and T1R1 + T1R3 umami (Li *et al.* 2002; Zuker, *et al.* 2009), although other candidate receptors for sweet and umami may exist (Damak, Rong, Yasumatsu, Kokrashvili, Varadarajan, Zou, Jiang, Ninomiya & Margolskee, 2003; Maruyama, Pereira, Margolskee, Chaudhari & Roper, 2006; Yasumatsu, Horio, Murata, Shirosaki, Ohkuri, Yoshida & Ninomiya, 2009). The family of GPCRs T2Rs, respond to a diversity of bitter taste molecules (Chandrashekar, Mueller, Hoon, Adler, Feng, Guo, Zuker & Ryba, 2000; Mueller, Hoon, Erlenbach, Chandrashekar, Zuker, Ryba, 2005; Meyerhof, Batram, Kuhn, Brockhoff, Chudoba, Bufe, Appendino & Behrens, 2010).

Taste perception varies greatly among individuals, strongly influencing food preferences and selection, and therefore nutritional status and health (Tepper, 2008). Although the individual differences in taste-related behaviors concern all taste qualities, in the last decades, the genetic predisposition to perceive the bitter taste of 6-n-propylthiouracil (PROP) has gained considerable attention as a prototypical taste stimulus and an oral marker of food preferences and eating

behavior that has an impact on body composition and health (Tepper, 2008). This assumption is based on data showing that individuals who perceive PROP as more bitter (super-tasters), compared with those who detect PROP only at high concentration or not at all (non-tasters), are also more responsive to various oral stimuli, including other bitter-tasting compounds (Bartoshuk, Duffy, Lucchina, Prutkin & Fast, 1998).

Methods of Sensory Evaluation

When humans communicate sensory experiences, it is difficult to describe their perceived sensations without a common domain or terminology usage (Hudson, 2011). Attempts to quantify sensations by applying numerical values led to the development of scales by psychologists and psychophysicists (Lawless & Heymann, 1999). These scaling techniques incorporate intensity descriptors, which are used as anchors in many psychophysical scales to quantify the perceived sensation. Scaling is particularly useful for measuring the intensity of tastes and smells in foods.

According to Hudson (2011), the older tradition of sensory evaluation depends on scales of various types labelled with adjective/adverb intensity descriptors. Most commonly, category and some labelled scales are very basic and can include the terms ‘weak’, ‘medium’, and ‘very strong (Lawless & Heymann, 1999). The newer tradition strays from these descriptors and focuses more on direct scaling methods. Direct scaling methods focus on ratio properties that originated with magnitude estimation, which are the basis of magnitude matching and hybrid labelled/ratio scales (Jones, Peryam & Thurstone, 1955).

These methods derive primarily from the work of S.S. Stevens, who significantly advanced scientific taste studies.

Since humans cannot share experiences, direct comparisons of sensory or hedonic (likeability) perceived intensities across individuals is difficult. Numerous studies have proven that the strongest taste experienced varies genetically while the strongest pain varies with experience (Bartoshuk *et al.,* 2004). For example, there are definite gender differences for the strongest pain since only women experience childbirth. In order to make indirect comparisons among varying experiences, it is necessary to identify a standard that is assumed to be equal for everyone.

Recent advanced scaling techniques such as the general Labelled Magnitude Scale (gLMS) attempt to solve this issue (Hudson, 2011). This scale allows for comparisons among groups of individuals (e.g., sex, age, race, clinical status, genetic status) (Bartoshuk *et al.,* 2004). The gLMS shows great use for taste research and other fields of study, especially since recent studies show genetic variation in taste.

Consumers Acceptability of Miracle Powder

The overall sensory experience of eating any food is influence by a combination of the five senses including hearing, sight, touch, taste, and smell (Lawless & Heymann 1999). Taste, or gustation, is the perception of basic taste qualities on the tongue. Smell, or olfaction, is the perception of odour molecules by a dual process olfactory system in the nasal cavity (Hudson, 2011).

Ortho-nasal olfaction results when these volatiles are sniffed through the nostrils. When the food undergoes mastication, which breaks down the food matrix, the release of this volatiles in the back of the mouth and throat results in retro nasal olfaction. These volatiles are the odours that are responsible for the overall flavour character (Bachmanov & Beauchamp, 2007). The combinations of taste and retro nasal olfaction produce flavour. Recent literature suggests that some individuals experience more intense taste perceptions than others based on their taste genetics and the number of taste buds, which may influence flavour (Bartoshuk, Duffy & Miller, 1994).

Taste enhancers are able to replace sugar totally or partially, but are rarely as satisfying as the full-sugar alternative because they fail to trigger physiological satiety mechanisms (Raben, Vasilaras, Moller & Astrup, 2002; Swithers, Martin & Davidson, 2010). Unlike sucrose, most taste enhancers exhibit undesirable off-tastes as concentration increases, shifting from pleasant (sweet) towards unpleasant (bitter/metallic) (Riera, Vogel, Simon & le Coutre, 2007), and are also associated with severe side effects, including psychological problems, mental disorders, bladder cancer, heart failure and brain tumors (Kant, 2005; Sun, Cui, Ma & Ezura, 2006).

Studies involving chimeric receptors, mutagenesis, and molecular modelling have revealed that the GPCRs are very susceptible to allosteric modulation (Beltramo, Dörong & Londner, 2018). By screening synthetic chemical libraries, PAMs has been identified for several members of the metabotropic glutamate receptor and GABA receptor (Koizumi *et al.,* 2011).

Every synthetic PAM identified for these receptors binds to the TDM to enhance activity and sometimes increases the affinity of the natural ligand (glutamate, aminobutyric acid or calcium) within the VFT. While taste enhancers bind near the hinge region to trigger the initial closure of the VFT, enhancers bind near the opening of the pocket and stabilize the closed conformation by strengthening the hydrophobic interactions between the two lobes and lowering entropic penalties of lobe closure. Upon binding to the receptors, PAMs exhibit little or no intrinsic agonist activity on their own or act both as agonists on their own and as enhancers for the endogenous agonists.

In order to improve the taste of HP taste enhancers, PAMs may bind to the sweetener receptor without activating it but do so in a manner that they cause carbohydrate taste enhancers such as sucrose, fructose, and glucose to bind with higher affinity. These molecules would be enhancers or PAMs of the sweetener receptor. As they possess no sweetness activity, formulations associating carbohydrate taste enhancers with PAMs should accurately replicate carbohydrate sweetener taste (Servant, Tachdjian, Li, & Karanewsky, 2011; DuBois & Prakash, 2012).

An evaluation of miracle berry has established that miracle berry has a sensory profile, which is similar to that of sucralose, an established and recognized sugar substitute (Rodrigues, Andrade, Bastos, Coelho & Pinheiro, 2016). With the similarity in the two food taste enhancers as same as sucralose, it would not be strange if consumers like miracle berry too. What matters is the food in which it is applied to taste sweet. Other situations may arise that the consumer

may reject miracle berry when it is put in food in his/her presence. Human beings in nature are resistant to change and taste to miracle sweetener.

Lipatova and Campolattaro (2016) to determine the sensation of miracle berry carried taste sensation study among students. The study used 19 undergraduate students who enrolled in a Sensation and Perception course at Christopher Newport University (CNU). The result revealed that one of the respondents did not experience any change in taste perception following consumption of the Miracle berry. The Miracle berry did not alter the perception of salty or bitter tastes. Paired t-tests analysis according to the study revealed that the bitterness rating of broccoli and saltiness rating of the Goldfish crackers did not change after the berry was consumed ($P > 0.05$). The perceived sweetness for each acidic food item (lemon, grapefruit, lime, sour candy and cider vinegar) significantly increased after the berry was eaten ($P < 0.01$), whereas sweetness perception of bitter (broccoli), salty (Goldfish crackers) and sweet (jellybean) tastes did not change at $p > 0.05$ (Lipatova & Campolattaro, 2016).

Differences in Miraculin Powder, Splenda, and Equal

Miraculin is a protein that does not taste sweet by itself but modifies taste receptors to make sour things taste sweet temporarily. Miraculin was first sequenced in 1989 and was found to be a glycoprotein consisting of 191 amino acids and some carbohydrate chains. Miraculin occurs as a tetramer, a combination of 4 monomers group by dimer. Within each dimer two miraculin glycoproteins are linked by a disulfide bridge (Kakhia, n. d.).

Miraculin powdered sachets have been developed into low-calorie cakes containing citric acid that can be eaten after using miracle berry/miraculin, presumably since (part of) the taste of citric acid will be perceived as sweet (Shimamura & Lin, 2007). There is a fundamental difference between miraculin and food additives because it is not necessary to add miraculin to the food itself. Hence, the amount of miraculin used may be low because you take a certain amount of miraculin and then everything tastes sweeter (in contrast with additives in which the amount you ingest is proportional to the amount of food product you eat) (Bartoshuk, 1974)

Miraculin could be used to make food taste better/sweeter though the food might not be palatable. In addition, miraculin is potential for patients who were forced to eat foods that were not palatable (Bartoshuk, 1974). Artificial food taste enhancer like Splenda is an odourless, white crystalline powder that was derived from two amino acids aspartic acid and phenylalanine. It is about 200 times as sweet as sugar and can be used as a tabletop sweetener or in frozen desserts, gelatins, beverages, and chewing gum. When cooked or stored at high temperature, Splenda breaks down into its constituent amino acids (Kakhia, n. d.). According to Kakhia (n. d.), Splenda is currently one of the most popular artificial enhancers (sweeteners) used in the food industry in the U. S.

CHAPTER THREE

RESEARCH METHODS

This chapter describes the study areas as well as information on the community where the fieldwork was carried out. It also outlines the research methodology and the procedures adopted for the laboratory work, management and analysis of data.

Research Design

The study adopted an experimental research design to investigate the utilization of miracle berries by students at the University of Cape Coast. The adopted design was appropriate because the experimental materials would be manipulated to see the different influence of miraculin powder as taste enhancer on consumers. The sample materials for the study were also homogeneous. Hence, they could be manipulated without any biases. Although there are disadvantages to this design, the advantages outweigh the disadvantages. The advantages of the completely randomized design are that there is complete flexibility in this design as to the number of treatments and replications that could be experimented on. In addition, the whole experimental material could be utilized if the need be.

The primary aim of adopting experimental design was to establish the correlation between the variables ‘miracle berries’ and ‘production of taste enhancer. The study also adopted a mixed method of data collection to explore much about the use of miracle berries in powdered form. The methodology applied involved philosophical assumptions that guide the direction of the

collection and analysis of data and a mixture of qualitative and quantitative approaches in research (Creswell, Hanson, & Clark-Plano & Morales 2007). The choice of a qualitative approach employed to aid in getting insight into the level of knowledge and perceptions of people on the use of miracle berry among the students in the Cape Coast University.

According to Creswell, *et al.,* 2007 some level of qualitative research methods would be employed to provide the researchers with knowledge of what people think about the subject and what makes their thinking differ to other people's thoughts. Therefore, learning how and why people preserve miracle berry in ever-changing world makes application of some level of qualitative research appropriate for the study. In addition, a sensory evaluation of the powdered miraculin was conducted by making use of a quantitative approach to qualitative data. As documented by Marsland, Wilson, Abeyasekera & Kleih, (2001) various strategies and ways can be employed. However, these strategies do not specifically show how qualitative information, which may relate to the senses of peoples, may be quantified.

Study Area

The study area refers to the place where the participants would provide a response to help the course of the study. In this instance, the Panellists that helped to do the sensory evaluation on the miracle berries powdered sachets were from the University of Cape Coast. The University of Cape Coast is in the Central Region of Ghana, West Africa. It is located along the shores of the Gulf of Guinea, which spans along the West Coast of Africa.

The University is within the Cape Coast Metropolis on the route to Elmina on the Accra Takoradi road. The University's main entrance is about 200metres from the Atlantic Ocean on the Cape Coast Takoradi high way. The University is on a hill facing the Atlantic Ocean and it has two campuses, the Southern Campus (Old Site) and the Northern Campus (New Site).

Source of Miracle Berries for the Study

The main material for the study was miracle berries, which were obtained in the Bunso Cocoa Research farm. The farm is located 120km north of Accra, 150km south of Kumasi and 30km west of Koforidua (Asiedu-Darko & Bekoe, 2014). Bunso is one of the 111 communities of the East Akim Municipal of the Eastern Region of Ghana. The Miracle berries were harvested with assistance from some of the workers on the farm. See photos taken during the harvest in Appendix C.

Processing of miracle fruits into freeze-dried Miraculin

Sample Preparation and Reagents

Ripe fruits of *Synsepalum dulcificum* were obtained from the Bunso Cocoa College farms of the Eastern Region of Ghana. The harvested fruits were transported at the cool of the evening in an iced chest with some iced blocks (this was done to reduce the heat of the day which tends to destroy the miraculin in the fruit) to the Bio Resource Company Limited in a truck and docked at the reception bay and offloaded the containers with the fruits unto the floor in a cold room. The inspector picked random samples of fruits according to the sampling plan and checked for maturity, wholesomeness, other quality indices based on

which some fruits were accepted, and others rejected. The sample was then manually sorted and cleaned to ensure that they were free from insects, dirt and other foreign materials.

Washing, sorting, and Sanitization

Washing and sanitization were done to rid fruits of dirt, soil particles, and insects and to reduce bacteria and another pathogen load prior to subsequent processing.

Washing and Sorting

After weighing, the fruits were then poured into a clean, clear and sanitized basin half-filled with water. The fruits were agitated to remove adhering debris, mould patches, extraneous materials, and soils particles. The unwholesome fruits floated in the soaked-water and therefore were separated from the wholesome ones. The process was repeated until the soak-water became clear, indicating that the debris was removed. After the soaking process, the fruits were transferred into a plastic basket with smaller holes filter adequate to hold the fruits for rinsing. The rinsing was done to get rid of the dirt and organic matter that were still stuck on the fruits especially those at the bottom of the basin.

Sanitization

10kg of the washed fruits were transferred into another sieve. The sieve, with the fruits, was dipped into a sanitizer solution of chlorine in a stainless-steel basin. After the sanitizer dipping, the sanitizer solution was drained off and the fruits were rinsed with potable water to wash off any excess chlorine residue. The

fruits were then air dried to inhibit the potential growth of any residual organisms; both spoilage or pathogenic. This process aimed at reducing microbial load.

Depulping

This is a process of removing the seeds of the miracle berry from the pulp. The Depulping instrument was washed and sanitized before use. This was first inspected and verified by a supervisor and the operator of the depulper. The parts of the depulper were assembled and a test-run was done on the depulper to ensure that it was working. The pulp collected from depulping was bagged, placed in a tray and frozen.

Description of the mechanical Depulper

The stainless steel mechanical depulper used in this research comprises of a perforated (approximately 2–7mm) static cylindrical screen that is positioned horizontally on its longitudinal axis. Inside the screen are two (2) paddles or scraper system, which is adapted to rotate. When the paddles are in motion the mass of miracle berry fruits are rotated and the friction removes the pulp from the seeds. The depulper is equipped with a hopper (inlet), a means for feeding the fresh miracle berry fruits, a means for removing the depulped miracle berry fruits at the front end of the cylindrical screen (outlet) and beneath the cylindrical screen a means to discharge pulp.

The samples were then stored at a temperature of 4°C to minimize the physiological and chemical changes that may occur (Karaaslan & Tuncer, 2008). Freeze-drying is a process, in which a product is first frozen and then dried by sublimation of the ice. The total process involves four steps: freezing, sublimation

of the ice, called main drying (MD), desorption of the water bound to the solid, called secondary drying (SD); and packed in containers to exclude absorption of water and/or oxygen from the atmosphere. By freeze-drying, a product unstable in water is transformed into a dry, stable product (Bellissent-Funel & Teixera, 1999).

The process had to be developed to satisfy four demands on the finished product: its volume remains that of the frozen substance, the structure and the biological activity of the dried solid correspond as far as possible to those of the original substance; the dried product remains stable during storage (Monger, 1997).

The following reagents were used for the extraction of the miraculin from the fruits; Acetone, sodium chloride, Tris (hydroxymethyl) aminoethane, hydrochloric acid, sodium dodecyl sulphate (SDS), sucrose, bromophenol blue, Coomassie brilliant blue R-250, glacial acetic acid, ammonium bicarbonate, sodium potassium tartrate, sodium hydroxide, and sodium carbonate were from Fluka Chemical Company (Buchs) and were of analytical grade.

Sodium dihydrogen phosphate, dipotassium hydrogen phosphate, NNN'N'-tetramethelenenediamine (TEMED), ammonium persulphate and glycine were obtained from Wako Chemical Company (Japan). N'N'Methelenebisacrylamide was from ServaFienbiochemica Company Ltd. (N.Y.). Analytical grade P-Nitroaniline was obtained from Hopkin and Williams Ltd. Essex (England). Ethanol, disodium hydrogen phosphate and ethanoic acid were obtained from Riedel-de Haen (ph.Eur). 2-mercaptoethanol and Folin-Ciocalteau reagent were

from BDH Chemical Company Ltd. (England) and were of analytical grade. Hydrated copper sulfate was obtained from Merck Company Ltd. (Germany).

Pumps, HIC columns 1 and 2 (pre-loaded with column resins), the column resins; SP-sepharose FF, sephacryl S-100, Sephadex G-25 and standard protein markers namely, transferrin, ovalbumin, Q-chymotrypsinogen and carbonic anhydrase were obtained from Pharmacia Biotech. AB (Sweden). The ovalbumin and transferrin were premixed. Unless otherwise indicated, all other chemicals were from Sigma Chemical Company St. Louis, MO., and were of analytical grade.

Extraction and purification of miraculin

Miracle berry was vacuum-dried and ground to a powder. The miracle berry powder (MFP) was extracted by 250 ml of water for 40 min, and the extraction solution was filtered, and the filtered liquid was vacuum-concentrated and stored at 20 ºC, it is referred to as miracle berry-water extract (MFWE). On the other hand, MFP was extracted with butanol and these extracts were partied by water (water fraction) after filtration. This water fraction was carried out the partition with butanol (butanol fraction), hexane (hexane fraction), and ethyl acetate (EA fraction) (Achel, 1996).

The extraction and purification procedure was a modification of the method of Theerasilp and Kurihara (1988). The washed pellet was re-suspended in 0.5M NaCl solution and homogenized for 15 to 20 minutes at 4°C in a Bransonic-92 sonication bath (Yamato Co. Ltd.). The homogenate was centrifuged at 8,500 rpm in a Hitachi 20PR-52D centrifuge (Hitachi Koki Co. Ltd.

Japan) at 4°C for 10 minutes and the supernatant fraction was recovered (Achel, 1996).

Preparation of the Miracle Berry Powdered Sachets

Powdered sachets are produced from powdered materials without modifying their physical nature of materials. This method is applicable for crystalline chemicals having good compressible characteristic and flow properties such as Potassium salt (chlorate, chloride, bromide), Sodium chloride, Ammonium chloride, Methylamine (Harbir, 2012). After a quantity of powdered or granulated powdered sachetting material flows into a dye, the upper and lower punches of the powdered sachet machine compress the material under high pressure.

Direct compression is used on most cases because it provides the shortest, most effective and least complex way to produce powdered sachets. However, it does require a very critical selection of excipients in comparison to granulation processes because the raw materials must demonstrate good flowability and compressibility for successful operation.

The flowchart processing steps involved in wet granulation compression is displayed in Figure 2.

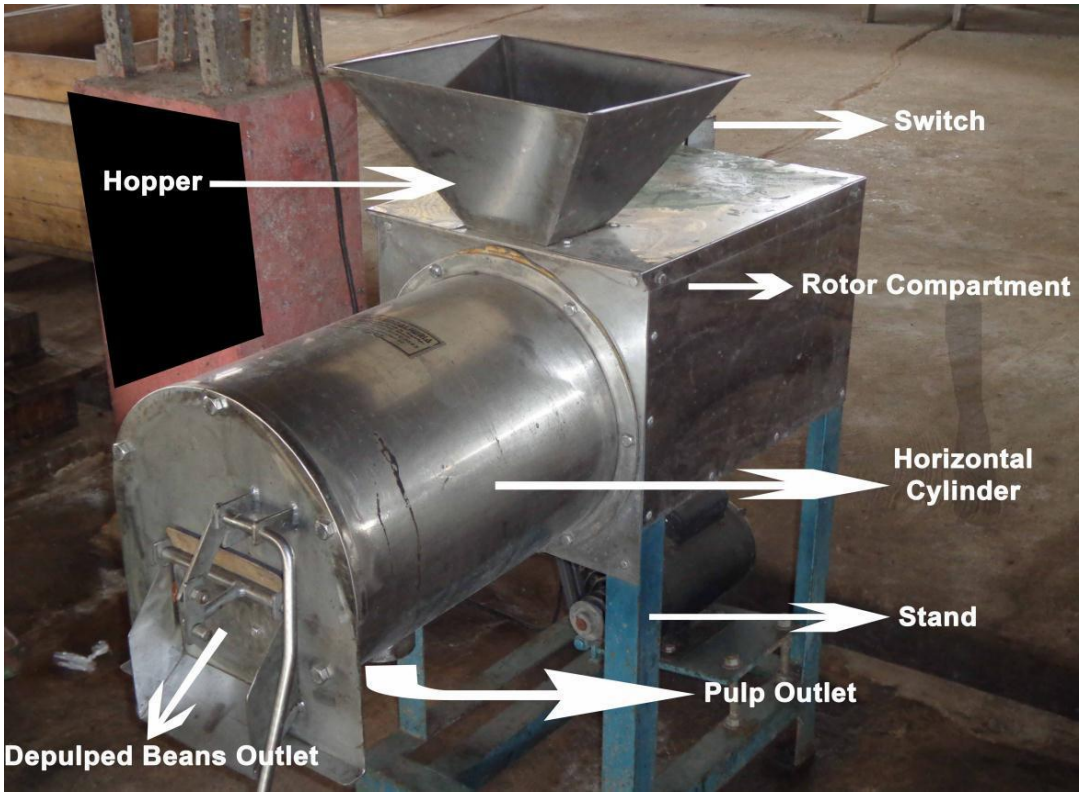

Figure 2: Mechanical Depulper

Weighing of the Product

Miracle berry powder was sent to the laboratory under the cold condition and weighed 1g each into aluminium foil since the other two samples are all 1g each.

Packaging of the Product

I designed a package where the miracle berry powder was put in and named it miracle natural enhancer. The nutritional facts were also stated on the package. The weighed powder in aluminium foil was then put in the paper package purposely designed to contain 1g each of the product and sealed. The product was put in the aluminium foil because it is hygroscopic, that is it absorbs moisture from the atmosphere.

Sensory Evaluation

Three taste enhancers in their powdered form were used for the sensory evaluation. Two artificial taste enhancers namely Splenda Artificial Sweetener and Equal Artificial Sweetener were coded as SAE and EAS respectfully. These artificial taste enhances are widely used in the food industry. The third taste enhancer namely Miracle Natural Enhancer, which was the natural taste enhancer was coded as MNE.

The participants tasted each of the powdered taste enhancers and scored their taste perception of each on a 1-5 point scale; 1-poor, 2-fair, 3-good, 4-very good, 5-Excellent. The analysis of the taste enhancers were based on the aroma, texture, colour taste smell and general acceptability. The participants were to taste the sample then drink lemon juice and record their findings.

The participants coated the entire membrane of their tongue with each taste enhancer in other to produce an effect on taste alteration. To do so, each student tasted the sample for approximately 45seconds. Then, the students again drink the lemon juice. Following each taste, the students rinsed their mouth with water to clean their palate and recorded the perceived taste intensity of each taste enhancer in the questionnaire table prior to tasting the next enhancer. Approximately, I minute elapsed between tasting each taste enhancer. Following the data collection, students also indicated in the comment section of the questionnaire on the taste enhancers.

Figure 3: Flowchart for processing Steps in Wet Granulation powdered sachets

Source: Harbir (2012)

Chemical Composition Analysis of Samples

Chemical components of samples and framed formulae were determined according to the methods defined by the Association of Official Analytical Chemists (AOAC) (Horwitz, 2000). All these were done in triplicates. The analyses were carried out at the School of Agriculture Laboratory of the University of Cape Coast.

Moisture content Determination

Porcelain crucibles were washed dried and weighed. 10grammes of fresh samples were placed into the crucible and weighed. The crucibles containing the fresh samples were placed in the oven at a temperature of 105ºCfor 48 hours. At

the end of the period, the crucibles were removed, cooled in a desiccator and weighed (Rowell, 1994).

Crude Protein Determination

Protein was determined by weighing 0.2g of the powdered sample into a numbered kjedahl digestion flask. About 4.5ml of digestion mixture was added and the sample was digested at 360 ºC for two hours as defined by the AOAC method (Cuniff, 1995). The digest was allowed to cool and diluted to 50ml with distilled water. Twenty millilitres (20ml) of the digested was immediately distilled after adding 10ml of alkali mixture using 5ml of boric acid as an indicator. 50ml of the distillate was collected and titrated against 0.00712M HCl until it turns to a pink colour, which determined the endpoint. The remaining diluted digest was reserved for the mineral determination as ascribed by the Food and Agriculture Organisation (FAO), (2008).

Percentage protein was calculated using the formula;

%N= [×14.007 x100]

%Protein=%Nx6.25

Fiber Determination

A sample of 0.5000g was weighed into a boiling flask and 100ml of 1.25% sulphuric acid solution was added and boiled for 30minutes. Filtration was carried out in a numbered sintered glass crucible. The residue was transferred into the boiling flask and 100ml of 1.25% sodium hydroxide solution added and boiled for 30 minutes. Filtration continues after the boiling and residue was washed with water and methanol (FAO, 2008). The crucible was dried in an oven overnight at

105ºc and weighed. The weighed crucible was placed in a furnace at 500ºc for 3 hours. The crucible was slowly cooled and weighed.

% Crude fiber = × 100

Mineral Determination

To determine the possible minerals in the Miracle Berry, different methodology with their needed reagents have been used (Rowell, 1994).

Calcium Determination

An aliquot of 10ml of the reserved digest was pipetted into a 250ml conical flask and 150ml of distilled water was added. I ml each of potassium cyanide, hydroxylamine hydrochloride, potassium ferrocyanide, and triethanolamine were added. 20ml of 10% sodium hydroxide was added to raise the pH and then 10 drops of the calcon indicator were added to the solution and titrated against 0.005M EDTA solution (Cuniff, 1995).

Dry matter Determination

After the moisture lost calculation, the dry sample weight was noted and expressed as a percentage of the fresh weight (Rowell, 1994).

Ash Determination

The dried samples in the crucibles were transferred to the hot plate charred over a period for the smoke to go out (Rowell, 1994). The charred samples were then transferred into a muffle furnace and ignited at 550ºCfor 5 hours. The crucibles containing the samples were then cooled in desiccators and weighed. The percentage of ash was then computed as:

% ASH = ×100

Carbohydrate Determination

Some of the milled sample was weighed into a 50ml conical flask and 30ml of distilled water added. The content was allowed to simmer gently on a hot place for 2 hours. It was toppled periodically to 30ml and allowed to cool after the 2 hours. The solution was filtered into a 50ml conical flask and topped to volume. The extract was kept for colour development. Two millilitres of glucose standard solution and the extract were pipetted into a set of boiling tubes, 10ml of anthrone solution was rapidly added to the boiling tubes mixed thoroughly and cooled under running tap water or ice bath.

The tubes were placed in a beaker containing boiling water in a dark fume cupboard for 10minutes. The tubes were allowed to cool in cooled water in the dark (FAO, 2008; Page, Miller & Keeney, 1982). The optical density of the standards and the sample solution was measured at 625min using the spectrophotometer. A calibration graph was prepared from the standards and used to obtain mg glucose in the sample aliquot.

% carbohydrate =

Where C (mg) = carbohydrate concentration from the graph.

Magnesium Determination

An aliquot of 10ml of the reserved digest solution was pipette with a 250ml conical flask. One hundred and fifty millilitres (150ml) of distilled water was added. Fifteen millilitres (15ml) of buffer solution was added and allowed to stand for a few minutes. One millilitre (1 ml) of each of potassium, cyanide,

hydroxylamine hydrochloride, potassium ferrocyanide, and triethanolamine were added. Ten (10) drops of trichrome Black T indicator was added and titrated against 0.005m EDTA solution (Page, Miller & Keeney, 1982).

Phosphorus Determination

Two millilitres of aliquot of the digested sample solutions was pipette into a 25ml volumetric flask. 2ml of the blank digest was also added to the 2ml of standard phosphorus solution to give it the same background as the digest. Ten militres of distilled water was added to the standards as well as the sample solutions. Four militres of reagent B made up of ascorbic acid and reagent (Page, Miller& Keeney, 1982). A reagent was added to the standard and sample solutions. Distilled water was added to the volumetric flask to make up to the volume of 25ml and allowed to stand for about 15 minutes for the colour to develop. After colour development, the absorbances of the standard and sample solutions were determined using a spectrophotometer at a wavelength of 882mm. A standard calibration curve was plotted using their concentration against absorbance.

Calculations

If C= ugP/ml obtained from the graph then ugP/g =

Sodium and Potassium Determination

Potassium and sodium concentrations in the digested samples were determined using the flame photometer. The following standard concentrations of both potassium and sodium were prepared 0, 2,4,6,8 and 10 ug/ml (Page, Miller, Keeney, 1982). Both the working standards and the sample solutions were

aspirated individually into the flame photometer and their emissions recorded. A calibration curve was plotted using the concentration and missions of the working standards. The concentration of potassium and sodium in the sample solution were extrapolated from the curve using their emissions

ug K/g or Na =

Iron, Copper and Zinc Determination

Standard solutions of 1,2 and 5ug/ml solutions of Fe, Cu and Zn were prepared (FAO, 2008). The standard solutions of Fe, Cu and Zn were aspirated into the atomic absorption spectrophotometer (AAS) and their respective curve plotted on the AAS. The sample solutions were also aspirated on the AAS with their respective concentrations provided by the AAS.

Fe/Cu/Zn ug/g =

Personal Protective Equipment

i. Lab coats and aprons
ii. Clogs
iii. Nose masks
iv. Hair Nets
v. Gloves
vi. Safety goggles

Potential Hazards and Safety Precautions

i. The freeze dryer is an electrical device and as such poses an electrical shock hazard if misused or faulty. Inspect for any problems before use

(e.g. problems with power supply, burns, sparks, and smell. Notify supervisors and or manufacturers.

ii. Freeze dryer generates low temperatures. Shelves may cause skin damage upon exposure. Gloves should be worn at all times when handling the freeze drier.

iii. Do not use freeze dryer for any other sample apart from miraculin pulp

iv. Wear gloves when changing vacuum pump oil or heat transfer fluid.

v. For the harvest right freeze dryer, the vacuum pump can squirt out oil especially when the oil level is too high or the pump is too hot. Safety goggles should be worn at all times especially when operating this freeze dryer.

Procedure and Working Instructions

Procedure

The Freeze dryer operator ensures that the freezer dryer is clean, sanitized and ready for a batch. This includes draining, filtering or changing the vacuum pump oil. The operator then turns the freeze dryer on and runs it for 30 minutes before taking the frozen miraculin pulp out of the freezer. The Frozen pulp materials are transferred out based on the FIFO (First in first out) inventory management approach. They are then prepared (broken down into smaller pieces) and placed on the freeze dryer trays. This is done to increase the surface area of the pulp to ensure an efficient freeze-drying process. The trays containing the frozen material are then placed on the freeze dryer shelving unit and the door locked.

Working Instruction

Cleaning the freeze drier

i. Remove the black rubber gasket the door seals against.

ii. Push the shelves forward until it is half-way out of the freeze dryer.

iii. Spray mild detergent over the shelves and allow settling on the surface for about 5 minutes.

iv. Rinse the surface with water thoroughly.

v. Repeat the detergent and water rinsing process at least three times.

vi. Allow the shelves to dry.

Draining, Filtering or Changing Vacuum Pump Oil

i. Take the vacuum pump oil filter and place it in position under the drain valve.

ii. Locate the drain valve for the oil reservoir located at the bottom of the reservoir.

iii. Use your hand to open up the valve as far as it will go.

iv. Drain the oil from the vacuum pump reservoir into your containers. Once drained close the vacuum.

v. Filter the oil.

vi. Refill the oil reservoir of the vacuum pump with filtered oil or use new oil.

vii. From experience, use new oil after filtering used oil for about 4 times.

viii. Section 3.2.3- Test-running the Vacuum Pump

ix. Press “START CUSTOM”.

x. Close the drain valve and press continue.

xi. Press the Blue clock on the top right corner; this changes the phase from

Freezing to Drying

i. Monitor the pump for 15 minutes; if the pump achieves the baseline pressure of 500mTorr or less with the 15 minutes time frame then the pump is working well and vice versa.

ii. The test run should be done every 4 to 5 batches.

Breaking frozen pulp down

i. Take out pre-frozen pulp material according to the FIFO plan and hit with a mallet (thoroughly cleaned and wiped with 70% alcohol) to break them into smaller parts.

ii. Weigh smaller portions of the frozen pulp on a freeze-drying tray and on to a mass balance.

iii. Continue to add until the desired weight for a tray is achieved. Do the same for the rest of the freeze-drying trays.

iv. Pick the mallet with a clean knife, cut the smaller pre-frozen pieces on the tray to increase the surface area for freeze-drying further.

v. Do not attempt to operate a freeze dryer without your personal protective equipment on.

Do Not Overload Freeze-Dryer

i. Harvest right – 4kg per batch

ii. Virtis – 12kg per batch

Running the harvest right Freeze dryer

i. Do not use the freeze dryer until an experienced user had trained you into detail on how to operate the machine.
ii. Press the "CUSTOMIZE' button and set the shelf temperature to 100°F (38°C).
iii. Tap on "ADJUST CYCLE TIMES", set the "FREEZE TIME" to 11 hours and the "FINAL DRY" to 20 hours.
iv. Press "START CUSTOM".
v. Close the drain valve and press continue.
vi. Wait for 30 minutes before placing pre-frozen material onto the shelves.
vii. Add the insulating pad for efficient freezing and drying, close the glass door tightly
viii. After the process is complete, open the drain valve and check the freeze-dried product.
ix. If the material is not well dried, press the "EXTRA DRY" button, normally you will have to wait for the pump to cool for one hour.
x. Defrost for 2 hours.
xi. Clean the freeze dryer and wait at least 12 hours before starting another batch.

Sensory Evaluation of the prepared Miracle berry Powdered Sachets

Sensory analysis (or sensory evaluation) is a scientific discipline that applies principles of experimental design and statistical analysis to the use of human senses (sight, smell, taste, touch, and hearing) for the purposes of

evaluating consumer products (Pangborn, 1981). By applying statistical techniques to the results, it is possible to make inferences and insights about the products under test. Most large consumer goods companies have departments dedicated to sensory analysis.

Sensory analysis can mainly be broken down into three sub-sections: Analytical testing (dealing with objective facts about products), Affective testing (dealing with subjective facts such as preferences) and Perception (the biochemical and psychological aspects of sensation) (*Morten,* Meilgaard, Civille & Carr, 2007).

In this study, analytical testing method of sensory evaluation was used to compare the Miracle berry taste enhancer powder with two artificial Enhancers called Splenda and Equal by the Panellist to determine which of them should be the ideal taste enhancer for human consumption. The researcher engaged a total of 25 individuals for the sensory evaluation. This activity was carried out at in VOTEC's Department laboratory. This purposive technique was deepening the evidential knowledge addition of the research work into the miracle berry plant.

Population

According to Fraenkel and Wallen (2006), the population is the extensive group from which the researcher obtains a sample for a study. The population in this context is all the students in the University of Cape Coast. Students in the university were eligible to participate in sensory evaluation of the Miracle Berries Powdered sachets. The estimated total number of population in the University of Cape Coast was 25, 490.

Target Population

The target population of the study was the students from the University of Cape Coast with a special focus on the Vocational and Technical Education (VOTEC) Department. The target population was all the undergraduates in the VOTEC Department for 2017/18 academic year. The target population was 471 students who were pursuing a first degree from the University of Cape Coast in the VOTEC Departments as presented in Table 1.

Table 1: Undergraduate Students in VOTEC

Level	Male	Female	Total
400 students	5	142	147
300 students	12	123	135
200 students	6	96	101
100 students	2	86	87
Total	**25**	**447**	**471**

Source: VOTEC, 2018

Sample and Sampling Procedure for Panellists

Random sampling technique was adopted to sample 214 students out of the total target population of 471 in the VOTEC Department of the University of Cape Coast. A sample of 214 was arrived at when the sample determination table of Krejcie and Morgan (1970) was used. The 214 students were sampled by dividing the total number of undergraduate students in the VOTEC Department to get 2.20. Every other two students from each level were sampled using their registered list provided by the Data Processing Unit (DPU) of the University of Cape Coast. The students involved were contacted through their Course Reps for the sensory evaluation.

Ethical Consideration

In avoiding any legal issues arising out this study, relevant permissions were sought from the University authorities which include the University of Cape Coast, Institutional Review Board (UCC-IRB) and VOTEC Department to enhance data collection (See Appendix B for details). The study involves consumables and to avoid food contamination, hand gloves were worn and hair covered when handling the Miracle Berry Tables.

In collecting the details of the respondents during the sensory evaluation, great care was taken in order not to inflate any passion or commenting unnecessarily on any information given out. The introduction section of the sensory evaluation form clearly indicated the reason for the study and the use of the findings. The Panellists were given clear information to either participate in the study or not. Also, there was a flexibility to continue with the study or withdraw from it while the study was ongoing.

Personal identity was not allowed to be disclosed in any sort on the sensory evaluation form (questionnaire) which could make it possible to trace any panellist with the sort of information provided. The sensory evaluation forms were coded after every form had been submitted in the absence of the Panellists. The collection of the forms from the Panellists was not done in any pattern to link any instrument to a particular Panel member for identification.

The collected data in its primary form would only be shared with the research supervisors and any mandated authority that has anything to do with the study. Any persons outside this domain would not have access to the data

collected for the purpose of this study. The sensory form that has been answered would be kept under lock and key for at least 5years after graduation. This is to enable any person having any legal issues bordering on this study could access it without a problem.

Research Instrument

The instrument used by the researcher in collecting information from the respondents for the study was a questionnaire. The questionnaire was considered appropriate in the sense that, it has the ability to secure factual information about practices and conditions. In other words, its usage permits wider coverage since respondents can be approached more easily during administration, and it offers greater assurance of anonymity to respondents (Kaiser, 2009).

The questionnaire was in two parts with the first part dealing with biographical information of respondents while the second part was on the Sensory evaluation of the Miraculin powder. The sensory evaluation questionnaire was self-designed based on appearance, taste, texture, aroma and overall acceptability. The scale for assessing the panellists was a 5-point hedonic scale as used by some researchers (Peryam & Pilgrim, 1957; Lim, 2011). The measuring scale on the instrument was as follow 1-Poor, 2-Fair, 3-Good, 4-Very good and 5-Excellent (Refer to Appendix A).

Validity and Reliability of Instrument

Sensory evaluation questionnaire was pilot tested using 20 respondents from the University of Cape Coast, VOTEC Department. Convenient sampling was used to access students after their lectures at ‘Science – New site’. According

to Osuala (2005), content validity of an instrument demonstrates that the items of that instrument are representative and comprehensive enough to represent and measure a presumed objective and variable.

Reliability, according to Fraenkel and Wallen (2000), is the consistency of an instrument for each respondent, from one administration to another and from one set of items to another. The supervisors of the study did facial validity of the instrument and after which a pilot test was conducted. The internal reliability of the sensory evaluation after the pilot testing of the instrument was 0.63 Cronbach's alpha coefficient. The reliability ratio obtained has established the fact that the instrument was good to collect reliable data from the panellists for the actual data for the study.

Data Collection Procedure

The data collection was in two parts. The first part was for the proximate analysis work in the chemical laboratory and the second part was the sensory evaluation of the powdered food enhancers.

The sensory evaluation data was collected from the students in the Vocational and Technical Department of the University of Cape Coast. The packaged food enhancers that was developed and the artificial one on the market was rebranded to disguise it from those who were familiar with it. The students were sampled to do the sensory evaluation in a lecture hall. They were seated as students at a lecture with comfort. The samples in their powdered form were placed on their tables in arranged order of label based on the questionnaire. A lemon juice in a disposable cup were provided to each student to sip after tasting

each taste enhancers. A bottled water and three disposable cups were also provided to each of the students. The purpose of the water was to enable them risen their mouth before every evaluation of the enhancer. The respondents (panellists) were briefed on how to use the evaluation form after briefing them on the sensory evaluation process. Photos were taken during the sensory evaluation and laboratory analysis work. These photos have been presented in Appendix B.

Data Processing and Analysis

Data obtained from the sensory evaluation was organized by editing and coding of the questionnaires. After the screening of the instrument, the data was entered into an IBM SPSS (version 25) computer software for Windows. Research questions one, two and four were analysed using a constant comparative approach as ascribed to by Shenton (2004). The sensory evaluation question (Research question three) was analysed using One-Way ANOVA.

CHAPTER FOUR

RESULTS AND DISCUSSION

Introduction

The results from the sensory evaluation have been presented in this chapter for analysis and discussion. The result is presented in two parts, which are demographical information and the result for the research objectives.

Demographical Information

The result of the Panellists who took part in the sensory evaluation has been presented in Table 2.

Table 2: Age of Sensory Panellists

Age range	Frequency	Percentage
21-25years	23	92.0
26-30years	2	8.0
Total	25	100.0

Source: Field data, Adebessah (2019)

The panellists that took part in the sensory evaluation were all female totalling 25. The age of the panellists as presented in Table 2 shows that most of the panellists have their age ranging from 21-25 years. The least age panellists also fell in the 26-30 years of age. Although the older panellists were few in number, the comparable young panellists too were above the age of 18years. The age of the panellists does reveal that they were of age and their judgment cannot be doubted. This, therefore, suggests that the judgment they would pass on the taste enhancer made from the miracle berry can be trustworthy.

Table 3: Acceptability of Miracle berry Enhancer Formulations Developed

Sample	Colour	Taste	Aroma/ Flavour	Texture	Overall Acceptability
SAE	3.92±0.81	3.48±0.95	2.44±1.04	3.28±0.89	3.44±0.71
EAE	3.04±1.02	3.88±0.67	2.88±0.97	3.04±0.98	3.32±0.75
MNE	3.60±0.82	3.64±0.86	3.48±0.77	3.12±0.78	4.24±0.52

Source: Field data, Adebessah (2019)
**Sample (SAE-Splenda Artificial Enhancer, EAE –Equal Artificial Enhancer & MNE-Miracle Natural Enhancer)
**Values are averages of triplicate determinations
**N = 25 **Data is represented as mean ± standard deviation

The result of the three formulated taste enhancers as in Table 3 indicates that the value of the three samples with respect to the colour varies. The mean value for EAE was the least follow by MNE and SAE. The mean difference between EAE and MNE is 0.56. The difference in the mean value realized between SAE and MNE is 0.32 and this difference was more than nine times the mean value of the least rated product (EAE) with respect to colour. The result thus shows that the colour of SAE was accepted more and this was followed by MNE enhancer.

Humans by nature use colour a lot and it helps in making choices of things when it is applicable. Miracle berry becomes red when it is ripped (Chen, Liu & Cheng, 2006) and this status of the fruit attracts humans and other living organisms to it. In finding colour of the Enhancer, sight becomes a significant medium the individual would use to differentiate in the colour of the taste enhancers. From the indications that have been illustrated, it can be deduced that the panellists were of concern to the colour of the formulated taste enhancers.

The implication of this result is that most people are likely to use colour as their basis to select SAE as their preferred food enhancer followed by MNE.

In assessing the taste of food enhancer in particular, the tongue becomes the most useful organ that one could use. The taste of EAE was ranked higher using the mean and standard deviation, and this was followed by MNE. The least mean value for SAE is 3.48. It is worth noting that mean difference between the second higher food enhancer (MNE) in terms of taste to the third-ranked sample (SAE) is minimal (0.16) compared to that of EAE which had a most preference. The difference in mean and standard deviation value between EAE and SAE is 0.40-0.28 respectively. Though taste in a person's mouth varies, the majority of the panellists had shown preference to EAE. The implication for this result is that when it comes to selecting food enhancers in the samples, most people may show preference to EAE and this would be followed by MNE.

The mean and standard deviation values for the food enhancers (SAE, EAE & MNE) have shown that the higher score is for MNE and the least value is for SAE in terms of aroma/flavour. The difference in mean value the most preferred enhancer (MNE) and the next preferred (EAE) is 0.6, which is more than the difference in the last two preferred enhancers (SAE & EAE) with value of 0.44. The appreciation of the samples by the panellists has indicated that the mean value with respect to the aroma/flavour increases by an average of 0.52 from SAE through to MNE as presented in Table 3. The mean difference between SAE and EAE is 0.44, that of EAE and MNE is 0.60 and between MNE

and SAE is 1.04. The mean difference between the most and least preferred enhancer is significant. This could be an influencing factor for panellists to make a positive decision in favour of MNE even before doing their evaluation. With respect to aroma/flavour, MNE was the most preferred food enhancer and the majority of people have accepted it based on what they have smelled during the sensory evaluation.

The texture of the formulated taste enhancers was another domain on which the panellists evaluated the samples. The mean value for SAE was more (0.16) than MNE being the second most preferred food enhancer. Meanwhile, the mean value between the second (MNE) and the third (EAE) food enhancers is 0.08 which is twice (0.16) the mean difference between the first (SAE) and the second (MNE) food enhancers. The texture is one of the means humans rely on to make choices that seems difficult to be assessed with eye, tongue, and nose.

The result as in Table 3 has indicated that MNE enhancer is the most accepted formulation, which was followed by SAE and lasts, but not the least being EAE. The mean difference between SAE and MNE is 0.61 which shows that the Panellists' acceptability for the two samples were far apart in terms of their mean/standard deviation value. The acceptability means/standard deviation value between the second and the third accepted food enhancers indicated that the value difference was not that much (0.08). The reasons behind the Panellists choice of a particular sample over the other were premised on the characteristics

and the Hedonic scale made available to them. They could have made other decisions, which might not be in tune with how they usually assess foods.

The comments as made by the Panellists have been presented under 'Colour', 'Taste', Aroma/Flavour' and 'Texture'. The comments on the samples after tasting the samples (SAE, EAE & MNE) were on an optional basis. It must also be emphasized that not all of the Panellists gave written comments after the sensory evaluation. Hence, the comments presented here does not cover all the 25 Panellists. The comments are presented in the form of quotes.

Comment of Panellists' on the formulated Food Taste Enhancers

The comments made by the Panellists with respect to the colour of the food taste enhancers are presented. The SAE sample's colour was seen to be good by one of the Panellists. One panellist said '*MNE has a good colour',* another *one also said 'MNE is so attractive'.* Two of the Panellists, however, have contrary views about MNE formulation.

'*On the contrary, MNE looks natural and the colour should be improved',* and '*MNE colour is not attractive'.*

The comments by some of the Panellists on the taste of the samples are presented as follow: On the taste of the sample, MNE was seen by four respondents to have a good taste. These were some of the comments, '*MNE has a good taste*', '*MNE has good taste and not bitter after taste'*, '*MNE taste well'*, '*MNE has a better taste'* and '*MNE has good taste when eaten raw'*. However, two of the Panellists had the opinion that 'MNE' needs to be improved. The sample labelled 'SAE' has a sharp taste and should be improved upon.

The comments by the Panellists on Aroma/flavour have been presented as below. Few (two) of the Panellists were of the view that the 'MNE' has good aroma and MNE has the best flavour. However, four of the Panellists had indicated MNE needs some improvement. For instance, they said '*the aroma of MNE should be reduced', 'MNE has strong aroma'*, '*The aroma of MNE should be improved*' and *'....the aroma for SAE should be improved'*. Meanwhile, one of the Panellists had the opinion that the aroma of SAE is mild. Another Panellist had the view that EAE product '*is not good'*.

On the issue of texture, 5 Panellists were of the view that '…MNE *should be a little smooth', '....MNE should be improved'. '....MNE feels slimy*', *'MNE texture must be improved' and* 'MNE is too moist and it is difficult to dissolve"

Four other Panellists said *"".....MNE needs improvement*'. Meanwhile, a different Panellist also said '*...MNE texture should be like that of SAE and EAE*'. Four other Panellists had the view that the texture of EAE needs to be improved upon. One Panellist had the opinion that SAE has better texture and another also said '*SAE is very coarse and need to be made smooth'*. The texture of SAE and EAE were commented upon by one Panellist to be '*very nice'.*

Overall comments on the three food taste enhancers

Fifteen (15) Panellists gave some specific reasons for accepting MNE sample. For instance, four of the Panellists have these to say about the sample coded MNE.

'... MNE has good appearance', '... MNE should be on the market', '... MNE is okay'. '... I prefer MNE because it tastes like natural fruit'.

Having analysed the result, it can, therefore, be concluded that the panellists generally accepted Splenda Artificial Enhancer (SAE) as the most preferred food Enhancer and this was followed by Miracle Natural Enhancer (MNE) and Equal Artificial Enhancer (EAE) was the least accepted food Enhancer.

Comparing Miraculin powder to two Artificial Taste Enhancers

In comparing the Miraculin powder to the two artificial taste enhancers, this was done by comparing the chemical constituents and the elements of the formulations as presented in Tables 4 and 5 respectively.

Table 4: Chemical Constituents of the Three Food Taste Enhancers

Sample	%DM	%Moisture	%Ash	%Protein	%Fat / Oil	%Fibre	%Glucose
MNE	81.16	18.84	19.90	4.91	1.41	0.23	40.62
SAE	88.26	11.47	0.09	0.57	0.00	0.00	22.40
EAE	88.43	11.57	0.05	1.13	0.00	0.00	19.61

Source: Field data, Adebessah (2019)
Values are averages of triplicate determinations-Data is represented as mean ± standard deviation
**Sample (SAE-Splenda Artificial Enhancer, EAE –Equal Artificial Enhancer & MNE-Miracle Natural Enhancer)
**Values are averages of triplicate determinations
**Data is represented as mean ± standard deviation

The percentage of dry matter in the three food taste enhancers ranged between 81.16 and 88.43. The highest value in terms of the dry matter was for EAE and it was followed by SAE. The Enhancer with the least value is for MNE. With respect to the percentage moisture, the values seem to be low relative to the percentage dry matter. The percentage of moisture is less in EAE and more in SAE. The value of the percentage of moisture is in the range of 11.57 and 18.84. The ash percentage in the samples under review has a minimum value of 0.05 and a maximum value of 19.90.

MNE has more ash content as compared to the other two samples as in Table 4. The range values of protein content in the food taste enhancers are from 0.57 to 4.91 and the least protein content was found in SAE while the most protein content was also found in MNE. Fat and oil as an essential constituent have its value ranging from 0.00 to 1.41 in terms of their percentage composition. It was only MNE that had the presence of fat/oil and the other two taste enhancers did not have any per the laboratory analysis. The two food taste enhancers that do not have any fat/oil content are the artificial taste enhancers.

The percentage fibre as found during the laboratory analysis has indicated that the value ranged from 0.00 to 0.23. It is also important to mention that it was only the MNE that has some presence of fibre and the other two did not have as well. The last but not the least constituent found in the analysis was glucose. In this case, all the three samples did have some amount of percentage glucose content. The glucose content as found ranges from 19.61 to 40.62. MNE

is the Enhancer has the highest glucose presence and it was followed by SAE and EAS in that order.

It could be noted from Table 4 that apart from the dry matter value, all other values for MNE with respect to moisture, ash, protein, fat/oil, fibre, and glucose are on the high side relatively. SAE and EAE have high values from the dry matter up to the moisture content per Table 5 and these figures start reducing from the ash content to the protein content. None of these food taste enhancers has fat/oil and fibre. However, the glucose content was relatively high. The total glucose content in SAE and EAE is not even up to that MNE alone. This is an indication that the percentage glucose was very high and thus suitable for use as the Enhancer. However, if the need for it use as an enhancer borders on glucose then MNE would be the most preferred one.

It can also be observed that the total values for SAE and EAE for ash and protein respectively was less than that of MNE alone. Generally, the chemical constituents in the three-food taste Enhancers indicate that aside from the dry matter contents all the other values were in favour of MNE. The significant values of the various constituents in Table 5 have indicated that there is significance between and within the groups at 0.05 of alpha.

It can, therefore, be concluded that MNE has more quantity of the chemical constituents compared to SAE and EAE. This finding has confirmed the earlier finding from Agblekpe, Osseyi, and Dossou (2016) that miracle berry has protein, sodium, and other chemical elements more than what has been found. This study has also confirmed Ekwueme and Njoku (2014) work that

miracle berry has protein, moisture content, ash, crude fibre, fat, and carbohydrate. Again, this is also in line with Jeremiah, Ilesanmi, and Ig (2015) who found the same elements as in the current study.

Table 5: ANOVA of Chemical Constituents in Taste Enhancers

		Sum of Squares	df	Mean Square	F	Sig.
%DM	Between Groups	98.14	2	49.07	4426.87	.00
	Within Groups	.07	6	.01		
%Moisture	Between Groups	98.14	2	49.07	4426.86	.00
	Within Groups	.07	6	.011		
%Ash	Between Groups	786.87	2	393.43	954144.56	.00
	Within Groups	.00	6	.000		
%Protein	Between Groups	33.44	2	16.72	977.14	.00
	Within Groups	.10	6	.017		
%Fat and Oil	Between Groups	3.95	2	1.98	64353.03	.00
	Within Groups	.00	6	.000		
%Fibre	Between Groups	.10	2	.05	1345.51	.00
	Within Groups	.00	6	.00		
%Glucose	Between Groups	1495.81	2	747.90	2577.79	.00
	Within Groups	1.74	6	.29		

Source: Field data, Adebessah (2019)

Chemical elements found in Food Taste Enhancers

Table 6: Chemical elements in Food Taste Enhancers

Sample	Fe (ug/g)	Cu (ug/g)	Zn (ug/g)	K (ug/g)	Na (ug/g)	P (ug/g)	%Ca	%Mg
MNE	272.6±70.98	23.87±0.77	44.04±0.73	10450.37±394.45	3003.85±7.90	2778.36±13.17	0.89±0.07	0.11±0.00
SAE	12.89±1.26	15.85±1.67	23.92±1.05	300.03±68.03	85.74 ±0.51	405.81±4.13	0.45±0.01	0.01±0.00
EAE	46.56±0.13	15.87±0.15	24.83±0.69	602.66±1.68	236.65±8.95	374.59±6.16	0.49±0.00	0.01±0.00

Source: Field data, Adebessah (2019)
*Values are averages of triplicate determinations-Data is represented as mean ± standard deviation
*Sample (SAE-Splenda Artificial Enhancer, EAE –Equal Artificial Enhancer & MNE-Miracle Natural Enhancer)
*Values are averages of triplicate determinations
*Data is represented as mean ± standard deviation

Table 7: ANOVA of Chemical Elements in Food Taste Enhancers

		Sum of Squares	df	Mean Square	F	Sig.
Fe ug/g	Between Groups	119741.58	2	59870.79	69388.56	.00
	Within Groups	5.18	6	.86		
Cu ug/g	Between Groups	128.35	2	64.18	56.68	.00
	Within Groups	6.79	6	1.13		
Zn ug/g	Between Groups	774.55	2	387.27	705.64	.00
	Within Groups	3.29	6	.55		
K ug/g	Between Groups	2.001E8	2	1.000E8	1873.32	.00
	Within Groups	320445.18	6	53407.53		
Na ug/g	Between Groups	1.620E7	2	8097709.55	94.14	.00
	Within Groups	516088.01	6	86014.67		
P ug/g	Between Groups	1.141E7	2	5704080.84	1330.45	.00
	Within Groups	25724.09	6	4287.35		
%Ca	Between Groups	.36	2	.18	122.83	.00
	Within Groups	.01	6	.00		
% Mg	Between Groups	.02	2	.01	3749.62	.00
	Within Groups	.00	6	.00		

Source: Field data, Adebessah (2019)
Values are averages of triplicate determinations-Data is represented as mean ± standard deviation
**Sample (SAE-Splenda Artificial Enhancer, EAE –Equal Artificial Enhancer & MNE-Miracle Natural Enhancer)
**Values are averages of triplicate determinations **Data is represented as mean ± standard deviation

The laboratory analysis of the three food taste enhancers has revealed that there are eight chemical elements (Iron, Copper, Zinc, Potassium, Sodium, Phosphorous, Calcium and Magnesium) as presented in Table 6. The Iron content in the food taste enhancers ranges from 11.63 to 273.65 and SAE is the Enhancer with the least iron content followed by EAE and MNE. The quantity of the iron in MNE is about four and half times the total value of SAE and EAE combined. In the case of the least enhancer (SAE), it is about nineteen times of the iron content in the MNE. It is obvious that MNE has so much iron contents compared to the other two food taste enhancers.

Cupper content detected from the laboratory analysis has shown that SAE and EAE have almost the same quantity. Though there is a difference of 1.5 in the Cupper quantity in the two samples (SAE & EAE) this value is not significant enough to cause any influence. MNE has the highest Cupper quantity of 24.64 about one and half times of the least quantity with respect to EAE. The Zinc quantity in MNE is about twice the quantity in either SAE or EAE. The Enhancer having the least quantity is SAE and the highest being MNE. The Potassium quantity in MNE seems to be an outlier of a relatively large value of 10, 45037394.45.

The curious thing also noted among SAE and EAE was that the Potassium quantity in EAE is about twice of SAE. However, the sum of Potassium quantity in SAE and that of EAE are nowhere close to that of what was found in MNE. The Sodium content as in the taste enhancers ranged from 85.23 to 3,511.74. The value of the Sodium in SAE and EAE put together is

about one-tenth of what was found in MNE. It can be concluded that MNE has so much deposit of Sodium in the Enhancer. Phosphorus value in MNE, SAE, and EAE are in descending order of magnitude. The value of MNE is higher in terms of the other two and EAE has the least quantity. The sum of Phosphorus quantities of SAE and EAE is 790.69; this quantity is not closer to that of MNE.

The calcium values for the three taste enhancers seem to be on the lower side relative to the earlier figures seen so far. The value ranges from 0.46 to 0.96; the least value is for SAE and the highest is for MNE. The quantity of Magnesium in SAE and EAE is the same (0.01 0.00). The highest value is for MNE, which is about eleven times of each of the taste enhancer's value.

The ANOVA result in Table 6 has indicated that the values between and within groups are significant at 0.05 (α). The significance of the result runs through all of the eight elements that have been detected from the taste enhancers. Having discussed the results in Tables 5 and 6, the figures were high in favour of MNE. It can be concluded that the eights elements found in the food taste enhancers have much quantity relative to the other two taste enhancers.

CHAPTER FIVE

SUMMARY, CONCLUSIONS, AND RECOMMENDATION

Introduction

This chapter summarises the findings of the study, conclusions, and recommendations. The purpose of the study was to assess the utilization of miracle berry and sensory evaluation of the fruit among the University of Cape Coast students. The relevant literature was reviewed to unearth the existing work done with respect to the problem for the study.

The experimental research design was used to guide the study. The data for the study was from two different sources. The fruits for the proximate analysis were harvested from Bunso Cocoa Research farm in the Eastern Region of Ghana. The data for the sensory analysis was collected from students in the University of Cape Coast. The sample size for the sensory analysis was a Panellist of 25 students from the Department of Vocational Technical Education (VOTEC, UCC) and a questionnaire was used to collect the data from the panellists.

The proximate analysis on the miracle berry was done at the University of Cape Coast Agriculture laboratory. The data from the proximate analysis and the questionnaire were analysed using frequency, percentage, means and standard deviation. The research question three was analysed using mean, Standard deviation and One-way ANOVA with the aid of IBM SPSS version 25 for Windows. Mean and the standard deviation was also used to analyse research question four.

Summary of Key Findings

The result of the study had revealed a lot of information per the research questions. However, the key findings have been summarised as follows:

1. Miraculin was extracted from the miracle berry using the depulper machine.
2. Miraculin juice from the miracle berry was converted into powder by the use of direct compression technology for easy packaging and transport.
3. The most accepted taste enhancer was Miracle Natural Enhancer (MNE) this was followed by Splenda Artificial Enhancer (SAE) and Equal Artificial Enhancer (EAE) respectively.
4. MNE has more quantity of the chemical constituents compared to SAE and EAE.
5. Eight mineral elements (Fe, Cu, Zn, K, Na, P, Ca & Mg) have been identified in the food taste enhancers.
6. Miracle Natural Enhancer (MNE) has much quantity of chemical elements than the other two food taste enhancers (EAE & SAE).

Conclusions

The extraction of Miraculin from fresh miracle berries and processing them into a packaged powdered sachet was successful achieved. The nutritive value of the miracle berry as compared to the two artificial taste enhancers (EAE & SAE) on the market showed a significant high proportion of chemical constituents and elements. The chemical elements found in the miracle berry were natural and have no negative adverse effects on the health of the

consumers. Notwithstanding, these natural elements are easily digestible into the bloodstream when consumed. In addition, MNE may have other elements that the body may require for other functioning, as the human body system needs many natural chemicals to function well.

The sensory evaluation from the study also proved that MNE was superior and must be recommended for use in the food industry, as it was natural and superior to enhance the taste of food items that are sour.

Recommendations

In view of the findings, the following recommendations have been made

1. Miracle food Enhancer factories should be established in Ghana to do mass production of the powder for commercial sale.
2. More improvement has to be done on Miracle Natural Enhancer (MNE) as suggested by the panellists during the data collection so that most people could accept the product.
3. Shelf life for the MNE has to be done to ensure public use of the powder.
4. Forestry Commission should collaborate with the Bunso Cocoa Research to make sure the miracle fruit tree is not destroyed but be planted on a larger scale.

Suggested Areas for Further Study

This study limits its scope to the University of Cape Coast in the Central Region of Ghana. Therefore, the study should be expanded to cover a broader area in scope outside the university in the region. The study should focus on the contribution of food Enhancer to diabetic patients in the Central Region of Ghana.

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APPENDICES

APPENDIX A

SENSORY EVALUATION QUESTIONAIRES

The purpose of this evaluation is to collect data to help with the promotion of natural sweetener from miracle berry fruit and its nutritional benefits. The study is for academic purpose therefore, your candid response would be of great help to the study. Any information given would be for the said purpose and be assured that your identity would not be revealed under any circumstance.

Date……. Panellist ID……

Biographical Information of Respondent

Please tick [ ] your age range

Age (years): 15-20[ ] 21-25[ ] 26-30[ ] 31-35[ ] 36-40[ ] 41-45[ ]

Please tick [ ] your gender

Gender: Male [ ] Female [ ]

Poor = 1, Fair =2, Good =3, Very good =4, Excellent =5

Sample	Colour	Taste	Aroma/Flavour	Texture	0verall Acceptability
SAE					
EAE					
MNE					

Comments……

Thank you for participation.

APPENDIX B

PHOTOS FROM THE FIELD