ASSESSMENT OF THE QUALITY AND STABILITY OF ETHANOL EXTRACTS OF *CONOIDES MILLER AND LONGUM NIGRUM* (*CAPSICUM ANNUUM).*

By

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DECLARATION

I declare that the work in this Dissertation entitled **"ASSESSMENT OF THE QUALITY AND STABILITY OF ETHANOL EXTRACTS OF *CONOIDES MILLER AND LONGUM* NIGRUM (*CAPSICUM ANNUUM)*"** has been carried out by me in the Department of Chemistry under the supervision of Professor E.B Agbaji and Professor V.O Ajibola. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this research was previously presented for another degree or diploma at any University or Institution.

Ugochi Judith Mmereole	……	…….
Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled "ASSESSMENT OF THE QUALITY AND STABILITY OF ETHANOL EXTRACTS OF *CONOIDES MILLER AND LONGUM NIGRUM* (*CAPSICUM ANNUUM)* " by Ugochi Judith Mmereole meets the regulation governing the award of the degree of Master of Science in Analytical Chemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to my family.

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ABSTRACT

Available synthetic colour additives used in food have been shown to exhibit genotoxic effects and pose several potential health hazards to human. Hence, the global increase in the demands for natural colour additives. This study therefore investigated the quality and stability of ethanolic extracts from *Capsicum annuum* for use as food colourants. Colour pigments and other constituents were extracted from bell pepper (*var. conoides miller*) and sweet pepper (*var.longum nigrum*) using ethanol. The extracts were subjected to pH adjustment and addition of ascorbic acid as stabilizer under varying conditions of light (white light, UV light and in the dark). The results showed that the extracts maintained their colour at an acidic pH. The yield of colour pigments and other constituents from both varieties were high (above 70%) and they also had good colouring ability as shown by their colour units of 24,460 for bell pepper *(var. conoides miller*) and 27,645 for sweet pepper (*var. longum nigrum*). The data from this study suggests that the most suitable condition to maintain stability is in acid media and 0.5% ascorbic acid kept in the dark. Also both extracts had a low energy value, they need to be fortified while being incorporated into food.

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ABBREVIATIONS, GLOSSARIES AND SYMBOLS

ASTA	-	American Spice Trade Association
DS	-	Dry Shombo (Sweet pepper (*var.longum nigrum*)
DT	-	Dry Tattasai (Bell pepper (*var.conoides miller*)
FTIR	-	Fourier Transform Infrared Spectroscopy
ICU	-	International Colour Units

CHAPTER ONE

1.0 INTRODUCTION

1.1 Food Additives

Food additives are substances usually added to foods in small quantities during the process of production, storage and/or packaging in order to improve the appearance, texture, taste, colour, smell, durability, or nutritional value (FI, 2010). In the United States, colours added to foods are referred to as food additives under the 1958 Food, Drug, and Cosmetics Act and are defined as "any dye, pigment or substance which imparts color when added or applied to a food, drug or cosmetic, or to the human body" (USFDCA 2014). Consumers naturally enjoy bright colours in food because, as the saying goes, people "taste" first with their eyes, then their mouths (Chaitanya, 2014; Matulka and Tardy, 2014). Colour is one of the most important sensory qualities as it helps us to accept or reject particular food items. It is used as a measure of the quality and nutrient content of foods (Chaitanya, 2014). Colour is important in consumer perception of food and it is often associated with a specific flavour and intensity of flavour (Abdeldaiem, 2014). People often associate certain colours with certain flavours, and the colour of food can influence the perceived flavour in anything from candy to wine (Jeannine, 2003). It is very unfortunate that most manufacturing process in food processing decreases the inherent colour(s) contained in food. For instance, the simple act of heating can destroy pigment molecules like anthocyanins that provide the attractive bright red and blue shades in fruits and berries (Laleh *et al.*, 2006).

Food colour is an additive in the form of any dye, pigment or any substance that is used to give foodstuff a more attractive look or impart colour. They come in numerous forms consisting of liquids, powders, gels and pastes. Some are natural colours, but most are artificial and may have toxic properties (Abdeldaiem, 2014; Eissa *et al.*, 2014). These additives are added to food

to replace or boost colour lost as a result of processing procedures such as exposure to light, temperature extremes, moisture and storage conditions.

Furthermore, they may be added to correct natural variations in food colour, to minimize batch variations in processing, enhance colours that occur naturally in food or provide colour to colourless and "fun" foods or make food more attractive, appetizing, informative and finally allow consumers to identify products on sight (Barrows *et al.,* 2009; Chaitanya, 2014; Saltmarsh and Insall, 2013). The importance of food colour additives cannot be over emphasized. For instance, without colour additives, colas would not be brown, margarine would not be yellow and mint ice cream would not be green (Katz, 2014). Colour additives are now recognized as an important part of practically all processed foods.

An increasing number of commercial colour additives, which are used as food colourants have been shown to exhibit genotoxic effects (Eissa *et al.,* 2014). Hence, the need for colour additive from natural sources, since the commercially available colour additives possess potential hazards to the human health (Kirti *et al.,* 2014; Chengaiah, *et al.,* 2011). Globally, there is an increasing demand towards the use of natural colour additives in food. Much awareness is created amongst consumers regarding natural products and adopting a more natural way of life. In recent times, it has been observed that people now prefer natural food, herbal medicines, natural curing practices and even organic farming i.e. without using chemical fertilizers and pesticides (Abdeldaiem, 2014; Kirti *et al.,* 2014). This is mostly due to the unbridled use of synthetic chemicals, colours, and derived products that has led to various human health hazards (Eissa *et al.,* 2014). As a result of the high demand of natural colourant in food and drinks, most food industries now replace synthetic colourants in their products with natural pigments (Kirti *et al.,* 2014).

Natural colourants for food are made from renewable sources. Nature is rich in colours obtained from fruits, vegetables, roots, minerals, plants, microalgae, and so forth, and due to their origin from biological materials they are often called "biocolours" (Pattnaik *et al*., 1997; Kirti *et al.*, 2014). Most often, the colourants are extracted from plant materials, but other sources such as insects, algae and fungi are used as well (Aberoumand, 2011; Abou- Arab, 2011).

Pepper (*Capsicum annuum)* is a typical example of a natural source of food colourant. It is used as spices in a broad variety of dishes in Nigeria and the entire world. Pepper belongs to the genus *Capsicum* with approximately 27 different species and to the *Solanaceae* family which includes the major types of chilli pepper. There are five domesticated species of pepper: the three most wide-spread are *C. annuum, C. frutescens, C. chinense,* together with *C. pubescens* and *C. baccatum* (Cantrill, 2008*).* The first to be introduced worldwide was *C. annuum,* originating from Mexico. It is divided into two categories: sweet (or mild) peppers and hot (or chilli) peppers. They are commonly called Chilli pepper, red or green pepper, or sweet pepper in Britain (Reddy and Sasikala 2013). The large wild form is called bell pepper in the U.S. and Canada (Reddy and Sasilaka 2013). It is called paprika in some other countries. In Nigeria, it is called "ose" in Igbo, "ata" in Yoruba and a host of names depending on the specie such as "*tattasai"*, "*atarugu", "borkonu"* in Hausa.

The fruits of *Capsicum* annum contain colouring pigments, pungent principles, resins, protein, cellulose, pentosans, mineral elements and very little volatile oil (Cantrill 2008). The paprika species contains dark-red colourful oil liquid, which can be used as a fine food colour with good mobility. This colour pigment is light-resistant, heat-resistant, acid-resistant, and alkali-resistant and will not be affected by metal ion (Reddy and Sasikala 2013). Capsaicin, the spicy chemical in peppers, has also been shown to be a potent anti-inflammatory *in vivo* (Sancho *et al*., 2002). In Nigeria, *Capsicum annuum* is used as a colour additive to give a bright reddish colour to food such as stew, *moi-moi* and *akara* made from ground beans and eaten in almost

every part of the country. The green specie of pepper is used as colourant to flavor foods like fried rice and vegetable salad. The pigments present in paprika are a mixture of carotenoids, in which capsanthin and capsorubin are the main compounds responsible for the red colour of the dye (Chaitanya, 2014; Chengaiah *et al.,* 2010).

As a result of the global increase in the demands for natural colour additives in food, this research therefore aims to extract the colour pigment and other constituents present in *Capsicum annuum*, as well as determine its quality and stability.

1.2 Statement of Research Problem

There has been a geometric increase in the demand for food colour globally over the last decade (Chaitanya 2014). In year 2000 the demand for food colour in the global market was 2400 metric tonnes and it increased to 3000 metric tonnes by the year 2005 and further to 8000 metric tonnes by the year 2010 and was expected to increase to 15000 metric tonnes by the year 2015 (Chaitanya, 2014).

The investment in natural food colour market worldwide has reached US $ 1 billion and is continuously growing as there is demand for natural food colours against synthetic food colours (Sahar *et al.*, 2012; Ree, 2006). Although there is no documented literature with respect to the demand for food colours in Nigeria, however, it is believed to be on the rise looking at the number of eateries, restaurants and commercial catering services.

Several reports have attributed the cause of cancer and other diseases of mankind to the synthetic chemicals used in food. Most of these chemicals exhibit genotoxic effects (Eissa *et al.,* 2014). The effect on health from the prolonged usage of synthetic and inorganic food colours on the quality of human health calls for urgent attention. Hence, the need for this research.

1.3 Justification

The advantages of using natural colourants are numerous as they are biodegradable, harmless, unsophisticated and harmonized with nature. They are obtained from renewable sources and their preparation involves a minimum possibility of chemical reactions (Abdeldaiem, 2014; Chengaiah, 2010). By and large, natural colourants do not cause health hazards; on the contrary, they sometimes act as health cures like turmeric and annatto (Abdeldaiem, 2014).

There is a growing need for natural colours to be used in foods, pharmaceuticals, cosmetic products and so on (Aberoumand , 2011; Chengaiah , 2010) Consumers tend to favour a more natural way of life because of health challenges posed by the excessive use of artificial or synthetic colours (Blanc, 2009; Chen and Wu 2009).

The use of natural dyes dates back to 2600 BC in China (Chaitanya, 2014) although archeologists believe the use of food colour dates back to 1500BC (Burrows 2009; McKone 1995). Addition of colourants to foods is reported in Europe during the Bronze Age (Madhava Naidu and Sowbhagya 2012; Chaitanya, 2014). Since 1500 BC, the Egyptian cities candy makers have been known to make use of natural extracts and wine to improve the appearance of food (Madhava Naidu and Sowbhagya 2012; Chaitanya, 2014).

Marketing strategy of food by major manufacturers is greatly influenced by colour (Sharma 2013), since human appetites are greatly influenced and stimulated by colour (Abou-Arab, 2011; Malik *et al*., 2012). Colour may sometimes discourage eating certain foods and diminish the desire for that food hence over the last 2 to 3 decades many food industries ensure that their food is not only tasty, but visually appealing. This brings out the importance of colour as it relates to food choices (Betina, *et al.*, 2012).

1.4 Aim and Objectives

1.4.1 Aim

To assess the quality and stability of the ethanol extracts of bell and sweet pepper for application as food colourant

1.4.2 Specific Objectives

The aim will be achieved through the following objectives:

1. Extraction of the colour pigments and their constituents from two varieties of *Capsicum annum var. conoides miller* (bell pepper) and *var.longum nigrum* (sweet pepper)
2. Determination of the yield of the extracts.
3. Evaluation of the stability of the extracts.
4. Determination of the functional groups present in the extracts using FTIR.
5. Evaluation of some of the nutritional components of the extracts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Background of Food Colour

The addition of colour to food is thought to have been used since 400 BC in Egypt where natural extracts were used to colour candy and wine (Dowham and Collins, 2000). In ancient Greece and Rome, wine was often artificially coloured and inspectors were appointed by the government to monitor this practice, as harmful substances found their way into food (McKone,1995). At a point, it was almost impossible to find any food, drink or medicine that had not been extensively contaminated. For example, cod liver oil was adulterated, almost to substitution with train oil mixed with iodine. Yellow tinged milk, coloured with lead (II) chromate ($PbCrO_4$), was so common that people refused to purchase white milk, thinking that the latter had been doctored (McKone 1995).

It has been said that we eat first with our eyes (Matulka and Tardy 2014) hence a person's appetite is dampened or stimulated by his perception of food presented to him which is influenced to a great extent by the blend of colour(s) in his plate (Dowham and Collins 2000). This goes to show the importance of the appearance of what we eat in other words, the colour. The colour of food gives a hint as to its flavor (as people associate different colours with different foods) and possibly its edibility in terms of freshness or decay (Abdeldaiem, 2014; Chaitanya, 2014; Malik *et al.,* 2012). According to food researchers, when early humans searched for food, they had to learn to avoid toxic or spoiled objects. Colour was the most readily accessible clue and such inedible items are often blue, black, or purple (Burrows, 2009). Sometimes, colour is used to enhance visual appeal and match consumers expectations. Natural food colourants have been in use in Japan in the Shosoin text of the Nara period (8^{th} century), which makes mention of colouring soybean and adzuki-bean cakes (Aberoumand 2011;

Rymbai *et al.*, 2011). The earliest written record of the use of natural dyes dates back to 2600 BC in China and addition of colourants to foods is reported in Europe during the Bronze Age (Chaitanya, 2014).

These colours from natural sources such as plants, animals and mineral sources had their own draw backs such as light, heat and pH instability (Chaitanya, 2014).

2.2 Classification of Food Colours

Food colours are classified into: Natural colours, Nature-identical colours, Synthetic and Inorganic colours (Abdeldaiem, 2014; Mortensen, 2006).

Natural food colours are extracted from edible food products (Chowdhury *et al.*, 2010) such as fruits, vegetables, seeds, roots, barks and also from microorganisms which are sometimes called biocolours (Chaitanya, 2014; Madhava Naidu and Sowbhagya, 2012). Plant pigments, by virtue of their natural occurrence in edible plants, are generally considered to be harmless (Abdeldaiem, 2014; Chen and Wu 2009) Nature produces a variety of brilliant coloured pigments viz., water soluble anthocynanins, betanins and fat soluble pigments like carotenoids and chlorophylls used for colouring foods.

Drawbacks of natural colours according to Sharma, (2014) includes;

i. Most sources of natural colours are seasonal in production – sources of natural colour are not available throughout the year, they are seasonal.
ii. Variation in quality and purity from source to source - Samples are usually collected from several sources with different growing conditions which in turn influences the quality and purity of the final product.
iii. Availability in limited shades -Natural colours do not have a wide range of shades like the synthetic colours. This has limited its use as consumers desire a wide range of colours to choose from.

iv. Low concentration in source material - A large quantity of source material is required to extract a reasonable quantity and quality of colour.

v. Difficulties in extraction from the source - The extraction procedure is most at times very tedious and time consuming.

vi. Instability during storage and use - Natural colours are susceptible to moisture, pH, temperature, oxidation, effects of light, storage conditions.

vii. High cost - The cost of processing natural colourants is very exorbitant as enough material has to be sourced and this will cost money.

Nature - identical colours are exactly the same molecules found in natural sources but they are made synthetically. Most of them dissolve in oil but do not dissolve in water. They are used in foods after processing them into their sodium or potassium salts. The main chemical classes are: flavanoids, indigoid and carotenoids (Delgado-Vargas *et al.*, 2000)

Synthetic colours are derived from chemical synthesis. They have been favoured for over the past 100 years. Examples are azo dyes, such as amaranth (colour for black currant jams), quinoline, indigoid (Mortensen 2006)

Inorganic colours - Examples are titanium dioxide, gold, silver, ultramarine (Aberoumand 2011)

2.3 Uses of Colour in Food and Drinks

Colour additives are added for one or more of the following reasons: (IFIC, 2010; Madhava Naidu and Sowbhagya., 2012; Rymbai, *et al*., 2011).

i. Offsetting colour loss due to light, air, extreme temperature, moisture and storage conditions. Natural colours are affected by the above conditions which lead to colour loss.

ii. Masking natural variations in colour-Samples from different sources will ultimately lead to variations in colour and a colour additive will help unify the various shades.

iii. Enhancing naturally occurring colours - A naturally colourful food can be further enhanced by adding colour from a natural source.

iv. Providing identity to foods – Most foods are identified by their colours. For example, colas are brown, mint is green, strawberry and vanilla have a distinct pink and yellow colour respectively. The colour of food also gives an idea as to how fresh or stale it is.

v. Protecting flavors and vitamins from damage by light – When foods are exposed to light, oxidation takes place which leads to colour fading.

vi. Decorative or artistic purposes – Foods like fried rice would not be attractive without the interplay of colours.

vii. Increase appetite appeal – the appearance of food increases or reduces ones desire for that particular food.

viii. To make less desirable food more desirable, Burrows (2009) recorded that in the 1^{8th} century, coffee and tea makers often took used or fake leaves, treated and coloured and sold them as genuine and new. He also stated that vegetable substances preserved in a state called pickles whose sale depended greatly on a fine lively green colour are sometimes impregnated with copper.

ix. To mask defects – Processing and storage of food sometimes leads to defects. Example burns from frying, baking etc

x. Additives may keep certain foods tasting fresher for long a time.

2.4 Pepper (*Capsicum annuum* L)

Pepper was in antiquity, a commodity paid for in gold and sometimes regarded as currency in its own right. It was regarded as a prized possession in the middle ages that a cleric named

Bede distributed it to his brethren on his death bed (Adamson 2004). Pepper is an important agricultural crop not only because of its economic importance but also due to the nutritional and medicinal value of its fruits as well as being an excellent source of natural colour and antioxidant compounds (Sanusi and Ayinde 2013; Howard *et al.*, 2000). It is the world's second important vegetable, ranking after tomatoes (Benson *et al.,* 2014) and it is the most produced type of spice flavouring and colouring for food while providing essential vitamins and minerals.

The genus *Capsicum*, which originates from Central and Southern America, belongs to the Solanaceae family and includes major types of chilli peppers. It includes all the peppers, from the mildest bell pepper to the hottest habanero. The first to be introduced worldwide was *C. annuum*, originating from Mexico. It is divided into two categories: sweet (or mild) peppers and hot (or chilli) peppers. The genus Capsicum has approximately 27 species, *Capsicum annuum,* which is cultivated mainly as a vegetable is the most widespread. Pepper is largely cultivated in most parts of Nigeria with the North as the major area of production (Sanusi and Ayinde 2013; Business day, 2007; Erinle, 1989). The fruits of capsicum plants have a variety of names which depend on place and type. They are commonly called chilli pepper, red or green pepper, or sweet pepper in Britain. The large wild form is called bell pepper in the U.S. and Canada. They are called paprika in some other countries (Mortensen, 2006).

2.4.1 Bell pepper

The bell pepper is a cultivar group of the species *Capsicum annuum* belonging to the night shade family. Cultivars of the plant produce fruits in different colours which include red, yellow, orange, green etc. They grow as annuals in temperate regions although they are perennials and are sensitive to low temperatures and can be grown commercially or in home gardens (Tuquero and Bamber 2016). They can be eaten as vegetables e.g in salads and

sandwiches, either raw or cooked and are also an essential ingredient in preparing stews, sauces, “moi-moi”.

There are numerous health benefits of bell pepper. They are rich in Vitamin C, Vitamin A, and B complex group of Vitamins including Vitamin B1 and Vitamin B6.

2.4.2 Sweet pepper

This belongs to *Capsicum annuum* variety longum nigrum which is peppery in nature. It is slender and not as fleshy as bell pepper. The spice “paprika” is derived from this variety of capsicum annuum and is used as a spice and colouring agent.

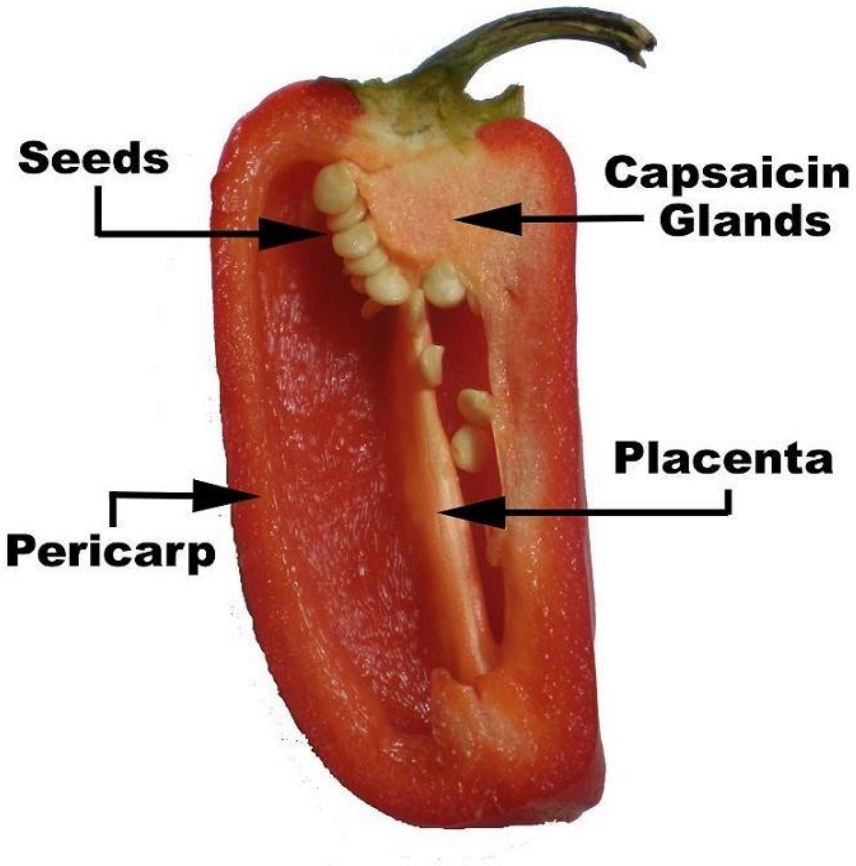


Plate 2.1: A Cross Section of Bell Pepper Fruit Showing Seeds, Placenta, Pericarp and Capsaicin Glands

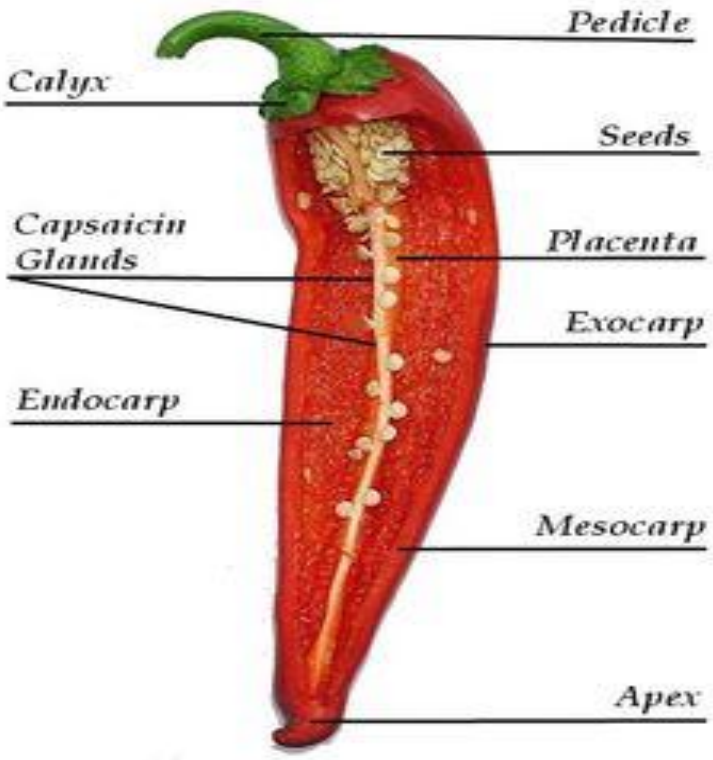


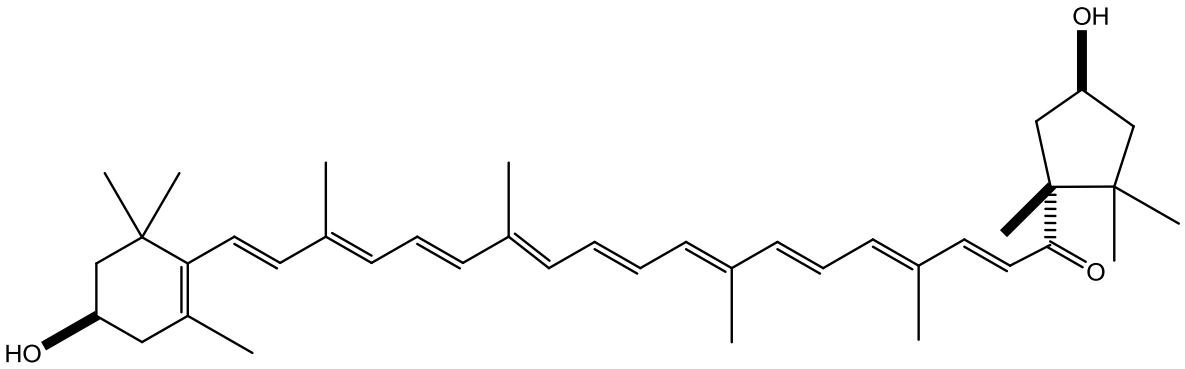
Plate 2.2: A Cross Section of Sweet Pepper Showing Pedicle, Seeds, Placenta, Calyx, Endocarp, Exocarp and Capsaicin Glands

2.5 Uses of Pepper

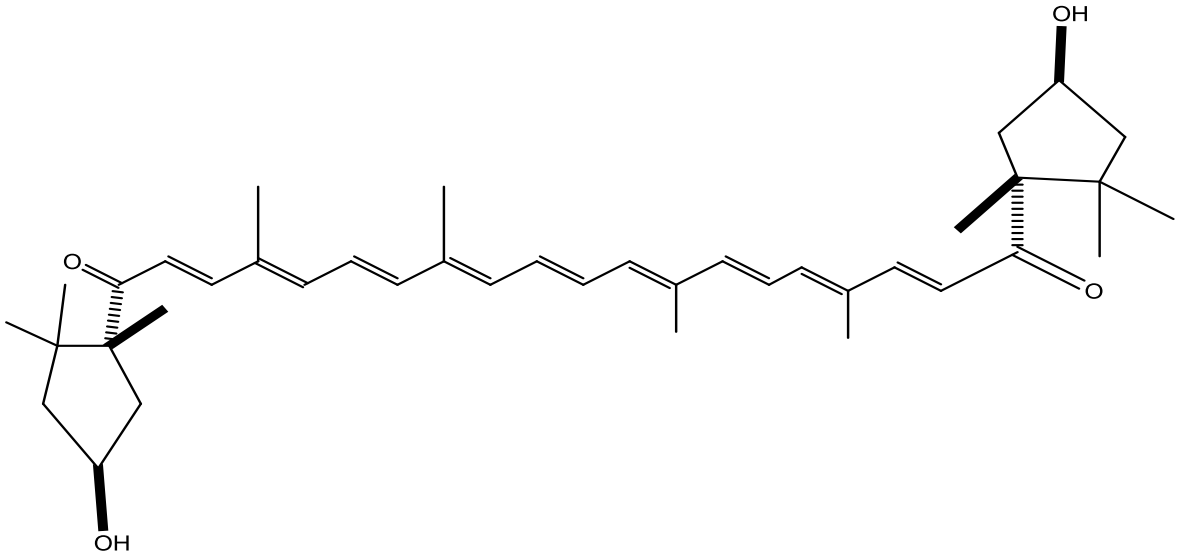
Pepper is used as an ingredient in a broad variety of dishes throughout the world. It is an ingredient that is mixed with meats and other spices. In the United States, pepper is frequently sprinkled on foods as a garnish, but the flavour is more effectively produced by heating it gently in oil.

For centuries in South America, peppers have been used to treat such ailments as gastrointestinal disorders, and it is also thought to benefit those suffering from circulatory diseases. Capsaicin, the spicy chemical in peppers, has also been shown to be a potent anti-inflammatory in vivo (Sancho *et al.,* 2002). In Nigeria, it is used to give a reddish colour to food such as stews, sauces, jollof rice, *moi-moi* and *akara* made from ground beans and eaten in almost every part of the country. The green specie of pepper is used to flavour food such as fried rice and also used in vegetable salad preparation. The pigments present in paprika are a mixture of carotenoids, in which capsanthin and capsorubin are the main compounds responsible for the red colour of the dye (Chengaiah *et al.,* 2010).

The red colour of red pepper is due to the presence of carotenoids, mainly capsanthin, capsorubin (Structure 2.5 i and ii) Other carotenoids zeaxanthin and cryptoxanthin, and the yellow colours are violalaxanthin, cucurbitaxanthin A, cis-zeaxanthin, criptoxanthin, carotene and cis-α-carotene (Inanc *et al.,* 2010). The bright colours of carotenoids serve as powerful visual attractants for birds and insects, which assist in the dispersal of pollen and seeds aiding plant reproduction (Cazzonelli, 2011). The red pepper fruit (*Capsicum annuum*) has been used for a very long time to add colour to food making it attractive, as a spice, a vegetable, and flavour enhancer.



(i) Capsanthin



(ii) Capsorubin

2.6 Carotenoids

Carotenoids are natural isoprenoid pigments that give leaves, fruits, vegetables and flowers their distinctive yellow, orange and some reddish colours as well as several aromas in plants (Cazonelli 2011). Their bright colours attract insects for pollination and seed dispersal. Carotenoids comprise a large family of C-40 polyenes (Biehler *et al.*, 2010; Rao and Rao 2007) They are synthesised by photosynthetic organisms, aphids, some bacteria and fungi. Carotenoids are commercially important in agriculture, food, health and the cosmetic industries. In plants, carotenoids are essential components required for photosynthesis, photo protection and the production of carotenoid-derived phytohormones.

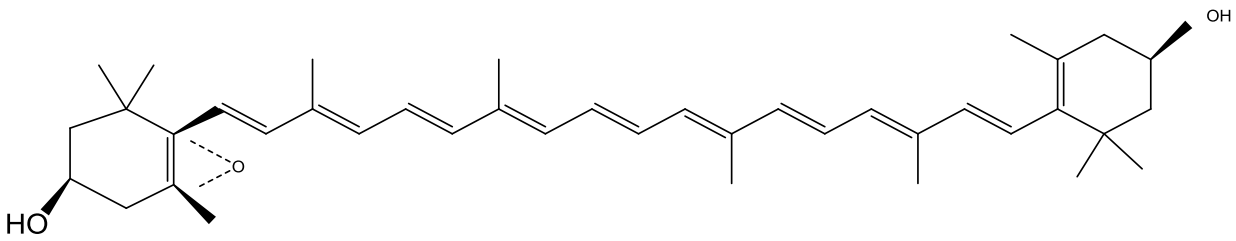
One of the most important classes of plant pigments that play a fundamental role in defining the quality parameters of fruit and vegetables are carotenoids (Eldahshan and Singab 2013). Rao and Rao, (2007) had reported that high consumption of carotenoids leads to lower risk of cancer and cardio-vascular diseases. Consequently, carotenoids have been a subject of research interest as potential antioxidants. The different colours of paprika such as yellow, orange and red originate from carotenoids and are synthesized during ripening (Mortensen, 2006; Rao and Rao 2007). The number of naturally occurring carotenoids has continued to be on the increase and so far more than 600 have been identified in nature (Eldahshan and Singab 2013; Rao and Rao 2007). Although, Mortensen (2006) reported that 750 structures have been identified.
Carotenoids are found mostly in plants, algae and photosynthetic bacteria, where they play a critical role in the photosynthetic process. They also occur in some non-photosynthetic bacteria, yeasts, and molds, where they may carry out a protective function against damage by light and

oxygen (Eldahshan, and Singab 2013). They are responsible for a lot of the red, orange and yellow hues of plant leaves, fruits and flowers, as well as the colours of some birds, insects, fish and crustaceans. Some familiar examples of carotenoid colouration are the oranges of

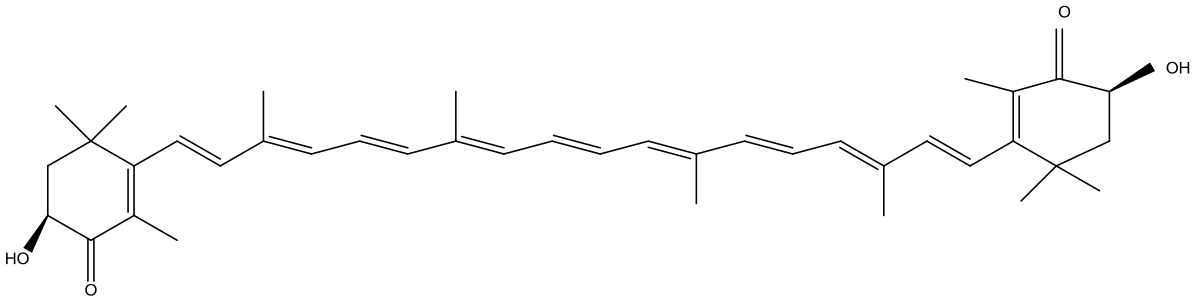
carrots and citrus fruits, the reds of peppers and tomatoes and the pinks of flamingos and salmon (Eldahshan and Singab 2013). Animals are not able to synthesize carotenoids, hence they obtain their carotenoids from their food, selectively or unselectively absorbed and accumulated unchanged or modified slightly into typical animal carotenoids (Rodriguez-Amaya, 2001; Cazzonelli, 2011).

2.6.1 Classification of carotenoids

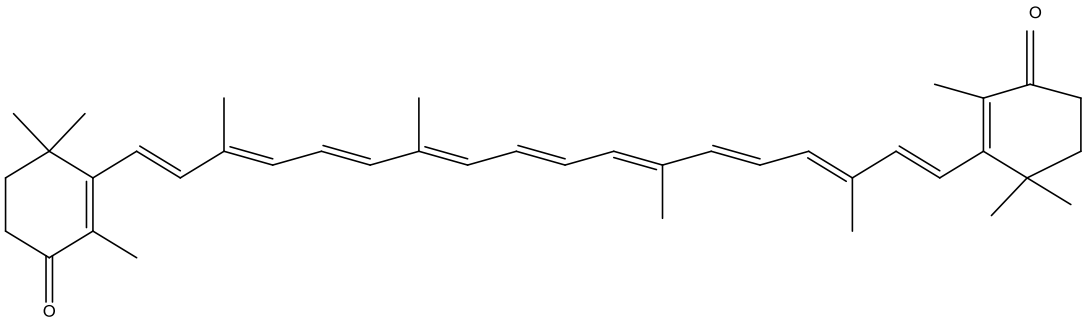
Carotenoids are classified based on the structure as shown below: The hydrocarbon carotenoids containing only carbon and hydrogen are known as carotenes example β-carotene and the oxygenated carotenoids which are derivatives of these hydrocarbons known as xanthophylls, examples of these compounds are zeaxanthin and lutein (hydroxy), spirilloxanthin (methoxy), echinenone (oxo), and antheraxanthin (epoxy). Carotenoids are lipid-soluble C40 tetraterpenoids. The majority carotenoids are derived from a 40-carbon polyene chain, which could be considered the backbone of the molecule. This chain may be terminated by cyclic end-groups (rings) and may be complemented with oxygen-containing functional groups. Below are structures of some carotenoids.



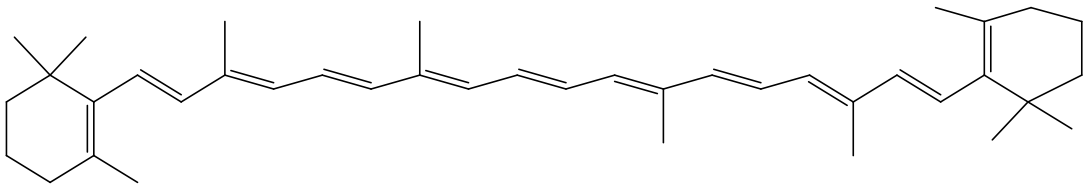
(iii) Antheraxanthin



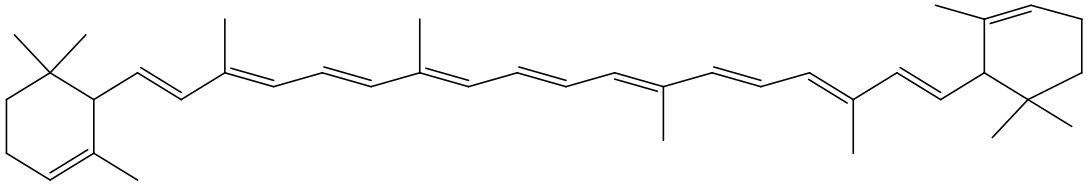
(iv) Astaxanthin



(v) α-Carotene



(vi) ß-Carotene



(vii) ε-Carotene

2.6.2 Factors influencing carotenoid composition

The following factors affect the amount of carotenoids in food:

i. Cultivar or variety- Differences in cultivar or variety can be only in terms of the quantitative composition, because essentially the same carotenoids are found in the different varieties (Rodriguez-Amaya, 2001).

ii. Part of the plant consumed- Carotenoids are not evenly distributed in the plant itself. Various investigators, for example, found that carotenoids are usually more concentrated in the peel than in the pulp of fruits and fruit vegetables (Rodriguez-Amaya, 2001).

iii. Stage of maturity - How mature the pepper is has an effect on the amount of carotenoids present. Carotenoids are more in ripe pepper than in unripe ones.

iv. Climate or geographic site of production - Pepper requires light soil such as sandy loam, well drained, rich in organic matter and with a pH between 6.5-7.5.

v. Harvesting and postharvest handling - Carotenoid losses during post harvest handling were recorded in some vegetables especially leaves. These losses were more pronounced under conditions favourable to high temperature, light exposure and wilting.

vi. Processing and storage - Carotenoids are prone to isomerization and oxidation during processing and storage, this leads to loss of colour and biological activity and the formation of volatile compounds that impart desirable or undesirable flavour in some foods. Oxidation depends on the presence of oxygen, metals, enzymes, unsaturated lipids, prooxidants, or antioxidants; exposure to light; type and physical state of carotenoid present; severity of the treatment (i.e., destruction of the ultrastructure that protects the carotenoids, increase of surface area, and duration and temperature of heat

treatment); packaging material; and storage conditions. Heating promotes *trans-cis* isomerization (Gross 1991, 1987 in Rodriguez-Amaya, 2001).

2.6.3 Functions of carotenoids

Carotenoids help in the enhancement of immune system function. This is done by the body converting carotenoids to vitamin A which helps in a healthy immune system protecting the body against invaders like bacteria and viruses (Thurnham, 1994) Different types of cancers can also be inhibited by carotenoids. Lycopene a carotenoid present in tomatoes has been proven to be effective in the prevention or reduction of several kinds of cancer (Morais, 2002). Studies have shown that there is an inverse relationship between the consumption of tomatoes and the risk of prostrate, breast, cervical, ovarian, liver and other organ sites cancers (Rao and Rao 2007). Prevention of age related macular degradation of the eye is ensured by two carotenoids Zeaxanthin and Lutein.

Carotenoids also ensure decreased risk of cataract formation, prevention of cardiovascular diseases and provitamin A activity. Quite a number of research has been carried on the processing, extraction, preserving of *capsicum annuum.*

Seliem *et al.,* (2015) extracted oleoresin from waste pepper fruits by supercritical fluid CO_2, evaluated the image of the extracts and studied the physical properties of both the extracts and its microencapsulated form. Other workers investigated the effects of Ultra violet radiation on some antioxidant compounds and enzymes in *capsicum annuum* L. Another method developed by some other researchers used fresh red peppers without drying, and ethanol was the solvent used for stage extraction of capsaicinoids and red colourants (Dong *et al.,* 2014). Minguez-Mosquera *et al,* (2008) have worked extensively on colour quality in red pepper (*Capsicum annuum* L) and derived products. Solvent extraction using different solvents and quality parameters of the extracts were discussed. Tepic and Vujicic (2004) studied the colour change

in pepper (*Capsicum annuum*) during storage and established a correlation between ASTA and apparent colour of the pulverized pepper.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

The following materials were used in this work:

(i) Weighing balance

(ii) Beakers

(iii) Pepper samples

(iv) Spatula

(v) Digital weighing balance

(vi) pH Meter

3.1.1 Reagents

All Reagents used were of Analytical grade.

(i) Sodium hydroxide pellets

(ii) Boric Acid

(iii) Hydrochloric Acid

(iv) Tetraoxosulphate (VI) Acid

3.2 Methods

3.2.1 Preparation of Reagents

3.2.1.1 Preparation of 1M HCl

Hydrochloric acid (1mol dm^{-3}) was prepared by transferring 20.9 cm^3 of the concentrated acid into a beaker containing 30 cm^3 of distilled water. The contents were properly stirred and quantitatively transferred into a 250 cm^3 volumetric flask and made up to mark with distilled water.

3.2.1.2 Preparation of 1M NaOH solution

To make a 1 M solution of sodium hydroxide, 40 g sodium hydroxide was added slowly to 500 ml distilled water in a 1000 ml volumetric flask. When all the solid is dissolved and the solution is at room temperature, it is made up to mark the flask inverted several times to mix.

3.2.1.3 Preparation of 0.25M H_2S0_4

The preparation of 0.25M H_2SO_4 was carried out by measuring 3.4 cm^3 of sulphuric acid into a beaker containing 30cm^3 of distilled water. The contents were stirred and quantitatively transferred into a 250 cm^3 volumetric flask and made up to mark with distilled water.

3.2.1.4 Preparation of 0.31M NaOH

Sodium hydroxide (0.31mol $dm^{-}3$) was prepared as follows: 3.1g of the salt was weighed into a beaker, 30cm^3 of distilled water was used to dissolve the salt. The contents were stirred and quantitatively transferred into a 250cm^3 volumetric flask and made up to mark with distilled water.

3.2.1.5 Preparation of 2% (v/v) boric acid

The preparation of 2% boric acid was carried out by measuring 2cm^3 of the acid using a measuring cylinder into a beaker. About 30ml of distilled water was added to the beaker, the contents of the beaker were shaken to attain homogeneity. This was quantitatively transferred into a 100ml volumetric flask and made up to mark.

3.2.1.6 Preparation of 4% (v/v) boric acid

Boric acid (4%) was prepared by measuring 4cm^3 of the acid with a measuring cylinder into a beaker; 30cm^3 of distilled water was added to properly mix the solution. The contents of the beaker were quantitatively transferred into a 100cm^3 volumetric flask and made up to mark.

3.2.1.7 Preparation of 40% (w/v) NaOH solution

Sodium hydroxide 40% was prepared by weighing 40g of sodium hydroxide salt into a beaker, about 30cm of distilled water was added to it to dissolve the salt. The contents of the beaker were quantitatively transferred into a 100cm^3 volumetric flask, and made up to mark with distilled water.

3.2.1.8 Preparation of 0.1M NaOH Solution

The preparation of 0.1M NaOH, was carried out by weighing 0.4g of NaOH into a beaker, about 30mls of distilled water was added to it to dissolve the salt. The contents of the beaker were quantitatively transferred into a 100cm^3 volumetric flask, and made up to mark with distilled water.

3.2.1.9 Preparation of 0.1M HCl

The preparation of 0.1M HCl was carried out by diluting 0.8cm^3 of HCl (specific gravity 1.18, % purity 37) in 30cm^3 of distilled water in a beaker and the contents were quantitatively transferred into a 100ml volumetric flask and made up to mark with distilled water.

3.2.1.10 Preparation of 0.25M HCl

The preparation of 0.25M was carried out by diluting 2.06cm^3 of HCl (specific gravity 1.18,purity 37%) in 30cm^3 of distilled water in a beaker and the contents were quantitatively transferred into a 100cm^3 volumetric flask and made up to mark with distilled water.

3.2.1.11 Preparation of ascorbic acid

In other to prepare 0.1%, 0.5% and 1% of ascorbic acid solution, 0.1g, 0.5g and 1g ascorbic acid salt were weighed respectively into clean beakers and dissolved with distilled water. This was then transferred quantitatively into a 100cm^3 volumetric flask and made to mark using distilled water.

3.3 Collection and Preparation of Pepper Sample

Ripe, healthy looking and fresh bell and sweet pepper samples were bought from several selling points in the dry season (December, 2015) from Samaru Market, Zaria. They were selected to remove the bad parts. The samples were identified and authenticated at the Herbarium of the Biological Sciences Department of Ahmadu Bello University, Zaria. The fresh pepper samples were washed to remove adhering soil and dirt, deseeded and the stalk removed. They were weighed and sliced into thin pieces and shade dried for two weeks. After drying, the samples were pulverized separately using a blender. The blended samples (paprika) were stored in amber bottles until they were to be used.

3.3.1 Extraction of colour and other constituents

The constituents of the pepper samples were extracted according to the method described by Chen and Wu, (2009). Briefly, pepper sample (70g) was extracted with the aid of a soxhlet extractor using ethanol at 78^{o}C for six (6) hours for bell pepper and nine (9) hours for sweet pepper. The ethanol extract was concentrated using a rotary evaporator at 40^{o}C. It was then air dried and the percentage yield calculated using equation 3.1

$$\% \text{ yield of pepper sample} = \frac{\text{Weight of capsicum red pigment(g)}}{\text{total mass of capsicum powder(g)}} \times 100 \qquad (3.1)$$

3.3.2 Stability Measurements of Extracts

3.3.2.1 Evaluation of stability to pH

This was carried out with slight modification to the method of Chen and Wu, (2009) where the initial pH of the extract of 4.5 was adjusted using 0.1M HCl and 0.1M NaOH to pH 3.0, 5.0 and 7.0. The extracts were kept in amber coloured bottles under white light, UV light and in the dark and the pH of each extract was measured weekly using pH metre.

3.3.2.2 Evaluation of stability to light

The extract was tested for its stability to light as follows: Vials containing the extract were exposed continuously to the direct light of a white fluorescent lamp (20 W) at room temperature, another set were put in UV chamber (254 nm) and a third set of vials were put in a dark cupboard. Absorbance at 460nm was taken weekly for eight weeks.

3.4 Evaluation of the Nutritional Components of the Extract

3.4.1 Determination of total ash

The AOAC (1980) method was used. The porcelain was dried in an oven at 100^0C for 10 minutes, cooled in a dessicator and weighed (W_1). Two grams sample was placed into the previously weighed porcelain crucible and reweighed (W_2). It was first ignited and then transfered into a furnace, which was then set at 550^0C. The sample was left in the furnace for eight hours to ensure proper ashing. The crucible containing the ash was then removed,cooled in the dessicator and weighed (W_3). The percentage ash content was calculated using equation 3.2

$$\% \text{ Ash Content} = \frac{W_3 - W_1}{W_2 - W_1} \times 10 \qquad (3.2)$$

3.4.2 Determination of crude fibre

The method described by AOAC (1980) was used. Two grams (2g) of sample was weighed out into a round bottom flask, 100cm^3 of 0.25M sulphuric acid solution was added and the mixture boiled under reflux for 30 mins. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it was acid free. It was quantitatively transfered into a Buckner flask and 100 ml of hot 0.31M sodium hydroxide solution was added and mixture boiled again under reflux for 30 mins and quickly filtered under suction. The insoluble residue was washed with boiling water until it was base free. It was dried to constant weight in the oven at 100^{o}C, cooled in a dessicator and weighed (C_1).

The weighed sample (C_1) was then incinerated in a muffle furnace at 550^0C for 2 hours, cooled in a dessicator and reweighed (C_2).

The loss in weight on incineration = $C_1 - C_2$. The calculation was carried out thus;

$$\% \text{ Crude fibre } = \frac{C_1 - C_2}{\text{Weight of original sample}} \times 100 \qquad (3.3)$$

3.4.3 Moisture content

The method described by AOAC (1980) was adopted. A clean crucible was dried to a constant weight in an air oven at 110^0C, cooled in a dessicator and weighed (W_1). Two grams of sample was accurately weighed into the previously labeled crucible and reweighed (W_2). The crucible containing the sample was dried in an oven to constant weight (W_3). The percentage moisture content was calculated thus:

$$\text{Moisture Content} = \frac{W2 - W3}{W2 - W1} \times 100 \qquad (3.4)$$

3.4.4 Total nitrogen

The method of AOAC,(1980) was used. Exactly 1.5g of the ground defatted sample in an ashless filter paper was dropped into 300ml kjeldahl flask. Twenty five millilitres conc H_2SO_4 and 3g of digesting mixed catalyst (weighed separately into ashless filter) was also dropped into the kjeldahl flask. The flask was then transferred to the Kjeldahl apparatus. The sample was digested until a clear green colour was obtained.

3.4.5 Distillation of the Digest

The digest was transferred to a 500cm^3 kjeldahl flask containing anti-bumping chips. Distilled water (250cm^3) was added (rinsing the 300cm^3 flask into the 500cm^3 flask with part of the 250ml water). About 70 – 120ml of 40% NaOH was slowly added by the side of the flask and then followed by 3 drops of 1% phenophthalein indicator. A 500cm^3 conical flask containing

a mixture of 125cm^3 (4%) boric acid and 4 drops of mixed indicator was used to trap the ammonia being liberated.

The conical flask and the kjeldahl distillation apparatus, with the tubes inserted into the conical flask and the kjeldahl flask. The distillation was carried out until 125ml of the distillate was trapped in the boric acid solution (to make a total volume of 250ml). The distillate was then titrated with 0.1M HCl.

$$\%\mathrm{N} = \frac{0.014 \times \mathrm{M} \times \mathrm{V} \times 100}{\text{weight of smple}} \qquad (3.5)$$

% Crude protein = % nitrogen (N_2) × 6.25

Where M = actual molarity of HCI acid, V = volume of HCl used

3.4.6 Determination of total carbohydrate

The total carbohydrate content was determined by difference. The sum of the percentage moisture, ash, crude lipid, crude protein and crude fibre was subtracted from 100 (Muller and Tobin, 1980). Carbohydrate is calculated by difference using the formula below.

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

3.5 Determination of Functional Groups in Extracts using FTIR Spectrometry

The FTIR spectra were recorded on a Shimadzu Fourier Transform Infrared (FTIR) Spectrometer 8400S. The principle of FTIR is absorption. Most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The ratio of the sample spectrum to the background spectrum is directly related to the sample's absorption spectrum. The sample was mixed with KBr and then ground to a homogenous blend and compressed to give a small transparent disc of 13 mm diameter. A background spectrum of pure KBr was run before the spectrum of any sample to account for any absorption due to impurities in the KBr. The disc containing the sample was

then placed in the infrared spectrometer and the spectrum obtained. A total of twenty (20) scans were collected for each measurement over the spectral range of 4000-400cm^{-1} with a resolution of 4cm^{-1}. All spectra were presented without baseline correction or normalization.

3.6 Extractable Colour Measurements in ASTA Units

The extractable colour measurements were carried out according to the method described by Vracar *et al.*, (2007) by weighing 0.07-0.11g paprika powder and transferred to a graduated 100 cm^3 flask. Acetone was then added to the mark. The mixture was homogenized and kept in the dark for 4 hours. An aliquot of the transparent decanted extract was taken and the absorbance was recorded at 460 nm, acetone was used as blank and ASTA colour units calculated using equation 3.6

$$\text{ASTA } 20 = \frac{A \times 164}{m} \text{ x } I_f \qquad (3.6)$$

Where

A = is the absorbance of acetone extract

I_f = is the correction factor for the instrument calculated from a standard solution of potassium dichromate.

M = is the mass of sample weighed.

International Colour Units (ICU) is calculated as

$$\text{ASTA} \times 40 \qquad (3.7).$$

3.7 Statistical Analysis

Experimental data was generated using Design Expert 7.0 Statistical software (Stat-Ease Inc. USA). Design of Experiment is a systematic series of tests in which purposeful changes are made to input factors so that causes for significant changes in the output response may be

identified. It studies multiple factors changing at once (parallel processing) and accounts for interactions between variables thereby maximizing information with minimum runs. A two level three factorial (2^3) design was adopted in this work to measure the interactions between the three factors of light (white light, UV light and in the dark), pH and percentage of ascorbic acid for eight weeks.

CHAPTER FOUR

4.0 RESULTS

4.1 Yield of Extracts

Figure 4.1 shows the results of the ethanolic extraction, bell pepper had a yield of 77.5% while sweet pepper had a yield of 79.5% .

4.2 Stability Studies of the Extracts.

4.2.1 Bell pepper

Figure 4.2 shows a plot of pH against duration (in weeks) of bell pepper extracts stored under white light, in the dark and under UV.

Figure 4.3 shows the 3D plot for factor interaction between time and stabilizer (ascorbic acid) against response (absorbance) for bell pepper extracts keeping the pH and light condition constant.

The coded equation for the model is given as:

$$R(Dt) = +0.95 - 0.016A - 0.13B - 0.066C1 - 0.037C2 - 0.70D + 0.40 + 0.36BC1 - 0.48BC2 + 0.12BD$$

Where:

A = pH

B = Stabilizer

C = Photo (Light, Dark, UV)

D = Time (weeks)

Fig 4.4 shows the 3D plot for factor interaction between stabilizer (ascorbic acid) and pH against response (absorbance) for bell pepper extracts keeping the time and light conditions constant.

Fig 4.5 shows the 3D plot for factor interaction between pH and time against response (absorbance) for bell pepper extracts keeping stabilizer (ascorbic acid) and light condition constant.

The results of the interaction are shown on Appendix II.

Fig 4.6 shows the spectrum of bell pepper extract in the visible region showing wavelength of maximum absorbance.

4.2.2 Sweet pepper

Fig 4.7 shows a plot of pH against duration (in weeks) of sweet pepper extracts stored under white light, in the dark and under UV.

Figure 4.8 shows factor interactions between time (in weeks) and stabilizer (ascorbic acid) against the response (absorbance) for sweet pepper.

The coded equation for the model is given as:

$$R(DS) = +0.49 - 0.22A - 0.085B - 0.055C1 + 0.017C2 - 0.21D - 0.32AB + 0.20AC1 + 0.18AC2 + 0.096AD + 0.063BC1 + 0.030BC2 + 0.083BD + 0.063C1D + 0.084C2$$

Where:

A = pH

B = Stabilizer

C = Photo (Light, Dark, UV)

D = Time (weeks)

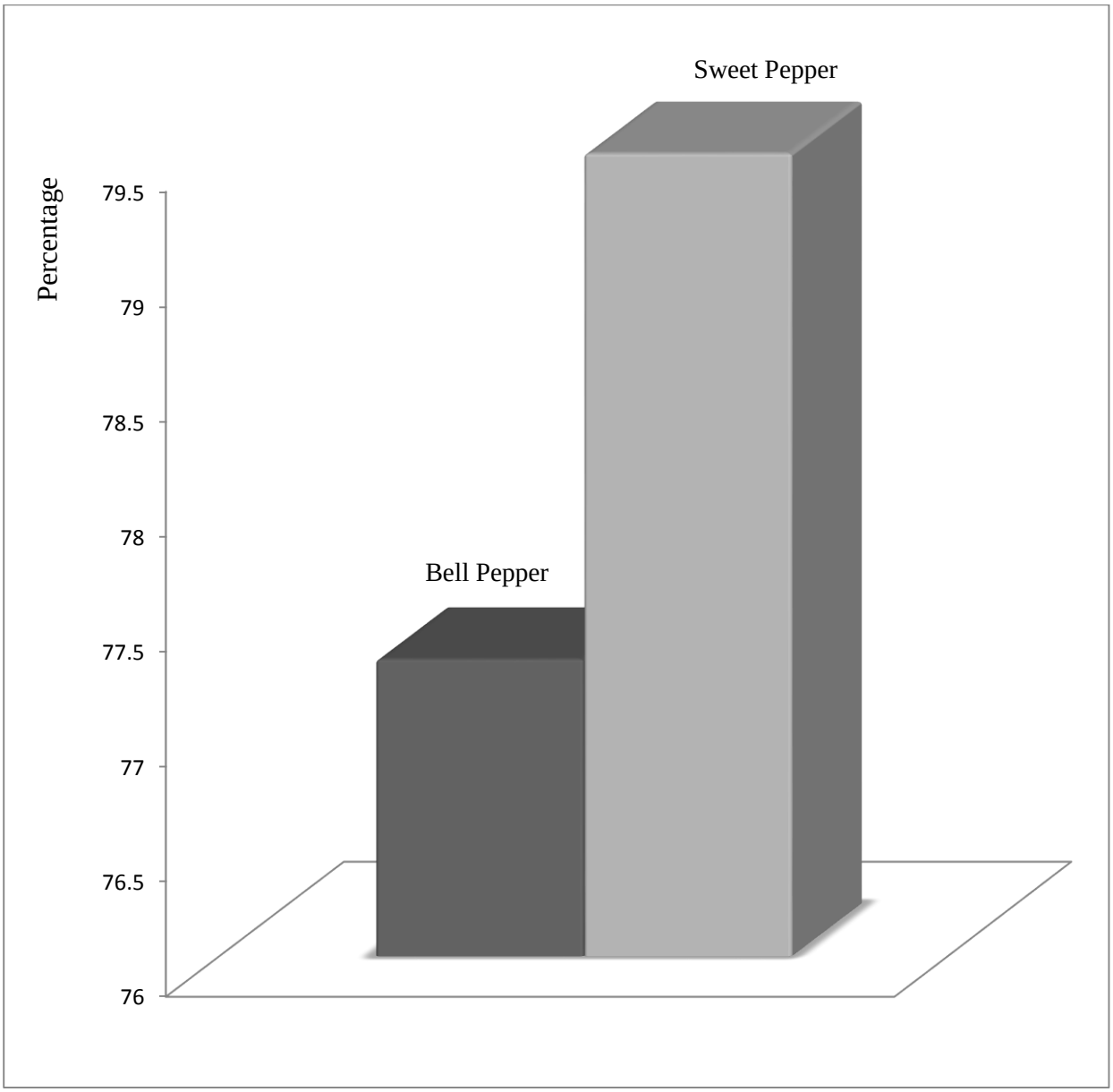


Figure 4.1: Percentage Yield of the Pepper Extracts.

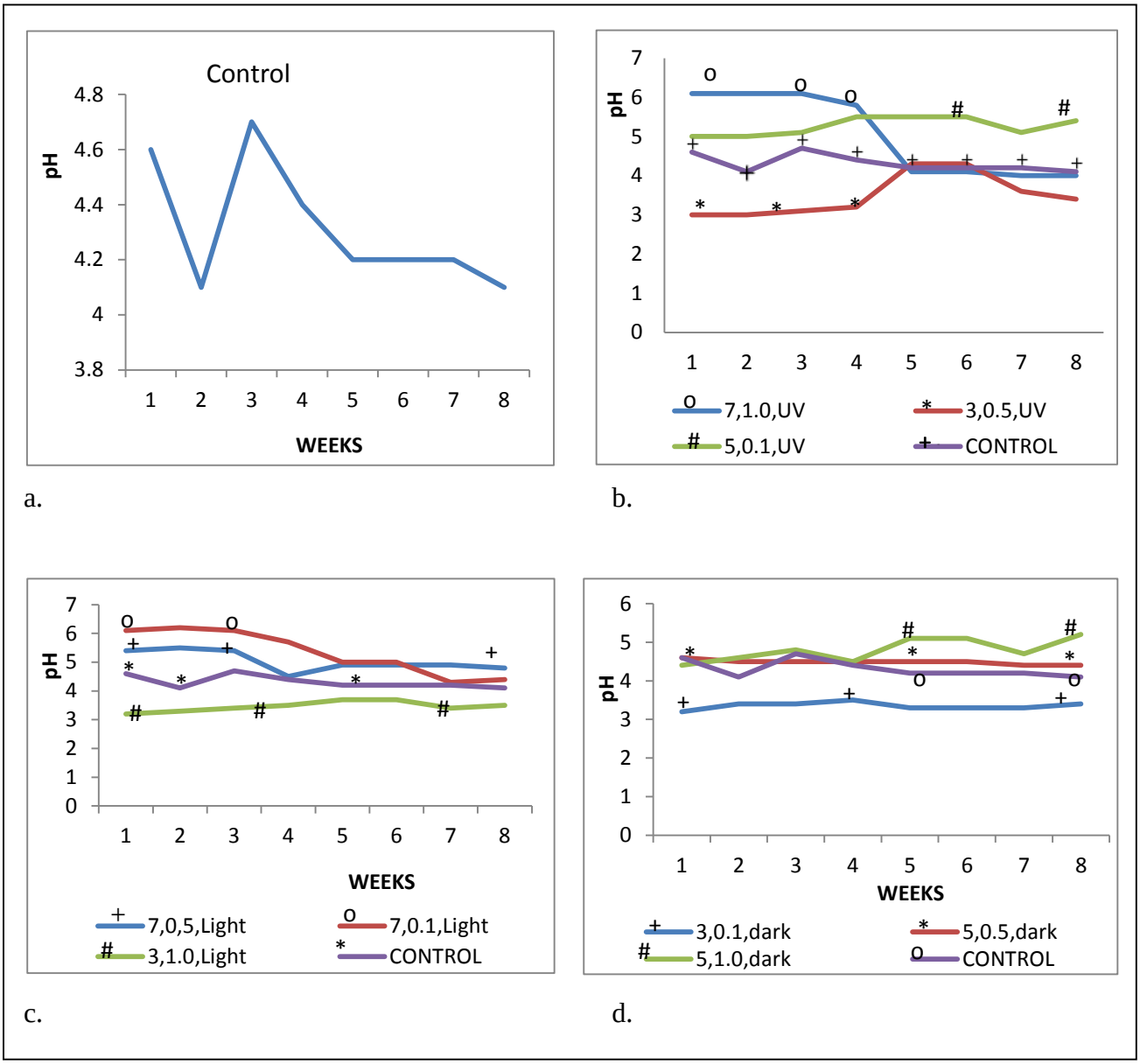


Figure 4.2: Results of Variation in pH of Bell Pepper under UV, White Light and in the Dark for Eight Weeks.

a = Control (Untreated extract)

b = Extract at pH 3,5,7 and ascorbic acid, 0.5,0.1,1.0% under UV light

c = Extract at pH 3,7 and ascorbic acid, 0.5,0.1,1.0% under white light

d = Extract at pH 3,5,5 and ascorbic acid, 0.1,0.1,1.0% in the dark.

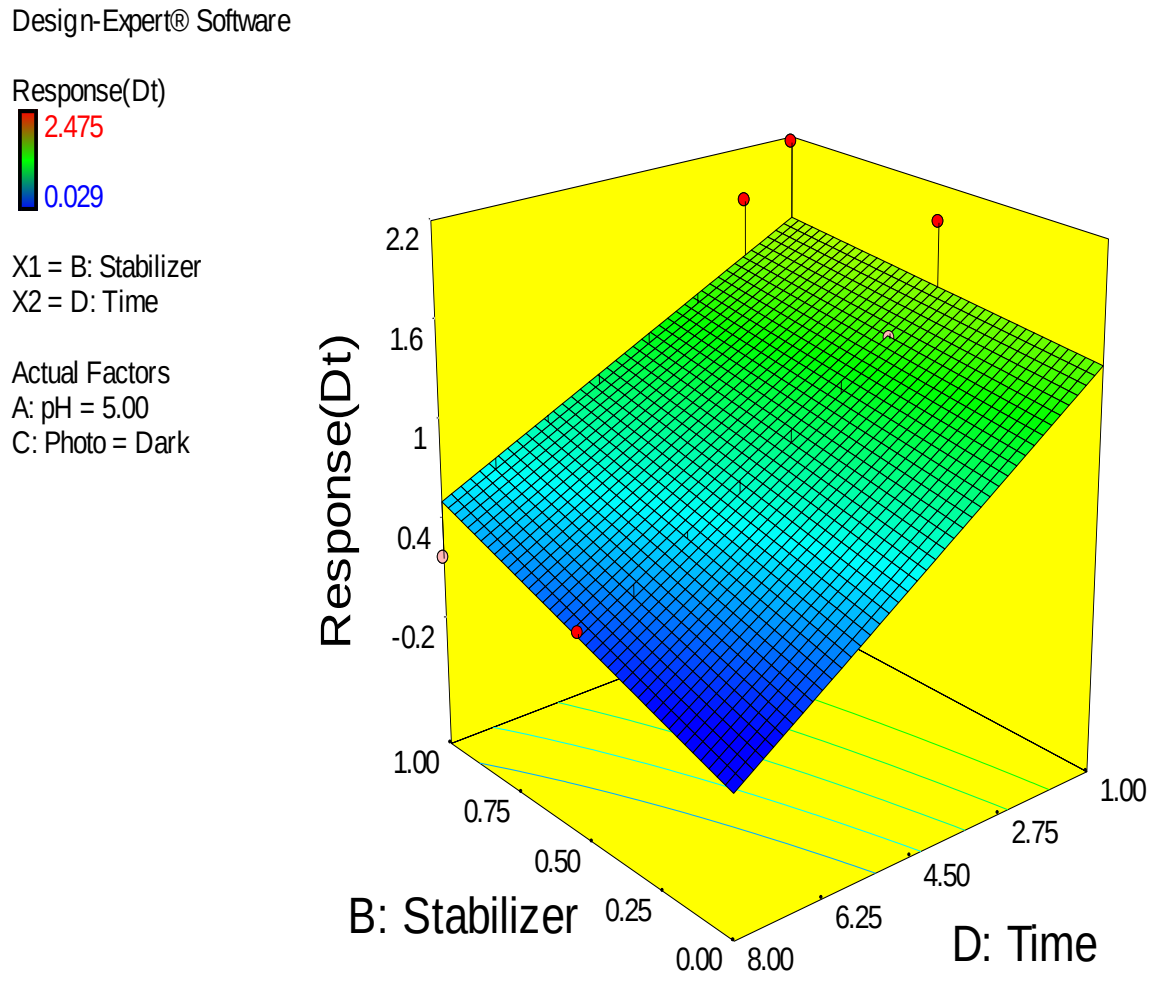


Figure 4.3: 3D Plot for Factor Interaction between Time and Stabilizer (Ascorbic Acid) against the Response (Absorbance) for Bell Pepper.

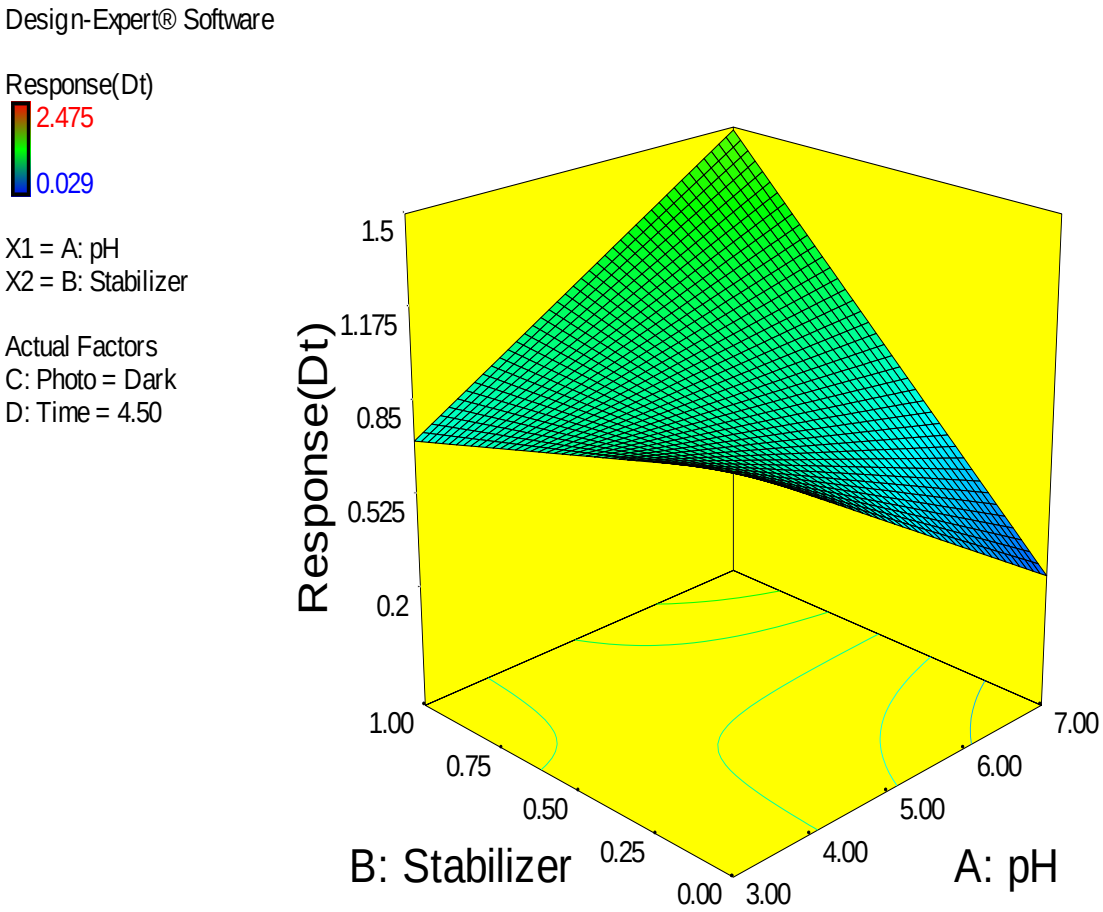


Figure 4.4: 3D Plot for Factor Interaction between pH and Stabilizer (Ascorbic Acid) against the Response (Absorbance) for Bell Pepper.

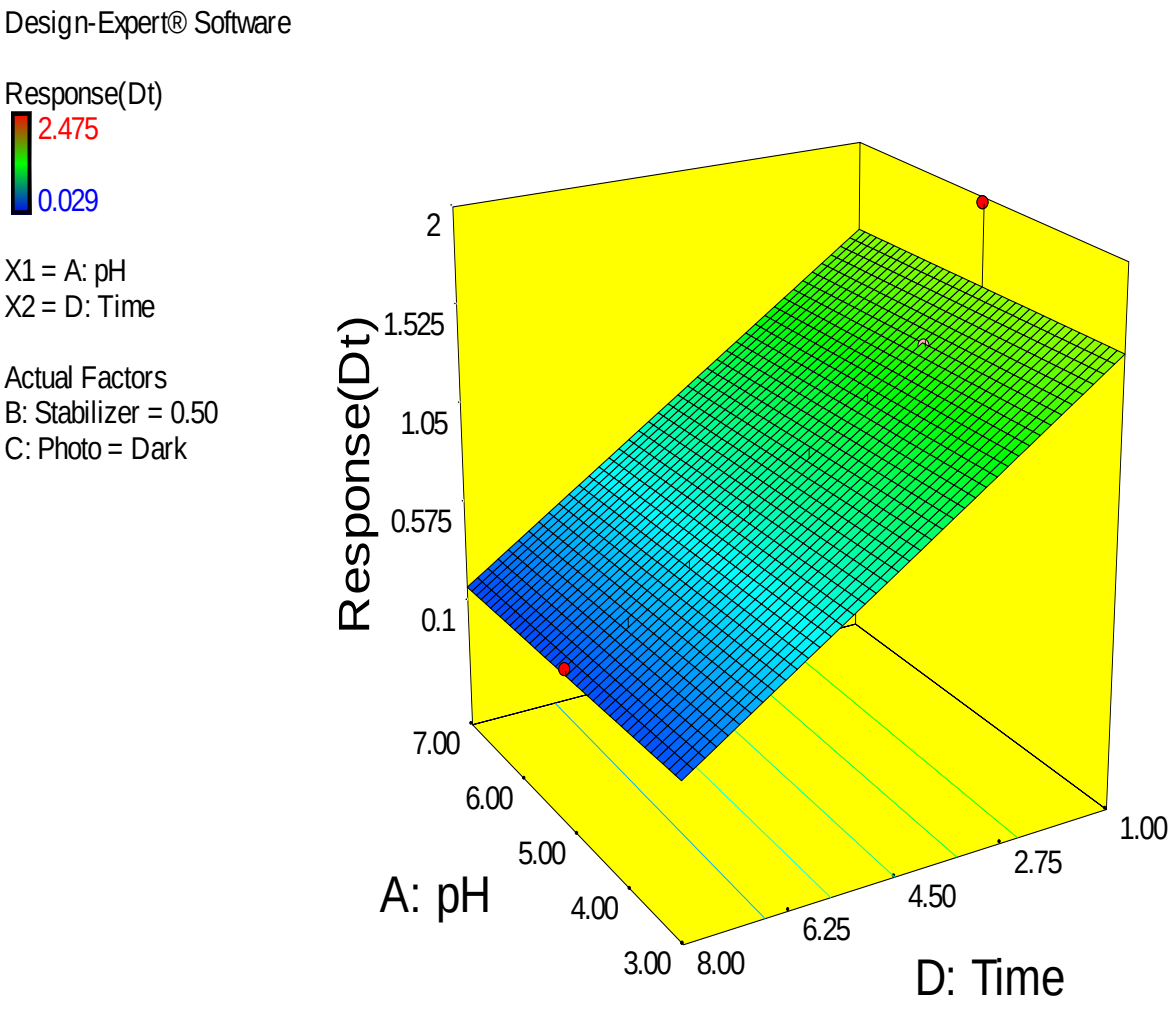


Figure 4.5: 3D Plot for Factor Interaction between pH and Time against the Response (Absorbance) for Bell Pepper.

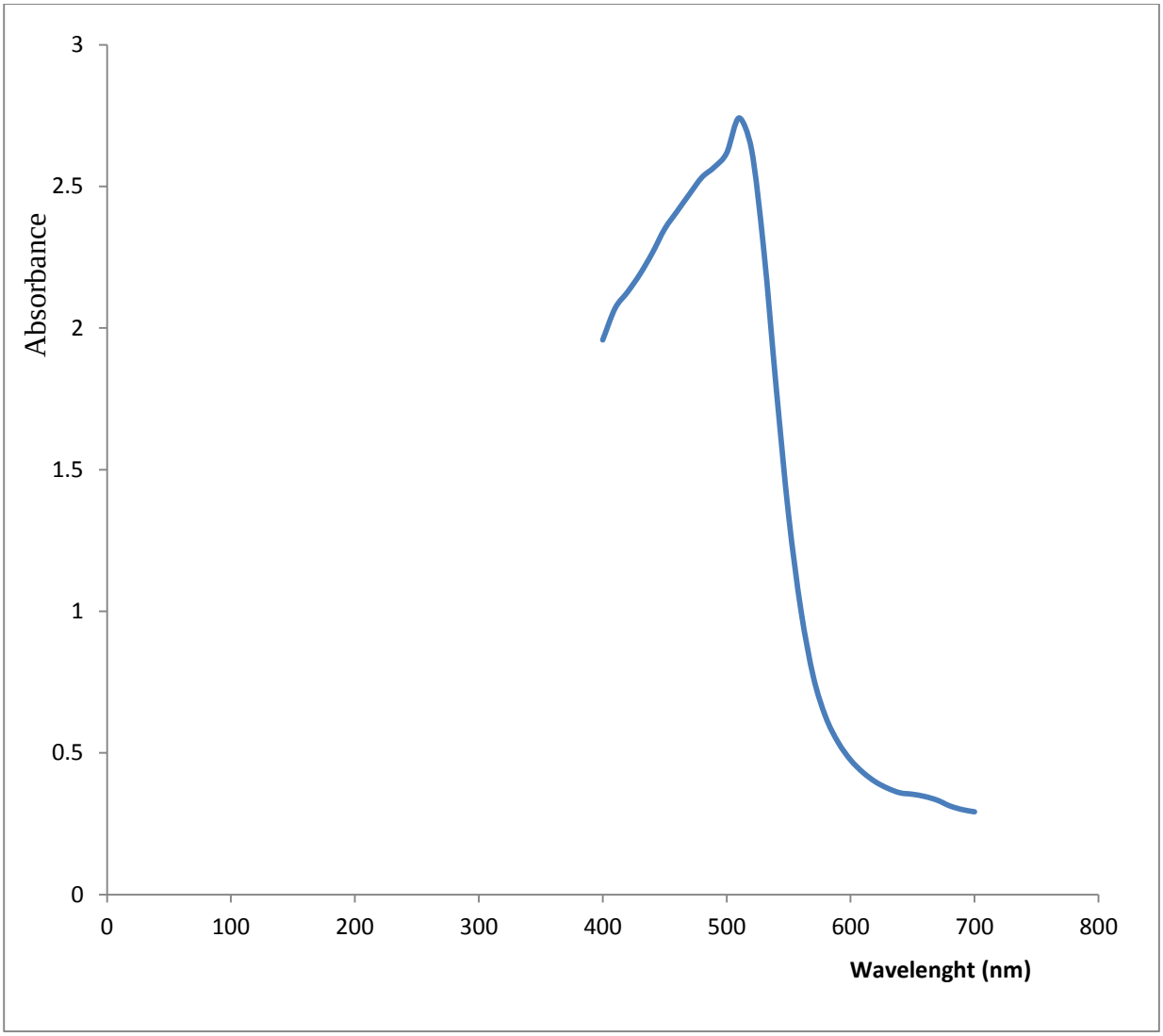


Figure 4.6: UV Spectrum of Bell Pepper Extract in the Visible Region Showing Wavelength of Maximum Absorbance.

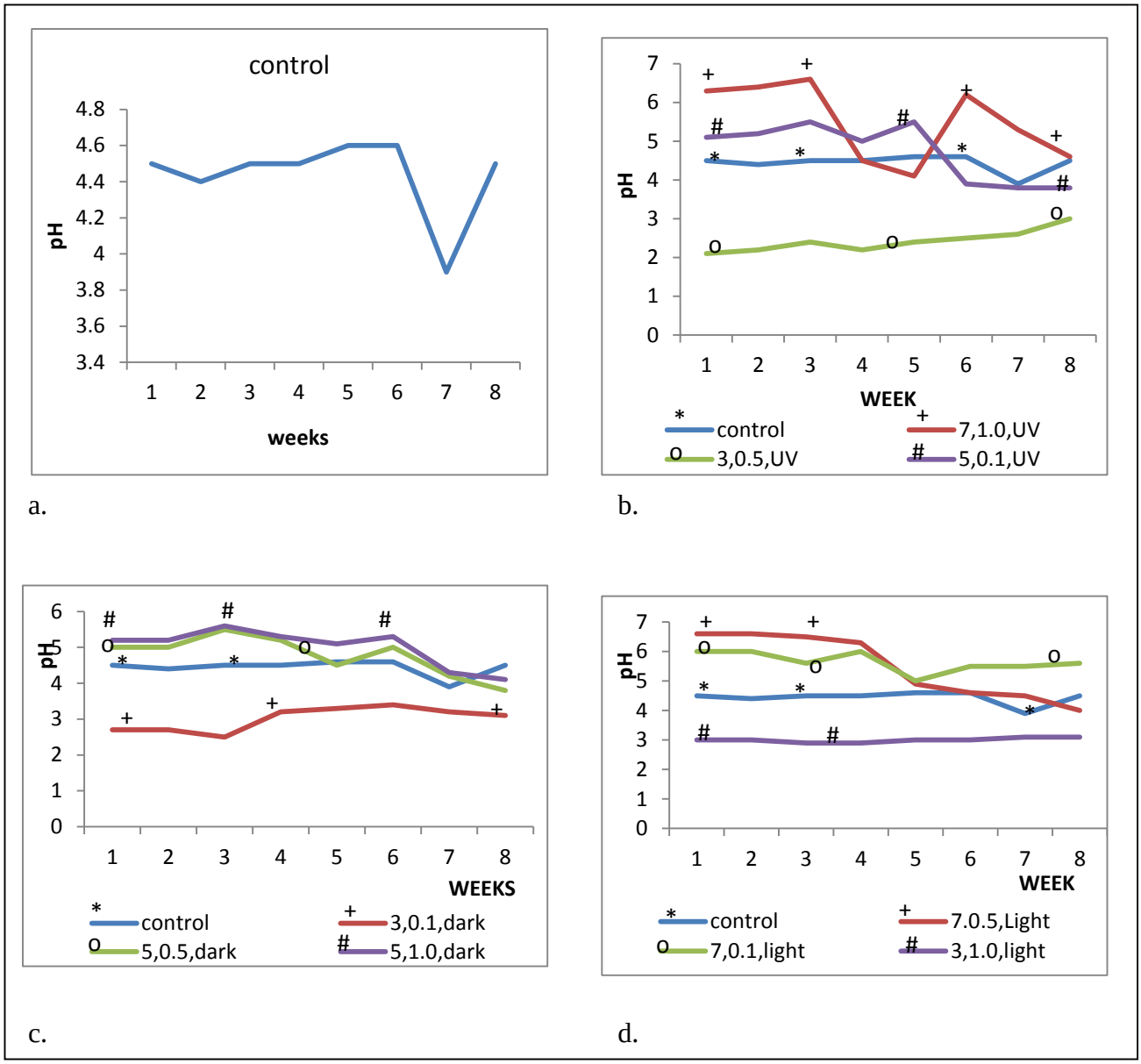


Figure 4.7: Variation in pH of Sweet Pepper under UV, White Light and in the dark for Eight Weeks.

Where:

a = Control (Untreated extract)

b = Extract at pH 3,5,7 and 0.5,0.1,1.0% ascorbic acid under UV light.

c = Extract at pH 3,5 and 0.5,0.1,1.0% ascorbic acid in the dark.

d = Extract at pH 3,7and 0.5,0.1,1.0% ascorbic acid under white light.

Figure 4.8 shows factor interactions between time and stabilizer (ascorbic acid) against the response (absorbance) for sweet pepper.

Fig 4.9 shows factor interactions between stabilizer (ascorbic acid) and pH against the response (absorbance) for sweet pepper.

Figure 4.10 shows factor interactions between time (in weeks) and pH against the response (absorbance) for sweet pepper.

The results of the interaction are shown in Appendix V.

Figure 4.11 shows the UV spectrum of Sweet pepper at wavelength of maximum absorbance.

4.3 Characteristics of Extracts

The functional groups present in the extracts and their corresponding wave numbers are shown in in Figures 4.12 - 4.31.

The ASTA and IC values and absorbance of bell and sweet pepper extracts at 460nm are shown in Table 4.1. Bell pepper extracts had lower values for ASTA and IC and absorbance at 460nm than extracts from Sweet pepper.

4.4 Nutritional Components of Extracts

The food value for the grounded peppers and their extracts are shown in table 4.2 and 4.3. The pepper powders had higher values for all the parameters considered except for crude lipid and moisture content of the extracts which was higher in value than the grounded pepper powders.

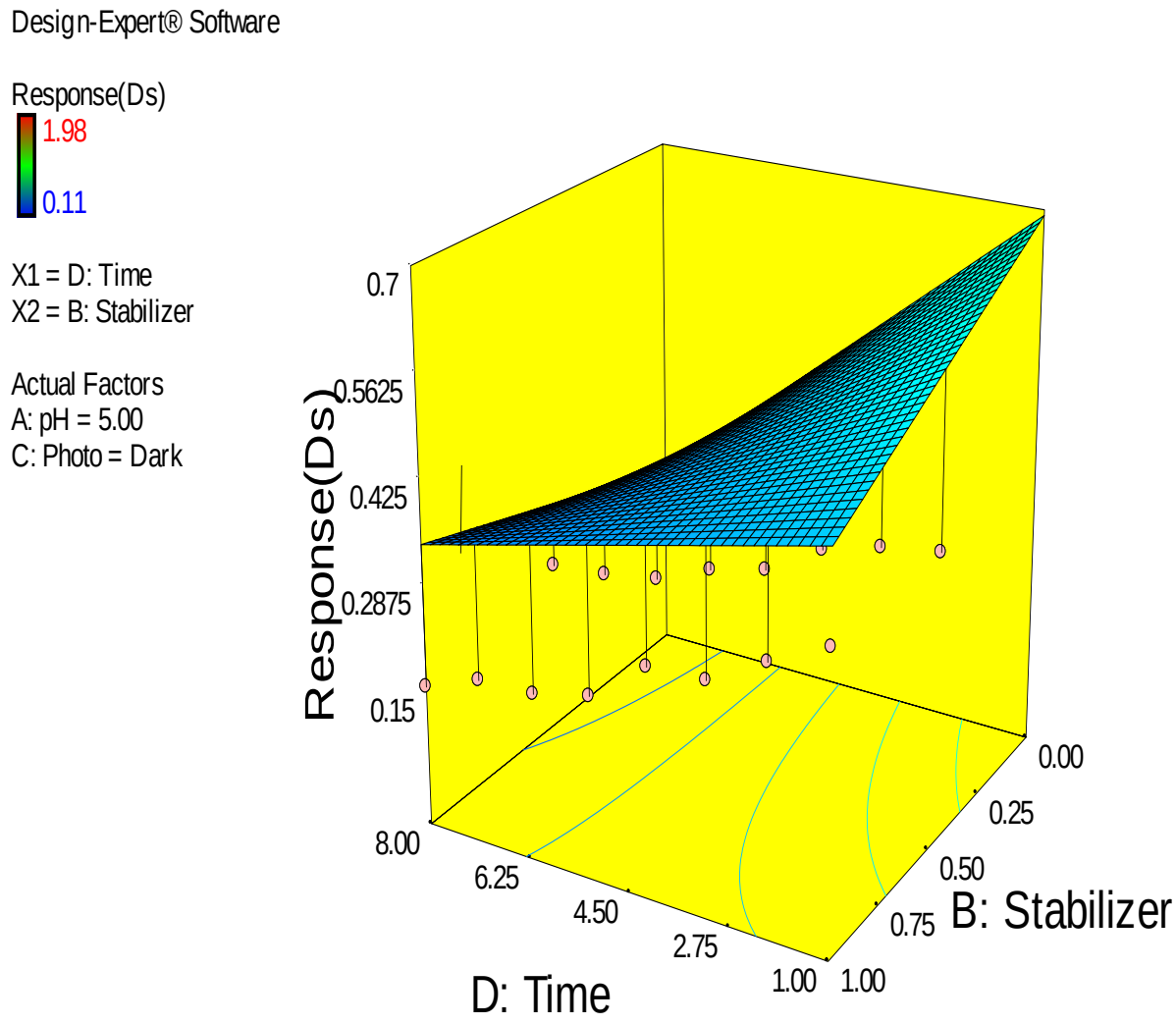


Figure 4.8: 3D Plot for Factor Interaction between Time and Stabilizer (Ascorbic Acid) against the Response (Absorbance) for Sweet Pepper.

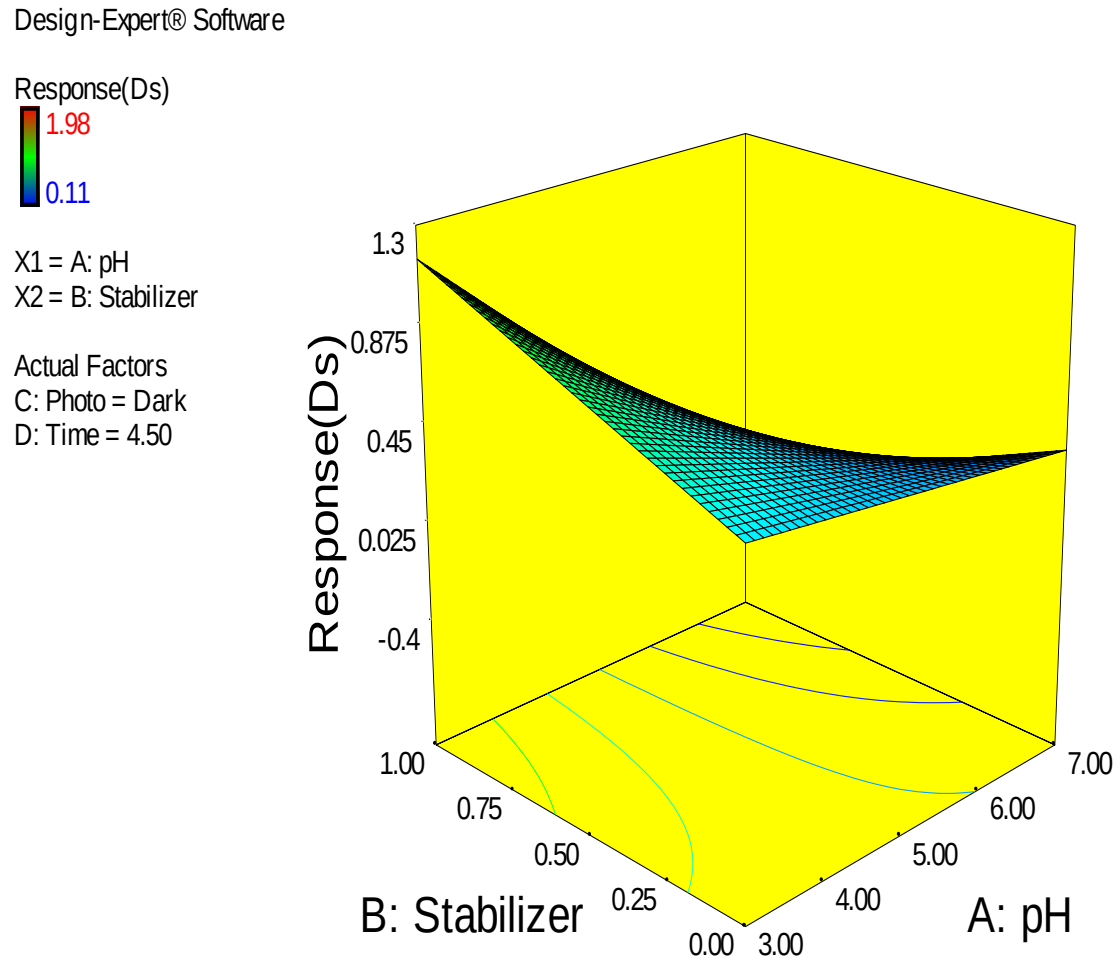


Figure 4.9: 3D Plot for Factor Interaction between Stabilizer (Ascorbic Acid) and pH against the Response (Absorbance) for Sweet Pepper.

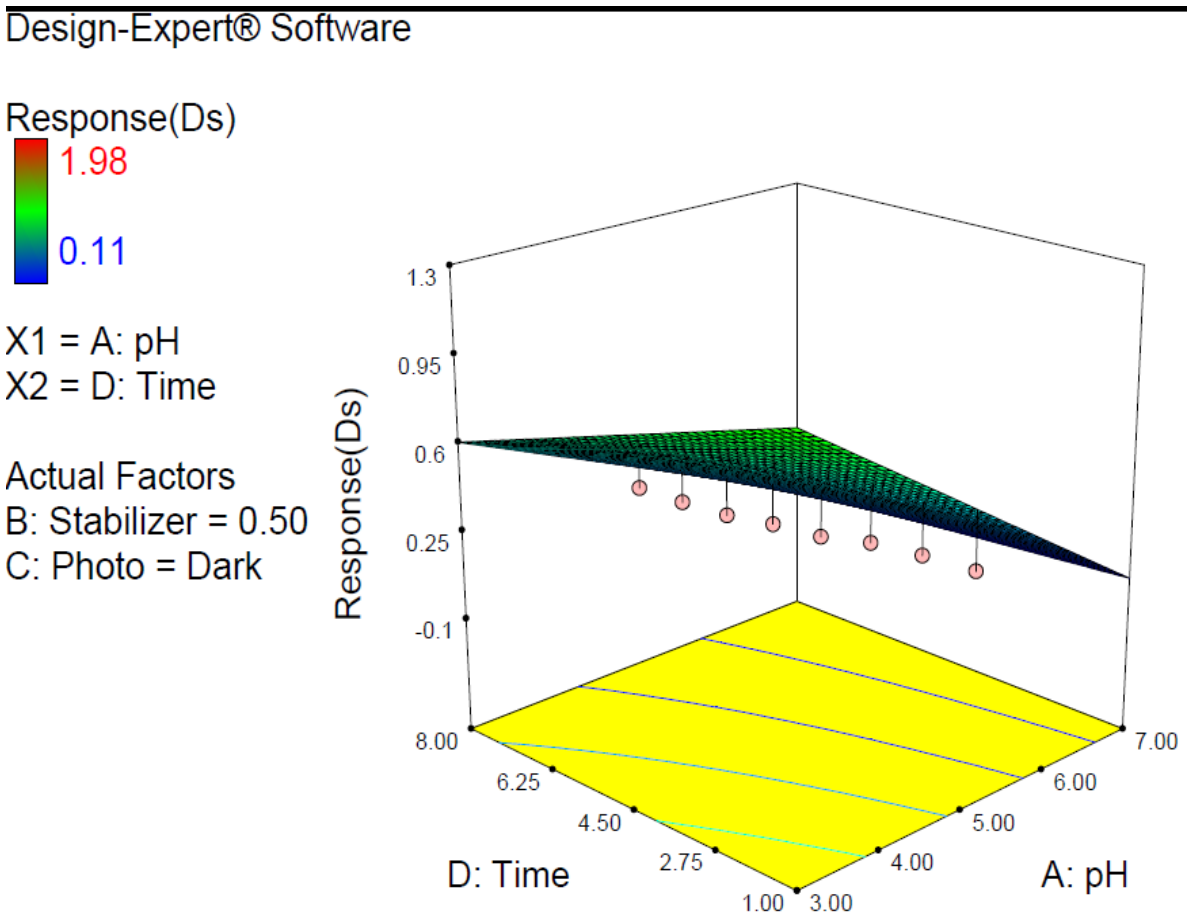


Figure 4.10: 3D Plot for Factor Interaction between Time and pH against the Response (Absorbance) for Sweet Pepper.

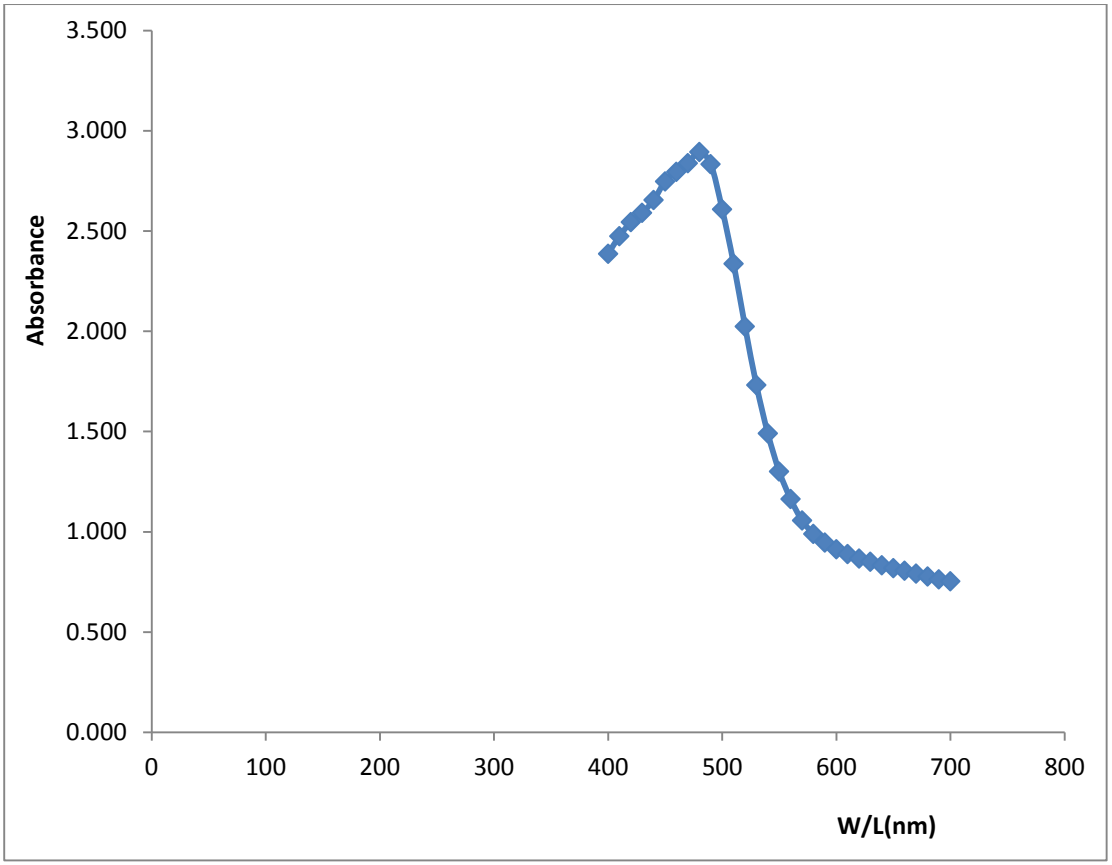


Figure 4.11: UV Spectrum of Sweet Pepper in the Visible Region Showing Wavelength of Maximum Absorbance.

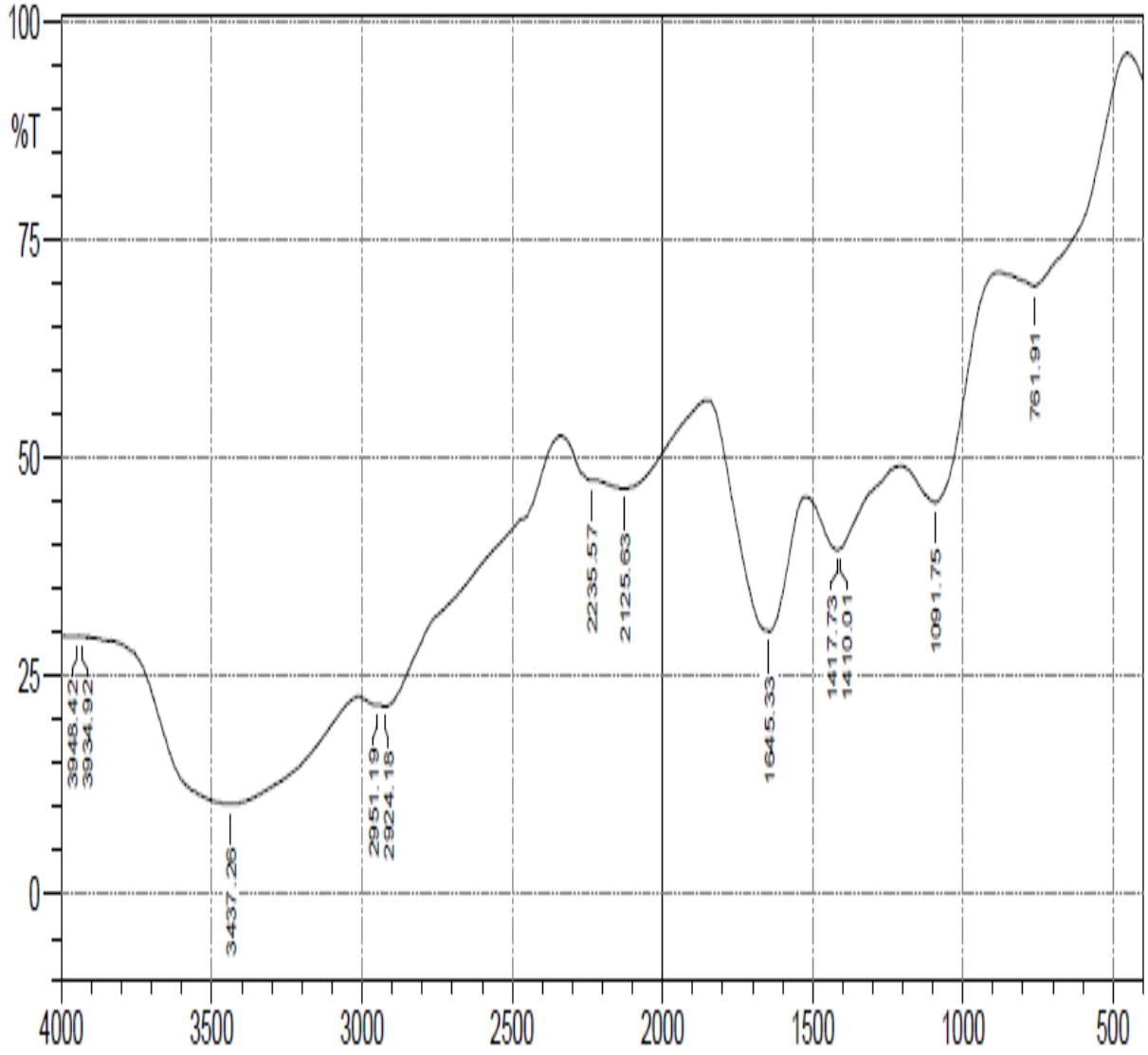


Figure 4.12: FTIR Spectrum of Bell Pepper Extract (Control)

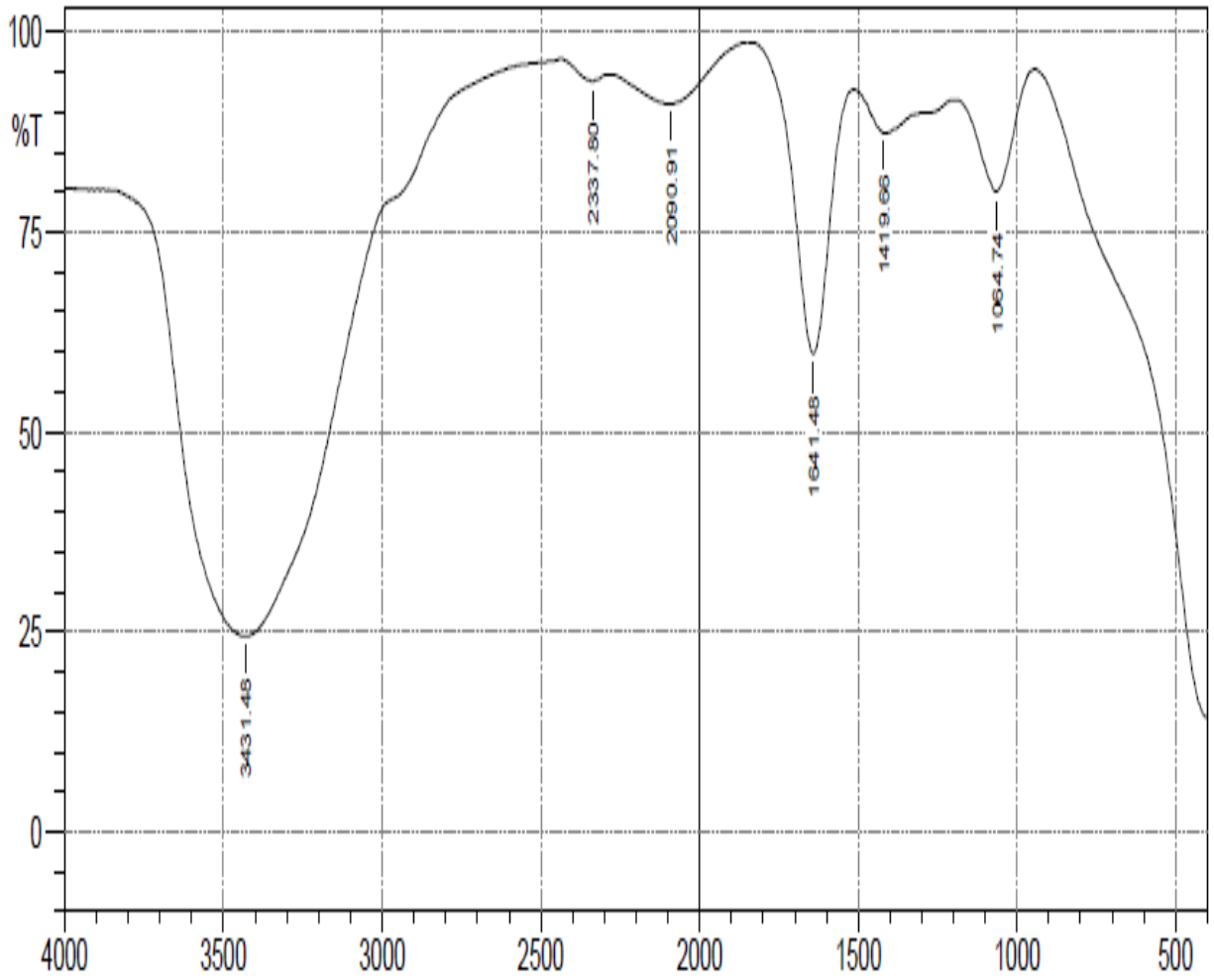


Figure 4.13 FTIR Spectrum of Bell Pepper Extract at pH 3 and 0.5% Stabilizer under UV Light

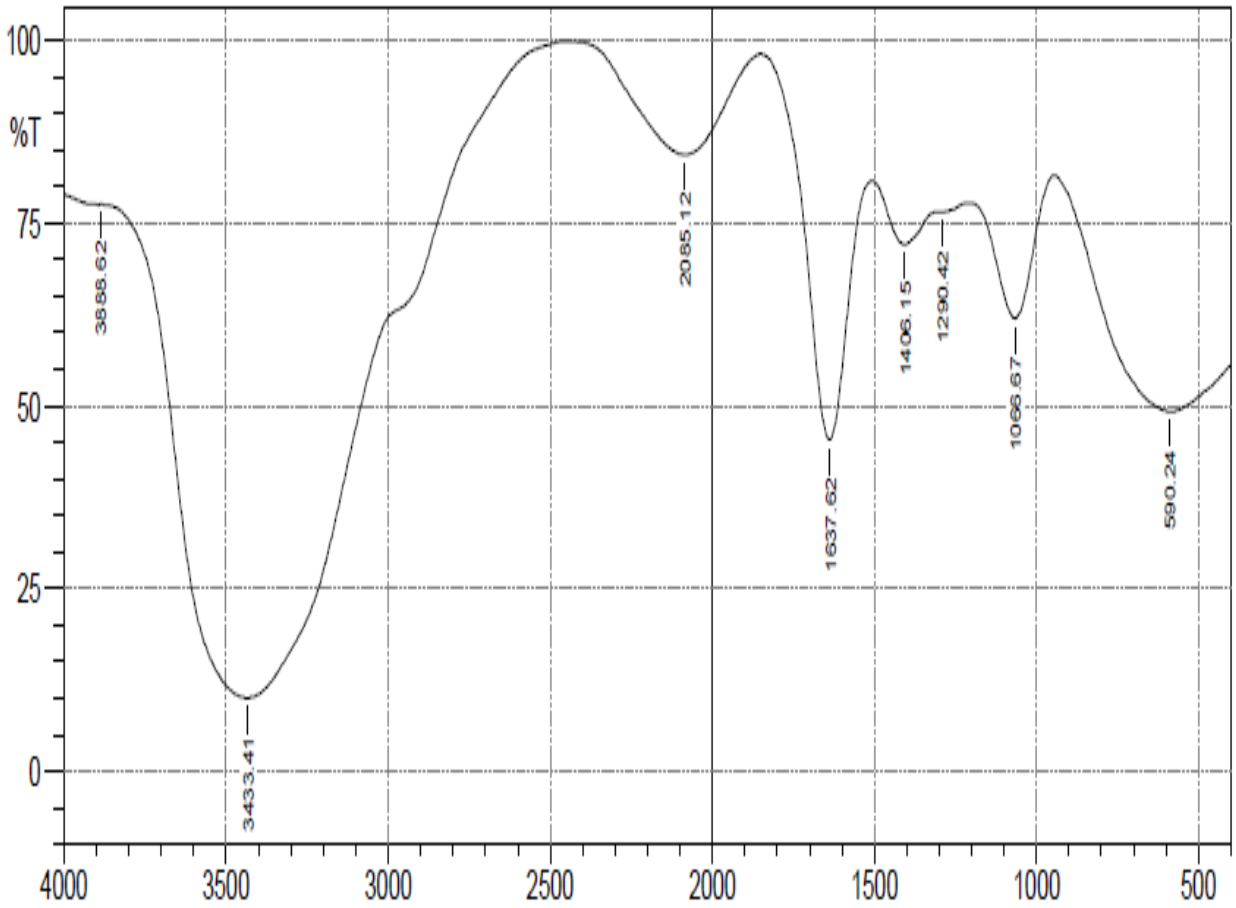


Figure 4.14: FTIR Spectrum of Bell Pepper Extract at pH 5 and 0.1% Stabilizer under UV Light.

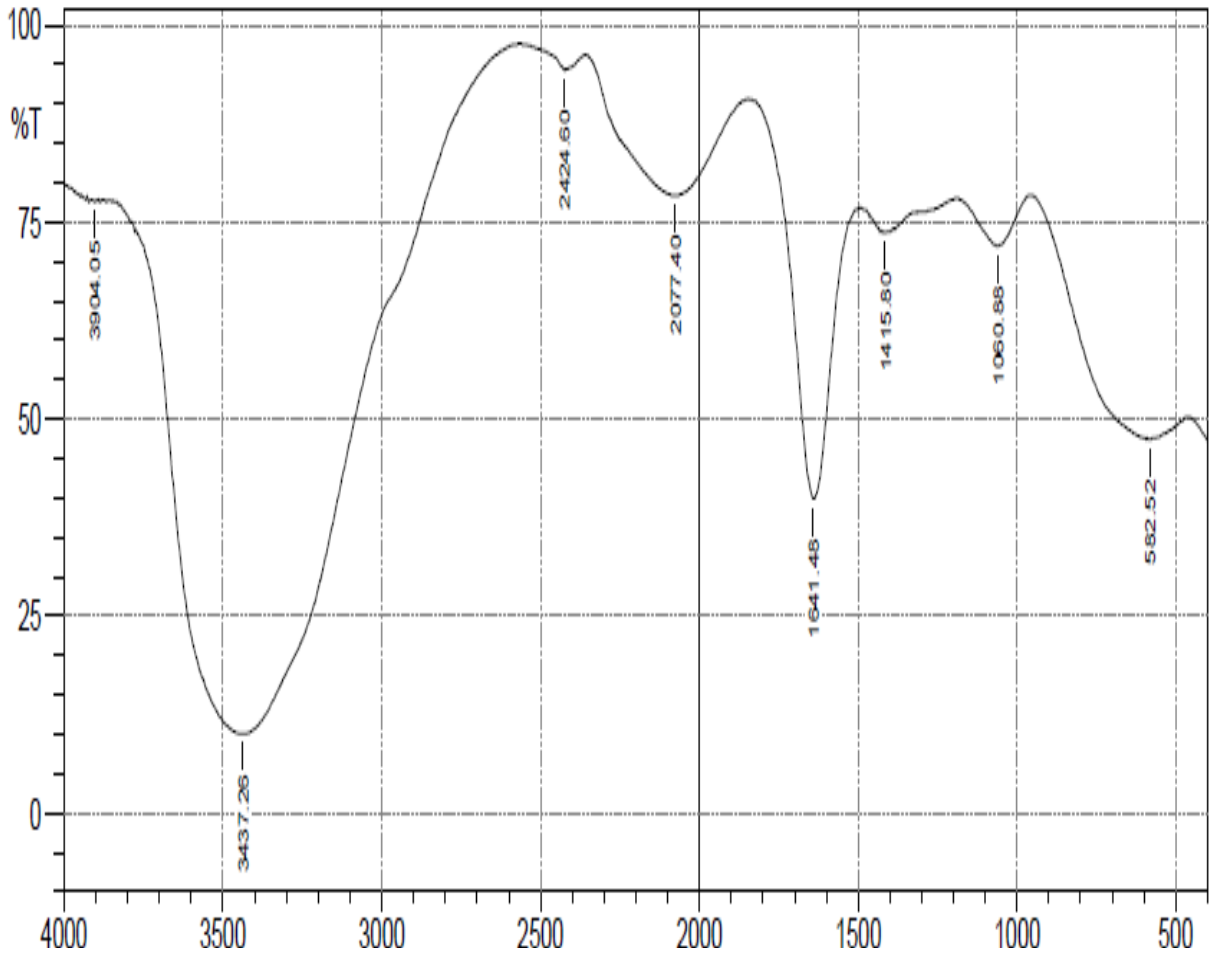


Figure 4.15: FTIR Spectrum of Bell Pepper Extract at pH 7and 1.0% Stabilizer under UV Light.

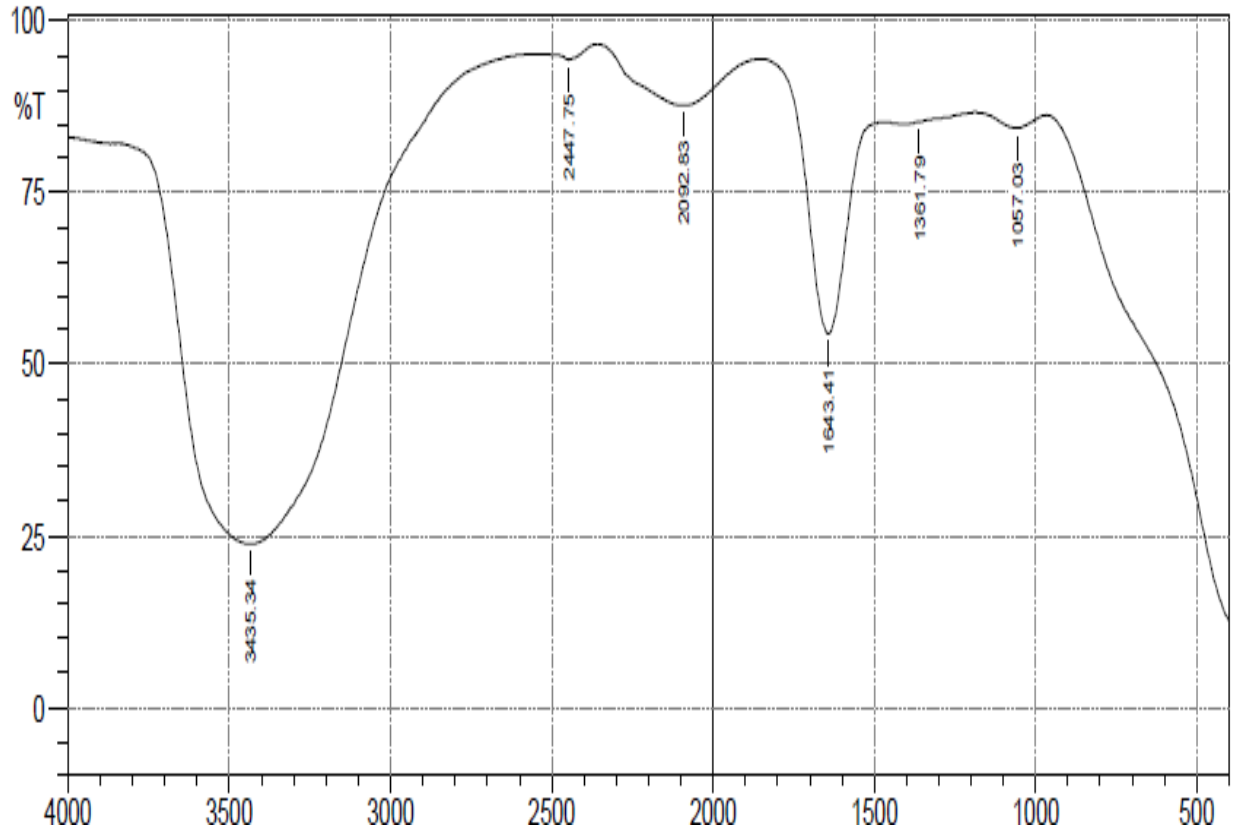


Figure 4.16: FTIR Spectrum of Bell Pepper Extract at pH 3 and 1.0% Stabilizer under White Light.

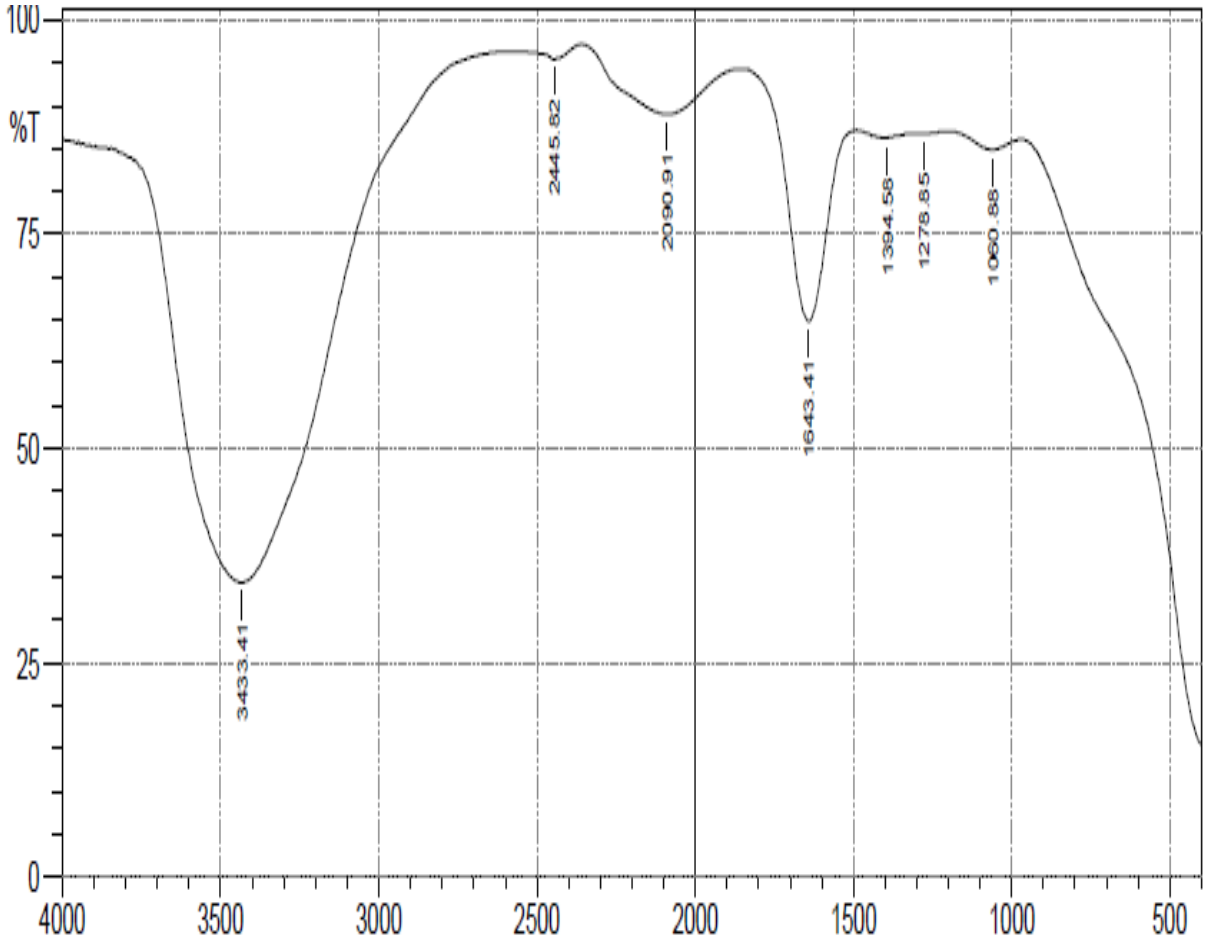


Figure 4.17: FTIR Spectrum of Bell Pepper at pH 7 and 0.1% Stabilizer under White Light

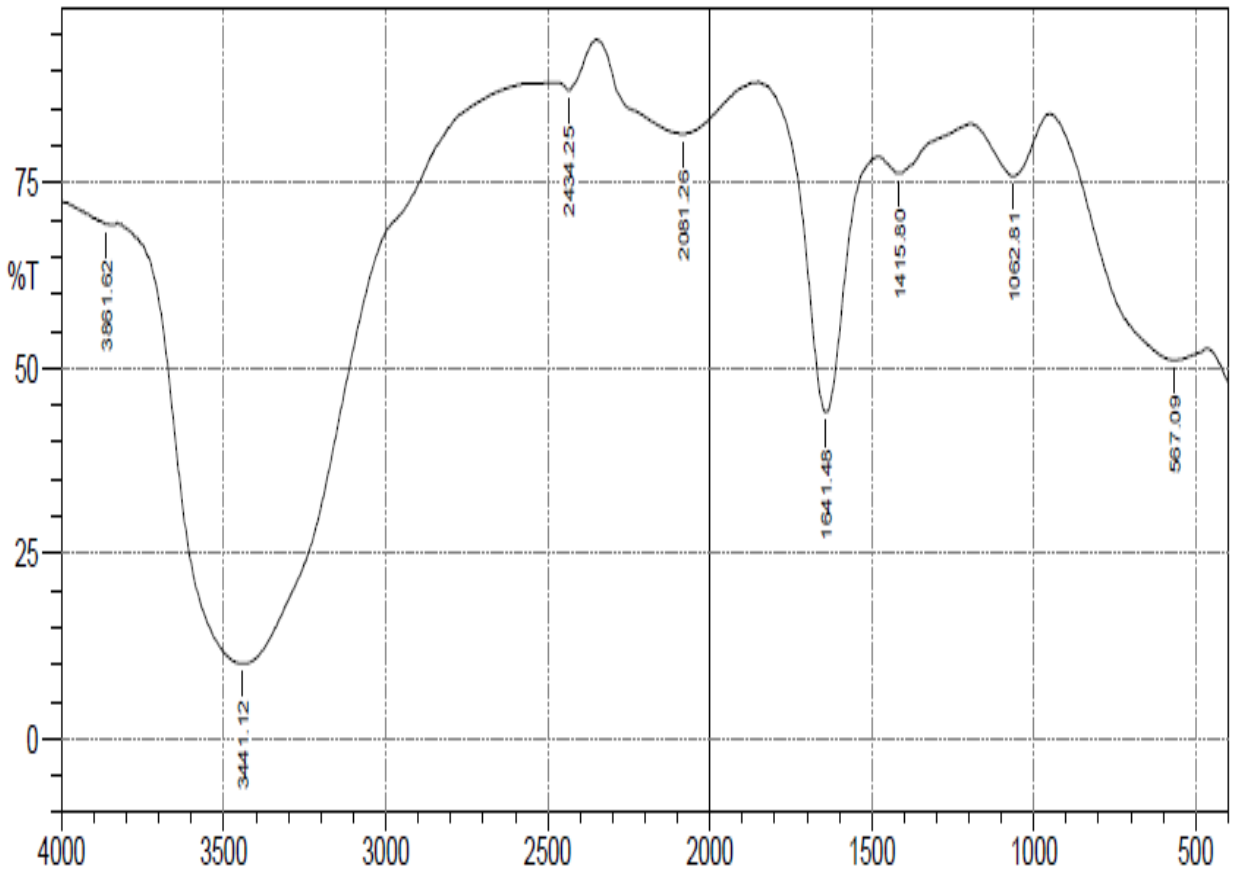


Figure 4.18: FTIR Spectrum of Bell Pepper Extract at pH 7 and 0.5% Stabilizer under White Light

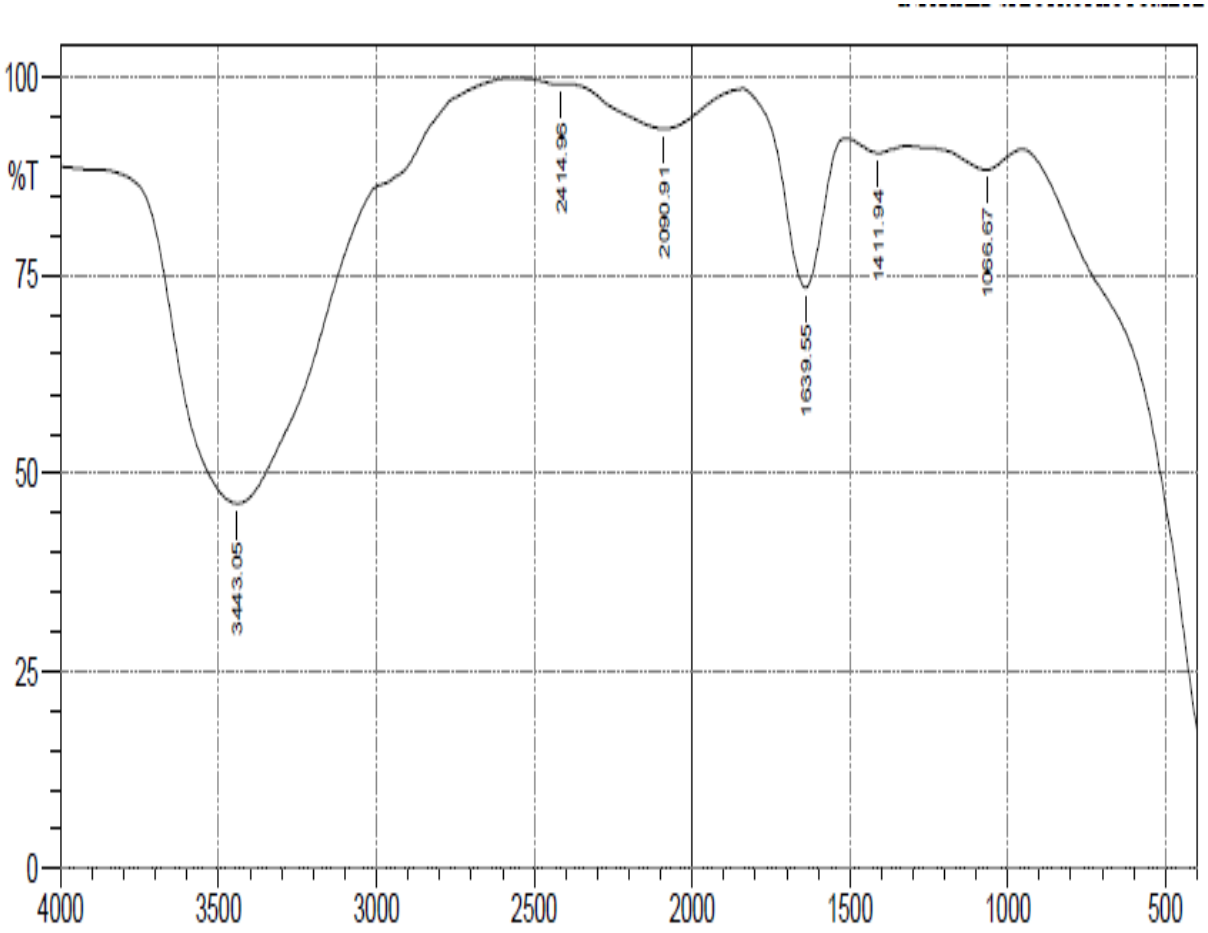


Figure 4.19: FTIR Spectrum of Bell Pepper Extract at pH 3 and 0.1% Stabilizer in the Dark

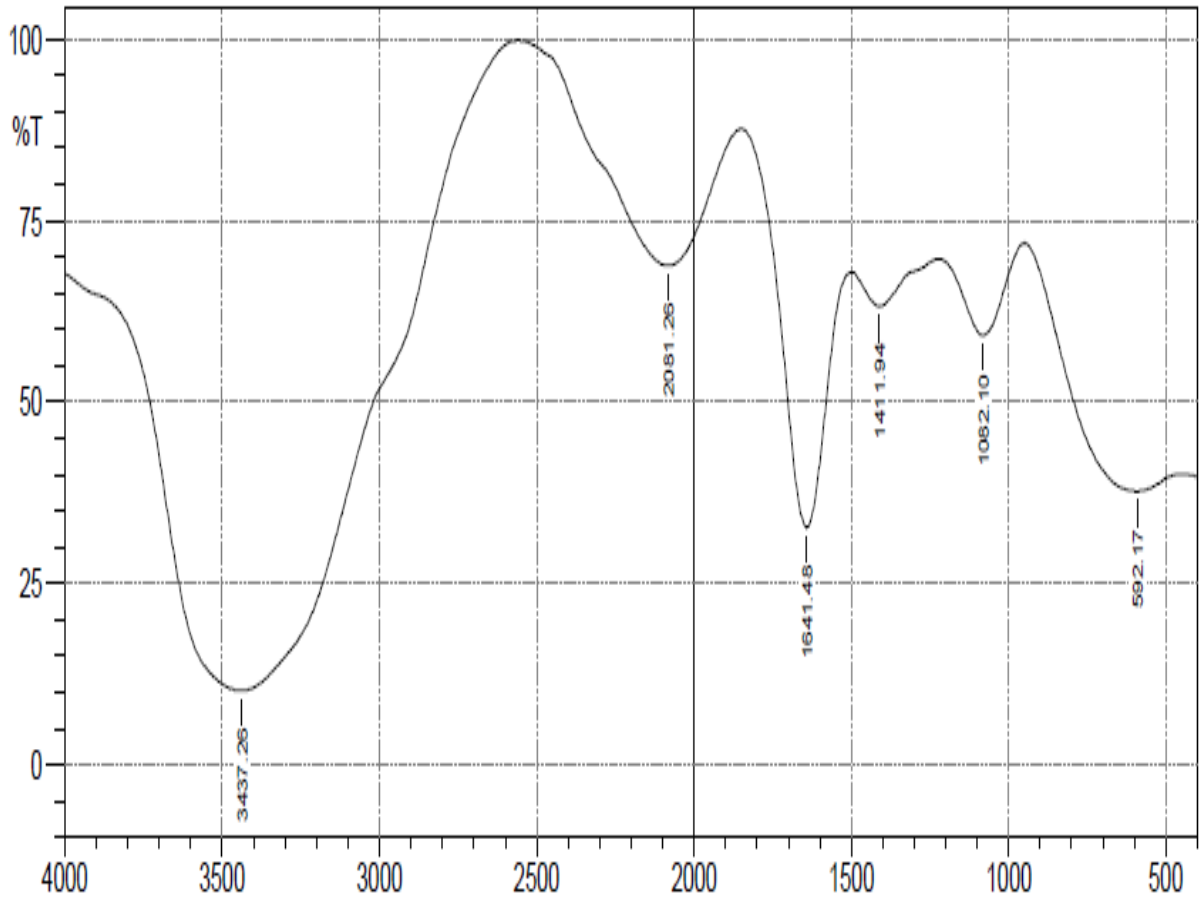


Figure 4.20: FTIR Spectrum for Bell Pepper Extract at pH 5 and 0.5% Stabilizer in the Dark

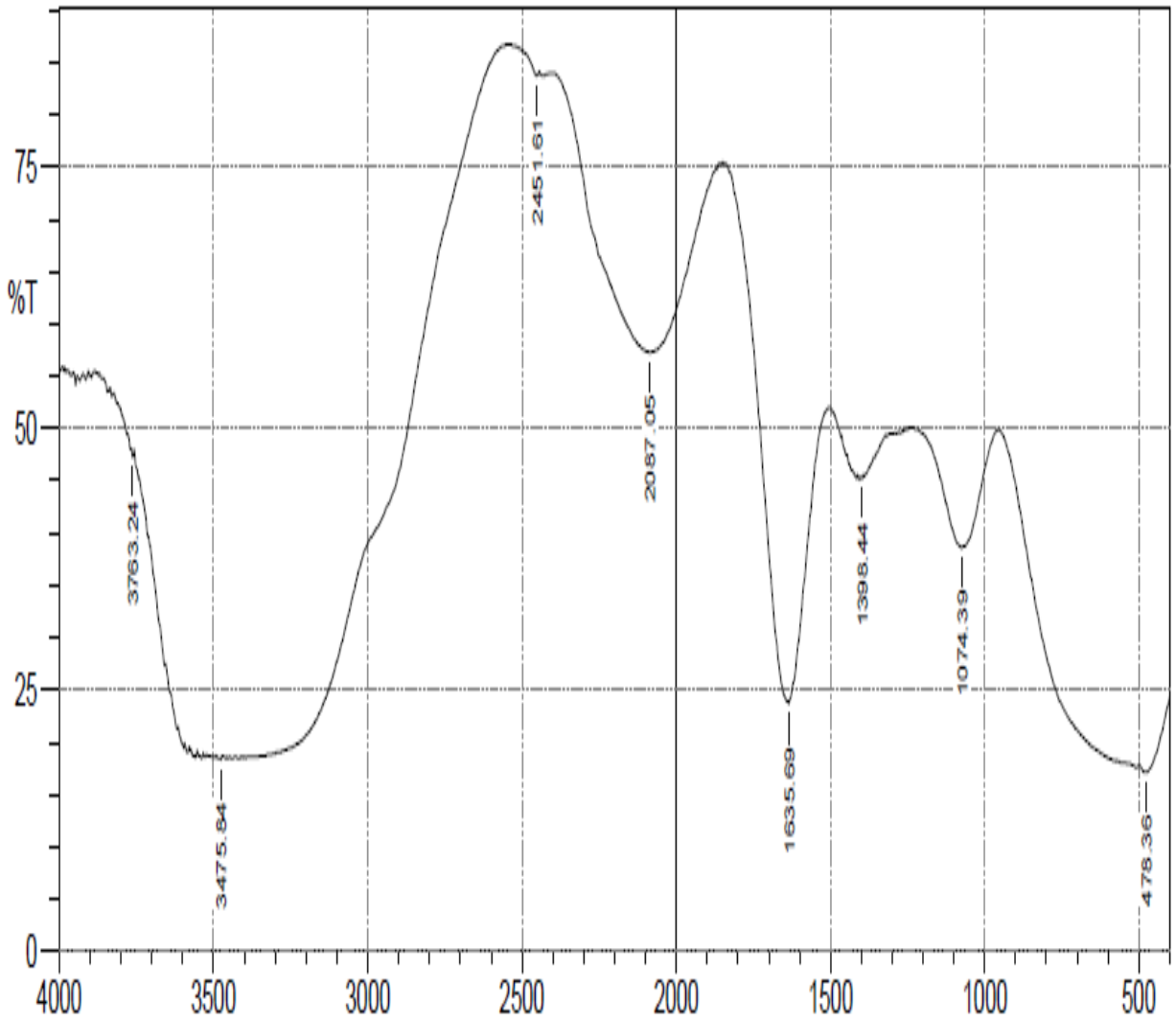


Figure 4.21: FTIR Spectrum of Bell Pepper Extract at pH 5 and 1.0% Stabilizer in the Dark

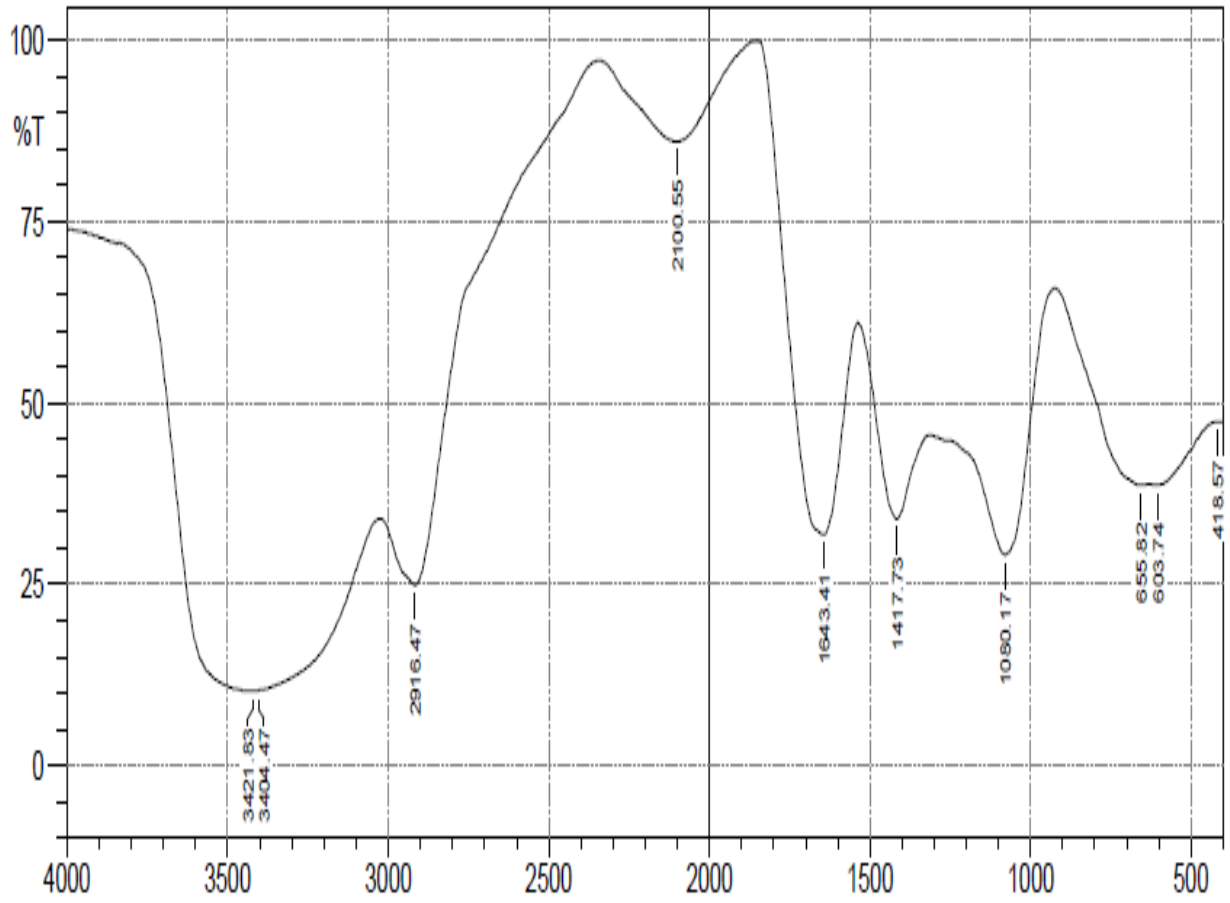


Figure 4.22: FTIR Spectrum of Sweet Pepper Extract (Control)

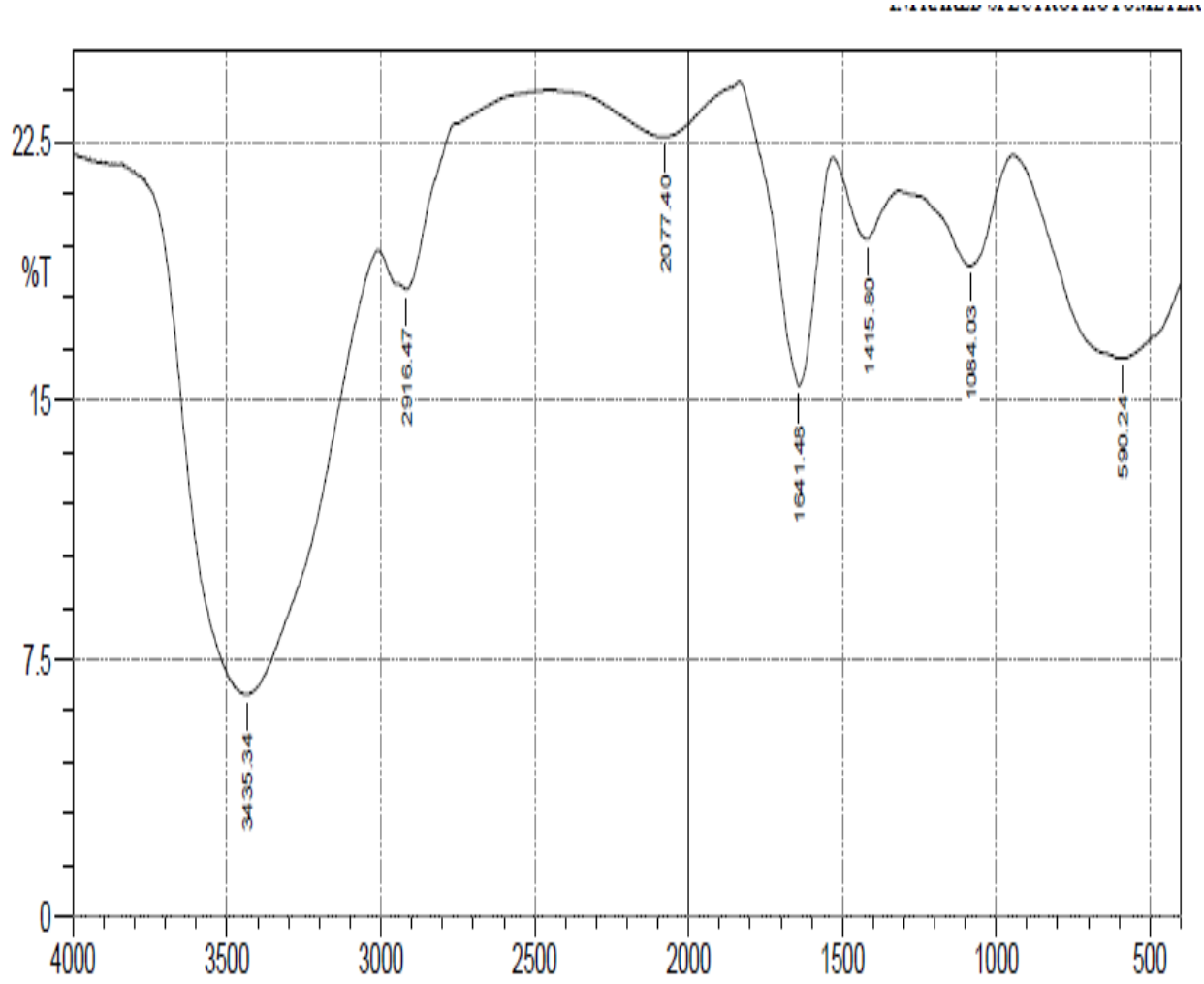


Figure 4.23: FTIR Spectrum of Sweet Pepper Extract at pH 3 and 0.5% Stabilizer under UV Light

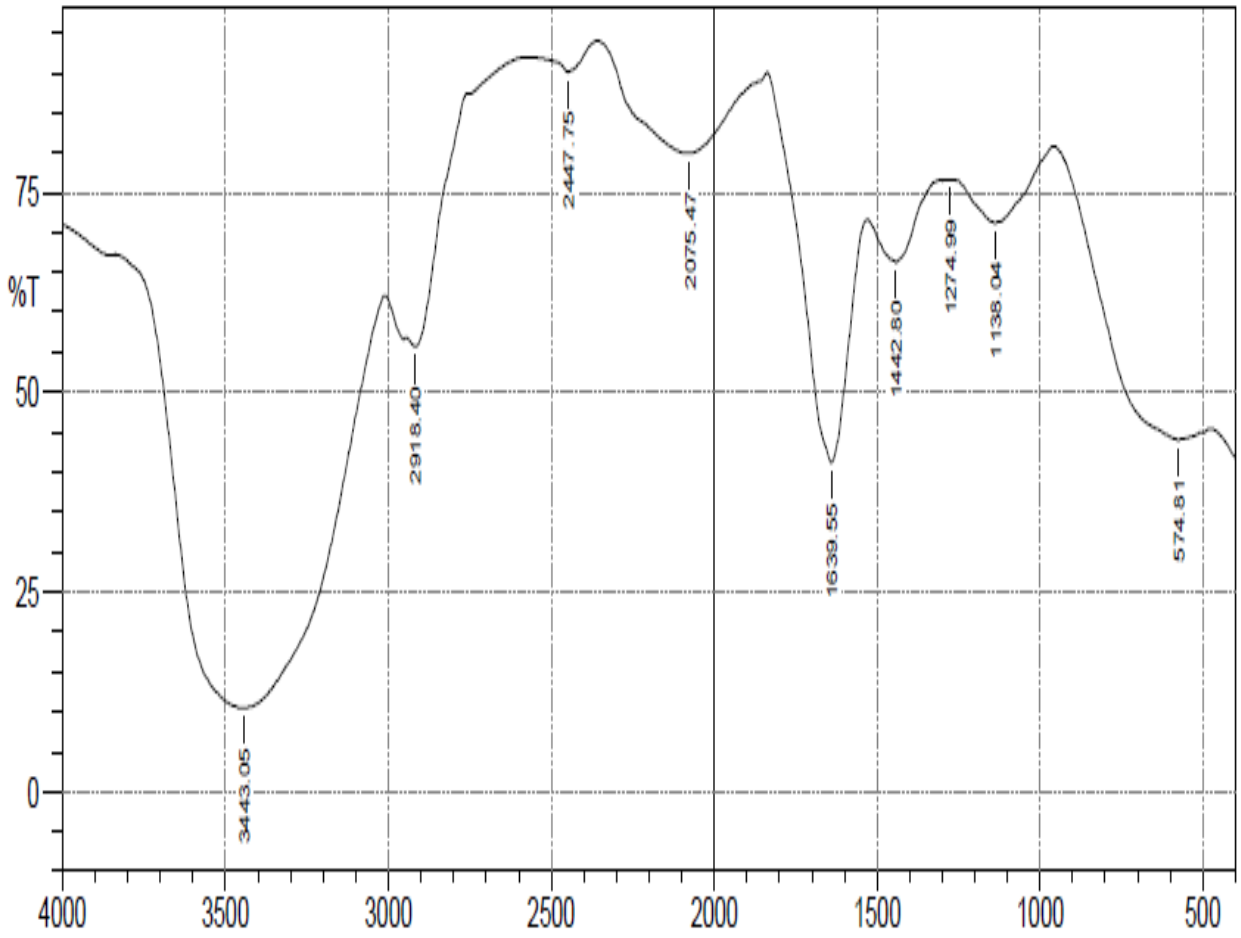


Figure 4.24: FTIR Spectrum of Sweet Pepper Extract at pH 5 and 0.1% Stabilizer under UV Light.

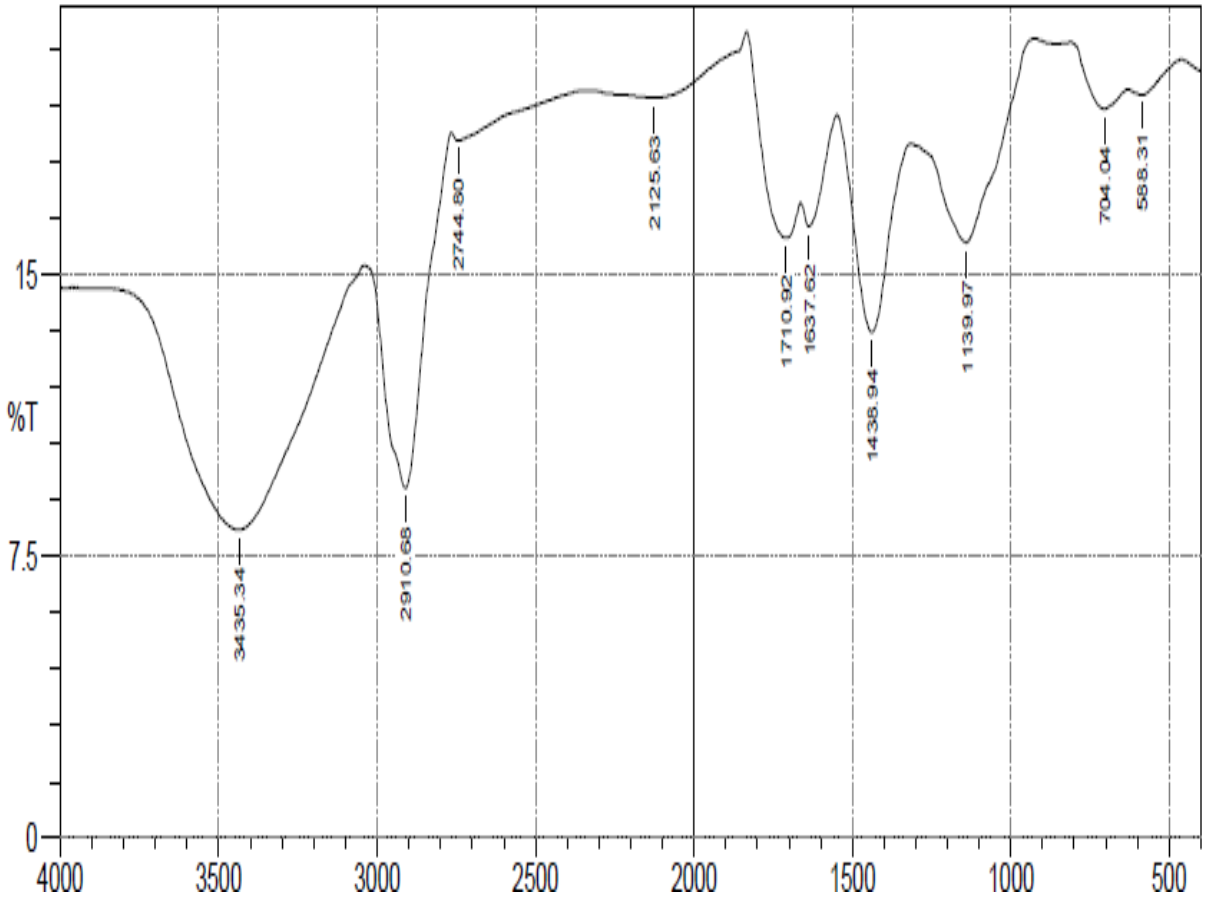


Figure 4.25: FTIR Spectrum of Sweet Pepper Extract at pH 7 and 0.1% Stabilizer under UV Light

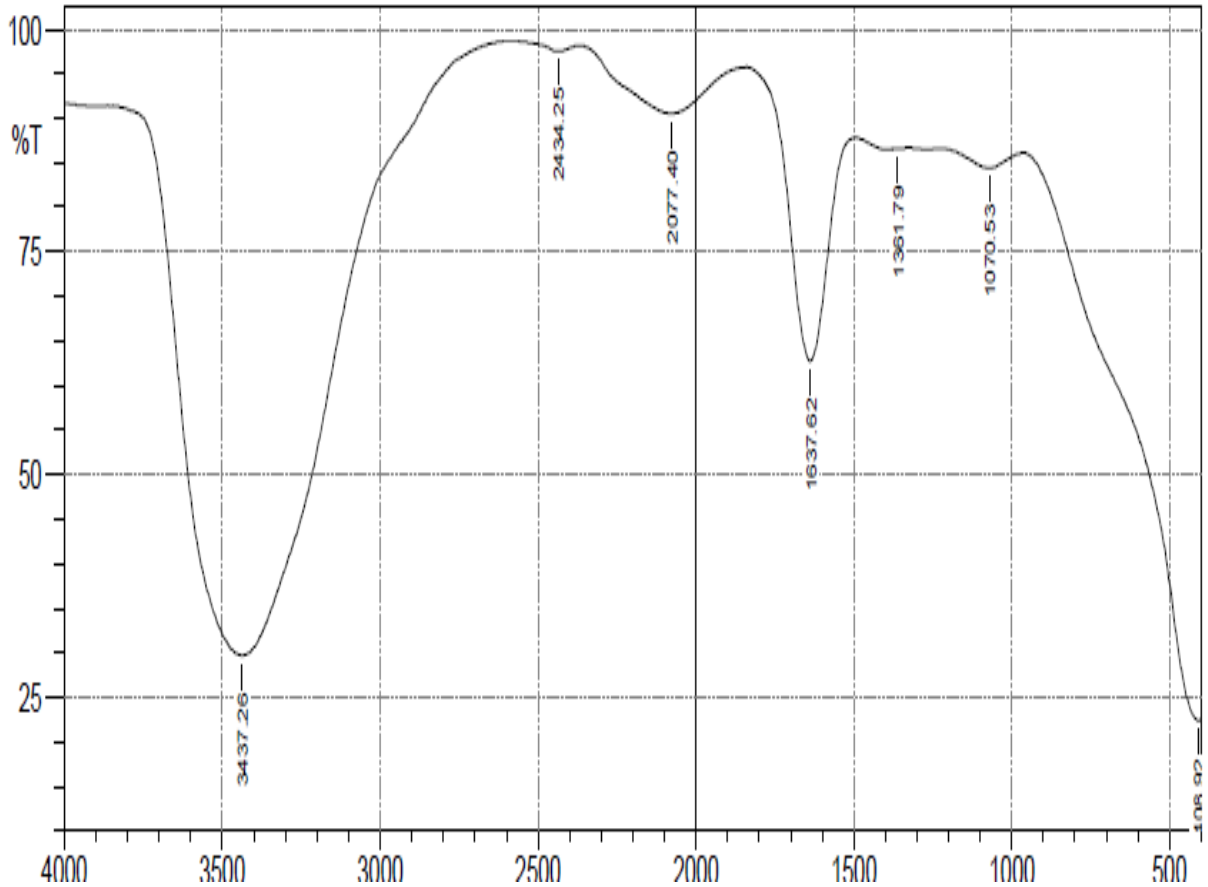


Figure 4.26: FTIR Spectrum of Sweet Pepper Extract at pH 7 and 0.5% Stabilizer under White Light

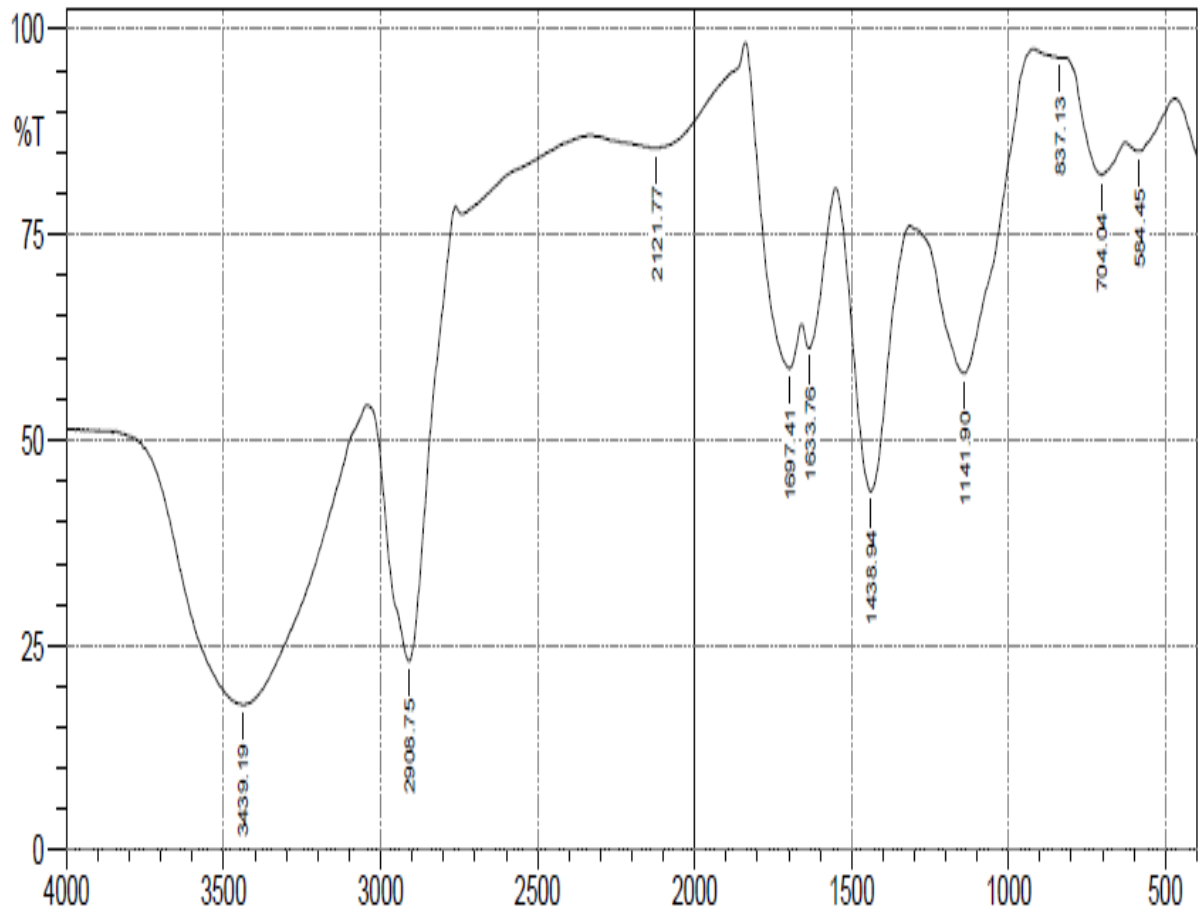


Figure 4.27: FTIR Spectrum of Sweet Pepper Extract at pH 7and 0.1% Stabilizer under White Light

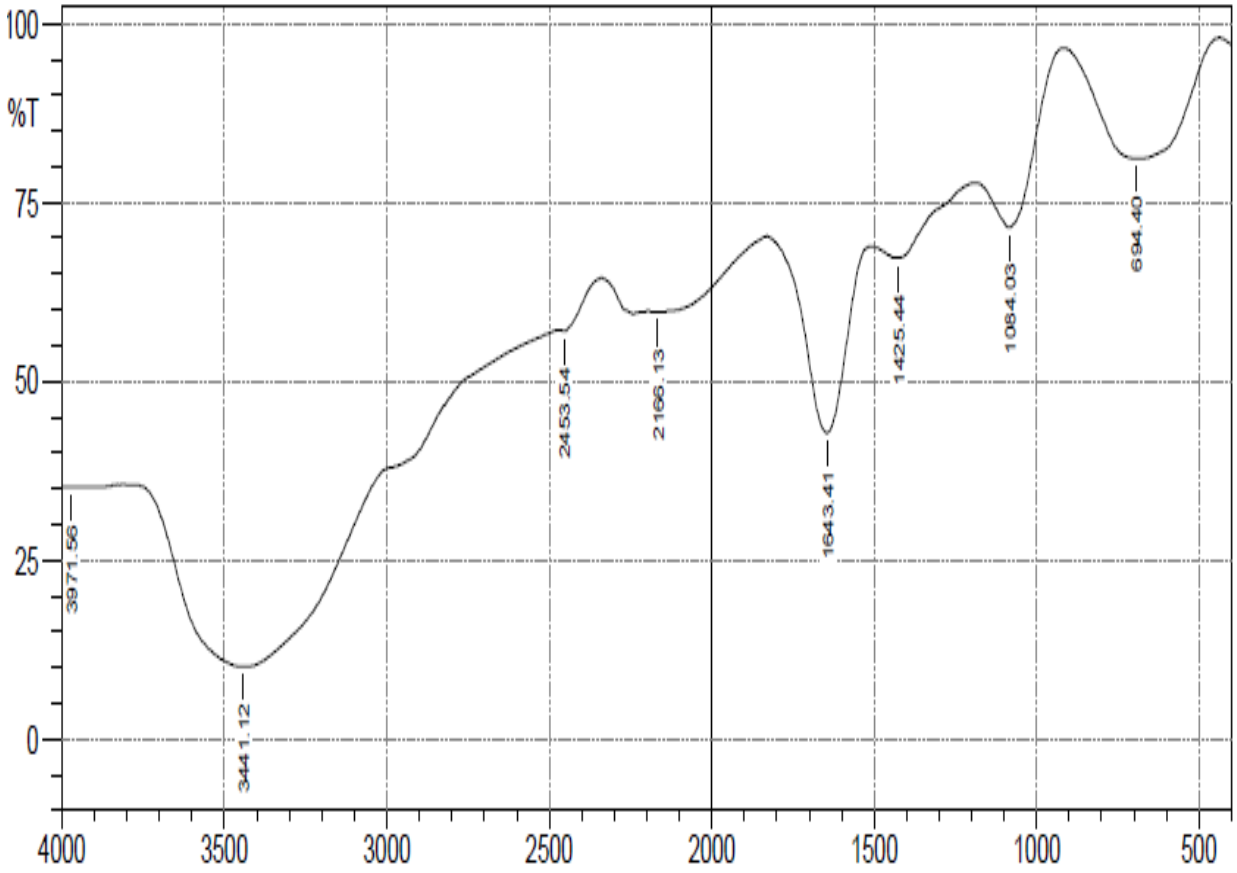


Figure 4.28: FTIR Spectrum of Sweet Pepper Extract at pH 3 and 1.0% Stabilizer under White Light

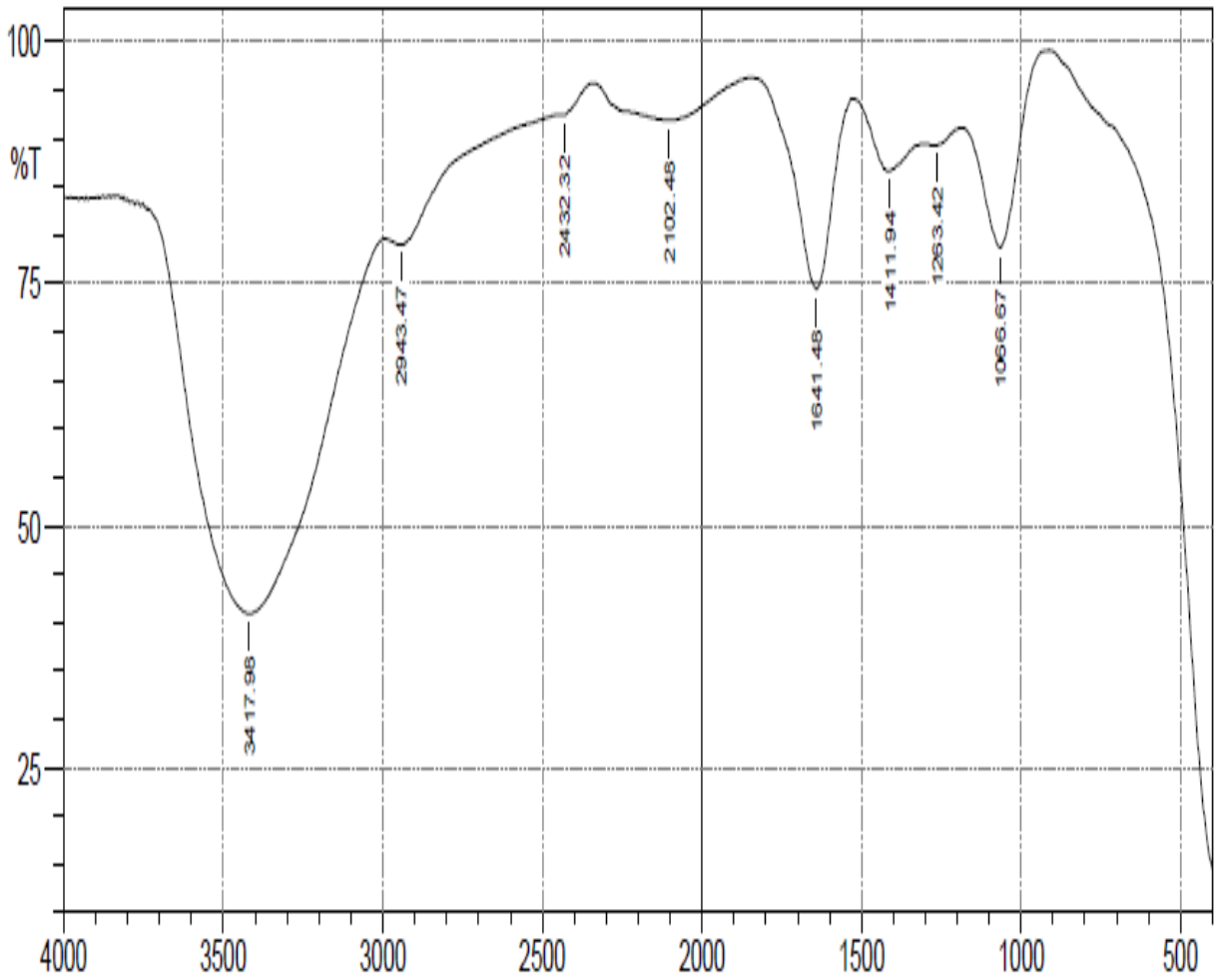


Figure 4.29: FTIR Spectrum of Sweet Pepper Extract at pH 3 and 0.1% Stabilizer in the Dark

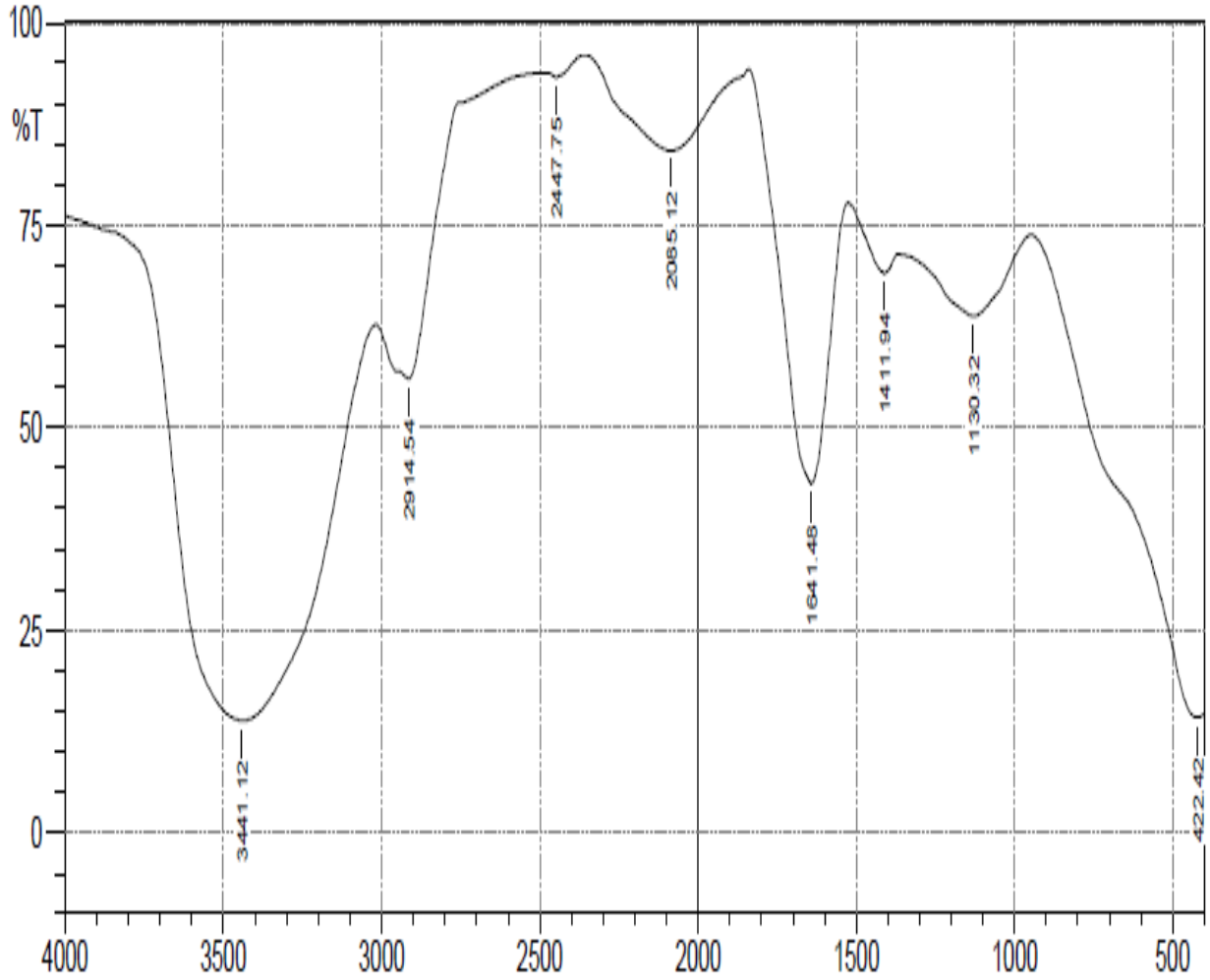


Figure 4.30: FTIR Spectrum of Sweet Pepper Extract at pH 5 and 0.5% Stabilizer in the Dark

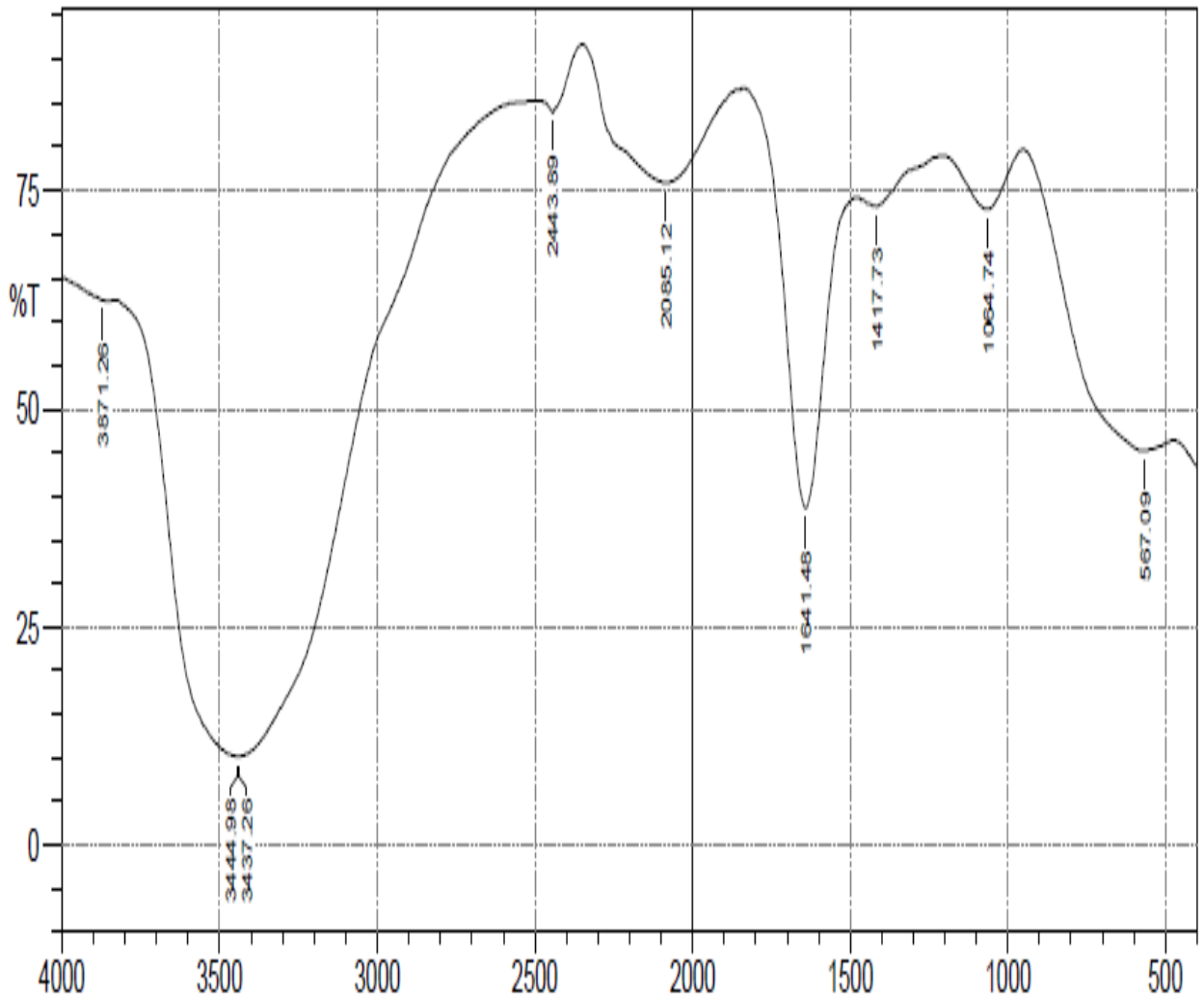


Figure 4.31: FTIR Spectrum of Sweet Pepper Extract at pH 5 and 1.0% Stabilizer in the Dark

Table 4.1: Results of Colour Value of Extracts

Sample	Absorbance (460nm)	ASTA	ICU
Bell pepper	0.261	611.5	24,460
Sweet pepper	0.295	691.1	27,646

Key

ASTA : American Spice Trade Association.

ICU International Colour Units.

Table 4.2: Results of Proximate Composition of Ground Bell Pepper and Extract

Parameter	Ground Pepper %	Extract %
Moisture	10.40 ± 1.38	45.00 ± 0.28
Ash	5.20 ± 0.30	0.90 ±0.24
Crude Fibre	12.00 ± 1.41	ND
Crude Protein	6.70 ± 1.25	2.30 ± 0.01
Crude Lipid	7.40 ± 0.74	7.50 ± 2.37
Carbohydrate	59.10 ± 0.79	44.30 ± 2.32

n= 2

ND: NOT DETECTED

Carbohydrate by difference

Table 4.3: Results of Proximate Composition of Ground Sweet Pepper and Extract

Parameter	Ground Pepper (%)	Extract (%)
Moisture	7.87 ± 0.88	51.60 ± 2.37
Ash	4.99 ± 0.44	0.70 ± 0.64
Crude Fibre	9.56 ± 0.60	ND
Crude Protein	5.99 ± 1.84	2.37 ± 0.37
Crude Lipid	8.03 ± 0.67	16.15 ± 1.56
Carbohydrate	61.75 ± 2.47	29.21 ± 3.66

n= 2

ND: NOT DETECTED

Carbohydrate by difference

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Yield of Extracts

The observed yield of colour pigment above 70% with ethanol, signifies that the pigments were very soluble in the solvent. This observation is in harmony with that reported by Dong *et al.*, (2014) that ethanol is the best solvent in processing food.

5.2 Variations in pH against Time

When pH remains stable for a considerable length of time, the extract is said to be stable. The extracts were stored under UV light, in the dark and under white light at room temperature and their pH monitored weekly for eight weeks. Bell pepper extracts were unstable under UV as their pH continued to change almost on a weekly basis.

Extracts from bell pepper stored under white light showed a greater stability at pH 3 in 1 percent ascorbic acid for the first four weeks. Same extracts at pH 7 were not stable as they deteriorated steadily from the first to the eight week. It can be deduced that under white light, a neutral pH will not effectively maintain the colour of bell pepper extracts. This may partly be attributed to maillard reactions as a result of the high content of reducing sugars and amino acids present in pepper which leads to a colour change or browning at higher pH values as there is a lower amount of unprotonated amino groups at lower pH values (Gogus and Eren 1998).

Bell pepper extracts stored in the dark were more stable at pH 5 when 0.5% ascorbic acid was used as the stabilizing agent. This shows that extracts stored in the dark preserved colour better since the pH remained the same for the period under investigation . Storing in the dark has been adjudged

the best condition for preserving colour (Chattopadhyay *et al.*, 2008); because natural colourants are sensitive to light. Hence for extracts from bell pepper an acid medium with 0.5% stabilizer kept in the dark best preserves colour.

Sweet pepper extracts stored under UV light showed no stability in the acidic, basic or neutral regions. Same extracts stored under white light were stable in the acidic region and 1.0% stabilizer to maintain colour for the entire period under investigation. Those stored in the dark did not maintain pH in acidic or basic media. The degradation of carotenoids is dependent on factors such as nature and composition of foods, processing methods, packaging and storage conditions, activity of lipoxygenase. Instability in carotenoids is caused by the polyene chain, carotenoid susceptibility to oxidation and geometric isomerization. Heat, light and acids promote isomerization of trans-carotenoids to the cis-form (Dutta *et al.*, 2005). Oxidation is affected by the availability of oxygen, the carotenoids involved and their physical conditions.

The 3D dimensional plot gave the following results for Bell and Sweet pepper.

i). Bell Pepper

The 3-Dimensional (3D) plot shows a decrease in response (absorbance) as pH increases and an increase in response as the stabilizer (ascorbic acid) decreases. The most favourable response is at pH of 3 and no stabilizer (0.00). This is very likely because acidity inhibits microbial growth which may lead to colour loss so the colour of the extract is maintained. All the plots showing interactions between different factors such as stabilizer and time , stabilizer and pH, show that the response is highest at low pH and stabilizer values. In other words, colour is retained more in the acidic region with no stabilizer when extracts are kept in the dark. The interaction between pH and time showed a steady decrease in response with time (week 1-8). This may be due to attack by microbes as the

pH increases. Practically, the intensity of colour of the extract in the different conditions of light, began to fade from week one to week eight. This agrees with Morais, (2002) whose findings showed that pigment concentration decreased with increasing length of storage especially for β-carotene.

(ii) Sweet Pepper

The 3-Dimensional (3D) plot for factor interaction between time and stabilizer against the response (absorbance) for sweet pepper shows that response increases as the value of stabilizer decreases. Other interactions such as Stabilizer and pH showed an increase in response (absorbance) when the value of pH drops and stabilizer increases. This defers from the trend observed in bell pepper. Other plots showing interaction between Time and pH showed the acidic media to be the region where colour of the extract is maintained.

5.3 FTIR Spectra of Extracts.

The characteristics of extracts were determined using Fourier Transform Infrared Spectroscopy (Model No.8400S by Shimadzu) which shows the functional groups present and their corresponding wave numbers. In the untreated extract for bell pepper, the prominent functional groups were OH, C-H and C=O. These functional groups were present in extract stored at pH of 5, 0.1% stabilizer under UV light. While those stored at pH 3 in 0.5% stabilizer had only OH and C=O and that at pH 7 and 1% stabilizer had only OH present. The trend followed by extracts kept under UV light showed a reaction in the acidic pH of 3 where all functional groups still remained in the extract. Bond deformations such as stretching or bending occur when IR radiation is absorbed by molecules. Stretching occurs when the excitation causes frequency of vibration changes in the bonded atoms along the bond axis and bending involves atoms moving out of the bonding axis.

Extracts of bell pepper stored under white light, and pH 7 in 0.5% ascorbic acid maintained the same functional groups as the untreated extracts except for those with pH 3 and 1% ascorbic acid and pH 7, 0.1% ascorbic acid where the C-H is missing. Extracts kept in the dark maintained all three functional groups present in untreated extract. It can therefore be said that the best condition to preserve bell pepper extract is in the dark and within an acidic pH range. This agrees with the earlier conclusion that storage in the dark is adjudged the best condition to retain the colour of the extract.

The following functional groups were present in the untreated (control) for Sweet pepper, OH, C-H (Strong) and C=O. These functional groups remained in all the different storage conditions but in varying amounts. Extracts kept under UV light at pH 3, 0.5% ascorbic acid and pH 5, 0.1% ascorbic acid had all three functional groups present. While the extract at pH 7, 0.1% ascorbic acid, under UV light had the three functional groups present with 2 carbonyl C=O bonds. Extracts kept under white light had all three functional groups present except for that stored at pH 7, 0.5% ascorbic acid which had OH, C=O and traces of C-H. Extract kept at pH 7, 0.1% ascorbic acid had OH, C-H and two C=O. The neutral media of pH 7. Extracts kept in the dark had all three functional groups present.

In an attempt to control colour degradation of paprika powders during storage, ascorbic acid which is an important chain-breaking antioxidant, is added to the paprika powder. Ascorbic acid reduces lipid hydroxyperoxides to alcohol inhibiting the propagation of free radicals and protects the antioxidant capacity of a-tocopherol (Morais, 2002)

Carotenoids are susceptible to isomerization, and oxidation during processing and storage this leads to loss of colour and biological activity and the formation of volatile compounds that impart undesirable flavour to food. Oxidation takes place depending on the availability of oxygen, metals, enzymes, unsaturated lipids, antioxidants, severity of treatment.

5.4 Colour Measurement

The American Spice Trade Association (ASTA) and International Colour (IC) are the industry reference methods for quantifying the colour of paprika and other oleoresins as defined in ASTA Method 20.1 – 1997. It is also a method used to measure extractable colour in capsicums and their oleoresins based on the spectral absorbance at 460nm.

The ASTA and IC values for bell pepper are 611.5 and 24460 respectively while that of sweet pepper are 691.1 and 27646 respectively. Hence both varieties can be used as food colour given the high IC value. This agrees with the report of Cantrill (2008) that extract from *Capsicum annuum* have high colour value.

5.5 Evaluation of Nutritional Components of Extracts.

Extract from bell pepper had a moisture content of 45% and 52% for sweet pepper. Ash contains essential mineral components which are necessary to nourish the blood and tissue. They are necessary for blood coagulation, treating wounds, stopping bleeding and treatment or prevention of other ailments. The ash content 0.9% in this work for bell pepper extract and 0.7% for sweet pepper shows low amount of minerals as against their respective powders of about 5%. This means that in other to retain minerals in both peppers using it in powder form is preferable.

The crude protein for both extracts were very similar 2.30% for bell pepper and 2.37% for sweet pepper extracts respectively. The crude lipid levels were higher for extracts from sweet pepper

with a value of 16.15% as against bell pepper with 7.50%. Though generally pepper has low lipid value. The carbohydrate value calculated by difference was higher in extracts from bell pepper which had a value of 44.3% than that from sweet pepper with a value of 29.2%.

On the other hand, FAO (2011) recorded the following values for dried peppers 13.4% moisture, 12.8% protein, 11.9% fat, 56.2% carbohydrate, 22.5% fiber and 5.7% ash. The results in this work are lower than the values recorded by FAO except for the carbohydrate which are higher than FAO values. Furthermore, the moisture content of the bell pepper was 10.40% and that of sweet pepper was 7.87%. Zaki *et al*., (2013) reported that dried pepper with moisture content from 10-14% could retard colour loss while a moisture content less than 4% causes excessive colour loss. Hence ground bell pepper will retard excessive loss of colour.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

Pepper commands a good economic return as an export crop with a significant market. In recent years, sales and marketing have opened up with a broader range of buyers for both rain fed and irrigated produce. In the world ranking of important vegetables, pepper comes second after tomatoes and it is the most produced type of spice used for flavouring and colouring food while providing vitamins and minerals (Esayas, 2011). The following can be deduced from this work;

i. Ethanol was able to extract the colour pigments and its constituents from bell and sweet pepper giving a good yield (above 70%) of colour pigments and other constituents
ii. The most suitable condition to store extract from sweet pepper is in acid media 1% ascorbic acid kept under white light for eight weeks.
iii. For bell pepper the most suitable condition to maintain stability is in acid media with 0.5% ascorbic acid, kept in the dark. This will remain stable for a period of eight weeks as the ascorbic acid slows down deterioration as observed in colour loss.
iv. Extracts of the two varieties of *capsicum annuum* have essentially the same FTIR spectra.
v. The extracts from the varieties of *Capsicum annuum* under investigation can be used as food colour as they have high colour unit of 24460 and 27646 for bell and sweet pepper respectively.
vi. The extracts from the two varieties of *Capsicum annuum* studied must be fortified before adding to food as their nutritional values are quite low as shown in the ash content, and carbohydrate values.

6.2 Recommendations

On the basis of this research finding, it is recommended that:

i. Other varieties of *Capsicum annuum* should be investigated to establish their stability profile.

ii. A second reagent such as food grade n- hexane could be used for the extraction to compare the percentage yield..

iii. The seeds could also be added to the flesh during extraction to determine if there will be any effect in stabilizing the colour.

iv. Another stabilizer such as citric acid could be introduced for comparison**.**

v. The addition of an artificial stabilizer should also be investigated and compared with our findings.

Original Contributions: The study established that:

i. The order of stability for bell pepper was found to be Dark > Light > UV at pH 3,5 and 7.
ii. The order of stability for sweet pepper was found to be Light > UV > Dark at pH 3 and 5 while at pH 7, the order of stability was found to be Dark > Light >UV.
iii. The extracts from bell and sweet peppers can only be used in foods with short shelf lives.
iv. The acidic media is the most suitable media for preserving the colour of extracts from bell and sweet pepper.
v. The extracts from sweet pepper had a lower carbohydrate content (29.21±3.66) than extracts from bell pepper with carbohydrate content value of 44.30±2.32.
vi. The extraction time for sweet pepper using ethanol is longer (9-12 hours) than the extraction time for bell pepper (5-9 hours).
vii.

REFERENCES

Abdeldaiem, M. H. (2014). Use of yellow pigment extracted from turmeric (Curcuma Longa) Rhizomes Powder as Natural Food Preservative. *American Journal of Food Science and Technology, 2* (1): 36-47.

Aberoumand, A. (2011). A Review Article on Edible Pigments Properties and Sources as Natural Biocolorants in Foodstuff and Food Industry. *World Journal of Dairy and Food Sciences, 6* (1): 71-78.

Abou-Arab, A. A., Abu-Salem, F. M., and Abou-Arab, E. A. (2011). Physiochemical Properties of Natural Pigments(Anthocyanins) Extracted from Roselle Calyces (Hibiscus Sabdariffa). *Journal of American Science, 7* (7): 445-446.

AOAC (1980) Official Methods of Analysis 13th edition. Association of Official Analytical Chemists.

Adamson, M. W. (2004). *Food in Medieval Times.* Westport,CT: Greenwood Press.

Barrows, J. N., Lipman, A. L., and Bailey, C. J. (2009). Colour Additives: FDA's Regulatory Process and Historical Pespectives.

Benson, G. A., Obadofin, A. A., and Adesina, J. M. (2014) Evaluation of Plant Extracts for Controlling Insect Pests of Pepper(capsicum spp) in Nigeria Humid Rain Forest. *New York Science Journal, 7* (1): 39-43.

Betina, P. F., Agnes G. and Charles, S. (2012). Assessing the influence of the colour of the plate on the perception of the complex food in a restaurant setting, *Flavour Journal*, 2:24

Biehler, E., Mayer, F., Hoffmann, L., Krause, E., and Bohn, T. (2010). Comparison of 3 spectrophotometric methods for carotenoid determination in frequently consumed fruits and vegetables. *Journal of food science*, *75*(1): C55-C61.

Blanc, P. J. (2009). Natural Food Colourants. In *Encyclopedia of Life Support System, Biotechnology.* Oxford, United Kingdom, 7:140

Burrows, J. D. (2009). Palette of our palates: a brief history of food coloring and its regulation. *Comprehensive Reviews in Food Science and Food Safety*, *8*(4): 394-408

Businessday (2007). Producing pepper for export market. Retrieved September 21, 2015 from http://www.businessdayonline.com

Cantrill, R. (2008). *Paprika Extract.* Chemical and Technical Assessment(CTA) presented at the 69th JEFCA.

Cazonelli, C. I. (2011). Carotenoids in Nature: Insights from Plants and Beyond. *Functional Plant Biology, 38*: 833-847.

Chaitanya, L. G. (2014). Food Colouring: The Natural Way. *Research Journal of Chemical Sciences , 4* (2): 87-96.

Chattopadhay, P., Chatterjei, S., and Sin, K. S. (2008). Biotechnological Potential of Natural Food Grade Biocolourants. *African Journal of Biotechnology, 7* (17): 2972-2985.

Chen D, and Wu .Z (2009). Study on the extraction and purification process of capsicum red pigment. *Journal of Agricultural Science,* 1: 2

Chengaiah, B., Mallikarjurarad, K., Mahesh, K. K., and Madhusudhana, C. C. (2010). Medicinal Importance of Natural Dyes - A Review. *International Journal of Pharmaceutical Research, 2* (1): 144-154.

Chowdhurya, A. I., Mollaa, M. D. A. I., Sarkerb, M., Ranaa, A. A., Rayc, S. K., Nurc, H. P.,and Karima, M. M. (2010). Preparation of edible grade dye and pigments from natural sources Bixa orellana Linn. *International Journal of Basic and Applied Sciences*, *10*(4): 7-21.

Delgado-Vargas, F., Jiménez, A. R., and Paredes-López, O. (2000). Natural pigments: carotenoids, anthocyanins, and betalains—characteristics, biosynthesis, processing, and stability. *Critical reviews in food science and nutrition*, *40*(3): 173-289.

Dowham, A., and Collins, P. (2000). Colouring Our Foods in the Last and Next Millenium. *International Journal of Food Science and Technology,* 35: 5-22.

Dong, X., Li, X., Ding, L., Cui, F., and Tang, Z. (2014). Stage Extraction of Cap saicinoids and Red Pigments from Fresh Red Pepper(Capsicum) Fruits with Ethanol as Solvent. *Food Science and Technology,* 59: 396-402.

Dutta, D., Chaudhuri U. R, and Chakraborty R. (2005). Structure, health benefits, antioxidant prpperty and processing and storage of carotenoids. *African Journal of Biotechnology,* 4(13):1510-1520

Eissa, H. A., Hassanane, M. M., and Sharaf, H. A. (2014). Effect of colorant on cytogenetic, biochemical and histochemical parameters and quality changes during storage of some commercial fruit drinks. *Journal of Nutrition and Food Sciences*, *4*(2): 1.

Eldahshan, O. A., and Singab, A. N. (2013). Carotenoids. *Journal of Pharmacognosy and Phytochemistry, 2* :1

Erinle, I. D. (1989). Present Status and Prospects for Increased Production of Tomato and Pepper in Nigeria. *Integrated Management Practices,* 536-547.

Esayas, K., Shimelis, A., Ashebir, F., Negessie, R., Tilahun, B. and Gulelat, D. (2010). A Short Communication in the Bulletin of Chemical Society of Ethiopa. 25(3): 451-454.

FAO, I., IMF, O., & UNCTAD, W. (2011). the World Bank, the WTO, IFPRI and the UN HLTF (2011). *Price Volatility in Food and Agricultural Markets: Policy Responses. Rome, FAO*.

International Food Information Council (IFIC, 2010) Food Insight, *Food Ingredients and Colours.*

Gogus, F. and Eren, S.(1998). Effect of Temperature and pH on non Enzymic Browning in minced dried pepper during storage. *Journal of Engineering and Environmental Sciences,* 22: 33-38

Howard, L. R., Talcott, S. T., Brenes, C. H., and Villalon, B. (2000). Changes in phytochemical and antioxidant activity of selected pepper cultivars (Capsicum species) as influenced by maturity. *Journal of Agricultural and Food Chemistry, 48*(5): 1713-1720

İnanç, L. A., Demirci, M., and Alpaslan, M. (2010). Effects of Vegetable Oils on The Quality Parameters of Red Pepper During Storage. *Journal of Tekirdag Agricultural Faculty*, *7*(1): 39-47.

Jeannine D (2003). The impact of perceptual interactions on perceived flavor. *Food Quality and Preference* 14 (2): 137–146.

Katz, L. (2014). How Safe are Colour Additives? Food Safety Science. Retrieved November 16, 2016, from https://www.foodsafety.gov.

Kirti, K., Amita, S., Priti, S., and Jyoti, S. (2014). Colorful world of microbes: carotenoids and their applications. *Advances in Biology*, 2014.

Laleh, G. H., Frydoonfar, H., Heidary, R., Jameei, R., and Zare, S. (2006). The effect of light, temperature, pH and species on stability of anthocyanin pigments in four Berberis species. *Pakistan Journal of Nutrition*, *5*(1): 90-92.

Malik, K., Tokkas, J., and Goyal, S. (2012). Microbial Pigments: A Review. *International Journal of Microbial Resource Technology,* 1 (4): 361-365.

Matulka, R. A., and Tardy, A. (2014). Global Focus: Food Colours Vs Colouring Foods in the USA, EU, China, Russia and Brazil. *Agrofood Industry Hi Tech, 25* (3): 7-9.

McKone, H. T. (1995). The Unadulterated History of Food Dyes. *Chemistry Matters,* 14:4.

Morais H, R. P. (2002). Effect of Ascorbic Acid on the Stability of β- carotene and capsanthin in Paprika (Capsicum annuum) powder. *Nahrung/Food, 46* (5): 308-310.

Mortensen, A. (2006). Carotenoids and Other Pigments as Natural Colourants. *Pure and Applied Chemistry,* 78 (8): 1477-1491.

Madhava Naidu, M., and Sowbhagya, H. B. (2012). Technological advances in food colours. *Chemical Industry Digest*, 79-88.

Minguez-Mosquera, M.I.,Perez-Galvez, A., and Honero.D.M.,(2008) Colour Quality in Red pepper (Capsicum annuum) and derived products In Amercan Chemical Society Symposium Series, *American Chemical Society.* 983: 311- 327

Pattnaik, P., Roy, U., and Jain, P. (1997). Biocolours: New Generation Additives for Food. *Indian Food Industry, 16* (5): 21-32.

Rao, A. V., and Rao, L. G. (2007). Carotenoids and Human Health. *Pharmacological Research , 55*, 207-216.

Reddy, M. V., and Sasikala, P. (2013). Capsaicin and Colour Extraction from Different Verities of Green and Red Chilli Peppers of Andhrapradesh. *International Journal of Advanced Scientific and Technical Research, 3* (2), 554.

Ree, E. (2006). Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to Lutein for use in foods for particular Nutritional Uses. *European Food Safety Authority, 315* (1) pp 1-12

Rodriguez-Amaya, D. B. (2001). *A Guide to Carotenoid Analysis in Foods (p.64).* Washington D.C: International Life Sciences Institute.

Rymbai, H., Sharma, R. R., and Manish, S. (2011). Biocolourants And its Application in the Health and Food Industry- A Review. *International Journal of Pharmaceutical Research, 3* (4), 2228-2244.

Sancho,R. C. Lucena, A. Macho, M. A. Calzado, M. Blanco-Molina, A. Minassi,G. Appendino, and E. Mun˜oz (2002), *European. Journal of Immunology*, 32, 1753–1763.

Sahar, S. A., Soltan, Manal, M. E., and Shehata, M. (2012). The Effects of Using Colour Foods of Children on Immunity Properties and Liver,Kidney on Rats. *Food and Nutrition Sciences, 3*, 897-904.

Saltmarsh, M. and Insall, L. (2013). Food Additives and Why They Are Used, In: Saltmarsh, M. (2013). *Essential guide to food additives, (4th ed). Royal Society of Chemistry. pp.1-13*. Doi: 10.1039/9781849734981-00001.

Sanusi, M. M., and Ayinde, I. A. (2013). Profitability of Pepper Production in Derived Savannah Zone of Ogun State, Nigeria. *International Journal of Agriculture and Forestry , 1 and 2*, 401-410.

Sancho,R. C. Lucena, A. Macho, M. A. Calzado, M. Blanco-Molina, A. Minassi,G. Appendino, and E. Mun˜oz (2002), *European. Journal of Immunology*, 32, 1753–1763.

Seliem, E. I., Mahmoud, K. F., Amin, A. A., and Salama, M. F. (2015). Extraction of oleoresin by supercritical fluid extraction, capsulation and its physiochemical properties. *International Journal of Current Research and Academic Review*, 3(5): 1-11.

Sharma, D. (2014). Understanding Biocolour-A Review. *International Journal of Scientific and Technology Research, 3*(1), 294-299.

Sharma, S. (2013). Natural Food Colours. *International Journal of Advanced Research in Pharmaceutical and Biosciences*, 3(2), 151-153.

Tepic, A. N., and Vujicic B.L. (2004). Colour Change in Pepper during Storage. *Acta Periodica Technologica, APTEFF; 35*, 1-280

Thurnham,D.I (1994). Carotenoids: Functions and fallacies. Proceedings of the Nutrition Society, 53:77-87.

Tuquero, J. and Bamba, J. (2016). Bell pepper (capsicum annuum), a potential commercial crop for Guam.College of Natural and Applied Sciences. University of Guam pp 1-5

US Federal Food, Drug and Cosmetic Act, 1958; USFDCA (2014). Retrieved June 3, 2016, from http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/default.htm.

Vracar, L. O., Tepic, A. N., Vujicic, B. L., and Solaja, S. (2007). Influence of the Heat Treatment on the Colour of Ground Pepper (Capsicum annuum). *Acta Periodica Technologica, 38*.

Zaki, N., Hakmaoui, A., Ouatmane, A., and Rernandez-Trujillo, J. P. (2013). Quality Characteristics of Moroccan Sweet Paprika (Capsicum annuum L) at Different Sampling Times. *Food Science and Technology, 33* (3): 577-585.

APPENDICES

APENDIX I

Variation in the pH of Bell Pepper at varying Concentrations of Ascorbic Acid and Light Conditions

Sample ID	Control	7,1.0, UV	3,0.5, UV	5,0.1, UV	7,0.5, Light	7,0.1, Light	3,1.0, Light	3,0.1 Dark	5,0.5, Dark	5,1.0, Dark
WK 1	4.6	6.1	3.0	5.0	5.4	6.1	3.2	3.2	4.6	4.4
WK 2	4.1	6.1	3.0	5.0	5.5	6.2	3.3	3.4	4.5	4.6
WK 3	4.7	6.1	3.1	5.1	5.4	6.1	3.4	3.4	4.5	4.8
WK 4	4.4	5.8	3.2	5.5	4.5	5.7	3.5	3.5	4.5	4.5
WK 5	4.2	4.1	4.3	5.5	4.9	5.0	3.7	3.3	4.5	5.1
WK 6	4.2	4.1	4.3	5.5	4.9	5.0	3.7	3.3	4.5	5.1
WK 7	4.2	4.0	3.6	5.1	4.9	4.3	3.4	3.3	4.4	4.7
WK 8	4.1	4.0	3.4	5.4	4.8	4.4	3.5	3.4	4.4	5.2

APPENDIX II

Design of Experiments results for bell pepper

Std	Run	Block	Factor 1 A:pH	Factor 2 B:Stabilizer %	Factor 3 C:Photo	Factor 4 D:Time Week	Response 1 Response(Dt)
8	37	Block 1	7.00	0.10	Light	1.00	0.693
63	38	Block 1	7.00	1.00	UV	7.00	0.15
48	39	Block 1	3.00	0.10	Dark	6.00	0.249
38	40	Block 1	3.00	0.10	Dark	5.00	0.274
7	41	Block 1	7.00	1.00	UV	1.00	0.82
35	42	Block 1	7.00	0.50	Light	4.00	0.285
32	43	Block 1	5.00	0.10	UV	4.00	0.55
75	44	Block 1	5.00	0.10	UV	8.00	0.23
15	45	Block 1	3.00	0.50	UV	2.00	1.354
14	46	Block 1	5.00	0.10	UV	2.00	2.395
50	47	Block 1	5.00	0.50	Dark	6.00	0.249
58	48	Block 1	3.00	0.10	Dark	7.00	0.237
72	49	Block 1	7.00	0.10	Light	8.00	0.181
66	50	Block 1	7.00	0.50	Light	7.00	0.25
2	51	Block 1	3.00	0.10	Dark	1.00	1.766
51	52	Block 1	3.00	0.50	UV	6.00	0.22
56	53	Block 1	7.00	0.50	Light	6.00	0.247
9	54	Block 1	7.00	0.50	Light	1.00	1.919
61	55	Block 1	3.00	0.50	UV	7.00	0.252
55	56	Block 1	7.00	0.10	Light	6.00	0.161
67	57	Block 1	4.60	0.00	Light	7.00	0.35
5	58	Block 1	5.00	0.10	UV	1.00	2.252
37	59	Block 1	3.00	1.00	Light	4.00	0.193
49	60	Block 1	5.00	1.00	Dark	6.00	0.28
64	61	Block 1	3.00	1.00	Light	7.00	0.125
23	62	Block 1	5.00	0.10	UV	3.00	2.106
59	63	Block 1	5.00	1.00	Dark	7.00	0.245
73	64	Block 1	7.00	0.50	Light	8.00	0.25
41	65	Block 1	3.00	0.50	UV	5.00	0.295
69	66	Block 1	5.00	0.50	Dark	8.00	0.212
71	67	Block 1	3.00	1.00	Light	8.00	0.038
42	68	Block 1	5.00	0.10	UV	5.00	0.428
26	69	Block 1	7.00	0.10	Light	3.00	0.318
68	70	Block 1	3.00	0.10	Dark	8.00	0.168
29	71	Block 1	3.00	0.10	Dark	4.00	0.4
40	72	Block 1	5.00	1.00	Dark	5.00	0.302
43	73	Block 1	7.00	1.00	UV	5.00	0.18
45	74	Block 1	7.00	0.10	Light	5.00	0.2
27	75	Block 1	7.00	0.50	Light	3.00	0.297

53	76	Block 1	7.00	1.00	UV	6.00	0.17
1	77	Block 2	4.60	0.00	Light	1.00	2.475
78	78	Block 2	4.60	0.00	Light	2.00	2.372
79	79	Block 2	4.60	0.00	Light	3.00	2.175
80	80	Block 2	4.60	0.00	Light	4.00	0.835

APPENDIX III

Variation in pH of Sweet Pepper under varying Concentrations of Ascorbic Acid and different Conditions of Light

Sample ID	Control	7,1.0, UV	3,0.5, UV	5,0.1, UV	7,0.5, Light	7,0.1, Light	3,1.0, Light	3,0.1, Dark	5,0.5, Dark	5,1.0, Dark
WK 1	4.5	6.3	2.1	5.1	6.6	6.0	3.0	2.7	5.0	5.2
WK 2	4.4	6.4	2.2	5.2	6.6	6.0	3.0	2.7	5.0	5.2
WK 3	4.5	6.6	2.4	5.5	6.5	5.6	2.9	2.5	5.5	5.6
WK 4	4.5	4.5	2.2	5.0	6.3	6.0	2.9	3.2	5.2	5.3
WK 5	4.6	4.1	4.3	5.5	4.9	5.0	3.7	3.3	4.5	5.1
WK 6	4.6	6.2	2.5	3.9	6.2	5.5	6.2	3.4	5.0	5.3
WK 7	3.9	5.3	2.6	3.8	4.5	5.5	3.1	3.2	4.2	4.3
WK 8	4.5	4.6	3.0	3.8	4.0	5.6	3.1	3.1	3.8	4.1

APPENDIX IV

Absorbance of Bell Pepper Extract at Wavelength of 460 nm

Sample ID	Control	7,1.0, UV	3,0.5, UV	5,0.1, UV	7,0.5, Light	7,0.1, Light	3,1.0, Light	3,0.1, Dark	5,0.5, Dark	5,1.0, Dark
WK 1	2.475	0.820	1.418	2.395	1.919	0.693	0.852	1.766	1.970	2.164
WK 2	2.372	0.792	1.394	2.106	1.692	0.609	0.718	1.611	1.347	1.863
WK 3	2.175	0.342	0.350	0.550	0.297	0.318	0.225	1.400	0.290	0.366
WK 4	0.835	0.197	0.350	0.428	0.285	0.275	0.193	0.400	0.258	0.323
WK 5	0.610	0.192	0.295	0.350	0.275	0.200	0.192	0.274	0.252	0.302
WK 6	0.461	0.180	0.252	0.306	0.250	0.183	0.190	0.249	0.249	0.280
WK 7	0.350	0.150	0.220	0.252	0.250	0.181	0.125	0.237	0.230	0.245
WK 8	0.193	0.029	0.075	0.230	0.249	0.161	0.038	0.168	0.258	0.178

APPENDIX V

Absorbance of Sweet Pepper Extract at Wavelength of 460 nm

Sample ID	Control	7,1.0, UV	3,0.5, UV	5,0.1, UV	7,0.5, Light	7,0.1, Light	3,1.0, Light	3,0.1, Dark	5,0.5, Dark	5,1.0, Dark
WK 1	1.980	0.280	0.850	0.190	0.204	1.270	1.000	0.850	0.353	0.190
WK 2	1.362	0.230	0.580	0.190	0.190	1.107	0.700	0.650	0.340	0.190
WK 3	1.250	0.192	0.350	0.186	0.174	1.100	1.489	0.632	0.317	0.186
WK 4	0.973	0.186	0.347	0.179	0.162	0.874	0.290	0.426	0.270	0.179
WK 5	0.627	0.185	0.275	0.179	0.145	0.750	0.263	0.314	0.250	0.179
WK 6	0.532	0.180	0.234	0.148	0.138	0.634	0.243	0.185	0.217	0.148
WK 7	0.495	0.177	0.206	0.136	0.136	0.255	0.239	0.178	0.203	0.136
WK 8	0.449	0.159	0.192	0.110	0.130	0.251	0.233	0.145	0.196	0.110

APPENDIX VI

Design of Experiments results for sweet pepper.

Std	Run	Block	Factor 1 A:pH	Factor 2 B:Stabilizer %	Factor 3 C:Photo	Factor 4 D:Time Week	Response 1 Response(Ds)
24	1	Block 1	3.00	0.50	UV	3.00	0.35
54	2	Block 1	3.00	1.00	Light	6.00	0.243
46	3	Block 1	7.00	0.50	Light	5.00	0.145
57	4	Block 1	4.60	0.00	Light	6.00	0.532
16	5	Block 1	7.00	1.00	UV	2.00	0.23
12	6	Block 1	5.00	0.50	Dark	2.00	0.34
28	7	Block 1	3.00	1.00	Light	3.00	0.489
25	8	Block 1	7.00	1.00	UV	3.00	0.192
21	9	Block 1	5.00	0.50	Dark	3.00	0.317
70	10	Block 1	5.00	1.00	Dark	8.00	0.155
19	11	Block 1	3.00	1.00	Light	2.00	0.7
11	12	Block 1	3.00	0.10	Dark	2.00	0.65
22	13	Block 1	5.00	1.00	Dark	3.00	0.275
33	14	Block 1	7.00	1.00	UV	4.00	0.186
60	15	Block 1	5.00	0.50	Dark	7.00	0.203
65	16	Block 1	7.00	0.10	Light	7.00	0.255
62	17	Block 1	5.00	0.10	UV	7.00	0.136
74	18	Block 1	3.00	0.50	UV	8.00	0.194
44	19	Block 1	3.00	1.00	Light	5.00	0.263
13	20	Block 1	5.00	1.00	Dark	2.00	0.32
18	21	Block 1	7.00	0.50	Light	2.00	0.19
31	22	Block 1	5.00	1.00	Dark	4.00	0.27
76	23	Block 1	7.00	1.00	UV	8.00	0.159
52	24	Block 1	5.00	0.10	UV	6.00	0.148
30	25	Block 1	5.00	0.50	Dark	4.00	0.27
39	26	Block 1	5.00	0.50	Dark	5.00	0.25
4	27	Block 1	5.00	1.00	Dark	1.00	0.36
34	28	Block 1	3.00	0.50	UV	4.00	0.347
47	29	Block 1	4.60	0.00	Light	5.00	0.627
17	30	Block 1	7.00	0.10	Light	2.00	1.107
10	31	Block 1	3.00	1.00	Light	1.00	1
36	32	Block 1	7.00	0.10	Light	4.00	0.874
3	33	Block 1	5.00	0.50	Dark	1.00	0.353
6	34	Block 1	3.00	0.50	UV	1.00	0.85
77	35	Block 1	4.60	0.00	Light	8.00	0.449
20	36	Block 1	3.00	0.10	Dark	3.00	0.632
8	37	Block 1	7.00	0.10	Light	1.00	1.27

63	38	Block 1	7.00	1.00	UV	7.00	0.177
48	39	Block 1	3.00	0.10	Dark	6.00	0.314
38	40	Block 1	3.00	0.10	Dark	5.00	0.426
7	41	Block 1	7.00	1.00	UV	1.00	0.28
35	42	Block 1	7.00	0.50	Light	4.00	0.162
32	43	Block 1	5.00	0.10	UV	4.00	0.179
75	44	Block 1	5.00	0.10	UV	8.00	0.11
15	45	Block 1	3.00	0.50	UV	2.00	0.58
14	46	Block 1	5.00	0.10	UV	2.00	0.19
50	47	Block 1	5.00	0.50	Dark	6.00	0.217
58	48	Block 1	3.00	0.10	Dark	7.00	0.178
72	49	Block 1	7.00	0.10	Light	8.00	0.251
66	50	Block 1	7.00	0.50	Light	7.00	0.136
2	51	Block 1	3.00	0.10	Dark	1.00	0.85
51	52	Block 1	3.00	0.50	UV	6.00	0.234
56	53	Block 1	7.00	0.50	Light	6.00	0.138
9	54	Block 1	7.00	0.50	Light	1.00	0.204
61	55	Block 1	3.00	0.50	UV	7.00	0.206
55	56	Block 1	7.00	0.10	Light	6.00	0.634
67	57	Block 1	4.60	0.00	Light	7.00	0.495
5	58	Block 1	5.00	0.10	UV	1.00	0.19
37	59	Block 1	3.00	1.00	Light	4.00	0.29
49	60	Block 1	5.00	1.00	Dark	6.00	0.19
64	61	Block 1	3.00	1.00	Light	7.00	0.239
23	62	Block 1	5.00	0.10	UV	3.00	0.186
59	63	Block 1	5.00	1.00	Dark	7.00	0.186
73	64	Block 1	7.00	0.50	Light	8.00	0.13
41	65	Block 1	3.00	0.50	UV	5.00	0.275
69	66	Block 1	5.00	0.50	Dark	8.00	0.196
71	67	Block 1	3.00	1.00	Light	8.00	0.233
42	68	Block 1	5.00	0.10	UV	5.00	0.179
26	69	Block 1	7.00	0.10	Light	3.00	1.1
68	70	Block 1	3.00	0.10	Dark	8.00	0.145
29	71	Block 1	3.00	0.10	Dark	4.00	0.44
40	72	Block 1	5.00	1.00	Dark	5.00	0.21
43	73	Block 1	7.00	1.00	UV	5.00	0.185
45	74	Block 1	7.00	0.10	Light	5.00	0.75
27	75	Block 1	7.00	0.50	Light	3.00	0.174
53	76	Block 1	7.00	1.00	UV	6.00	0.18
1	77	Block 2	4.60	0.00	Light	1.00	1.98
78	78	Block 2	4.60	0.00	Light	2.00	1.362
79	79	Block 2	4.60	0.00	Light	3.00	1.25
80	80	Block 2	4.60	0.00	Light	4.00	0.973

APPENDIX VII

FTIR Table of Assignment for Characterization of Bell Pepper Extract (Control)

Frequency (cm^{-1})	Vibration
1085-1125	C-O
750-810	C-H
1640-1670	C=C
2240-2260	C=N
2850-3000	C-H

APPENDIX VIII

FTIR Table of Assignment for Characterization of Bell Pepper at pH 3, 0.1% Ascorbic Acid in the Dark.

Frequency (cm^{-1})	Vibration
3443.05	OH
2000.91	HC=CH (Stretch)
1999.55	C=O (Stretch)
1411.94	CH_2 (deformation), H-C-H
1066.67	OH bending

APPENDIX IX

FTIR Table of Assignment for Characterization of Bell Pepper at pH 5, 0.5 Ascorbic Acid in the Dark

Frequency (cm^{-1})	Vibration
3437.26	OH stretch
1641.48	C=O
1411.94	CH_2 (deformation), H-C-H (bend)
1082.10	OH bending

APPENDIX X

FTIR Table of Assignment for Characterization of Bell Pepper Extract at pH 5, 1% Ascorbic Acid in the Dark.

Frequency (cm^{-1})	Vibration
3475.84	OH Stretch
2087.05	HC=CH (stretch)
1635.69	C=O (stretch)
1398.44	C=O (stretch), OH
1074.39	OH bending

APPENDIX XI

FTIR Table of Assignment for Characterization of Bell Pepper Extract at pH7, 0.5% Ascorbic Acid under White Light.

Frequency(cm^{-1})	Vibration
3443.05	OH(stretch)
2414.96	HC=CH(stretch)
1641.48	C=O(stretch)
1415.80	OH(bending)
1062.81	C-N stretch aliphatic, OH bending

APPENDIX XII

FTIR Table of Assignment for Characterization of Bell Pepper Extract at pH 7, 0.1% Ascorbic Acid under White Light.

Frequency(cm^{-1})	Vibration
3433.41	OH stretch
2080.91	HC=CH(stretch)
1643.41	C=O
1394.58	C=O(stretch), OH bending
1278.85	OH bending
1060.88	C-O(stretch), OH bending

APPENDIX XIII

FTIR Table of Assignment for Characterization of Bell Pepper Extract at pH 3, 1% Ascorbic Acid under White Light.

Frequency(cm^{-1})	Vibration
3436.34	OH stretch
2002.83	HC=CH(stretch)
1643.41	C=O stretch
1361.79	C=O(stretch), OH bending
1057.03	OH bending

APPENDIX XIV

FTIR Table of Assignment for Characterization of Bell Pepper Extract at pH 7, 1% Ascorbic Acid under UV Light.

Frequency(cm^{-1})	Vibration
3457.26	OH(stretch)
2424.50	C=N
2077.44	HC=CH(stretch)
1641.48	C=O (stretch)
1060.88	C-O (stretch)

APPENDIX XV

FTIR Table of Assignment for Characterization of Bell Pepper Extract at pH 3, 0.5% Ascorbic Acid under UV Light.

Frequency(cm^{-1})	Vibration
3431.48	OH stretch
2000.01	HC=CH(stretch)
1641.48	C=O (stretch)
1054.74	C-O(stretch)

APPENDIX XVI

FTIR Table of Assignment for Characterization of Bell Pepper Extract at pH 5, 0.1% Ascorbic Acid under UV Light

Frequency(cm^{-1})	Vibration
38838.62	OH(stretch)
2085.12	HC=CH(stretch)
1637.62	C=O(stretch)
1406.15	OH bending
1280.42	OH bending
1066.67	C-O stretch

APPENDIX XVII

FTIR Table of Assignment for Characterization of Sweet Pepper Extract (Control)

Frequency (cm^{-1})	Vibration
500-680	C-Br
2500-3500	O-H
2850-3000	C-H
1050-1085	C-O
1630-1680	C=O
3400-3500	N-H

APPENDIX XVIII

FTIR Table of Assignment for Characterization of Sweet Pepper Extract at pH 7, 0.5% Ascorbic Acid under White Light.

Frequency (cm^{-1})	Vibration
3437.26	OH(stretch)
1637.62	C=O
1361.79	CO (stretch) OH bending
1070.53	CO (stretch) OH bending

APPENDIX XIX

FTIR Table of Assignment for Characterization of Sweet Pepper Extract at pH 7, 0.1% Ascorbic Acid under White Light.

Band Position(cm^{-1})	Vibration
3439.19	OH(stretch)
2908.75	C-H(stretch)
2121.77	HC=CH(stretch)
1697.41	C=O(stretch)
1633.76	C=O,C=C,C-N(stretch), NH_2(deformation)
1439.94	H-C=O,OH(Bend)
1141.90	C-O,C-N(stretch)
837.13	C-H(deformation)
704.04	C-H(deformation)

APPENDIX XX

FTIR Table of Assignment for Characterization of Sweet Pepper Extract at pH 3, 1% Ascorbic Acid under White Light

Frequency (cm^{-1})	Vibration
3441.12	OH(stretch)
2166.13	HC=CH(stretch)
1643.41	C=O(stretch)
1425.44	OH(bend)
1084.03	CO(stretch), OH bend
694.40	C-H(deformation)

APPENDIX XXI

FTIR Table of Assignment for Characterization of Sweet Pepper Extract at pH 5, 0.1% Ascorbic Acid in the Dark

Frequency(cm^{-1})	Vibration
3417.98	OH(stretch)
2943.47	C-H(stretch)
2102.48	HC=CH(stretch)
1641.49	C=O(stretch)
1411.94	CH_2(deformation),H-C-H(bend)
11263.42	OH(Bend)
1066.67	OH(bend)

APPENDIX XXII

FTIR Table of Assignment for Characterization of Sweet Pepper Extract at pH 5, 0.5% Ascorbic Acid in the Dark

Frequency(cm^{-1})	Vibration
3441.12	OH(stretch)
2914.54	C-H(stretch)
2085.12	HC=CH(stretch)
1641.48	C=O(stretch)
1411.94	CH_2(deformation), H-C-H(bend)
1130.32	OH(bend)

APPENDIX XXIII

FTIR Table of Assignment for Characterization of Sweet Pepper Extract at pH5, 1% Ascorbic Acid in the Dark

Frequency(cm^{-1})	Vibration
3444.98	OH(stretch)
3437.26	OH(stretch)
2005.12	HC=CH(stretch)
1641.40	C=O(stretch)
1417.73	H-C-H(bend)
1084.74	OH(bend)

APPENDIX XXIV

FTIR Table of Assignment for Characterization of Sweet Pepper Extract at pH 3, 0.5% Ascorbic Acid under UV Light

Frequency (cm^{-1})	Vibration
3435.34	OH(bending)
2916.47	CH(stretch)
2077.40	HC=CH(stretch)
1641.49	C=O
1415.80	CH_2(deformation) H-C-H(bending)
1004.03	OH(bending)

APPENDIX XXV

FTIR Table of Assignment for Characterization of Sweet Pepper Extract at pH 5, 0.1% Ascorbic Acid under UV Light

Frequency (cm^{-1})	Vibration
34443.05	OH (stretch)
2918.40	CH(stretch)
2076.47	HC=CH(stretch)
1639.55	C=O
1442.00	H-C-H(bending)
1274.99	OH(bend)
1139.04	C-O,C-N(stretch)