EFFECT OF OSMOTIC DRYING ON BETA CAROTENE CONTENT IN MANGO

By
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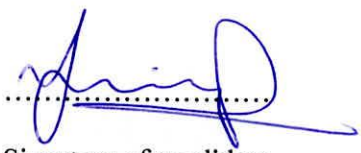
Declaration

The work described in this thesis was carried out by me under the supervision of Dr. K.K.D.S. Ranaweera, Head, Department of Food Science and Technology, University of Sri Jayewardenepura, Nugegoda, Sri Lanka and Mr. M. A. J. Wansapala, Lecturer, Department of Food Science and Technology, University of Sri Jayewardenepura, Nugegoda, Sri Lanka and report on this has not been submitted in whole or in part to any university or any other institution for another degree or diploma.



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I / we certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the university for purpose of evaluation.

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Effect of Osmotic Drying on Beta Carotene Content in 'Karuthacolomban' Mango

By
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ABSTRACT

Fresh ripened mango has higher percentage of β-carotene. One molecule of β-carotene gives two molecule of vitamin A, and hence it is good source of vitamin A. Nowadays people use various technologies to increase the variety of products being produced. As result demand for the processed fruits is increased rapidly. Osmotic drying is one of the methods of preserve mango and produces various types' products using this technique.

Experiments were conducted to find out, the effect of osmotic drying on β-carotene content of 'Karuthacolomban' mango. 'Karuthacolomban' mango is very popular variety in Sri Lanka and highly available. The β-carotene content of fresh ripened mango was determined by open column chromatographic method. The content of β-carotene in fresh ripened 'Karuthacolomban' mango was compared with the osmotically dried mangoes. When syruping of mango for a short period of time, the effects on β-carotene content was observed. Further mangoes were kept in three different sugar solutions; the effect on β-carotene was estimated. For keeping mangoes for a long time, about one month, the effects on β-carotene was determined.

There is no any significant loss of β-carotene in 'Karuthacolcmban' mango when osmotically drying up to four weeks. Within this period of time no significant change in β-carotene content in mango with increasing the concentration of sugar solutions from 30^0Brix and up to 50^0Brix. But there is a significant loss of β-carotene content when keeping mangoes in sugar solution after four weeks. When processing various types of mango products, losses of β-carotene should be avoided because it reduces the vitamin A in the final product.

Chapter 1

1.0 Introduction

Everybody likes to eat fruits. Various types of fruits are available in Sri Lanka. Out of these varieties mango is considered as the king of all other fruits. Mango fruit has significant taste, fragrance and easily available in every part of the country. Sri Lankan people use to grow even one or more mango tree around their home garden as a habit in ancient time. So Sri Lankan people habitually consume fresh mango when ever possible.

But nowadays there are so many developed varieties around us and use to produce various types of products to attract consumers. There are jams, cordials, squashes, pickles, fruit preserves and etc. in the market having various characteristics. All products enter the market has very attractive appearance. The taste, colour product design and many other features are changed dramatically to get consumer attraction to the product. As an example there are many osmotically dried preserve fruits in the market. Sometimes the appearance of these products may similar with fresh fruits. But nutritionally these processed fruits are not good as fresh form.

Therefore my interest was to find out if there any change in carotene in mango after osmotic drying. For this study I selected 'Karuthacolomban' mango variety because it is highly used in canning processes and it is highly popular among consumers and easily find in the market. Some people identify the 'Karuthacolomban' mango as Jaffna mango.

Carotenoids are a group of yellow, orange and orange red fat soluble pigments widely distributed in fruits and vegetables. There are a class of nearly four hundred known naturally occurring pigments found in fruit and vegetables. The carotenoids are important in nutrition very much because they consider as precursors for the synthesis of vitamin A. so it is call as pro vitamin A. The yellow, orange colour pigment β-carotene is the most common form of

pro vitamin A. therefore if we determine the β-carotene in 'Karuthacolomban' mango it gives an idea about vitamin A availability.

So my study is determination of β-carotene in fresh 'Karuthacolomban' mango and the β-carotene content after 'Karuthacolomban' mango is keeping in sugar syrups. When mango slices are put in the sugar solution the transfer of water molecule from mango slices to the sugar solution and transfer of solute molecule from sugar solution into mango slices is take place. Due to lowering of available water in the mango slices are preserved. Therefore microorganism can not live in mango slices with having lower water activity.

Chapter 2

2.0 Literature Survey

2.0 What is carotenoids

The colour of fruits and vegetables is very important from the point view of ultimate quality of the product. The chief pigment of fruits and vegetables which are imparting the colour are caronetoids, chlorophylls, anthoxanthinns and anthocyanins.

The carotenoids are a group of yellow, orange and red fat soluble pigments widly distributed in nature. The green colour of the chlorophyll masks the yellow to red colour of the carotenes except in very young leaves in which the chlorophyll content is less. These pigments are also present mango, papaya, peach, tomato, red peper, carrot etc.. The name carotenoid is applied to all pigments chemically related th the carotens which were first isolated. The carotenoids are either hydrocarbons or derivatives of hydrocarbons and are composed of isoprene units. Carotenoids which contain hydroxyl groups are called xanthophylls and are often associated with carotenes. (Rangana S, 1986)

2.2 Nature of carotenoids in foods

Food carotenoids are usually C_{40} tetraterpenoids built from eight C 5 isoprenoid units, joined so that the sequence is reversed at the center.the basic linera symmetrical skeleton, which can be cyclized at one or both ends, has lateral methyl gsix C atoms at the center and five C atoms elsewhere. Cyclization and other modification, such as hydrogenation, isomerization, double bond migration, chain shortening or elongation, rearrangement, isomeriztion, introduction of oxygen functions or combination of these processes result in a myriad of structures. A disinative characteristic is an extensive conjugated double bond system, which serves as the light absorbing chromophore responsible for the yellow, orange, red colour that these compounds impart to many foods. Because plants are able to

synthesize carotenoids, the carotenoid compositions of plant food is enriched by the presence of small or trace amounts of biosynthetic precursors, along with derivatives of the main components. Animals are incapable of carotenoid biosynthesis thus their caronenoids are diet derived, selectively or unselectively absorbed and accumulated unchanged or modified slightly into typical animal caronenoids (Amaya D. B. R. 1999).

2.3 Functions of carotenoids

The many various biological properties and functions of the carotenoids are besides their ubiquitous occurrence the main reason for the importance of this class of compounds and the main aspects have recently been reviewed (Krinsky, 1994).

In photosynthesis the energy transfer involves the direct excitation of carotenoids by light to form the first excited singlet state, and the subsequent transfer of this excitation energy to chlorophyll to initiate the process of photosynthesis. This type of process can effectively extend the wavelength of light available to an organism for photosynthesis.

2.3.1 Carotenoids also play a major role in the photoprotection of cells and tissues

This ability is the result of energy transfer reactions in which the energy of triplet state sensitizers or singlet oxygen is transferred to carotenoid molecules in the ground state, forming triplet state carotenoid molecules. The energy acquired by the carotenoids is then lost as heat and the ground state carotenoid is regenerated to undergo another cycle of photoprotection.

2.3.2 As precursors of vitamin A

In humans and in those animals that require vitamin A for normal growth and development the most important source is the ingestion and metabolism of carotenoids that can be converted to vitamin A, i.e. compounds with an unsubstituted beta-ring, especially beta-carotene. According to an estimate of the World Health Organization (WHO), 250'000-500'000 children go blind every year due to a deficiency of vitamin A. It was demonstrated that the formation of vitamin A from beta-carotene can occur either by central or by excentric cleavage of beta-carotene.

2.3.3 Act as antioxidants

The ability of carotenoids to act as antioxidants has been known for a long time and at the moment it is of great interest whether carotenoids behave as antioxidants in low-density lipoproteins (LDL), such as the oxidation of LDL is now considered to be an important causative agent in coronary heart disease, but the results of studying carotenoid involvement in preventing LDL oxidation remain controversial.

2.3.4 Improving fertility or reproduction capacity in animals

There have been many reports of a positive effect of dietary carotenoids on improving fertility or reproduction capacity in a number of animals, but additional evidence is still required for this proposed function of the carotenoids

2.3.5 Detoxification of carcinogens

There are a few examples where it was demonstrated that carotenoids can alter the activity of a specific enzyme (e.g. aryl hydrocarbon hydroxylase) and this could be of importance in view of the detoxification of potential carcinogens.

2.3.6 Decrease of malignant transformation of cells

Some years ago it was demonstrated that various carotenoids, such as lycopene , beta-carotene , alpha-carotene , lutein and canthaxanthin can decrease the extent of malignant transformation of cells. It was shown that the molecular actions occur via up-regulation of the connexin43 gene, the gene responsible for the production of one of the important components of the gap junction.

2.3.7 Decrease risks for degenerative diseases.

Based on epidemiological data it can be assumed that diets rich in carotenoid-containing fruits and vegetables are associated with significantly decreased risks for a variety of degenerative diseases. However in dietary epidemiology it is always difficult to pinpoint the components which may be related to the lowered risk.

2.3.8 Decrease the risk of cataract formation

Several epidemiological studies have supported the observation that a high content of blood carotenoids decrease the risk of cataract formation. This is important in view of dietary aspects, particularly of the growing elderly population.

2.3.9 Age-related macular degeneration

Age-related macular degeneration (ARMD), associated with aging can lead to total blindness in otherwise healthy people. A significant reverse relationship between the incidence of ARMD and the ingestion of fruits and vegetables rich in provitamin A carotenoids was demonstrated and it was shown that there are very significant reductions in the risk of developing neovascular ARMD as a function of plasma levels of alpha-carotene, beta-carotene , cryptoxanthin and lutein)/zeaxanthin .

Coronary heart disease (CHD) remains the major cause of death in many countries. There is epidemiological evidence for an inverse association between serum levels and ischemic heart disease, but it is still unclear whether a single antioxidant plays the essential role or whether the sum of the antioxidants are responsible for preventing this disease in humans (Britton G. et al.,).

2.4 Food carotenoids

Most common acyclic carotenoids are lycopene and carotein. Lycopene is the principal pigment of any red flesh fruits and vegetable ζ-carotein is more ubiquitous but it is usally present at low levels. Phytoene and phytofluene are more widely distributed than reported, because they are both colourless and vitamin A inactive their presence may often be overlooked.neurosporene has limited occurance and is normally found in small amounts. The bicyclic β-carotein is the most common widespread of all carotenoids in foods either as a minor or as the major constituent (carrot, mango) . The hydroxyl derivatives of lycopene, lycoxanthin and lycophyll are rarely encountered, they are found in trace amount of tomatoes. The xanthophylls α-cryptoxanthin and zeinoxanthin are widely distributed. β-cryptoxanthin is the main pigment of many orange fleshed fruits (Amaya D. B. R. 1999).

2.4.1 Composition of carotenoid in foods

Leaves have a strikingly constant carotenoid pattern, often referred to as the chloroplast carotenoid pattern, the main carotenoids being lutiein, β-carotene, violaxanthin and neoxanthin. In contrast to leafy and other green fruits and vegetables are known for their complex and variable carotenoid composition. Carotenoids are not widely distributed in root crops. (Amaya D. B. R. 1999)

2.4.2 Occurrence of carotenoids in foods

How and in which chemical and physical form carotenoids occur in nature are questions of importance, particularly to food technologists involved in the processing and development of new food products. They are source also important to nutritionists because fruits have been declared the world's source of vitamins and minerals. Therefore content of carotenoids in varies food stuff is important. The table 2.1 shows carotene content of some food items (Bauernfeind J.C.1981).

The colour of fruits and fruit juices is important for characterizing thede products and one of the criteria used for commercial standards. However many factors affect to the Carotenoids , such as colour, including variety of fruit, maturity, place of origin, seasonal and climatic changes, and processing method. In ripening fruit the decrease in chlorophyll content is frequently accompanied by an increase in carotenoid concentration and in the ratio of carotenes to oxycarotenoids. α-carotene, γ-carotene and lycopene are the common fruit hydrocarbon carotenoids. α-carotene, γ-carotene occur more frequently in fruit than in leaves. Carotenes are quit widely distributed in some fruits, in a range of 1-60% of the total carotenoid content. The oxycarotenoids of fruits are often esterified. Oxygen is required for maximal carotenoid production, and the temperature range is critical. Light however is not required during maturation for carotenoid synthesis (Bauernfeind J.C.1981).

Table: 2.1 Carotene content of some food items

Food	Carotene (mg/100 gm)
Banana raw	0.03-0.22
Mangoes raw	2.07
Pineapple raw	0.03
Avocado raw	0.61
Plums dried	1.0
Apple raw	0.02-0.30
Oranges and juice raw	0.03-0.05
Melons raw	1.1-1.2
Mandarins	0.13-0.28

Source: Carotenoids as colorants and vitamin a precursors, 1981

2.4.3 β- carotene as provitamin A

Structurally vitamin-A is essentially one half of the molecule of β-carotein with an added molecule of water at the end of the lateral polyene chain. Thus β-carotein is a potent pro vitamin-A to which 100% activity is assigned. An unsubstituted β-ring with a C11 polyene chain is the minimum requirement for vitamin A activity. β-carotein is also called as pro vitamin A, because it can be converted to vitamin A in the body . At least ten percent of the carotene found in plant can be converted with varying efficiencies into vitamin A. Four of these carotenoids α- carotene, β- carotene, γ- carotene and cryptoxanthin are of particular importance due to their pro vitamin A activity. Of the four, β- carotene has the highest vitamin A activity and provides about two thirds of the vitamin A necessary for human nutrition.

Food supply vitamin A in the form of vitamin A, vitamin A esters and carotenes. Almost no absorption of vitamin A occurs in the stomach. In the small intestine, vitamin A and β-

carotene are emulsified with bile salts and products of fat digestion and absorbed in the intestinal mucosa. Here much of the conversion of β- carotene to vitamin A(retinol) take place. There are wide difference in species and individuals as to how well they utilize the carotenoids (Ersminger et al., 1994).

2.5 Factors influencing carotenoid composition

The carotenoid composition of foods are affected by factors such as cultivar or variety , part of the plant consumed, stage of maturity, climate or geographic site of production, harvesting and post harvesting handling, processing and storage . A close look at some published values revels discrepancies that surpass those expected from the effects of these factors, indicating analytic inaccuracy. The analyst must take utmost care to differentiate between and analytic variations.

In carotegenic fruits ripening is usually accompanied by enhanced caratogenesis as chlorophylls decompose and the chloroplast carotenoid pattern gives way to a complex composition, the carotenoids increasing dramatically in number and quantity. Increased carotenogenesis with maturation ripening was also found. The one factor that decisively affects the carotenoid content is the maturity of the plant food when harvested and afforded for consumption.

Carotenogenesis may continue even after harvest as long as fruit or vegetable remains intact. Carotenoid biosynthesis in the flesh of ripening mango was observed to be maximal at tropical ambient temperature (23^0-28^0C). Storage at 7-20 ^{0}C for 16-43 days caused a substantial decrease in total carotenoid content even when the fruits were substantially ripened at optimal conditions (Amaya D. B. R. 1999).

2.6 **Biosynthesis of carotenoids**

Carotenoids are synthesized in nature by plants and many microorganisms. Animals can metabolize carotenoids in a characteristic manner, but they are not able to synthesize carotenoids.

Carotenoids, being terpenoids, are synthesized from the basic C5-terpenoid precursor, isopentenyl diphosphate (IPP) (XVII) (Fig 2.1). This compound is converted to geranylgeranyl diphosphate (C20) (XVIII). The dimerization of XVIII leads to phytoene (7,8,11,12,7',8',11',12'-octahydro-gamma,gamma-carotene) (XIX) and the stepwise dehydrogenation via phytofluene (15Z,7,8,11,12,7',8'-hexahydro-gamma,gamma-carotene (XX), zeta-carotene (7,8,7',8'-tetrahydro-gamma,gamma-carotene) (XXI), and neurosporene (7,8-dihydro-gamma,gamma-carotene) (XXII) gives lycopene (I). Subsequent cyclizations, dehydrogenations, oxidations, etc., lead to the individual naturally occurring carotenoids, but little is known about the biochemistry of the many interesting final structural modifications that give rise to the hundreds of diverse natural carotenoids.

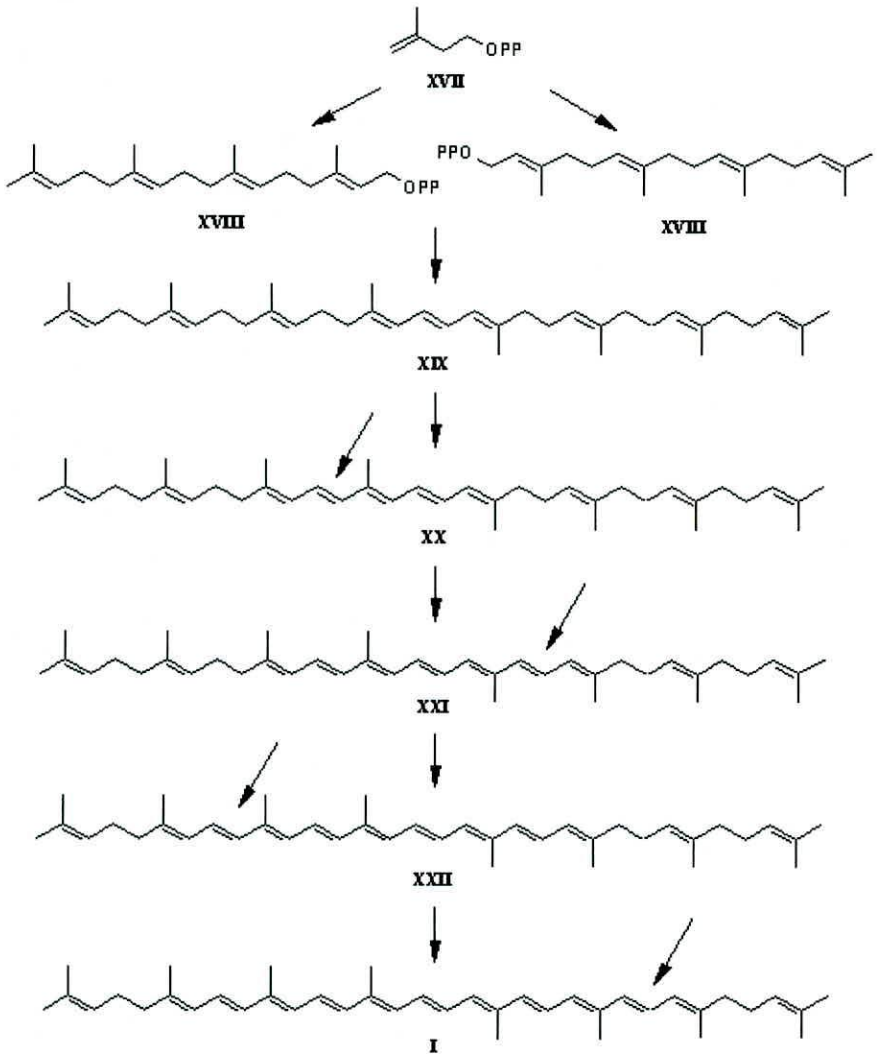

Figure: 2.1 Biosynthesis of carotenoids

There are now exciting prospects for rapid progress through the application of molecular genetics techniques in combination with other biochemical and chemical approaches. The benefits of this are not purely academic. The industrial production of natural carotenoids through microbial biotechnology is already established and expanding, mainly through the exploitation of some micro algae (particularly *Dunaliella*) which can synthesize large amount of carotenoid.

2.7 Absorption of vitamin A

Their absorption is affected by several factors, including the presence in the small intestine of bile, ditery fat and antioxidants. Bile aids emulsification, fat must be absorbed simultaneously and antioxidants, such as alpha tocopherol and lecithin, decrease the oxidation of carotene. Also presence of enough protein of good quality enhance the conversion of carotene to vitamin A. This is main concern factor country like Sri Lanka where protein is limited in both quantity and quality. The absorption of vitamin A is adversely affected by the presence of mineral oil in the intestinal tract. Since mineral oil is not absorbed and since it holds carotene and vitamin A in solution, carotene and vitamin a are lost through excretion. Therefore mineral oil should never be used as substitute for regular fats in food preparation. In the blood vitamin A esters are transported in association with a retinol binding protein whereas the carotenoids are associated with the lipid bearing protein (Ersminger et al., 1994).

2.8 Retinol equivalent (RE)

At one time the amounts of most nutrients were in international units (IUs). These were usually based on the different growth rates exhibited by animals fed varying amounts of a specific nutrient or food. Today we can directly measure very small quantities of nutrients more precisely; consequently, milligrams (1/1000 of a gram) and microgram (1/1000000 of a gram) have replace international units of measure. For vitamin A current unit of measurements is the retinol equivalent (RE) which is basically 1 microgram (μg) of retinol. In this system it is assumed that 6 μg of β- carotene yield 1 μg of vitamin A activity and that 12 μg of other carotenoids yields 1 μg of vitamin A activity. The correction factor of 6 and 12 are estimates, based on incomplete knowledge and primarily compensate for the poorer absorption of β- carotene and other carotenoids compared with

preformed vitamin A as well as their incomplete conversion to the active form. The total retinol equivalent value for a food is calculated by adding the actual weight of retinol and the adjusted equivalent weights of the pro vitamin A carotenoids present in the food. Table: ii is a handy tool for converting amounts of vitamin A and carotenes expressed in one unit of measure into another unit of measure (Gordon and Paul, 1993).

Table: 2.2 Conversion values of for vitamin A

Compound with vitamin A activity	**µg =**	**RE =**	**IU**
Retinol	1	1	3.3
β- carotene	6	1	10
Other carotenoids (α, δ, etc.)	12	1	10
Mixture of both preformed and pro vitamin	_	1	5

Source: Perspective in nutrition, 1993

2.9 Mango as a high carotene containing fruit

The mango is the apple of the tropics, and one of the most commonly eaten fruits in tropical countries around the world. So mango can be considered as one of the most important and popular Asian fruits in Sr lanka due to its beautiful colour, attractive fragrance, delisious taste, and health giving properties. The fruits grow at the end of a long, string like stem (the former panicle), with sometimes two or more fruits to a stem. The fruits are 2 to 9 inches long and may be kidney shaped, ovate or (rarely) round. They range in size from 8 ounces to around 24 ounces. Mango is grown every part of the country. The major cultivars grown in Sri Lanka are 'Karuthacolomban', 'Vellaicolomban' and 'Willard'. The 'Karuthacolcmban' and 'Vellaicolomban'which can be grown both in the intermediate zone and dry zone and 'Willard' which can even be grown in the wet zone are popular as fresh fruits. But there are many other varieties

such as 'Betti amba','Parrot mango', 'Rata amba', 'Mee amba'and etc. not produce for commercial purposes (Department of agriculture,1993).

2.9.1 Food Uses of mango

Mangos should always be washed to remove any sap residue, before handling. Some seedling mangos are so fibrous that they cannot be sliced; instead, they are massaged, the stem-end is cut off, and the juice squeezed from the fruit into the mouth. Non-fibrous mangos may be cut in half to the stone, the two halves twisted in opposite directions to free the stone which is then removed, and the halves served for eating as appetizers or dessert. Or the two "cheeks" may be cut off, following the contour of the stone, for similar use; then the remaining side "fingers" of flesh are cut off for use in fruit cups, etc. Small mangos can be peeled and mounted on the fork and eaten in the same manner. If the fruit is slightly fibrous especially near the stone, it is best to peel and slice the flesh and serve it as dessert, in fruit salad, on dry cereal, or in gelatin or custards, or on ice cream.

The ripe flesh may be spiced and preserved in jars. Surplus ripe mangos are peeled, sliced and canned in syrup, or made into jam, marmalade, jelly or nectar. Mango juice may be spray-dried and powdered and used in infant and invalid foods, or reconstituted and drunk as a beverage. The dried juice, blended with wheat flour has been made into "cereal" flakes; a dehydrated mango custard powder has also been developed in India, especially for use in baby foods.

Ripe mangos may be frozen whole or peeled, sliced and packed in sugar (1 part sugar to 10 parts mango by weight) and quick-frozen in moisture-proof containers. The diced flesh of ripe mangos, bathed in sweetened or unsweetened lime juice, to prevent discoloration,

can be quick-frozen, as can sweetened ripe or green mango puree. Immature mangos are often blown down by spring winds. Half-ripe or green mangos are peeled and sliced as filling for pie, used for jelly, or made into sauce which, with added milk and egg whites, can be converted into mango sherbet. Green mangos are peeled, sliced, parboiled, and then combined with sugar, salt, and various spices and cooked, sometimes with raisins or other fruits, to make chutney; or they may be salted, sun-dried and kept for use in chutney and pickles. Thin slices, seasoned with turmeric, are dried, and sometimes powdered, and used to impart an acid flavor to chutneys, vegetables and soup. The fat extracted from the kernel is white, solid like cocoa butter and tallow, edible, and has been proposed as a substitute for cocoa butter in chocolate.

Table: 2.3 Food value per 100 g of ripe mango flesh

Component	Amount
Calories	62.1-63.7
Moisture	78.9-82.8 g
Protein	0.36-0.40 g
Fat	0.30-0.53 g
Carbohydrates	16.20-17.18 g
Fiber	0.85-1.06 g
Ash	0.34-0.52 g
Calcium	6.1-12.8 mg
Phosphorus	5.5-17.9 mg
Iron	0.20-0.63 mg
Vitamin A (carotene)	0.135-1.872 mg
Thiamine	0.020-0.073 mg
Riboflavin	0.025-0.068 mg
Niacin	0.025-0.707 mg
Ascorbic Acid	7.8-172.0 mg
Tryptophan	3-6 mg
Methionine	4 mg
Lysine	32-37 mg

Source: Minimum and maximum levels of food constituents derived from various analyses made in Cuba, Central America, Africa and India

2.9. 2 Mango - traditional medicine value

All parts of the mango plant from the seeds and flowers to the leaves and gum are used in traditional South Asian medicine, but the fruits are most important. Unani physicians hold mangos in very high esteem because of their many medicinal virtues. They are used for strengthening the nervous and blood systems, ridding the body of toxins and treating

anaemia. In Ayurveda, dried mango flowers are used to cure dysentery, diarrhoea and inflammation of the urinary tract.

2.9.3 Carotenoids in mango

Like other fruits, mangoes vary in their carotenoid content. The β-carotene content forming the largest propotion of total carotenoid in mango fruit with oxy carotenoids present. There are 16 different carotenoids, including β-carotene, γ-carotene, monoepoxy β-carotene and cryptoxanthin in the fully ripe mango. The level of oxy carotenoids in ripe fruit was higher than in partially ripened fruit. In partially ripened fruit, the hydrocarbon carotenes (phytofluene, β-carotene, and phytoene) constitute the major part (about 85%) of the cartenoids.

The isolation of carotein-protein complex in the mango was done. The total carotenoid values of 0.05-0.9, 1.2-2.3 and 2.3-4.3 mg per 100 gm for the stages of green, yellow and yellow-orange fruit respectively. β-carotene values for five varieties of mangoes ranged from 2.4 to 8.3 mg per 100 gm in the study of five mango cultivars. The greatest total carotenoid content existing mango variety had the best fruit colour and flavour and the best overall quality in canned or frozen form. Storage temperature below 25^0C adversely affected the development of aroma, flavour and carotenoid formation of mangoes upon ripening. The carotene always exceeded (60-70%) the level of oxycarotenoids of the total pulp carotenoids (Bauernfeind J.C.1981).

2.9.4. Mango processing technologies

Mangoes are processed at two stages of maturity. Green fruit is used to make chutney, pickles, curries and dehydrated products. The green fruit should be freshly picked from the tree. Fruit that is bruised, damaged, or that has prematurely fallen to the ground

should not be used. Ripe mangoes are processed as canned and frozen slices, purée, juices, nectar and various dried products. Mangoes are processed into many other products for home use and by cottage industry (Dauthy M. E.1995).

2.10. Osmotic drying

This method of drying involves immersing pieces of food, mainly fruit in a solution with higher osmotic pressure and hence lower water activity than the food. Water will pass from the food pieces into the solution under the influence of osmotic pressure. For fruits like mangoes sugar solutions with or without the addition of small quantity of salt are used. In this process the cell walls of the food act as a semi-permeable membrane. However these membranes are not completely selective and there may also be movement of solute in both directions. Up to 50% reduction in the fresh weight of fruits may be brought by osmosis. The process is more accurately described as concentration rather than drying. In osmotic drying the rate of diffusion is rapid initially but slows up significantly after 60-120 minutes.

The operating conditions which most influence this process are solute concentration and temperature. Different solutes in the osmotic solutions e.g. sucrose, fructose, glucose and corn starch give rise different drying patterns. The addition of small quantities 0.5-2.0 % of salt to sugar solutions increases the drying process. Normally osmotically dried fruits are shelf stable without further treatment (Brennan J.G.1994).

2.10.1 Preservation with sugar

The principle of this technology is to add sugar in a quantity that is necessary to augment the osmotic pressure of the product's liquid phase at a level which will prevent microorganism development.From a practical point of view, however, it is usual to

partially remove water (by boiling) from the product to be preserved, with the objective of obtaining a higher sugar concentration. In concentrations of 60% in the finished products, the sugar generally assures food preservation.

It is important to know the ratio between the total sugar quantity in the finished product and the total sugar concentration in the liquid phase because this determines, in practice, the sugar preserving action. The percent composition of a product preserved with sugar, for example marmalade, can be expressed as follows: [i + S + s + n + w] = 100;

i = insoluble substance s = sugar from fruits S = added sucrose;

n = soluble "non sugar" w = water.

In this case, total sugar concentration, in the liquid phase, of the finished product is:

$$X = 100 (S + s) / 100 - (n + i) [\%]$$

Therefore, in the case of a standard marmalade with 55 % sugar added (calculated on the finished product basis), the real concentration in the liquid phase is for example:

$$X = 100 (55 + 8) / 100 - (5 + 3) = 68.5\%$$

In the food preservation with sugar, the water activity cannot be reduced below 0.845; this value is sufficient for bacteria and meosmophile yeast inhibition but does not prevent mould attack. For this reason, various means are used to avoid mould development:

- finished product pasteurization (jams, jellies, etc.);
- use of chemical preservatives in order to obtain the antiseptisation of the product surface.

It is very important from a practical point of view to avoid any product contamination after boiling and to assure a hygienic operation of the whole technological process (this will contribute to the prevention of product molding or fermentation). Storage of the finished products in good conditions can only be achieved by ensuring the above level of water activity (Dauthy M. E.1995).

2.11 Processing of mango slices in syrup

Fully mature, unripe mangos are ripened in the cannery to optimum canning ripeness. Mangos high in flavour, with more flesh and low in fiber are always recommended for canning. Sound ripe mangos are soaked in antiseptic and water, brush washed and then conveyed to the preparation tables and hand peeled. "Cheeks" of the peeled mangos are sliced off and longitudinally cut into two or three slices. Side cuts are packed separately. Slices are then conveyed to the filling tables where they are graded for size, colour and maturity and filled into sterile cans. Filled cans are syruped, steam exhausted, sealed, processed in boiling water, cooled, labeled and packaged (Dauthy M. E.1995).

Chapter 3

3. 0 Experimental

3.1 Sample collection

Only 'Karuthacolomban' variety were used for whole experiment. Always similar mangoes were selected for the analysis. The colour, maturity stage and physical appearance always consider when selecting mangoes. Edible portion of every mango sample were cut into small pieces. From this homogeneous sample 50 g was taken for the analysis of β-carotene in raw stage. Remaining mangoes were blanched in hot water for 15 seconds and immersed in three sugar syrups having Brix values 0f 30^0, 40^0 and 50^0 respectively. β-carotene of this three syrup samples were analyzed after one day, after two weeks and finally after four weeks time. The experiments were conducted in a Completely Randomized Design. The data was subjected to ANOVA in SAS statistical package.

3.2 Determination of β-carotene

3.2.1 Materials

3.2.1.1 Reagents and apparatus

Acetone

Motor and pestle

Buchner funnel

Separatory funnel with stopcock

Chromatographic glass tube

Adsorbent tube with 250 mm length and 19 mm inside diameter was taken which was made out of borosilicate.

Adsorbent Mix magnesium oxide and hyflosupercel (1:2 w/w) this two were mixed well .this was done manually by shaking the adsorbent in a container big enough to allow through mixing. The adsorbent was activated by heating in the oven at 110 ^{0}C for four hours.

Petroleum ether, (reagent grade b.p. 30-60^{0}C)

Anhydrous sodium sulfate

Potassium hydroxide solution-10% in methanol

Rotary evaporator

Ultraviolet-visible recording spectrophotometer; cuvette, 1 cm inside diameter glass cuvettes were used in spectrophotometric analysis.

Volumetric flasks

Beakers

Erlenmeyer flasks

Pipettes

And other common glassware

3.2.2 Method

3.2.2.1 Preparation of raw mango extraction.

50 g of homogeneous sample was measured.

Samples were grinded in motor and pestle with acetone. To facilitate the grinding small amount of celite was added.

It was filter through a Buchner flask by using suction pump

Motor and funnel was washed with small amount of acetone further to receiving the washing in the suction flask with the extract.

The residue was returned to the motor and macerated again.

The extraction and filtration was repeated until the residue was out of any colour and washing was colourless.

3.2.2.2 Partitioning the extract to petroleum ether.

100ml of petroleum ether was added into a separating funnel.

Small portion of acetone extract was added into that separating funnel.

Distilled water was slowly added to the walls of the funnel to avoided formation of an emulsion.

The funnel was kept for sometimes to separate two phases and discarded the lower aqueous acetone layer.

3.2.2.3 Concentrating of the extract.

The extract was decanted to a round bottom flask and rinsed the receiving flask and sodium sulphate with small amount of petroleum ether.

The round bottom flask was connected the rotary evaporator and concentrated the solution to about 10 ml the temperature not exceeding 40 ^{0}C

3.2.2.4 Chromatographic separation.

3.2.2.4.1 Preparation of the column.

Chromatographic glass tube was mounted on a suction flask.

Small glass wool plug was placed at the bottom of chromatographic tube.

Adsorbent was added loosely up to a height from 20 cm and apply a moderate vacuum from a water aspirator.

A flat tampering rod was used to press down the absorbent and flatten the surface.

The column was packed about 10-12 cm high.

The column was topped with 1 cm layer of anhydrous sodium sulphate to ensure that no residual water gets into the adsorbent.

One bed volume of petroleum ether was passed through the column and adjusted the vacuum so that the solvent flow was about 2-3 drops per second.

Once the petroleum ether was added to the column kept the top of the column covered with solvent until the chromatography was completed.

3.2.2.4.2 Developing the column

The carotenoid solution was poured into column and let the sample layer go down almost to the surface of the sodium sulphate layer before adding the rinse from the round bottom flask.

Once the petroleum ether rinse almost was reached the surface of the sodium sulphate layer, developed the column successively with 50 ml each of 1%,2%, and 5% ethyl ether and than 2%,5%,8%,10%,15% and 20% acetone in petroleum ether.

Separations of carotenoids were visually monitored and collected first fraction as it leaves the column.

Spectrum was recorded at 450 nm wave length in the "Thermospectronic" spectrophotometer.

3.2.2.4. Preparation of osmotic drying mango samples.

Three sugar solutions were prepared having Brix value of 30^0, 40^0 and 50^0. Previously cut mango pieces were blanched and put into this three sugar solution and tightly sealed the cans. 50 g of sample was taken out from the osmotic dried cans after 24 hours and analysis of β-carotene was done as in the procedures in 3.2.2.2 & 3.2.2.4.

3.2.3 Calculation

Calculate the concentration of β-carotene according to the following formula.

$$X\ (\mu g) = \frac{A^* y\ (ml) * 10^6}{A^{1\%}_{1cm} * 100}$$

$$X\ (\mu g/g) = \frac{X\ (\mu g)}{\text{Wt of the sample (g)}}$$

Where x is the weight or concentration of the carotenoid, y is the volume of the is solution that gives an absorbance of A at a specified wave length, $A^{1\%}_{1cm}$ is the absorption coefficient of the carotenoid in the solvent used.

For β-carotene in petroleum ether the absorbance (A) at 450 nm and an $A^{1\%}_{1cm}$ of 2592 should be used.

Chapter 4

4 Results and Discussion

4.1 Results

4.1.1 The spectrum of the first fraction of the first band

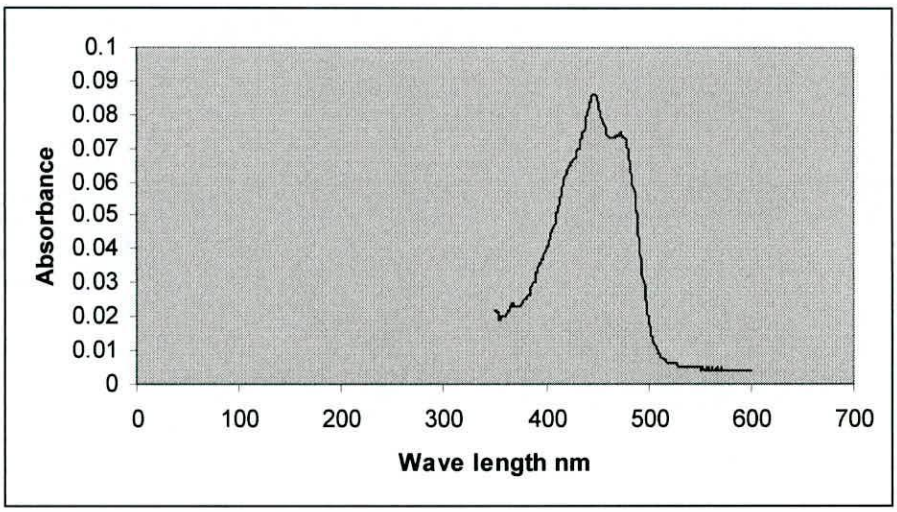

Figure 4.1

4.1.2 The spectrum of middle fraction of the first band

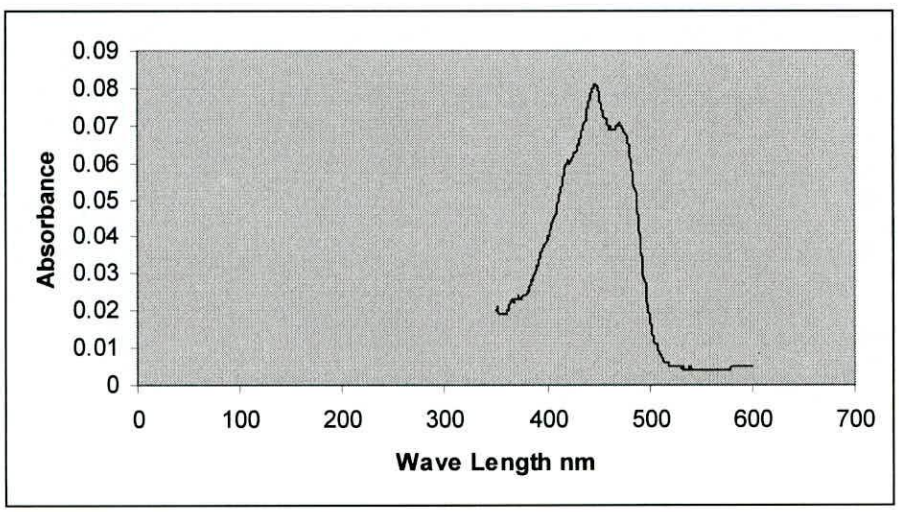

Figure 4.2

4.1.3 The spectrum of final fraction of the first band

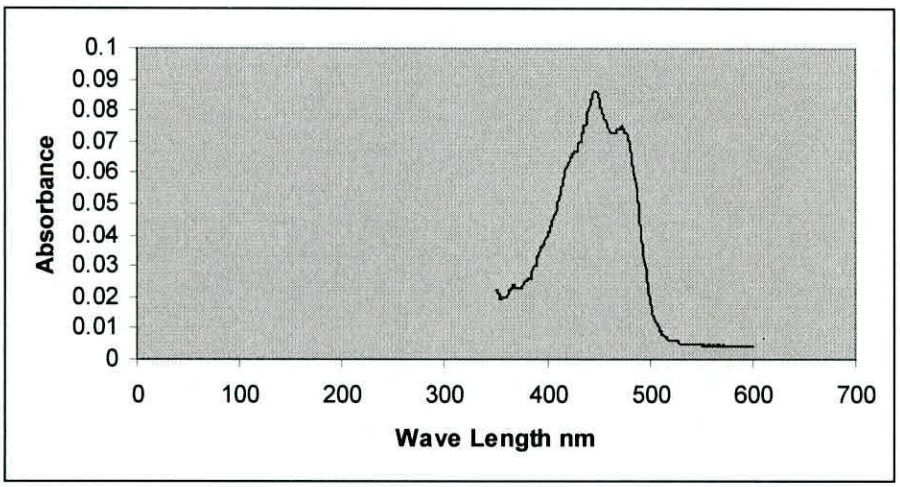

Figure 4.3

4.1.4 The spectrum after the first band separation

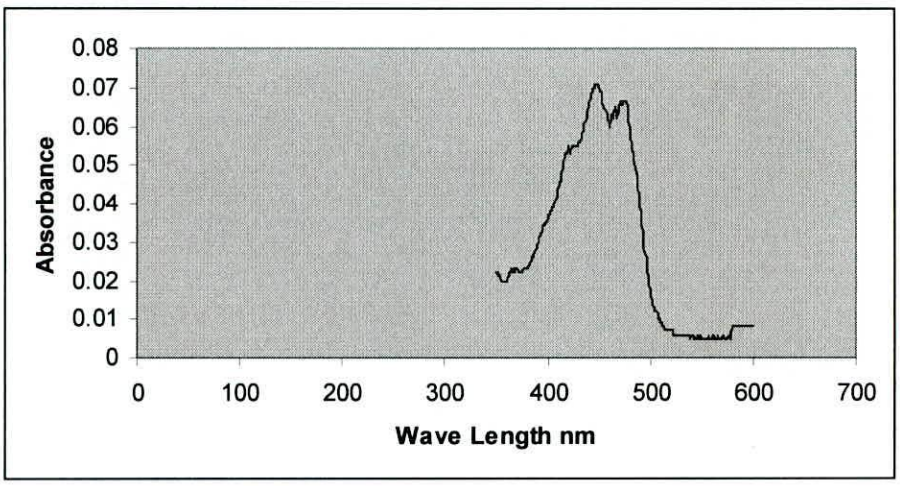

Figure 4.4

4.1.5 β-carotene content of fresh mango and osmotic dried sample after keeping one day (Table: 4.1)

Sample	Content of β-carotene
Fresh mango	0.292
Fresh mango	0.247
Fresh mango	0.305
30^0 Brix	0.240
30^0 Brix	0.240
30^0 Brix	0.296
40^0 Brix	0.276
40^0 Brix	0.236
40^0 Brix	0.287
50^0 Brix	0.260
50^0 Brix	0.229
50^0 Brix	0.282

4.1.6 β-carotene content of osmotic dried sample after keeping two weeks (Table:4.2)

Sample	Content of β-carotene
30^0 Brix	0.257
30^0 Brix	0.222
30^0 Brix	0.275
40^0 Brix	0.246
40^0 Brix	0.216
40^0 Brix	0.269
50^0 Brix	0.245
50^0 Brix	0.200
50^0 Brix	0.247

4.1.7 β-carotene content of osmotic dried sample after 4 weeks (Table: 4.3)

Sample	Content of β-carotene
30^0 Brix	0.090
30^0 Brix	0.079
30^0 Brix	0.100
40^0 Brix	0.088
40^0 Brix	0.078
40^0 Brix	0.093
50^0 Brix	0.062
50^0 Brix	0.041
50^0 Brix	0.086

4.2 Discussions

Separated first yellow colour band was identified as the beta carotene by getting the spectrum of the fraction in the UV-visible spectrophotometer. The shape of spectrum was compared with the reference spectrum of beta carotene. It confirms the initial separated fraction as beta carotene. After finishing the separation of beta carotene the next colourless fraction was also subjected to UV-visible spectrophotometer. This fraction was far more different from the spectrum of beta carotene. As a result it was easily calculate the beta carotene without any problem.

. The results were analyzed by using computer aided SAS statistical package. The analysis of variance was carrying out in randomized block design. The probability value of analysis of variance for osmotic dried samples and the fresh 'Karuthacolomban' mango samples was 0.4088. (ANOVA was done to find out if there is a significant difference between the three Brix solutions and the fresh raw mango samples.) The probability value is more than 0.05 at 5 % level of significance. As a result the null hypothesis was accepted. (i.e. H_0: there is no significant different between the beta carotene content of raw mango and osmotic dried mango, H_1: there is significant different between the beta carotene content raw mango and the osmotic dried mango.)

Alternative hypothesis was rejected therefore there is no any affect on the beta carotene content in the 'Karuthacolomban' mango variety with increasing Brix value of the sugar solutions. Changing sugar concentration in osmotic drying of 'Karuthacolomban' mango was not happened according to my results. But when considering effect of time factor for beta carotene content Karuthacolomban' mango when syruping is similar as it.

To find out if there is an effect on time factor for beta carotene content in 'Karuthacolomban' mango analysis of variance was done for results. (H_0: there is no

significant different with time on beta carotene content in 'Karuthacolomban' mango, H_1: there is a significant different with time on beta carotene content in 'Karuthacolomban' mango). The probability value of analysis variance for time factor was obtained as 0.0001. This probability value is less than 0.05 at 5 % level of significance. As a result the null hypothesis was rejected and alternative hypothesis was accepted. Therefore there is a significant effect on beta carotene and time factor in osmotic dried 'Karuthacolomban' mango.

It is important to find out, what extend effect on beta carotene is happened, in 'Karuthacolomban' mango under osmotic drying process. To prove that further mean separation is done according to Duncan's multiple range test. (DUNCAN) according to DUNCAN test lowest mean value was obtained to the beta carotene determined at four weeks time. The beta carotene content of fresh 'Karuthacolomban' mango after one day of osmosis and after two weeks of osmosis were not significantly different. But the beta carotene content of 'Karuthacolomban' mango after four weeks during osmosis has significant changed. Therefore when 'Karuthacolomban' mango were osmotically drying the significant changed was happened in beta carotene content of 'Karuthacolomban' mango after four weeks time. Up to four weeks the loss of beta carotene content of 'Karuthacolcmban' mango is not significant. But after four weeks the loss of beta carotene content was significant.

Carotenoid analysis can be performed for a number of reasons. A major reason for carotenoid analysis is to determined vitamin A value. Although more than fifty caroteniods possess vitamin A activity only ten are of real significance out these large amounts of carotenoids. Beta carotene is the most abundant and it theoretically yields two molecule of vitamin A per molecule. The other yields only one for one.

Therefore my study shows, what happened when "Karuthacolomban" mango keep in sugar surups. It gives an indirect clue for the vitamin A availability of fruits in sugar syrups. Due to sophisticated modern world, people don't have time to harvest fresh fruits from the trees. Nowadays trend is to buy everything from the market. In market there are various types of preserved fruit product such as jam, cordials, squashes, nectar and as sugar preservers. When consuming this type of preserved fruits, we can not expect all nutrients exist in the processed fruits as in fresh fruits.

My study shows there is a significant loss of beta carotene content in "Karuthacolomban" mango, after four weeks on osmoatically dried mango. It gives an idea about vitamin A content of the product.

A carotene present in the extraction can be largely destroyed by photochemical oxidation and the presence of chlorophylls makes carotene even light sensitive. Therefore neither initial extraction nor the chromatographic separation should be carried out in direct sunlight. Excessive aeration during extraction and subsequent washing of the extract should be avoided. The extract should be stored in the refrigerator when not being used. Also chromatographic separation and spectrophotometric determination should be carried out as soon as possible.

A sample for analysis must represent the material from which it is taken. Carotenoids are not uniformly distributed in tissues or present in all types of tissues that may be closely associated with each other. The nature and the quantity of carotenoids in plant tissue are usually a function of maturity and may vary from one part of a tissue mass to another. So when selecting the mango samples for analysis it should be selected a uniform and homogeneous as possible. In my study I always tried to select same colour, same matured

"Karuthacolomban" mango whenever possible. It helps to give uniformly of each and every sample.

The solvent used in the analysis must free of oxidizing compounds, acids and free of halogens. The colour of carotenoids is due to conjugated double bonds. Most naturally occurring carotenoids, posses a trans configuration for conjugated double bonds. These double bonds can easily oxidized and so solvent must free of oxidizing compounds

Filtration is a common part of carotenoid analysis and is frequently complicated by the presence of large hydrophilic molecules, which clog filters. Addition diatomaceous earth filter eliminate clogging.

The carotenoid content of one particular cultivar may different from another cultivar. My study was based on as assuming that mango samples having similar carotenoid content and the colour, maturity stage and the physical appearance is similar to each other.

Carotenoid content of any fruit commodity is affected by the climate or geographic site of production. These factors affect on carotenoid biosynthesis, so if one sample is coming one particular place for example from Anuradhapura may different from a mango coming from another place like Ambilipitiya.

Mango fruits are being easily perishable commodity the post harvest handling practices has significant effect on the overall quality of the fruits. Therefore from harvesting to consumption without knowing how it was handled is very difficult to predict the similarity of the mango samples. So always tired to select mango from one place and the overall appearance could have similar characteristics. Therefore, for my study I always bought mangoes from Petta market.

The temperature of mango is stored also effect on the carotenoid content of any fruit. If one particular mango is selected from a super market and another mango sample is selected from open market, the condition of storage is different from each other.

Light exposure to the fruit also affects on carotenoid in mango. Therefore it is important store mango without exposure to direct sun light. With sun light beta carotene is distrusted. Therefore I was tried always to select mangoes from the market without exposure to direct sun light.

Chapter 5

5.0 Conclusion

The beta carotene content of "Karuthacolomban" mango bas not altered in the process of osmotic drying even the sugar concentration is increased from 30^{0} Brix to 50^{0} Brix. When keeping the "Karuthacolomban" mango in sugar syrup for very short time there is no significant loss of beta carotene content. But when keeping the "Karuthacolomban" mango in sugar syrup long time (more than 4 weeeks) has a significant loss of beta carotene.

Therefore when somatically processed "Karuthacolomban" mango is kept for more than four weeks careful attention should be given to prevent the loss of beta carotene, because it is on the other hand, gives the vitamin A content of the fruit.

6.0 References

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7.0 Appendix

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Obs	days	sample	carotene
1	1	raw	0.292
2	1	raw	0.247
3	1	raw	0.305
4	2	30	0.240
5	2	30	0.240
6	2	30	0.296
7	2	40	0.276
8	2	40	0.236
9	2	40	0.287
10	2	50	0.260
11	2	50	0.229
12	2	50	0.282
13	14	30	0.257
14	14	30	0.222
15	14	30	0.275
16	14	40	0.246
17	14	40	0.216
18	14	40	0.269
19	14	50	0.245
20	14	50	0.200
21	14	50	0.247
22	30	30	0.090
23	30	30	0.079
24	30	30	0.100
25	30	40	0.088
26	30	40	0.078
27	30	40	0.093
28	30	50	0.062
29	30	50	0.041
30	30	50	0.086

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The ANOVA Procedure

Class Level Information

Class	Levels	Values
days	4	1 2 14 30

Number of observations 30

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The ANOVA Procedure

Dependent Variable: carotene

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.19884724	0.06628241	118.84	<.0001
Error	26	0.01450156	0.00055775		
Corrected Total	29	0.21334880			

R-Square	Coeff Var	Root MSE	carotene Mean
0.932029	11.64535	0.023617	0.202800

Source	DF	Anova SS	Mean Square	F Value	Pr > F
days	3	0.19884724	0.06628241	118.84	<.0001

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The ANOVA Procedure

Level of days	N	carotene Mean	carotene Std Dev
1	3	0.28133333	0.03043572
2	9	0.26066667	0.02524381
14	9	0.24188889	0.02483166
30	9	0.07966667	0.01809005

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The ANOVA Procedure

Class Level Information

Class	Levels	Values
sample	4	30 40 50 raw

Number of observations 30

The SAS System 22:48 Friday, June 11, 1993 6

The ANOVA Procedure

Dependent Variable: carotene

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.02205747	0.00735249	1.00	0.4088
Error	26	0.19129133	0.00735736		
Corrected Total	29	0.21334880			

R-Square	Coeff Var	Root MSE	carotene Mean
0.103387	42.29539	0.085775	0.202800

Source	DF	Anova SS	Mean Square	F Value	Pr > F
sample	3	0.02205747	0.00735249	1.00	0.4088

The SAS System 22:48 Friday, June 11, 1993 7

The ANOVA Procedure

Level of Sample	N	carotene Mean	carotene Std Dev
30	9	0.19988889	0.08552404
40	9	0.19877778	0.08706144
50	9	0.18355556	0.09373248
raw	3	0.28133333	0.03043572

The SAS System 22:48 Friday, June 11, 1993 8

The ANOVA Procedure

Class Level Information

Class	Levels	Values
Days	4	1 2 14 30

Number of observations 30

The ANOVA Procedure

Dependent Variable: carotene

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.19884724	0.06628241	118.84	<.0001
Error	26	0.01450156	0.00055775		
Corrected Total	29	0.21334880			

R-Square	Coeff Var	Root MSE	carotene Mean
0.932029	11.64535	0.023617	0.202800

Source	DF	Anova SS	Mean Square	F Value	Pr > F
days	3	0.19884724	0.06628241	118.84	<.0001

The ANOVA Procedure

Duncan's Multiple Range Test for carotene

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	26
Error Mean Square	0.000558
Harmonic Mean of Cell Sizes	6

NOTE: Cell sizes are not equal.

Number of Means	2	3	4
Critical Range	.02803	.02944	.03036

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	days
A	0.28133	3	1
A			
B A	0.26067	9	2
B			
B	0.24189	9	14
C	0.07967	9	30