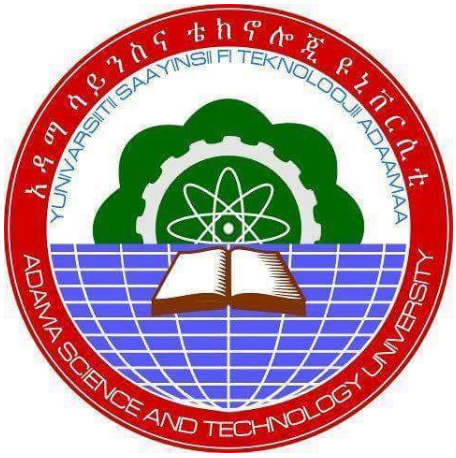
Determination of Mango and Orange Fruit Juice Quality by UV-Visible and Fluorescence Spectroscopy



By
Muktar Gebishu

A Thesis Submitted to Department of Applied Physics
School of Applied Natural Science

Office of Graduate Studies

Adama Science and Technology University

November 2, 2020,
Adama, Ethiopia

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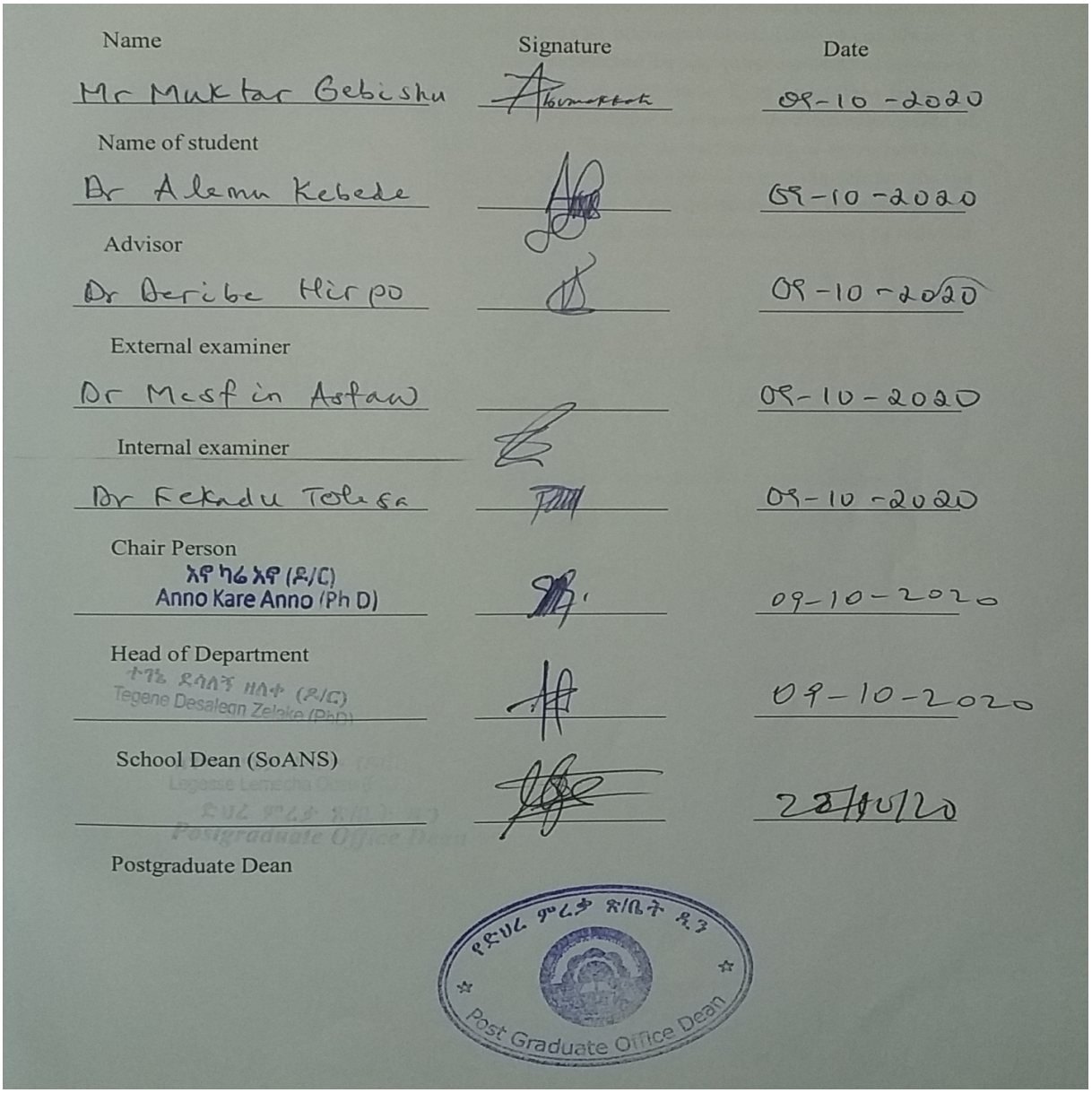
Approval Page

We, the undersigned, members of the Board of Examiners of the final open defense by Muktar Gebishu have read and evaluated his thesis entitled **"Determination of Mango and Orange Fruit Juice Quality by UV-Visible and Fluorescence Spectroscopy"** and Examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirement of the degree of Master of Science in Applied in Physics (Laser Spectroscopy).



Declaration and Statement of the Candidate

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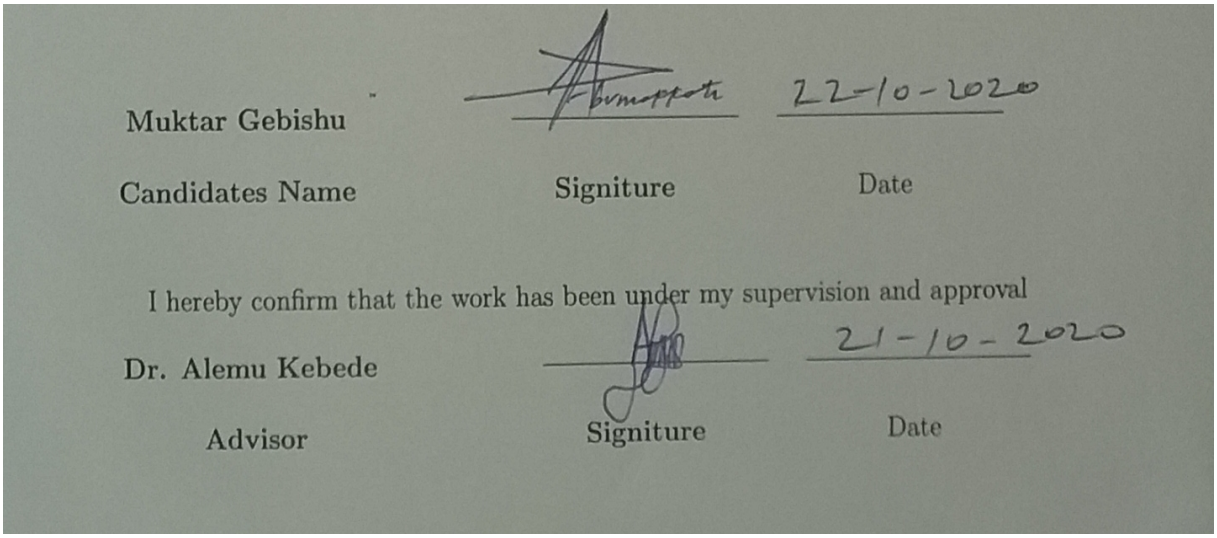

Dedication

I dedicate this thesis manuscript to my father, Gebishu Kasim and my mother Hawa Hussein, for the foundation they laid in the success of my life, and my wife Kulthuma Jundi who sacrificed all her time, love and benefits for my success.

Acknowledgment

I am grateful to the almighty Allah who has blessed me so much in life, including the opportunity to do this research.The completion of the thesis would not have been possible without the help and support of my supervisor, Dr. Alemu Kebede. Your encouragement and guidance has been indispensable to my work. So, I have a special indebted thank for your generously supervise from the beginning up to the end by sharing your knowledge and experience to steer me in the right direction to complete my research work.It is difficult to overstate the role my my family Kulthuma Jundi with my lovely girls Makkah Muktar, for their encouragement and financial supports throughout and also allowing me to put aside the time necessary to complete my Master's Degree.I couldn't have been achieved without the support and encouragement of my brother Ma'aruf Gebishu and all my family members . Last but not least, my heartfelt gratitude goes to Maths department student Sadam Dawud helped me in use of latex in the process of writing this thesis.

Adama Science and Technology University

Muktar Gebishu
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Abstract

In this study, the quality of mango and orange juices was determined using UVvisible and a fluorescence spectrophotometer. The juice were extracted with a home juice extractor. Subsequently the juice were filtered and each sample were separated in to three portions. Juices sample that were measured immediately marked as 'Fresh' those stored at 22^0C for 8 days (were marked as stored) and the other portion heated-stored in a water bath at $(40^0, 60^0, 80^0)C$ for 10 min. Results of Uv-visible absorption spectra of fresh mango juice were had peak at 455 nm . The absorption spectra of stored mango juice had peaks at 270 nm and 460 nm. The heated-stored at (40^0) mango juices had peak at 320 nm but no peaks found in heated-stored at 60^0 and 80^0C because this could be the effect of temperature dependence. The peak appearing at 270 nm corresponds to polymethoxyflavons. The peak at 460 nm corresponds to chlorophyll. The absorption spectra of fresh orange peaks at 330 nm and 390 nm . The stored orange juice had peaks at 260 nm and 320 nm. The absorption spectra peaks of heated-stored at 40^0C and 60^0C were found in 320 nm and 260 nm respectively, but no peaks at heated stored at 80^0C. The peak at 260 nm corresponds to vitamin C. The peak at 330 nm and 390 nm were assigned to cuomerin and vitamin A respectively. The emission spectra of fresh mango juice had peaks on 454 nm ,540 nm and 700 nm. The stored mango juice had peaks at 460 nm and 700 nm. Heated-stored mango juice at 40^0C have bands at 420-500 nm and peak at 700 nm. The heated-stored at 60^0C and 80^0C mango juice had peak only at 700 nm. The peak at 454 nm corresponds to total carotenoids. The emission spectra peaks at 546 nm and 700 nm corresponds to polymethoxyflavons and chlorophyll respectively. The emission spectra of fresh orange juice had peaks at 455 nm, 500 nm and 700 nm. The stored orange juice band around 460-500 nm and peak at 700 nm ,but heated-stored orange juice had peak only at 700 nm. The absecence of some absorption and fluorescence peaks in heated orange and mango juice above 40^0C indicated that essential vitamins in the sample were degraded, but heating also resulted in emergence of new peaks that could result from oxidation and heat solubility of vitamins. The pH value of mango and orange juice were 4.02-4.72,3.52-3.73 respectively. The effect of pH value of mango and orange juice on fluorescence spectra were differ from portion to portion of fruit samples. The fluorescence quantum yield of chlorophyll found in mango and orange juice were 0.10 and 0.37 respectively. The fluorescence lifetime value of two fruit juices were measured. The measured lifetime of two samples were 102.8 ms.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

The quality of fruits is mainly due to the taste (such as sweetness, acidness and bitterness), feeling in mouth characterized by the structure, aroma and so on. Though the quality is attributed to the balance of the above factors, the taste such as sweetness, acidness and bitterness would be one of the most interesting factors for consumers. Especially, the acid and sugar content in fruit and their ratio are very important factors for the quality evaluation by consumers. The acidity and sugar content are conventionally evaluated with the acidimeter and Brix scale based on the titration and refractometry, respectively. But the predicted values indicate only the overall acidity and sugar content, and do not have enough accuracy.Therefore to understand the amount and the sources of the acid and sugar spectroscopic techniques such as ultraviolet (UV), visible (VIS), near-infrared (NIR), mid-infrared (MlR) and fluorescent spectroscopic characterization techniques must be employed[1].

Fruit quality such as taste ,color and sugar content etc. may depend on latitudinal,climate, soil and post harvest management variation [2, 3]. Shelf life of products are determined by dates and are the responsibilities of manufacturer and regulatory agencies. These are dependent on thresholds of either microbiological safety,physical condition or organoleptic qualities. Many factors that influence shelf-life, could be categorized into intrinsic (water activity, pH, acidity,preservatives, biochemical and microbial compositions) and extrinsic factors (Timetemperature, pressure, relative humidity, ultrasonic lights, packaging material, and handling) procedures. Shelf-life of most home-made products unlike industrial products, are not standardized and are predisposed to uncertainty factors. To the best of our knowledge the quality of some fruit juice were determined by spectroscopic method

in Japan , China , Denmark and in other country but not in Ethiopia. Thus in this works the quality of mango and orange fruit juice will be determined by using fluorescence and Uv-visible spectroscopy.

1.2 Statement of the Problem

According to Ethiopian Investment Agency (1998), fresh and processed fruits have a large domestic market in Ethiopia despite the fact that the quality and safety were not determined by spectroscopic method. So the researcher was interested to do research on the determination of mango and orange fruit juice quality by Uv-Visible and Fluorescence spectroscopy.

1.3 Objectives of the Study

1.3.1 General Objective of the Study

The main objective of this study is to determine the quality of mango and orange fruit juice by UV-Visible and Fluorescence spectroscopy.

1.3.2 Specific Objectives

The specific objectives are to:

- Determine the excitation and emission wavelength
- Calculate the fluorescence quantum yield
- Calculate the Fluorescence life time
- Determine absorption wavelength

1.4 Significance of the Study

This study helps vendor to have better understanding and awareness about quality and stay competent in the market and also they benefit consumer by preventing disease spreading through consumption of fruit juice. Government regulatory bodies (Ethiopian Standard authority and Ethiopian Food, Medicine and Health Care Administration and Control Authority) can use the study result for designing appropriate disease prevention strategies. Moreover, the study will be used as an initial data for future researches on quality of fruit juice. It helps to aware people about the health risks that possibly associated with consuming deteriorated juice.

CHAPTER 2

LITERATURE REVIEW

2.1 Fruits

Fruit refers to the mature ovary of a plant, including its seeds, covering and connected tissue. This includes both fleshy and dry fruits[4]. More than 1000 species of tropical fruits are found in the tropical Americas, versus about 500 in Asia, about 300 in the Indian subcontinent, and about 1200 in Africa [5]. Currently, fruit juices are widely consumed by most people (wide age group) as meal and dessert. Fruit juices are also consumed at fruit juice houses, cafeterias and restaurants. From nutritional point of view, fruits are low energy-dense food relatively rich in vitamins, minerals and other bioactive compounds as well as good sources of fiber [6]. Fruit, in botanical terms is freshly or dry ripened ovary of a plant, which encloses the seed or seeds. The fleshy component, which is normally the portion eaten, serve to protect and eventually nourish the seed as part of the natural development of the original plants progeny [7]. Fruits, either fresh or processed, form an important part of our daily diet, and demand is increasing in all over the world. Recent advances in agricultural technology have contributed significantly to the production of fruits throughout the world. Fruits are very perishable in nature because they are living beings and carry out transpiration, respiration, ripening and other biochemical activities which adversely affect the quality. In addition, because of their high moisture content fruits are inherently liable to deteriorate, especially under tropical conditions, and finally become unmarketable [8].

Fruit is consumed both as fresh and processed form. The storage life of fruit depends on the stage of maturity at which the fruit is harvested.The production marketing and consumption of fruit are restricted due to improper handling, inadequate transport and storage facility, disease problem and sensitivity to low storage. Heating fruit can have

several advantages. It can delay ripening and control decay and insect attack. Heating fruit is one the methods for quality and and shelf life.

2.1.1 Mango Fruit

Mango (Mangifera indica L.) is an important tropical fruit having a huge demand in world markets. India produces around 1104 million tonnes of mangoes annually while contributing meagrely in the world market, mainly because of lack of precision in sorting methods based on internal quality. Consumer preference is mainly driven by sweetness, which is dependent on physiological parameters described by various researchers [9]. Increase in total soluble solids (TSS), carotenoid pigments and decrease in acidity are some indicators of sweetness of mango [10]. Presently most consumers determine these by experiencing surface firmness, gloss, aroma, flavour, etc., which is often misleading[11]. Many workers have worked for maturity and quality indices of otherfruits and vegetables too [12], but most of them are of a chemical or physiological nature, and their determination involves very laborious laboratory techniques.

Currently, non-destructive techniques for quality evaluation have gained momentum[13]. These techniques,particularly for fruits and vegetables, are quick and easy to use. Many physical characteristics of fruits and vegetables have been determined non-destructively[14]. India produces around 1104 million tonnes of mangoes annually while contributing meagrely in the world market, mainly because of lack of precision in sorting methods based on internal quality. Consumer preference is mainly driven by sweetness, which is dependent on physiological parameters described by various researchers[15]. Increase in total soluble solids (TSS), carotenoid pigments and decrease in acidity are some indicators of sweetness of mango. Presently most consumers determine these by experiencing surface firmness, gloss, aroma, flavour, etc., which is often misleading. Many workers have worked for maturity and quality indices of otherfruits and vegetables too, but most of them are of a chemical or physiological nature, and their determination involves very laborious laboratory techniques.Currently, non-destructive techniques for quality evaluation have gained momentum. These techniques, particularly for fruits and vegetables, are quick and easy to use. Many physical characteristics of fruits and vegetables have been determined non-destructively[16]

Fruit crops play an important role in the national food security of people around the world. They are generally delicious and highly nutritious, mainly of vitamins and miner-

als that can balance cereal-based diets. Fruits supply raw materials for local industries and could be sources of foreign currency. Moreover, the development of fruit industry will create employment opportunities, particularly for farming communities. In general, Ethiopia has great potential and encouraging policy to expand fruit production for fresh market and processing both for domestic and export markets. Besides, fruit crops are friendly to nature, sustain the environment, provide shade, and can easily be incorporated in any agro-forestry programs [17]. The mango, because of its attractive appearance and the very pleasant taste of selected cultivars, is claimed to be the most important fruit of the tropics and has been touted as 'king of all fruits. The fruit contains almost all the known vitamins and many essential minerals. The protein content is generally a little higher than that of other fruits except the avocado. Mangos are also a fairly good source of thiamine and niacin and contain some calcium and iron [18]. According to CSA (2012/2013), about 61,972.6 hectares of land is under fruit crops in Ethiopia; mangoes contributed 14.2% of the area. Moreover, out of 479,336 tons of fruits produced in the country, mangoes accounted 14.5% fruit production. It is grown in several parts of the country where the western and eastern Ethiopia are among the major producing belt that accounts> 50% of the total mango production in Ethiopia [19]. However, none of them identified the farmers mango cultivars and the depth of generated information with regard to pre and post production practices and marketing especially in the east and western Ethiopia was not sufficient to alleviate the challenges.

2.1.2 Orange Fruit

The sweet orange (Citrus sinensis) is a member of the citrus family (Rutaceae), along with other fruits such as mandarins, lemons, grapefruits and limes. Oranges account for the greatest value followed by grapefruits, lemons, mandarins and limes. In the pre-historic era,sweet orange was cultivated in several locations including areas now occupied by the modern China, India, Bhutan, Burma, and Malaysia [20]. Globally, the leading producer of sweet oranges is Brazil followed by the European Union and China. In 2014, Brazil produced 17 340 MT followed by China [7 600 MT], United States [6 291 MT], and European Union [6 075 MT]. In Africa, Egypt was leading with a production of 2 570 MT followed by South Africa [1600 MT] and Morocco [1 000 MT] [21]. Orange production in Southern Africa is ranked the third regarding importance after vegetables and deciduous fruits with commercial production mainly concentrated in South Africa, Swaziland, Mozambique, and Zimbabwe (RSA, 2011). Zimbabwes geographic position and climate makes it ideal to produce early maturing varieties of oranges which reach the target mar-

kets earlier than neighbouring competing countries [22]. Oranges are mainly produced in areas within or surrounding Limpopo Valley, Save Valley, Mazowe Valley, and Rusitu Valley in Zimbabwe [23].

Orange is one of the most economically important fruit crops grown by smallholders and commercial farmers in Ethiopia[24]. The total area coverage and the annual production of citrus were estimated 5,947 ha and 77,087 tons, respectively [25, 26]. However, citrus production and productivity in Ethiopia is seriously threatened by various diseases including leaf and fruit spot disease [27], which is caused by the fungus Pseudocercospora angolensis [28].

2.2 Fruit Juices

Fruit juice are defined in the most general sense as the extractable fluid contents or tissues of the fruit or aqueous liquid squeezed or extracted usually from one or more fruits [29]. Fruit juices are prepared mechanically by squeezing or macerating the pulp 10 of fresh fruits or vegetables without application of heat or solvent to give an unfermented cloud, un-clarified and untreated juice ready for consumption. A common practice like diluting or blending in fruit juices preparation determine the strength of acidity or flavor [30]. However, quality deterioration temporarily appears during the processing and storage. Enzymatic browning of damaged tissues of fruits during postharvest handling and processing is one of the main causes for quality loss of fresh fruit produce [31]. Since quality is supremely important for fruit juice, deterioration has to be controlled during processing and storage. Therefore, the fast and real-time detection of the extent of quality changes would provide a valuable tool.

Chlorophylls a and b are the only chlorophylls found in higher plants and green algae, a minor variant of chlorophyll a (chlorophyll a') is found in the reaction centre of photosystem I. Carotenoids are very strong absorbing molecules, with an extinction coefficient that can exceed 150,000 mor l cm^{-l} (the extinction coefficient is a simple measure of the lightabsorbing power of a molecule; an extinction coefficient of I mort cm^{-1} would mean that a 1 cm thick layer of a 1 mol dm^{-3} solution would have an absorbance of 1; chlorophyll has an extinction of about 100,000 mort cm^{-1}'in ether at 660 nm). High carotenoid content could provide a high vitamin A value and anti-oxidative capacity to people living in vitamin A-deficient areas. Carotenoids absorb the shorter wavelengths of light,typically less than 500 nm, and therefore, in solution they appear yellow, red

or orange to the human eye. Though all based upon the linear polyene structures, the structures of the carotenoids are very diverse, due to the diverse groups that terminate the chain. The presence of carotenoid pigments next to chlorophylls in the chlorophyll binding pigment-protein complexes, and the antioxidant tocopherol (vitamin E) within the thylakoid membrane. Chlorophyll fluorescence, it is worth spending some time looking at the properties of chlorophyll a in vitro. Chlorophylls are bound to proteins in vivo, and thus in vitro properties do not accurately reflect those found in photosynthetic tissue. However, the in vitro properties do act as a reference for the properties of chlorophyll a in vivo. The excitation spectra, better known as the absorption spectra, differ for the two chlorophylls (Figure 2.1) [32].

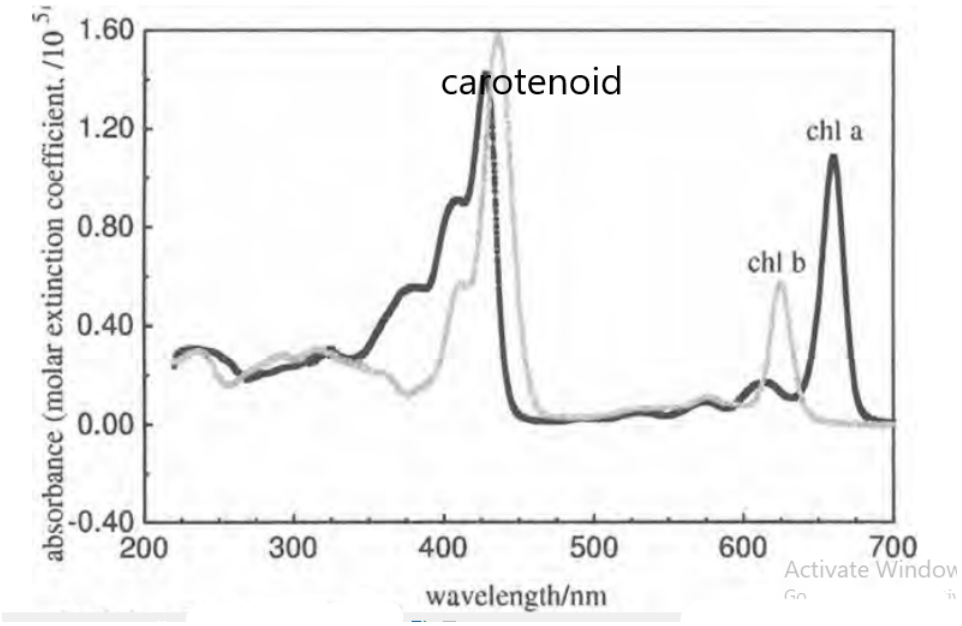


Figure 2.1: The absorption, or excitation, spectra of the carotenoids and chlorophylls

The red absorption peak for chlorophyll b is at shorter wavelengths than that for chlorophyll a, and this has major consequences for the way that excitation energy is transferred between chlorophyll a and chlorophyll b and for the chlorophyll fluorescence spectrum that is observed in vivo. Before we discuss how energy can move between molecules, we need to understand something about how the relaxation of excited molecules. The absorption (and fluorescence and phosphorescence) spectrum is shown in a rotated format to the left of the diagram, and the energy levels corresponding to the absorbance peaks, and their interconversion routes, are shown to the right [32].

2.3 Factors Affecting Postharvest Quality of Fruits

2.3.1 Pre-Harvest Factors Affecting Post Harvest Quality of Fruits

Postharvest management starts with preharvest managements. Once the fruits are harvested,the overall quality of fresh fruits can hardly be improved but it can be maintained. The final market value of the produce and acceptance by the consumers depends upon the growers ability to apply best available preharvest technology followed by harvesting and then to apply best available postharvest handling practices. The preharvest factors influencing postharvest quality are frequency of irrigation, use of fertilizers, pest control, growth regulators, climatic conditions like wet and windy weather, natural climates such as hailing, high wind velocity, heavy rainfall, and tree conditions (age, training pruning, light penetration, etc), which influences overall fruit quality and suitability for storage by modifying physiology, chemical composition, and morphology of fruits. One such preharvest factor is spray of Gibberellic acid (10 ppm), if applied at color break stage, results in delay in color development and maintains firmness. This is important because it in extending harvesting period.

i. Cultural Operations

The capacity of leaf photosynthesis depends on the incidence of light, whereby the shaded parts of the canopy assimilate less and need more leaves than the well illuminated part for optimal fruit development. Growers can rely on a number of methods which directly or indirectly influence photosynthesis and sink activity (fruit growth). Among these, the most important are tree height, distance, fruit thinning, pruning, fertilization, and application of growth regulators, irrigation and phytosanitary control [33].

ii. Mineral Nutrition

The effect of soil on fruit quality is largely dependent on plant nutrient availability [34, 35]. Differences in soil patterns also affect the internal quality of pears. Fruit from sandy soils have lower firmness and TSS levels [36]. Plant nutrition is an important factor that potentially affects both the quality and postharvest life of fruit. Optimum plant performance depends on a balanced availability of mineral nutrients that can be limited in many soils around the world [37]. Nitrogen (N) and potassium (K) are the principal nutrients needed by plants [38].

iii. Climatic Factors

Environmental factors such as light, CO, relative humidity, temperature and water availability are major direct or indirect constraints for plant photosynthesis. Environmental factors affect the content of bioactive compounds indirectly by giving the prerequisites for photosynthesis, and thereby providing energy or precursors of the synthesis of the bioactive compounds. Further, the syntheses of these compounds are also affected directly by various environmental factors [37]. Abiotic conditions, i.e. soil fertility and water availability, vary from year to year and site to site, and can affect the level and quality of fruit after harvest [39]. Increased exposure to light increases fruit size [40], total soluble solids and flesh firmness [41].

iv. Genetic Factors

The cultivar of the fruit species is one of the most important factors in determining the variation in, e.g., the fruits soluble solids content and acidity [42]. Nowadays, horticultural breeding and biotechnology could play a significant role in improving and maintaining postharvest quality and the safety of fresh produce. Moreover, the growers have the choice of selecting preferred cultivars prior to planting crops [43].

2.3.2 Post-Harvest Factors Affecting Post Harvest Quality of Fruits

i. Maturity Stage

This is the starting point of postharvest quality management. Therefore, it must be ensured that properly matured fruits should be harvested. It must be harvested when it attains the appropriate stage of development based on physiological and horticultural maturity. Harvest maturity varies in accordance with the crop concerned. The fruit is harvested at different stages of maturity depending on how far the fruit will be transported, how long it will be kept in storage and the requirements for the specific market [44]. Maturity always has a considerable influence on the quality of fresh produce as well as the storage potential and occurrence of many storage disorders [45]. Maturity at harvest has a major impact on quality and postharvest life potential of fruits and vegetables [46]. All fruits with a few exceptions avocados, bananas and pears reach their best quality stage when fully ripen on tree.

ii. Methods of Harvesting

Selection of suitable method for harvesting of the produce is necessary otherwise bruises or injuries during harvesting may later manifest as black or brown patches making them unattractive. Latex coming out of stem in mango should not be

allowed to fall on fruits as it creates a black spot. Injury to peel may become an entry point for microorganisms, causing rotting. Some harvesting gadgets have been developed, e.g. mango harvester in Luck now (CISH). There are basically three methods most commonly used for harvesting any fruits.

a) Harvesting individual fruits with hand by pulling or twisting the fruit pedicel

b) Harvesting individual fruits or fruit bunch with the help of fruit clippers/secateurs/scissors

c) With harvester specially designed for harvesting

iii. Time of Harvesting

Harvesting time also affects quality. Fruits harvested before 10 am in the morning and transported to pack house for sorting, grading, and packing yield better quality and lasts longer [47]. It is desirable that the fruits are harvested during the cooler parts of the day to reduce the risk of heat injury and sunburn [48]. Therefore, morning harvesting and within 10 am transportation to destination pack house or market is always preferred in order to control damage due to high temperature. In case of grapes harvesting in India, it starts at 6 oclock in the morning and harvested produce reach pack house by 10 am. It facilitates faster precooling also and yield better quality.

iv. Precooling

The quality of fresh fruits largely depends on precooling before storage and marketing [47]. This is a compulsory postharvest treatment followed in developed countries for almost all perishable commodities. The rapid cooling of fresh produce from field temperature (pulp temperature at the time of harvesting) to its best storage temperature is called precooling. It is an important postharvest operation recommended in almost all flowers, fruits, and few vegetables. Fruits and vegetables which require on farm precooling if transport time to reach them to cold storage is more than a few hours. It is desirable that fresh produce like grapes, mandarins, berries,cherries, leeches, melons, stone fruits, okra, tomatoes, capsicum, chili peppers, cucumbers, green beans, peas, spinach should be cooled as rapidly as possible [49]. The main objective of any precooling operation is to remove field temperature (field heat). This is important because it increases shell life of the produce. Removing field heat reduces rate of respiration and all biochemical reactions from newly harvested produce.The act of cooling immediately after harvest is important to remove the field heat before the items are handled further. It is important that the cooling occurs as

soon as possible after harvest. Delays in the precooling will reduce the final quality and shorten the postharvest life [50]. Precooling to remove field heat as quickly as possible after harvest is essential for slowing down the rate of deterioration of highly perishable products. The method chosen is largely determined by the type of product in question and the cost to benefit ratio [51, 52].

v. Sorting and Grading

This is one of the most important postharvest operations after harvesting. This is done primarily for quality packing and removal of diseased and defective produce from the lot. Proper sorting and grading gives assurance of quality produce [47]. This is either done in the farmers field or in the pack houses. Both manual and mechanical graders are used for grading. All roundshaped fruits and vegetables are easily graded by mechanical graders. Grading may be based on color,size, and extent of defects, while sorting is totally dependent on man power for removal of diseased, defected, and damaged fruits. Grading is done by simple to highly sophisticated graders. Today, many sophisticated graders are in use for fresh produce such as GREEFA. Both size and color grading simultaneously is possible and is being used on commercial scale in apples.

vi. Packaging and Packaging Materials

Fruits are fragile products and therefore need packaging to protect them from mechanical damage [53]. The packages should also be well ventilated [54]. It is important to avoid compression damages on the fruit during storage and transportation [55]. The packages should also hold a weight of maximum 20 kg, as the fruit can be damaged when a heavy box is dropped on top of another. When fruits are transported the main goal should be to have as low amount of losses as possible [53].Packages used are big and heavy and result in significant fruit injury because of weight compression of upper fruits. Anwar, et al. also reported that most mangoes packed in wooden crates which apart from causing physical injuries and bruises during transit are being restricted in international markets on account of quarantine concerns and special disinfestations treatments necessary for international trade [55]. Both packing and packaging materials play many important roles in quality maintenance of fresh produce. Packing starts with placing the produce in the box. While placing, care must be taken to place in line, pedicel end of all fruits should be in one direction, separation layers or trays must be used where it is necessary. The box should not be underfilled or overfilled.

vii. Storage

These practices are supported by Liu who reported that in developing countries the common storage facilities are aircooled common storage houses which rely on natural cold air [56]. Almost all fruits are seasonal in nature. Every year, harvesting season falls during a fixed period,say 23 months. This may be little early or late due to prevailing weather conditions during growing periods. The demand of any fruit beyond the harvesting season is called offseason demand. This demand can be fulfilled only if fruits are stored in the harvesting season and sold during off season. The management of temperature, ventilation, and relative humidity are the three most important factors that affect postharvest quality and storage life of horticultural produce. Recommended storage temperature and relative humidity for cabbage, lettuce and carrots are $0 to 2^0 C$ and 95 to 100 % respectively. Recommended storage temperature and relative humidity for mangoes, avocados, papayas and potatoes are $1315^0 C$ and 8590%,respectively [49].

viii. Temperature

Temperature is usually the most important environmental factor limiting shelf life of fresh fruits [57]. Since fruits are alive after harvest, all physiological processes continue after harvest such as respiration and transpiration (water loss), and supply of nutrient and water is not possible since produce is no more attached to the parent plant. Respiration results in produce deterioration, including loss of nutritional value, changes in texture and flavor, and loss of weight by transpiration. These processes cannot be stopped, but they can be reduced significantly by careful management of temperature and relative humidity during storage and transportation [47]. According to Hofman, et al. low temperature conditioning is more effective than heat treatment to prevent chilling injuries and increase [54] the quality of fruits [54]. Growth and multiplication of microorganism responsible for rotting and spoilage are also associated with low temperature. At sufficiently low temperature, many disease causing microbes stop growth and multiplication. Respiration rates vary tremendously for different products. It can also be affected by environmental conditions, mostly by temperature.

viiii. Transportation

According to the FAO transportation is a big and often the most important factor in the marketing of fresh produce. Ideally, transport would take produce from the

grower directly to the consumer [58] . Kader stated that in most developing countries, roads are not adequate for proper transport of horticultural crops [59]. Also, transport vehicles and other modes, especially, those suited for fresh horticultural perishables are in short supply for local and export to other countries and the majority of producers have small holdings and cannot afford to own their own vehicles. Transportation is done in two phasesnamely, from the field to the homestead and from the home/company collection area to the market. Transportation for small scale farmers is relatively safe, because the product is either carried to the market or simply transported on carts or bicycles, rather than on trucks. However, for mediumscale farmers or groups of farmers, the transportation of produce is more complicated, and the produce is more susceptible to mechanical and heat damage. The fruits are either loaded onto trucks on wooden stacks, or simply piled onto the trucks (Figure 1). Mechanical damage (fatigue) occurs during transportation because of vibrations that occur while traveling what are typically long distances, usually over untarred roads [60, 61]. High temperatures and the buildup of gases that accelerate enzyme activity (and thus cause overripening or softening) and microbial activity are factors that contribute to the deterioration of fruit harvests [**?**]. Road condition and duration of transportation both road condition and duration of transportation affect quality of fresh produce. In hilly tracks and rough road surface, more touching and bruising take place as compared to smooth surface. Longer duration during transportation also affects quality. Reefer van should not be hold unnecessary. It not only increases the cost of produce, but also affects quality.

x. Pattern of Loading

Pattern of loading also plays crucial role in maintaining quality of fresh produce. Here pattern of loading means number of packed boxes in one layer (stacking height). In case of fresh produce, stacking height depends on extent of perishable nature of packed commodities and strength of packing materials. If produce are more perishable or box strength is weak, stacking height is kept low and vice versa. For example, height of grape boxes is kept low or it is packed in five ply corrugated boxes or thermocol boxes. This precaution must be taken to preserve postharvest quality of this highly perishable commodity. While loading, another important criteria is interlocking between the boxes. Loading and unloading fruits directly affect quality of fresh produce. It can be done either by hand or with the aid of a forklift. Forklift is used for palletized boxes and shipping containers only. Generally, fruits are stacked on pallets to ease the loading and unloading process and to prevent damage to the

product and packages. Exposure to sun while awaiting loading at local mandis or transport can reduce quality drastically. The exposed portion turns black or brown and starts decaying. It is advised for nonreefer transport to move continuously while under sunlight and stop and park your vehicle under a tree shade, especially during sunny days.

2.4 Spectroscopy

Spectroscopy deals with the interaction between matter and radiated energy. Historically,spectroscopy originated through the study of visible light dispersion according to its wavelength, by a prism. Later the concept was expanded greatly to comprise any interaction with radiative energy as a function of its wavelength or frequency. Spectroscopic data is often represented by a spectrum, a plot of the response of interest as a function of wavelength or frequency. Spectroscopic studies were central to the development of quantum mechanics included with Max Plancks explanation of black body radiation; Albert Einsteins explanation of the photoelectric effect and Niels Bohrs explanation of atomic structure and spectra.Spectroscopy is used in physical and analytical chemistry because atoms and molecules have unique spectra. As a result, these spectra can be used to detect, identify and quantify information about the atoms and molecules. Spectroscopy is also used in astronomy and remote sensing on earth. Most research telescopes have spectrographs. The measured spectra are used to determine the chemical composition and physical properties of astronomical objects (such as their temperature and velocity). One of the central concepts in spectroscopy is a resonance and its corresponding resonant frequency. Resonances were first characterized in mechanical systems such as pendulums. Mechanical systems that vibrate or oscillate will experience large amplitude oscillations when they are driven at their resonant frequency. A plot of amplitude Vs excitation frequency will have a peak centered at the resonance frequency. This plot is one type of spectrum; with the peak often referred to as a spectral line, and most spectral lines have a similar appearance. There are different kinds of spectroscopy: Atomic Absorption Spectroscopy (Energy absorbed by the sample is used to assess its characteristics), Fluorescence Spectroscopy (the absorbed energy causes light to be released from the sample), Fourier Transform Spectroscopy (the sample is irradiated by all relevant wavelengths simultaneously for a short period of time), Raman Spectroscopy (scattering of light by molecules), X-ray, Spectroscopy, Spectroscopy (excitation of inner electrons of atoms), Multiplex or Frequency-Modulated Spectroscopy (optical wavelength that recorded is encoded with an audio frequency), Mass Spectrometry (analyzing the dispersion of ions

when they interact with the sample), Gamma-ray Spectroscopy (activation analysis by gamma radiation) and Astronomical Spectroscopy (Energy from celestial objects is used to analyze their chemical composition, density, pressure, temperature, magnetic fields, velocity, and other characteristics): etc... are some examples of spectroscopy.

2.4.1 Fluorescence Spectroscopy

Fluorescence is the emission of light subsequent to absorption of ultraviolet or visible light of a fluorescent molecule or substructure, called a fluorophore. Thus, the fluorophore absorbs energy in the form of light at a specific wavelength and liberate energy in the form of emission of light at a higher wavelength. The general principles can be illustrated by a Jablonski diagram [62] as shown in Figure2.2. The first step (1) is the excitation where light is absorbed by the molecule which is transferred to an electronically excited state meaning that an electron goes from the ground singlet states, S_0, to an excited singlet state, S_1'. This is followed by a vibrational relaxation or interna (2) where the molecule undergoes a transition from an upper electronically excited state to a lower one S_1 without any radiation. Finally, the emission occurs (3) typically 10^{-8} sec after the excitation, when the electron returns to its more stable ground state, S_0,emitting light at a wavelength according to the difference in energy between the two electronic states. This explanation is somewhat simplified. In molecules, each electronical state has several associated vibrational states. In the ground state, almost all molecules occupy the lowest vibrational level. By excitation with ultraviolet or visible light, it is possible to promote the molecule of interest to one of several vibrational levels for the given electronically excited level. This implies that absorption and fluorescence emission does not only occur at one single wavelength, but rather over a distribution of wavelengths corresponding to several vibrational transitions as components of a single electronic transition. This is why excitation and emission spectra are obtained to describe the detailed fluorescence characteristics of molecules. In fact, fluorescence is characterized by two wavelength parameters that significantly improve the specificity of the method, compared to spectroscopic techniques based only on absorption.

2.4.1.1 Quantum Yield (Efficiency)

Each molecule presents a specific property, which is described by number, named quantum yield or quantum efficiency (Φ).

$$\Phi = \frac{number\ \ of\ \ quanta\ \ emitted}{number\ \ of\ \ quanta\quad absorbed} = quantum\ \ yield \tag{2.4.1}$$

As illustrated in equation (2.4.1), the higher the value of the greater the fluorescence of a compound (*e.g.chlorophyll*). A practically non-fluorescent molecule ($e.g, carotenoids$) is one whose quantum efficiency is zero or so close to zero that the fluorescence is not measurable. All energy absorbed by such a molecule is rapidly lost by collision deactivation. Absolute values are calculated using the standard samples which have a fixed and known fluorescence quantum yield value, according to the following equation:

$$\phi_X = \phi_{ST}(\frac{Grad_X}{Grad_{ST}})(\frac{\eta_X^2}{\eta_{ST}^2}) \tag{2.4.2}$$

Where the subscripts ST and X denote standard and test respectively, ϕ is the fluorescence quantum yield, Grad the gradient from the plot of integrated fluorescence intensity vs absorbance,and η the refractive index of the solvent. The fluorescence lifetime and quantum yield are perhaps the most important characteristics of a fluorophore. Quantum yield is the number of emitted photons relative to the number of absorbed photons. Substances with the largest quantum yields, approaching unity, such as rhodamines, display the brightest emissions. The lifetime is also important, as it determines the time available for the fluorophore.The fluorescence quantum yield is the ratio of the number of photons emitted to the number absorbed. The rate constants Γ and k_{nr} both depopulate the excited state. The fraction of fluorophores that decay through emission, and hence the quantum yield, is given by

$$Q = \frac{\Gamma}{\Gamma + k_{nr}} \tag{2.4.3}$$

The quantum yield can be close to unity if the radiationless decay rate is much smaller than the rate of radiative decay, that is $k_{nr} < \Gamma$ We note that the energy yield of fluorescence is always less than unity because of Stokes losses. For convenience we have grouped all possible non-radiative decay processes with the single rate constant k_{nr}. The lifetime of the excited state is defined by the average time the molecule spends in the excited state prior to return to the ground state. Generally, fluorescence lifetimes are near 10 ns. For the fluorophore illustrated in Figure 2.2 the lifetime is

$$\tau = \frac{1}{\Gamma + k_{nr}} \tag{2.4.4}$$

The lifetime of the fluorophore in the absence of nonradiative processes is called the intrinsic or natural lifetime, and is given by

$$\tau_n = \frac{1}{\Gamma} \tag{2.4.5}$$

In principle, the natural lifetime τ_n can be calculated from the absorption spectra, extinction coefficient, and emission spectra of the fluorophore.[63]

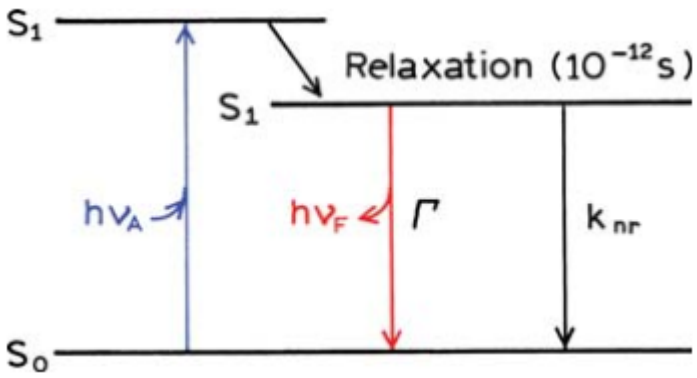


Figure 2.2: A simplified Jablonski diagram to illustrate the meaning of quantum yields and lifetimes.

$$\Gamma = 2.88 * 10^9 n^2 \frac{\int F(v)dv}{\int F(v)dv/v^3} \int \frac{\varepsilon(v)}{v} dv = 2.88 * 10^9 n^2 < v^{-3} > \int \frac{\varepsilon(v)}{v} dv \qquad (2.4.6)$$

where $F(v)$ is the emission spectrum plotted on the wavenumber cm^{-1} scale $\varepsilon(v)$ is the absorption spectrum,and n is the refractive index of the medium. The integrals are calculated over the $S_0 - S_1$ absorption and emission spectra.This expression assumes no interaction with the solvent, does not consider changes in the refractive index (n) between the absorption and emission wavelength, and assumes no change in excited-state geometry. A more complete form of equation (2.4.5) (not shown) includes a factor G = gl/gu on the right-hand side, where gl and gu are the degeneracies of the lower and upper states, respectively. For fluorescence transitions G = 1, for phosphorescence transitions G = 1/3. The natural lifetime can be calculated from the measured lifetime (τ) and quantum yield

$$\tau n = \tau / Q \qquad (2.4.7)$$

which can be derived from equations (2.4.3) and (2.4.4) . Many biochemical fluorophores do not behave as predictably as unsubstituted aromatic compounds. Hence, there is often poor agreement between the value of τ_n calculated from equation (2.4.6) and that calculated from its absorption and emission spectra equation (2.4.5). These discrepancies occur for a variety of unknown and known reasons, such as a fraction of the fluorophores located next to quenching groups, which sometimes occurs for tryptophan residues in proteins. The quantum yield and lifetime can be modified by factors that affect either of the rate constants (Γ or k_{nr}). For example, a molecule may be non-fluorescent as a result of a large rate of internal conversion or a slow rate of emission. Scintillators are generally chosen for their high quantum yields. These high yields are a result of large Γ values.Hence, the lifetimes are generally short: near 1 ns.

2.4.1.2 Relationship Between the Emission Spectrum and Excitation Wavelength

Emission occurs from the excited state S1, and so is in principle independent from the excitation wavelength.Since not all the molecules present at the excited states will participate in the fluorescence process, a quantum yield ϕ_F

$$\phi_F = \frac{I_F}{I_0} \tag{2.4.8}$$

$\phi_F < 1$ Since

$$I_F = I_0 - I_A \tag{2.4.9}$$

$$I_A = I_0 e^{-\varepsilon lc} \tag{2.4.10}$$

$$I_F = I_0 - I_0 e^{-\varepsilon lc} = I_0(1 - e^{-\varepsilon lc}) \tag{2.4.11}$$

$$\phi_F = \frac{I_0(1 - e^{-\varepsilon lc})}{I_0} \tag{2.4.12}$$

$$\phi_F = (1 - e^{-\varepsilon lc}) \tag{2.4.13}$$

i. **Excitation Spectrum:** The excitation spectrum is defined as the relative efficiency of different wavelengths of exciting radiation in causing fluorescence. The shape of the excitation spectrum should be identical to that of the absorption spectrum of the molecule and independent of the wavelengths at which fluorescence is measured. However, this is seldom the case because the sensitivity and the bandwidth of the spectrophotometer (absorbance spectrum) and the spectrofluorimeter (excitation spectrum) are different. In addition, for many food samples, scattering properties and energy transfer between neighboring molecules could contribute to this difference. A general rule of thumb is that the strongest (generally the longest) wavelength peak in the excitation spectrum is chosen for excitation of the sample. This minimizes possible decomposition caused by the shorter wavelength, higher energy radiation.

ii. **Emission Spectrum**

The emission spectrum of a compound results from the radiation absorbed by the molecule. The emission spectrum is the relative intensity of radiation emitted at various wavelength

2.4.1.3 Instrumentation for Fluorescence Spectroscopy

The success of fluorescence experiments requires attention to experimental details and an understanding of the instrumentation. There are also many potential artifacts that can distort the data. Light can be detected with high sensitivity. As a result, the gain or amplification of instruments can usually be increased to obtain observable signals, even if the sample is nearly nonfluorescent. These signals seen at high amplification may not originate with the fluorophoreof interest. Instead, the interference can be due to background fluorescence from the solvents, light leaks in the instrumentation, emission from the optical components,stray light passing through the optics, light scattered by turbid solutions, and Rayleigh and/or Raman scatter, to name a few interference sources [64]. An additional complication is that there is no ideal spectrofluorometer. The available instruments do not yield true excitation or emission spectra. This is because of the nonuniform spectral output of the light sources and the wavelength-dependent efficiency of the monochromators and detector tubes. The polarization or anisotropy of the emitted light can also affect the measured fluorescence intensities because the efficiency of gratings depends on polarization. It is important to understand and control these numerous factors. In this chapter we will discuss the properties of the individual components in a spectrofluorometer, and how these properties affect the observed spectral data.

These instrumental factors can affect the excitation and emission spectra, as well as the measurement of fluorescence lifetimes and anisotropies. Additionally, the optical properties of the samplessuch as optical density and turbiditycan also affect the spectral data. Specific examples are given to clarify these effects and the means to avoid them.

i. Spectrofluorometer

With most spectrofluorometers it is possible to record both excitation and emission spectra. An emission spectrum is the wavelength distribution of an emission measured at a single constant excitation wavelength. Conversely, an excitation spectrum is the dependence of emission intensity, measured at a single emission wavelength, upon scanning the excitation wavelength. Such spectra can be presented on either a wavelength scale or a wavenumber scale. Light of a given energy can be described in terms of its wavelength λ,frequency ν or wavenumber. The usual units for wavelength are nanometers, and wavenumbers are given in units of cm^{-1}. Wavelengths and wavenumbers are easily interconverted by taking the reciprocal of each value. For example, 400 nm corresponds to $(400 \times 10^{-7} cm)^{-1} = 25,000 cm^{-1}$.

However,most commercially available instrumentation yields spectra on the wavelength scale, and such spectra are more familiar and thus easier to interpret visually. Since corrected spectra are not needed on a routine basis, and since accurately corrected spectra are difficult to obtain, we prefer to use the directly recorded technical or uncorrected spectra on the wavelength scale.For an ideal instrument, the directly recorded emission spectra would represent the photon emission rate or power emitted at each wavelength, over a wavelength interval determined by the slit widths and dispersion of the emission

Figure 2.3: Agilent Cary Eclipse Fluorescence Spectrophotometer

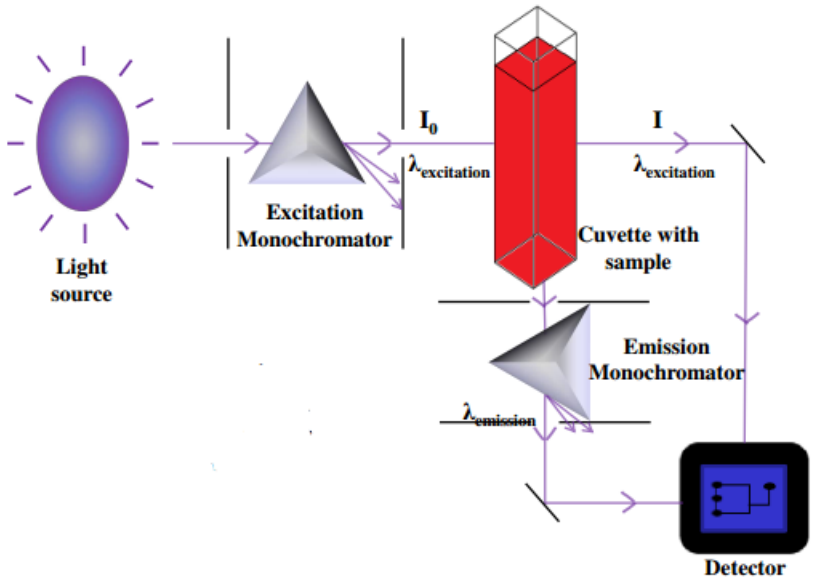


Figure 2.4: Schematic diagram of a spectrofluorometer

ii. Light Sources

We now describe the individual components of a spectrofluorometer. The general characteristics of these components are considered along with the reason for choosing specific components. Understanding the characteristics of these components allows one to understand the capabilities and limitations of spectrofluorometers. Commonly employed sources in fluorescence spectrometry have spectral outputs either as a continuum of energy over a wide range or as a series of discrete lines.An example of the first type is the tungsten-halogen lamp and of the latter, a mercury lamp. Mercury lamps are the most commonly employed line sources and have the property that their spectral output depends upon the pressure of the filler gas. The output from a low-pressure mercury lamp is concentrated in the UV range, whereas the most commonly employed lamps, of medium and high pressure, have an output covering the whole UV-visible spectrum.Although in many cases the output from a line source will be adequate, it is rare that an available line will exactly coincide with the optimum excitation wavelength of the sample. It is therefore advantageous to employ a source whose output is a continuum and the most commonly employed type is the xenon arc. Xenon arc sources can be operated either on a continuous DC basis or stroboscopically; the latter method offers advantages in the size and

cost of lamps. Arc lamps are inherently more unstable than discharge sources and for long term stability a method of compensating for drift is advisable. The most satisfactory method of doing this is to split the excitation energy so that a small portion is led to a reference detector. The signal from this reference detector is ratioed with the signal from the detector observing the sample. Such a ratio-recording system is therefore independent of changes in the source intensity. All sources of UV radiation will produce ozone from atmospheric oxygen, which should be dispersed, since it is not only toxic, but also absorbs strongly in the region below 300 nm. For this reason, most lamps will be operated in a current of air and, if the supply fan fails, the lamp should be extinguished immediately. Lamps must be handled with great care since fingermarks willseriously decrease the UV output.

iii. Monochromators

Monochromators are used to disperse polychromatic or white light into the various colors or wavelengths. This dispersion can be accomplished using prisms or diffraction gratings. The monochromators in most spectrofluorometers use diffraction gratings rather than prisms. The performance specifications of a monochromator include dispersion,efficiency, and stray light levels. Dispersion is usually given in nm/mm. The slit width is sometimes expressed in mm, which requires knowledge of the dispersion. A monochromator for fluorescence spectroscopy should have low stray light levels to avoid problems due to scattered or stray light.By stray light we mean light transmitted by the monochromator at wavelengths outside the chosen wavelength and bandpass.

Monochromators are also chosen for high efficiency to maximize the ability to detect low light levels. Resolution is usually of secondary importance since emission spectra rarely have peaks with line widths less than 5 nm. The slit widths are generally variable, and a typical monochromator will have both an entrance and exit slit. The light intensity that passes through a monochromator is approximately proportional to the square of the slit width. Larger slit widths yield increased signal levels, and therefore higher signal-to-noise ratios. Smaller slit widths yield higher resolution, but at the expense of light intensity. If the entrance slit of the excitation monochromator is already wide enough to accept the focused image of the arc, then the intensity will not be increased significantly with a wider slit width. If photobleaching of the sample is a problem, this factor can sometimes be minimized by

decreasing the excitation intensity. Gentle stirring of the sample can also minimize photobleaching. This is because only a fraction of the sample is illuminated and the bleached portion of the sample is continuously replaced by fresh solution.

iv. Detectors

All commercial fluorescence instruments use photomultiplier tubes as detectors and a wide variety of types are available. The material from which the photocathode is made determines the spectral range of the photomultiplier and generally two tubes are required to cover the complete UV-visible range. The S5 type can be used to detect fluorescence out to approximately 650 nm, but if it is necessary to measure emission at longer wavelengths, a special red sensitive,S20, photomultiplier should be employed. The limit of sensitivity of a photomultiplier is normally governed by the level of dark current (which is the signal derived from the tube with no light falling on it). The dark current is caused by thermal activation and can usually be reduced by cooling the photomultiplier. Another method of minimizing dark current is to use a stroboscopic source since the ratio of dark current to fluorescence will be very small during each high intensity flash. During the periods between flashes when the dark current is relatively high, the photomultiplier output can be disconnected. The overall result is that the dark current no longer becomes the limitation to sensitivity. The spectral response of all photomultipliers varies with wavelength, but it is sometimes necessary to determine the actual quantum intensity of the incident radiation and a detector insensitive to changes in wavelength is required. A suitable quantum counter can be made from a concentrated solution of Rhodamine 101 in ethylene glycol which has the property of emitting the same number of quanta of light as it absorbs, but over a very wide wavelength range. Thus, by measuring the output of the quantum counter at one wavelength, the number of incident quanta over a wide wavelength range can be measured.

v. Read-out Devices

The output from the detector is amplified and displayed on a readout device which may be a meter or digital display. It should be possible to change the sensitivity of the amplifier in a series of discrete steps so that samples of widely differing concentration can be compared. A continuous sensitivity adjustment is also useful so that the display can be made to read directly in concentration units. Digital displays are most legible and free from misinterpretation. Improvement in precision is obtained by the use of integration techniques where the average value over a period

of a few seconds is displayed as an unchanging signal. Microprocessor electronics provide outputs directly compatible with printer systems and computers, eliminating any possibility of operator error in transferring data.

2.4.2 Uv-Visible Spectroscopy

UV-Visible spectroscopy is a method that can monitor and measure the interactions of UV and visible light with different chemical compounds in the wavelength range between 200 and 780nm. The technique exploits different physical responses of light and analytes within the sample such as absorption, scattering, diffraction, refraction, and reflection . The phenomenon of UV and visible light absorption is restricted to specific chromophores and several chemical species with defined molecular functional groups [65]. Consequently, the characteristic absorption spectra may be obtained for single molecules because electrons within these chromophores are excited . Quantitative analysis based on Uv-Vis spectroscopy is ultimately described by the BeerL-ambert law and is the correlation between the quantity of the incident light absorbed by the molecule, the sample, the light path length, and the concentration of the absorbing compound or molecule in the matrix [65]. Beers law can be expressed as A = ε l c, where A is the absorbance, ε is absorptivity, l is path length through the solution, and c is the concentration of the species absorbing the light. However, it is important to note that Beers law is only obeyed for very dilute solutions up to 10 mM in most cases. Furthermore, Beers law is obeyed when the incident light is monochromatic, and not polychromatic [66]. Absorption of Uv light is very accurate, and the linear relationship between absorbance and concentration has seen Uv-Vis spectroscopy emerge as a workhorse technique in analytical chemistry.The main advantages of Uv-Vis spectroscopy are it can be employed for analysis of a wide range of compounds and is a sensitive and nondestructive technique.

2.4.2.1 Beer-Lamberts Law

The beer-Lambert's Law, also called the Beer-Lambert-Bouguer's Law or simply Beer's law, is the linear relationship between absorbance and concentration of an absorbed of electromagnetic radiation. The general Beer-Lambert's law is written as:

$$A = a_\lambda bC \tag{2.4.14}$$

where A the measured absorbance a_λ is a Wavelength-dependent absorptive coefficient, b is the path-length and c is the analyze concentration. When working in concentration

units of molality, the Beer-Lambert's laws are written as:

$$A = \epsilon_\lambda bc \tag{2.4.15}$$

where ϵ is the wavelength-dependent molar absorptive coefficient with units of $M^1 cm^{-1}$ the wavelength subscript is often dropped with the understanding that a value of is for a specific wavelength. If multiple species that absorb light at a given wavelength are present in a sample, the total absorbance at that wavelength is the sum due to all absorbers.

$$A = \epsilon_1 C_1 b + \epsilon_2 C_2 b = \epsilon_i C_i b \tag{2.4.16}$$

where, the subscripts refer to the molar absorptive and concentration of the different absorbing species that are present. Experimental measurements are usually made of transmittance (T) and absorbance (A) which are defined as follows

2.4.2.2 Derivation of Beer-Lamberts Law

The beer-Lambert law can be derived from an approximation for the absorption coefficient for a molecule by approximating the molecule by an opaque disk whose crosssectional areas σ, represents the effective area seen by a photon of frequencyγ . If the frequency of the light is far from resonance frequency, the area is approximately zero, and if γ is close to resonance frequency the area is a maximum.

Quantitative Analysis: The fractional change in light intensity ($\frac{dI}{I}$=αdx) where α=ϵc Then, the absorbance on optical density A,is given by

$$A = -\ln \frac{I_0}{I_t} = \epsilon cl \tag{2.4.17}$$

where: I_0- is the intensity of the incident beam I- is the intensity of the transmitted beam l- is the absorbing path length c- is the concentration of the absorbing species. Absorbance (A)and Transmittance (T) can be defined in terms ofI_0 and I

$$T = \frac{P}{P_0} \tag{2.4.18}$$

or Symbolized as

$$T = \frac{I}{I_0} \tag{2.4.19}$$

where P or I is the power (Intensity) of light after it passes through the sample and IO is the initial light power (intensity). This is simply a measure of how many photons pass through the sample without being absorbed. This is then related to the absorbance by:

$$A = -\log(T) = -\log \frac{P}{P_0} \tag{2.4.20}$$

When performing an analysis it is important that the standards and unknowns are analyzed at the same wavelength, and are matched for solvent and, where necessary, pH, otherwise results will be incorrect. The Beer-Lambert's law connects concentration and light intensity as shown

$$I = I_0 * 10^{-\epsilon lc} \tag{2.4.21}$$

Where,ϵ- is the molar absorptive (formerly known as the molar extinction coefficient), l-is path length and c- the concentration of the absorbing species. Putting this equation together with that connecting absorbance and light intensity gives the familiar expression called Beer-Lambert Law: Thus, the magnitude of the Absorbance will depend directly on the concentration (c) of the absorbing species, the Path Length (l) of the light through the cell and the ability of the species to absorb radiation at the given wavelength, the extinction coefficient (ϵ). These quantities are related to the absorbance via the Beers Lambert Law:

$$A = \epsilon lc \tag{2.4.22}$$

where, Molar absorptive ϵ in $M^{-1}cm^{-1}$, Path length (l) in cm, Concentration (C) in $moldm^{-3}$

2.4.2.3 Components of Optical Spectrometers

i. Light Sources

Radiation sources need to be continuous over the range of wavelengths of interest. The earliest sources were simply tungsten filament lamps (light-bulbs!) but these have since been replaced by tungsten-halogen lamps. Such light sources cover the wavelength range from 300-900 nm. To reach further into the UV an additional source is needed. This is usually a deuterium arc lamp, which has a continuous spectrum below 400 nm.

ii. Monochromator

A monochromator is used to select the wavelength at which an absorption measurement is made. In fact, it is not possible to select a single wavelength, but rather a narrow range of wavelengths, which defines the spectral resolution of the spectrometer.

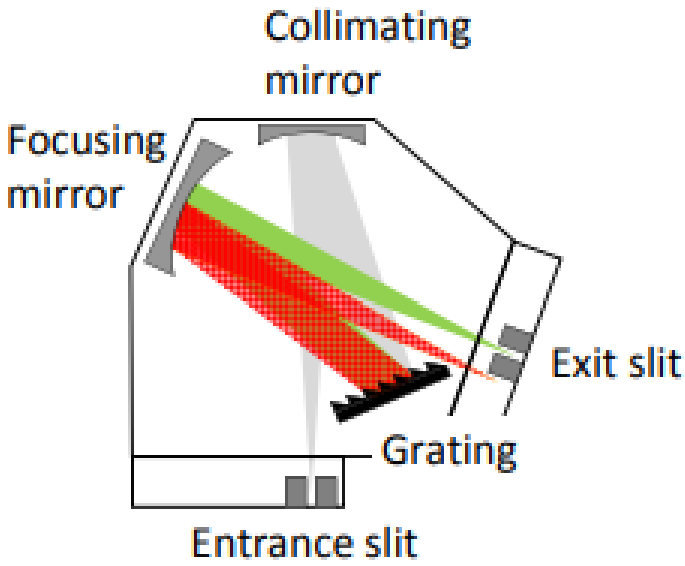


Figure 2.5: Schematic representation of a grating monochromator

There are two main choices for dispersing light into its different components: a prism, or a diffraction grating. Most modern instruments employ gratings, because it is easier to achieve high spectral resolution. However, gratings have the disadvantage of giving rise to more than one order of diffraction. This means that if the monochromator is set to 600 nm for example, then it will also pass 300 nm (second order) radiation. This problem is easily overcome by the use of additional filters to remove the unwanted radiation. It consists of the diffraction grating (dispersing element), slits, and curved mirrors, which image the entrance slit onto the exit slit and produce a parallel beam at the grating. During a scan, the grating is slowly rotated, and light of different wavelengths will emerge from the exit slit and pass through the sample to the detector. Thus the spectrum is obtained sequentially as the grating is rotated to select the wavelength and the detector observes the transmitted radiation intensity. The spectral resolution can be varied by changing the size of the slits. Narrower slits allow for higher resolution at the expense of light intensity, which can result in larger noise.

iii. Detectors

The following detectors are commonly used in Uv-Visible spectroscopy:

1. Photomultipliers:

A photomultiplier consists of a photocathode and a series of dynodes in an evacuated glass enclosure. Light that strikes the photo cathode causes the ejection of electrons due to the photoelectric effect. The electrons are accelerated towards a series of additional electrodes called dynodes. These electrodes are each maintained at a more positive potential. Additional electrons are generated at each dynode. This cascading effect creates 105 to 107 electrons for each photon hitting the first cathode depending on the number of dynodes and the accelerating voltage. This amplified signal is finally collected at the anode where it can be measured.

2. Semiconductor Photodiodes:

When a photon strikes a semiconductor, it can promote an electron from the valence band (filled orbitals) to the conduction band (unfilled orbitals) creating an electron(-) - hole(+) pair.

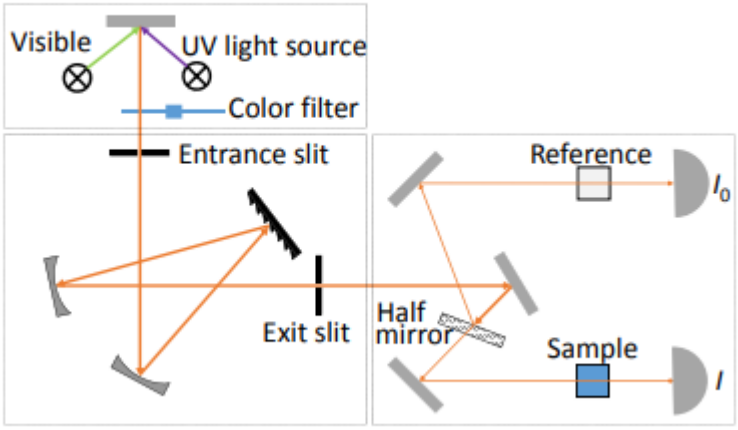


Figure 2.6: Schematic view of a dual beam spectrophotometer

The concentration of these electron-hole pairs is dependent on the amount of light striking the semiconductor, making the semiconductor suitable as an optical detector. Photovoltaic detectors contain a p-n junction that causes the electron-hole pairs to separate to produce a voltage that can be measured. Photodiode detectors are not as sensitive as PMTs but they are small, cheap and robust.

3. Charge-coupled devices (CCD):

A CCD is an integrated-circuit chip that contains an array of capacitors that store charge when light creates electron-hole pairs. The charge accumulates and is read

in a fixed time interval. CCDs are used in similar applications as arrays of photodiodes but the CCD is much more sensitive for measurement of low light levels. They can replace the exit slit of a monochromator which disperses light only after it has passed a sample. In this way, full spectra can be accumulated very quickly without moving any optics.

CHAPTER 3

MATERIALS AND METHOD

3.1 Materials Used

3.1.1 Instruments and Apparatus

The experiment were carried out with the help of the following devices: beakers, spatula, column tube, flasks, digital electronic beam balance to measure the mass of the sample, plastic cuvette to hold the sample for spectroscopic analysis, filter-tip pipette, conical vial, home juice extractor, computer linked UV-visible spectrophotometer and a fluorescence spectrophotometer. Water baths and refregerator were used. The distilled water were used.

3.1.2 Samples

Mango and orange fruits sample with differences in color and size for capturing a wide range of pigments were purchased from market in Adama town.

3.2 Methods

The collected fruit were preserving at 6^0C. The juice were extracted with a home juice extractor. Fruits with similar color were considered as one sample and extracted. Totally, fruits juice samples were prepared. The core were eliminated, while the skin were reserved.The juice were centrifuged for 10 min with a speed of 3600 rpm and room temperature (22^0C) . Subsequently the juice were filtered and each sample were separated in to two portions. One portion were measured immediately (marked as 'Fresh') and were stored at 22^0C for 8 days (were marked as stored). The other portion were heated in a water bath at 40^0C , 60^0C and 80^0C for 10 min.The juice were cooled rapidly with an ice-

water bath, and stored at 22^0C. The spectra and reference values were measured (marked as heated-stored). UV-visible spectrophotometers(Double Beam Spectrofluoremeter) and a fluorescence spectrophotometer(Agilent Cary Eclipse Spectrofluorimeter(Instrument Serial Number MY18490002) were used for absorption spectra and fluorescence measurements, respectively.

(a) Mango Juice

(b) Orange Juice

Figure 3.1: Mango and Orange Juice Sample

CHAPTER 4

RESULTS AND DISCUSSION

This chapter deals with the analysis, discussion and the results of the thesis. The quality of mango juice and and orange juice were analyzed using Uv-Visible spectroscopy and Fluorescence spectroscopy.

4.1 Uv-Visible Spectroscopy

i. Mango Juice:

The mango juice absorption spectra were measured by separating in three portion. One portion fresh mango juice and the second portion were stored mango juice also heated stored mango juice at 40^0C , 60^0C and 80^0C were the third portion. Fresh mango juice had peaks on 455 nm. Stored mango juice had peaks on 270 nm and 460 nm. Heated-stored mango juice at 40^0C had peaks on 320nm and the heated stored mango juice had no peaks found on 60^0C and 80^0C as shown in the Figure 4.1 below. This means the chemical composition found in mango juice was burned when it was heated at higher temperature. This shows temprature can affect shelf life of fruit.The absortption spectra peaks appearing at 320 nm assigned to coumarin. The peak appering at 270 nm corresponds to polymethoxyflavons. The peak appering on 460 nm corresponds to chlorophyll.

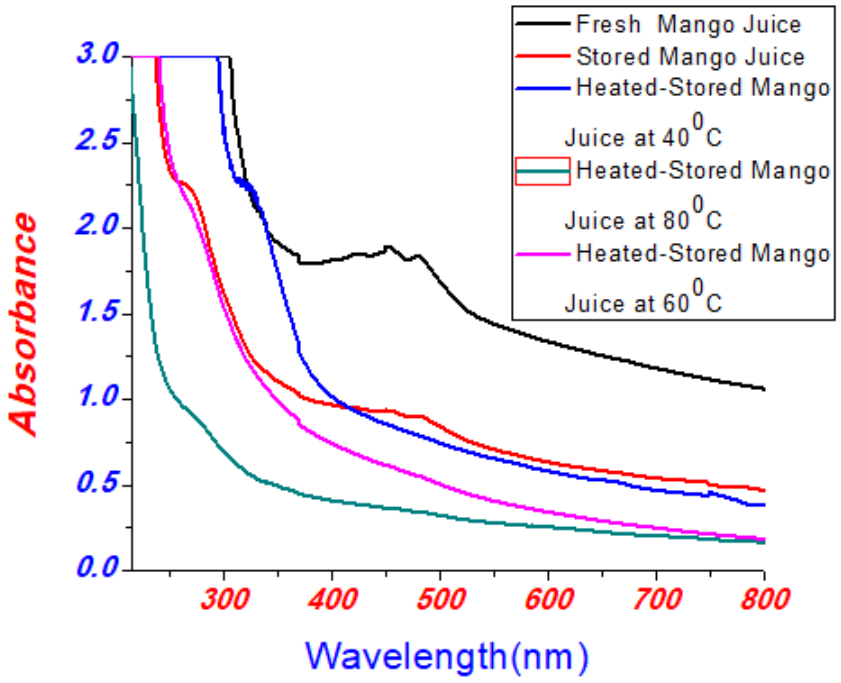


Figure 4.1: The absorption spectra of mango juice

ii. Orange Juice:

The orange juice absorption spectra were measured in the same procedure with mango juice. Fresh orange juice had peaks at 330 nm and 390 nm. Stored Orange Juice had peaks at 260 nm and 326 nm. Heated stored orange juiceat 40^0C had peaks at 320 nm and heated stored juice at 60^0C had peak at 260 nm but no peaks found in heated stored at 80^0C. The band found 260 nm to 280 corresponds to vitamin C. The band appearing at 330 nm corresponds to coumarin. Coumarin is a secondary plant metabolite that is a native inhibitor, also known to be a plant growth regulator. The band appearing on 390 nm corresponds to vitamin A. The band found at 450 nm has been assigned to carotenoid.

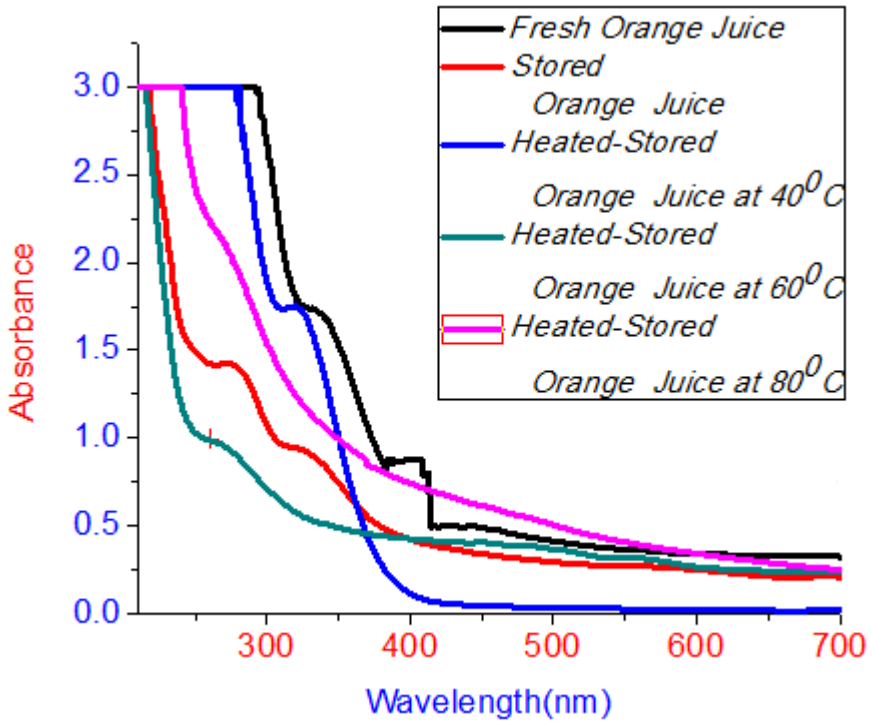


Figure 4.2: The absorption spectra of orange juice

4.2 Fluorescence Spectroscopy

i. Mango Juice:

The excitation (Ex) wavelength was fixed at 350 nm and the emission (Em) wavelength was scanned from 370 to 1000 nm at 1-nm increments.The excitation and emission slits were maintained at 5 nm, with a scan speed of 1200 nm/min and response time of 50 ms for all measurements. The mango juice were prepared in fresh, stored and heated stored forms. Fresh mango juice had peaks at 454 nm , 546 nm , and 700 nm also had shoulder at 480 nm. Stored mango had peaks at 457 nm and 700 nm. Heated-stored mango juice at 40^0C had bands around 420-500 nm and peak at 700 nm. The heated-stored at 60^0C and 80^0C mango juice had peak only at 700 nm. The peaks at 454 nm, 457 nm and 480 nm was assigned to total carotenoids. The peak at 546 nm was assigned polymethoxyflavons. PMFs are a subclass of flavonoids, which are richer in the peel than in the flesh. The peak at 700 nm corresponds to chlorophyll.

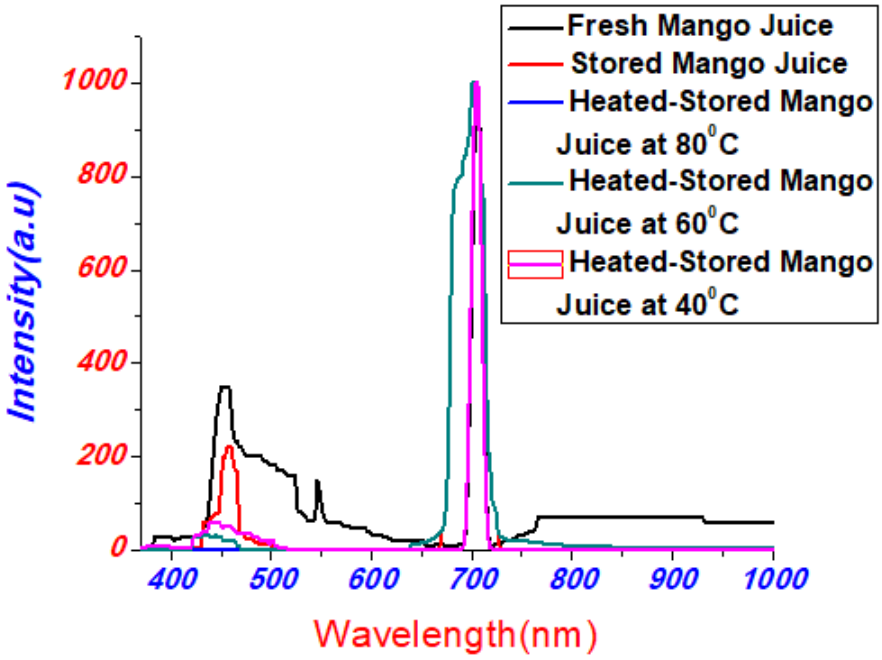


Figure 4.3: The emission spectra of mango juice

ii. Orange Juice:

The Excitation and Emission spectra of orange juice were measured which prepared in fresh, stored and heated stored forms. The excitation spectra of orange juice were fixed at 350nm. Fresh orange juice had peaks at 455 nm, 500 nm, and 700 nm. Stored orange had peaks at 480 nm and 700 nm. Heated stored orange juice had peak at 700 nm only.The peaks at 455 nm 480 nm and 500 nm assigned to total carotenoids.The peak at 455 nm corresponds to total phenolic compounds. The peak at 700 nm were assigned to chlorophyll.

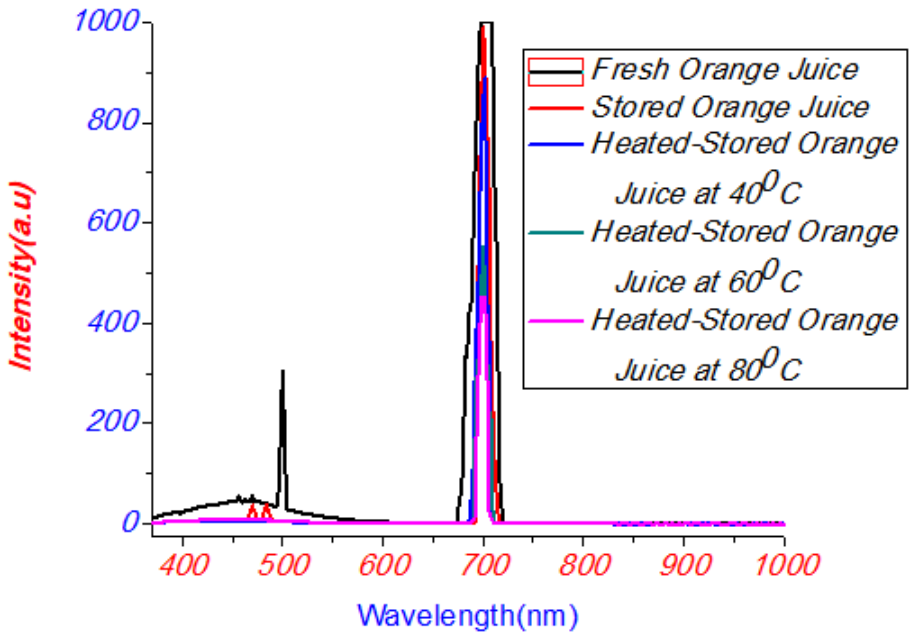


Figure 4.4: The emission spectra of orange juice

iii. Fluorescence Quantum Yields of Mango Juice:

The Fluorescence quantum yield of chlorophyll found in fresh and stored mango juice were measured. When the quantum yield of chlorophyll found fresh mango juice were measured the stored mango juice sample were taken as standard. Firstly the quantum yield of chlorophyll found stored mango juice were calculated by using equation (2.4.13) and its value were 0.26. The value of quantum yield of chlorophyll found in fresh mango juice were 0.10 depending on the equation (2.4.2).But for this quantum yield measurements our refractive indexes of solvents are the same so its value are one according to equation (2.4.13). By the same procedure the chlorophyll of fresh mango juice were taken as standard because no standard value of chlorophyll. Similarly using equation (2.4.2) the quantum yield of chlorophyll of stored mango juice were $0.254 \sim 0.26$. This value is the same with which were calculated from equation (2.4.13).

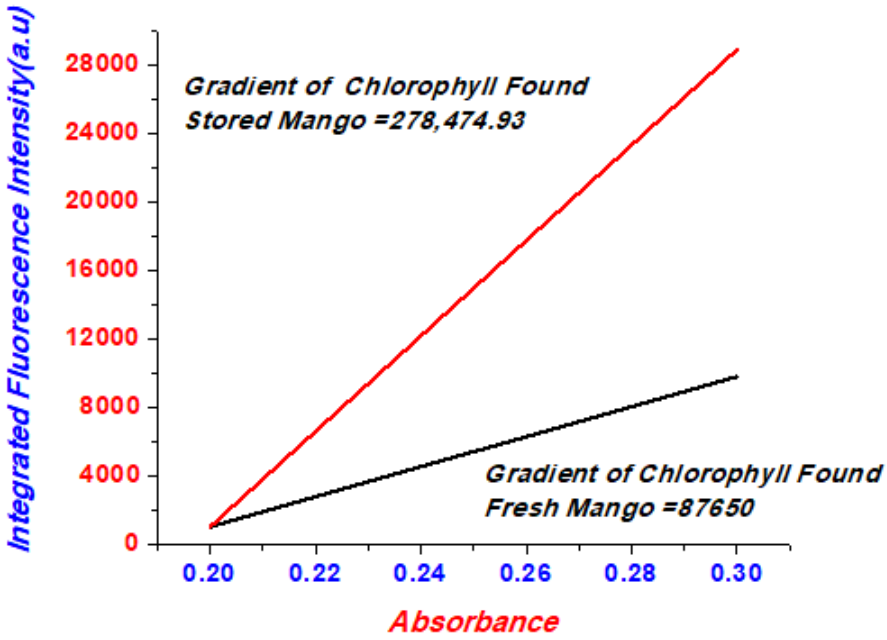


Figure 4.5: The gradient for each sample is proportional to that samples fluorescence quantum yield of chlorophyll found in mango juice

iv. Fluorescence Quantum Yields of Orange Juice:

Within the same procedure of mango juice the fluorescence quantum yield of chlorophyll found in fresh and stored Orange juice were measured. When the quantum yield of chlorophyll found fresh Orange juice were measured the stored Orange juice sample were taken as standard. Firstly the quantum yield of chlorophyll found stored Orange juice were calculated by using equation (2.4.13) and its value were 0.26.The value of quantum yield of chlorophyll found in fresh Orange juice were 0.37 depending on the equation (2.4.2).But for this quantum yield measurements our refractive indexes of solvents are the same so the value of refractive index were one according to equation (2.4.13). By the same procedure the chlorophyll of fresh orange juice were taken as standard. Similarly using equation (2.4.2) the quantum yield of chlorophyll of stored orange juice were 0.26. this value is the same with which were calculated from equation (2.4.13). The gradients of the graphs obtained in Figure 4.6.

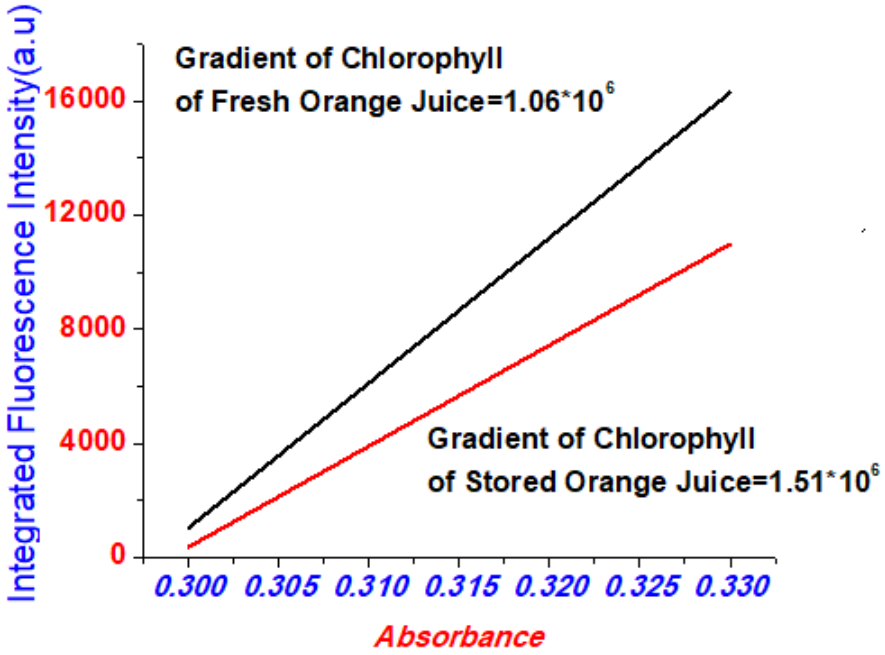


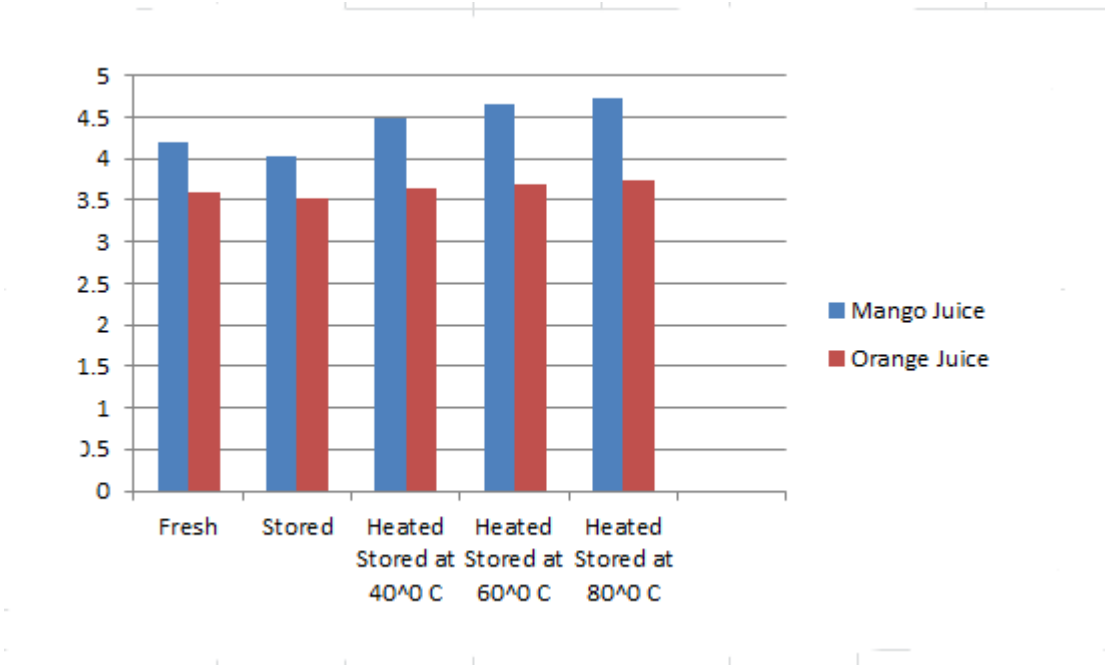
Figure 4.6: The gradient for each sample is proportional to that samples fluorescence quantum yield of chlorophyll found in orange juice

v. Fluorescence Lifetime:

The mango and orange fruit juice fluorescence lifetime were measured. The measured lifetime of two samples were 102.8. From the equation (2.4.7) the natural lifetime of fresh and stored mango juice were 1028 ms , 395.38 ms respectively . Natural lifetime of fresh and stored orange juice also 277.8, 395.38 respectively.

4.3 Fruit Juice pH Value

The pH value of mango and orange juice were measured by using pH-meter. The digital reading was allowed to stabilize for a few seconds and the pH reading was taken.In between readings, the electrode was cleaned with distilled water and placed in a standard solution of pH 70 and pH 4.0 Buffer solution. The pH value mango and orange juice were measured in three form ; fresh , stored and stored-heated at $(40^0C, 60^0C, 80^0C)$ respectively as shown in graph below .Between mango and orange fruits in the study, orange fruit juice showed pH less than 4 (more acidic). Fresh Orange juice (3.6) , stored orange juice (3.52), heated-stored orange juice at 40^0C(3.65),heated-stored orange juice at 60^0C(3.69), heated-stored orange juice at 80^0C(3.73) and fresh mango (4.2) , stored mango juice (4.02) ,heated-stored mango juice at 40^0C(4.5), heated-stored mango juice at 60^0C(4.65) ,heated-stored



mango juice at $80^0C(4.72)$. The pH value were affect peaks of the emission spectra of mango and orange juice. This effect of pH value of mango and orange juice on fluorescence spectra were differ from portion to portion of fruit samples. Table 4.1 is showing the pH value at different portion of mango and orange juice preparation.

Figure 4.7: pH value of fruit juices

Table 4.1: pH value of mango and orange juice at different portion

Fruit Juice	Fresh Juice	Stored Juice	Heated-Stored at 40^0C	Heated-Stored at 60^0C	Heated-Stored at 80^0C
Mango	4.2	4.02	4.5	4.65	4.72
Orange	3.6	3.52	3.65	3.69	3.73

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

From the result of this experiment it was concluded that the fruit juice quality could be determined by using Uv-Visible and Fluorescence spectroscopy. Fluorescence spectra and Uv-Visible spectra were to classify the physical properties of the fresh juice, the stored juice and heated-stored juice. The result from absoption spectra and emission spectra indicates that the measured absorption spectra of fresh mango juice had peak at 455 nm, the absorption spectra of stored mango juice had peaks at 270 nm and 460 nm and the heated-stored at (40^0) mango juices had peaks at 320 nm but no peaks found in heated-stored at 60^0 and 80^0C. The absortption spectra peak appearing 320 nm was assigned to coumarin. The peak appering at 270 nm was corresponds to polymethoxyflavons. The peak appering at 460 nm corresponds to chlorophyll. On the other hand the peaks of absorption spectra of fresh orange juice were founds at 330 nm and 390 nm, the stored orange juice had peaks at 260 nm and 320 nm. The absorption spectra peaks of heated-stored at 40^0C and 60^0C were found in 320 nm and 260 nm respectively, but no peaks at 80^0C. The band found 260-280 nm corresponds to vitamin C. The peak appearing at 330 nm and 390 nm were assigned to cuomerin and vitamin A respectively. The emission spectra of fresh mango juice had peaks at 454 nm, 540 nm and 700 nm, the stored mango juice had peaks at 460 nm and 700 nm. Whereas heated-stored mango juice at 40^0C had bands around 420-500 nm and peak at 700 nm. At 60^0C and 80^0C heated-stored mango juice had peak only at 700 nm. The emission spectra peak appearing at 454 nm corresponds to total carotenoids. The emission spectra peak at 546 nm assigned to polymethoxyflavons. The peak at 700 nm was corresponds to chlorophyll.

The emission spectra of fresh orange juice had peaks at 455 nm, 500 nm and 700 nm ; the stored orange juice had band around 460-500 nm (corresponds to carotenoids) and

had peak 700 nm (corresponds to chlorophyll). Whereas the heated-stored orange juice at $(40^0, 60^0, 80^0)C$ had peaks only on 700 nm. In mango and orange juices the prominant peaks founded between 670-720 nm corresponds to chlorophyll for three portion.The other peaks founded between 400-550 nm was corresponds carotenoids. The less amount of carotenoid were found at heated-stored at $(60^0, 80^0)C$. More carotenoid found in fresh juice for mango and orange juices. The stored and heated-stored at (40^0C) mango juices carotenoid founded than heated-stored at (60^0C) and (80^0C) indicates the carotenoid and vitamins founded in mango fruits were degraded at higher temperature. In orange the carotenoid and vitamins founded only in fresh orange juices but small peaks found in stored orange juices. In third portion of heated-stored orange carotenoids and vitamins were degraded means heating orange juice were not good for quality of juices. Heating fruits at higher temperature degrades their quality. From the measured absorption and emission spectra the orange are more affected at higher temperature. In this spectroscopical method the fluorescence quantum yield of the sample found in mango and orange juice were measured. The fluorescence quantum yield of chlorophyll found in mango and orange juice were 0.10 and 0.37 respectively. The fluorescence lifetime value of two fruit juices were measured. The measured lifetime of two samples were 102.8 ms. The pH value of mango and orange juice were measured by pH-meter. The pH value of mango juice were 4.02-4.72 for three portion and the pH value of orange juice were 3.52-3.73. The pH value were found to affect peaks of the emission spectra of mango and orange juice. This effect of pH value of mango and orange juice on fluorescence spectra were differ from portion to portion of fruit samples.

5.2 Recommendation

The quality deterioration in fruits and ready to eat juices implies that the prevailing pre-harvest and post-harvest handling practices are insufficient in controlling deterioration. Hence, several cultural related pre and postharvest practices should be improved. Regular monitoring of the quality of fruits, vegetables and its products for human consumption must be introduced to avoid any quality looses. The fruit juices house owners should focus on food safety practices by giving trainings (orientations) and other safety related issues besides focusing profit maximization. Vendors in corporation with Ethiopian Standard Agency should adopt rules and regulations on RTE foods and take regular fruits inspection and its product handling. Unless strict handling of fruit juices are used, fruit juices need to be prepared while customer is there to use, if not refrigerator use for storage should be mandatory. However,government health agencies must adopt measures to educate the vendors on food safety and hygienic practices. Regular monitoring of the quality of fruit juices for human consumption must also be enforced. There is need to educate the juice makers and retailers on the hazards associated with the cultivation of nonchalant attitudes to hygienic processing, display and packaging of these juices. There should, also, be regular training/retraining and health education of handlers handlers in all aspects of food hygiene and safety. More research is needed to improve and optimizes the current procedure and method of handling fruit and fruit juice for better quality improvement. Generally, in our country the study regarding fruits in spectroscopic method is on the ground and too young. So, the researchers are recommend to do more.

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