BIOCHEMICAL CHANGES IN *KHASI* MANDARIN (*Citrus reticulata* Blanco) DURING RIPENING

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MASTER OF SCIENCE (*Agriculture*)

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By

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CERTIFICATE – I

This is to certify that the thesis entitled "***Biochemical Changes in Khasi Mandarin (Citrus reticulate* Blanco*) During Ripening***"submitted to the Faculty of Agriculture, Assam Agricultural University, in partial fulfillment for the degree of ***Master of Science (Agriculture)*** *in* ***Agricultural Biochemistry*** is a record of research work carried out by ***Mr. Surjit Moni Deka*** **(Roll No. 2017–AMJ–38)** under my personal supervision and guidance.

All helps received by him have been duly acknowledged.

No part of this thesis has been reproduced elsewhere for any degree.

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CERTIFICATE – II

This is to certify that the thesis entitled **“Biochemical Changes in *Khasi* Mandarin (*Citrus reticulate* Blanco) during Ripening”** submitted by **Mr. Surjit Moni Deka, Roll No.2017-AMJ-38** to the Assam Agricultural University, in partial fulfilment of the requirements for the degree of **Master of Science (Agriculture)** in the discipline of **Biochemistry and Agricultural Chemistry** has been examined and approved by the Student’s Advisory Committee after viva-voce.

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ABSTRACT

Physical and biochemical constituents of the fruit *khasi* mandarin (*Citrus reticulata* Blanco) cultivated in Assam was studied during different stages of development after 90 days after fruit setting (DAFS) to 210 DAFS. The fruits were analysed for physical parameters such as fruit weight, diameter, volume, pulp weight, peel weight and pulp-peel ratio as well as moisture, juice percentage, TSS, titratable acidity, sugar, juice pH, organic acid, ash and minerals (Na, K, P, Ca).

Fruit weight increased from 23.46 g at 90 DAFS to 78.32 g 210 DAFS. Fruit diameter increased from 3.13 cm at 90 DAFS to 5.20 cm at 210 DAFS. Fruit volume also increased from 24.66 cc at 90 DAFS to 78.67 cc at 210 DAFS. Pulp and peel weight increased form 16.98 g and 6.48 g respectively at 90 DAFS to 61.06 g and 17.26 g at 210 DAFS respectively. Pulp: peel ratio also increased from 2.62 at early stage to 3.52 at last stage of observation. Juice content increased from 29.23 % at 90 DAFS to 46.03 % at 210 DAFS. The highest moisture content 87.37percent was observed at 210 DAFS which was significantly higher than that of moisture content at 90 DAFS (74.48 %). Lowest TSS content 5.83 percent was observed at 90 DAFS then it increased to 8.84 percent at last stage of observation (210 DAFS). There was a decreasing trend of titratable acidity from 3.19 percent at 90 DAFS to 0.85 percent at 210 DAFS. TSS/ Acidity ratio showed increasing trend in a significant manner towards maturity. Reducing, non reducing and total sugar increased from 1.65, 0.87 and 2.52 percent respectively at 90 DAFS to 2.85, 3.86 and 6.7 percent respectively at 210 DAFS. Citric acid content was found to decrease from 644.17 to 94.24 mg per 100 ml fresh juice. Oxalic acid content in the fruit was observed to be increased up to 150 DAFS then it decreased. Highest fumeric acid content was recorded at 90 DAFS as 20.78 mg/100ml of fresh juice. Highest ascorbic acid content 83.88 mg/ 100 g was observed at 210 DAFS. Ash content decreased from highest 5.14 per cent at 90 DAFS to the lowest 1.94 percent. Calcium content in the fruit decreased from 0.63 to 0.39 g/100 g. Potassium content also decreased gradually from 90 DAFS to 210 DAFS. Highest Sodium content was found at 90DAFS and found to decrease upto 180 DAFS. Phosphorus content of the pulp increase from 0.15g/100g (90 DAFS) to 0.26g/100g (150 DAFS) then decreased to 0.15 g/100g (180 DAFS) and then again increased to 0.17 g/100g (210 DAFS)

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LIST OF ABBRIVIATIONS

AOAC	:	Association of official Analytical Chemists
°B	:	Degree Brix
CD	:	Critical Difference
CAE	:	Catechin Equivalent
CRD	:	Completely Randomized Design
°C	:	Degree centigrade
DAF	:	Days after flowering
DAFS	:	Days after fruit setting
et al	:	Et alli (and others)
etc.	:	and the rest
Fig.	:	Figure
g	:	Gram
hr	:	Hour
i.e.	:	That is
L	:	Litre
M	:	Molar
µl	:	Microliter
mg	:	Miligram
ml	:	Millilitre
N	:	Normal
%	:	Per cent
/	:	Per
rpm	:	Revolution per minute
pH	:	$-\log[H^+]$
S. Ed	:	Standard Error Deviation
Viz.	:	Videlicet (Namely)

CHAPTER I

INTRODUCTION

Citrus fruits are one of the world's most economically important fruit crops cultivated throughout the tropical and subtropical regions and are recognized as an important parts of human diet owing to its high content of vitamin C, folic acid, potassium, flavonoid etc. The genus *citrus* belongs to the family Rutaceae and sub family Aurantiodae, consisting of large number of species and varieties. The north eastern region of India stretches from 21˚57 N to 29˚28 N and from 89˚ 40 E, is considered as a natural home of citrus fruit cultivation. Assam and its neighbouring states with diversity of soil ranging from alluvium in the plain to the lateritic in the hills, rainfall varying from 100 to 1250 cm, altitude ranging from 35 m to 2700 m and varied humidity and temperature in different parts offer ideal condition for several citrus varieties to grow (Bharali, 2013). No other region in India harbours such diverse form of citrus fruits. The genus citrus includes various commercially important fruits like mandarin, sweet orange, sour orange, grape fruit, pummelo, lemon, lime, citron etc. According to Swingle and Recee (1967) the genus *citrus* has only three basic true species namely Citron (*Citrus medica* L.), Mandarin (*Citrus reticulate* Blanco) and Pummelo (*Citrus grandis* L. Osbeck), while the other species are hybrid derivatives of any one of the true species and the species belonging to the sub genus *Papeda.* The major citrus growing areas in India are Tamil Nadu, Madhya Pradesh, Punjab, Karnataka and southern slope of Cherrapunji, hills of Assam, Northern Orissa, Maharashtra and Uttar Pradesh. Bhattacharya and Dutta hold that two species in *Eucitrus*, *Citrus indica* Tan. and *Citrus assamensis* and three species of *Papenda viz., C. ichangenous* Swing, *C. latips* Tan and *C. macroptera* Moutr are definitely indigenous to Assam. According to them *citrus aurantium, citrus magaloxycarpa* and *citrus reticulata* may also be added to the list (Singh, 2003). Citrus is mainly grown in Brazil, China, USA, Spain, Mexico, India, Italy, and Egypt. The most important factor for growing citrus is climate. It generally favours a dry climate with well-defined wet and dry season. Mandarin species are generally well adopted at higher elevation of 500 to 1100 meter. Citrus can tolerate maximum temperature of 46-50˚. Temperature should be between 14-40˚C. The temperature for the best growth and performance of plant should be around 29 -35˚ (Yadav, 2007).

In India among all, citrus mandarin occupies first position with respect to area and production (Yadav, 2007). Mandarins are well known for their unique nutritional quality in all over the world. *Khasi* mandarin is widely cultivated and commercialized variety of mandarin grown in the North-eastern region of India. Assam and Meghalaya occupy the maximum area under the production of Khasi mandarin (Singh 2016). The *Khasi* mandarin produced in North eastern region is popular for its superior quality in respect of its flavour, juice content, sugar and acidity ratio (Deka, 2018). It covers nearly 38.2% of the total citrus area and contributes about 43.6% of the total citrus fruits produced in India. Popularly it is known as komola or Humoptira in *Assamese,* Sohniamptra in *Khasi* and komola in *Bengali* (Singh *et al.* 2016). India is the fourth largest producer of oranges in the world. In India mandarin is cultivated in 3.11 lakhs hectare area with the production of 29.06 lakh tones. Mandarin Fruits are depressed, globose to oblate, medium size, loosed skinned, bright orange yellow in colour, surface smooth, glossy, rind thick to medium, rind and segment easily separable, segments moderate in numbers, juice abundant, orange colour with sour-sweet blend and has good keeping quality. The fruits weigh 10.31 to 109.90 g, total soluble solids 7-14°Brix, acidity 0.67-1.31 (%), 1.83to 3.95 pH, total sugar 2.07 to 4.65 (%) (Hangsing *et al.,* 2016). The tree flowers once under humid tropical climate because of cold winter i.e. spring blossom (February-April) and crop is ready for harvesting during October-December. (Deshmukh *et al.,* 2016).

Mandarin is widely consumed around the world for its delicious qualities and it also has medicinal values that have long been used in traditional herbal medicine (Zhang *et al.,* 2012).

In the conventional Chinese medicine, the dried mature fruit peels have been widely used for centuries as remedies for the treatment of indigestion and to improve inflammatory syndromes of the respiratory tract (Yung-Sheng *et al.,* 2010). Extracts from *C. reticulata* seeds provide protection to the uroepithelium against *E. coli* infection (Vollmerhausen *et al.* 2013). In traditional Chinese medicine, the dried peel is used to increase digestion, and to reduce phlegm, and its various parts are used for the treatment of cutaneous problem, hemiplegia, snake bite, fever, loss of taste, chronic rheumatism, stomach ache, menorrhagia, splenomegaly, edema and cardiac diseases, bronchitis and asthma (Xian *et al.,* 2007, Min-Sheng *et al.,* 2008). In India, citrus fruits are grown on a wide range of soil and under divergent climatic condition, which exercise significant influence on soil fertility and nutritional factor. Rokaya *et al.* (2016) reported that The most important and reliable judging criteria of fruit maturity in mandarin *i.e.* fruit weight, external fruit colour, firmness, TSS, acidity, and TSS/Acid ratio, and vitamin C. The different chemical components also changed in different season. At low temperature sugar converts to acid and many other organic acids are formed and at low temperature soil water condition also influence on the size of the fruit (Nitisch, 1953). Devkota *et al.* (1982) reported that the maturity standards vary from region to region. According to Deshmukh *et al.* (2016) under different altitudes the determination of the criteria for fruit maturation is quite complex as it depends upon the internal changes occurring in the fruit pulp and external colouration takes place on fruit peel. Deka *et al.* (2006) reported that the maturity indices of khasi mandarin as Harvesting of fruits 230-250 days after flowering, TSS (≥ 10ºBrix), content of juice (≥ 49.0%) and TSS: acid ratio (≥ 12.0). But it is found that in citrus fruit the commercial maturity indices are highly variable and dependent on growing region, market demand and varieties (Lado *et al.,* 2014). Maturity indices may also depend on the demand of the consumer. Appropriate stage of maturity for harvesting, methods of harvesting, storage condition and their treatments should be developed to prolong the shelf life of the fruits. Mandarin oranges are desirable to harvest as early as possible because they do not last long, otherwise they shrink, lose weight and may eventually drop off (Rajput, 2014). Strief (1983) reported that the fruits which are picked late have a good internal quality but have shorter shelf life and on the other hand the fruits picked early have better shelf life but poor eating quality.

Many chemical constituents start to accumulate with development of fruit. As fruits size increase, there is often a gradual decrease of some constituents (Bain, 1958). Considerable changes in the physical characteristics as well as chemical constituents of citrus take place from the time the fruit is small. In fact, changes in these components bring the fruit to maturity. Citrus fruit doesn’t continue to mature when detached from the mother plant and placed in storage, because it has no carbohydrate reserve. However, the stages of development at which citrus fruit is harvested have considerable effect on total acid and other quality aspects (Soni and Randhawa, 1969).

Keeping in view the above facts, changes in chemical constituents during ripening of fruits may be taken as important criteria for harvesting time for commercial use of the crop. Information regarding the changes in chemical composition of *khasi* mandarin fruit grown in Assam, during ripening or development for fixation of maturity standard is scanty. Therefore, the present investigation has been planned with the following objective: To study the biochemical changes in *khasi* mandarin during ripening

CHAPTER II

REVIEW OF LITARATURE

The studies of biochemical changes during development of fruits are useful in determining the stages of maturity. Most of the fruits are harvested when they are ripe. The ripening of fruit may be defined as the sequence of changes in colour, flavour and texture which lead to the state at which the fruit is acceptable for consumption. This doesn't necessarily mean that this is a fixed physiological state; it varies from fruit to fruit. In some cases, the changes may be even run in opposite direction (Kidd *et al*, 1952). There are of course several methods of determining the maturity of fruits on the basis of their appearance but these are subjected to human errors in judgment and can be applicable to only few fruits. Different biochemical constituents also change in different seasons due to variation in climatic condition, soil condition and root stocks used. Biochemical studies during the development and ripening of fruits have been considered as suitable criteria for determining the maturity of a particular fruit.

Information regarding changes in biochemical constituents in *khasi* mandarin fruit during different stages of development in Assam climatic condition is not far available; therefore present investigation has been carried out.

2.1 Physical parameters

The weight of the Fruit varies with the maturity and the different altitude. Fruit weight is directly related to the growth and development of the fruits. The fruit weight and diameter was found to be increased from 230 DAFS (days after fruit set) to 260 DAFS and thereafter it slowed down in the mandarin fruits in the different altitudes. However, the highest fruit weight was observed at upper altitude than the lower altitude (Rokaya *et al.*, 2016).

However, Deshmukh *et al.* (2016) reported that both the weight and diameter of the *khasi* mandarin fruits gradually increased from stage- I (fruit development, 180 DAFS) to Stage -V (advanced fruit maturity, 260 DAFS). Ram and Kumar (2012) found that there was a gradual increase in *Nagpur* mandarin fruit weight from 18.74 g (marble size) to 114.14 (mature fruit). Ladaniya and Mahalle (2011) also found the same in case of Sweet Orange cv. *Mosambi*. Bollard (1970) reported that the increase in fruit weight may be due to an increase in the size of cell and accumulation of food substances in the intercellular spaces in fruits. The increase in the fruit weight was also reported by Deka *et al*., (2006) in *khasi* mandarin, Bal and Chauhan (1987) in *kinnow* mandarin, Bakhshi *et al.* (1967) in sweet orange and Mahajan *et al.* (2004) in mandarin.

Bhatnagar *et al.* (2012) revealed that the weight and volume of fruit continuously increased with advancement of fruit maturity whereas slow growth of these parameters was observed in the last date of picking; however, the varietal variations in the change of the volume were observed. However, a study by Ram (2001) revealed that the fruit weight and size gradually increased with the advancement of maturity up to last stage (stage IV-mature deep orange colour). Copeman (1931) found that during the period of ripening the weight of oranges increased and this change was accompanied by an increase in the weight of the pulp.

Josan *et al.* (1988) observed that the fruit growth of *wilking* mandarin as measured by fruit breath and fruit weight followed a sigmoid growth pattern. Similar observations have been reported in *valencia* (Bain, 1958), and *tahiti* (Hittalmani *et al.* 1977). Randhawa *et al.* (1964) found that in *marsh* seedless and pink grapefruit, fruit growth as represented by length and diameter changes was rapid up to the beginning of October, increased gradually up to December and thereafter remained more or less constant.

Baruah and Mohan (1985) reported that in sapida fruit, the weight of fruit, peel and pulp, volume and diameter of fruits increased till maturity. The rate of growth of the citrus fruit is quite rapid during the early period. Usually the greatest increase in diameter precedes the attainment of prime eating quality (Harding and Sunday, 1949). It was also observed that the fruit attains ripeness, the increase in diameter is less rapid, climatic condition and other factor such as soil, age of tree, cultural practices and rootstocks influences the size of the fruit.

Sinha *et al.* (1962) found that in Hamlin orange the rate of fruit growth was rapid till middle October, slowed down in November to attain near fruit maturity and then no change in the diameter. In *valencia* late orange the rate of increase was rapid till the end of November. The volume and weight of the fruit increased simultaneously, although volume increased was not proportional to that of weight, especially when the fruits were mature or approaching maturity. In Hamlin, weight decreased after the middle of November, perhaps due to granulation since the juice vesicles were observed to be almost dry. Thus it is observed that during seven or eight month after blossoming, rapid growth occurred in which fruit weight and volume increased accompanied by corresponding increase in fruit diameter.

2.2 Moisture content

Gloria *et al.*,(2010) opined that the moisture content of orange fruits of Tanzania was always higher than 68.8% throughout the season and even after fully ripening, the moisture content of fruits varied significantly with time of season but the content decreased during storage ripening under normal room temperature conditions. The moisture content of Valencia orange and Navel orange decreased from 86.7 to 85.1% and 80.2 to 69.4% respectively during storage ripening. Moisture content determines the nutritional quality of various food materials; the high moisture contents (>80%) suggests a low energy value for these fruits. Fasoyiro *et al.*, (2005) reported that the Nigerian orange has high moisture content. A natural laxative property which is also important for human body nutrition is provided by the moisture content in fruits. Soni and Randhawa (1969) reported that moisture present in pulp increased markedly with advancement of maturity in Eureka and Lisbon lemon, but there was a decrease in moisture content during post maturity period.

2.3 pH of the juice

Juice pH is the index of acidity of fruit. Acidity is an important attribute as the sour taste is a major factor in acceptability of citrus fruits. Sinclair and Ramsey (1994) reported that as the fruit matured, the percentage of acid in the free state decreased and the pH of the juice increased. The pH of citrus juices provides the information about the state of acidity and basicity. Riaz *et al.* (2015) observed that, pH increased up to mid stage of maturity after that it started decreasing till the late stage. The increase in pH might be due to decrease in acidity with the maturity and decrease in pH indicates the increased acidity of the fruit and this might be due to the formation of acidic compounds due to degradation of reducing sugars. Juice pH also slightly decreases on winter season due to formation of organic acids (Nitsch *et al.*, 1953).

2.4 Juice content

Bhatnagar *et al.* (2012) observed the juice content of *Nagpur* mandarin went on increasing in the course of development from first stage to last stage of harvesting (41.40% to 48.80%). Deshmukh *et al.* (2016) reported that the juice percentage in *khasi* mandarin increased with the increasing level of maturity stages, juice yield was found highest at fruit maturity stage 46.64% ,49.11%, 50.90% and 51.15% at 500-600m, 700-800m, 900-1000m, 1300-1400m altitude respectively. Kumatkar *et al.* (20017) revealed that with an increase in fruit size, the weight of juice per fruit increased in *mosambi*, there was a rapid increase in juice content of *mosamb*i observed from 30th August to 15th September (26.94% to 32.73%) and again from 15th October to 15th November (35.98% to 41.03%). Deka *et al.* (2006) and Joolka *et al.* (1982) reported that the juice content increased with delayed harvesting in *khasi* mandarin and *kinnow* mandarin respectively. Juice content increased as fruit matures and reachs to maximum value at full maturity and decreased afterwards in citrus (Ladaniya, 1996 and Lado *et al.*, 2014).

Ladaniya and Mahalle (2011) also observed that there was increase in juice yield of *mosambi* between 230 and 240 days after fruit set. Bhatnagar *et al.* (2012) observed that juice percentage increased with the development of fruit up to second fortnight of November in mandarin, there was a Rapid rate of increase in juice content observed when fruit reached near maturity stage. Rakaya *et a.* (2016) revealed that the percentage of juice content continued to increase rapidly up to 20th November and then it showed constant or declined in case of different altitudes. Ram (2001) found the juice percentage 37.79%, 43.30%, 45.49%, and 50.39% in stage I, stage II, stage III and stage IV in *kinnow* mandarin fruits during ripening on the tree.

With the development of fruit growth, the increased juice content was observed in *khasi* mandarin (Barua *et al.,* 1993), Florida orange (Harding and Lewis, 1941) and *wilking* mandarin (Josan *et al.,* 1988). Porras *et al.* (1992) observed the increase in the content of juice during fruit maturation of grapefruit and become stable toward maturity. Riaz *et al.* (2015) revealed that the Juice contents in citrus fruits were dependent upon many factors, generally increase towards maturity and then decrease when fruits become over mature.

2.5 Total soluble solid (TSS)

Deshmukh *et al.* (2016) observed (210 DAFS to 260 DAFS) that there was a gradual increase in the total soluble solid in the *khasi* mandarin in various altitude during the maturation period, the lowest TSS content was observed in stage I (8.07˚B) which then gradually increased up to stage V (10.30˚B) in 500-600 m altitude and in 700-800 m altitude the TSS content increased from 7.86˚B to 10.17˚B. TSS was found slightly higher (11.63 ˚B) at 1300 m altitude than lower altitudes because of late maturity and later dryness in the fruits at 1300 m altitude.

Bhatnagar *et al.* (2012) also found that the TSS content in the juice increased with the fruit maturation in the *Nagpur* mandarin, the TSS content in *Ambia Bahar* increased from 6.37 to 8.90˚ Brix and 8.00 to 10.90 ˚Brix in case of *Mrig Bahar*. TSS content was reported to be related to the position of the fruits on the tree (Fallahi *et al.,* 1989). Within the canopy, fruits even at different positions showed significant differences in soluble solids contents (Kedar, 1979). The increased in total soluble solid during maturity may be due to hydrolysis of acids and deposition of polysaccharides (Ram *et al.,* 2003).

In initial stage (230 days after flowering, *i.e.,* DAF), TSS was found as 6.81, 7.52, and 8.32 ˚B and in maturity stage of development (280 DAF) TSS was found as 11.63, 11.05 and 10.82 ˚B in 1300 m, 1000 m, and 700 m altitudes respectively (Rokaya *et al.,* 2016). These increased trends in the TSS were also recorded by Khokhar and Sharma (1984) in sweet orange. The increase in TSS with ripening was reported by singh *et al.* (1998) from 3.8 to 11 percent during fruit set to ripening in *kinnow* mandarin. Ladaniya and singh (1998) reported that the increase in juice TSS of mandarin fruit from 8.6 % to 10 %. Samson (1986) observed that TSS increased rapidly at first and then it increased at slower rate towards the end of the maturity. Harding and Sunday (1949) noted that in tangerines a gradual increase in total soluble solid was found as the fruit ripened and TSS content exhibit a tendency to remain constant in very ripe fruits in the late season.

2.5 Titratable acidity

Rokaya *et al.* (2016) opined that the among all organic acids citric acids in citrus are the main reserve source of energy, the acidity decreased with the advancement of the fruit maturity in all altitude. At initial stage of maturity, the acidity percentage was higher (2.59) in the fruit of 1300 m altitude followed by 1000 m (2.39) and minimum percentage (2.15) in 700 m altitude. At the final stage of maturity, the titratable acidity (TA) percentage decreased to 0.66, 0.67 and 0.77 in the all altitude respectively. Decreased acidity in the ripening might be due to rapid utilization of acids in respiration (TCA cycle). The gradual decrease in the TA during maturation was also reported by Bal and Chauhan (1987) in *kinnow* mandarin, Bastakoti and Gautam (2007) and Thapa and Gautam (2002) in mandarin.

Deshmukh *et al.* (2016) observed the highest TA percentage in the initial stage in the all altitude then it gradually decreased towards the advance fruit maturity, at initial stage the TA acidity was recorded as 1.12 % and at final stage it was recorded as 0.64% in 500-600m altitude, like that in all attitudes the TA percentage was found to be decreased gradually. Bhatnagar *et al*. (2012) also found the highest TA percentage in early stage of maturity (1.25%) in *Ambia Bahar* variety and 1.0% in case of *Mrig Bahar* variety, in the last stage of maturity the TA was recorded as 0.88% in *Ambia Bahar* and 0.80 % in *Mrig Bahar* of *Nagpur* mandarin. However, the total acidity content was lower in the initial stages and there was a rapid and constant increase up to maturity followed by a slight decrease in some cases like *Columbia* and *Hill lemon* (Soni and Randhawn, 1969).

2.6 Ratio of TSS to Acidity

TSS to acid ratio is also called as permitted maturity parameter. Reported that TSS: Acidity ratio is best measure for quality determination and could be considered to identify the harvesting date to give the fruits of better nutritive quality (Syvertsen *et al*., 1980).

Sinclair *et al.* (1944) reported that its value increased toward the advancement of maturity period and decreased as the fruit become over mature. Similarly, Syvertsen *et al*. (1980) in grapefruits, Singh *et al.* (1998) in *kinnow* mandarin and Grewal *et al*. (2000) in *kinnow* and *feutrell* mandarin also observed increased this ratio towards the maturity. Rokaya *et al.* (2016) opined TSS/ Acidity ratio as a reliable index for determination of maturity of mandarin fruits. Bal and Chauhan (1987) and Jawanda *et al*. (1987) found the TSS/ Acidity ratio in the increasing trends in *kinnow* mandarin. In case of *Coorg* mandarin, the maturity indices for main and monsoon crops, the recommended TSS: acid ratio are 13.2 and 9.1 respectively (Ramana *et al.*, 1980). Bose *et al.* (2001) reported that the TSS: Acidity ratio 8.1 was the optimum for marketable fruits.

2.7 Sugar content

Ting and Attaway, (1971) observed that the sugar content in citrus juice during fruit development and maturation range from 1-2.3% glucose, 1-2.8% fructose, and 2-6 % sucrose for orange and tangerines; and in grape fruit reducing sugar 2-5 % and sucrose 2-3 %. Deshmukh *et al.* (2016) found that the reducing sugar and the total sugar content of *khasi* mandarin increased gradually from 1st stage of maturity to the last stage in all altitudes, and the maximum reducing sugar and the total sugar content was recorded as 3.88-4.03% and 6.01-6.12% respectively in the full maturity stage. The reason behind the increase in sugar content during ripening might be due to depolymerisation of polysaccharides and conversion of fruit starch to sugar.

Hangsingh *et al.*(2016) found 1.04 - 2.81% reducing sugar and 7.10-8.99 % total sugar in *khasi* mandarin of Garo Hill in fully mature fruits. Richardson *et al.* (1997) reported that the relative level of different sugars content and acids changes during citrus fruit development appeared to be influenced by temperature variation. The increase trend of sugar content was also observed in *Khasi* Mandarin (Deka *et al.*, 2006) and guava (Patel *et al.* 2013). There are several types of sugars present in fruits, among which prominent in citrus juice are namely sucrose, glucose and fructose (Ting *et al.*, 1959).

Anwar *et al.* (1999) reported that the increase in total sugar content might be due to a concurrent increase in the sucrose contents which was then hydrolyzed to simple sugars that affected both the taste and the texture of the fruit and the rise in sugars makes the fruit much sweeter, but at the end of the season the decrease in the sugar content might be due to the formation of acidic compounds owing to degradation of reducing sugars. Riaz *et al.* (2015) reported that the difference in the proportions of sugar contents were not only related to its variety, but are also greatly depended on rootstock, geographical location, weather and cultural practices.

2.8 Organic acid content

Citrus fruits fall under the classification of acid fruits, since their soluble solids are composed mainly of organic acids and sugars. Acids and sugars are used as the main index of maturity and one of the most important analytical measures of flavor quality. The main acids of citrus fruits are citric and malic acids with trace amounts of tartaric, benzoic, oxalic and succinic acids. (Karadeniz, 2004).

Baldwin (1993) reported that citric acid accounts for most of the titratable acidity in fruit juice (80-90%) which also contains malate (9-15%) and minor quantities of succinate and isocitrate. Matsumoto and Shiraishi (1981) found that citric acid was major organic acid followed by malic Acid, they also found other organic acid in very minute amount in case of *Sastuma* mandarin fruits, and reported that the concentration of organic acid increased rapidly at very young stage of *Satuma* mandarin may be due to transformation of sugars into malic acid by addition of carbon-di-oxide to pyruvate. Albertini *et al.* (2006) identified seven organic acids in citrus fruit pulp namely citric acid, malic acid, quinic acid, tartaric acid, succinic acid, oxalic acid, and ascorbic acid. And they found that during early stage of development quinic acid was the major organic acid. It contributed 47-64% of the total organic acids, while citric acid was observed less than 14% of the total organic acids. Nitsch (1953) reported that most of organic acids are formed at low temperature. In high temperature acidity is reduced due to formation of potassium salts.

2.9 Ash and minerals content (Ca, K, Na and P)

Ash is an index of mineral content of a biological material. Soni and Radhawa (1976) reported that in pulp and peel, total ash content slightly decrease towards maturity, the concentration of N, P, K also decrease gradually towards maturity. Concentration of phosphorus was maximum in the immature fruits which decline until November followed by a non-significant rise during the rest of the period. Harding and Fisher (1945) reported that total ash of grape fruits for immature fruits was the highest. Minerals analysis of the fruit can be a good indicator of the nutritional status of the fruits. Gallasch *et al.* (1984) reported that mineral analysis is used as a diagnostics tool for scheduling fertilizer need. Mineral analysis useful for ranking the stress tolerance (Levy and Shalhevet, 1990), environmental stress (Syvertsen and Smith, 1983) and also for fruit maturity indices (Singh *et al.*, 1998 and Ladaniya *et al.*, 1998). Sheng *et al.*, (2009) found that in pulp, concentrations of Ca, K, Mg, and Mn declined gradually with time. Similarly, Storey *et al.* (2000) also reported that Potassium and P concentrations increased in the pulp during stage I then decreased during stages II and III, the Ca concentration of the pulp changed little during stage I and then decreased more than four-fold by the final harvest. Kalita (1992) reported that ash content of lemon fruit gradually decreased from the highest 4.08 percent at 90 DAF to lowest 3.43 percent at 190 DAF. Similarly, Ca, K and P content also differed at different stage of maturity but Na content remained almost same.

CHAPTER III
MATERIALS AND METHODS

3.1 Materials

The present investigation was carried out to study the changes in biochemical constituents of *khasi* mandarin fruits during ripening after 90 days of fruit setting. The fruits of *khasi* mandarin fruits were collected (approx. 10 years old plant) from Nagajanka village of Jorhat district from a homestead garden ownership of Mr. Jagat Borah located at 155 m above mean sea level.

3.1.1 Description of the crops

The mandarin tree is a medium to large plant, height 2 to 8 m, leaves are small, ovate, elliptical or lanceolate usually crenate, flowers are white, fruits are depressed-globuse, 5 to 8 cm in diameter, pulp sweet and juicy, orange in colour, peel separate easily from the segments and loose skinned, it contains 10 to 15 segments, each segment contain 2 to 3 seeds, seeds poly-embryonic.

3.2 Season of the experiment

The experiment was done during August 2018 to December 2018. The type of soil was sandy loam and the pH around 5.5. No fertilizers and chemicals were applied to the crop.

3.3 Collection of sample

First batch of samples were collected after 90 days after fruit set (DAFS) and subsequent batches were collected at 120, 150, 180 and 210 days after fruit set. Sampling was done in the morning around 9 A.M. Observations on physical parameters viz., weight, volume, diameter, pulp weight, peel weight, pulp: peel ratio of the fruit were recorded before chemical analysis. After removing the peels, the fruits were sliced and mixed well. A part of the composite sample was immediately used for the analysis of chemical constituents viz., moisture, titratable acidity, sugars, total soluble solids, juice pH and organic acid content of the fruits.

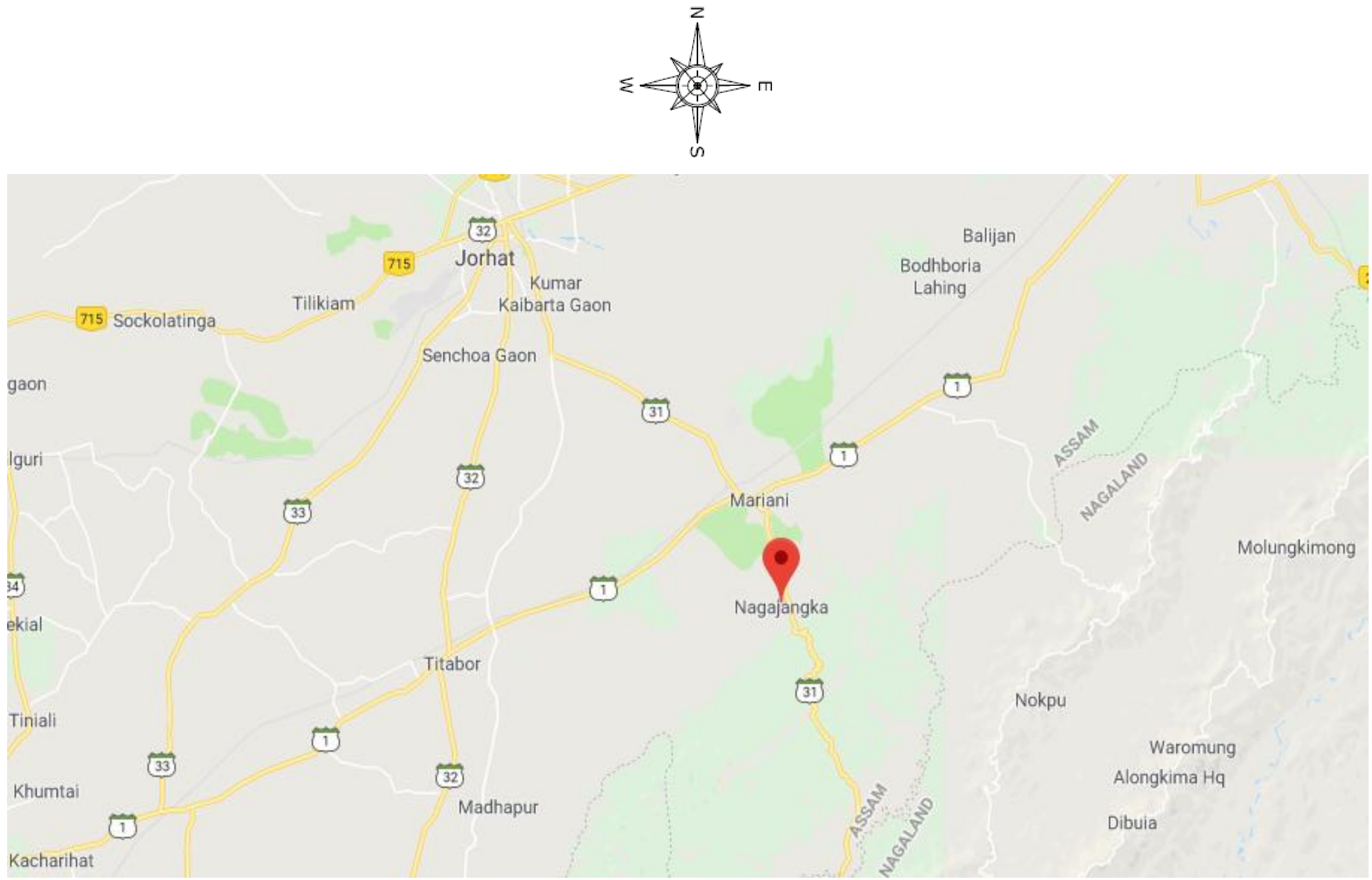

Plate 3.1: location of the Nagajangka village on google map

Pate 3.2.1: Khasi mandarin fruits with crops

90 DAFS | 120 DAFS | 150 DAFS

180 DAFS | 210 DAFS

Plate 3.2.2: Samples collected at different stages of fruit development

The remaining portion of the sample was oven dried at 55˚ C for 3 to 4 days until constant weight was obtained and then ground into fine powder in a grinder. The ground material was stored in a desiccator till used for further analysis. All the estimations were done in triplicate and mean values were recorded.

3.4 Method

3.4.1 Determination of fruit weight

The fruit weight was recorded on the basis of three representative fruits and the mean was expressed in gram. (Bharali, 2013).

3.4.2 Determination of fruit diameter

The fruit diameters were measured at is widest part by vernier calipers and average was expressed in cm. (Bharali, 2013).

3.4.3 Determination of fruit volume

Fruit volume was determined by water displacement methods and mean was expressed in cc. (Bharali, 2013). The volume of fruit was determined by subtracting the initial water level in the measuring cylinder before dipping the fruit from the final water level in the measuring cylinder with dipped fruit.

3.4.4Determination of pulp and peel weights

The pulp and peel weights were recorded separately and expressed in gram. (Bhuyan, 1996)

3.4.5 Determination of pulp : peel Ratio

The pulp: peel ratio was calculated out as follows: (Bhuyan, 1996).

$$\text{Pulp : Peel} = \frac{\text{Pulp weight (g)}}{\text{Peel weight (g)}}$$

3.4.6 Determination volume of juice per fruit

After peeling the fruit, juice vesicles were separated and juice extractions were done using muslin cloth**.** The error occurred during the extraction of juice due to the adhering to muslin cloths was nullified by necessary measurements. Amount of juice was measured using measuring cylinder and mean was recorded in ml.

3.4.7 Determination of juice percentages

The percentage of juice per fruit was determined by dividing the volume of the fruit juice by weight. (Bharali, 2013).

$$\text{Juice percentage} = \frac{\text{Juice (mL)}}{\text{Fruit weight (g)}}$$

3.4.8 Determination of Total soluble solids

The TSS content of fruit juice was determined by Digital refractrometer (Model: PR-32α, Make: ATAGO) and the results were expressed in percentages.

3.4.9 Determination of juice pH

The fruit juice pH was determined bydigital pH meter and average was recorded.

3.4.10 Determination of moisture contents

Moisture content was determined by the method of Rangana(1986). 5gm of fresh sample were accurately weighed in aluminium moisture boxes and dried in a hot air oven at 55 degrees centigrade for 3 to 4 days until constant weight was obtained. Moisture content was calculated using the following relationship

$$\text{Moisture content (\%)} = \frac{\text{Initial weight (g) Final weight(g)}}{\text{Weight of the sample(g)}}$$

3.4.11 Titratable acidity

The titratable acidity was determined by adopting the standard method of analytical chemists (A.O.A.C 1980). 10 ml of clear juice was taken in a 100 ml volumetric flask and volume was made up with distilled water. 10 ml of aliquot was titrated against 50 ml of 0.1 N NaOH using 1to 2 drop of phenolphthalein (1%). Titratable acidity as anhydrous citric acid was calculated by the following formula:

$$\text{Titratable Acidity (\%)} = \frac{\text{Titrate Value} \times \text{Normality of alkali} \times \text{Volume made up} \times \text{Eq. Weight of Citric acid} \times 100}{\text{Volume of sample} \times \text{Alliqut} \times 1000}$$

3.4.12 Reducing sugar

Reducing sugar was estimated following the Fehling's copper reduction method as described by Usha *et al.* (2015). 10 ml of juice was taken in 100 ml volumetric flask. To it 2 ml of lead acetate solution (45%) was added and allowed to stand for about 10 minutes. 4ml of potassium oxalate solution (22%) was added to it to remove the excess lead and volume made up to 100 ml with distilled water and centrifuged. After centrifugation, the supernatant was added drop wise from burette to the boiling mixture of 5 ml each of Fehling's solution A and B containing methylene blue indicator. The end point was indicated by the whole reaction liquid becoming brick red colour. Reducing sugar was determined by the following formula:

$$\text{Reducing sugar (\%)} = \frac{\text{Factor (0.05)} \times \text{Dilution} \times 100}{\text{Titre value} \times \text{volume of juice sample}}$$

Factor: 10 ml of Fehlings solution (A+B) is equal to 0.05 g of glucose

3.4.13 Total sugar

Total sugar was estimated by the method of described by Usha *et al*. (2015). 25 ml of diluted fruit juice used for estimation of reducing sugar was taken in a conical flask and 2.5 ml of concentrated HCL was added to it for inversion and kept overnight. The solution was then neutralized with 1N NaOH using phenolphthalein indicator till pink colour developed, indicating the end point and volume was made up to 75 ml with distilled water. The solution was then titrated against boiling mixture of 5 ml each of Fehling's solution A and B till the reaction liquid turns brick red in colour indicating the end points. The total sugar was calculated by following formula:

(a) $$\text{Total sugar as invert sugar (\%)} = \frac{\text{Factor*} \times \text{Dilution} \times \text{Volume made after neutralization} \times 100}{\text{Titre value} \times \text{volume of juice} \times \text{Aliquote taken}}$$

(b) Non reducing sugar as sucrose = (total invert sugar – Reducing sugar) x Factor**

(c)Total sugar (%) = Reducing sugar + Non reducing sugar

*Factor: 10 ml of Fehling's solution (A+B) is equivalent to 0.05 g of glucose

**Factor: 0.95 g of sucrose on hydrolysis will yield 1g of monosaccharide (glucose + fructose)

3.4.14 Determination of organic acid

Organic acid was determined by UHPLC (Make:Dionex, Model: Ultimate 3000 UHPLC; Detector: PDA, DAD3000, Dionex) in the Department of Biochemistry & Agricultural Chemistry, AAU, Jorhat.

3.4.14.1 Sample extraction:

1 ml of fresh fruit juice sample was thawed at ambient temperature and then 10 ml water-methanol mixture solution (75:25 v/v) was added and the mixture was centrifuged at 25˚ C and 3500 rpm/min for 30 minutes. The upper phase was filtered through filter paper (Whatmann No. 2, blue stripe). The centrifugation was repeated

three times and the filtrates were collected. The combined filtrates were filtered again through Nylon 0.2 micro meter filter (Agilent).

3.4.14.2 Chemicals:

Organic acids (or their sodium salts) were purchased from Supelco, Bellefonte, USA. Sodium sulphate and methanesulphonic acids were of analytical purity or for chromatographic use. Methanol, acetronitrile and water of HPLC grade were used during estimation of organic acids.

3.4.14.3 Preparation of Organic acid standards:

The standard stock solutions were prepared containing 10 mM of each of the organic acids. The stock solution and the corresponding dilution was made with HPLC grade water and stored at 4˚C in dark. The eluent was filtered through 0.2µm filters and degassed under pressure in an ultrasonic bath.

3.4.14.4 UHPLC operational conditions:

The analysis was performed with a UHPLC comprising a photodiode array detector (PDA). The separation of organic acid was performed using an Acclaim Organic acid (OA) column (OA, 5µm, 120Å, 4.0 × 250 mm, ThermoFisher) which were detected by absorbance and quantified with external calibration graph. For simultaneous detection of the analytes, the detector was set at λ =210 nm for all the organic acids. The determination was made in isocratic elution at 30˚C, using a mobile phase of 40 mM sodium sulphate solution prepared by dissolving 5.68g sodium sulphate in 900 ml water with the pH adjusted to 2.68 with methanesulphonic acid and then filled to 1000 ml with water and filtered through nylon filter (0.2 µm). The flow rate of mobile phase was 1ml/min for all chromatographic separation and the total run time 10 minutes. The separation column was balanced with mobile phase until the baseline was stabilized after which sample injection was done. The volume injected was 20 µl for either prepared sample or standard solution. The HPLC peaks were identified by comparing the retention time and spectral data obtained from standards.

3.4.14.5 Calibration:

For obtaining the calibration curves, mixtures of the standards of selected concentration were injected into UHPLC and their chromatograph were obtained. After the injection of the samples, chromatographic peaks were identified by comparing the retention times of the samples with those of the known standards. The quantities of organic acids were estimated from the peak areas, injecting knowun amounts of the standards. In the calculation of the amounts of organic acids, the dilution ratio was taken into consideration.

3.4.15 Determination of ash content:

The ash content was determined by the method as described in the AOAC (1970). 5g of dry powdered sample was taken in a silica crucible, charred in low Bunsen flame and finally ignited at 600°C for 6hrs in the muffle furnace.

Calculation:

$$\text{Ash content } \left(\frac{\text{g}}{100\text{g}}\right) = \frac{\text{weight of the ash (g)}}{\text{weight of the sample (g)}} \times 100$$

The estimation was done in triplicate and their mean was recorded as percent ash content on moisture free basis.

3.4.16 Preparation of Mineral Solution

The mineral solution was prepared according to the method described in AOAC (1970). For this, 0.5g-1g of ash was dissolved in HCl (1:1) on a water bath at 100°C and the solution was evaporated to dryness. After that 4ml HCl and 2ml distilled water were added, warmed and the acid solution portion obtained after filtration was made up to 50 ml in a volumetric flask with distilled water. This solution was used for the estimation of mineral viz. calcium, sodium, phosphorous and potassium.

3.4.17 Determination of phosphorus

Phosphorus content was determined by spectro-photometric method (AOAC, 1980)**.** To an aliquot (0.1 ml) of the mineral solution 1 ml each of ammonium molybdate, hydroquinone and sodium sulphite solution was added in this order, mixing well after each addition. The volume was then made 5 ml with distilled water and the solution was thoroughly mixed. After 30 minutes, the absorbance was measured in the spectrophotometer against a reagent blank at 660 nm wave length and amount of phosphorus was calculated out from the standard curve.

3.4.17 Determination of calcium

Calcium content was determined by flame photometric method (AOAC, 1980). Standards of 50, 100, 150, 200 and 250 ppm of calcium solution were prepared from calcium chloride and subjected to flame photometric estimation along with the mineral solution. A standard graph was prepared. The concentration of the calcium was determined from the graph and the estimation was done in triplicate and their mean was recorded. Calcium content in the sample was expressed as g of calcium/100g of sample.

3.4.19 Determination of sodium content:

Sodium content was determined by flame photometric method (AOAC, 1980). For sodium estimation 5, 10, 20, 30 and 40ppm of sodium standard solution was prepared from sodium chloride and subjected to flame photometric estimation along with the mineral solution by putting the reading in the standard curve. The amounts of sodium concentration in the samples were determined from the mineral solution by putting the reading in the standard curve. The estimation was done in triplicate and their mean was recorded as g of Sodium per 100 g sample.

3.4.20 Determination of potassium content:

Potassium content was determined by flame photometric method (AOAC, 1980). Standards of 5, 10, 20, 30 and 40ppm were prepared from potassium chloride and the flame photometric results were plotted in a graph to prepare standard curve. The amount of potassium concentrations in the samples were determined from the mineral solution by diluting to 20 times. The obtained results were plotted in the graph and the concentration of potassium in the sample was calculated. The estimation was done in triplicate and their mean was recorded as g of Potassium per 100 g sample.

3.4.21 Statistical Analysis

The data were analyzed throughComplete Randomized Design (CRD) and subjected to analysis of variance due to treatment by ‘F’ test (Panse and Sukhatme, 1978). The standard error of the mean difference (S. Ed.±) was calculated by using the following expression. (Snedecor and Cochran, 1967).

$$\text{S. Ed.} = \sqrt{\frac{2EMS}{r}}$$

Where, r = No. of replications.

EMS = Error Mean Square.

The significance of specific mean difference was determined by calculating the critical difference (C.D) at 5% probability level.

$$\text{C.D.} = \text{S. Ed.} \times t_{0.05}$$

Where, t = tabulated value of t at 5% level of significance at error degree of freedom.

CHAPTER IV

EXPERIMENTAL FINDING

Result obtained from the present investigation on biochemical changes in *khasi* mandarin during ripening are presented in different tables and figures in this chapter. Data pertaining to fruit weight, fruit diameter, fruit volume, peel weight, pulp weight, pulp: peel, pulp percentage, peel percentage, juice percentage, moisture content, TSS, titratable acidity, TSS: acidity ratio, juice pH, sugar content, ash, minerals (calcium, potassium, sodium, phosphorus) and organic acid (citric acid, oxalic acid, fumeric acid and ascorbic acid) content are presented in the Table 1, 2, 3, 4, 5 and figures (1 to 13)

4.1 Physical parameters

Data pertaining to physical parameters such as fruit weight, fruit diameter, fruit volume peel weight, pulp weight, pulp: peel ratio is presented in table 1

4.1.1 Fruit weight

Data presented in table 1 revealed that significantly highest fruit weight 78.32 g was observed at 210 DAFS while the lowest 23.46 g was observed at 90 DAFS. However, the rate of change in fruit weight was highly significant in all stages up to 150 DAFS and the rate of increase slightly slowed down towards the subsequent stages up to 210 DAFS.

4.1.2 Fruit diameter

Data presented in Table 1 revealed that the fruit diameter increased significantly in all stages of fruit development towards the maturity. The highest fruit diameter 5.20 cm was observed at 210 DAFS and the lowest fruit diameter was recorded at very early stage (90 DAFS) of fruit development *i.e*. 3.13 cm.

4.1.3 Fruit volume

Fruit volume recorded highest 78.67cc at 210 DAFS that was in the month of December and the lowest fruit volume 24.66 cc was observed at 90 DAFS in the month of August. Fruit volume increased significantly in all the stages of fruit development. (Table 1)

4.1.4 Peel weight, pulp weight and pulp: peel ratio

Data presented in the Table 1 revealed that peel weight, pulp weight, and pulp: peel ratio differed significantly in the different seasons. Pulp weight, peel weight and pulp: peel ratio recorded highest in the month of December i.e. 210 DAFS (61.05g, 17.26g, and 3.52 respectively) and lowest value was observed in the month of August i.e. 90 DAFS. All the three parameters increased significantly from first stage to last stage of maturity. The pulp percentage increased significantly towards the fruit maturity with decrease in peel percentage. The highest pulp percentage (77.96%) was recorded at last stage and highest peel percentage (27.61%) was recorded at early stage of maturity.

4.2 Chemical constituents

Data pertaining to juice content, moisture content, TSS, titratable acidity, TSS: acidity ratio, pH, reducing sugar, non reducing sugar, total sugar, organic acid (citric acid, oxalic acid, fumeric and ascorbic acid) ash, calcium, potassium, sodium and phosphorus are presented in the Table 2, 3, 4, and 5.

4.2.1 Juice content

Juice content was found to be increased significantly from 90 DAFS to 210 DAFS in the order 6.87, 12.63, 22.2, 28.63, 36.03 ml. lowest juice content 6.87 ml was recorded at 90 DAFS and highest 36.03 ml at 210 DAFS. Similarly juice percentage also increased from 29.23 percent to the highest 46.06 percent towards the fruit maturity. Highest juice percentage was recorded at 210 DAFS. (Table 1)

4.2.2 Moisture content

The data presented in Table 1 revealed that the differences in the moisture content on fresh pulp weight basis was significant at all stages of fruit development. The highest 87.37 per cent and the lowest 74.48 percent moisture content were recorded at 210 DAFS and 90 DAFS respectively. The moisture content gradually increased towards maturity.

4.2.3 Total soluble solids (TSS)

TSS content gradually but significantly increased in all stages of development towards maturity. The highest TSS 8.84 percent was observed at 210 DAF i.e. in the month of December and the lowest TSS 5.83 percent was recorded at 90 DAFS i.e. in the month of August. (Table 2)

4.2.4 Titratable acidity

Data portrayed in Table 2 showed a significant decrease in titratable acidity with the advancement of maturity. The highest titratable acidity 3.9 was recorded at very early stage of maturity at 90 DAFS and lowest 0.85 percent was observed at 210 DAFS i.e. in the month of December.

4.2.5 TSS: acidity ratio

The TSS: acidity ratio was found to be decreased significantly from early stage to last sage of maturity in the order 1.83, 2.20, 2.52, 6.93 and 10.40 at 90, 120, 150, 180, 210 DAFS respectively. The highest TSS: acidity ratio was observed at 210 DAFS, i.e. 10.40 and the lowest ratio was recorded at 90 DAFS i.e. 1.83.

4.2.6 pH

The pH content of juice significantly increased towards the fruit ripening. The highest value was observed at 210 DAFS i.e. 3.80 and the lowest value of pH was found at early stage of maturity at 90 DAFS. The pH value was found to be increased in the order 2.85, 2.90, 3.01, 3.12 and 3.85 at 90,120, 150, 180 and 210 DAFS respectively.

4.2.7 Reducing sugar, non reducing sugar and total sugar content

Data presented in table 3revealed that the reducing, non-reducing and total sugar increased significantly towards the fruit maturity. Reducing sugar, non reducing sugar and total sugar content were observed to be highest at 210 DAFS as 2.85, 3.86 and 6.7 percent respectively and lowest were observed at 90 DAFS i.e. 1.65, 0.87 and 2.52 percent respectively.

4.2.8 Organic acid

Data presented in Table 4 showed decrease in citric acid content in fruit juice with the advancement of maturity. Citric acid content was found to be decreased in the order 644.17, 198.75 164.44, 161.37, 94.24 mg per 100 ml fresh juice in 90, 120, 150, 180 and 210 DAFS respectively. Oxalic acid content in the fruit was observed to be increased up to 150 DAFS then it decreased towards the maturity. However, the highest oxalic acid 34.21 mg/100 ml was observed at 150 DAFS and lowest oxalic acid content was found at 210 DAFS in the month of December. Highest fumeric acid content was recorded at 90 DAFS as 20.78 mg/100ml of fresh juice. Ascorbic acid content was observed as 1.13 mg/100 ml at 90 DAFS then it increased to 2.89 mg/100 ml at 120

DAFS and interestingly again decreased to 1.64 mg/100 ml then again increased to 83.88 mg/100ml at 210 DAFS.

4.2.9 Ash and minerals content

A significant difference in ash content was noticed at different stages of maturity (Table 5). It gradually decreased from highest 5.14 per cent at 90 DAFS to the lowest 1.94 per cent at 210 DAFS. Similarly, calcium, potassium, sodium, and phosphorus content also differed at different stages of development. Calcium content in the fruit decreased in the order 0.63, 0.52, 0.49, 0.42, 0.39 g/100 g at 90, 120, 150, 180 and 210 DAFS respectively. The highest calcium content was obtained at 90 DAFS and lowest content was obtained at last stage of observation i.e. at 210 DAFS. Similarly, potassium content also decreased gradually from 90 DAFS to 210 DAFS in the order 1.03, 0.83, 0.78, 0.72 and 0.65 g/100 g. Sodium content decreased up to 180DAFS, highest sodium content was found at 90 DAFS i.e. 0.024 g/100g. The lowest sodium content 0.005 g/100g was observed at 180 DAFS. At 210 DAFS the sodium content was recorded as 0.008 g/100g. Phosphorus content of the pulp increase from 0.15g/100g to 0.26g/100g then decreased to 0.15 g/100g and then again increased to 0.17 g/100g.

CHAPTER V

DISCUSSION

The present investigation was carried out with the objective to study "Biochemical changes in *khasi* mandarin during ripening." The experimental results of the investigation have been discussed in this chapter.

5.1 Physical parameters

Fruit weight is a basic parameter of quality and should be measured precisely on digital balance/weighing machine. Volume is an important parameter that indicates the growth of fruit and also space required for the handling and packing of fruit. The ratio of weight and volume indicates internal quality such as juiciness or dryness. (Ladaniya, 2008).

The present study revealed that the fruit weight, fruit volume and fruit diameter increased progressively and significantly with maturity of *khasi* mandarin fruits. The increase in fruit weight may be due to accumulation of juice in the fruit. Again the increase in fruit weight and fruit volume could be the result of the increase in size of the cell and accumulation of food substances in the inter cellular spaces in fruit (Bollard, 1970). Similar increasing trends were also reported by Deshmukh *et al*. (2016) in *khasi* mandarin from 180 DAFS to 260 DAFS at different altitude, who observed that fruit weight increased from116.87g to 136.45g (500-600m),111.56g to 135.01g (700-800m) 116.36 to101.14g to 131.14g (900-1000m) and 100.23 to 133.15(1300-1400m)

Increasing trend of fruit volume was recorded by Bhatnagar *et al.* (2012) who observed that fruit volume increased from 87cc to 176cc in Nagpur mandarin from 1st August to 1st November however, the fruit volume slightly decreased after 15th August.

In the present investigation fruit diameter has been observed to be significantly increased towards the maturity. This increase in fruit diameter may be due to cell division and dry matter accumulation in the fruit. Similar increasing trend in fruit diameter was reported by Deshmukh *et al*. (2016) in *khasi* mandarin from 180 DAFS to 260 DAFS at different altitude, who observed that fruit diameter increased from 4.92cm to 5.86cm (500-600m), 5.3cm to 6.10cm(700-800m), 4.70cm to 5.43cm (900-1000m) and 5.01cm to 5.71cm (1300-1400m).

Pulp weight was low at initial stage i.e., at 90 DAFS. The percentage of pulp increased with decrease in the peel percentage with advancement of fruit maturity. Throughout the fruit development the pulp-peel ratio increased gradually. This is probably due to the increase in juice content in the pulp. Similarly, Bhuyan (1996) observed that peel weight increased from 14.48g (60 Days after flowering, DAF) to 21.30g (180 DAF), peel percentage decreased from 28.97 percent to 25.91 percent, pulp weight increased from 71.02g to 74.08g and pulp percentage increased from 71.02 percent to 74.08 percent in Assam lemon.

5.2 Moisture content

Moisture content refers to the presence of water, often in trace amount. Water is an important part of all cells and fluids in the body. It carries nutrients to waste products from cell in the body helps in digestion and absorption of food and regulates body temperature (Johnson, 1996).

The moisture content is not a fixed property as it is dependent upon many factors like soil moisture content, cultivar, proportionate amount of chemical compound and also environmental condition. In the present investigation the moisture content increased significantly from first date (90 DAFS) of harvesting to last date of harvesting (210 DAFS). The increase in moisture content towards maturity of the fruit may be due to the high soil moisture content. Bartholmew (1923) in lemon fruits and Bain (1958) in pulp segments of *Valenica* orange reported similar increasing trend of moisture content.

5.3 Juice content

Fruit juice is mainly the liquid expressed from fruit cell vacuoles, but also includes insoluble particles and bits of fruit tissue. Though primarily water, this organic medley contains sugars such as glucose, fructose, and sucrose, organic acids (malic, citric, and tartaric), fats, proteins, various volatile compounds and vitamins (Nagy *et al.*, 1993). Taste and flavor qualities are formed by the sugars, organic acids and aroma compounds present in juice. Sugars and organic acids make up the bulk of the soluble solids fraction, and a proper balance between the concentrations of both are important in the palatability of the juice. Thus, organic acid additions are only reasonable within the scope of maintaining an acceptable sugar/acid ratio. Aroma arises from a number of volatile compounds essential to juice quality.

Present investigation revealed that juice content increased with the development of fruit. The highest juice content (36.03ml) and highest juice percentage (46.03%) were recorded at last season of harvesting. The juice content increased till last date of observation (210DAFS). Similar increasing trend in juice content was also reported by Deshmukh *et.al.* (2016) in *khasi* mandarin from 180 DAFS to 260 DAFS at different altitude, who observed that juice content increased from 31.11 to 50.90% (900-1000m) and 36.18 to 51.15% (1300-1400m). Similarly, Bhatnagar *et al.* (2012) in Nagpur mandarin also observed that juice content increased from 41.40 % to 48.80% in Ambiabahar during August to November. Such increase in juice content may be due to the accumulation of water and solutes in the juice vesicles.

5.4 Total soluble solid (TSS)

Total soluble solids comprise 10-20% of the fresh weight of the fruit, and consist mainly of carbohydrates (70-80%), and relatively minor quantities of organic acids, proteins, lipids and minerals (Monselise, 1986).

In the present investigation the total soluble solids content increased towards the fruit maturity. The highest TSS 8.84 % was recorded at the last stage of observation (210DAFS). The early stage of maturation (90 DAFS) recorded lowest TSS 5.83 %. Such increase in TSS content in the fruit can be attributed to the fact that sugar deposited in the fruit as carbohydrate at the initial stage started getting converted into soluble sugars by various hydrolytic enzyme which apparently increased the TSS content. It may also be due to hydrolysis of acid to sugars. Similar increasing trends were also obtained by Deshmukh *et al.* (2016) in *khasi* mandarin from 180 DAFS to 260 DAFS at different altitude who observed that TSS content increased from 8.07˚B to 10.30˚B (500-600m), 7.86˚B to 10.17˚B (700-800m), 7.54˚B to 10.10˚B (900-1000m) and 7.36˚B to 10.18˚B (1300-1400m) and Bhatnagar *et al.* (2012) in Nagpur mandarin (6.30˚B to 8.90˚B) during 210 DAFS to 260 DAFS

5.5 Titratable acidity

The measurement of titratable acidity in fruit juices measures the concentration of titratable hydrogen ions contained in the fruit juice samples by neutralization with strong base solution to a fixed pH. This value includes all the substances of acidic nature in the fruit juice: free hydrogen ions, organic acids, acid salts and cataions. Percentage of sourness is due to the hydrogen ion concentration, while there also exist many factors which affect the intensity of the perceived sourness. (Richards, 1898). CoSeteng *et al.* (1989) opined that the interaction of titratable acidity and pH level was also statistically significant for citric acid, malic acid and tartaric acids. This interaction form indicates titratable acidity and pH does not independently influence sourness but rather both factors play a role in the intensity of sourness of solution). Total acidity percentage of citrus juice can be considered as a limiting factor in overall juice quality parameters and in determining the harvest time in several citrus producing regions.

Titratable acidity content in juice was observed highest at early stage of fruit maturation (90DAFS). Then it decreased significantly till the last stage of maturity (210 DAFS). A significant variation was observed for acidity content of the fruit juice among all the stages of development. Such decrease in acidity may be due to dilution effect or hydrolysis of acid into sugars. Such a decreasing trend obtained from present investigation was quite similar to those obtained by Bhatnagar *et al.* (2012) who observed that acidity content decrease from 1.25 to 0.88% for Ambiabahar (August to November) and from 1.00 to 0.80 for Mrigbahar (December to March) of Nagpur mandarin and Rokaya *et al.* (2016) in mandarin who observed acidity decreased from 2.59% to 0.66 % (230 to 280 DAFS). However, considering the position of the fruit on the tree, Jawanda *et al.* (1973) reported highest acidity concentration in the outer fruit than the inner one.

5.6 TSS: acidity ratio

TSS to acid ratio is also called as permitted maturity parameter. TSS: Acidity ratio is the best measure for quality determination and could be considered to identify the harvesting time to give fruits of better nutritive quality (Syvertsen *et al*. 1980). In citrus fruits that are used for table purposes (such as fresh fruits) and that are processed into juices, maturity is determined mainly on the basis of the ratio of total soluble solids to titratable acidity, the relative sweetness or sourness of citrus fruit is determined by its ratio of sugars to acids. Since most soluble solids in oranges, mandarins, and grapefruits are constituted by sugars, the ratio of the soluble solids to acid is used for convenience. This is a maturity index and used to determine the legal maturity of oranges, mandarins, grapefruit, pummelos, and their hybrids for fresh-fruit (dessert) purposes (Ladanyiya, 2008).

In the present investigation, a significant variation in TSS: Acidity ratio has been observed among the all stages of fruit development. The highest TSS: Acidity ratio was observed at last stage of observation (210 DAFS). 90 DAF recorded the lowest TSS: Acidity ratio. TSS: Acidity ratio significantly increased from first stage to last stage of maturity. Such increase in TSS: acid ratio may be due to the increasing rate of deposition of polysaccharides and decreasing rate of acidity with the advancement of maturity period. The data obtained from present investigation were in agreement with those of Rokaya *et al.*(2016) for mandarin at different altitudes who found that TSS: acidity ratio increased from 2.65 to 17.76 (1300m), 3.14 to 16.52 (1000m) and 3.58 to 14.34 (700m) and Deshmukh *et al.* (2006) in Nagpur mandarin (5.35-12.87%) from 180 DAFS to 260 DAFS.

5.8 pH content

pH is a measure of the hydrogen ion concentration or activity of an aqueous solution and every aqueous solution can be measured to determine its pH value. This value ranges from 0-14 with values below pH 7 exhibiting acidic properties and values above pH 7 exhibiting basic or alkaline properties.

In the present investigation the pH content of the fresh juice increased significantly throughout the season till last stage of the observation. Highest pH content has been observed at the last stage of the observation and the lowest pH was observed at early stage of maturation. In the present investigation, a significant difference in juice pH was observed among the all stages of fruit maturation. Such increase in pH content may be the result of the corresponding decrease in the organic acid mainly citric acid. The results of present investigation well comparable with the findings of Sinclair *et al.* (1994) in Valencia orange who observed that pH of juice increased from 2.72 to 3.11 during investigation period from October to May. However, Riaz *et al.* (2015) observed that pH content of juice first increased from 3.30 to 4.40 towards the maturity then it slightly decreased at late stage of fruit development to 4.20.

5.9 Reducing sugar, non reducing sugar and total sugar

Reducing sugar, non reducing sugar and total sugar increased significantly throughout the fruit development. In the early stage, the sugar content was very less then it increased till the last stage of observation. In the present investigation the highest total sugar content (6.7%) highest reducing sugar (2.85%) and highest non reducing sugar (3.86) were obtained for 210 DAFS. A significant difference has been observed among all the stages of fruit development. The total sugar content of the fruit juice increases from 2.52 percent to 6.7 percent during maturation period. The increase in sugar content in the maturing fruit may be attributed to the translocation of phtosynthates from leave to fruit as well as hydrolytic activities of various polysaccharide degrading enzymes. Similar trend of increase in sugar content during fruit development was reported by Ram (2001) for *kinnow* mandarin fruits who observed that reducing sugar, non reducing sugar and total sugar increased from 2.20 - 3.60%, 2.16-4.39% and 4.48-8.22% respectively (210 -290 DAFS).

Deshmukh *et al.*(2016) for *khasi* mandarin also reported similar increasing trend of sugar content at different altitudes who observed that non reducing sugar increased from 3.14 to 3.96%, 2.83 to 3.88% , 2.54 to 4.00% and 2.88 to 4.03 % and total sugar increased from 4.80 to 6.01%, 4.52 to 6.03%, 4.15 to 6.12% and 4.65 to 6.11% at 500 - 600m, 700-800m, 900-1000m and 1300 to 1400m altitude respectively.In the present investigation it has also been observed that towards the last stage of development the non-reducing reducing sugar content is more than that of reducing sugar. It might be due to the enhanced rate of sucrose synthesis due to high activity of metabolizing enzymes towards the fruit maturity. Similar findings were reported by Ram (2001) for *kinnow* mandarins.

5.10 Organic acid

Organic acids are a useful index of authenticity in fruit products, since they have lower susceptibility to change during processing and storage than other components of fruits (Camara *et al*., 1994). Accurate knowledge of organic acid levels (and ratios) might be useful for determining the percentage juice content of juice products, and also for detecting misbranding and/or adulteration in this food class (Coppola and Starr, 1986), since each fruit has a unique pattern of organic acids (Wrolstad, 1981). The organic acid composition of fruits is also of interest because of its important influence on the sensory properties of fruits and fruit juices. Even though they are minor components of fruits, in combination with sugars, they are important attributes of the sensory quality of raw and processed fruits (Wang *et al*., 1993). At the same time, some organic acids may be used as indicators of ripeness (Palmer and List, 1973), bacterial activity and adulteration (Evans *et al*., 1983; Blanco *et al*., 1996). Citrus fruits are classified as acid fruits, since their soluble solids are composed mainly of organic acids and sugars (Kale and Adsule, 1995), which are used as the main index of maturity and one of the major analytical measures of flavor quality (Fellers, 1991). The main acids of citrus fruits are citric and malic acids. In addition, traces of tartaric, benzoic, oxalic and succinic acids have also been reported (Kale and Adsule, 1995).

Organic acids are essential in the control of fruit growth via cell enlargement through water uptake (Liu *et al*., 2007). Accumulation of organic acids during the early stages of fruit development is directly linked to the supply of substrates for the maintenance of respiration processes during the development of fruits (Seymour *et al*., 2013).

Citric acid (2-hydroxy-1,2,3-propanetricar-boxylic acid) is a weak tricarboxylic acid that is naturally concentrated in citrus fruits. At physiologic blood pH, and to a lesser extent in urine, it exists mainly as the trivalent anion. Citric acid is frequently used as a food additive to provide acidity and sour taste to foods and beverages. Citrate salts of various metals are used to deliver minerals in biologically-available forms; examples include dietary supplements and medications. Among fruits, citric acid is most concentrated in lemons and limes, (Muller *et al.* 1996) comprising as much as 8% of the dry fruit weight. A major source of citric acid *in vivo* results from endogenous metabolism in the mitochondria via the production of ATP in the citric acid cycle. Gastrointestinal absorption of citric acid from dietary sources has been associated with a modest increase in urinary citrate excretion (Seltzer *et al*. 1996; Kang *et al*. 2007; Penninston *et al*. 2007). Citric acid is important in prevention and elimination of kidney stones, so lemons and limes, due to high concentration of this acid, can be used as a therapy against this disease (Penniston *et al*., 2007). Malic acid possesses many health-related benefits such as boosting immunity, maintaining oral health, reducing the risk of poisoning from a build-up of toxic metals, promoting smoother and firmer skin and helps reducing symptoms of fibromyalgia (Abraham and Flechas, 1992).

Insoluble oxalate is excreted in the faces while the soluble oxalate is absorbed by the body. Soluble oxalate forms strong chelates with dietary calcium, rendering it unavailable for absorption and assimilation. However high dietary intake of soluble oxalate can lead to formation of kidney stones. On the other hand, oxalic acid is considered an "undesired compound" (Franceschi and Horner, 1980; Webb, 1999; Perera *et al.,* 1990).

High concentration of ascorbic acids in plant samples might associate with attractive free radical scavenging capacity and health benefit like anti-carcinogenic and anti-atyerogenic (Lui *et al.,* 2008). Ascorbic acids contribute to the antioxidant properties by protecting the membrane erythrocyte, maintain the blood vessels flexibility and improving blood circulation in arteries of smokers as well as facilitating the absorption of iron in the blood (Adegunwa *et al.*, 2011).

Organic acids are supposed to be important factors for determination of the taste of the fruits. In the present investigation various organic acids content in the fresh fruit juice were observed in UHPLC at various stages of fruit development. It has been observed that citric acid content decreased from 644.17 mg/ 100 ml to 94.23 mg/ 100 ml from the first to last stage of observation. Similarly, in the present investigation fumeric acid and oxalic acid content of the juice also decreased towards the maturity period. During early stage oxalic acid content increased significantly till 150 DAFS then it decreased significantly towards last stages of observation. Similar decreasing trends of citric acid and oxalic acid contents were also reported by Albertni *et al.* (2006). Highest content of fumericacid was observed at early stage at 90 DAFS and then decreased. Fumeric acid peaks were not observed at 150DAFS and 180 DAFS. No report is available regarding changes in fumeric acid content during fruit development. Decrease in organic acid content in the fruit juice towards the maturity could be attributed to the supply of organic acids as substrates for the maintenance of respiration processes and also may be due to conversion of acids to sugars during the development of fruits.

In the present investigation it has been observed that ascorbic acid content increased during the fruit maturity till the last stage of observation. Ascorbic acid content increased significantly and rapidly towards the maturity period. The highest ascorbic acid content was observed at 210 DAFS. This active synthesis of ascorbic acid content in last stage of maturity may be attributed to inactivation of ascorbic acid oxidase enzyme due to increasing rate of phenols in juice. Increase in ascorbic acid content during fruit development has also been reported by Ram (2001) for *kinnow* mandarin and Patel *et al.* (2014) in *Megha purple* and *Nagaland purple* genotype of passion fruit up to 90DAFS.

5.11 Ash and minerals (Ca, K, Na, P) content

Ash is the inorganic residue remaining after water and organic matter have been removed by heating in the presence of oxidizing agent. It provides a measure of the total amount of minerals within a food (Mc Clement, 2003). Higher ash content predicts the presence of an array of minerals elements as well as high molecular weight elements

(Onot *et al.,* 2007). Ash content can originate from the biomass itself. Ash is one of the components in the proximate analysis of biological materials, consisting mainly of salty, inorganic constituents, metal salts which are important for process requiring ions such as Na^+(Sodium), K^+ (Potassium) and Ca^{2+}(Calcium).

A significant difference has been observed for ash content in the fresh pulp among all stages of development. Ash content decreased towards the maturity of the fruit. Highest ash content was observed at immature stage of development and then it decreased towards the maturity. The regular decreasing of ash content might be due to the continuous decreasing rate of uptake of minerals from the soil or may be due to the distribution of the minerals to the various parts of the plant. Another reasons for decreasing minerals may be due to the dilution effect due to increase in the juice and moisture content of the fruits. Similar decreasing trend of ash content was also reported by Soni and Randhawa (1976) in lemon fruit, Harding and Fisher (1945) in grape fruits. This finding of present investigation was also in agreement with those of Kermasha *et al.* (1987) for pineapple, Saikia (1993) in pineapple fruit and Bhuyan (1996) in Assam lemon.

5.11.1 Calcium content

Calcium is an important constituent of bones and teeth and is involved in regulation of nerve and muscle function. In blood coagulation, calcium activates the conversion of pro-thrombin to thrombin. It plays a vital role in enzyme activation. Calcium activates large number of enzymes such as adenosine triphosphatase (ATPase), succinic dehydrogenase and lipase. It is also required for membrane permeability, involved in muscle contraction, normal transmission of nerve impulses and in neuromuscular excitability (Soetan *et al.,* 2010). Reduced extracellular blood calcium increases the irritability of nerve tissue, and very low levels may cause spontaneous discharges of nerve impulses leading to tetany and convulsions (Hays and Swenson, 1985). Calcium absorption requires calcium-binding proteins and is regulated by vitamin D, sunlight, parathyroid hormone and thyrocalcitonin. Thyrocalcitonin decreases plasma calcium and phosphate levels whereas parathyroid hormone increases them.

In children, calcium deficiency causes rickets due to insufficient calcification by calcium phosphate of the bones in growing children. The bones therefore remain soft and deformed by the body weight. In adults, it causes osteomalacia, a generalized demineralization of bones. It may also contribute to osteoporosis, a metabolic disorder resulting in decalcification of bone with a high incidence of fracture, that is, a condition where calcium is withdrawn from the bones and the bones become weak and porous and then breaks (Hays and Swenson, 1985; Malhotra, 1998; Murray *et al.,* 2000). Calcium deficiency also affects the dentition of both children and adult. Toxicity symptoms occur with excess absorption due to hypervitaminosis D or hypercalcaemia due to hyperparathyroidism, or idiopathic hypercalcaemia.

Excess calcium depresses cardiac activity and leads to respiratory and cardiac failure; it may cause the heart to stop in systole, although, normally, calcium ions increase the strength and duration of cardiac muscle contraction. Excess calcium and phosphorus are excreted by the kidney. Ca and P excreted in faces are largely the unabsorbed dietary minerals; some comes from the digestive juices, including bile (Hays and Swenson, 1985). Growing, pregnant and especially lactating humans and animals require liberal amounts of calcium and phosphorus. Parturient paresis, or milk fever, in cows is associated with calcium metabolism. This illness usually occurs with the onset of profuse lactation and the most common abnormality is acute hypocalcaemia with decline in blood calcium level from normal. Serum magnesium levels may be elevated or depressed, low levels being accompanied by tetany and high levels by a flaccid paralysis.

In the present investigation calcium content in the fruit pulp significantly decreased towards the fruit maturity. The highest calcium content was observed at 90 DAFS then it significantly decreased to 0.39g/100g. A significant difference has been observed among all the stages of development. The decreased in Ca content leads to the softening of the fruit by destabilizing the middle lamella. Similar decreasing pattern was also reported by Singh *et al.* (2015) in grapefruit up to 180 days after fruit set and Sheng *et al*. (2009) in Navel oranges. However, after 180 DAFS Singh *et al.* (2015) observed a increasing trend of calcium content. Storey and Treeby (1999) also reported the similar decreasing pattern of calcium content in Navel orange.

5.11.2 Potassium content

Potassium is the principal cation in intracellular fluid and functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle, cell membrane function and Na^{+}/K^{+}-ATPase. Potassium is also required during glycogenesis. It also helps in the transfer of phosphate from ATP to pyruvic acid and probably has a role in many other basic cellular enzymatic reactions. Its metabolism is regulated by aldosterone. Hyperkalaemia is increased level in serum potassium and this occurs in Addison's disease, advanced chronic renal failure, shock and dehydration (Soetan *et al.,* 2010). Toxicity disease or symptoms include dilatation of the heart, cardiac arrest, small bowel ulcers. Hypokalaemia is low level of serum potassium and this occurs in diarrhoea, metabolic alkalosis and familial periodic paralysis. Deficiency disease or symptoms occurs secondary to illness, functional and structural abnormalities including impaired neuromuscular functions of skeletal, smooth, and cardiac muscle, muscular weakness, paralysis, mental confusion (Others are cardiac arrythmias, impaired carbohydrate tolerance, altered electrocardiogram in calves. Potassium deficiency affects the collecting tubules of the kidney, resulting in the inability to concentrate urine, and also causes alterations of gastric secretions and intestinal motility.

Potassium is a key circulating electrolyte which is also involved in the regulation of ATP dependent channels along with sodium. These channels are the Na+/K+ –ATPases and their primary function is in the transmission of nerve impulses in the brain. Sodium and potassium maintains osmotic and water balance as well as membrane potentials. The Na/K ratio in the body is of great concern for prevention of high blood pressure. Na/K ratio less than one is recommended (Akubugwo *et al.,* 2007). Regular consumption of these leafy vegetables may assist in preventing hypertension and cardiovascular diseases, as dietary potassium is an important cation in regulating blood pressure and attenuating platelet reactivity, which is the major causative factor of vascular occlusion (He and MacGregor, 2008). Furthermore, consumption of wild vegetables with high potassium content enhances the bioavailability of calcium in body and promotes bone health by preventing the occurrences of calciuria.

In the present investigation it has been found that the potassium content in the pulp decreased significantly towards the fruit maturity. Highest potassium content observed at 90 DAFS and the lowest was observed at 210 DAFS. Such decreasing pattern of potassium has been observed by Sheng *et al*. (2009) in Navel oranges. However, Singh *et al*. (2015) observed that the potassium content first decreased from 90 days after fruit set to 150 days after fruit set then it increased till the month of December (240 DAFS) in grapefruit development.

5.11.3 Sodium content

Sodium is important for fluid distribution, blood pressure, cellular work and electrical activity. Sodium is involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction (Okon and Akpanyung, 2005). .

In the present investigationsodium content significantly decreased throughout the growing season up to 210 DAFS. Then it slightly increased at 210 DAFS. Highest sodium content was observed at 90 DAFS and lowest content was observed at 180 DAFS.No data were available in theliterature with which to comparethe current data of seasonal changes in sodium content.

5.11.4 Phosphorus content

Phosphorus is a part of every cell of the body and is vitally concerned with many metabolic processes, including those involving the buffers in body fluids (Hays and Swenson, 1985). It functions as a constituent of bones, teeth, adenosine triphosphate (ATP), phosphorylated metabolic intermediates and nucleic acids. It as aids the buffering system (phosphate buffers, functions in the formation of high energy compounds), and is involved in the synthesis of phospholipids and phosphor-proteins. Practically, every form of energy exchange inside living cells involves the forming or breaking of high-energy bonds that link oxides of phosphorus to carbon or to carbon-nitrogen compounds.

In the present investigation phosphorus content followed an increasing pattern from 90 DAFS (0.15 g/100g) to 210 DAFS (0.17 g/100g) although at 180 DAFS (0.15g/100g) it slightly decreased. There was no significant difference between the 90 and 180 DAFS. Similar pattern of changes during fruit development was also reported by Singh *et al.* (2015) for grape fruit.

CHAPTER VI

SUMMARY AND CONCLUSION

An experiment on Biochemical changes in *khasi* mandarin (*Citrus reticulata* Blanco) during ripening was conducted with the aim of evaluating the physical parameters such as fruit weigh, fruit volume, peel weigh, pulp weight, pulp:peel ratio, peel percentage, pulp percentage, juice content, moisture, titratable acidity, juice pH, TSS, TSS/Acidity ratio, reducing sugar, non-reducing sugar, total sugar, organic acids, ash and minerals (Na, K, P, Ca). For this purpose, *khasi* mandarin fruits were collected in monthly intervals from 90 DAFS to 210 DAFS from a homestead garden situated at Nagajangka village of Jorhat district of Assam. The chemical experiment was conducted in the laboratory of the Department of Biochemistry and Agricultural Chemistry and Horticulture AAU, Jorhat-13. The salient findings are summarized below

- Significant variation in fruit weight among all the stages of development was observed. The fruit weight increased from 23.46g to 78.32g. Highest fruit weight was recorded at 210 DAFS.
- Significant variation in fruit diameter among all the stages of development was recorded. The highest fruit diameter was recorded at 210 DAFS i.e. 5.20 cm
- The fruit volume (24.66cc) was lowest at 90 DAFS and which increased significantly to 78.67 cc at 210 DAFS.
- The peel weight gradually increased from 90 DAFS to 210 DAFS. The maximum peel weight was recorded at 210 DAFS i.e. 17.26g.
- Peel percentage gradually decreased from 90 DAFS to 210 DAFS. The lowest peel percentage was recorded at last stage of harvesting i.e. 22.04 %.
- Pulp weight increased from 90 DAFS to 210 DAFS. The maximum pulp weight was recorded at 210 DAFS i.e. 61.06g.
- Pulp percentage gradually increased from 72.39% at 90 DAFS to 77.96 % at 210 DAFS.
- Pulp: peel ratio gradually increased from 90 DAFS to 210 DAFS. The maximum pulp: peel ratio was recorded as 3.52 at last stage of harvesting.

- Juice content significantly increases from 90 DAFS to 210 DAFS. The maximum juice content was recorded at 210 DAFS i.e. 46.06 percent and lowest was observed as 29.23 percent at 90 DAFS.
- The moisture content increased from 74.48 percent at 90 DAFS to 87.37 percent at 210 DAFS.
- The lowest TSS content 5.83 percent was observed at 90 DAFS and then it gradually increased to the highest 8.84 percent at 210 DAFS.
- The highest titratable acidity 3.19 percent was observed at 90 DAFS which then decreased to 0.85 percent at 210 DAFS.
- TSS / acidity ratio showed an increasing trend from 1.83 to 10.40 during fruit development from 90 DAFS to 210 DAFS.
- pH content of juice increased from 2.85 to 3.80 during the development the fruit. The highest pH content was recorded at 210 DAFS.
- Reducing sugar, non reducing sugar and total sugar content in the fruit juice increased towards the fruit maturity. Reducing sugar, non reducing sugar and total sugar content were observed to be highest at 210 DAFS as 2.85, 3.86 and 6.7 percent respectively.
- Citric acid content decreased from 644.17mg/100ml at 90 DAFS to 94.24 mg/100 ml fresh juice at 210 DAFS. Oxalic acid content in the fruit juice increased up to 150 DAFS then it decreased towards the maturity. The highest fumeric acid content was observed at early stage of maturity i.e. 20.78mg/100ml. Highest ascorbic acid content was observed at last stage of fruit development i.e. 83.88mg/100ml.
- Ash content showed a decreasing pattern towards the fruit maturity. Ca and K content decreased towards the maturity. Na content also decreased towards the maturity but slightly increased at last stage of harvesting. Phosphorus content first increased to 150 DAFS then decreased at 180 DAFS and then again slightly increased at 210 DAFS.

From the present investigation it can be concluded that the fruits harvesting at 210 DAFS contained the highest sugar content, fruit weight, highest vitamin C content and lowest acidity content which are very essential judging criteria for determination of maturity of the mandarin fruits. At this stage the TSS: Acidity ratio was also maximum. TSS: Acidity ratio is best measure for quality determination and can be used to identify the harvesting date to give the fruits of better nutritive quality. High TSS: acidity ratio determines the sweetness of the fruit and thus effect the marketability of the fruits. All the constituents attained a desirable level in this stage of development. Therefore, it is suggestive not to harvest the fruits before the 210 DAFS.

There is further scope for investigation in order to have complete assessment of the composition of organic acids, sugar and minerals, vitamins along with the colour and flavour and also evaluation of biochemical composition and quality of the mandarin fruits, grown in different altitude with different varieties during different stages of development.

It also desirable to study the activities of various enzymes like sucrose phosphate synthase, citrate synthase, polygalacturonase etc. in mandarin fruits which play important role in attributing quality.

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Table: 4.1

Changes in physical characters of *khasi* mandarin fruit during ripening

(Average of three replications)

Days after fruit set(DAFS)	*Whole Fruit weight (g)*	*Fruit diameter (cm)*	*Fruit volume (cc)*	*Peel weight (g)*	*Peel (%) of whole fruit*	*Pulp weight (g)*	*Pulp (%) of whole fruit*	*Pulp : Peel ratio*
90	23.46	3.13	24.66	6.48	27.61	16.98	72.39	2.62
120	37.51	3.97	38.33	9.19	24.51	28.32	75.49	3.08
150	58.35	4.33	59.33	13.45	23.05	44.9	76.95	3.33
180	69.23	4.77	69.67	15.48	22.37	53.74	77.63	3.47
210	78.32	5.20	78.67	17.26	22.04	61.06	77.96	3.52
S.Ed (±)	0.2316	0.1333	0.4714	0.1448	0.3359	0.2248	0.3359	0.0543
CD (0.05)	0.5161	0.2970	1.0503	0.3227	0.7486	0.5010	0.7486	0.1211

Table :4.2

Changes in chemical constituents of *khasi* mandarin fruit during ripening

(Average of three replications)

Days after fruit set(DAFS)	*Juice content per fruit (ml)*	*Juice percentage per fruit(%)*	*Moisture content (%) (Excluding peels)*	*Total soluble solid(%)*	*Titratable acidity (%)*	*TSS/ Acidity ratio*	*pH*
90	6.87	29.23	74.48	5.83	3.19	1.83	2.85
120	12.63	33.67	80.87	6.23	2.83	2.20	2.90
150	22.20	38.04	84.25	6.75	2.54	2.52	3.80
180	28.63	41.36	86.14	7.83	1.13	6.93	3.12
210	36.03	46.03	87.37	8.84	0.85	10.40	3.80
S.Ed (±)	0.4184	0.7219	0.5711	0.0181	0.0111	0.1102	0.0141
CD (0.05)	0.9323	1.6085	1.2726	0.0404	0.0248	0.2457	0.0315

Table: 4.3

Changes in chemical constituents (in fresh juice) of *khasi* mandarin fruit during ripening

(Average of three replications)

Days after fruit set(DAFS)	*Reducing sugar (%)*	*Non reducing sugar(%)*	*Total sugar (%)*
90	1.65	0.87	2.52
120	1.91	1.30	3.21
150	2.14	1.84	3.99
180	2.19	3.24	5.43
210	2.85	3.86	6.70
S.Ed (±)	0.0109	0.0117	0.0126
CD (0.05)	0.0244	0.0261	0.0281

Table: 4. 4

Changes in organic acid content in *khasi* mandarin fruit during ripening

(Average of three replications)

Days after fruit set(DAFS)	*Citric acid (mg/100ml)*	*Oxalic acid (mg/100ml)*	*Fumeric acid (mg/100ml)*	*Ascorbic acid (mg/100ml)*
90	644.17	10.31	20.78	1.13
120	198.75	27.31	1.00	2.88
150	164.44	34.21	-	1.64
180	161.37	32.47	-	4.30
210	94.24	8.23	1.13	83.88
S.Ed (±)	0.2015	0.0985	0.0111	0.0257
CD (0.05)	0.4491	0.2194	0.0249	0.0573

Table: 4.5

Changes in Ash and Minerals content of *khasi* mandarin fruit pulp during ripening

(Average of three replications; dry weight basis)

Days after fruit set(DAFS)	*Ash (%)*	*Calcium (g/100g)*	*Potassium (g/100g)*	*Sodium (g/100g)*	*Phosphorus (g/100g)*
90	5.14	0.63	1.02	0.024	0.15
120	3.62	0.53	0.83	0.018	0.23
150	2.68	0.49	0.78	0.012	0.26
180	2.45	0.42	0.72	0.005	0.15
210	1.94	0.39	0.65	0.008	0.17
S.Ed (±)	0.1180	0.0011	0.0125	0.0008	0.0012
CD (0.05)	0.2630	0.0248	0.0278	0.0018	0.0026

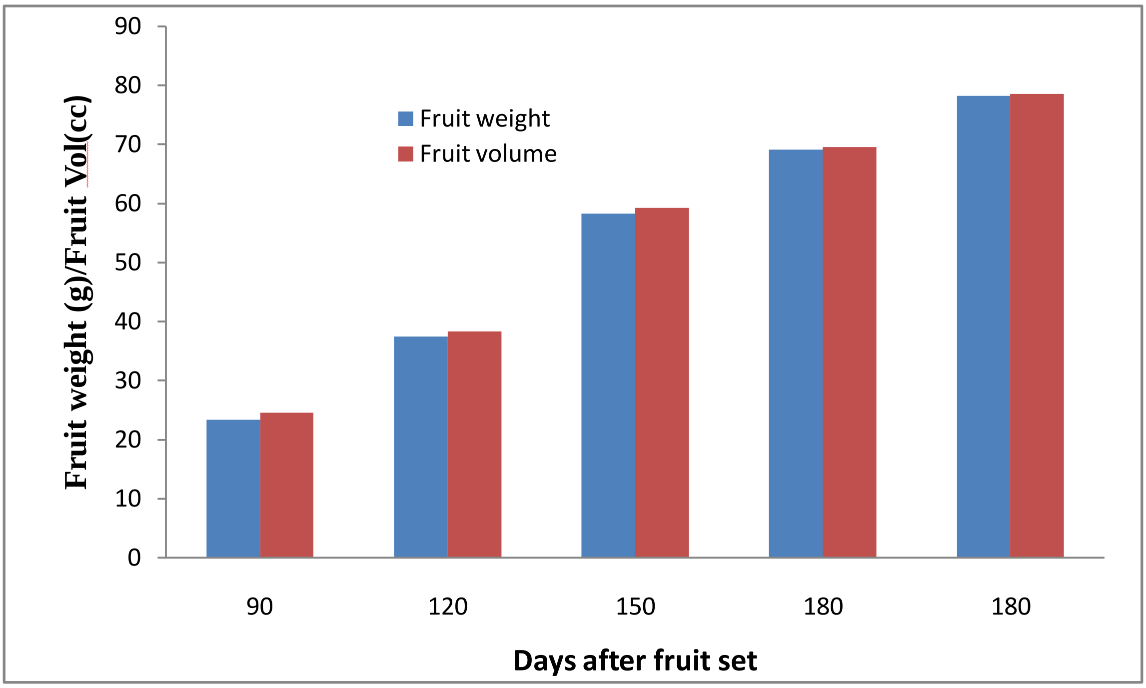

Fig. 4.1: Changes in fruit weight and volume in *khasi* mandarin during ripening

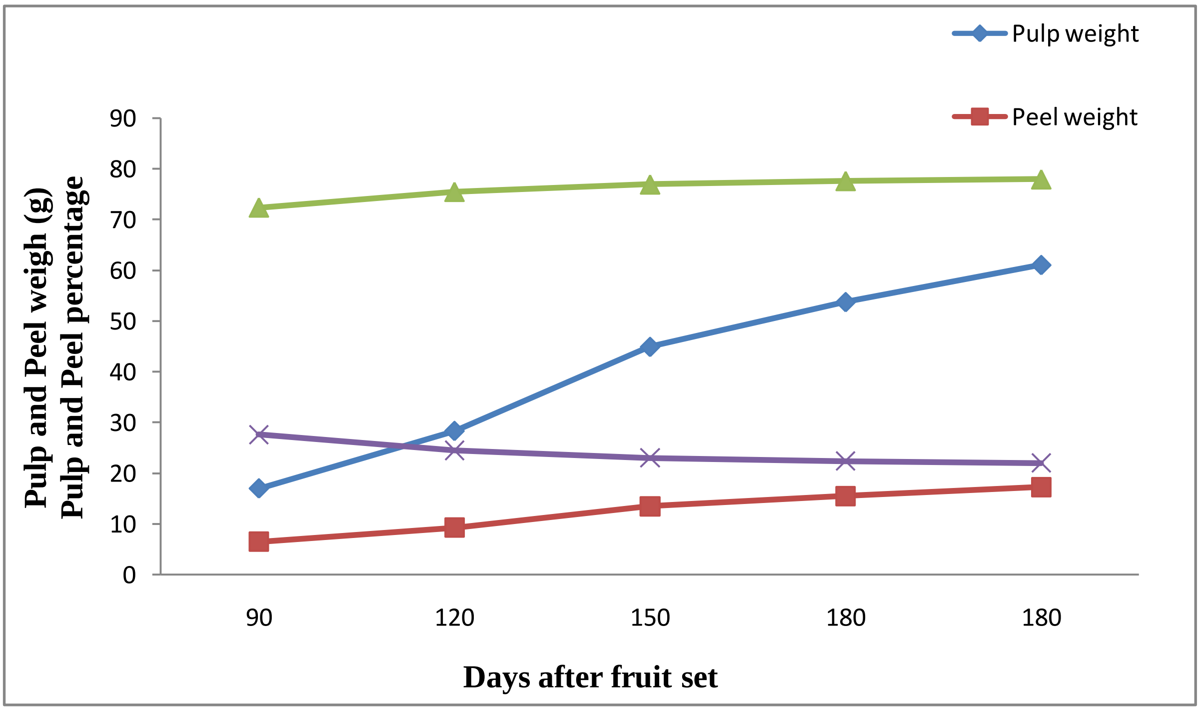

Fig. 4.2: Changes in pulp and peel weigh pulp and peel percentage in *khasi* mandarin during ripening

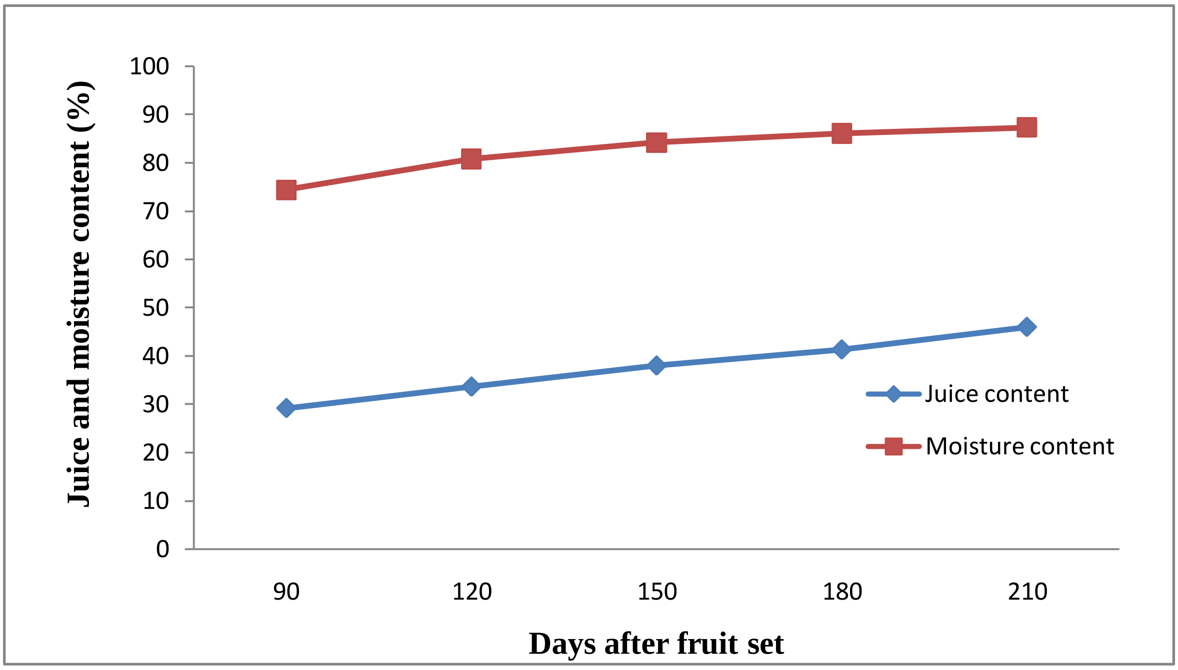

Fig. 4.3: Changes in juice and moisture content (%) in *khasi* mandarin during ripening

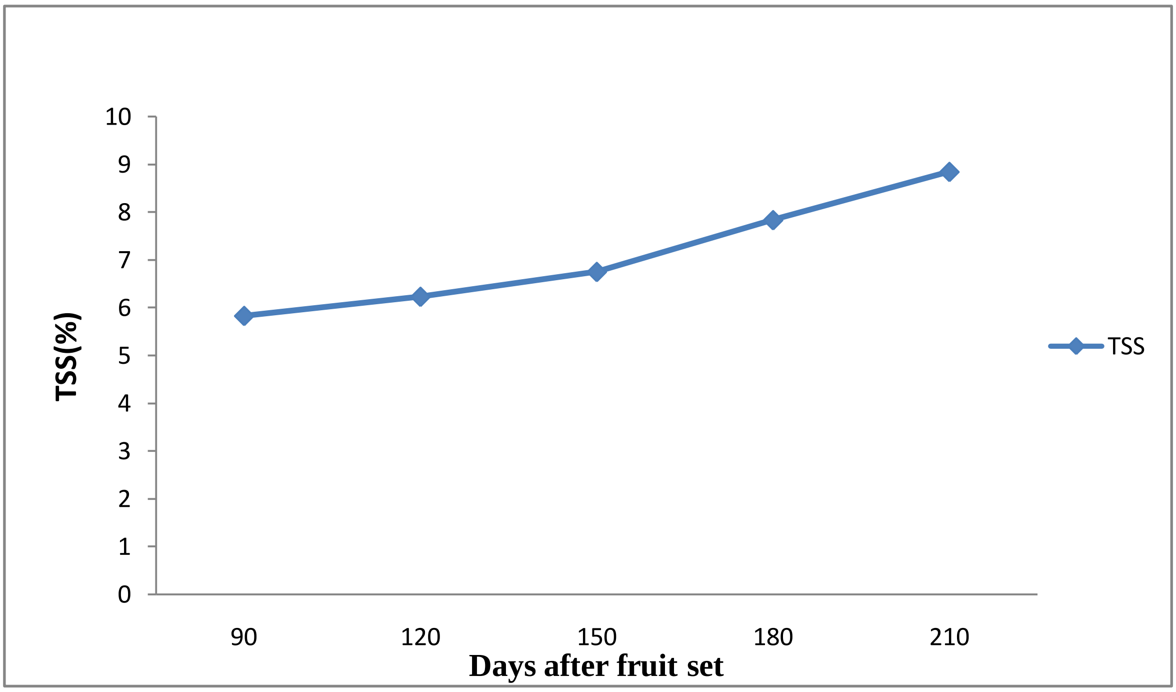

Fig. 4.4: Changes in total soluble solid content (%) in *khasi* mandarin during ripening

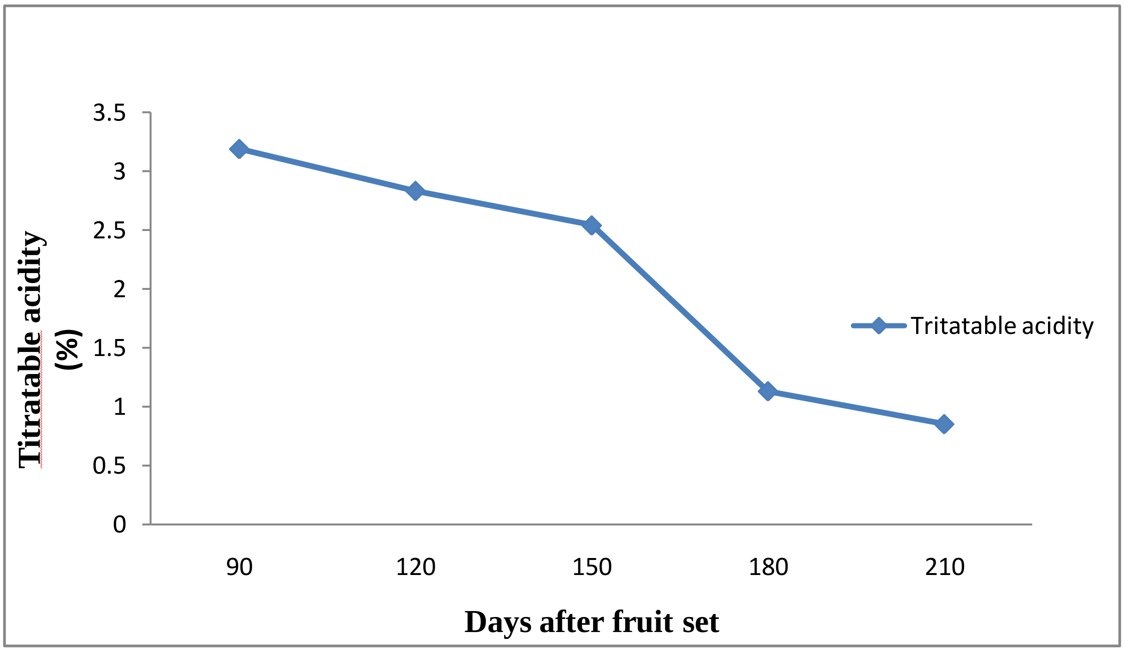

Fig. 4.5: Changes in titratable acidity (%) in *khasi* mandarin during ripening

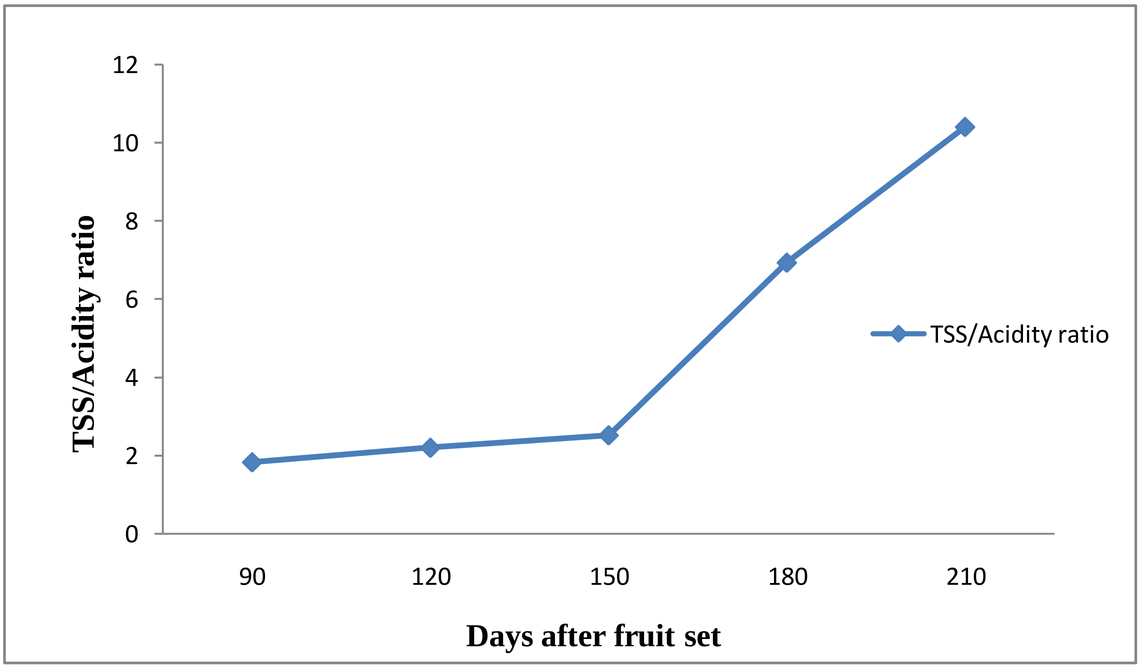

Fig. 4.6: Changes in TSS:acidity ratio(%) in *khasi* mandarin during ripening

Fig. 4.7: Changes in pH content in *khasi* mandarin during ripening

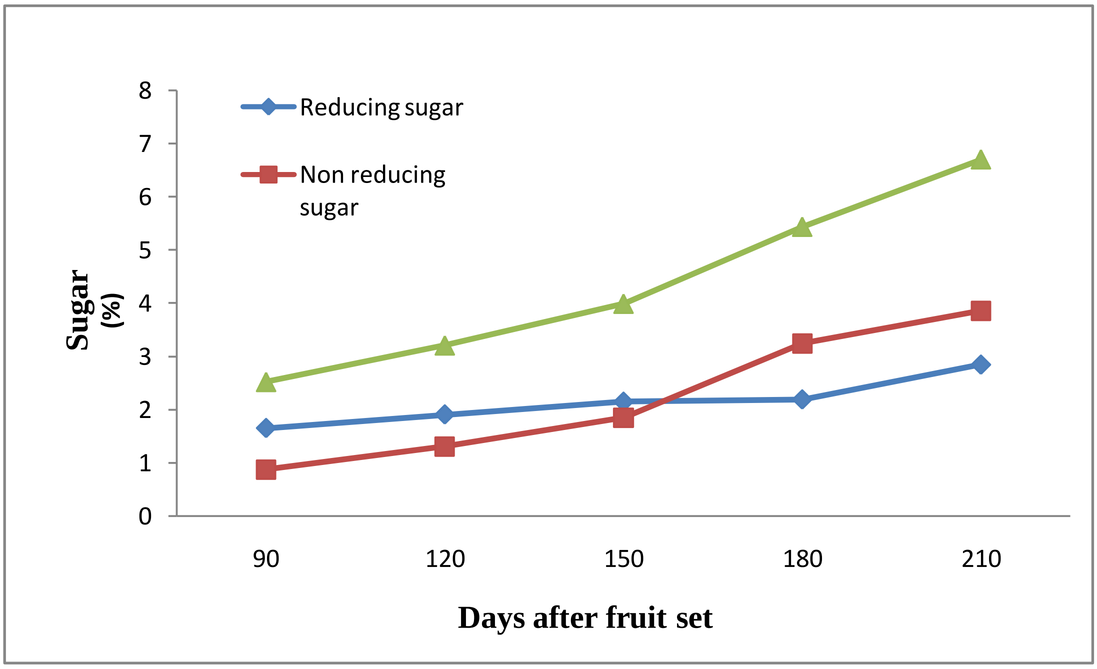

Fig. 4.8: Changes in sugar content (%) in *khasi* mandarin during ripening

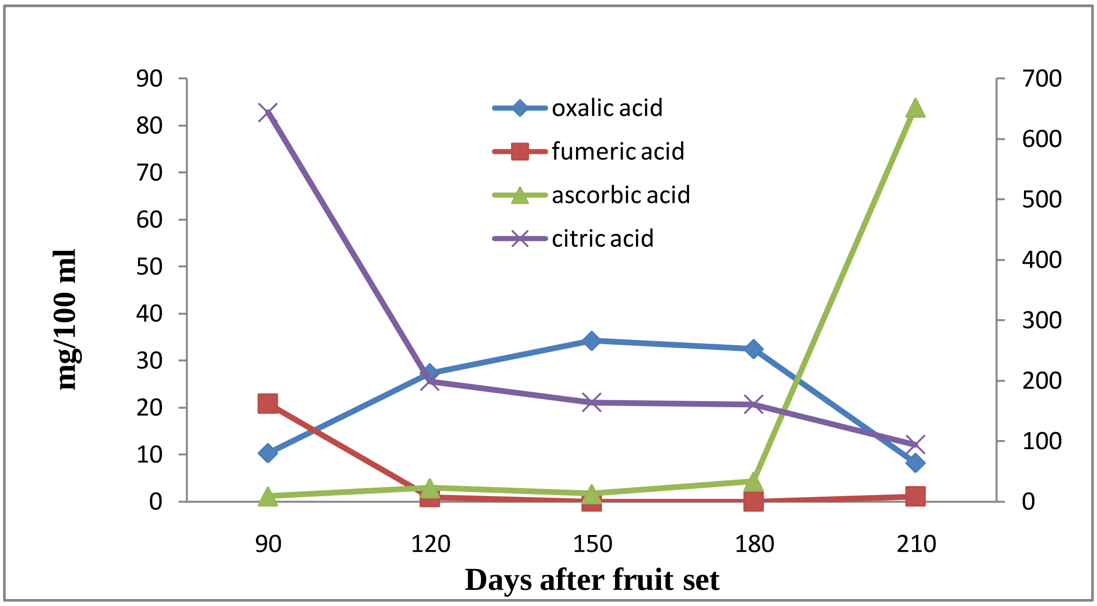

Fig. 4.9: Changes in organic acids content in *khasi* mandarin during ripening

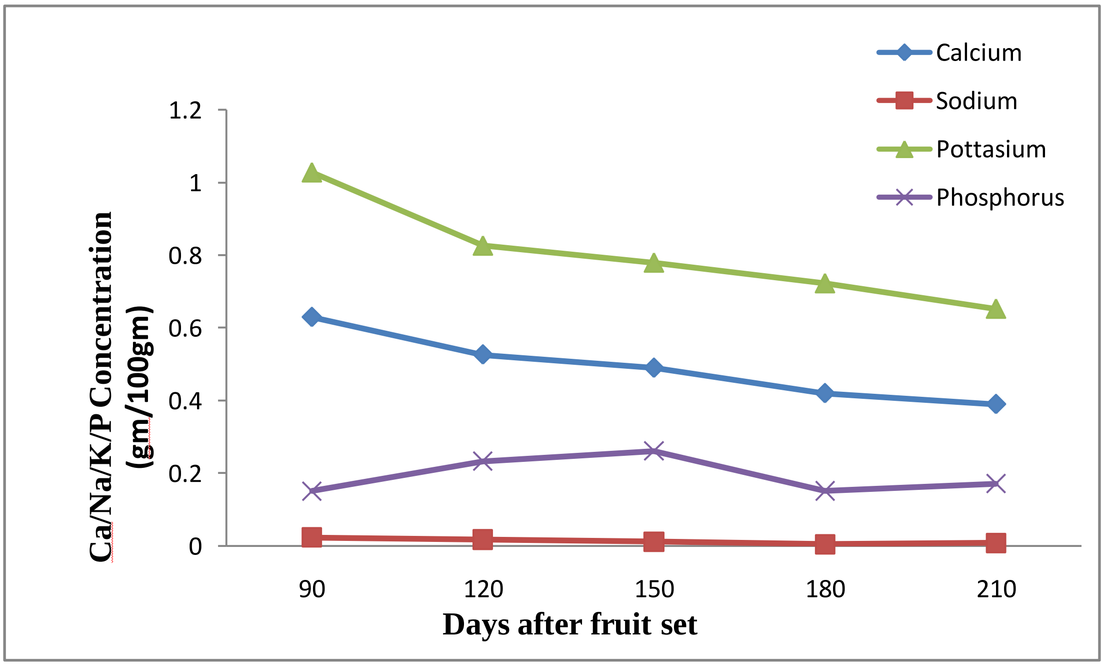

Fig. 4.10: Changes in minerals content in *khasi* mandarin during ripening

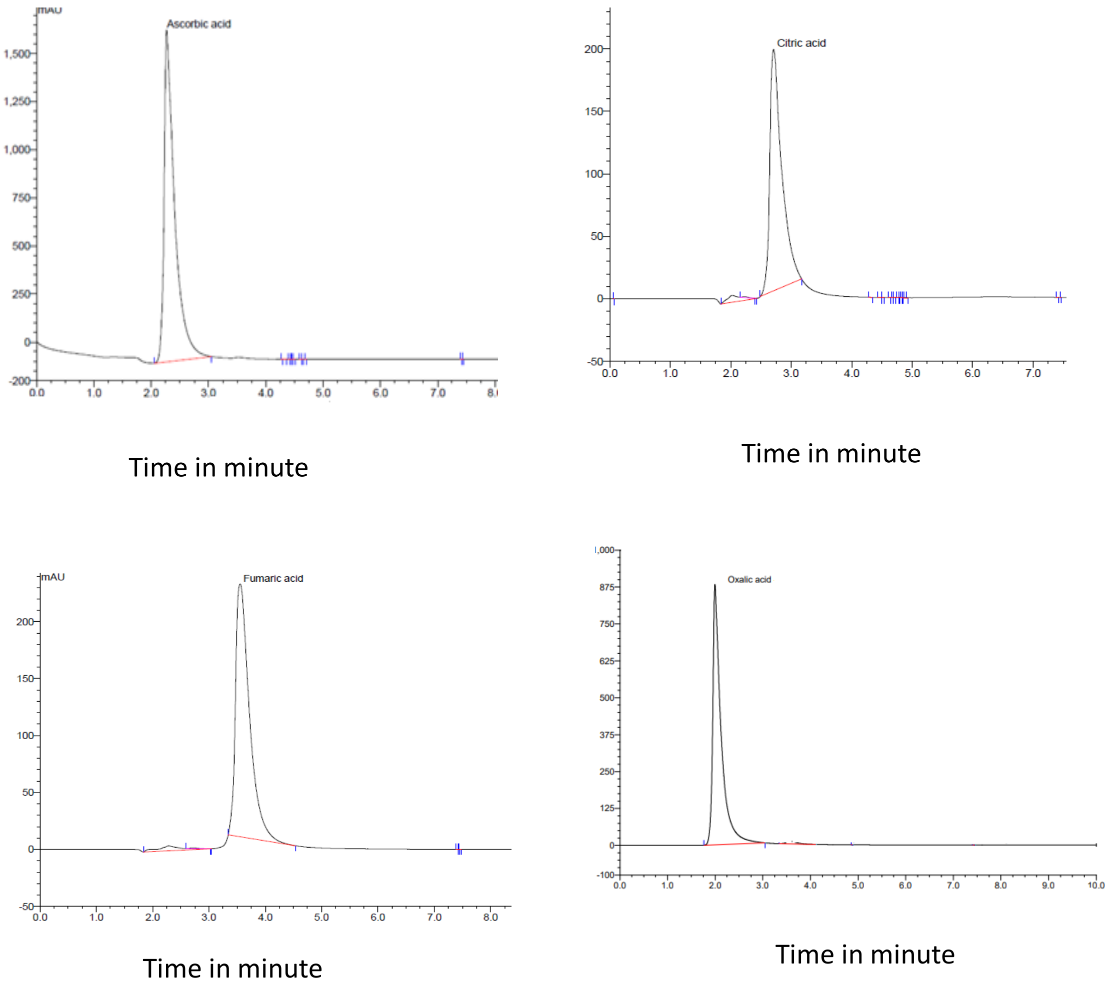

Fig. 4. 11: Chromatogram of organic acids standards

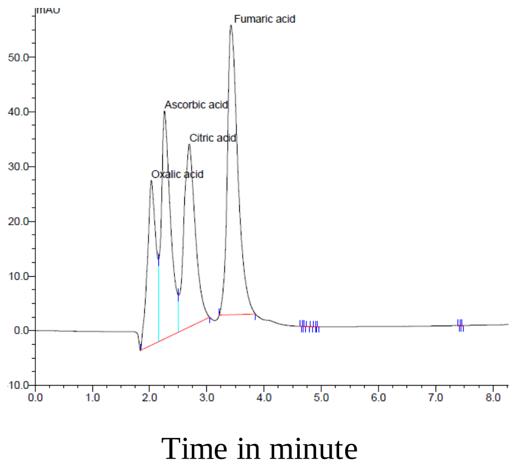

Fig. 4.12: Chromatogram of mixed organic acids standards

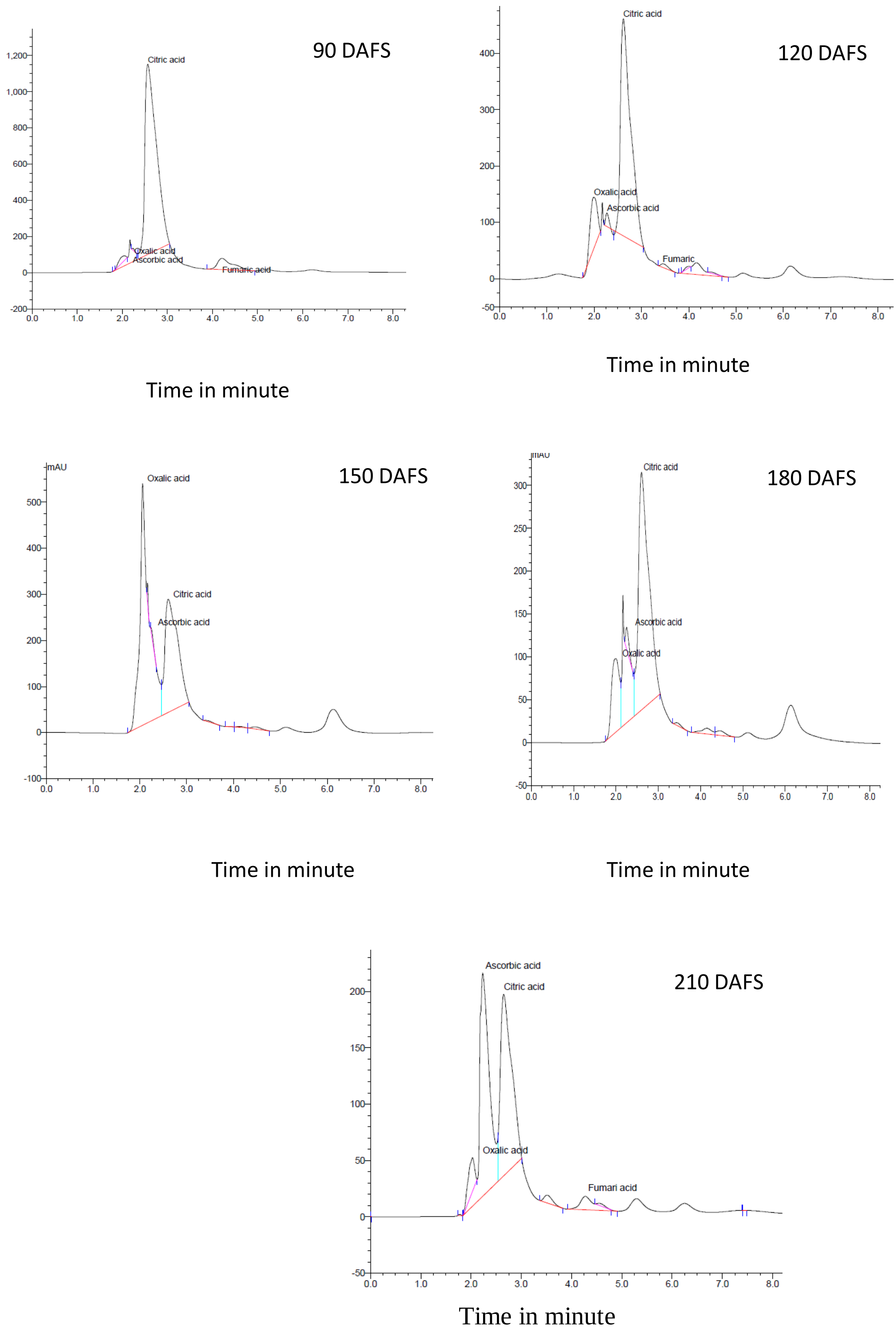

Fig. 4.13: Chromatogram of organic acids in juice sample at different development stages