DEVELOPMENT OF OPTICAL FIBER VIS-NIR SPECTROSCOPY SYSTEM FOR METROLOGICAL ANALYSIS ON INTRINSIC QUALITIES OF AVERRHOA CARAMBOLA L.

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BY

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To my late Father, who taught me the true meaning of knowledge

To my Mother
To my Family

To myself

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May this work will be an insight for many others related work that will one day, well-benefited humanity.

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List of Symbols and Abbreviations

αa	absorption coefficient
αs	scattering coefficient
λ	wavelength
ε_λ	extinction coefficient of the absorbing species at wavelength λ.
A_λ	absorbance at wavelength λ
BS	basic stamp
^{o}C	degree celsius
c	concentration
D	Dark intensity at wavelength λ
g/L	gram per litre
I_o	light intensity at point 0 (origin)
kgf	kilogram force
l	optical path length of the absorption.
mcd	millicandela
ml	millilitre
NIR	near infrared
nm	nanometre
R	reference intensity at wavelength λ
R	correlation coefficient
R_λ	reflectance at wavelength λ
R^2	coefficient of determination
RMSEC	root mean square error of calibration
RMSEP	root mean square error of prediction
S	Sample intensity at wavelength λ
SSC	soluble solids content
UV	ultraviolet
Vis	visible

PEMBANGUNAN SISTEM SPEKTROSKOPI GENTIAN OPTIK VIS-NIR UNTUK ANALISA METROLOGIKAL KE ATAS KUALITI INTRINSIK AVERRHOA CARAMBOLA L.

ABSTRAK

Teknik pengukuran optik adalah salah satu kaedah yang telah menampakkan kejayaan awal dalam penilaian kualiti tanpa musnah. Dalam kajian ini, beberapa pendekatan kuantitatif dalam mengukur kualiti dalaman buah-buahan telah berjaya dilakukan melalui teknik serapan, pantulan dan interaksi melalui spektrum yang boleh dilihat dan inframerah hamper beserta sistem pengukuran monokromatik. Penyelidikan bermula dengan taksiran Aquaphotomics (serapan NIR) larutan air-gula (sukrosa, glukosa dan fruktosa) dan larutan air-asid (sitrik, tartarik, malik dan oksalik) yang merupakan komposisi biokimia yang biasa terdapat di dalam buah-buahan. Tujuannya adalah untuk mengenal pasti jarak gelombang optik yang terbaik dalam mengukur parameter yang berkait rapat dengan kualiti dalaman buah-buahan. Peringkat penyelidikan seterusnya telah dijalankan ke atas sampel buah-buahan yang telah dikategorikan kepada sampel awal (anggur, limau dan belimbing) yang dijalankan melalui spektroskopi pantulan bagi pengukuran kandungan pepejal larut dan pH (keasidan) dan sampel intensif (belimbing premium B10 - kualiti eksport) untuk mengukur kandungan pepejal larut, pH (keasidan) dan kekerasan yang dijalankan melalui spektroskopi pantulan dan interaksi. Kajian ini telah memperkenalkan analisis spektrum baru yang dinamakan sebagai pelinearan spektrum dan anjakan kecerunan. Teknik-teknik ini telah digunakan semula ke atas semua data spektrum yang diperolehi daripada analisis air-bahan terlarut serta ukuran spektroskopi belimbing B10. Hasil yang paling ketara diperolehi adalah dalam pengukuran kandungan pepejal larut dengan menggunakan interaksi NIR pelinearan spektrum di mana hasil yang diperoleh adalah yang terbaik jika dibandingkan dengan teknik regresi lelurus berganda dari semua julat panjang gelombang. Sepanjang keseluruhan eksperimen spektroskopi yang telah dijalankan dalam penyelidikan ini, dapatlah dirumuskan bahawa panjang gelombang 950nm, 960nm, 965nm, 970nm dan 975nm telah dikenal pasti berkait rapat dengan serapan oleh air, manakala panjang gelombang antara 909nm dan 915nm bagi serapan oleh gula dan panjang gelombang 918-925nm dan 990-996nm untuk serapan yang berkaitan dengan pH sampel. Peringkat terakhir kajian adalah pada pembangunan penderia gentian optik 12-bit yang beroperasi dengan mod monokromatik dan merupakan objektif utama kajian ini. Dua reka bentuk yang berbeza telah dikemukakan dan dikenali sebagai Sistem Gentian Optik Merah dan Sistem Gentian Optik Inframerah Hampir. Sistem yang baru dibangunkan ini telah digunakan untuk aplikasi baru dalam pengukuran pH dan kekerasan belimbing B10 dan juga ke atas pH jus belimbing. Keputusan yang diperolehi dengan menggunakan penderia gentian optik adalah setanding dengan spektrometer komersial, tetapi dengan kos pembangunan yang lebih rendah, mesra pengguna dan keputusan yang dihasilkan adalah lebih cepat tanpa perlu untuk pemprosesan data yang kompleks.

DEVELOPMENT OF OPTICAL FIBER VIS-NIR SPECTROSCOPY SYSTEM FOR METROLOGICAL ANALYSIS ON INTRINSIC QUALITIES OF AVERRHOA CARAMBOLA L.

ABSTRACT

Optical measuring techniques are one of the methods that have shown promising results in non-destructive quality evaluation. In this research, several approach in quantifying fruits intrinsic quality have been successfully performed through absorbance, reflectance and interactance techniques via visible and near infrared spectral as well as novel application of monochromatic measurement systems. The research begins by Aquaphotomics assessment (NIR absorbance) of water-sugars (sucrose, glucose and fructose) and water-acids (citric, tartaric, malic and oxalic) solutions which are common biochemical composition in fruits. The intention was to identify optical wavelengths that serve the best in quantifying parameters that are closely related to intrinsic quality of fruits. The next stage of research was conducted on intact fruits samples that have been categorised into preliminary samples (grapes, limes, and carambola fruits) conducted through reflectance spectroscopy for the measurement of SSC and pH (acidity) and intensive samples (premium export quality of B10 carambola fruits) for the measurement of SSC, pH (acidity) and firmness conducted through reflectance and interactance spectroscopy. This research has successfully introduced a novel spectral analysis named as spectral linearisation and gradient shift. These techniques were reapplied on all spectral data obtained from water-solutes analysis as well as the spectroscopy measurement on B10 carambola. The most significant result obtained was in the measurement of SSC using NIR interactance spectral linearisation where the result produced is the best if compared to multiple linear regression techniques from all range of wavelengths. Throughout the entire spectroscopy experiment conducted in this research it can be summarised that wavelengths 950nm, 960nm, 965nm, 970nm and 975nm have been identified to be strongly related to absorbance of water, wavelengths between 909nm and 915nm for absorbance of sugars and wavelengths 918-925nm and 990-996nm for absorbance related to pH of samples. The final research stage which serves as the main research objective was on the development of 12-bit optical fiber sensors that operates on monochromatic mode. Two different designs have been presented and known as Optical Fiber Red System (OF-RS) and Optical Fiber Near Infrared System (OF-NIRS). These newly developed systems have been used for its novel application in the measurement of pH and firmness of intact B10 carambola as well as on pH of its juice. The results obtained using optical fiber sensors are at par with commercial spectrometer, but with much lower cost of development, user friendly and faster results can be produced without the need for complex data processing.

Chapter 1
Introduction

Fruits and vegetables are important in world food production and also for human nutrition consumption and health benefits (Dris and Jain, 2004; Joffe and Robertson, 2001). World Health Organization (WHO) recommends a daily intake of fruits and vegetables of more than 400g per person due to its importance in reducing many diseases (Joffe and Robertson, 2001). Serious diseases such as cardiovascular disease, diabetes and some particular cancers can be prevented by taking sufficient amount of fruits and vegetables. A research conducted in Denmark shows that intake of appropriate amount of fruits and vegetables will lower the risk of lung cancer for some group of people (Sorensen *et al*, 2007).

Botany, the scientific study of plant, defines fruit as the ripened ovaries of flowering plants (Biologyreference.com, 2008). From botanical point of view, vegetables such as cucumbers and tomatoes are also considered as fruits (Biologyreference.com). Most fruits have the edible portion at the fleshy part of the pericarp or vessel that surrounds the seeds (Dauthy, 1995). The pericarp itself is typically made up of three distinct layers as shown in Figure 1.1 and describe below:

a. The exocarp is the most outside layer or peel which bears oil glands and pigments such as chlorophyll and carotenoids.
b. The mesocarp is the middle layer or pith which usually the part of the fruit that is eaten.
c. The endocarp is the inner layer surrounding the hollowed ovary or the containing seeds.

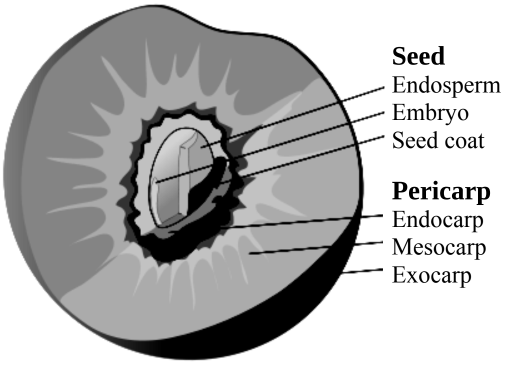

Figure 1.1 Typical drupe (peach) layout showing both fruit and seed.

In general, fruit is acidic and sugary and are grouped into several major divisions, depending mainly upon their botanical structure, chemical composition and climatic requirements. Berries are fruit which are usually small and rather fragile. Grapes are also physically fragile and grow in clusters. Melons, in contrast, are large and have a tough outer rind. Drupes or also known as stone fruit contain single pits such as apricots, cherries, peaches and plums. Pomes contain many pits, and are represented by apples, quince and pears. Citrus fruit are high in citric acid like oranges, grapefruit and lemons. Tropical and sub-tropical fruits include bananas, dates, figs, pineapples, mangoes and others require warm climates, but exclude the separate group of citrus fruits (Dauthy, 1995).

In the commercialisation of fresh fruits, the quality of the fruits can be deteriorated at certain stages especially when reaching the market for consumers. One of these critical stages is at the point when the fruits come out of the producer facilities and reaches the wholesaler chambers. This transition, however, may sometimes go through the quality-controlled procedures. The fresh produce that arrives at the market may have very different conditions that those observed at field. This may due to bad handling of fruits during packaging or transporting which causes impact or bruises on the fruits. Besides, the level of fruits ripeness may also changes more or less during this period of time (Valero and Ruiz-Altisent, 2000). Woodcock *et al*. (2008) stated that quality changes in foods product between production and consumption are due to contamination by dust, dirt, chemicals and weeds, mechanical injury during harvesting or processing, physico-chemical changes by weather conditions, contamination or spoilage by microorganisms, insects or rodents and biochemical changes due to enzymatic activity. Therefore, the quality assessment system is required to ensure that the fruit that the consumers are purchasing posses the required quality standard (Valero and Ruiz-Altisent, 2000).

Consumers are now looking for trusted grades and quality standards on fruits and vegetables products hence rejecting products with adulteration. In addition, consumers are also choosing to buy healthful and non-contaminated products. Accordingly, the fruit and vegetable industries require more research on technology in determining the quality of fruit. For fruits industries, the determination of internal qualities are the important indicators for harvesting, transportation, storage and other handlings before the product launches into the market. For that reason, necessary measurements have to be taken to meet these demands (Lin and Ying, 2009). There are many efforts being made to establish the standard quality parameters for fresh produce and the development of instrumentations that meet these expectations. For instance, the Physical Properties Laboratory (LPF) directed by Prof. Margarita Ruiz-Altisent has been working on fruit quality assessment both on theoretical and practical basis concerning the quality specifications as well as instrumental measurement of quality in fruits (Dris and Jain, 2004). In the field of nutrient research and the regulatory commercial requirement, indices for internal quality parameters such as soluble solids contents, total acids and firmness are becoming the focus subjects (Reid *et al*, 2006). However, assessing these internal quality parameters of fruits usually involve destructive procedures and require much labour and time consuming. Therefore, a much simpler, faster and highly accurate measurement method is required (Temma *et al*, 2002). Employing non-destructive sensing techniques in fruits industry ensure the quality and wholesomeness of fruit. This would increase consumer satisfaction and acceptance, and enhancing industry competitiveness and profitability. Various non-destructive sensing techniques have been studied and implemented for predicting internal quality of fresh fruits. For instance, light-based sensing techniques or the so-called spectroscopy offers great prospect for measuring the firmness and sugar or soluble solids content (SSC) of fruits.

The interaction between radiation and matter has been proven useful in many research labs (Valero and Ruiz-Altisent, 2000). Spectroscopy has progressively contributing as a source of information in various fields of research particularly in biology (Tsenkova, 2010). In post-harvest quality assessment of fruits, spectroscopy measuring technique is gaining a remarkable increase in attention. Spectroscopy, which can be divided in terms of electromagnetic spectral range such as ultraviolet (UV: 200-400 nm), visible (VIS: 400-750 nm) and near infrared (NIR: 750-2500 nm) is an established technique to examine the chemical constituents of agricultural products. This technique is comparable to the currently available measurement performed through different physical methods (Carlini *et al*, 2000). The absorbance (or conversely, reflectance) spectrum are the result of complex pattern of scattering and absorption by various structural and biochemical composition of the fruits. The information content of a sample’s UV-Vis-NIR spectrum provides brief and rich summaries of the overall biochemical components of the sample (Richardson *et al*, 2003; Ocean Optics, 2008). Upon this inspiration, analysis on existing techniques, design of specialised optical instrumentations for biochemical interpretation and new optical analysis will be conducted in this research. These will be derived, with intention to bring the subjects of fruits quality measurement and spectroscopy analysis and optical instrumental design up to a different level of understanding.

1.1 Fruits Quality Assurance

The word quality originates from the Latin language which means property or characteristic. In food industry, the word quality originally is used as a synonym for freshness and unspoilt. DIN EN ISO 8402 (1989) define quality "as the sum of characteristics, properties and attributes of a product or commodity which is aimed to fulfill the established or presumed customer requirements" (Shewfelt, 2000). The term quality has become one of the most emphasised factors in the field of food and food production since the last two decades due to the importance of quality and quality management systems which is tools for food safety production and economy (Buckenhueskes, 2007). Since the last years, consumers' perception on product quality has changed significantly. The high competition from surplus production of horticultural crops has led the changing in the consumers' purchasing behaviour which demands only the highest quality of products. This change is also attributed by the rising awareness on environmental, nutritional and health concerns among society (Huyskens-Keil and Schreiner, 2004).

The word quality is being used in various ways in defining fresh fruits and vegetables. These include the term such as market quality, utilisation quality, sensory quality, nutritional quality, ecological quality, external and internal quality, shipping quality. Most of these various terminologies are being applied in different ways to classify and describe the quality items of a product. However, these usually meant different things to different customer groups. Figure 1.2 summarises different quality attributes from different group of customers (Huyskens-Keil and Schreiner, 2004). In summary, the quality specifications of fruits can be divided into three different categories which are legal quality, consumer quality as well as company quality (Barreiro *et al*, 2004).

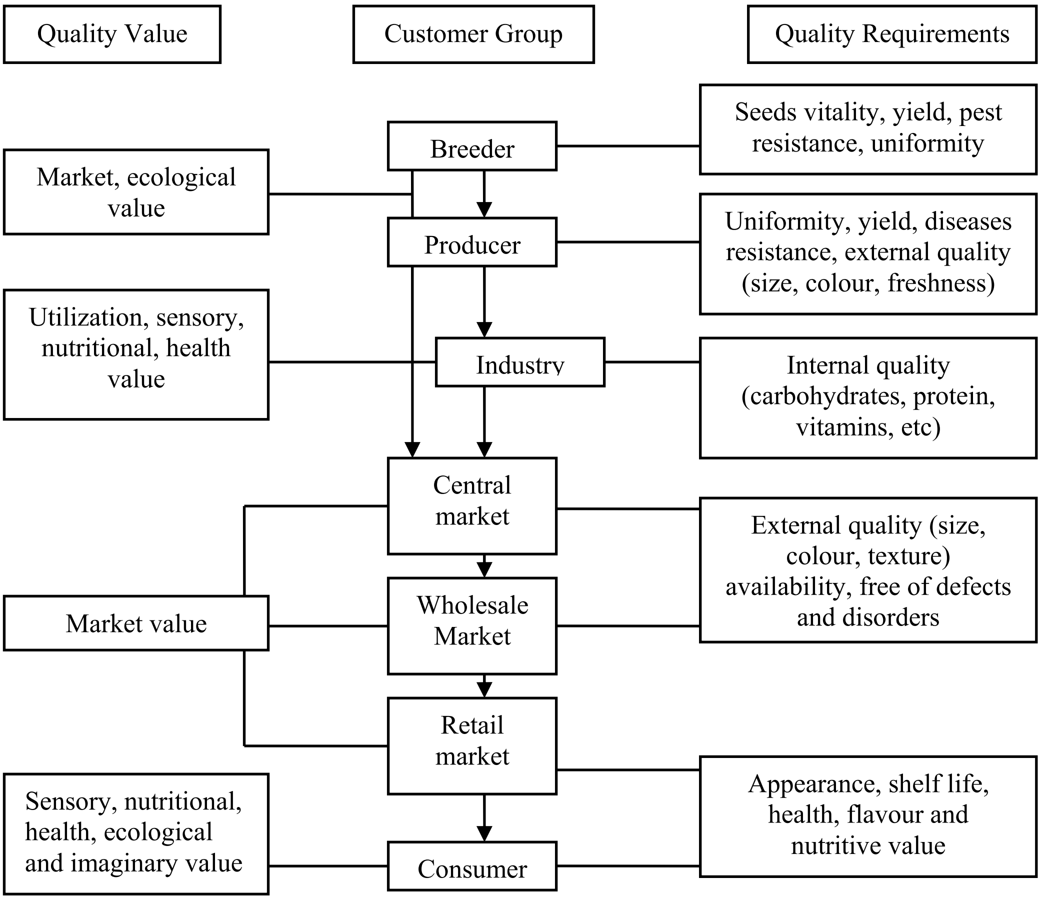

Figure 1.2 Quality values defined by actors in the supply chain (customer groups) and their requirements (Huyskens-Keil and Schreiner, 2004).

1.1.1 Fruits Standards

Product from the fruit and vegetable industries can be classified based on two major end uses, that are fresh market or processing such as canning, freezing, juicing and dried/dehydrating. Fruits and vegetables harvested for fresh use that do not meet quality standards will be sold for processing (Lucier *et al*, 2006). At the present time, quality properties, classification and evaluation of fresh fruits and vegetables is conducted to comply with the criteria of the official quality grades and standards set by the United Nation Economic Commission for Europe (UN/ECE) or the United States Department of Agriculture (USDA) standards which are based on the Codex alimentation of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) (Huyskens-Keil and Schreiner, 2004; Barreiro *et al*, 2004). These quality standards are used for the national and international trade as a measure of the economically important fresh horticultural product. However, the current official product quality standard is based upon the subjective assessment which priorities the visual and external product attributes mainly to satisfy technological concerns of trade. Consequently, the product properties which reflect the growing consumer requirements relating to environmental, health and sensory benefits such as chemical composition are not considered (Huyskens-Keil and Schreiner, 2004). In Malaysia, the standard set by Federal Agricultural Marketing Authority (FAMA) also emphasise on the external attribute of the fruits such as size, colour, shape and percentage of visible defect on the fruits (FAMA, 2011).

As an example, in order to expand the globalisation of fresh produce market, UN ECE has drawn standards for fresh fruits and vegetables, E.91.II.E.42, which every product in the market has to comply with. The properties of the product which could be standardised are based on the magnitude which can be measured such as size, shape, presence and size of external damages. Some other properties which may be included are based on the subjective assessment such as colour and its distribution and also occurrence of off-shape. On the contrary, this regulation does not include properties which can not be measured with definite procedure. As a result, it is common that this situation has led the fresh produce market to a point where many fruits and vegetables do not satisfy the consumers' quality expectations. For instance, there are many cases where beautiful fruits, such as peaches and pears are completely inedible or tasteless. The practice of 'degreening' oranges at the beginning of the season through a treatment process, changes the peels into a beautiful orange colour from originally green. Present standards established the minimum colour for the mandarins. With this degreening technique, the fruits are suitable for market, but the sugar content may be non-existence. Therefore, growers and distributors are now developing the specifications ahead of the legal quality, summarising the relevant intrinsic properties that the consumers will accept: such as firmness, sugar and acid contents, aromas (juice content has been established as a comparatively standard measurement) and also vitamins (Barreiro *et al*, 2004). There are various components of quality that are being used to evaluate fruits and vegetables. Quality can be categorised into external and internal component as shown in Table 1.1. There are five quality factors which are normally recognised for fruits and vegetables. Those are appearance, flavour, texture, nutritive value and defect factors.

Table 1.1 External and internal quality factors for fruits and vegetables (Noh and Choi, 2006; United Nations, 2007).

External Qualities		Size: weight, volume, dimension Shape: diameter, depth ratio Colour: uniformity, intensity (measurement can be made by visual guides and colorimeters) Defect: bruise, stab, spot (measurement can be made by mechanical methods (e.g. ultrasound)
Internal Quality	**Flavour**	Sweetness, Sourness, Bitterness, Saltiness, Astringency, Aromas (Mostly qualitative and subjective evaluation through smelling or can be measured by technical method such as gas chromatography). Taste compounds can be technically quantified through chromatography.
	Texture	Firmness, Crispness, Juiciness, Tenderness, Crunchiness, Chewiness, Fibrousnesses which are measured by applying force to the produce. Textural characteristics are evaluated as mouth feel.
	Nutrition	Fat, Carbohydrate, Proteins, Vitamins, Minerals Functional Property and other substances that influence human well-being.
	Defect	Internal Cavity, Water Core, Frost Damage, Rotten
	Safety	Can be determined through the examination on fruits items based on their pathogenic microbial load, content of chemical contaminants or presence of physical foreign matter in the fresh produce.

Table 1.2 summarises the most relevant quality attributes for several fruits gathered from a survey in a European Project (FAIR CT 95 0302 'Mealiness in fruits') participated by 818 consumers, 77 producers and 26 warehousemen and gathered from other parallel sources of information. Almost in all cases, the most significant quality attribute is an internal property such as firmness or taste. This is however related to the fact that other important quality attributes such as size, shape and cleanness are already met (Barreiro *et al*, 2004).

Table 1.2 Summary of most relevant quality attributes for several fruits gathered (Barreiro *et al*, 2004).

Fruits	Most Desirable Quality Attribute	Second	Third
Apple	Firmness/Texture	Bruises	Sugar and Acidity
Apricot	Firmness	Sugar	Colour
Citrus	Rots-molds	Blemishes or Bruises	Sugar and Acidity
Melon	Sugar	Colour	-
Peach	Firmness	Sugar and Acidity	Bruises
Pear	Firmness	Sugar and Acidity	Bruises
Tomato	Colour	Firmness	Sugar and Acidity

Also from this survey, there appears the prospect of developing instrumentation to measure the properties of any fresh produce that can be used to define more precisely the choice of the consumer. Hence, the properties which the consumer relates the perception of quality when eating fresh produce are indeed the intrinsic properties of the product itself. This opens the possibility for analysing these intrinsic properties of fruits and move forward to its quality measuring instrumentation (Barreiro *et al*, 2004). According to Shewfelt (1999), consumers judge quality base on the appearance at the time of initial purchase and consider good quality of fresh fruits and vegetables to be those that have a good appearance, firm and offer high flavour and nutrition (Shewfelt, 1999). A research has been conducted to identify the common consumer complaint on peach purchases. It was found that 30% complain was on the little flavour of the fruits, 21% on too hard, 5% on too soft and 13% on mealy (Crisosto, 2008).

1.1.2 Fruits Maturity and Ripeness

Maturity is the character of the fruits on the tree that grows to its intended size and shape. Maturity at harvest is the most important factor that determines the fruit quality to the consumer and the storage-life of the fruits (Lamp, 1997). Ripeness is the subsequent process happen to the fruits within a week or so after it matures (Wischik, 2008) and result in developing changes in composite colour, texture or other sensory attributes of the fruits (Kader, 1999). The fruits generally ripen through the following criteria:

a. Aroma: A bitter and astringent phenol which was initially to discourage animals before the seed was ready. This will fade away and a nice aromas are produced which will then used to encourage animals (Wischik, 2008).

b. Taste (Sweetness & Sourness): Fruits sweetness is in the form of sucrose or fructose (Wischik, 2008). The ripening of the fruits is associated with changes in the composition of carbohydrates. In some fruits such as apple, pear, kiwifruit and banana, the key characteristic of the ripening is the nearly complete conversion of insoluble starch (which is a large component of the fruit at harvest time) into soluble solids where simple sugars (glucose, fructose, and sucrose) represent the largest contribution (Beever and Hopkirk, 1990; Esau, 1972). In fruit without reserve starch, such as plum, peach and citrus, the ripening is characterised by a decrease in acid content and an increase in sugars. In the ripening avocado, the sugar content decreases and fat content increases (Esau, 1972). Sugars and acidity are two key elements which determine the flavour of fruit. Fruit contains natural acids, such as citric acid in citrus fruits like oranges and lemons, malic acid in stone fruits like apples and kiwifruit, tartaric acid in grapes (Garner *et al*, 2008; Dauthy, 1995) and ascorbic acid (source of vitamin C) in citrus fruits, tomatoes, cabbage and green peppers. These acids will give the fruits tartness which helps in slowing down bacterial spoilage. The sugar/acid ratio is a very frequently used indicator to give a technological characterisation of fruits and of some vegetables (Dauthy, 1995). Fruit are sweetest when allowed to ripen on the tree (Crane, 1994). Fruit ripens from the inside out and during this maturation process; there is a gradient in the properties such as sugar content. Even mature fruit has natural variation in properties such as sugar and acid content from one side to another (Ozanich, 2001). For instance, total carbohydrate concentration (sugar and starch) will mainly determine the final soluble solids concentration of a fruit. The soluble solids content at harvest may vary significantly between fruit, locally, within or between vines and also between orchards and seasons even if they are harvested at the same apparent maturity. These variations suggest different patterns of sugar and starch formation inside fruit grown under different conditions (MacRae *et al*, 1989). One example of the high variation of sugar content within a fruit is a blush Golden Delicious apple. Although the localised and near-surface region of the blush can be 3 to 5% higher ^{o}Brix, the overall contribution to the whole apple ^{o}Brix average is usually relatively small (Ozanich, 2001). From the context of a citrus fruits, it is known that sugar and acid content vary as a function of storage time and temperature. They reported more pronounced changes at higher temperatures and a more significant change in sucrose than glucose and fructose. Citric acid declined over 4 to 9 weeks storage time (Miller and Zude, 2002).

c. Juiciness and softness: Cell wall structure of fruit tissue is the determining factor for fruit firmness and is composed of cellulose microfibrils that are embedded in a matrix of pectins and hemicelluloses or lignin. An immature fleshy fruit wall initially has a firm texture, but it becomes softer as the fruit starts to ripe. The cell wall structure is partly dissolved as enzymatic degradation of the cell walls takes place during fruit ripening which results in softening of the tissue. Chemical changes in the cell contents and in the structure of the walls are responsible for the softening (Oey *et al*, 2007; Esau, 1972). The enzyme polygalacturonase attacks pectin in the cell walls making cells slide around (softness) and may even become dissociated from each other (Wischik, 2008; Esau, 1972) and spill their contents (juiciness). Acids are used up in this, making the fruit less sour (Wischik, 2008). Besides cell wall structure, turgor pressure is having a major influence on tissue strength and macroscopic fruit firmness. Turgor is put forth by intracellular liquids on the cellular membrane and cell wall that contribute in imparting turgidity, rigidity, crispness and a fresh appearance to the plant tissue. Fruits will loose its turgor when they are deprived of water through transpiration or stop from respire and causes the tissue to wilt and have a dry appearance through loss of gloss and colour (Aguilera and Stanley, 1999).

d. Colour: Colour, exist within visible electromagnetic spectrum from 380 nm to 780 nm is the human visual perception of light reflected, transmitted or emitted from fruit. The key aspect in the distribution of light energy reflected from the fruit is the presence and concentration of pigments such as carotenoids, anthocyanins and other flavonoids, betalains, and chlorophylls in the skin of the fruits (Gross, 1987; Mazza and Miniati, 1993). The pigments and colour originator of fruit and vegetables take place in the most part of the cellular plastic enclosure such as the chloroplasts and other chromoplasts, and to a minor degree dissolved in fat droplets or water within the cell protoplast and vacuoles (Dauthy, 1995). Immature fruits have numbers of chloroplasts in the outermost cells which are thus green in colour. The development of carotenoid pigments, which are fat-soluble and the disappearance in chlorophyll, produces a change to a yellow, orange or red colour, as observed in tomato (Esau, 1972; Dauthy, 1995). Ripening fruit may generate anthocyanins which will give the tissue a red, purple or blue colour (Esau, 1972). One of the properties of the anthocyanins is to transform colours with pH. Hence, many of the anthocyanins which is either purple or blue in alkaline media become red upon addition of acid. Many plant pigments are natural pH indicators since organic acids influence the colour of foods (Dauthy, 1995). These pigments may be spread to the entire fruit wall, as in some cherries. However, they may also be limited to peripheral parts of the fruit wall as in the plum or Concord grape. The outer epidermis of fruit usually accumulates tannins (Esau, 1972).

Apart from the above listed properties of ripen fruits, vegetal cells contain an important amounts of water which play a significant task in the evolution, reproduction cycle and in physiological processes. Fruits' water content affects the storage period and the consumption of tissue reserve substances. Useful storage life for fruits is from one to seven days while for leafy vegetables, it is within one or two days (Desrosier and Desrosier, 1977). Fruits' typical water content is between 80 and 90% while vegetables contain generally 90-96% water (Dauthy, 1995). Table 1.3 summarises the typical composition percentage of banana, orange, apple and strawberries.

Table 1.3 Percentage of carbohydrate, protein, fat, ash and water in common fruits (Dauthy, 1995).

Fruits	Carbohydrate	Protein	Fat	Ash	Water
Banana	24.0	1.3	0.4	0.8	73.5
Orange	11.3	0.9	0.2	0.5	87.1
Apple	15.0	0.3	0.4	0.3	84.0
Strawberries	8.3	0.8	0.5	0.5	89.9

Immature fruits have higher tendency to shrivel and are of lower quality when ripe. Overripe fruits, on the other hand, are likely to become soft and mealy soon after harvest. Fruits picked at the proper time are more likely to have a longer storage-life than those picked either too early or too late in the season. Fruits, with a few exceptions such as pears, avocados, and bananas, will arrive to their best eating quality when allowed to ripen on the tree or plant (Lamp, 1997). Some fruits are picked when it is mature but unripe (early stage of ripening) to better withstand handling and they do not reach full flavour and aroma.. This is very important especially for soft fruit like cherries, peaches and nectarines which will undergo rapid ripening and become very soft when fully ripe and can easily get damaged by the act of picking itself that can leads to losses in the marketing chain (Dauthy, 1995; Lamp, 1997; Ziosi *et al*, 2008). For peach, there is a close relation between on-tree physiological maturity and development of key traits responsible for its quality. A delayed harvest could improve fruit organoleptic characteristics. This is due to the fact that sugars and flavour components increase while total acids decrease during late ripening (Vizzotto *et al*, 1996; Visai and Vanoli, 1997; Etienne *et al*, 2002). Furthermore, this is also important since many types of fruits continue to ripen off the tree and some may become overripe before they could be utilised if picked at peak ripeness (Dauthy, 1995). Besides, this will make the fruits able to withstand the post-harvest handling when transported for long distance. Most fruits are picked at a time between the best eating quality for the consumer and that which can provide the required flexibility for marketing purposes. Fruits can be divided into two groups. The first type are the fruits that are stop ripening once removed from the plant and second is the fruits that can be harvested mature and continue to ripen off the plant (Lamp, 1997). The examples of fruits which belong to each group are:

Group 1: Berries (such as blackberry, raspberry, strawberry), cherry, citrus (grapefruit, lemon, line, orange, mandarin, and tangerine), grape, lychee, pineapple, pomegranate, tamarillo.

Group 2: Apple, pear, quince, persimmon, apricot, nectarine, peach, plum, kiwifruit, avocado, banana, mango, papaya, cherimoya, sapote, quava, passion fruit.

As fruits ripen, they will produce ethylene (C_2H_4) which is the ripening hormone that coordinates the ripening process of fruits (Wischik, 2008). For fruits such as peaches and nectarines, like any other climacteric fruit, display a quick rise in ethylene production at the beginning of ripening, paralleled by dramatic changes in the transcriptional profile of genes, many of which are regulated by the hormone. Coordination and programmed modulation of gene expression leads to changes in fruit aroma, flavour texture and colour that contribute to overall fruit quality (Trainotti *et al*, 2003, 2006). Fruits in the first group generate very small amount of ethylene and do not further react to additional exposure to ethylene. These types of fruits should be picked when fully ripe to guarantee good flavour quality. On the other hand, fruits in Group 2 produce higher quantities of ethylene as they ripen. Further exposure to ethylene will result in faster and more uniform ripening (Lamp, 1997). Table 1.4 lists the optimum stages of maturity for several common fruits. The suitable maturity stage will rely on the chosen market and different cultivars have different harvest maturities, too.

Table 1.4 Harvest maturity indicators for common fruits (Gast, 1994; Kader, 1999).

Fruits	Indicators
Apple	Industry standards for soluble solids are at least 12 percent Ground colour a change to a yellowish cast Iodine-starch test 60 percent of the area blue-black in colour Firmness should be less than 20 pounds and more than 12 pounds
Apricot	Colour of external surface area is ¾ or larger yellowish green or less than ½ yellow.
Blueberries	Fruit should be blue in colour without any green Soluble solids of 10 to 15 percent pH 3.43 to 3.73
Cherries	Sweet cherry should have the characteristic skin colour for the variety, which can range from yellow to black red. Sour cherries should be bright red. Soluble solids should be at least 14 to 16 percent depending on cultivar.
Grapes	Wine and juice markets require grapes with specific soluble solids and acid content. Fresh market grape will depend on the flavour and aroma. Grapes will often colour up before they are ripe, so soluble solids and the colour change of the stems from green to brown may be a better indicator. Soluble solids of 14 to 17.5 percent depending on cultivar and production area or soluble solids to acidity ratio of 20 or higher.
Grapefruit	Soluble solids to acidity ratio of 5.5 to 6.0 in desert area and 2/3 of fruit surface is yellow in colour.
Kiwifruit	Soluble solids of 6.5 percent.
Lemon	30 percent of juice by volume.
Nectarine & Peach	Surface ground colour change from green to yellow and the shape is in fullness of shoulders and suture.
Pears	Soluble solids are not usually used but should be at least 13 percent for marketability Pear firmness should be 23 pounds, but can be less if the soluble solids are less than 13 percent Ground colour changes from a green to yellowish green Iodine-starch test 60 percent or less of the area blue-black in colour
Plums	Each variety has its own characteristic colour change, familiarity with standards for planted varieties is important Soluble solids should be at least 17 percent and pressure testing may be useful
Pomegranate	Red juice colour and below 1.85 percent of acid content in juice.
Strawberry	More than 2/3 of fruit surface is showing pink or red colour.
Tangerine	Soluble solids to acidity ratio of 6.5 and yellow, orange or red colour on 75 percent of the fruit surface.

1.2 Spectroscopy

Spectroscopy is an optical analysis in identifying the absorption and emission of light spectra and other radiation by matter, which in this point of research is fruit. This process is dependence on the wavelength of the radiation. At present, the definition of spectroscopy has been stretched to also include the study of the interactions between particles (such as electrons, protons and ions) as well as their interaction with other particles as a function of their collision energy. Spectroscopy has been applied widely from astronomy, in measuring the chemical composition and physical properties of astronomical objects to medicine, in identifying cancerous tissue. The ability of spectroscopy to identify and quantify biochemical composition makes it very important in the applications in multiple fields of study. In food industry, spectroscopy has been applied in checking the quality and freshness of food, including fruits and vegetables. The application of spectroscopy is a promising tool in overcoming existing destructive measuring techniques. The integration between spectroscopy instrumentation with optical fiber probe makes the spectroscopy application more flexible and can be applied in different environmental conditions. Thus, this research is putting forward spectroscopic analysis into its detail application in identifying and quantifying fruits quality parameters. In order to achieve that, it is necessary to examine and understand the phenomena related to spectroscopic analysis which is bounded by fundamental optical nature.

1.2.1 Spectroscopy Essentials

Spectroscopy is the study of spectra, which is the result of dispersing a ray of light into its constituent colours. Through spectroscopy, the light is broken into its components or spectral features that consist of absorption and emission lines

(Caussade, 2004). Spectroscopy analysis can be applied to determine the identity, the structure and the environment of the atoms and molecules which can be done through analysis of the radiation emitted or absorbed by them. The light produced from a gaseous discharge will form a spectrum and is found to consist of discrete lines and bands when analysed by wavelength, conceivably overlying a continuum. Each line or band on the spectrum is having a characteristic of a particular atom or molecule. The analysis can be made quantitative by measuring relative intensities as well as wavelengths (Thorne, 1974). When light rays hit a medium, the light wave is forced to deviate from a straight line path by the roughness of the medium. This phenomenon is called as diffuse reflectance. However, if the light path diverged through a predicted angle and unscattered, it is called as specular reflection which obey the law of reflection (Omar, 2008). Specular reflection causes gloss (McGlone *et al*, 1997) and does not carry any information about the material since it never penetrates the medium. While for the diffusely reflected light, it occurs when the light penetrates the medium, being absorbed and undergone multiple scattering before the light is making its way back to the surface of the medium (Enderle *et al*, 2000). McGlone *et al.* (1997) separate the definition between diffuse reflection and scattering. They stated that diffuse reflection is induced by rough surfaces while scattering results from multiple refractions at phase changes inside the material. Figure 1.3 shows the possible resultant optical phenomena in the form of distribution of the incident light into its constituent components after hitting an object.

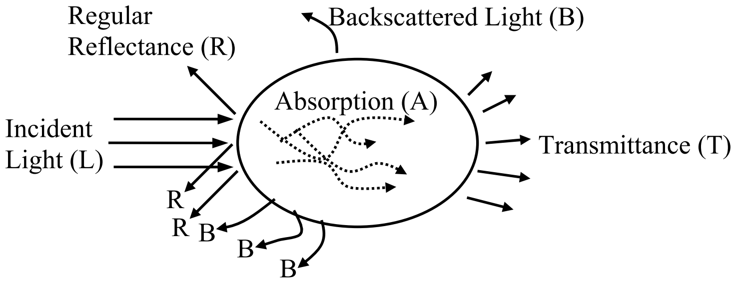

Figure 1.3 Distribution of an incident light on an object.

The optical scattering phenomena can be further divided into elastic and inelastic scattering. In elastic scattering, the energy (frequency/wavelength) of the incident particles is conserved but their direction of propagation is modified, such as in the application of UV-Vis-NIR (Ultraviolet-Visible-Near Infrared) reflection/absorption spectroscopy. Elastic scattering can also be described by Mie theory (and Rayleigh theory for particles with size smaller than wavelength of light) which the intensity of the scattered radiation can be associated with the concentration, size and shape of the particles. While for the inelastic scattering, the frequency (wavelength) of the excitation light will be shifted after interacting with the sample such as in Raman and Flourescence spectroscopy (Enderle *et al*, 2000).

Spectroscopy is the most vital technique in obtaining comprehensive information on the structure and dynamics of atoms and molecules. The fundamental aspects in any application of spectroscopic technique are the sensitivity and the attainable spectral resolution. The sensitivity of a spectroscopic system is defined as the minimum number of photons detectable during absorption or emission on a transition process. In other words, this is also a measurement for the minimum number of atoms or molecules which can be monitored by the spectroscopic system. UV-Vis-NIR spectroscopy technique is widely being applied such as in the variety discrimination, grading system and for non-destructive estimation of the internal attributes. The advantages of using this technique are low cost, non-destructive and limited sample preparation (Huang *et al*, 2006). In agriculture industry, visible and near infrared (Vis-NIR) spectroscopy is an optical technology that has been widely used and now this technique is gaining higher interest in the field of post-harvest quality evaluation (Carlini *et al*, 2000). Visible range of wavelength is currently being used as an absorbed energy during photosynthesis by pigments such as chlorophylls and carotenoids. While NIR wavelength is being used in the characterisation of starch, protein, and carbohydrates which are a consequence of stretching and vibration by the C-H, N-H and O-H groups of which they are composed. However, even the interpretation of the NIR spectral characteristics of the different compounds are unique, they are also broad and thus often overlap (Richardson *et al*, 2003). Besides, UV spectroscopy has been applied in the measurement acidic and basic substances such as the experiment conducted by Hamada *et al* (2003) in the identification of Fe^{3+}/citric acid and Cr^{3+}/citric acid pH level (Hamada *et al*, 2003).

1.2.2 Optical Fiber Sensor

In general, an optical fiber sensory system consists of a light source, optical fiber, a sensing element (transducer) and a detector. The operating principle of a fiber based sensory system is that the transducer modulates some parameter of the optical system such as intensity, wavelength, polarisation or phase of light signal. This will gives rise to a change in the characteristics of the optical signal received at the detector. The fiber sensor can be either in intrinsic or extrinsic form. Intrinsic means that the modulation of signal takes place directly in the fiber while for the extrinsic, the modulation is performed by some external transducer (Micron Optics). Fiber optic technology presents many degrees of freedom and some advantages such as no moving parts, produces absolute measurement, high stability (immunity to electromagnetic interference), having excellent resolution and range, passive operation and intrinsically safe, resistant towards water and corrosion, compact (rugged, small size and light weight), can be multiplexed in parallel or in series and having modest cost per channel (Villatoro *et al*, 2009; Micron Optics).

In the design of the optical fiber sensor for metrological application, fiber optic probe will be used as the light transferring medium between light source, sample and detector. The light signal will be transmitted to the sample and the resultant reflected light will be retrieved from the sample and send it to the optical detector. The fiber optic cable may contain a core, cladding, protective tube, buffer and jacket as shown in Figure 1.4. Fundamentally, there are 3 types of optical fiber available (Tomasi, 1998):

a. Plastic core and cladding (often known as POF – Plastic Optical Fiber)
b. Glass core with plastic cladding (often called PCS fiber, plastic-clad silica)
c. Glass core and glass cladding (often called SCS, silica-clad silica)

Plastic fiber has a few advantages over glass fiber. It is more flexible, can be bent at 90° with no reduction of light transmission and rugged than glass. Plastic fiber is easy to install and can better withstand stress. Besides, it’s weigh 60% less than glass. However, since it has the highest attenuation, plastic fiber is most suitable to be used in short distance (Tomasi, 1998).

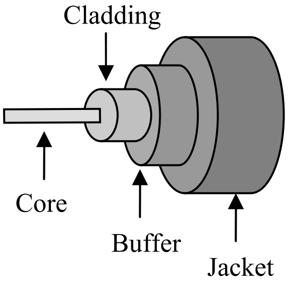

Figure 1.4 Typical component layer of fiber optic cable (Tomasi, 1998).

In one design and application of optical fiber sensor, Michal (2007) have developed an intelligent fiber optic sensor for estimating the concentration of a solution. The sensor operates based on a stepwise measuring procedure which includes sensor's head submerging, submersion, emerging and emergence from the examined solution (Borecki and Kruszewski, 2001). The deviation of the amplitude of the measured signal against time offers information about the type of the liquid (Michal, 2007). Fiber optic also has been widely applied as a low cost strain sensor for structural monitoring. In this application however, the fiber has undergone some modification to make it having higher sensitivity for the specified application. For instance, fiber Bragg grating (FBG) sensors which comprise an optical fiber with diffraction gratings incorporated into its core. The passage of light through this type of optical fiber will be affected by stretching it. Existing electrical strain gauges that serve for the same purposes suffer from sensitivity to electromagnetic interference, whereas FBG sensors do not. Despite of having an excellent sensitivity and versatility, FBG is comparatively expensive and not very mechanically robust (Poisel, 2008). In addition to the examples given, optical fiber sensor has also been applied as interferometric sensors in which the output beam of the sensing waveguide interferes with a reference beam. There are a well established sensors based on interference concept such as Mach-Zehnder interferometers, Young interferometer and Michelson. Besides, resonator type sensors are also relying on interference such as ring resonator and Fabry-Perot resonator. These classes of sensors are having typical resolution in the order of 10^{-7} to 10^{-5} refractive index units (RIUs) (Mayeh *et al*, 2009). Thus, the technology of fiber optic system is continuously emerging especially in the upgrading of its configurations and the material used in adding its sensitivity especially for a specific sensory application.

The application of fiber optic in spectroscopic analysis provides the capability of taking the spectrometer to the sample despite the conventional method of taking the sample to the spectrometer (Melling and Thomson, 2002). This is because fiber optic cables can provide a flexible interface between the samples to be examined in-situ with the spectroscopy devices. The introduction of fibre optics into spectroscopic system contributed to an enormous expansion for remote measurements especially for products which are difficult to handle such as fruits, vegetables, meat and fish. The miniaturisation of spectrometers is an added advantage is making them powerful analytical tool where robust systems are required in hostile environments (Buning-Pfaue, 2003). In the medical applications, the integration between the current optical examinations with spectroscopy devices has a potential to significantly improve clinical process (Utzinger, 2001). In another example, Omar and MatJafri (2008^a) have successfully designed an optical fiber sensor for water quality measurement that are based on two wavelengths' sensitive optical devices that are 470 nm (blue) and 635 nm (red). The system has the sensitivity to measure capacity of TSS (total suspended solids) in water with resolution of 10 mg/L (Omar and MatJafri, 2008^a). On-line analysis is the most effective means for obtaining process feedback and control. This is due to the fact that reactant and product concentrations can quickly be determined and the process is not disturbed by sampling. The fiber optic based spectroscopy is the application by which these criteria can be met (Omar, 2008). Moreover, the fiber optic probe allows flexible delivery and collection of scattered or reflected light even in hard to reach areas (Papaioannou, 2003).

1.3 Research Objectives

Current standard set by UN-ECE and Malaysian local authority such as FAMA emphasising on the external quality of fruits such as size, colour, shape and percentage of visible defect on the fruits as the benchmark to grade and market the fresh products. This research will present an innovative approach in quantitatively evaluating fruits quality according to their intrinsic properties through optical sensory systems. One of the main focuses is on producing measuring technique and spectral analysis that are capable of producing consistent result from sample with dynamic and uncertain external properties. The introduced instrumentation, measuring technique and spectral analysis are expected to show their unique strength in eliminating the setback in measuring fruits quality and unveil the effective function of optical physics in analytical chemistry and biology. Hence, the following objectives are derived.

1. To develop multiple wavelengths Optical Fiber Sensors for the measurement of intrinsic qualities of carambola.
2. To develop a novel spectral algorithms for quantitative quality analysis.
3. To determine optical parameters of various aqueous sugars and acids present in fruits and defines its intrinsic quality.
4. To analyse the peak responsivity wavelengths that able to quantitatively interpreting intrinsic qualities parameters of intact carambola using Vis-NIR (400 nm to 1100 nm) spectroscopy..

The strength and reliability of the results presented in this research will be evaluated by the precision of the generated calibration algorithms for every analysed variable through different optical methodologies and its accuracy in predicting individual quality attributes.