

**THE EFFECT OF ASPIRIN IN DELAYING POSTHARVEST RIPENING
OF PAPAYA (*Carica papaya* L.) FRUIT**

M. Sc THESIS RESEARCH

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**The Effect of Aspirin in Delaying Postharvest Ripening of Papaya (*Carica
papaya* L.) Fruits**

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As thesis Research advisors, we hereby certify that we have read and evaluated this Thesis, prepared, under our guidance by Jima Abebie entitled: **The effect of Aspirin in Delaying Postharvest Ripening of Papaya (*Carica papaya* L.) Fruit.** We recommend that it be submitted as fulfilling the thesis requirement.

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DEDICATION

This piece of work is dedicated to my beloved family: my Father Abebie Biru, my mother Askale Faye and all my sisters and brothers.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this M.Sc Thesis is my own work. I have followed all ethical and technical principles of scholarship in conducting studies, data collection, data analysis, and compilation of this Thesis. Any scholar matter that is included in the Thesis has been given recognition through citation.

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BIOGRAPHICAL SKETCH

The author, Jima Abebie, was born from his father Abebie Biru and his mother Askale Faye on November 13, 1978 in Madifo Gora, Amigna Wereda, Arsi Zone, Oromia Regional State, Ethiopia. He attended his elementary school at Madifo Gora Elementary School from 1988 to 1995 then he attended his secondary and preparatory school from 1996 to 1998 at Amigna Gasigar Secondary and Preparatory School. After completion of his Preparatory in 2001, he joined Debre Birehen University in 2000. He graduated, in July 2003 with B.Sc. degree in Biology. After graduating, he was employed by Oromia Education Bureau as a teacher at Hirna Secondary and Preparatory School, East Hararghe. After four year of service he join Haramaya University for his Master of Science in Biology in 2016.

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ABBREVIATION /ACRONYMS

ASA	Acetylsalicylic acid
PME	Pectinemethylesterase
GTP	Growth and Transformation Plan
HSP	Heat Shock Proteins
LAR	Local Acquired Resistance
LOX	Lipoxygenase
MeSA	Methyl salicylic Acid
PG	Polygalacturonase
RUBP	Ribulose-1,5-Bisphosphate
SA	Salicylic acid
SAR	System Acquired Resistance
TMV	Tobacco Mosaic Virus
TSS	Total Soluble Sugar

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The Effect of Aspirin in Delaying Postharvest Ripening of Papaya (*Carica papaya* L.) Fruit

ABSTRACT

*Papaya (*Carica papaya* L.) fruit is rich in vitamin A papain content has mild laxative property. However, it has very short shelf life faces huge postharvest losses. Salicylic acid is reported as a natural and safe phenolic compound which has been found to generate a wide range of metabolic and physiological responses in plants and act as potential bio agent in controlling post harvest loss of horticultural crops and delay in ripening through inhibition of ethylene biosynthesis or action. This study was planned to investigate the role of aspirin (acetylsalicylic acid) treatment in improving post harvest quality, shelf life, sugar and antioxidant (as carotenoid) content of papaya fruit. The experiment was laid in completely randomized design (CRD) in two replications. Quantitative data were collected for weight loss, vitamin C (ascorbic acid) from 0.47 to 0.90 total chlorophyll 0.01 to 0.05 and carotenoid content 0.01 to 0.24 are raised by using of ASA and reducing sugars 0.02 to 1.2 contents reduced from 0 day to 15 days. total titratable acidity 1.2 to 3.8 are increased during treatments and The result indicated that there were significance differences between control and acetylsalicylic acid treated papaya fruit samples for all the studied parameters except for weight loss. Quantitative analysis of organoleptic properties of the fruit has shown that the overall increase in reducing sugars and carotenoid contents while chlorophyll, total acidity, vitamin C and weight loss decrease during post harvest ripening of papaya fruit. Further studies are required on the effect of storage conditions, cultivar differences and environments on fruit quality parameters.*

Keywords: Carotenoid content, Organoleptic property, Reducing sugars, shelf life, Total acidity, Vitamin C.

1. INTRODUCTION

Papaya (*Carica papaya* L.) is one of the important tropical fruit of caricaceae family having good market demand as table fruit and also for its papain content. Besides, the fruit is rich in vitamin A and has mild laxative property. For successful marketing of the crop, the main drawback is, that it has very short shelf life. It was reported that papaya faced postharvest loss up to 75% by wholesaler and retailers in USA (Paull and Chen, 2014). As a tropical fruit, papaya fruit has various limitations in export due to insect and disease attack, chilling sensitivity during transportation and storage and rapid ripening on the market. Especially in domestic market, most papaya fruit have been sold under ambient temperature in which deterioration of the fruit is stimulated, due to increased ripening and the incidence of fungi pathogen attack.

The acceptability of fruits and vegetables depends largely on how they are packaged and presented; but quality is more than packaging or anything else. Success in marketing is equally important. It is made up of a great many characteristics; some external, internal, physical or mechanical. The first impression of quality judgment is appearance i.e. shape, size, colour, free from of blemish and dirt. This then calls for sorting and presentation. It also makes the difference in the price people are willing to pay for the good (Samson, 1986).

Papaya is one of the fruits that can be consumed locally or exported. It also contributed significantly to the economic development of the international markets. They also get papain which is used as meat tenderizers in industries and for other medical purposes (Foyer, 197. It contains about 88.8% of water. All the parts of the plant contain latex (Samson, 1986).

Maturity at harvest, some harvesting conditions and practices coupled with time of harvest are some important factors that determine the acceptability of the papaya fruit (Samson, 1986). Immature fruits, over ripe fruits and methods of harvesting all determine the post harvesting behaviors of the fruit. Many fruits are harvested with some length of the peduncle still attached to the fruit (Maxwell and Betty, 1984). Some of these fruits include the egg plant, squash, pumpkin, and okra. The above fruits mentioned may not be more susceptible to bruises as compared to the papaya fruit. The peduncle is the first point of detachment of the fruit from the mother plant. Depending on how and where it is cut may both have effect on the shelf life and

the ripening quality of the fruit. Again the peduncle is part of the papaya fruit in the package that occupy space. The peduncle could cause bruises on other fruits in the package on transit. This may cause quick ripening of the fruits and may shorten its shelf life and subsequently market value thereby increasing cost on export. The peduncle of the papaya can be cut at any length during harvest and could affect the postharvest characteristics quality ripening. Horticultural production is profitable. Farmers involved in horticultural production usually earn much higher farm income as compared to cereal producers and per capita farm income has been reported to be five times higher (Lumpkin *et al.*, 2005).

At the unripe stage, the fruit is consumed as a cooked vegetable in countries where papaya is widely grown (Mendoza, 2007, Mano *et al.*, 2009). In Thailand, unripe fruits are used as ingredients in papaya salads and cooked dishes (Sone *et al.*, 1998,). In Puerto Rico, unripe fruits are canned in sugar syrup and sold either in local markets or exported (Morton, 1987). Unripe fruits must be cooked prior to consumption to denature the papain in the latex (Odu *et al.*, 2006; Morton, 1987). The ripe fruit is consumed fresh for desert and in fruit salad or processed. It is highly accepted worldwide and the demand for fresh papaya fruit is increasing for its high content of vitamin C and pro vitamin A, which has protective effect against cancer, and its low-calorie status that is recommended for low hypo caloric diets (Lobo and Cano, 1998).

In addition, papaya fruit is a good source of papain and chymo papain. Both are digestive proteolytic .Papaya seeds are sometimes used to adulterate whole black pepper (Morton, 1987). The leaves of papaya contain papain, a strong proteolytic enzyme. Crushed leaves may be used to tenderizing enzyme that digest protein and used as meat tenderizers, as digestive medicine in

Pharmaceutical, brewing, and tanning industries, and in manufacture of chewing gum (Nakasone And Paul,1998). The pure is an important immediate product in the manufacture of several products such as beverages, ice cream, jam and jelly (Brekke *et al.*, 1972, Ahmed *et al.*, 2002).Powdered or dried papaya can be used as a meat tenderizer or as an ingredient in soup mixes (Singfield, 1998). Papaya pomace, skins, leaves and other by-products of papaya processing may find use in animal feed application (Babu *et al.*, 2003; Aloba, 2003, Ulloa, 2004). Papaya is considered to be a rich source of provitamin and ascorbic acid (Nakasone And Paul, 1998).

While the vitamin is generally associated with carotene, the yellow pigment in the papaya is not carotene but caricaxanthin composition and food value of ripe papaya fruit per 100g of edible flesh is given as 88% moisture, carbohydrates 10%, protein 0.5%, fat 0.1%, acid 0.1%, fibre 0.7%, and ash 0.6%. It also has a calorific value of 40 (Singfield, 1998).

Even though heat treatment is reported to control disease and maintain postharvest quality of fruits, increase in the metabolic activities of the treated fruits are apt to ultimately lead to quality deterioration. So, refrigeration becomes a requirement for prolonged storage of fruits. But ethylene concentration in fruits induces flesh softening which limits long term cold storage. Thus a better alternative to enhance the post harvest quality of fruits is treatment with acetylsalicylic acid (ASA). Salicylic acid is reported as a natural and safe phenolic compound which has been found to generate a wide range of metabolic and physiological responses in plants and act as potential bio agent in controlling post harvest loss of horticultural crops and delay in ripening through inhibition of ethylene biosynthesis (Asghari and Aghdam, 2010).

Acetyl salicylic acid undergoes hydrolysis to salicylic acid (SA) up on exogenous application. salicylic acid is a plant hormone inhibiting the biosynthesis of ethylene and thus delay senescence. The activities of major cell wall degrading enzymes such as cellulase, poly galacturonase and xylanase decreased in response to SA. The major enzymatic antioxidants, catalase and peroxidase also decrease in response to SA during fruit ripening. So, SA induced fruit ripening delay may be through such inhibition.

Post-harvest losses and quality deterioration of horticultural crops are mostly caused by pests, microbial infection, natural ripening processes and environmental conditions such as heat, and improper post-harvest handling (Idah *et al.*, 2007; Olayemi *et al.*, 2010). In Ethiopia, limited studies are found on postharvest management of horticultural crops despite huge potential for production of horticultural crops like papaya. The present study was undertaken with the aim of investigating the effect of acetyl salicylic acid in delaying the quality deterioration of papaya fruits after harvest .This study had in mind the numerous fruits venders selling papaya in the streets of east Hararghe town .to this end the study involved four ASA concentration for investigation some variables that determine postharvest quality of papaya.

General Objective:

- To study the effect of aspirin in reducing postharvest quality loss of papaya fruit.

Specific Objectives:

- To assess postharvest shelf life of papaya fruit;
- To evaluate post harvest quality of papaya fruit by use of ASA;
- To determine carotenoid (antioxidant) content of papaya fruit;
- To determine sugar content of papaya fruit.

2. LITERATURE REVIEW

2.1. Postharvest management of fruits and Vegetables

Plants continuously remain exposed to the challenging threats of a variety of pathogenic attacks. For many years synthetic fungicides were used to control post-harvest decay but, the public concerns about fungicide residues in fresh horticultural crops and the harmful effects of chemicals on human health and environment have recently caused scientists to search for new alternatives to chemical fungicides (Babalar *et al.*, 2007). Malamy *et al.* (1990) showed that large amount of SA accumulates in the leaves of tobacco mosaic virus (TMV) resistant tobacco variety *Nicotiana tabaccum cv. Xanthi* upon inoculation with TMV. Further, SA or acetyl salicylic acid (ASA), a synthetic analogue of SA, when applied exogenously induced the expression of PR (pathogenesis related) genes and also conferred resistance against various pathogens (Malamy and Klessig, 1992). Exogenous application of SA at nontoxic concentrations to susceptible fruits and vegetables could enhance resistance to pathogens and control post-harvest decay (Asghari *et al.*, 2009).

Salicylic acid (SA) and methyl salicylate (MeSA) are endogenous signal molecules, playing pivotal roles in regulating stress responses and plant developmental processes including heat production or thermo genesis, photosynthesis, stomatal conductance, transpiration, ion uptake and transport, disease resistance, seed germination, sex polarization, crop yield and glycolysis (Klessig and Malamy, 1994). Recently, SA has received a particular attention because it is a key signal molecule for expression of multiple modes of plant stress resistance. Although the focus has been mainly on the roles of SA on biotic stresses, several studies also support major roles of salicylates in modulation of the plant response to several abiotic stresses, such as UV light, drought, salinity, chilling stress and heat shock (Ding and Wang, 2003). Salicylates delay the ripening of fruits, probably through inhibition of ethylene biosynthesis or action, and maintain post-harvest quality (Srivastava and Dwivedi, 2000).

Methyl salicylate triggers disease resistance and mediates the expression of defense related genes in neighboring plants and in healthy tissue of infected plants. SA also effectively enhances the biocontrol efficacy of antagonist yeasts (Vlot *et al.*, 2009). Plants protect themselves against the pathogen attacks by activating some kinds of defense mechanisms such as local acquired

resistance (LAR) and systemic acquired resistance (SAR) (Vlot *et al.*, 2009). SA can induce the accumulation of hydrogen peroxide (H_2O_2) levels in plant tissues which acts as a signal activating the SAR (Tian *et al.*, 2007). Mutant and transgenic plants that are impaired in SA signaling are incapable of developing SAR and don't show PR gene activation upon pathogen infection, which indicates that SA is a necessary intermediate in the SAR signaling pathway (Durrant and Dong, 2004).

According to Zeng *et al.* (2006), in a study on mangoes, have found that the level of hydrogen peroxide (H_2O_2) and the rate of superoxide radical (O_2^-) generation in SA-treated fruits were higher than that in controls after 8 days of treatment. Thus, SA may also facilitate H_2O_2 accumulation during the oxidative burst induced by infection with the virulent pathogens. The increased resistance associated with the oxidative burst may contribute to resistance via several mechanisms, including directly killing the invading pathogen and/or activating cell wall cross linking and lignifications, thereby strengthening the cell wall and helping confine the pathogen to the infection site) (Vlot *et al.*, 2009).

2.2. The Role of Salicylic Acid in Delaying Postharvest Ripening of Fruits

The first indication for a physiological effect of SA was the discovery of flower-inducing action and bud formation in tobacco cell cultures (Eberhard *et al.*, 1989). The stimulatory effect of SA on flowering was latter demonstrated in other plant species and this was ground for suggesting that SA functions as an endogenous regulator of flowering (Cleland and Ajami, 1974). The SA effect was not specific and it promoted flowering in combination with other plant regulators (e.g. gibberellins). Besides flowering, SA also affected multiplication rate, anthocyanin and chlorophyll contents in *Spirodella polyrrhiza*. High concentration of SA (grater than 10^{-6} M) retarded the growth of fronds. SA treated fronds became gibbous with large air-chambers. Maximum gibbosity was observed in the 5×10^{-5} M SA-treated fronds of *Spirodela* (Khurana and Maheshiwari, 1980).

Fruit ripening and senescence are accompanied by changes in several quality aspects such as softening, decrease in total acidity and increase in sugar contents, color development, aroma production and etc. (Wills *et al.*, 1998). Softening of fruits is a main and critical quality change. MeSA, in a concentration dependent manner from 0 to 32 ml/L, maintained firmness of kiwifruit

during storage (Aghdam *et al.*, 2009). Srivastava and Dwivedi (2000) reported that in bananas treated with SA fruit softening markedly decreased. It has been demonstrated that SA decreases ethylene production and inhibits cell wall and membrane degrading enzymes such as polygalacturonase (PG), lipoxygenase (LOX), cellulase and pectinmethylesterase (PME) leading to decreasing the fruit softening rate (Srivastava and Dwivedi, 2000; Zhang *et al.*, 2003).

Total soluble sugars and soluble sugars may increase during fruit ripening due to the action of sucrose-phosphate synthase, a key enzyme in sucrose biosynthesis (Hubbard *et al.*, 1991). This enzyme is activated by ethylene and the ripening process itself during storage (Langenkamper *et al.*, 1998). Recently, an increase in sucrose-phosphate synthase and invertase activities and a decrease in sucrose synthase activity have been reported during ripening of some fruits (Cordenunsi and Lajolo, 1995). Treatment of kiwifruits with MeSA of 32 ml/L maintained a lower TSS content than the control fruits at the end of cold storage (Aghdam *et al.*, 2009). The authors proposed that MeSA reduced ethylene production may results to decreased sucrose phosphate synthase enzyme activity leading to decrease in sucrose synthesis.

The ripening of banana fruit is accompanied by increase in pulp to peel ratio. Rise in pulp to peel ratio during fruit ripening may be due to change in sugar concentration in the two tissues. A rapid increase in sugar contents in the pulp than those in the peel leads to a change in osmotic pressure, as a result of which water is withdrawn from the peel and hence pulp to peel ratio increases accordingly. According to the findings of Srivastava and Dwivedi (2000), SA treatment, in a concentration dependent manner, reduces this increase in pulp to peel ratio, leading to a delay in banana fruit ripening. The result showed that the invertase activity is concomitant with decrease in non reducing sugar content. The level of reducing sugars is increased and non reducing sugars is decreased during Beaudry ripening and senescence. This accumulation of reducing sugars may be due to increased breakdown of starch during ripening as reported by *et al.* (1989). Salicylic acid treatment resulted in decreased levels of invertase and reducing sugar contents while an opposite effect on non reducing sugar content, suggesting that SA delays banana fruit ripening. On the other hand, cell walls contain large amounts of polysaccharides, mainly pectins and cellulose, and are digested due to the activity of the cell wall degrading enzymes leading to a significant increase in TSS content. Then any factor preventing these enzymes will prevent from a dramatic increase in TSS content. Salicylic acid effectively

protects cell walls by decreasing the expression of degrading enzymes and as a consequence prevents from dramatic increase in TSS content of the cells.

Ethylene plays a key role in fruit ripening and senescence. This hormone triggers the induction of cell wall hydrolyzing enzymes leading to increase in respiration rate, fruit softening and senescence (Wills *et al.*, 1998). Salicylic acid effectively decreases ethylene production in several horticultural crops. It has been shown that Me SA treatment significantly decreases ethylene production in kiwifruits (Aghdam *et al.*, 2009). Both SA and ASA have been shown to inhibit ethylene production in cultured pear cells, mung bean hypocotyls, apple and pear fruit tissue discs, carrot cell suspension cultures and some fruits (Babalar *et al.*, 2007). Srivastava and Dwivedi (2000) reported that SA has delayed the ripening of banana fruit, probably through inhibition of ethylene biosynthesis action. Zhang *et al.*, (2003) reported that postharvest treatment of kiwifruit with ASA resulted in decreased ethylene production during the early stages of fruit ripening

There is evidence for a positive correlation between cell wall and membrane degrading enzymes, free radicals production and ethylene biosynthesis in fruit tissue (Marcelle, 1991). SA inhibited wound induced transcription, and decreased cell wall degrading enzyme activity in disks of kiwifruit leading to the consequent reduction in the production of free radicals and ethylene biosynthesis (Zhang *et al.*, 2003). Ding and Wang (2003) showed that ripening process in mature green tomatoes was enhanced by 0.1 mmol/L MeSA and by 0.01 mmol/L during breaker stage. But in fruit at turning stage even 0.01 mmol/L SA inhibited the ripening process. 0.5 mmol/L SA prevented red color development and increased ethylene production and respiration rate in all maturity stages.

2.3. Postharvest Ripening of papaya fruits

During their growth, papaya steadily accumulates starch. First, they elongate then increase in width. The increase in width continues as long as the fruit are not harvested so that they become rounded or oval (Tucker and Grierson, 1987). Papaya is picked green and ripens under controlled, temperature, relative humidity and ventilation to prevent accumulation of carbon dioxide(which blackens the peel). Normal ripening will occur at 5⁰C or higher concentration of oxygen. (McGrath and Karahadian, 1994). Ripening of papaya is represented by a sequence of

changes in the colour of the peels from green to yellow, as defined by a colorimetric scale from 1 to 7 (Anon., 1980) and the texture and flavour of the pulp. These developments are linked to changes in metabolism and biochemical composition. Of the latter, the transformation of starch into sugar, and the evolution of aroma are the most noticeable.

Numerous enzyme systems, which has not been all elucidated, controlled the co-ordination of the reactions involved. Biales (1960), reported that, fruits detached from the branch with the peduncle attached ripened later than when it was removed, the peduncle and stem supply a ripening inhibitor to the fruit or the stem may act as a sink for ripening hormone produced in the fruit. The presence of 6 cm long peduncle on avocado delayed onset of the climacteric by 2 days and also caused reduced ethylene production from all parts of the fruit (Tingwa and Young, 1975).

2.4. Some chemical and physical changes that takes place during quality ripening

2.4.1. Conversion of starch into sugar

There are two main types of carbohydrates in papaya fruit, cell wall polysaccharides and soluble sugars. Best quality fruit is determined largely by sugar content (Storey, 1972). During early stage of fruit development, glucose is the main sugar. The sucrose content increases during the ripening process (Hulme, 1971). The most striking post-harvest chemical change which occur during the post-harvest ripening of papaya is the hydrolysis of starch and the accumulation of sugar (i.e. sucrose, glucose and fructose; Loesecke (1950), Palmer (1971) which are responsible for the sweetening of the fruit as it ripens. In papaya the breakdown of starch and the synthesis of sugar are usually completed at full ripeness and continue in over-ripe and senescent fruit (Marriott *et al.*, 1981). During ripening, the sucrose content was shown to increase from 13.9+₋5.0mg/g fresh weight in green fruits to 29.8+₋4.0mg/g fresh weight in ripe fruits (Gomez *et al.*, 2002). Chan *et al.*, 1970 also confirms that sucrose is the main sugar of *Carica papaya* (80% of total soluble solids in full ripened fruit)

2.4.2. Firmness

Assessment of firmness is important in the evaluation of fruit susceptibility to physical and mechanical damage which can adversely affect the ripening quality of the fruit (Kramer, 1964). Under normal storage conditions, papaya undergoes significant textural transformation as they

pass through the ripening process. The crisp, hard and green fruit turns into yellow fruit with tender and soft internal pulp at optimum ripening stage, and becomes mushy as it advances toward senescence. Loss of pulp firmness during ripening varies with cultivars. Pulp firmness is often inversely related to ripening; implying that, as ripening progresses, pulp firmness declines (Smith *et al.*, 1989). Loss of pulp firmness or softening during ripening has been associated with two or three processes. The first is the breakdown of starch to form sugar. The second is the breakdown of the cell wall or reduction in the middle lamella cohesion due to solubilization of pectin substances (Smith *et al.*, 1989). The third is the movement of water from the peel to the pulp during ripening due to osmosis.

2.4.3. Change in total soluble solids (TSS) content

During ripening in papaya, the total soluble solids increase (Chan *et al.*, 1979). In ripe papaya, sugar forms the main component of the total soluble solids (Gomez *et al.*, 1979) since the amount of sugar in fruits usually increases as the fruit matures and ripens, the soluble solid content of the fruit can be a useful index of stage and quality of ripeness. To attain maximum total soluble solids in solo type papaya the yellow colour must cover 6% of the fruit surface skin (Akamine and Goo, 1971).

2.4.4. Change in Total Titratable Acidity (TTA)

Usually organic acids decline during ripening as they are respired or converted to sugar (Wills *et al.*, 1989). Organic acid are important in giving a desired sugar-to- acid balance which result in pleasing fruit taste during ripening. Acidity measured as titratable acidity, in the pulp tissues of papaya shows large increases during ripening or as ripening progresses (Akamine and Goo, 1971). Therefore titratable acidity could be used as an index of quality ripening during fruit ripening, titratable acidity was reported to increase up to the climacteric peak and declined afterward in papaya (Selvarag *et al.*, 1982).

2.4.5. Change in pH

Pulp pH is an important post-harvest quality attribute in the assessment of fruit ripening quality. Pulp pH rapidly declines in response to increasing ripeness (Chan *et al.*, 1979). Generally when fruits are harvested at matured green stage, the pulp pH is high but as ripening progresses pH drops (Chan *et al.*, 1979). Thus the pulp pH could be used as an index of quality ripening.

2.4.6. Change in Peel and Pulp Color

The color of papaya contributes more to the assessment of quality by the consumer than any other single factor. Therefore peel and pulp color of papaya is important post-harvest selection criteria. The color of the fruit could give an indication of the state of deterioration, disease incidence and/or contamination. The peel color is often the major post-harvest criterion used by Researchers, growers and consumers to determine whether the fruit is ripe or unripe (Medlicott *et al.*, 1992). Colour is critical as the first assessment of the quality of papaya fruit. Consumers associate the colour of the peel with specific taste or uses and they will usually buy if the colour is suited to the required purpose or desire. In some countries like Ghana, if the pulp colour of papaya is white, consumers feel that, the fruit is immature and if the pulp colour is orange or light yellow it indicates that the fruit is matured. Therefore, assessment of peel and pulp colour is important in the post-harvest ripening quality determination. Colour charts or colour measuring instruments are used for this purpose (Knee, 1980).

2.4.7. Shelf Life

Shelf life is simply the time period that a fruit can be expected to maintain a predetermined level of quality under specified storage conditions. Shelf life begins immediately the green-life of the fruit ends (Aked, J. 2000). Shelf life of fruit is dependent on textural firmness which is due to cell wall modification resulting in structural changes in starch and non-starch polysaccharides (Yashoda *et al.*, 2006). Shelf life is directly proportional to firmness. This means that as firmness reduces shelf life also reduces. Again, fruit softening rate is a character that determines fruit shelf life and thought to be the result of cell wall degradation (Brummell and Harpster, 2001).

3. MATERIALS AND METHODS

3.1. Study Area

The experiment was conducted in Biotechnology Laboratory, Haramaya University which is found at about 5010 km East of Addis Ababa. The University is located at 9°24'N latitude and 42° 01' E longitude with an elevation of 2047 m.a.s.l. The area is characterized by a sub-humid type of climate with an average annual rainfall of about 790 mm, annual mean minimum and maximum temperatures of 20°C and 25.5°C, respectively.

3.2. Experimental Materials

Papaya fruit sample was collected from farm land in Hirna district and sample of papaya collect from 20 trees West Hararghe, Ethiopia. The mature green fruits were collected and immediately brought in a polystyrene bag to Biotechnology Laboratory of the University. Fruits were left for 22 hrs (after harvest) at room temperature in order to stabilize ethylene.

3.3. Experimental Design and Treatments

The experimental design was completely randomized design in two replications Of the collected papaya fruit sample, 20 fruits of uniform size with no bruises or damage were selected. Fruit sample was surface sterilized with sodium hypochlorite solution (500 ppm) for 10 minutes so as to reduce fungal infection and air dried for approximately 15 minutes. After surface sterilization, the fruit sample was immersed for five minutes in solutions of 0mM, 1mM, 2mM, and 4mM aspirin solution for five papaya in each treatments and was air dried at room temperature for 1h following the method used by Dibbisa *et al.* (2016) and Duguma *et al.* (2016). The surface dried fruit samples were individually packaged in perforated plastic bags to maintain relative humidity. Each bag was packaged in a 1L disposable container. All packages were sealed and stored for 10 days followed by 2 days shelf life at 20 °C. The treated groups were evaluated in each treatment at 1, 10 and 15 days of treatment. Thereafter, fruits were assessed for different quality parameters such as physiological weight loss, vitamin C (ascorbic acid) content, titratable acidity, total carotenoids, total chlorophylls, reducing sugars at 1, 10 and 15 days.

3.4. Data Collection and Analysis

3.4.1. Physiological Loss of Weight

Weight loss was determined by using method indicated by Akbudak, (2007) periodically (on 1st, 10th, and 15 days of storage) by weighing papaya fruit samples in each treatment using digital balance (Denver Instrument XL-1810). Percentage weight loss was calculated using the following formula:

$$\% \text{WL} = \frac{W_i - W_f}{W_i} \times 100$$

Where: WL=Weight loss; W_i = Initial weight; W_f = Final weight

3.4.2. Ascorbic Acid Analysis

The ascorbic acid content was determined by the 2, 6- dichlorophenol indophenol (DCPIP) dye method (AOAC, 2000). 5 ml of the standard ascorbic acid solution was pipetted into a 100 ml conical flask and 5ml of the 3% HPO_3 solution was added. The ascorbic acid solution was titrated with the dye solution to a pink colour, which should persist for 15sec. The titre value was recorded. The dye factor was calculated by dividing 5ml volume of ascorbic acid solution taken for titration by titrant volume of dye solution. Dye factor was expressed as mg of ascorbic acid per ml of the dye. Since 5ml of the standard ascorbic acid solution contains 0.5 mg ascorbic acid:

$$\text{Dye factor (mg ascorbic acid per dye)} = \frac{0.5\text{mg}}{\text{titrant volume}}$$

An aliquot of 5ml papaya fruit juice extract was diluted to 50 ml with 3% meta phosphoric acid in a 50 ml volumetric flask. The aliquot was then centrifuged (Model, Z300, 580W, 3052 Nm, German) for 15 minutes and titrated with the standard dye to a pink end point (persisting for 15 seconds). The ascorbic acid content was calculated from the titration value, dye factor, dilution and volume of the sample as:

$$\% \text{A.A} = \frac{(\text{ABRs} - \text{ABRb}) \times \text{dye factor} \times \text{volume of initial test solution}}{\text{volume of test solution titrated}} \times 100$$

Where: A.A=Ascorbic Acid; ABR= Average Burett

3.4.3. Measurement of Total Acidity

The total acidity was determined by a standard titrimetric method. For the determination of total acidity, 5 grams of extracted papaya fruit juice was mixed with 100ml of distilled water. In the presence of phenolphthalein as an indicator, the mixture was titrated by adding 0.1 N NaOH until the break of light pink color (pH 8.2) observed for 15 seconds. The volume of NaOH added to the solution was multiplied by the correction factor of 0.064 for the calculation of titratable acidity as %age of citric acid. Titratable acidity was expressed as % age of citric acid (or total acidity) (AOAC, 2000).

$$\% \text{ acid} = \frac{\text{titrant volume} \times 0.1 \text{N NaOH} \times \text{acid factor} \times \text{titration volume}}{\text{weight of the sample}} \times 100$$

3.4.4. Determination of Chlorophylls and Total Carotenoids

Chlorophylls and carotenoid contents were determined using spectrophotometric method following procedure of Nagata (1992) for the simultaneous determination of chlorophylls and total carotenoids in papaya fruit. 16ml of acetone–hexane (4:6) solvent was added to 1g of papaya fruit juice homogenates. For this, the homogenous sample was prepared by Kenwood blender, (Model, BC311 P.R.C. China). The homogenate was centrifuged at 5000 rpm using centrifuge (Model, Z300, 580W, 3052 Nm, German) for 10 minutes at 20°C. Then after absorbance was measured at 663, 645 and 470nm in a Jenway model 6100 spectrophotometer. Total chlorophyll and carotenoid contents were calculated according to the equations indicated below, and the final results was expressed as µg/g for chlorophylls and mg/g for antioxidants.

$$\text{Chlorophyll a } (\mu\text{g/g}) = 0.999A_{663} - 0.989A_{645}$$

$$\text{Chlorophyll b } (\mu\text{g/g}) = 1.77A_{645} - 0.328A_{663}$$

$$\text{Total Chlorophyll (a+b)} (\mu\text{g/g}) = \text{chla} + \text{chlb}$$

$$\text{TC (mg/g)} = \frac{1000A_{470} - 2.27(\text{chla}) - 81.4(\text{chlb})}{227}$$

Where: A = absorbance; TC=Total carotenoids

3.4.5. Sugar Analysis

3.4.5.1. Determination of standard glucose concentration

Sugar analysis was conducted by spectrophotometric Nelson-Somogyi method using glucose a standard. The standard stock glucose solution was prepared by dissolving 100mg glucose in 100ml distilled water from which the working standard was prepared by taking 10 ml of stock solution and diluting it to 100ml with distilled water. Thus, the concentration of the standard solution was 0.1 mg/ml. The glucose standard curve was prepared using modified procedure of Gros *et al.* (2010) as follow: the blank solution (as a control) was prepared by taking 2 ml of distilled water. The standard glucose concentration was prepared by making five different known concentrations of standard glucose solution (0.4ml, 0.6 ml, 0.8 ml, 1.0 ml, 1.2 ml) in the first five test tubes (Appendix Table 1). The volume was made to 2 ml with distilled water in each tube so that it was same volume with that of blank solution. Then 1ml of alkaline copper reagent was added and mixed well until color develops. The solution was heated over a water bath with a temperature of 100 °C for 20 minutes, and cooled to 25 °C, then the solution was stirred by adding 1ml sodium acetate reagent and allowed to stand for 25 minutes. Absorbance was read with a visible spectrophotometer at maximum wavelength of 540nm. Then, glucose standard graph (Appendix figure 1) showing the relationship between concentration and absorbance was drawn.

3.4.5.1. Determination of sugar in a papaya fruit juice sample solution

Total reducing sugar was estimated by using the technique used by Gros *et al* (2010) with some modifications. For extraction of total reducing sugars from the papaya fruit juice sample, 5g of homogenized juice sample was dissolved in 15ml of 80% ethanol, then mixed and heated in boiling water bath for sufficient time until the ethanol odor went off. Then, the solution was filtrated by adding 1ml of saturated Pb (CH₃COO)₂ and 1.5ml of NaHPO₄ and the content was mixed by gentle shaking on Vortex Shaker. After filtration, the extract was made to 1: 10 dilution with distilled water. From this solution 0.8ml and 1.2ml (Table 3.1) of sample solution was taken and made up to 2ml with distilled water in labeled test tubes. Thereafter 1ml of copper reagent was added to both solutions and heated for 20 minutes in a boiling water bath. After heating, the contents were cooled under running tap water without shaking. Then, 1ml of sodium acetate was added (as a color reagent), mixed well, and left for about 10 minutes to allow color

development. Then after, the absorbance was read using spectrophotometer at 540 nm. Finally, the content of sugars in the unknown and standard solution (in mg/ml and mg %) was estimated by using the standard curve. The calculation of the concentration on the sample was done using the linear regression equation of standard solution obtained $y = ax + b$ where Y is the absorbance of the measured sample solution, b is the y-intercept and X is the concentration of the sample solution (mg/ml):

$$X = \frac{Y-b}{a}$$

$$\text{Conc of sugar in sample soln} = \frac{\text{OD of test sample} - \text{OD of blank}}{\text{OD of std} - \text{OD of blank}} \times \text{conc of std}$$

$$\text{Mg of reducing sugar} = \frac{\text{OD of test sample} - \text{OD of blank}}{\text{OD of std} - \text{OD of blank}} * \text{conc of std glucose}$$

$$\text{Dilution factor} = \frac{\text{final dilution volume}}{\text{original volume of substance being diluted (aliquote vol)}}$$

$$\% \text{ reducing sugar} = \frac{\text{mg of reducing sugar}}{\text{mg of original sample}} \times 100$$

$$\text{mg std glucose} = \text{concentration of working std solution} \times \text{volume of standard solution}$$

3.5. Data Analysis

Data were subjected to ANOVA (Analysis of Variance) and mean separation based on DMRT (Duncan's Multiple Range Test) using SAS 9.1.2 statistical software. All significance tests were made at ($P \leq 0.01$ and/or 0.05) levels.

4. RESULT AND DISCUSSION

4.1. Weight loss and Vitamin C content

Mean comparison based on Duncan's Multiple Range Test (DMRT) for weight loss and vitamin C content was shown in Table 1. It was observed that there was no significance difference in weight loss during postharvest ripening of papaya fruit as treated by acetylsalicylic acid. For vitamin C content, there was also no significance difference among acetyl salicylic acid (ASA) treated and control (0mM) groups in freshly harvested fruits (test at 0 day). However, significance differences were observed after 10th days of postharvest treatment of papaya fruit. There was also significance difference among control and acetylsalicylic acid treated groups in during 15th days of treatment. It was also observed from mean values in Table 1 that vitamin C content was decreasing during postharvest ripening of papaya fruit. The highest mean vitamin C content (for ripened fruit) was observed for 4mM ASA treated group indicating that ASA treatment can delay ripening (increase shelf life) of papaya fruit.

Table 1: Percentage weight loss and vitamin C (ascorbic acid) content during post harvest ripening of papaya fruit as treated by different concentrations of acetyl salicylic acid (ASA)

Treatment	Average Weight loss	Change in composition of Vitamin C during ripening		
		0 day	10days	15 days
0Mm	2.88a	6.30a	5.54a	2.50b
1mM	2.76a	5.99a	4.03c	3.60ab
2mM	2.00a	6.20a	4.79b	3.80a
4mM	1.98a	6.85a	3.15d	2.68a
Overall mean	2.41±0.52	6.34±0.47	4.38±0.97	3.65±0.90

Means with the same letter within a column are not significantly different.

4.2. Total chlorophyll

Mean comparison based on Duncan's Multiple Range Test (DMRT) for total chlorophyll content was shown in Table 2. It was observed that there was no significance difference in total chlorophylls contents between control and ASA treated groups in freshly harvested (0 day treatment) papaya fruit. However, significance differences were observed for both parameters chlorophylls after 10th days of treatment chlorophyll content was decreasing during post harvest ripening.

Table 2: Mean comparison for Chlorophyll content during post harvest ripening of papaya fruit as treated by different concentrations of acetyl salicylic acid (ASA)

Treatment	Composition of Chlorophyll		
	0 day	10days	15 days
0mM	0.64a	0.34c	0.21b
1mM	0.64a	0.40bc	0.25ab
2mM	0.63a	0.47ab	0.30a
4mM	0.63a	0.50a	0.31a
Overall mean	0.64±0.01	0.43±0.07	0.27±0.05

Means with the same letter within a column are not significantly different.

4.3. Total carotenoids

Mean comparison based on Duncan's Multiple Range Test (DMRT) for total carotenoid content was shown in Table 3. It was observed that there was no significance difference in total carotenoid contents between control and ASA treated groups in freshly harvested (0 day treatment) papaya fruit. However, significance differences were observed for parameters carotenoids after 10th days of treatment. It was also observed from mean values in Table 3 that carotenoid content of papaya fruit was increasing while content was decreasing during post harvest ripening. The highest mean carotenoid content was observed for control group showing that treatment with ASA solution can delay ripening of papaya fruit. Finally, when fruit extremely ripened carotenoid content was decreasing showing deterioration in fruit quality at 15 days.

Table 3: Mean compares carotenoid content during post harvest ripening of papaya fruit as treated by different concentrations of acetyl salicylic acid (ASA)

Treatment	Composition of carotenoid		
	0 day	10days	15 days
0mM	0.16a	2.14a	1.74a
1mM	0.17a	2.01a	1.63a
2mM	0.17a	1.83b	1.64a
4mM	0.16a	1.55c	1.16b
Overall mean	0.17±0.01	1.88±0.24	1.54±0.24

Means with the same letter within a column are not significantly different.

4.4. Total acidity

Mean comparison based on Duncan's Multiple Range Test (DMRT) for total acidity was shown in Table 4. It was observed that there was no significance difference in total acidity (predominantly malic acid) between control and ASA treated groups in zero day treatment of papaya fruit. However, significance differences between control and ASA treated groups were observed in fully ripened fruits. It was also observed from mean values in Table 5 that total titratable acidity was decreasing during postharvest ripening of papaya fruit. The highest mean total acidity was observed for treated groups showing that treatment with ASA solution slowing reduction of total acidity in papaya fruit. This indicates that ASA treatment can delay postharvest ripening (increase shelf life) of papaya fruit.

Table 4: Mean comparison for total acidity during post harvest ripening of papaya fruit as treated by different concentrations of acetyl salicylic acid (ASA)

Treatment	Composition of total acidity during ripening		
	0 day	10days	15 days
0mM	39.20a	47.24b	34.51a
1mM	38.19a	53.94a	31.16b
2mM	39.53a	34.17c	26.47c
4mM	38.53a	26.13d	25.80c
Overall mean	38.86±1.2	40.37±11.6	29.48±3.8

Means with the same letter within a column are not significantly different.

4.5. Total Reducing Sugars

Mean comparison based on Duncan's Multiple Range Test (DMRT) for total reducing sugars was shown in Table 5. It was observed that there was no significance difference in total reducing

Sugars between control and ASA treated groups in zero day treatment of papaya fruit. However, significance differences in sugar content between control and ASA treated groups were observed in fully ripened (after 10th days of treatment) fruits. It was also observed from mean values in Table 5. that total reducing sugars was increasing during postharvest ripening of papaya fruit. The highest mean reducing sugar was observed for control group showing that treatment with ASA solution reducing total reducing sugar in papaya fruit. The low sugar content in ASA treated group showing that ASA delays postharvest ripening (increases shelf life) of papaya fruit,

Table 5: Percentage reducing sugar during post harvest ripening of papaya fruit as treated by different concentrations of acetyl salicylic acid (ASA)

Treatment	Composition of total reducing sugar during ripening		
	0 day	10 days	15 days
0mM	0.50a	14.46a	6.39a
1mM	0.53a	11.53b	4.03bc
2mM	0.51a	8.23c	4.48b
4Mm	0.50a	6.66d	3.45c
Overall mean	0.51±0.02	10.22±3.2	4.59±1.2

Means with the same letter within a column are not significantly different.

The principal component analysis (PCA) for six fruit quality parameters measured during post harvest ripening of papaya fruit was indicated in Table 6. The first principal component retained information contained in 4.55 of the original variables while the second component retained only 1.22 of the original variable. Only the first component (PC1) having Eigen value greater than one, accounting for 76% of the variable suggesting that this PCA might be sufficient to explain the relationship between variables in the original data. The PCA in the present study was shown that the first component has got high negative loading from total reducing sugars and carotenoid (antioxidant content) while high positive component loadings were obtained for total

chlorophyll, vitamin C, total acidity and weight loss. Factors that load high positive or negative loading have opposite effects as suggested by Ajay *et al* (2012).

Table 6: Principal component analysis of fruit quality parameters measured for papaya fruit

Parameter	PC1	PC2
Eigen value	4.55	1.22
Proportion	0.76	0.20
Cumulative	0.76	0.96
Weight loss	0.43	0.28
Vitamin C	0.44	-0.30
Chlorophyll	0.46	-0.17
Carotenoid	-0.46	-0.14
Total acidity	0.13	0.86
Total reducing sugar	-0.44	0.19

Based on the plot for PC2 vs PC1 for D statistics (Fig.1), reducing sugars and carotenoid contents having close PC1 and PC2 scores (with vector angle $<90^0$) showing similar/correlated effects while reducing sugars and/or carotenoids with chlorophyll content, vitamin C, weight loss, and total acidity contents have vector angle greater than 90^0 showing opposite effects or more divergence. Furthermore, total acidity, vitamin C, chlorophyll content and weight loss have similar effect since their vector angle $<90^0$. That is reducing sugars and carotenoid contents increase while chlorophyll, total acidity, vitamin C and weight loss decrease during post harvest ripening of papaya fruit.

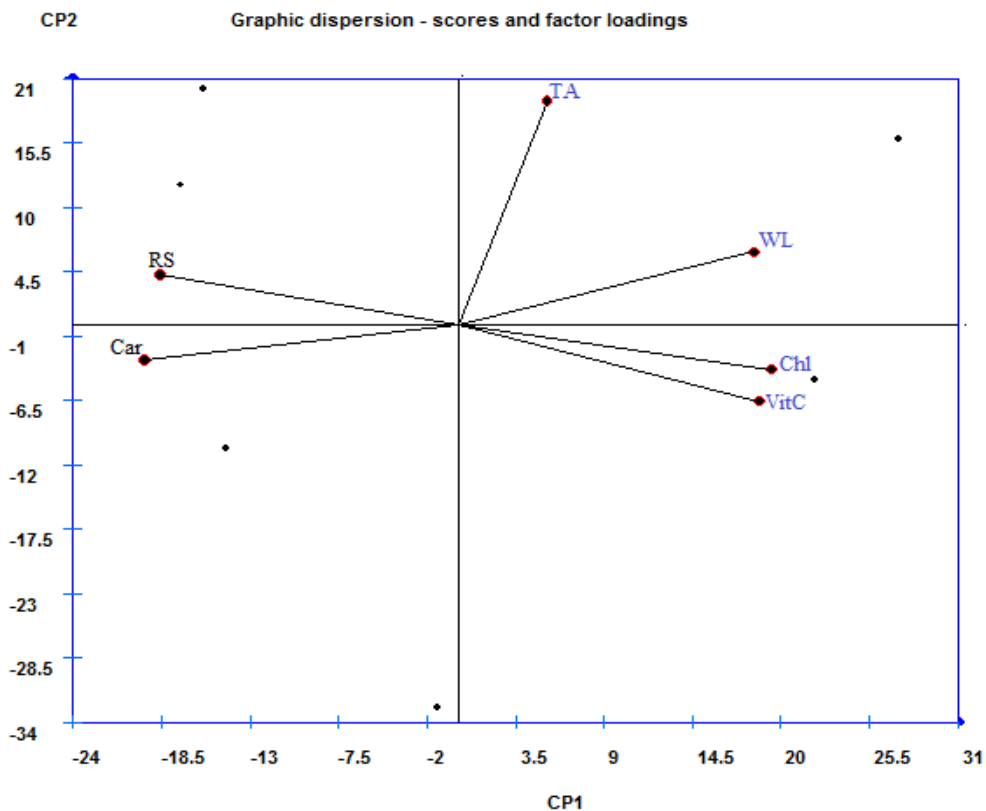


Figure 1. PCA scores for six fruit quality parameters measured for papaya fruit. RS: total reducing sugars; Car: carotenoid content; TA: total titratable acidity; VitC: vitamin C content; WL: weight loss; and Chl: total chlorophyll content.

5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1. Summary and Conclusions

ANOVA for papaya fruit quality parameters have shown significance differences most of the studied fruit quality parameters including vitamin C content, Total acidity, chlorophyll, carotenoid (antioxidant) and reducing sugars. However, ANOVA was not significant for weight loss. The PCA has shown the relationship between organoleptic property of papaya fruit the reducing sugars and carotenoid having similar/correlated effects while reducing sugars and/or carotenoids with chlorophyll content, vitamin C, total acidity and weight loss have shown opposite effects. On the other hand total acidity, vitamin C, chlorophyll content and weight loss have similar effect since their vector angle $<90^0$. From the PCA it was shown that reducing sugars and carotenoid contents increase while chlorophyll, vitamin C, total acidity and weight loss decrease during post harvest ripening of papaya fruit.

5.2. Recommendations

- The present study has generated quantitative data on major papaya fruit quality parameters including weight loss, vitamin C content, total chlorophyll and carotenoids, total acidity and reducing sugars. However, due to resource limitation fruit sample was taken from one location. Further studies are required by considering fruits grown in diverse environments;
- The farmer can be used acetylic salicylic acid for delaying post harvest papaya fruit ;
- There may also differences among cultivars in organoleptic properties. Studies are also required to evaluate genetic variations in organoleptic properties of papaya fruit;
- There are various methods of determination of organoleptic properties of fruits. Thus, reliable result should have to be obtained among the different methods of determination of carotenoid content, sugar analysis and vitamin C content;
- The farmer cultivate of post harvest papaya fruit around their locality and supply for market without deterioration ;

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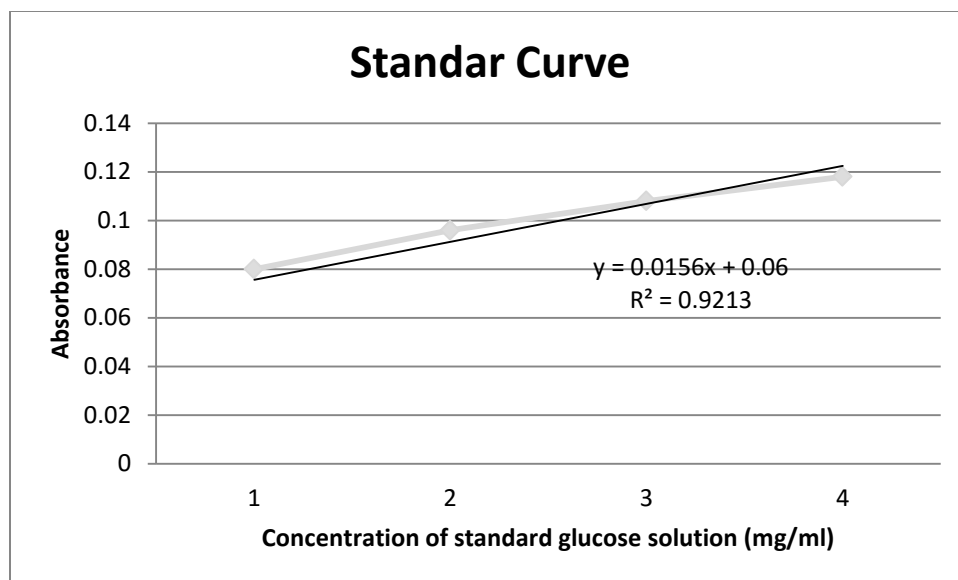
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7. APPENDICES

Appendix Table 1. Determination of sugar in standard solution

Vol of std	Distilled water	Conc of std	Alkaline	Sodium acetate	Optical
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slon (ml)	(ml)	glucose Soln	copper reagent (ml)	Soln (ml) or (NaAc)	density at 540nm
0.00	2.00	----	1	1	0.078
0.4	1.6 (2-0.4)	0.02	1	1	0.08
0.6	1.4 (2-0.6)	0.03	1	1	0.096
0.8	1.2	0.04	1	1	0.108
1.0	1.0	0.05	1	1	0.118
Vol of sample soln	Distilled water (ml)	Amount of glucose in juice sample(mg)	Alkaline copper reagent (ml)	Sodium acetate Soln (ml)	Optical density at 540nm
0.8	1.2 (2-0.8)	----	1	1	
1.2	0.8	----	1	1	



Appendix figure 1. Standard graph for glucose solution