Estimation of lycopene (A major carotenoid) in Tomato (*Lycopersicon esculentum*) in different conditions of ripening and storage.

By

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Thesis submitted in partial fulfillment of the requirement for the degree of bachelor of Science in Food science and technology of the faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, Sri Lanka.

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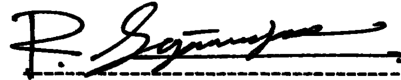
Declaration

The work is describe in this thesis was carried out by me at the faculty of Applied science and Food research unit Gannoruwa under the supervision of Dr.K.K.D.S.Ranaweera and Dr. (Mrs.) S.F.Hussain. The report on this thesis has not been submitted to any other university for another degree.



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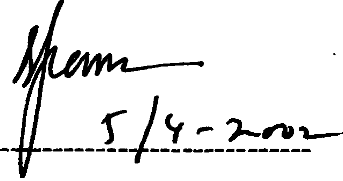

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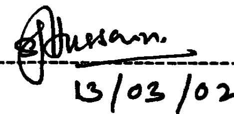

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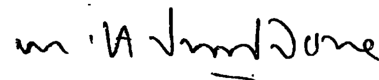

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To my Parents & teachers

For all the good things they taught to me.

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Abstract.

Colour of tomato (*Lycopersicon esculentum*) is one of the most important quality factor. That is mainly depend upon the quantity of carotenoid available in the fruit. Lycopene is the simple carotenoid found in tomato.

In tomatoes lycopene biosynthesis increase dramatically during the ripening process. Temperature is one of the most important factors for the biosynthesis of lycopene in tomato. There for the objective of this study was to identify the optimum ripening condition of lycopene and estimation of it.

Two common varieties *Thilina* and *Marglobe* were ripened in ambient conditions (23 – 35 ^{0} C and 85-95% Rh) and cold room conditions (15 – 18 ^{0} C and 85-95% Rh). Sets of preliminary quality were checked to identify the colour, pH, Moisture and TSS after ripening process was completed. Juice, puree and powder was prepared from well ripened fruits.

Statistically evaluated results said that cold room ripening has a trend of lycopene development. *Marglobe* showed 10 to 12 % of lycopene development in cold room ripening and variety *Thilina* showed 4 to 6% of lycopene development than Ambient ripening condition. Quantitatively verity *Marglobe* has high amount of lycopene than *Thilina.* Reduction of lycopene in tomato powder and puree is depend on the amount of isomaration and auto oxidation.

Therefore maximum development of lycopene is only carried out at low temperature, unless it converts to other forms of carotenes. Control the temperature at harvest and storage is essential for maximum development lycopene.

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Chapter1.

Introduction.

Tomato (***Lycopersicon esculentum***) belongs to the genus ***Lycopersicon*** of the family ***Solanaceae***. Usually it is herbaceous, annual to perennial, prostrate, and sexually propagated. Occasionally, however, it is also asexually propagated. A full-grown plant can produce a large number of seeds. Tomato has become one of the most popular and widely cultivated vegetables in the world due to the several reasons. Tomatoes are grown at many latitudes and under a wide range of soil types, with different climatic conditions. Tomato is not particularly nutritious, but it can be a major source of minerals and vitamins if its consumption is encouraged (Ngym, 1999).

Tomato is accepted by the consumer according to its unique sensory qualities including flavour, aroma, colour and other sensory attributes. The colour of tomato fruits and its products is the most important quality factor. It is probably the first character to be captured by the consumer's eyes. The degree of colour quality practically represents an essential component of the total quality. Therefore it is very essential that the fruit or tomato based product contain a standard colour which is familiar to the consumer which should satisfy the consumer.

Colour of the tomato mainly depends upon the quantity of carotenoid amount available in the fruit itself. As far as carotenoids found in tomato are concern lycopene is the principle member. Lycopene is a phyto chemical synthesised by plants and microorganisms. This pigment, after ingestion with tomato-based products, can be found in human serum and other vital organs (Stal and Sies 1996).

Lycopene is predominantly found in the chromoplast of plant tissues. Lycopene can be characterized into two types; globular chromoplast containing mainly β carotene and located in the jelly part of the pericarp and chromoplast in the outer part of the pericarp contain voluminous sheets of lycopene (Mac Gilvery et al, 1929). In tomato, lycopene biosynthesis is increased dramatically during the ripening process because at this stage, chloroplasts are converted to chromoplasts.

It has been found that serum level of lycopene and dietary intake of lycopene through tomato and its products were inversely related to occurrence of certain types of cancer such as prostate cancer, digestive track cancer and lung cancers and heart diseases-athrogenasis. Lycopene, with its ability to act as an antioxidant and scavenger of free

radicals that are often associated with carcinogenasis, is potentially a key compound in preventing such cancers thereby being beneficial for the human health. It may also inhibit the formation of LDL cholesterol level in the blood. Therefore the amount of lycopene in diet has a lot of positive effects to human body. (http \\ www:lycopene.com)

Different storage conditions differently affect the production of lycopene both in flesh and peel. Temperature is the most important factor to affect the biosynthesis of carotene including lycopene. Generally the optimum temperature for carotenogenesis in plants is relatively low. The synthesis of certain pigments was shown to be temperature sensitive varieties from plant to plant. At higher temperature, it is not formed and fruits become yellow (Gaylord, *et* al 1981).

In order to ensure the stability of both quality and quantity of lycopene and to minimize the loss of colour in the fruit, storage conditions are to be controlled. It is also necessity to study the effect of environmental factors such as temperature on the biosynthesis of lycopene in tomato.

The present study was aimed at finding out the effect of temperature on development of lycopene and estimating it. Experimental part of the study was conducted at Department of Agriculture, Food Research Unit, Gannoruwa and at the Food Laboratory in Faculty of Applied Science. Buttala.

In this study, two common varieties were used (***Marglob*** and ***Thilina***) and tomato based products namely tomato juice, puree and powder were investigated for lycopene content.

Objectives of the study were;

1. To check the preliminary parameters on different storage conditions under
    - Ambient ripening.
    - Cold room ripening
2. To estimate lycopene content under different storage conditions.
3. Find out the best variety for the maximum development of lycopene.
4. Estimation of lycopene retention in different tomato products.

Chapter 2.
Literature Review.

2.1 Botany of tomato.

Tomato belongs to the genus ***Lycopersicon*** of the family Solanaceae. This genus includes many species including some polymorphic types. Usually it is herbaceous, annual to perennial, prostrate, and sexually propagated. Occasionally, however, it is also asexually propagated. A full-grown plant can produce a large number of seeds per time (Word G.M. 1964)

2.1.1 Origin and Distribution.

Although the tomato's origin and the early history of its domestication are obscure, the weight of evidence suggests that Mexico was the probable center of origin from where it was transported to Europe and Asia. The most likely ancestor is the wild cherry tomato (*Lycopersicon esculentum)* found first throughout tropical and sub tropical American and then the tropics of Africa (Word G.M. 1964).

2.1.2 The fruit.

The fruit is a soft berry. Glandular hairs and glands are usually present on the small fruit that degenerate in the advanced stage. The fruits of some varieties do not have glandular hairs and glands. There are various shapes of fruit.

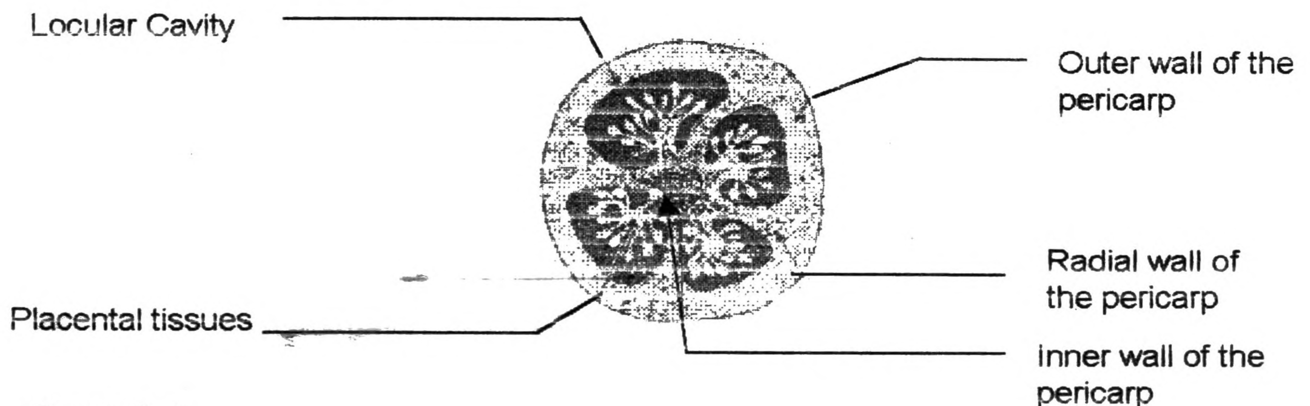


Figure 2. 1
Cross- section of tomato.

The transverse section of the fruit (in figure 2.1) indicates that there are five main parts in the fruit walls (outer and inner) skin locular tissues, pulp and seed. The pericarp constitutes the wall of the fruit and to considerable extent the quality of the fruit depends on the pericarp. The layers of the mesocarp increases the size of the cell resulting in the growth of the pericarp there is much variation in the number of locules. The radial wall of the pericarp known also as columella divides the locules. Each locule has a lot of seeds and the jelly like substance surrounds the seeds. The substance consists of parenchymatous cells that develop round the ovule. Jeana Gross (1978),

2.2 Tomato Composition.

Tomato is a rich source of minerals and vitamins. There are various types of flavouring compound found in the fruits that enrich their taste. The attractive red colour of the fruit is due to lycopene, and the yellow colour is due to carotenes. Carotenes are a good source of vitamin A. The tomato fruits comprise mainly pericarp, radial wall, and locular tissues. The chemical composition of these parts is influenced by the variety, the age of the fruits, and certain external and internal factors. (Williams and Bevenue, 1954)

Table 2.1: Nutritive values of raw and processed tomatoes.
(100-gram of edible portion)

Nutrient	Raw	Canned*	Catsup	Juice
Water (%)	94	94	69	94
Food energy (cal)	19	21	106	19
Protein (g)	0.7	0.8	1.8	0.8
Fat (g)	trace	trace	0.4	trace
Carbohydrate (g)	4	4	25	4
Calcium (mg)	12	6**	22	7
Phosphorous (mg)	24	19	50	18
Iron (mg)	0.4	0.5	0.8	0.9
Potassium (mg)	222	217	363	227
Vitamin A value (i.u.)	822	900	1399	798
Thiamin (mg)	0.05	0.05	0.09	0.05
Riboflavin (mg)	0.04	0.03	0.07	0.03
Ascorbic acid (mg)	21	17	15	16

* solids & liquieds
** applies to products without adding calcium salts

source USDA home and garden bulletin, no. 72.

2.2.1 Pigments and Colour.

The red colour of the tomato is due to availability of major carotene, lycopene which is found at least up to 90% of the total carotenoid content. The total carotenoid content of tomato varies between 70 -190 μg (g fresh wt). Carotenoids are predominantly carotenes, consisting of 90 to 95 % out of the total carotenoids. The common red tomato also contain the colourless precursors phytone and pyhtofluene (15 – 30 %) and minor pigments such as β carotene, ζ carotene, γ carotene and neuresparone.

The carotenoids are not uniformly distributed within the fruit. The outer pericarp has the highest concentration of pigments. β Carotene is equally distributed throughout the fruit, lycopene predominate in the flesh in which the total carotenoid contain is six fold higher than in the pulp (Olsion M.k et al, 1978).

2.3 Lycopene.

2.3.1 Importance of Lycopene.

Lycopene, a carotenoid in the same family as beta-carotene, is what gives tomatoes, pink grapefruit, apricots, red oranges, watermelon and guava their red color. Lycopene is not merely a pigment. It is a powerful antioxidant that has been shown to neutralize free radicals especially those derived from oxygen, thereby conferring protection against lots of diseases. In addition, preliminary research suggests lycopene may reduce the risk of macular degenerative disease, serum lipid oxidation and blindness in old age risk of exercise-induced asthma (EIA) and cancers of the lung, bladder, cervix, and skin. (www//Biocem.com)

Plants and microorganisms synthesize Lycopene but not by animals. It is an acyclic isomer of beta-carotene. This highly unsaturated hydrocarbon contains 11 conjugated and 2 unconjugated double bonds, making it longer than any other carotenoid. Human cannot produce lycopene and must ingest from fruits and vegetables then absorb the lycopene, and process it for use in the body. (Bern et al .(1987).)

Lycopene
Molecular Weight = 536.89
Exact Mass = 536
Molecular Formula = $C_{40}H_{56}$
Molecular Composition = C 89.49% H 10.51%

Fig 2.2: Structure of lycopene.

Although best known as an antioxidant, both oxidative and non-oxidative mechanisms are involved in lycopene's bio protective activity. Since lycopene lacks a beta-ionone ring structure, it cannot form vitamin A. Lycopene configuration enables it to inactivate free radicals. Because free radicals are electrochemically unbalanced molecules, they are highly aggressive, ready to react with cell components and cause permanent damage. Oxygen-derived free radicals are the most reactive species. These toxic chemicals are formed naturally as by-products during oxidative cellular metabolism. As an antioxidant, lycopene has a good-oxygen-quenching ability twice as high as that of beta-carotene (vitamin A relative) and ten times higher than that of alpha-tocopherol (vitamin E relative).

Lycopene participates in a host of chemical reactions hypothesized to prevent carcinogenasis and atherogenesis by protecting critical cellular biomolecules, including lipids, proteins, and DNA.(www \Lycored.com)

Lycopene is the most predominant carotenoid in human plasma, present naturally in greater amounts than beta-carotene and other dietary carotenoids. This perhaps indicates its greater biological significance in the human defense system. Its level is affected by several biological and lifestyle factors. Because of its lipophilic nature, lycopene concentrates in low-density and very-low-density lipoprotein fractions of the serum. Lycopene is also found to concentrate in the adrenal, liver, testes, and prostate. However, unlike other carotenoids, lycopene levels in serum or tissues do not correlate well with overall intake of fruits and vegetables. (Davis. 1997).

Scientists believe that excess oxidative stress plays an important role in the initiation and cancer and other degenerative diseases. Exposure to environmental perils, such as smoking, pollution or irradiation, increases the oxidative stress beyond the ability of the organism's defense system to fight with it. The natural mechanism, which protects us from free radicals, weakens with age. Therefore, the elderly, smokers and those exposed to environmental hazards, are more susceptible to degenerative diseases. (Clinton, -S.K.1998).

2.3.2: Absorption of lycopene.

Research shows that lycopene can be absorbed more efficiently by the body after it has been processed into juice, sauce, paste, or ketchup. In fresh fruit, lycopene is enclosed in the fruit tissue. Therefore, only a portion of the lycopene that is present in fresh fruit is absorbed. Processing fruit makes the lycopene more bio available by increasing the surface area available for digestion. More significantly, the chemical form of lycopene is altered by the temperature changes involved in processing to make it more easily absorbed by the body. Also lycopene is fat-soluble (as are vitamins, A, D, E, and beta-carotene), absorption into tissues is improved when oil is added to the diet. Although lycopene is available in supplement form, it is likely there is a synergistic effect when it is obtained from the whole fruit instead, where other components of the fruit enhance lycopene effectiveness. (www/lyco on helthwell.com).

Table 2.2: Approximate Lycopene Content of Various Foods.
(mg/100g of wet weight)

Apricot, dried	0.86	Tomato sauce	6.20
Grapefruit, raw pink	3.36	Tomato paste	5.40-150.00
Guava, fresh	5.40	Tomato soup, condensed	7.99
Guava juice	3.34	Tomato powder, drum or spray dried	112.63-126.49
Papaya, fresh	2.00-5.30	Tomato juice	5.00-11.60
Tomatoes, fresh	0.88-4.20	Sun-dried tomato in oil	46.50
Tomatoes, cooked	3.70	Watermelon, fresh	2.30-7.20

Source: Clinton, -S.K.1998. Lycopene: Chemistry, Biology, and Implications for human health and disease, Nutrition Review, 56(2) P35-51.

2.4 Biosynthesis of colour pigments.

2.4.1 Pigment changes during ripening.

Tomato is one of the most important carotenogenic fruits. During ripening the colour of the red tomato turns gradually from green to red as chloroplast are transformed to chromoplast, chlorophylls disappear, and carotenogenasis is taking place.

Green fruits under light conditions contain chlorophyll a and b; however, under dark conditions, they remain colourless; both types undergo change to red colour formation under favourable conditions (Kader. 1978) Green to white to yellow to Pink to red.

Carotene were followed in unripe, half ripe and fully ripe fruits. Concentration of all pigments increased, particularly lycopene, which showed a 70-fold increase and β carotene, which showed a lower 5-fold increase (Jhan. 1975)

2.4.2 Molecular changes in ripening.

The chloroplasts of mature green fruits of the cultivated varieties are starchy and vacuolated. The primary major components of mature green fruits are chloroplasts, which undergo various step-wise changes resulting in chromoplast or red colour. As chlorophyll decreases, the fruits become colourless, pale yellow and finally red under favourable conditions. The grana part of the chloroplast plays a vital roll in the formation of red colour.

The osmophilic globules yellow in colour appear first as carotenoid. Later in the granum part the carotenoids are synthesized and in the advanced stage likewise a large crystal of lycopene is synthesized covering a major part of the chromoplast. Lycopene crystalloids are formed in association with thylakoid. By ripening lycopene content is remarkably increased (Table 2.3).

Table 2.3: Changes of major carotenes in ripening of Marglobe.

	Percent of total Carotenoid.					
Carotenoid Pattern	**RS1**	**RS2**	**RS3**	**RS4**	**RS5**	**RS6**
Phytone	-	-	20	10.7	10.8	9.0
Phytofluene	-	-	2.5	2.1	2.6	2.4
β carotene	100	75.0	55.0	17.0	9.8	2.6
Lycopene	-	16.7	10.0	64.3	72.9	84.7
Total carotene	1.2	2.4	4.0	14.0	30.6	97.7

*RS= Ripping Stage. 1= Mature green; 2= Break; 3= Turning; 4 =Pink; 5=light red; 6=red.

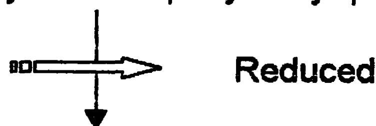
2.4.3 Biosynthetic path way of lycopene.

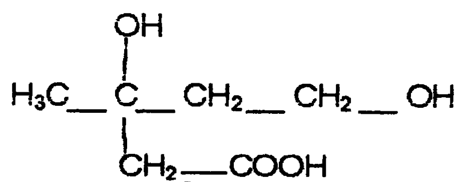
Porter and Anderson (1977) have described the detailed procedures of the biosynthesis of carotenoids and lycopene. In plants, carotenoid C_{40} tetraterpenses are biosynthesised by the terpenoid pathway. Terpenoid compounds are built up from C_5 Mevalonic acid. Mevalonic acid (MVA, 3,5-dihydroxy-3-methylpentanoic acid) is the first specific terpenoid precursor, which, in turn, is converted into carotene and lycopene. In the carotenoid biosynthesis, the terpenoid chain is built up to the C_{20} level, and two C_{20} units condense to give the typical C_{40} carotenoid skeleton. Lycopene biosynthesis involves in fore major stages:

1. Formation of Mevalonic acid.
2. Formation of Geranyl Geranyl pyrophosphate.
3. Formation of Phytone.
4. Desaturation of Phytone.

2.4.3.1 Formation of Mevalonic Acid.

Mevalonic acid (MVA) itself is formed through the condensation of three molecules of acetyl CoA, acetoacetyl CoA and β-hydroxy β methylglutaryl CoA, the latter being reduced to MVA. (fig 2.3)





Mevalonic Acid

Fig 2.3: Formation of mevelonic acid

The biological isoprene precursor of carotenoids and all terpenoids is isopentenyl pyrophosphate (IPP). It is formed from mevalonjc acid (MVA), which is successively phosphorylated into mevalonjc acid-S-phosphate and 5-pyrophosphate by kinase enzymes and ATP. MVA-5-pyrophosphate is then decarboxylated to give the isopreneunitIPP (fig 2.4)

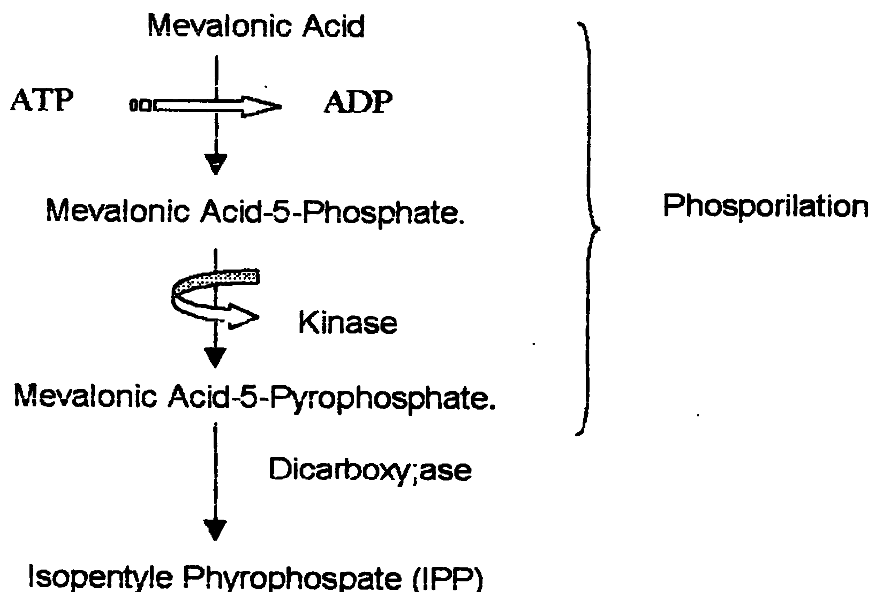


Fig 2.4: Formation of Isopentyle Phyrophospate

2.4.3.2 Formation of Geranyl Geranyl Pyrophosphate.

The chain elongates from IPP (C_5) to geranyl geranyl pyrophosphate (C_{20}), which is catalysed by the prenyl transferase enzyme.

IPP is first isomerised into dimethylallyl pyrophosphate (DMAPP). DMAPP undergoes condensation with IPP to give the C_{10} intermediate. geranyl pyrophosphate (GPP). Successive addition of 2 molecules of IPP gives the C_{15} intermediate farneslyl pyrophosphate (FPP) and the C_{20} geranylgeranyl pyrophosphate (GGPP). GGPP may be used to form diterpenes such as phytol, the side chain of chlorophyll, or C_{40} carotenoid.(Fig. 2.5).

Isopentenyl pyrophosphate isomerase, which catalyses the isomerisation of IPP into DMAPP, and prenyl transferase, which catalyses the successive condensation of 3 molecules of IPP with DMAPP, GPP, and FPP, both present in tomato plastids (Spurgeon et al., 1984).

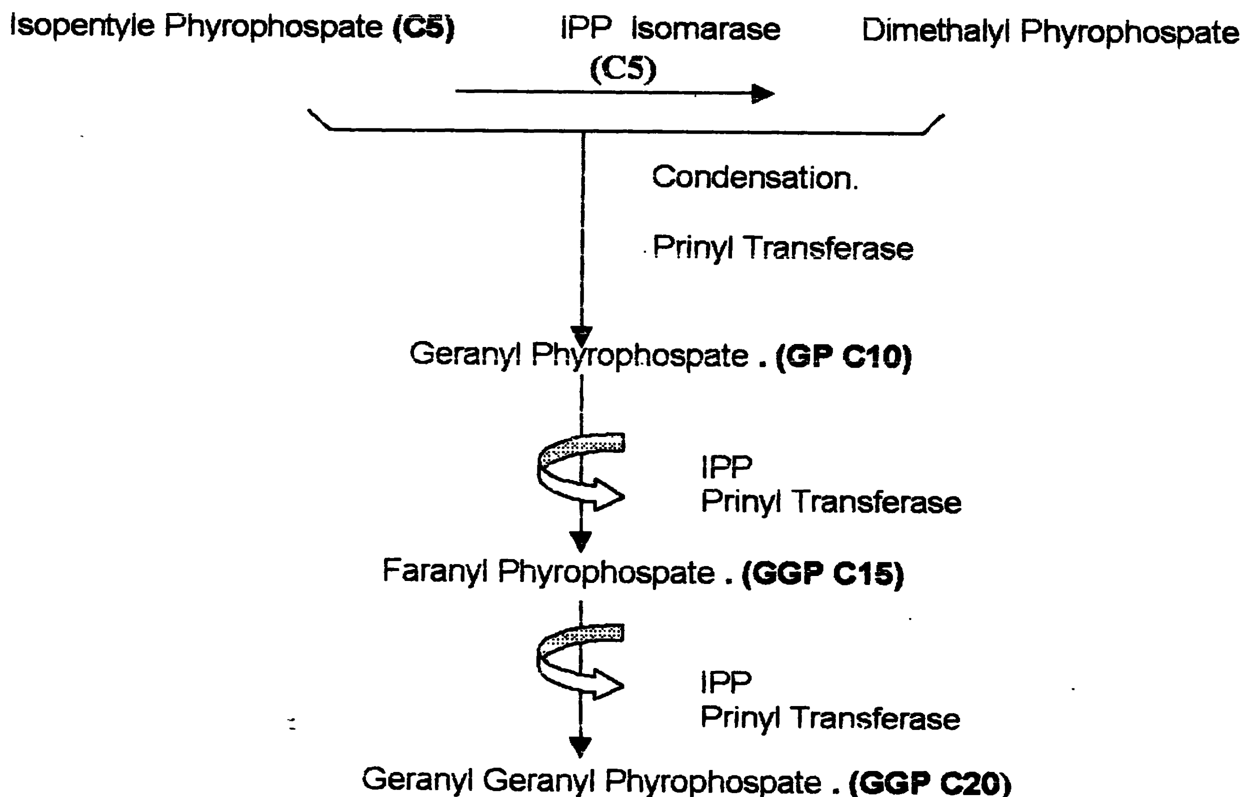


Fig 2.5: Formation of Fig Geranyl Geranyl Phyrophospate.

2.4.3.3 Formation of Phytoene.

The first step unique to carotenoid biosynthesis is the condensation of 2 molecules of GGPP to form phytoene. Phytoene is formed via the C_{40} cyclopropane intermediate, prephytoene pyrophosphate (PPPP) (Jordan., 1978). In higher plants 15-cis-phytoene is generally found, the *trans* isomer occurring in other organisms. The formation of one isomer or another depends on the stereochemistry of the hydrogen removal involved in the condensation of GGPP (Fig 2.6).

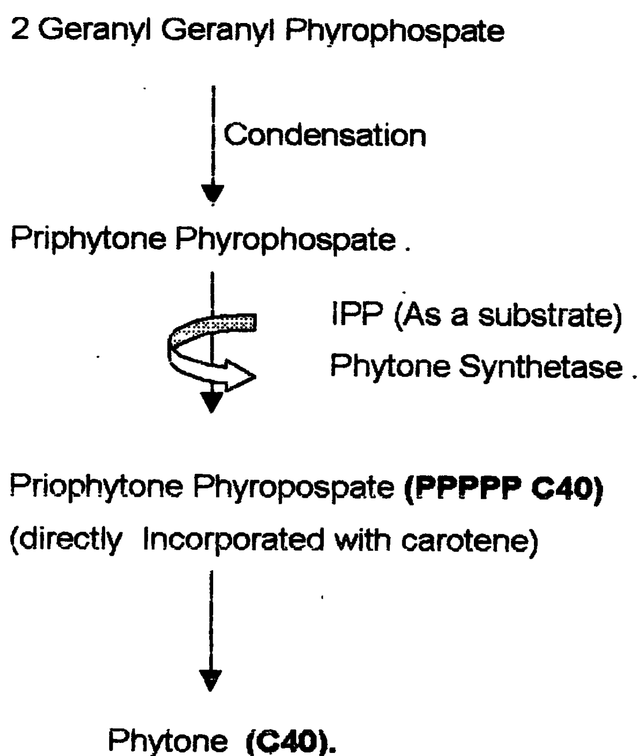


Fig 2.6: Formation of Phytone

2.4.3.4 Desaturation of Phytone.

From phytoene, according to the Porter-Lincoln pathway, lycopene is formed through stepwise dehydrogenation. Through each dehydrogenation sequence the chromophore is extended by two double bonds alternately from either side. Phytoene is converted into lycopene, via phytofluene, ξ-carotene, and neurosporene (fig 2.7). Since all the coloured carotenes are *trans* isomers, a *cis-trans* isomerisation must occur at the phytoene or phytofluene level. (Spurgeon.,1984)

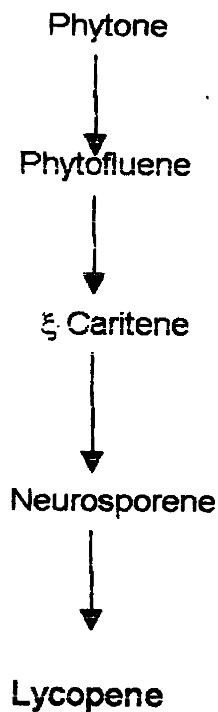


Fig 2.7: Formation of lycopene

2.5 Factors affecting the biosynthesis of carotenoid.

2.5.1 Photohormones.

The different effects of photohormones, which also involve pigment changes. Plant carotenoids are influenced reversibly by various endogenous and exogenous plant growth regulators.

Ethylene, the fruit-ripening hormone, is extensively used to promote ripening. The quality of ethylene-ripened tomatoes, including the carotenoid changes, was repeatedly investigated (Davis ,1986). One effect was an increased lycopene content in some varieties. Veegan tomatoes, picked at 90% development and treated with ethylene, showed an accelerated rate of chlorophyll degradation and a rapid synthesis of carotene (Spurgeon,1984). The same effect of ethylene was observed in a range of varieties, but the reaction to ethrel differed among cultivars.

2.5.2 Light.

Carotenoids are synthesised within fruits, tubers, and roots where only low light intensity can penetrate. For practical reasons the influence of light on colour development in tomatoes was thoroughly studied. It has been shown that with red light being more effective than white or green light (Kader, 1978).

In fact, red-light-induced carotenoid synthesis and far-red light inhibited it in ripening tomatoes, thus suggesting a phytochrome-mediated response (Kader, 1975a,b). Comparative effects of light and ethephon on the ripening of detached tomatoes indicated that carotenoid biosynthesis is light-dependent and that ethephon has no effect on the total carotenoid content (Olsion and Len, 1976).

2.5.3 Temperature.

Generally the optimum temperature for carotenogenesis in plants is relatively low. The synthesis of certain pigments has been shown to be temperature sensitive: the temperature sensitivity varies from plant to plant. Temperature sensitivity was studied thoroughly in connection with lycopene formation in tomato. Very early, the effect of environmental factors on tomato colour was studied (Vogele, 1937). The optimal temperature for lycopene formation in the tomato is 16°C to 21 °C; above 30°C it is not formed. Detached fruits, ripened at temperatures between 32°C and 38°C, became yellow; after being returned to lower temperatures (20—24°C), they developed normally (Went et al, 1942).

Koskitalo and Ormrod (1972) investigated the influence of sub-optimal temperatures on colour quality and pigment composition of ripening tomatoes. Fruits exposed to a diurnal regime of 18°C to 26°C had the highest pigment content, with lycopene accounting for 90% of the carotenes At this regime the carotenoid biosynthesis rate was highest, whereas in fruits exposed to a diurnal regime of lower temperature the ripening process was delayed.

Neuresparone is the major precursor of all carotenoids. In different environmental conditions it changes the original characteristics. At higher temperatures (20 – 32°C) neuresparone produce β-ring structure, as well as in 20 – 25 it produce e – ring structure respectively. Under low temperature neuresparone produce lycopene. The optimum temperature for the development of lycopene is in-between 15 – 22 ° C (Olsion, 1978).

The monocyclic intermediates of ε- and β-carotene are δ-carotene and γ-carotene, respectively. Cyclization may also occur at the neurosporene level, as indicated by the existence of α-zeacarotene and β-zeacarotene. Cyclization occurs in carotenes that have at least a double bond at C-7,8.

At lower temperatures, only lycopene can be developed properly unless it is converted either to γ carotene or δ carotene under present temperature. Therefore, fruits should be kept in correct temperatures during the harvest, storage and handling in order to keep bright red colour in the fruit. If γ carotene or δ carotene present in the chromoplast the other reactions happens spontaneously. Both γ carotene and δ carotene have an ability to produce α-carotene, but the most predominant reaction is the formation of β--carotene from γ carotene (Camara and Dogbo, 1986) (Fig. 2.8).

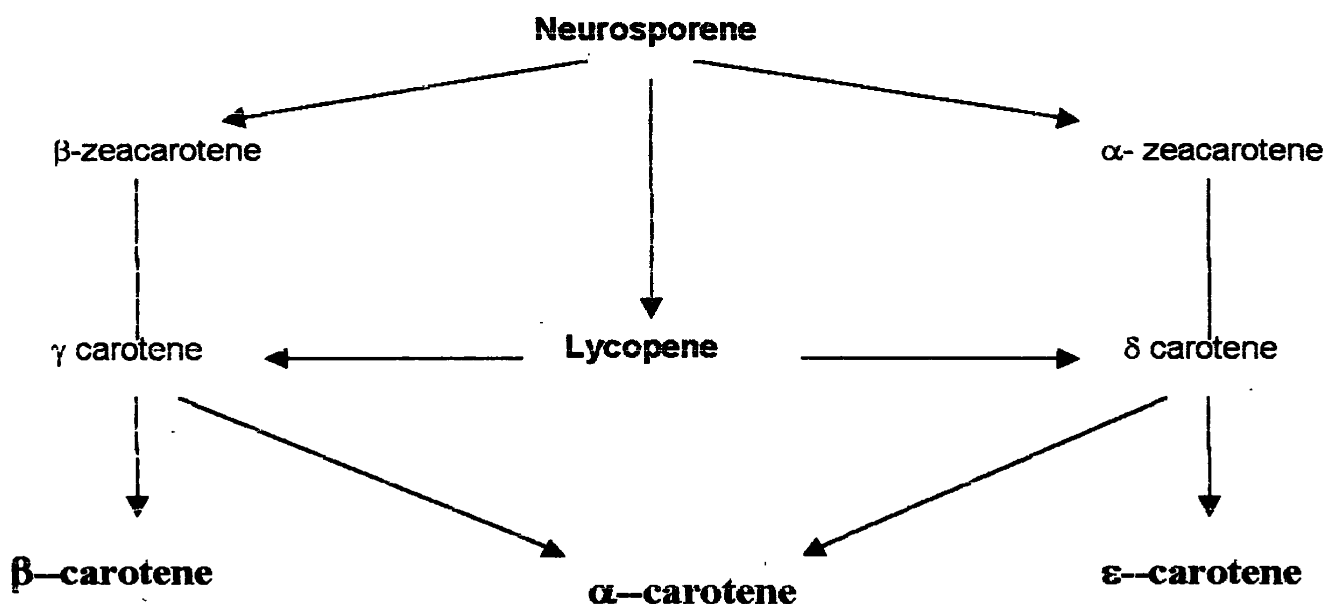


Fig 2.8: Temperature effect on carotenogenasis

2.6 Changes of carotene During Storage and processing

Changes in β-carotene and lycopene levels, during a long storage of tomatoes were investigated (Dalal et al, 1978).The fluctuations differed among cultivars. The content of β-carotene was unchained during first week, but lycopene decreased more rapidly up to 10% of the initial content at the end of the storage period.

2.6.1 The effect of dehydration and temperature.

The oxidative destruction of lycopene during the manufacture of tomato puree was studied. The factor affecting lycopene losses during the concentration of tomato juice seemed to be the length of heating rather than the temperature (Mac, 1995)

The stability of lycopene during heating of tomato pulp was studied (Cole and Koper, 1954). The rate of lycopene breakdown varied according to availability of oxygen, temperature and intensity of illumination. Heating tomato pulp at 100 ^{0}C in daylight in the presence of oxygen led to a loss of 15% of lycopene after 1h and 33% after 3h. After heating for 3h in the presence of oxygen and in light, the loss increased with temperature: 22% and 57% in lycopene tomato pulp.

During storage of tomato puree, both the contents of β-carotene and lycopene decreased. One year of storage reduce the content of both pigments to half of the initial values, which were 190 and 380 $\mu g\ g^{-1}$ *cis-trans* isomeration of lycopene and colour stability of form-mat-dried tomato powder during storage were studied. The main cause of colour fading was the oxidation of lycopene as well as trans-cis isomeration of lycopene. Excessive desiccation strongly promoted oxidation losses. Storage in an inert atmosphere improved colour retention. (Spurgeon et al 1984).

2.7 Properties of lycopene.

2.7.1 Physical properties.

Carotenoid are soluble in lipids that is, they are liposoluble and in fat solvents, such as acetone alcohol diethyl ether, and chloroform. The Carotenes are soluble in apolar solvents such as petroleum ether and hexane, Except for the most unsaturated carotenes (phytoene, Phytofluene, and i-carotene), the Carotenoid are solids at room temperature and can be crystallized in various forms,

2.7.2 Spectroscopic Properties.

Carotenoids can specifically absorb light in the ultraviolet (UV) and visible region of the spectrum. Rest is transmitted or reflected, and they appear colored. The structural feature responsible for light absorption is the chromophore, which in Carotenoid is the system of conjugated double bonds. Each Carotenoid is characterized by an electronic absorption spectrum. In Consequence, absorption spectroscopy is an important technique in tomato. The progressive bathocromic shift (movement to longer wave length) is illustrated by the absorption spectra of the acyclic Carotenoid of increasing chromophore length. (fig 2.9)

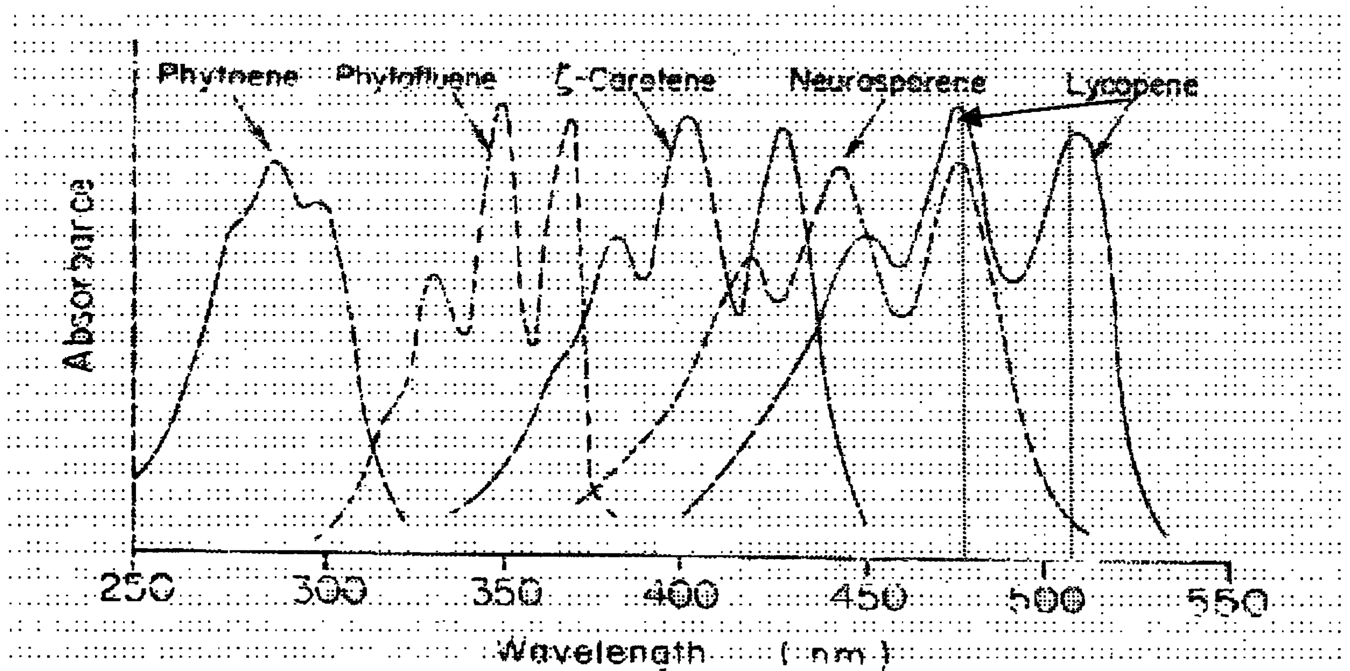


Fig 2.9: Absorption Maximal of carotenes.

Carotenoid absorb mainly in the blue (430 – 470 nm) region, but they also absorb in the blue region (470 – 500 nm) and green (500 – 530 nm) region of the spectrum. The solvent used influence the position of the absorption maximal, petroleum ether and methanol have little or no effect. Lycopene shoes the maximum absorption at 473 and 503 nm.

2.7.3 Chemical properties.

Natural Carotenoids (lycopene and beta carotene) consist of carbon, hydrogen, and oxygen. They are hydrocarbon or derivatives of hydrocarbons. Those are consisting isoprene unit. Because the functional groups are oxygen functions, their characterization can be carried out on a spectroscopic scale requiring only small amount of sample, and a usually routine analysis. due to the system of conjugated double bonds in the molecule, the lycopene (a major carotenoid in tomato) are easily destroyed by oxidative degradation.

2.7.3.1 Chemical Oxidation.

The various oxidants are oxygen, ozone, alkaline permanganate, chromic acid. The nature of degradation products depend on the location at which the attack occurs. By ozonolysis, oxidative cleavage of the carbon-carbon bond occurs, and the form carboxylic acid allow the characterization of the carotenoid end group.

2.7.3.2 Auto oxidation.

Auto oxidation is the spontaneous combination of substance with oxygen in the air at room temperature. Both in solution and crystallized. The carotenoid undergo auto oxidation in the presence of oxygen, a free radical chain process. The process is stimulated by temperature, light, humidity and some metals. Because lycopene oxidation occurs widely during processing and storage.

2.7.3.3 Effect of water content.

It is known that carotenoids are more rapidly oxidized in dehydrated products, because water attached on the food surface forms a protecting film. Agrawal studied the effect of water on carotenoid stability in low moisture system. Presence of antioxidant inhibits the rate of oxidation. The presence of oxygen in the headspace is a crucial factor in lycopene degradation. Even a low concentration of 1% to 2 % leads to a significant loss of pigment. The presence of free radicals also accelerate the degradation rate

2.7.3.4 Photo oxidation (light effect).

Photo oxidation is the oxidative bleaching produce by the oxygen in the air. The kinetics of carotenoid photodegradaiton in a tomato juice were studied (Boskovicl 1979). The photo degradation followed first order kinetics, And alpha and beta carotene were degraded much faster than lycopene.

2.8 Analytical Method.

Analytical method have been describe by S. Rangana (1985).The analysis of carotinoid is complicated because of their instability, their tendancy to undergo stereomutation, their photo and thermolability, and the readiness with which they undergo oxidation. Carotenoids are altered by acid and partially destroyed enzymatically, especially by lipoxiganase.

Solvents must be purified, the temperature no higher than 40 °C, and samples must be stored at about –20 °C .Estimation of Carotenoids involve extraction, Separation by chromatography and spectroscopic data analysis on the basis of absorbency.

2.8.1 Extraction.

Materials should be fresh and undamaged, and a sample of it must be representative. Extraction should be done as rapidly as possible to ovoid oxidative or enzymatic degradation. Before extraction the material must be ground or cut in to small pieces to facilitate complete extraction. Since the lycopene liposoluble they are extracted with organic solvents. All solvents used must be pure and free of oxidizing compounds, acids or halogens.

For fresh tissues, which contain a high percentage of water miscible polar organic solvents (acetone, methanol, ethanol, usually a mixture of acetone and methanol) are used. Dry material must be extracted with water – imsicible solvents such as diethyl ether or petroleum ether. The extraction procedure is repeated until the pigment is totally extracted.

2.8.2 Chromatography.

Chromatography is the most important technique for separation and purifying Carotenoids. A multitude of absorbent is used for carotenoid chromatography, among them sucrose, cellulose, starch, $CaCO_3$, MgO, $ZnCo_3$, Al_2O_3, Silica gel, kieselghur, which are all used as mixtures. The absorbents are used according to the type of Carotenoid to be separated, because different absorbents achieve the separation in different ways. Thus, group of different polarity hydrocarbons, monohydroxy and polyhydroxy carotenoids can be

separated on alumina or silica, with the most polar carotenoids being more strongly adsorbed.

For MgO and $Ca(OH)_2$ for example separation is determined by the number and type of double bond in the molecule. In the carotene series lycopene is the most strongly absorbed and is followed by neuresparone and ξ- carotene . The column may be pack with adsorbent such as alumina or silica gel as a slurry in petroleum ether. The pigments are eluted with solvent of increasing polarity (diethyl ether in petroleum ether). The polarity of the solvent is changed after the colored band is completely eluted. Open column chromatography on MgO and super cell (1:3 by weight) is the most widely applied method for investigation carotenoid in vegetables.

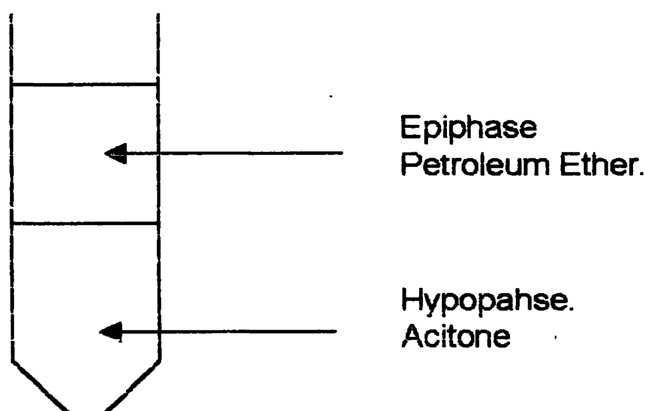


Fig 2.10: Phase separation

Phase separation (fig 2.10) is used to obtain a band separation according to it's molecular weight so called this is size exclusion chromatography. It has been applied in the carotenoid investigation in tomato juice, tomato puree and tomato powder (Rymal and Nakyaman, 1974 *et al*) . separation in to Epiphasic and hypophassic carotenoid may be achieved by solvent partition between petroleum ether (PE) and aqueous methanol (85 – 95 %). When the PE extract is shaken in a separating funnel with an equal volume of aqueous methanol the epiphase (PE) contain the nonpolar compounds namely chlorophyll, carotenoids and their epoxides, and the totally esterified carotenols, monocarotenoids, monoketones and monoles are evenly distributed in both phases. Polar xanthophills, diols and polyois are found in the Hypopahse methanol.

2.8.3 Identification.

Carotenoids can be identified on the basis of chromatography and spectroscopic behavior. The length of the chromophore and the polarity of the functional group are determined by the absorption affinity. The slightly difference values may be appear the literature are inherent to the spectroscopic mete accuracy. For quantitative determination of each pigment, the extinction is read at the maximal absorption and reported extinction coefficient is used in the calculation.

Chapter 3.

Material and Methodology.

3.1 Material.

3.1.1 Refractometer.

Erma hand Refractometer (o – 32^0 Brix) was used to measure the Total Soluble Solids of tomato juice and tomato puree.

3.1.2 pH Meter.

Hanna, H 185 19 pH meter was used to measure the pH value of juice, in preliminary quality parameter checking.

3.1.3 Colour Codes.

Royal Horticultural Society colour codes were used to tally the colour of tomato peel, tomato flesh, tomato juice and pasteurized tomato juice.

3.1.4 Chromatographic Column.

Absorbent tube with 250 mm length and 19 mm inside diameter made out of brosi cilicate I was taken. This is constructed at one end to attach 3 mm glass tubing and was taken to pack the chromatographic column.

3.1.5 Suction pump.

D 21 CA Electronic suction pump was used to make a pressure in order to generate a vacuum in the column.

3.1.6 Spectrophotometer.

Milton Roy Spectronic 21 D UV Visible spectrophotometer was taken to check the absorbency. All samples were checked at 473nm wave length.

3.1.7 Glass weare.

Brosil petridishes, test tubes, boiling tubes, burettes, pipettes, titration flask were used in the preliminary quality parameter checking.

Separating funnel, Buchner funnel, Glass rods, beakers, mortar and jars were used in the extraction procedure.

Chromatographic column and Buchner funnel, were used in the chromatography. 1 cm inside diameter glass cuvette were used in spectrophotometric analysis to record the absorbence.

3.1.8 Miscellaneous Items

Rubber bushes, Rubber tubes, Plunger, tiles, Oven (momernt 34 – 250 ^{0}c), Cookers, knife, peeler, muslin cloth, Blotting papers were used

3.1.9 Place.

The project was conducted at the Food science laboratory of the Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka and laboratory, department of agriculture, food research unit, Gannoruwa.

3.1.10 Chemicals.

Preliminary Parameters.

0.1 M NaOH, Pinolpthalin Solution, For acidity determination
Standard pH 7 and pH 4 solutions for pH determination

Pigment Extraction.

Acetone
Petroleum ether
5 % $NaSO_4$
Distilled water.
Anhydrous Sodium Sulfate.

Chromatography.

Magnesium Oxide
Super Cell (Kiesel Gher)
Anhydrous Sodium Sulfate.
3 % Acetone in Petroleum ether.

3.2. Methodology .

3.2.1. Sample collection .

Tomatoes were collected from the main market at Kandy and Agriculture research field Gannoruwa at the colour brake stage. Among the varieties available Marglob andThilina varieties were subjected for the project. Fruits selected were free from decease and rots. Purchased tomatoes were transported to food research unit Gannoruwa at ambient temperature (23 - 25 °C). Tomatoes were washed with running tap water to remove extraneous materials and cleaned with a dry peace of cloth. Each verities were divided in to two portions and stacked on plastic creates. One portion of it was ripened at cold room (15 – 17 ° C) and the other sample was ripened at ambient temperature (23 - 27 ° C).

3.2.2. Storage handling.

Marglob and Thilina were submitted to Ambient ripening and cold room ripening until colour developed from colour break stage to fully mature deep red colour. Ambient ripening storage handling was taken out at 21 - 28 °C and in between 85 - 95 % relative humidity. The cold room was maintained at 17 – 20 °C and between 85 - 95 % relative humidity. Samples were observed throughout the storage period to identify the disease rots and other defects. Defected samples were removed immediately to maintain a good sanitation in storage creates. Healthy fruits were selected from the sample to proceed with next operation.

3.2.3. Preliminary quality parameters.

A Set of preliminary quality parameters was conducted in the project work prior to do the chromatography separation and spectroscopic analysis such as total soluble solid, acidity .pH, moisture and general colour qualities of the tomato fruit.

3.2.3.1 Total Soluble Solids Value

Brix was measured by Erma hand Refractometer(o – 32 Brix) at room temperature. Samples for brix value were taken from each party of juice prepared separately. Taking records from a single party was repeated 3 times.

3.2.3.2 Acidity.(AOAC Method).

Known amount of tomato juice was measured and put some distilled water to dilute it, then titrated with 0.1 % NaOH with Pinolpthalin as an Indicator. Colour change occurs from colour less to slight pink.

The calculation was determined by this way.

$$Z = \frac{V * N * M.vt}{S.W}$$

V = NaOH Concentration.

M.vt = Molecular weight of citric acid (64d)

Z = Acidity

S.W = Sample weight.

N = Volume of Sodium hydroxide.

3.2.3.3 pH

pH was measured from Hanna, H 185 19 pH meter. Prior to analysing the pH, meter was calibrated in standard pH 7 and pH 4 solutions. Then the appropriate pH was measured by placing the electrode on the prepared sample. Electronic digital display shows the accurate pH value.

3.2.3.4 Moisture.

Oven dry method was used on the wet basis. Samples with known weight were placed in the oven at 105 °C. The samples were weighed within 30 minuets of Intervals until no weight change in consecutive two readings. The moisture content was represented as the percentage of its original weight.

A = Sample weight.

B = Sample weight after dried.

$$\text{Moisture Percentage} = \frac{A - B}{A} * 100$$

3.2.3.5 The colour of Peel.

Peel colour was observed and tallied with the colour charts royal horticultural society. The appropriate colour grade was noted in the preliminary report .

3.2.3.6 The colour of Flesh.

Fruit was cut in to two pieces and observed the colour in inner wall of the pericarp and tallied with colour charts. The appropriate colour grade was noted down in the preliminary report .

3.2.3.7 The Juice colour.

Ripened fruit was blended and sieved to remove the fibrous particles and juice was extracted by using a muslin cloth. Colour was observed in juice and compared with the r colour charts. The appropriate colour grade was recorded in the preliminary report

3.2.3.8 Pasteurized juice colour.

Ripened fruit was blended and sieved to remove the fibrous particles and juice was extracted through a muslin cloth. Juice was heated up to 73 °C for 15 minuets and cool down to room temperature, The Colour was then observed in juice and tallied with colour charts. The appropriate colour grade was noted down in the preliminary report.

3.2.4. Chromatography and Spectrophotometry.

3.2.4.1 Principle.

Lycopene has absorption maximum at 473 nm and 503 nm. The molecular extinction coefficient for all *trans*-lycopene at 473 nm is $18.6 \cdot 10^4$ and at 503 nm $17.2 \cdot 10^6$. As the reading taken at 503nm need to calibrate to rectify the energy deference. The most suitable and accurate way use to measure the total absorbence at 473 nm.

3.2.4.2. Extraction.

About 5 to 10 grams of juice puree or powder were weighted and Extracted repeatedly with acetone in a pestle and mortar until the residue become colorless. The acetone extract was Transferred to a separating funnel containing 10 to 15 ml of petroleum ether and gently mixed. Carotene pigments was taken in to petroleum ether by diluting the acetone (lower phase) with water or water containing 5% anhydrous sodium sulfate. The lower phase was transferred to another separating funnel and the petroleum ether extract containing the Carotenoid pigments was taken to an amber colour bottle. The extraction from the acetone phase was Repeated with petroleum ether until it become colourless. Acetone phase was discarded. Small quantity of anhydrous sodium sulfate was added to the petroleum ether extract and transferred it to a 50 ml volumetric flask.

3.2.4.3 Chromatographic separation.

The absorption tube was attached to a Buchner flask and placed a plug of non-absorbent cotton in to the constriction. Vacuum was applied and added enough absorbent (Magnesium oxide: Silica gel = 1:3) to make the column 2 – 2.5 cm in length. Absorbent was pressed down by plunger , once or twice. the surface of the absorbent was loosen around the edges with a thin edged spatula and more absorbent was added. The above steps were repeated until the column reached to 10 cm in length approximately. 1cm of sodium sulfate was placed on the top of the column.

The column was wetted with 25 to 50 ml of petroleum ether .Vacuum was disconnected and transferred the absorption column to a clean dry Buchner flask and applied suction. Column was washed continuously with eluent (3% acetone in petroleum ether) and Added successive portion of the eluent was added. Elution was continued until all the Carotenoid pigments except lycopene (Which remain Absorbed at the top) have moved off And eluent become colour less. Content of the flask was transferred to a separating funnel and washed off the acitone with water. Petroleum ether was transferred to a funnel containing anhydrous sodium sulfate in to a 100-ml volumetric flak. Anhydrous sodium sulfate was washed with petroleum ether.

3.2.4.4. Spectrophotometry.

Colour was measured by the spectrophotometer at 473 nm in extracted sample using a 1cm cell and petroleum ether as control reading (A). A similar aliquot was pipetted out as that was taken from column chromatographic separation (5 or 10 ml) and diluted to same volume as the one made after chromatography. Colour was measured similarly of the total carotenoid pigment at 473 nm and recorded the reading as "B". From the absorbence reading at 473 nm for the total carotenoid pigments, deducted the absorbence due to the carotene alone at 473 nm (B – A). The values obtain from deduction is the absorbence due to lycopene.

The lycopene content was calculated of the sample as given below using the relation ship that an optical density (OD) 1 = 2.887 μg of lycopene per Ml

E Mole/ 1 Cm at 473 nm = $18.6 * 10^4$

The molecular weight of lycopene is 536.85 which when dissolved in 1L and measured in spectrophotometer at 473 nm by using 1 cm cell gives the above optical density (S.Rangana, 1989).

536.85 g / 100 ml	=	$18.6 * 10^4$
536.85 mg / ml	=	$18.6 * 10^4$
533.85 mg / µg	=	$(18.6 * 10^4) / 10^3$
	=	186 OD
OD of 1	=	536.85 / 186
	=	2.887 µg / ml.

This amount was added to the final calculation.

$$\text{Mg of Lycopene in 100g} = \frac{2.887 * \text{OD of sample} * \text{Dilution} * 100}{1 * \text{Weight of Sample} * 1000}$$

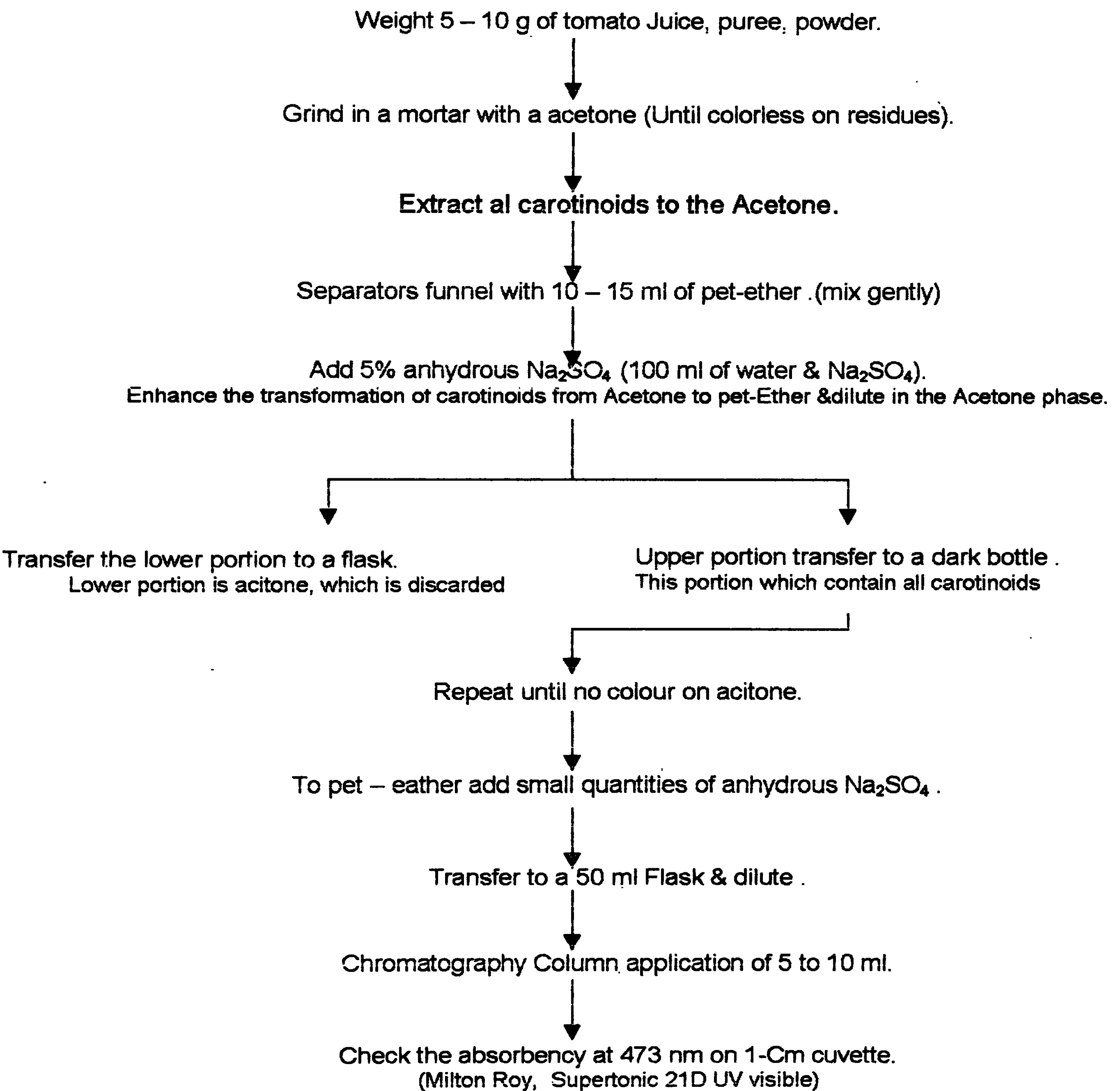


Fig 3.1: Extraction of Lycopene.

3.3 Sample Analysis.

The experimental format used known as factorial experiment. This type of experiment allows to study two or more factors simultaneously. A factor is a kind of treatment and that have levels in a factorial experiment not only we investigate the effects of the individual factors, investigation which also enables to study the interaction between factors.

Experiment were design in such a manner that both varieties kept in different storage conditions (Cold room and Ambient) were analysed for lycopene content for different forms (Juice, puree and powder). However these two analysis were done in 2 varieties (Experimental level 1 and 2). However the discussion of results obtain are confined to level 1, because the data obtain for 3 different forms were calculated in to single form viz. Reading lycopene content on wet basis

Chapter 4.

Results and discussion.

4.1 Preliminary quality parameters.

Sets of test were conducted to measure quality parameters like pH, Acidity, Moisture, Total soluble solids, flesh colour, peel colour, pasteurized juice colour were conducted to identify the general qualities in the fruit ripening in different storage conditions. The above preliminary qualities were checked to all the collected samples at the correct stage. (Table 4.1 and 4.2).

4.1.1 Total soluble solids.

The total soluble solids is a successful indicator in measuring the quality of tomato fruit. There is a direct relationship between the soluble solid content of tomato and the refractive index of the solution. This index gives an idea on the amount of sucrose present in the tomato juice.

The total sugar percent are 2 to 5 in ripe fruit and free sugar including fructose, glucose, sucrose present in the fruit represent more than 60 percent in the solid. (Williem and Beven. 1978). During maturing and ripening of the fruit, changes can be taken place in total soluble solids. This is increased from mature green stage to red ripe stage. (Winsor et al, 1962). The terning stage of the fruit has the highest sugar content. Maximum sugar can be found 4 days after the pink stage fruit (Rosa, 1926).

In both varieties total soluble solids content were about 4 to 5 % and no change in total soluble solids was observed in fruites.

Genotypes, light, temperature and nutrition in the fruit development and storage effect on the quantity of total soluble solid. Light and temperature increase the total soluble solids. Application of nitrogen decreases the sugar content while phosphorus increase it(Davis and Winzop, 1967).

Table 4.1: Preliminary quality parameter determination of Marglob.

Sample description	Marglob	Marglob
Place of brought	Kandy main Market	Kandy main Market
Cultivated field	Udadumbara, Gannoruwa	Udadumbara, Gannoruwa
Ripening Procedure	Ambient Temperature	Cold room
Date (purchased)	5.10.2001	5.10.2001
Date (Analyzed)	10.10.2001	5.11.2001
Total Soluble Solid	3.5	3
Acidity	0.43	0.412
pH	4.23	4.38
Moisture	95.5	95.5
Peel colour	Orange red , Group 33-B	Orange red , Group 33-B
Flesh colour	Orange red , Group 33-D	Orange red , Group 34-C
Juice colour	Red, Group 37-A	Red, Group 35-A
Pasteurized juice colour	Grayed Orange, Group 169-A	Grayed Orange, Group 169-A

Table 4.2: Preliminary quality parameter determination of Thilina

Sample description	Thilina		Thilina	
Place of brought	Kandy main Market and Agriculture research field.		Kandy main Market and Agriculture research field.	
Cultivated field	Hewahata, Gannoruwa		Hewahata, Gannoruwa	
Ripening Procedure	Ambient Temperature		Cold room	
Date (purchased)	7.10.2001		7.10.2001	
Date (Analyzed)	14.10.2001		7.11.2001	
Total Soluble Solid	4.5		4.5	
Acidity	0.61		0.62	
pH	4.15		4.11	
Moisture	95.32		93.2	
Peel colour	Orange red ,	Group 33-A	Orange red ,	Group 33-A
Flesh colour	Orange red ,	Group 34-B	Orange red ,	Group 34-C
Juice colour	Red,	Group 34-C	Red,	Group 35-B
Pasteurized juice colour	Grayed Orange, Group 169-A		Grayed Orange, Group 169-A	

Table 4.3: Range of water and solid in Tomato.

Tomato fraction	Content in percentage.
Total Soluble solids	3.5 – 5.0
Water Content	90 - 96

According to the results obtain from the preliminary studies there were no changes in total soluble solids in fruits ripen in both storage conditions.

4.1.2 Acidity and pH.

Tomato contain acid or a mixture of acids which may occur naturally. The acid portion is mainly responsible for the tart or sour flavour. Determination of Total acid is useful in measuring the tartness.

In order to measure the pH value of an unknown sample it is necessary to convert the sample in to liquid form by chopping to a uniform fleshy content and subsequently filtering through muslin a cloth to the determination pH.

Maximum acidity was found at the pink stage of the fruit and quantity of the acid present in the fruit during this stage, citric acid activity was the highest. Titrable acidity is associated with pH and citrate is largely contribute to it (Sands, 1972).

According to data obtain the acidity of Thilina was found to be higher than Marglobe. The reason for this variation can probably be due to variety, farmer practices, soil conditions and the other agro ecological factors. No much change of acidity in both two storage conditions were observed. (table 4.6).

Table 4.4: Acidity in different Storage conditions.

Storage Condition	Marglobe	Thilina
Ambient Ripening	0.43	0.62
Cold room Ripening	0.41	0.61

4.2 Colour development and Estimation of Lycopene.

4.2.1 Colour development.

Table 4.5: Colour development in fruits during storage.

Duration (Days)	Ambient Ripening	Duration (Weeks)	Cold Room Ripening
1-4	Green	1-2	Green
4-5	White	2-3	White
5-7	yellowish pink	2 -3	Reddish Pink
7-9	Red	3-4	Red

Green fruits under light conditions contain chlorophyll a and b. Chloroplasts of mature green fruits of the cultivated varieties are starchy and voculated. Chloroplasts undergoing various stepwise changes, form in to chromoplast having red colour. Chlorophyll degradation and carotenoid biosynthesis are the two main operations and the sequence of changes of the colour is an under green, white, pink. Yellow and Red. (Green, 1976).

Both Marglobe and Thilina showed the same colour development pattern. But Thilina required little longer duration to its maximum colour development than variety marglobe. As chlorophyll decreases, the fruit become colour less pink and finally red under favorable conditions.

Chlorophyll degradation is mainly take in place at turning stage from green to white. Colour changes from White to red involves carotenoid biosynthesis (Khudaria, 1972). The granum parts of the chloroplast play a vital role in the formation of red colour. There is a loss of chlorophyll in the plastids too (Haris, 1978). Moreover the granum part of the chloroplast changes drastically and disappear. Some times the grana become larger due to swelling. The osmophilic globules yellow in colour, appear first as carotenoid. Later in the granum part the carotenoids are synthesised and in the advanced stage larger crystals of lycopene is synthesised covering a major part of the chromoplast.(Porter and Anderson, 1977). .Lycopene crystalloid are formed in associated with lycopen.

The typical colour development was shown in both varieties but the development of colour in cold room storage took much longer duration than compared the ambient ripening storage probably due to fact that under cold room storage conditions fruits take a longer time to complete the biosynthesis of carotenoid. Colour changes from white to rèd was found to be the quicker than change of colour from green to white in both varieties. Colour development is very distinct and changes are clear at ambient ripening storage condition.

Lycopene precursors such as geranyl geranyl phyrophospate, phytoflein, phytone are synthesis at the very beginning stages of carotenoid biosynthesis. Chlorophyll degradation from green to white involves chlorophyll degradation and white to red involves carotene biosynthesis. (Khudaria, 1972). There for ripening fruits at a white stage has no chlorophyll any more. At this place by the action of enzyme, phytone destruction is increased, simultaneously neuresparone is the major precursors of lycopene in turning fruits at this stage show pink in colour. Accumulation of neuresparone which enhance the forward reaction of lycopene biosynthesis under favorable conditions like low temperature increase this process (Goodwin.1977).

During cold room storage ripening of fruit the above favorable conditions were given to both varieties. Therefore red colour development was higher due to the accumulation of neuresparone.

Table 4.6

Approximate Lycopene Content (mg/100g wet weight)

	MARGLOB		THILINA	
	Ambient ripening	Cold room ripening	Ambient ripening	Cold room ripening
Juice 95% moisture	3.114 3.246 3.626	3.962 4.123 4.452	2.66 2.78 2.99	3.25 3.65 3.85
Puree 85% moisture	15.9 16.2 16.8	17.39 17.89 18.9	14.32 14.82 14.92	15.29 15.33 15.45
Powder 5% moisture	91.6 92.62 93.21	99.54 100.26 100.32	88.87 88.96 88.97	94.22 94.32 94.32

Table 4.7

Approximate Lycopene Content (mg/100g Edible portion)

	MRGLOB		THILINA	
	Ambient ripening	**Cold room ripening**	**Ambient ripening**	**Cold room ripening**
Juice	5.418	5.992	5.112	5.432
	5.422	5.883	5.002	5.322
	5.132	5.831	4.961	5.323
Puree	5.107	5.747	4.773	5.050
	5.043	5.703	4.750	4.943
	4.943	5.617	4.710	4.970
Powder	4.887	5.288	4.645	4.860
	4.867	5.243	4.621	4.809
	4.795	5.211	4.537	4.801

4.2.1 Estimation of lycopene.

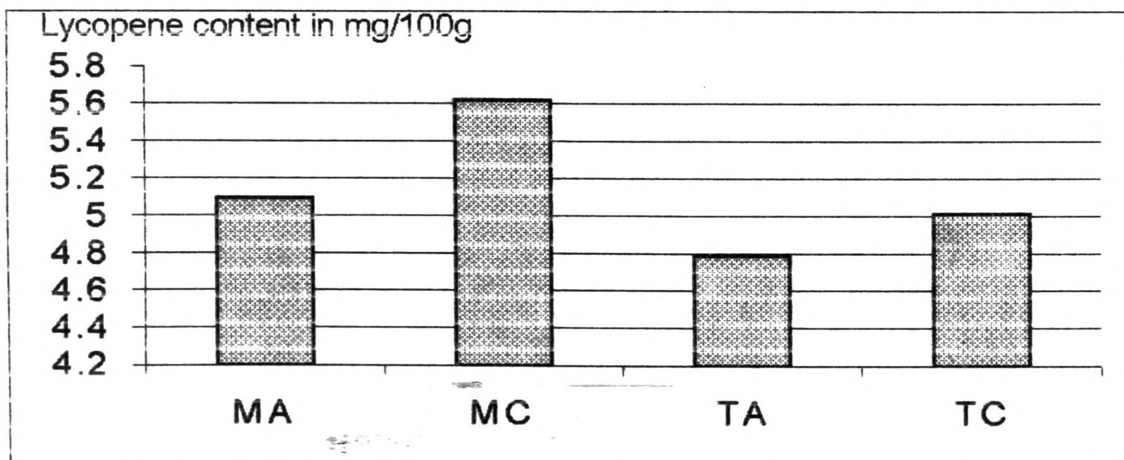
To identify the major changes taken place in tomato products namely juice, puree and powder results obtain in the present study were analysed in 2^2 factorial design. In order to comparer lycopene content in deferent products which contain different moisture content Preliminary data were converted in to wet basis. According to the given results obtain for both factors (Marglobe and Thilina) and treatments (cold room ripening and ambient ripening) were statistically different in 95% significant level (Appendix 1).

It was statistically revealed that the total lycopene content is higher in the variety Marglobe than Thilina (Appendix 1). According to the descriptive statistics the total lycopene distribution is summarised in the table 4.6.

Table 4.8: Lycopene content in 2 common varieties

Variety	Lycopene content
Marglob	5.35
Thilina	4.92

For further clarification least square design test was carried out to confirm the true effect on lycopene development. The lycopene development is actually high in both varieties kept under cold room storage condition for ripening. (Appendix 2).



MA = Marglobe ambient, MC = Marglobe cold room, TA = Thilina Ambient, TC = Thilina cold

Fig 4.1: Approximate lycopene content in 4 samples

Changes in the total lycopene content during fruit ripening can be observed in the figure 41. As the total value of tomato is determined by the intensity of red colour, the development of lycopene is important. The consumer usually relates certain colour characteristics to preference like fresh fruits and wholesomeness of the products.

Colour in the tomato is mainly due to the presence of carotenoid and carotenols. The most abundant carotene is lycopene in tomato, which contain 90% of those pigments (wogle et al 1975). Tomatoes that are grown in temperate countries have more lycopene than those cultivated in tropical countries (Went et al., 1978). Under cold room ripening a trend was found the lycopene development was higher than in ambient ripening. (Apendix1). This could be due to the environmental factors which were applied to treat the fruit. According to the present study other factors are the same except temperature. Therefore temperature plays an vital role in biosynthesis of temperature. Variety Marglobe showed 10.12% of increasing of lycopene content in cold room ripening and Thilina showed 4.65% of development of lycopene under cold room storage condition.(Apendix2)

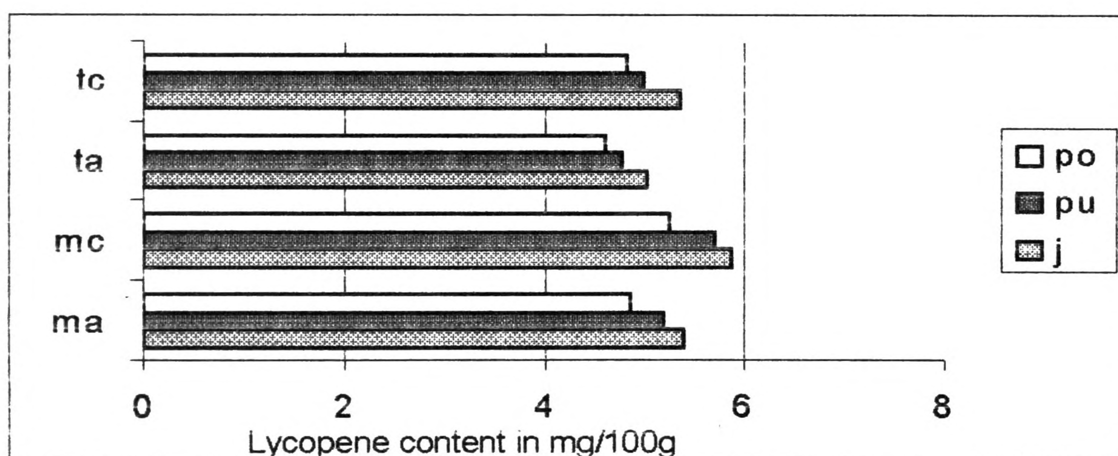
Koskitalo and Ormrod (1972) investigated the influence of suboptimal temperatures on colour quality and pigment composition of ripening tomatoes. Fruits exposed to a diurnal regime of 18°C to 20°C had the highest pigment content, with lycopene accounting for 90% of the carotenes. In this condition the carotenoid biosynthesis rate was the highest, whereas in fruits exposed to a diurnal regime of lower temperature the ripening process was delayed.

In different environmental conditions Neuresparone, major precursor of all carotinoids. it changes the original characteristics. At higher temperatures (20 – 32°C) neuresparone produce β-ring structure and in 20 – 25 °C e – ring structure. Under low temperature neuresparone produces lycopene. The optimum temperature for the development of lycopene is between 15 – 22 ° C. (Camara and Dogbo, 1986).

The monocyclic intermediates of ε- and β-carotene are δ-carotene and γ-carotene, respectively. Cyclization may also occur at the neurosporene level, as indicated by the existence of α-zeacarotene and β-zeacarotene. Cyclization occurs in carotenes that have at least a double bond at C-7,8. If γ carotene or δ carotene present in the chromoplast the other reactions happens spontaneously. Both γ carotene and δ carotene have an ability to produce α-carotene, but the most predominant reaction is the formation of β--carotene from γ carotene. (Camara and Dogbo, 1986).

Lower temperatures lycopene is develop properly but at higher temperature it might be converted to beta-carotene or its precursors and remain small amount of lycopene. According to the current research cold room temperature was given as appropriate conditions to promote maximum lycopene development. In ambient ripening conditions resulted for them room temperatures (22 - 28 °C) were much higher than the optimum temperature of lycopene development. Both Marglobe and Thilina ripen in ambient temperatures showed little lower in lycopene content than cold room ripened samples. Therefore to keep bright red colour we have to maintain the correct temperature in storage handling.

4.3 Degradation of lycopene.



Po = Powder; Pu= Puree; J= juice

tc= Thilina cold room ripening; ta= Thilina ambient ripening; mc = Marglobe cold room ripening, ma = Marglobe ambient ripening.

Fig 4.2: Lycopene content in three different products (Appendix 3).

Lycopene degradation can be attributed to both oxidation and isomaration. According to the above results the degradation of lycopene is clearly seen. The highest degradation of lycopene was found in tomato powder due to low moisture (around 7 10%). Lycopene oxidation is high in tomato powder, because it contain less water attached to the food surface forms a protecting film and prevents contact with oxygen. Tomato powder contains only 5%of moisture. this amount is not enough to form a protecting layer. There for oxidation is enhanced.

The various oxidants are found such as oxygen, ozone, alkaline permanganates and chromic acid. As well as in tomato powder auto oxidation is occurs due to the free radical

chain process. Temperature, light, and humidity stimulate this process because lycopene oxidation occurs widely during food processing And storage (Kem et al, 1996).

In case of tomato puree, there is a slight reduction of lycopene by comparing to the tomato juice. This reduction is around 3 – 10% in both varieties [(table 4.6) (appendix 2)]. Nobel (1975) found that heat concentration of tomato pulp resulted in up to 57% loss of lycopene. It was observed reduction of all trans lycopene content could go up to 20%.

In the tomato puree, reduction of lycopene is totally due to oxygen and temperature combination effect. Most predominant lycopene is trans lycopene. At a higher temperature it is converted to *cis* lycopene. (Capore 1977a,b). Sis form of lycopene is not much stable. Sis lycopene is rapidly converted to the *tans* lycopene again. Continuous reaction of this at higher temperatures helps to degradate the total lycopene content in tomato puree.

The oxidative degradation of lycopene during the manufacture of tomato puree and powder were also observed in the present study. Temperature and oxygen are the major factors to effect the degradation of lycopene. The rate of lycopene degradation is also depends upone the amount of moisture percent in dehydrated tomato products as well.

Chapter 5.

Conclusion.

The colour of tomato fruit or its product is most important quality factor. Colour of the tomato is mainly depend upon the quantity of carotenoid available in the fruit. Lycopene is the simple carotenoid found in tomato fruit. Therefore development of tomato colour is important

- Lover temperature only lycopene (the major carotenoid in tomato) develop properly. At higher temperature it might convert to other forms of carotenoids.
- Both harvesting and storage temperature should be low for proper devolopment of lycopene.
- Storage temperature at 15 – 17 ° C and 85 – 95 % of relative humidity can enhance the proper lycopene production of lycopene than ambient ripening conditions.
- The best source of lycopene is tomato powder which is prepared form variety Marglob
- Due to oxidation, lycopene degradation is high in low moisture products like tomato powder.
- Isomaration of lycopene at higher temperature is the major effect on lycopene degradation in tomato puree.

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Appendix 1.

Factorial Analysis test.

Dependent Variable: LYCOPENE

Source	DF	Anova SS	Mean Square	F Value	Pr > F
STORAGE	1	1.83964011	1.83964011	24.91	0.0001
VARIETY	1	1.23580278	1.23580278	16.73	0.0003

Level of	-----------LYCOPENE----------		
STORAGE	N	Mean	SD
Marglobe	18	5.35400000	0.37681841
Thilina	18	4.90188889	0.27217293

Level of	-----------LYCOPENE----------		
VARIETY	N	Mean	SD
Ambient	18	4.94266667	0.26930018
Cold	18	5.31322222	0.42316365

Appendix 2

Lest square design test

Dependent Variable: LYCOPENE

Source	DF	Anova SS	Mean Square	F Value	Pr > F
STORAGE	1	1.83964011	1.83964011	26.25	0.0001
VARIETY	1	1.23580278	1.23580278	17.63	0.0002
STORAGE*VARIETY	1	0.19448100	0.19448100	2.77	0.1055

T Grouping	Mean	N	STORAGE
A	5.35400	18	Marglobe
B	4.90189	18	Thilina

T Grouping	Mean	N	VARIETY
A	5.31322	18	Cold
B	4.94267	18	Ambient

Level of	Level of		-----------LYCOPENE----------	
Storage	Variety	N	Mean	SD
Marglobe	Ambient	9	5.09522222	0.25352849
Marglobe	Cold	9	5.61277778	0.29458987
Thilina	Ambient	9	4.79011111	0.19356940
Thilina	Cold	9	5.01366667	0.30304249

Appendix 3.

Descriptive statistics of 3 deferent products.

Variable	N	Mean	Std Dev	Minimum	Maximum
MAJ	3	5.3240000	0.1662889	5.1320000	5.4220000
MAPU	3	5.0310000	0.0826559	4.9430000	5.1070000
MAPO	3	4.8496667	0.0483873	4.7950000	4.8870000
MCJ	3	5.7920000	0.1155465	5.6620000	5.8830000
MCPU	3	5.6890000	0.0661211	5.6170000	5.7470000
MCPO	3	5.2473333	0.0386825	5.2110000	5.2880000
TAJ	3	5.0250000	0.0780833	4.9610000	5.1120000
TAPU	3	4.7443333	0.0318800	4.7100000	4.7730000
TAPO	3	4.6010000	0.0567098	4.5370000	4.6450000
TCJ	3	5.3623333	0.0605007	5.3230000	5.4320000
TCPU	3	4.9876667	0.0556447	4.9430000	5.0500000
TCPO	3	4.8233333	0.0320052	4.8010000	4.8600000

MAJ = Marglobe ambient ripening juice.
MAPU = Marglobe ambient ripening puree.
MAPO = Marglobe ambient ripening powder.
MCJ = Marglobe cold room ripening juice.
MCPU = Marglobe cold room ripening puree.
MCPO = Marglobe cold room ripening powder.
TAJ = Thilina ambient ripening juice.
TAPU = Thilina ambient ripening puree.
TAPO = Thilina ambient ripening powder.
TCJ = Thilina cold room ripening juice.
TCPU = Thilina cold room ripening puree.
TCPO = Thilina cold room ripening powder.

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