UNIVERSITI PUTRA MALAYSIA

CHANGES IN SELECTED QUALITY CHARACTERISTICS, POLYPHENOLOXIDASE ACTIVITY AND PROTEIN PROFILE IN RELATION TO ASCORBIC ACID TREATMENT OF MINIMALLY PROCESSED CARAMBOLA (AVERRHOA CARAMBOLA L.)

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Thesis Submitted in Fulfillment of the Requirement for the Degree of Master Science in the Faculty of Agriculture Universiti Putra Malaysia

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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Chairperson: Siti Hajar Ahmad, Ph.D.

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A study on the changes in selected quality characteristics, polyphenoloxidase activity (PPO) and protein profile in relation to ascorbic acid treatment of minimally processed carambola was conducted. 'B10' carambola at maturity stages 3 and 4 were packed in boxes and precooled at 10°C in a cold room. After 24 hrs the fruits were washed, the edges trimmed-off, sliced transversely into 1-cm-thick slices and dipped in 0, 15 and 30 $mg.L^{-1}$ of 4°C ascorbic acid solution for 2 min. The slices were dried using tissue paper, placed on TP2 polystyrene foam tray, cling-wrapped and stored at 7°C for 0, 3 and 5 days. The experiment was conducted using a randomized complete block design (RCBD) with a factorial arrangement of treatments (3 ascorbic acid x 2 maturity index x 3 storage days) and four replications. Data from measurements of the skin colour (L*, C* and h°), flesh firmness, soluble solids concentration, vitamin C content, pH, sensory evaluation, browning degree and PPO activity were analyzed using analysis of variance (ANOVA) and differences within each factor were determined by Least Significant

Difference (LSD). Ascorbic acid treatment did not affect all the selected quality characteristics of minimally processed carambola i.e. skin colour (L*, C* and h°), flesh firmness, soluble solids concentration, vitamin C content and pH. Skin colour (C* and h°) and pH of minimally processed carambola were affected significantly by maturity index of fruit, while storage day affected skin colour (C* and h°), flesh firmness and vitamin C of minimally processed carambola significantly. The flesh firmness of minimally processed carambola was affected by the interaction between ascorbic acid x storage day. In the sensory evaluation, flesh colour, sweetness, flavour and overall taste were significantly affected by ascorbic acid treatment especially at 15 $mg.L^{-1}$. Sensory panelists preferred the colour of fruit maturity index of 4 compared to 3. However the flavour of minimally processed carambola showed opposite result. As storage day progressed, the sensory evaluation of minimally processed carambola decreased. Sensory evaluation was also affected significantly by interaction between ascorbic acid x maturity index. Browning degree of minimally processed carambola was affected significantly by ascorbic acid and storage day, but not by maturity index. The PPO activity increased significantly as storage day progressed. The electrophoretic pattern of minimally processed carambola revealed that proteolysis and tissue breakdown occurred at storage day 5. All the five sensory attributes (flesh colour, sweetness, texture, flavour and overall taste) positively correlated to each other but negatively correlated with browning degree. PPO activity positively correlated with browning degree ($r = 0.61$).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PERUBAHAN CIRI-CIRI KUALITI YANG TERPILIH, AKTIVITI POLIFENOLOKSIDASE DAN PROFIL PROTEIN KE ATAS HIRISAN CARAMBOLA (*AVERRHOA CARAMBOLA* L.) YANG DIRAWAT DENGAN ASID ASKORBIK

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Suatu kajian tentang ciri-ciri kualiti yang terpilih, aktiviti polifenoloksidase (PPO) and profil protein ke atas hirisan carambola yang dirawat dengan asid askorbik telah dijalankan. Buah carambola pada peringkat kematangan 3 dan 4 diprasejukkan terlebih dahulu di dalam 10°C bilik sejuk. Selepas 24 jam, buah dibasuh, pinggir buah dibuang, dihiris rentas untuk memperolehi hirisan setebal 1 cm dan direndam dalam 0, 15 dan 30 mg.L^{-1} 4°C larutan asid askorbik selama 2 minit. Hirisan itu dikeringkan dengan kertas tisu, 5 hirisan diatur pada dulang polistirena TP2, dibalut ketat dan disimpan pada suhu 7°C selama 5 hari. Ujikaji ini dijalankan dengan menggunakan rekabentuk blok rawak lengkap (RCBD) di mana rawatannya disusun secara faktorial (3 asid askorbik x 2 peringkat kematangan x 3 hari penyimpanan) dan mengandungi 4 replikasi. Data yang diperolehi dari warna kulit buah (L*, C* dan h°), kekerasan isi, kandungan jumlah pepejal terlarut, kandungan vitamin C, pH, penilaian sensori, darjah keperangan dan aktiviti PPO dianalisa dengan menggunakan kaedah analisis varian (ANOVA) dan perbezaan antara setiap

faktor ditentukan dengan menggunakan kaedah *least significant difference* (LSD). Rawatan asid askorbik tidak mempengaruhi kesemua ciri-ciri kualiti terpilih hirisan carambola iaitu warna kulit (L*, C* dan h°), kekerasan isi, kandungan jumlah pepejal terlarut, kandungan vitamin C dan pH. Warna kulit (C* dan h°) dan pH hirisan carambola dipengaruhi dengan berkesannya oleh peringkat kematangan buah. Sementara hari penyimpanan mempengaruhi warna kulit (C* dan h°), kekerasan isi dan kandungan vitamin C hirisan carambola dengan berkesannya. Kekerasan isi hirisan carambola juga dipengaruhi oleh interaksi di antara asid askorbik dan hari penyimpanan. Di dalam penilaian sensori, warna isi, kemanisan, keharuman dan rasa keseluruahan hirisan carambola telah dipengaurhi oleh rawatan asid askorbik, khasnya pada 15 $mg.L^{-1}$. Panel sensori lebih suka warna isi buah pada peringkat kematangan 4 daripada 3. Tetapi keharuman buah menunjukkan keputusan yang sebaliknya. Penilaian sensori juga dipengaruhi dengan berkesannya oleh interaksi antara aisd askorbik x peringkat kematangan. Darjah keperangan hirisan carambola dipengaruhi secara berkesan oleh asid askorbik dan hari penyimpanan, tetapi tidak dipengarubi oleh peringkat kematangan. Aktiviti PPO bertambah secara berkesan mengikut kelanjutan hari penyimpanan. Dari corak elektroforetik hirisan carambola didapati proteolisis dan keruntuhan tisu telah berlaku pada hari penyimpanan ke-5. Kelima-lima sifat sensori (warna isi, kemanisan, tekstur, keharuman dan rasa keseluruahn) berkorelasi postif antara sesama sendiri tetapi berkorelasi negatif dengan darjah keperangan. Manakala aktiviti PPO berkorelasi positif dengan darjah keperangan ($r = 0.61$).

CHAPTER I

INTRODUCTION

The carambola or starfruit (*Averrhoa carambola* L.) which is native to South-East Asia, is a popular dessert fruit. Carambola, belonging to the Oxalidaceace family, is an oblong fruit with five prominent longitudinal ribs. The skin is thin, smooth, glossy, waxy and is from yellow to deep yellow in colour at the late stage of ripening. The flesh is yellow, juicy and fairly crispy in texture with minimum fibre. There may be a few pale seeds, but more often they are absent. When cut transversely the fruit is star-shaped. Among the tropical fruits, carambola is one of the richest in niacin, vitamin C and carotene, and moderate in vitamin A, thiamin and riboflavin (Giri et al., 1980; Malaysian Fruit Industry Directory, 1989). The above properties of carambola make it a popular dessert fruit especially in Malaysia and it has been exported to overseas markets such as Hong Kong, Singapore and Australia (Mardi, 1992).

Currently, a lot of fresh fruits and vegetables have been minimally processed into ready-to-eat form. The fruits and vegetables have either been peeled, cored or sliced and packed in polybag or polystyrene tray sealed with polymeric film. The polymeric film provides a clear appearance of the fresh products to the consumers. In addition, the polymeric film modifies atmosphere of the packaged fresh fruits and vegetables (Saijo, 1988). Modified atmosphere packaging (MAP) has been found to improve the storability of perishable commodities such as fresh herbs, broccoli, green-onion, radishes (Aharoni et al., 1996), citrus, bell pepper, mango (Ben-Yehoshua et al., 1996), avocado (Meir et al., 1996) and minimally processed apple (Nicoli et al., 1994).

These minimally processed products are highly perishable with a very short post-cutting life due to enzymatic browning and/or excessive tissue softening (Gorny et al., 1996) where the retention of original and typical fresh colour has become a matter of great importance. The brown colour has been attributed to the action of polyphenoloxidase (PPO), E.C. 1.14.18.1 on the natural phenolic substances or compounds of the fruits (Corse, 1964). The brown pigment is called melanoidin (Eskin et al., 1971). The browning is due to the destruction of fruit cellular comparmentation and allows the phenolic substrates to be accessible to PPO which catalyzes phenolic oxidation (Mayer and Harel, 1979). The concentration and composition of phenolic compounds and/or the activity of PPO are often the major factors determining tissue browning development and intensity (Mathew and Parpia, 1971; Mayer and Harel, 1979).

Storage in refrigerated conditions can only reduce the rate of these degrading reactions and thus suitable treatments are necessary to retard tissue decay and to extend the product shelf life. The prevention of enzymatic browning cannot be achieved by traditional methods such as using heat to denature browning enzyme PPO and exclusion of oxygen; in fact, blanching can cause marked textural changes. Therefore inhibitors of enzymatic browning such as ascorbic acid and its isomer, erythrobic acid, cinnamic acids, sodium chloride (Nicoli et al., 1991), L-cysteine, ethanol and sulfites (Nicoli et al., 1994) have been used in food processing. However, the use of browning inhibitors is restricted by special requirements such as non-toxicity, wholesomeness, and effect on taste, flavour and texture. The use of sulfites such as sulfur dioxide and sulfurous acid is limited because of their toxicity (Iyengar and McEvily, 1992).

The objective of this study was to assess the effects of ascorbic acid at 0, 15 and 30 $mg.L^{-1}$ on the selected quality characteristics (skin colour, flesh soluble solids concentration, vitamin C and pH), sensory evaluation (flesh colour, texture, sweetness, flavour and overall taste) and browning characteristics (browning degree, PPO activity and protein profile) of the minimally processed carambola at maturity indices of 3 and 4.

CHAPTER II

LITERATURE REVIEW

Carambola

The plant's scientific name, *Averrhoa*, is derived from Averroës, the renowned Arabian philosopher and physician borned in Spain in the twelfth century (Jacqueline, 1989). Carambola grows wild in the forest of Indonesia, and has spread throughout the tropics. In the Philippines it is termed "balimbing" as well as "carambola". In Malaysia, it is called "belimbing besi", "belimbing segi" or "belimbing manis" and the main areas of production are in Johor, Selangor, Kedah, Perak, Melaka and Negeri Sembilan (Mardi, 1982).

Carambola has two or more regular seasons annually and their peak harvests occur in April - June and October - December with smaller crops interspersed throughout the year. This plant has a typically short juvenile period, maturing from 18 months to three years from planting. Because of its early maturity and regular fruiting seasons, extended drought periods will adversely affect plant growth and regularity in cropping. Agro-ecological region suitable for this fruit is that it must has the uniform precipitation throughout the year without extended dry spells. Locally, the region around Selangor extending north to Batang Padang, a major part of Johore and Central Pahang are agro-ecological regions suitable for this plant (Mardi, 1982).

The carambola tree is small, and pyramid-shaped when young but round-shaped when old. This plant suits well to the tropical climate. It needs plenty of rainfall and dry weather. It grows very well in friable and well-drained soil, and even on the coastal tin tailings. This upright growing tree has an open style of branching and the long slender branchlets and twigs assume a pendulous form, as the tree grows older. The tree reaches a height of 5 - 12 m in good soil and is a rapid grower. The leaves are pinnate with broad, sharply pointed, tender leaflets. The upper surface of the leaf is smooth and is yellowish green in colour. The lower surface is dark green in colour. The leaf is 2 - 4 cm wide and 2 - 9 cm long (Sturrock, 1959).

The fruit grows in groups of three or four, after the clusters of deep pink or lilac flowers with 5 sepals, petals and stamens have fallen. The fruits are born laterally on the twigs and young branchlets throughout the top of the tree. The shape of the fruit is unusual, it is so deeply angled as to be star-shaped in transverse section. The fruit varies from 5 cm to 17 cm in length and from 8 cm to 10 cm in width. The very thin skin covers a firm juicy flesh containing a few small seeds. The flavour is quincelike, becoming more pronounced as ripening proceeds. The fruit is green when unripe, turning yellow or almost orange and slightly translucent or waxy when ripe. When ripe the fruit make a firm, cloudy, yellow jelly with a pronounced quince flavour. The fully ripe fruit may be made into jam or pressed for juice to make "ades". Chilled and sliced into very thin transverse sections the ripe fruits make an interesting addition to salads (Sturrock, 1959).

Carambola has been said to increase the milk-flow of nursing mothers. The crushed leaves and shoots are made into a lotion to soothe chicken-pox, while the Chinese and Vietnamese use it as an eye-salve; high blood pressure can be ameliorated by carambola. In some parts of South-East Asia, carambola are said to cause hiccoughs; in others to cure it! Carambola is also used to clean brassware (Jacqueline, 1989).

Nutrient analysis by Mardi (1992) gives the following content, per 100 grams of edible portions:-

Calorie	24.00 kcal.
Protein	0.70 g
Fat	1.80 g
Calcium	7.00 mg
Iron	0.40 mg
Vitamin A	26.00 mg
Vitamin B1	0.07 mg
Vitamin B2	0.07 mg
Vitamin C	25.80 mg

Minimally Processed Horticultural Products

The availability of certain foods and the consumer's purchasing power have changed the pattern of food consumption. Today the emphasis has changed from ensuring an adequate supply of calories to an increased emphasis on quality and convenience. This is due to the upgraded living standard and an increased number of households consisting of two adults holding part or full time jobs. Demands from employment and other activities place a premium on time, including food shopping and preparation time. Thus, the change of lifestyle has increased the demand of convenient and fresh (or fresh-like) products, which has led to a relatively new area of food preservation i.e. minimally processed foods. The products are produced by cleaning, peeling, trimming, shredding, slicing and packaging to provide convenience to the user (King and Bolin, 1989).

Quality of Minimally Processed Fruits and Vegetables

Quality is the composite of those characteristics that differentiate individual units of a product, and have significance in determining the degree of acceptability of that unit by the buyer (Kramer and Twigg, 1970). Ultimate consumers will include quality of fruits and vegetables as purchase and consumption attributes. Purchase attributes are properties such as appearance, firmness and aroma that are important to the consumer in the buying decision. Consumption attributes such as flavour and mouthfeel influence the degree of liking during eating. In addition, hidden attributes such as nutrient composition and microbiological status also contribute to quality but are not readily determined by the consumer (Shewfelt, 1994).

Minimally processed products must be fresh in appearance, with acceptable colour and reasonably free of defects. Consumers rarely choose fruits and vegetables according to their nutritional value. Their choices are strongly influenced by esthetic and price considerations. This is because visual evaluation by buyers and consumers is a major factor in the purchase decision where decrease in quality is based primarily on appearance. Consumers use appearance as a nondestructive means of assessing ripeness and absence of disease or insect damage. Firmness-to-the-touch is an external textural attribute to the consumer in the purchase decision. It is an indicator of the ripeness of softening fruit. Aroma, which serves as a differentiating characteristic to consumers, is an indicator of fruit ripeness and vegetable decay. Consumers also use it in the purchase decision (Shewfelt, 1987).

Many factors influence the quality of minimally processed fruits and vegetables including growing conditions and cultural practices, cultivar and maturity at harvest, harvesting and handling methods, inspection standards, storage temperature and duration, and packaging conditions. Therefore a fully integrated handling and production system is needed to provide greater control over quality factors (Shewfelt, 1987).

Effects of Minimally Processed

In food technology, a long-standing goal is to extend the shelf life of fresh products. Until recently food processing techniques have been primarily devoted to stabilize products and lengthen their storage shelf life. Whereas in minimally processed fruits and vegetables, these techniques can increase their perishability.

In extending shelf life of minimally processed fruits and vegetables, two basic problems have been encountered. First, vegetative tissue is living and respiring in which many chemical reactions are interacting. Some of the reactions, if not controlled, can lead to rapid senescence or quality changes. Second, microbial proliferation must be retarded. The growth of disease-causing microorganisms is a food safety concern. Visible growth or odour caused by microorganisms is aesthetically unacceptable. Therefore controlling the physiology of vegetative tissue and microbial growth is critical to minimally processed fruits and vegetables. The tissue must remain alive and the quality being maintained with reasonable storage life (King and Bolin, 1989).

Minimal processing generally increases the rates of metabolic processes that cause deterioration of fresh products. The physical damage or wound caused by preparation increases respiration and ethylene production, and other biochemical reactions responsible for changes in colour (browning), flavour, texture and nutritional quality (vitamin loss). The greater the degree of processing, the greater the wound response (Cantwell, 1986).

Consequences of Mechanical Injury

Mechanical wound induces a diverse array of metabolic pathways and hence brings about changes in metabolism. These changes include localized increased respiration at the site of injury, stressed ethylene production, accumulation of secondary metabolites, and cellular disruption leading to decompartmentation of enzymes and substrates. These responses are brought about subsequent to a series of reactions designed to bring about membrane restoration in plant tissues (Rolle and Chism, 1987).

The membranes of plant cells and of subcellular organelles are lipoprotein structure in which the lipid components are predominantly phospholipids and galactolipids (Galliard, 1978). Wound or injury damages membrane systems of cells (Mazliak, 1983) causing membrane components to undergo extensive enzymatic degradation (Yapa et al., 1986), and to be depleted of their lipid components. Consequently, a series of biochemical reactions mediated by the action of lipid acyl hydrolyses take place and these result in the production of wound respiration. The wound also induced ethylene to be released (Mattoo and Anderson, 1984). Yang and Pratt (1978) concluded that the biosynthetic pathway for ethylene under such conditions is similar to the pathway for ethylene synthesis in ripening fruit.

Ethylene production in small disk cutting of tomatoes to be about 20-fold higher than that of the whole fruit and the increase was noted 15 - 20 min after cutting (Lee et al., 1970). Respiration rate of peeling and slicing to 1 cm thickness of kiwifruit doubles to about 50 $mg.kg^{-1}.hr^{-1}$ at 20°C in comparison to the intact fruit (Watada et al., 1990). In green bananas, ethylene production is extremely low, less than 50 $nL.kg^{-1}.hr^{-1}$, but slicing causes the rate of production to be several times higher than the rate in the intact fruit (McGlasson, 1970).

Textural Changes

Besides hasten senescence, wound ethylene also changes the texture of minimally processed fruits and vegetables where firmness is desired for storage and transit of the produce. In addition, softening is essential for sensory acceptance (Watada et al., 1990). Softening rate of kiwifruit is dependent on ethylene and temperature. Softening was accelerated by 0.5 $mg.L^{-1}$ ethylene at 0°C and the rate increased with temperature (Arpaia et al., 1985, 1986).

Glucan, galactan and water-insoluble pectinic acid are the major cell wall components considered to be responsible for texture changes (Tavakoli and Wiley, 1968). Conversion of insoluble protopectin to pectin; decrease in cellulose crystallinity; decrease in galacturonic acid; reduction in cell volume; thinning of cell walls; and folding of cell walls are changes occur in cells during senescence. Labavitch (1981) found that during softening, a decrease in wall-bound uronic acid and an increase in soluble uronide occur. According to Rolle and Chism (1987), membrane integrity must be maintained and the onset of senescence must be delayed to maintain the quality of minimally processed fruits and vegetables.

Polygalacturonase (PG) and ß-galactosidase hydrolyze pectic compounds cause the loss of cellular turgor. Plant tissue sensitivity to enzymatic hydrolysis varies considerably among cultivars, and even with tissue maturity or mineral composition. Enzymes such as ß-galactosidase and exo-PG initiate most of the undesirable textural changes. Both solubilize pectin and degrade cell walls (King and Bolin, 1989).

Appearance

Enzymatic reactions also initiate appearance changes in minimally processed fruits and vegetables. Cellular enzymes from ruptured cells are free to mix with substrate, producing dark-coloured compounds. The major enzyme associated with darkening in fruits is PPO (Luh and Phithakpol, 1972). PPO reacts with some substrates, such as *o*-dihydroxy phenols, producing brown-pigmented materials. There is a direct correlation between PPO and activity and browning in apples (Drake and Fridlund, 1986).

Factors Affecting Quality of Minimally Processed Fruits and Vegetables

Temperature

Minimally processed products are generally more perishable than intact products because they have been subjected to severe physical stress, such as peeling, cutting, slicing, shredding, trimming, and/or coring, and removal of protective epidermal cells (Watada et al., 1996). Most minimally processed products are prepared, shipped and stored at a lower temperature than that recommended for intact commodities. This is because respiration rates of minimally processed products were higher than the intact product. Slicing of muskmelon fruit had increased respiration rate (McGlasson and Pratt, 1964). In addition, the respiration rate of minimally processed products increased with temperature, and the degree of increase differed with the commodity. Chilling-sensitive minimally processed products could be held at a chilling temperature where injury from chilling will be of less consequence than the deterioration that results at non-chilling temperature (Watada et al., 1996). Storage at 0°C to 10°C can hasten deterioration substantially because Q_{10} of biological reactions range from 3 to 4 within this temperature region (Schlimme, 1995).