

A CRITICAL REVIEW ON PHYSIOLOGICAL CHANGES DURING GROWTH, MATURATION, AND RIPENING OF CITRUS FRUITS

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ABSTRACT

Citrus is one of the major fruit crops in the world and widely recognized by their nutritional, organoleptic and health-related benefits of fresh fruit. The genetic diversity among the genus and independent changes in peel and pulp, make the definition of standard maturity indexes of fruit quality. Commercial maturity indexes in the citrus industry are usually based on peel coloration, soluble solids, pH but their relevance may differ among varieties and the specific requirements of the markets. Citrus fruits are excellent source of many phytochemical, including ascorbic acid, antioxidant, tannin, etc., which greatly contribute to the health-related benefits of citrus fruits. Criteria and definition of the main maturity indexes for citrus fruit worldwide are described, as well as changes during fruit maturation in key components affecting organoleptic and nutritional properties. Citrus fruits were analyzed at different maturity stages. This review is aimed to characterize the physiological maturity of the fruit across the different developmental stage which has not been well reported in literature till now.

1. INTRODUCTION

Fruit have been a matter of extensive research in last few decades' because of their importance to agriculture and the human diet. However, research on fleshy fruit has focused primarily on climacteric fruits such as tomato while other fruit models are not so well known. Citrus fruits belong to the family Rutaceae and genus citrus, non-climatic fruit as their respiration rate and ethylene production rate is very low [1]. Citrus fruits showed minor changes in physical and chemical characteristics, as they detached from the tree. Citrus is one of the most popular and widely grown fruit crops in the world. Citrus and its products are a rich source of vitamins, minerals and dietary fibre that are essential for the overall nutritional well-being. Among the most commonly cultivated types, oranges account for more than half of world citrus production followed by tangerines, lemons and grapefruits. Among the continents, Asia is the major producer of citrus fruits (80 million MT) and thereafter Northern America and Africa also

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contributed with the production of 28.1 million MT and 20.3 million MT, respectively. China, Brazil, the USA, India, Mexico, and Spain are the world's top six citrus fruit-producing countries, accounting two-thirds of the global production. India is the fourth major citrus producing country with the production of 14 million tones and the production increased with the average annual growth rate of 5.16%. The volume of mandarin produced was the highest among citrus fruits in India at over six million metric tons. Sweet oranges, commonly known as Mosambi in the sub-continent came in second at about 4.25 million metric tons in this year [2].

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Citrus fruits are well known for their refreshing smell, thirst-quenching potential and ascorbic acid content. Citrus fruits are richest source of limonoids, carotenoids and flavones. Flavonoids are phenolic derivatives possessing remarkable health-promoting effects, a consequence of their marked antioxidant ability, and are generally found in fruits, vegetables, herbs, tea as secondary metabolites: Citrus juices are among the richest dietary sources of flavonoids. They are often found in Citrus juices as their glycoside derivatives and more specifically as their O-glycosides and C-glycosides. Citrus fruits are economically major fruit crops with excessive nutritional value which depends on the external characteristics like color, firmness and internal features like total soluble solids, total acid, and juice content and naringin content[3].

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Citrus fruits life cycle is divided into three different phases followed sigmoidal growth curve [4]. The first phase represents slow cell growth and cell division, while second phase represents rapid cell enlargement and water accumulation [5] and third phase represents maturation. Many physical, chemical, nutritional and functional quality changes are occurred during different phases of fruit life cycle. Due to the perishability of fruit, its storage in the atmospheric condition is limited after harvesting, which is accompanied by the browning of skin, loss of ascorbic acid content, and loss of glossiness [6,7]. Therefore, harvesting of fruit at proper maturity stage is pre-eminent for achieving desirable attributes. Maturity indices like skin color, juice content, total soluble solids (TSS) and TSS: acid ratio and other internal constituents are the key indicators for selection of optimum harvesting time of citrus fruits and ensure their maximum acceptability level by the consumers [8]. In citrus, the harvesting period of fruits vary depending upon the species, variety and purpose of consumption. Information on biochemical changes and correlation among different fruit characters at various stages of fruit development is important in determining the optimum harvesting period to meet the demand of fruit for a specific purpose.

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The quality and shelf-life of the citrus fruits are largely influenced by the various physico-chemical and biochemical changes, which occur during the various physiological stages of fruits. Very few studies on some citrus fruits have been reported concurrent changes in physicochemical properties during fruit development and ripening in Kinnow mandarin [9] and guava [10]. The information pertaining to the physico-chemical, biochemical and functional changes of most citrus fruits at different physiological stages is very scanty. Therefore, this study summarizes the comprehensive knowledge related to changes occurred in physical, chemical and functional properties of citrus fruits during their physiological stages.

2. PHYSIOLOGICAL PHASES OF CITRUS FRUITS

Citrus plants generally are evergreen shrubs or small trees, bearing flowers, which yield a strong scent. Fruit growth, development and maturation are the three major physiological phases of citrus fruits (Fig. 1). The first phase (unripe stage), a cell division phase of slow growth, environmental signals and the specific genetic background are the factors responsible for activating the genes involved in the biochemical and physiological changes that lead to floral induction and development, allowing the transition from vegetative to reproductive development. The second phase (semi-ripe stage) of cell enlargement of major increase in size and weight by growth of juice sacs from the pulp, changes in color are controlled by climatic conditions, temperature and rainfall regime [11] and third phase (fully mature stage) during which fruit growth is maximum and transformation of attributes of fruit maturation take place, such as sugar accumulation, acid degradation, starch hydrolysis etc. [4]. Defining the criteria to accurately and consistently determine citrus fruit maturation is not an easy task since it involves physiological changes occurring in two different and independent tissues: color transformation taking place in the fruit peel and compositional changes occurring in the flesh. Ordinary citrus fruit such as sweet oranges, mandarins, lemons and grapefruits are considered to be mature for fresh consumption when their external coloration, juice content and soluble solids: acidity ratio and other internal constituents reached to a minimum for visual acceptance or palatability. Accumulation of soluble sugars and the decline in acid content (mostly citric and malic acids, depending on the different citrus species) are typical changes taking place in the pulp during maturation of citrus fruit.

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Citrus fruit are non-climacteric fruits; displaying a progressive reduction in the rate of respiration and low ethylene production during the whole development process [12]. Despite this behavior, physiological and molecular evidences indicate the involvement of the phyto-hormone in the control of some ripening-associated processes [13] Therefore, the changes taking place during developmental and maturation that are essential to define maturity indexes are regulated by a complex interplay of endogenous hormonal and nutritional signal, and highly influenced by both environmental factors and agronomic practices.

3. PHYSICOCHEMICAL ATTRIBUTES OF CITRUS FRUITS

Citrus fruits belong to hesperidium, which is made up of two different morphologically separate regions, the pericarp of the fruit and endocarp that is the edible part of the citrus fruits. The quality of citrus fruits depends upon the physical attributes include shape, size, texture, color, peel ability, and the number of seeds and chemical attributes includes acids, volatiles, flavor and other nutraceutical compounds. These attributes eventually depend on the regulation of the physiological and biochemical characteristics of citrus fruits. The citrus ripening is allocated upon conversion of chloroplast and continuous loss in the chlorophyll molecule that results in accumulation of carotenoids in the fruit. The fruit growth regulators (phytohormones) play a major role in the development and maturation of fruit. It also includes physiological disorders as well as nutritional disorders that have been found in tropical and subtropical fruits [14]. The determination of the conscience fruit maturation is slightly complex as it depends upon the internal changes occurring in the fruit flesh as well as the external fruit peel coloration which occur during fruit growth, development and maturity [15]. According to Pathek& Srivastava[16] seasonal growth of citrus fruits has been divided into three stages- unripe (early stage), semi-ripe (mid-stage), ripe (late-stage). During the growth of citrus fruits showed changes in their physical and chemical attributes. These attributes assist in the proper storage and retention of nutrients which lost during storage.

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4. PHYSICO-CHEMICAL CHANGES DURING PHYSIOLOGICAL STAGES OF FRUITS

4.1 Fruit weight and size

The importance of fruit size as a parameter of quality of citrus fruits has increased markedly in recent times. The fruit weight is generally increased up to maturation phase and constant during ripening and thereafter slightly decreased during over ripening might be due to escaping out volatile constituents. The fruit weight of Assam lemon showed double sigmoid pattern of growth. Assam lemon fruit size change from 1.6 cm to 9.2 cm as fruit reached to final maturity stage (Table 1). The change in the parameters could be attributed to an increase in the size of the cell and accumulation of food substances in the intercellular spaces in fruit. The authors reported similar trends in grape fruit growth, development and maturation, fruit size increased rapidly from unripe to semi ripe stage due to enlargement of cell mesocarp[17]. In mandarin fruit size increased 21.09 cm – 29.31 cm at end of the fully mature stage, similar trend was also found in sweet orange [18].

Size enlargement is due to the extension of the fruit pulp. Water is stockpile in the pulp segment, the juice sacs growing promptly during this phase. The pulp axis increased from 20-67% of the total fresh weight [19]. As the fruiting season start, the growth of the fruit was slow but the rapid growth was seen during maturation. During the dormancy stage, optimal level auxin might arrest the fruit development as fruit start to grow auxin level decreased while cytokinin level increased which stimulate cell division and cell expansion in fruit [20]. Like, fruit weight and size, dry matter of fruit also increased from 0.97gm to 8.62 gm during fruit maturation. Mostly dry matter accumulated in the last phase of fruit development. It follows a sigmoidal growth curve and some cases double sigmoid growth curve[21]. In citrus fruit, Tangerine, grapefruit, Kinnow, and others citrus fruit, weight and length increase at a faster rate in early development condition as fruit get maturity its weight and length reach to its peak [22, 23]. During the fully ripe condition, moisture loss and respiration rate was high; and fruit weight and size decreased slightly. Increased fruit size is desirable for orange fruits. As fruit starts to mature, mesocarp cell accumulate water and starch, therefore cell size increased. Maximum fruit size and weight was observed at fully mature stage [24]. Khan M M [25] reported a rapid increase in weight of orange fruit, from 180 days onwards till 220 days after fruit set; followed by a slow increase up to 250 days and thereafter, slightly decreasing trend was observed, which might be attributed to higher rate of respiration [26].

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4.2 Fruit color

Color is important parameters in citrus fruit quality which influences consumer expectation and perception. Citrus peel is a complex source of carotenoids. The great diversity of fruit coloration among citrus varieties is directly correlated with carotenoid composition. Fruits tissues contain wide diversity in carotenoids of citrus fruit and their specific accumulation patterns are responsible for the broad range of colors changes during the fruit life cycle. Although the color characteristic of citrus varieties is mainly govern by the accumulation of (9Z)-violaxanthin and β -cryptoxanthin. Apart from these two coloring compounds, other citrus-specific apocarotenoids such as β -citraurin and β -citraurinene also develop an attractive coloration from the pale yellow to red in the case of white and red grapefruits, dark yellow in lemons and orange, an intense orange coloration in some mandarins. Recently, it has been reveal that apocarotenoids are exclusive to citrus genus and their content is controlled by specific enzymatic activity which directs the accumulation of these apocarotenoids in the peel of fruits in different proportions, imparting an intense orange-reddish coloration to some varieties of oranges, mandarins and hybrids [27, 28]. Color development is especially affected by climatic conditions such as temperature and light. In equator regions, where temperature remains high, citrus peel coloration is usually paler than colder region. In citrus fruits, the harvesting maturity is depends upon the fruit color [29]. The changes of fruit color of during physiological phases of different citrus fruits have been represented in Table 2. The L^* , a^* and b^* values of Assam lemon fruit increased from 36.10 to 61.21, -5.67 to -3.31 and 10.70 to 29.67 respectively during physiological stages of unripe to fully ripe conditions [21]. Moreover, in tangerine, change in peel color from green to yellow-orange was observed throughout fruit development. During growth, L , and hue angle values remained almost constant up to 16-24 weeks after full bloom [22]. Then, peel L values gradually increased and the hue angle value decreased after 28-34 weeks after full bloom. During maturation (35-37 weeks after full bloom), peel L^* values reached the highest levels while hue angle value decreased to the lowest levels. The hue angle value decreased throughout the fruit development as a result chlorophyll pigment starts to degenerate and carotenoid pigments start to accumulate. Whereas, in grape fruit color started to change and became deeper, as fruit reaches maturity condition. In non-climatic citrus fruit, ethylene production plays a major role in the natural color break but its low-level synthesis is continued throughout the process of growth [30]. In some citrus fruits, sucrose stimulates color change, while nitrogen delays chromoplast formation [31]. As the fruit mature its chlorophyll pigment vanished and carotene and

xanthophylls pigments start to appear. One of the primary sources of red color in mandarin fruit is increased at low temperature [32]. Grape fruits color change was observed in L* value and it has increased from 44.32 to 57.20. As the fruit mature its chlorophyll pigment vanished and carotene and xanthophylls pigments start to appear [33]. Similarly, in Pomelo the lightness value increased during ripening from 55.22 to 57.35 [34]. Moreover, in Feutrells, the lightness value and redness value of the fruits increased during maturation and ripeness of fruits [35]. Anonymous results were observed in case of Kinnow fruit, in which the chromameter values of L, a, b increased from dull greenish to bright yellow after ripening. Also, in mandarin fruit, as fruit start to grow its color change with burnt orange in November and it's become deeper and deeper as fruit matures [36]. And in case of oranges, during growth, the lightness values decreased (59.6 to 55.3) with an increase in redness (2.24 to 21.4) and yellowness (27.86 to 30.43) upon ripening [37].

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4.3 Total Soluble Solid (TSS)

Total soluble solids (TSS) content is an important index of internal quality of the fruits and an accurate basis for determining the harvest of the fruit. This measurement has been long used for maturity index among citrus growers since a portable refractometer can be easily used and provides an accurate measurement of fruit maturity in the field. An increase in sugars is usually accompanied by an increment in TSS value, which is normally expressed as °Brix or percentage. The TSS contents during the early fruit development, were minimum (9.25°Brix) and increased gradually; ultimately reached maximum value of 11.8°Brix in tangerine fruits. The increase in TSS value during the development processes is the result of starch degradation and metabolic transformation into soluble sugars in the fruits as polysaccharides during final maturity stages. The TSS levels were found to be lower in grape fruits at unripe stages of harvest (Table 3). The levels increased as the fruits matured. TSS content was maximum at semi ripe stage 8.87°Brix. Increase in TSS was tightly related with decrease in acidity was commonly observed in fruits as the fruit develops. TSS represents about 80% of sugars (mainly glucose, sucrose and fructose), 10% acids (mainly citric, oxalic and mallic acids) and 10% nitrogenous compounds (amino acids). Most citrus fruits are considered as ready for harvesting when their TSS level reaches 8.5% [38]. The degradation of polymers like starch and metabolism of organic acids into soluble sugar is thought to be the source of a higher level of total soluble solid [39]. During

growth and development of Assam lemon maximum TSS value was present at early stage of development, as fruit mature its TSS value continuously decreased. The decline of TSS could be attributed to the utilization of sugar as a substrate for increased rate of respiration. In kinnow maximum level of TSS was at semi ripe stage (9.20°Brix) was the consequence of translocation of photosynthase from leaves to fruits [40] and slightly decreased to fully ripe stage (8.80°Brix). TSS value > 10 by harvest is an acquiring value for utilization of citrus fruit. In many citrus fruits, like fruettrell showed an increase in TSS value till semi ripe stage (8.90°Brix). Whereas, in case of Mandarin, the TSS increased [6.81-11.63° Brix during fruit development. Most citrus fruits showed constant rise in TSS content till semi ripe condition due to synthesis of sucrose which then are hydrolyzed to simple sugar [41]. TSS increased slowly in oranges and it contains 12.1 °Brix at fully mature stage after 240 days from fruit set. . Nearly 75% to 85% of TSS in orange juice is sugars. While TSS in Fruettrell rose from 6.80 to 7.40 upon maturing from unripe stage to fully ripen stage [42].

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4.4 pH content

The pH of citrus fruits ranged from 3.50 to 4.33 and it is maximum either in semi ripe or fully ripe stage [43]. In tangerine fruit, the pH values increased to 3.6 at semi ripe stage and afterwards its value decreased to 3.2 at fully ripe stage due to the change in titratable acidity of fruits during ripening (Table 4). Similar finding was observed in kinnow and fruettrells where minimum pH was reported in semi ripe stage 3.30 and 3.60 respectively, it continuously increased to semi ripe stage and then started to decrease due to degradation of acid and increase level of sugar. In grapefruit pH was reported 3.67 at fully mature condition [44]. Matteo & Simeone [45] reported, pH value of Assam lemon was lowest at unripe stage (2.24) and it linearly increased till fully mature stage (2.35). Similar result was observed in tangerine and mandarin, wherein the pH value was increased about 1.5 fold from unripe stage to fully ripe stage, which might be due to accumulation of sugar in pulp of the fruit [15, 46]. Moreover, the pH increased from 3.08 to 3.32 in oranges during ripening [47].

4.5 Titratable acidity

Citrus fruit acidity not only stimulates the sourness perception to consumers, but it is also a key determinant for the commercial acceptability since its balance with the appropriate sugar levels

and provides the delightful and typical citrus taste to the consumer. During the maturation stage, the acidity of citrus fruits was affected by alteration in sugar concentration and other components of the fruits and improve their quality by decreasing the percentage acidity of the fruits [17]. High materialistic attributes such as sugar and organic acid and their ratio play a significant role in the quality of citrus fruit. In tangerine, titratable acidity decreased from 4.08- 0.86 % during development and maturation of the fruits (Table 4). The decrease with maturity might be due to the presence of limonate dehydrogenase, which turns limonin tonon-bitter, compound limonoid glucosides at late harvesting time and reduces the bitterness in the fruits. The maturity stages, has significantly influenced on the titratable acidity of grapefruit (Table 4). In unripe stage, TA was 1.88 % which was maximum 1.92 % at semi ripe stage then continuously decreased till fully ripe condition (1.39%) of the fruits. The visa-versa trend was observed in the TA of assam lemon, as it was lowest at unripe stage and continuously increased during maturity and ripening stage [48]. Kinnow fruit maturation is directly linked with peel pigmentations to represent color in green to yellow or orange. Green peel colored fruits possessed more titratable acidity 1.10% and decreased at ripening 0.70 % as acid is converted into sugar. In fruttrell maximum TA was observed in early growth stage was 0.65 %. As fruit started to grow its TA was decreased to 0.50% because acid change in sugar by TCA cycle. During physiological growth, fruits contain some other acids, which increased the TA in fully mature conditions. In mandarin fruit titratable acidity has been reported from 2.59 to 0.66. It was found that during the growth, development and maturation, titratable acidity decreased continuously to fully mature stage of the fruit. Mostly in citrus fruit glucose, fructose and sucrose sugar are the common sugars present in the fruits. Citrus fruits possesses bitter taste that may derived by high density of ellagitannin present during unripe condition; as fruit develop its level lessen. In sweet cultivars of citrus fruit mostly in Kinnow acidity, initially rapid increase in TA value followed by a continuous decrease due to enlargement of size mainly due to the higher water content of the fruit because of the dilution effect [36]. In Orange Maximum Titratable acidity was present at unripe stage (22.4g/l) and gradually started to decrease at fully mature stage. The loss of acidity during fruit maturation and storage appears to result, at least in part, to the use of acids as a respiratory substrate [47].

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4.6 Ascorbic acid

Citrus fruits is an important source of many vitamins, with vitamin C (ascorbic and dehydroascorbic acid) being the most important for their contribution to the antioxidant properties and nutritional benefits [49, 50] acid content is considered a primary mark of the quality evaluation of citrus [24]. Different citrus fruit like lemon and orange, ascorbic acid content remained high at the unripe stage. The ascorbic acid content of fruit is declined due to the exertion in the metabolic pathway (Table 5). In mandarin fruit, ascorbic acid was accumulated in both peel and pulp of the fruits. It was maximum at an immature stage at 30 days after fruiting (37.12 mg/100ml) and decreased in semi-ripe conditions at 120 days after fruiting (33.77 mg/100ml) and fully ripe condition at 230 days after fruiting (27.62mg/100ml). The ascorbic acid content was highest in the mandarin fruit during growth and development stage and the maximum differences was observed in semi-ripe and fully ripe (120 and 230 DAF) physiological stage of fruit. In citrus fruits, a reducing trend was observed in ascorbic acid during the ripening stage due to rise in temperature which suits to oxidation and consequently experience degradation of ascorbic acid. In Mandarin, Kinnow, and Fruterell's, the maximum amount of ascorbic acid was present in the early stage of maturation, and subsequently decreased during the maturation of these fruits [42]. Citrus fruits possess less ascorbic acid at maturity along with decreasing level of acidity and also utilize acids in respiration process [tricarboxylic acid (TCA) cycle] in citrus fruit ripening process.

4.7 Tannins

The highest amount of tannins was found at the early stage and as the fruit becomes mature the tannin level decreased in the citrus fruit [51]. As the fruit ripens the tannin level decreased and became a part of the pulp. In fruit reported to contain 2 types of hydrolyzable tannins, Emblicannin A and Emblicannin B with pedunculagin and punigluconin [52]. Most of the recognizable and dominant tannins compounds present at the early stage are isgallotannins type having sugar like glucose and gallic acid molecule. In pamele tannin content was maximum at early development stage (6.53mgcatcehin) at 100 DAF (Days after fruit set) and its value decreased continuously as fruit reached to fully ripe condition (0.02 mg catcehin) at 260 DAF (Table 6) [53]. Diba & Bultosa[54]reported decreased in tannin content with an increase of

maturity of fig fruit. Astringency reduces with fruit maturation, generally accompanied by a drop in tannin content or physicochemical changes in the molecules. Fully mature stage causes tannins to polymerize due to the action of acetaldehyde, which converts tannin into sugars or consumed during respiration [55]. The tannins are present in the fruits in the form of non-polymerized tannin and these compounds tend to convert into a condensed form, ensuring more stability with proteins. This explanation portrays the decreased value of condensed tannin (astringency) in the fruit during growth and development [56]. Tannin content in citrus varieties ranged from 0.12 to 0.37 mg CE/g DW with bitter orange being richer than mandarin 0.12 to 0.19 mg CE/g DW [57]. Bitter orange tannin content varied significantly during the development stages, in fact, the highest value was reached in the semi ripe stage and was slightly reduced in the mature stage while the smallest value was found at the immature stage. Additionally, in case of grape fruit the tannin content decreased from 4.45 to 3.50, which might be attributed to the dilution effect [58]. Similar observation might be expected in case of Assam lemons[59].

4.8 Pectin content

Pectin is a hetero polysaccharide that is mainly present in the cell wall of plants that contribute gelling power in the jam, jelly, and other food products. Citrus peel is an important source of pectin because its albedo may contain up to 20% of the total fruit pectin [60]. The change in pectin and total chlorophyll help in obtaining fruits maturity. Pectin is localized in the primary and middle lamella of all higher plants. Intracellular pectin provides a gateway for the passage of water and nutrients. All citrus fruits contain a rich amount of pectin. In Assam lemon total pectin content increased 1.36- 3.07% during the early to mid-stage of fruit ripening and thereafter it starts to decline 0.89% as the fruit reaches its fully mature stage [21]. Increase in pectin content during fruit growth, soluble pectin content increased due to enzymatic degradation of pectin [61]. In orange, pectin content increase linearly from unripe stage 0.46% to fully mature stage 0.56% (Table 6). In grapefruits pectin content decreased with the advancement of the fruit, protopectin being more consistent than the soluble form of pectin. The amount of pectin present in the middle lamella of grapefruit is varied according to growth stages. Simpson & egyankor[62] reported that the highest pectin content 4.10% was present at fully mature stage of grape fruit. Moreover, the pectin content increased in case of kinnow and orange fruit, which might be

attributed to presence of pectin degrading enzyme inhibitors [63, 65]. Similar findings are expected in case of tangerine fruits [64].

4.9 Naringin content

Naringin is the flavonoid commonly present naturally in the citrus fruits, especially in grapefruit. It is responsible for the bitter taste of the fruits. In tangerine and pomelo highest naringin content was observed in unripe stage 181mg/l and 40.27µg/ml respectively. As fruit started to grow its content continuously decreases due to the dilution effect and increase in fruit size. In grape fruits, most bitter compound are limonin and naringin that contribute to the unpleasant taste and reduce the acceptability among the consumers. The Table 7 represents the naringin content in fruits present at different maturity stages. In grapefruits naringin was found maximum (12102(mg/100gFW) in the immature stage. It can be seen that naringin was mainly synthesized during early stage of fruit maturation [66]. In mandarin peel naringin content was strongly affected by fruit ripening stage. Maximum naringin content was present in unripe stage (15.31µg/g) of fruits, as fruit mature naringin content decreases but slight increase was observed in fully ripe stage (4.31µg/g) shown in Table- 7. Concerning bitter orange, 18 compounds were identified and the extracts were characterized by the predominance of the flavanones glycosides, naringin and neohesperidin were found to be the major compounds during ripening and highest naringin content was recorded at fully mature stage (19.29%). Naringin, hesperidin and neohesperidin are flavanones glycosides known to accumulate specifically in citrus species. Naringin and neohesperidin are neohesperidosides with a bitter taste due to the sugar neohesperidose while the sugar rutinose causes the hesperidin to have a neutral taste [67]. Juice of immature chinotto showed highest value (19.95mg/l) of naringin, and continuously decrease as fruit reached to final maturity stage. Naringin content decreased in most of the citrus fruits owing to its hydrolysis by naringinase [68, 70-72], however, presence of inhibitors might result in its increase, as in case of bitter orange [69].

5. CONCLUSION

Citrus fruit is one the most nutritious fruit in world and its medical properties have long been known. Many processed products have been standardized by these fruits, but much of its physiology and basic biology is not known. Citrus fruits with its multi-faceted properties are occupying a prominent position in the herbal medicinal system. They contain much amount of ascorbic acid and many other nutrients such as polyphenols, pectin, starch, sugar etc. Fruit growth is faster at an initial stage and slows down in the last stage. In citrus fruits, most of the fruits like tangerine, grapefruits, kinnow shows increasing trend in TSS as reach to its fully mature condition. The pH of citrus fruit gives the details about the state of acidity. In the present investigation represent that, pH increased till mild stage of development after that it started to reduce till fully developed stage. Fruits softening are enzyme dependent activity that mostly catalyzed by pectin degrading enzyme, pectinmethylesterase(PME) and polygalactournease(PG).

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Comment [C17]: Follow same spacing in references

Table 1. Changes in fruit length and weight during physiological stages of citrus fruits

| Citrus fruit | Unripe stage | Semi ripe stage | Fully ripe stage | Reference |
|--------------------------|---------------------|------------------------|-------------------------|------------------|
| Fruit length (cm) | | | | |
| Assam lemon | 16.72 | 78.97 | 92.04 | [17] |
| Grape fruit | 2.50 | 6.50 | 9.70 | [21] |
| Tangerin | 39.90 | 41.66 | 64.00 | [22] |
| Kinnow | 5.34 | 5.74 | 6.00 | [23] |
| Fruettrell | 5.04 | 5.39 | 5.37 | [23] |
| Mandarin | 21.09 | 27.15 | 29.31 | [24] |
| Orange | - | - | 67.12 | [25] |
| Fruit Weight (g) | | | | |
| Assam lemon | 0.42 | 68.32 | 146.57 | [17] |
| Grape fruit | 94.50 | 260.97 | 355.29 | [21] |
| Tangerin | 18.03 | 73.82 | 141.07 | [22] |
| Kinnow | 125.50 | 126.90 | 129.20 | [23] |
| Fruettrell | 5.04 | 5.39 | 5.37 | [23] |
| Mandarin | 61.80 | 79.40 | 94.20 | [24] |
| Orange | 200 | 258 | 231 | [26] |

Table 2. Changes in fruit color during physiological stages of citrus fruits

| Fruits | Unripe stage | | | Semi ripe stage | | | Fully ripe stage | | | References |
|-------------|--------------|--------|-------|-----------------|-------|-------|------------------|-------|-------|------------|
| | L | a | b | L | a | b | L | a | b | |
| Assam Lemon | 36.10 | -5.67 | 10.70 | 38.27 | -7.58 | 13.24 | 61.21 | -3.31 | 29.67 | [21] |
| Grape fruit | 44.32 | -6.53 | 19.67 | 55.12 | 10.59 | 27.57 | 57.20 | 20.82 | 35.45 | [33] |
| Tangerine | 39.19 | - | - | 41.66 | - | - | 64.00 | - | - | [22] |
| Kinnow | 47.1 | -25.06 | 20.05 | - | - | - | 77.28 | 31.6 | 69.62 | [35] |
| Fruetrells | 68.01 | -8.51 | 50.48 | 73.92 | 4.72 | 62.86 | 71.93 | 30.12 | 69.35 | [35] |
| Mandarin | 31.7 | -11.1 | 22.1 | 44.2 | -13.5 | 31.4 | 56.9 | 18.4 | 37.9 | [36] |
| Orange | 59.6 | 2.24 | 8.82 | 56.2 | 16.0 | 9.13 | 55.3 | 21.4 | 18.2 | [37] |
| Pomelo | 55.22 | -6.75 | 27.86 | 57.28 | -7.06 | 31.27 | 57.35 | -6.60 | 30.43 | [57] |

Table 3. Changes in Total soluble solids (°Brix) during physiological stages of citrus fruits

| Citrus fruit | Unripe stage | Semi ripe stage | Fully ripe stage | Reference |
|---------------------|---------------------|------------------------|-------------------------|------------------|
| Assam lemon | 6.73 | 6.47 | 5.57 | [21] |
| Grape fruit | 7.87 | 8.87 | 8.07 | [17] |
| Tangerin | 9.25 | 9.75 | 11.38 | [22] |
| Kinnow | 7.50 | 9.20 | 8.80 | [42] |
| Fruettrell | 6.80 | 8.90 | 7.40 | [42] |
| Mandarin | 6.81 | 8.72 | 11.63 | [24] |
| Orange | - | - | 12.1 | [25] |

Table 4. Changes in fruit pH and Titratable acidity during physiological stages of citrus fruits

| Citrus fruit | Unripe stage | Semi ripe stage | Fully ripe stage | Reference |
|--------------------------------|--------------|-----------------|------------------|-----------|
| pH | | | | |
| Assam lemon | 2.24 | 2.32 | 2.35 | [45] |
| Grape fruit | - | - | 3.67 | [44] |
| Tangerin | 2.3 | 3.6 | 3.2 | [46] |
| Kinnow | 3.30 | 4.40 | 4.20 | [42] |
| Fruettrell | 3.60 | 4.90 | 4.20 | [42] |
| Mandarin | 3.05 | 3.42 | 4.72 | [15] |
| Orange | 3.08 | 3.28 | 3.32 | [47] |
| Titrateable acidity (%) | | | | |
| Assam lemon | 1.17 | 3.86 | 4.86 | [21] |
| Grape fruit | 1.88 | 1.92 | 1.39 | [17] |
| Tangerin | 4.08 | 2.80 | 0.86 | [22] |
| Kinnow | 1.10 | 0.55 | 0.70 | [42] |
| Fruettrell | 0.65 | 0.50 | 0.60 | [42] |
| Mandarin | 2.59 | 1.49 | 0.66 | [24] |
| Orange | 2.24 | 1.43 | 1.25 | [47] |

Table 5. Changes in ascorbic acid (mg/100g) content during physiological stages of citrus fruits

| Citrus fruit | Unripe stage | Semi ripe stage | Fully ripe stage | Reference |
|---------------------|---------------------|------------------------|-------------------------|------------------|
| Assam lemon | 90.45 | 44.75 | 11.11 | [21] |
| Grape fruit | 55.72 | 45.15 | 41.34 | [17] |
| Tangerin | 0.57 | 0.24 | 0.34 | [22] |
| Kinnow | 46.60- 52.10 | 41.50- 43.10 | 42.15- 45.30 | [42] |
| Fruettrell | 43.92- 48.30 | 38.10- 40.25 | 38.40- 40.40 | [42] |
| Manderin | 37.12 | 33.77 | 27.62 | [24] |
| Orange | - | - | 38.13 | [47] |

Table 6. Changes in Tannin and pectin content during physiological stages of citrus fruits

| Citrus fruit | Unripe stage | Semi ripe stage | Fully ripe stage | Reference |
|---------------------------|---------------------|------------------------|-------------------------|------------------|
| Tannin content | | | | |
| Assam lemon (%) | - | - | 0.35 | [59] |
| Grape fruit (mg catechin) | 4.45 | - | 3.50 | [58] |
| Mandarin (%) | 0.12 | - | 0.19 | [57] |
| Orange (%) | 0.12 | - | 0.37 | [57] |
| Pectin content (%) | | | | |
| Assam lemon (%) | 1.36 | 3.07 | 0.87 | [21] |
| Grape fruit (%) | - | - | 4.10 | [62] |
| Tangerin (%) | - | - | 7.71 | [64] |
| Kinnow (%) | 8.20 | 12.29 | 14.24 | [65] |
| Orange (%) | 0.46 | 0.52 | 0.56 | [63] |

Table 7. Changes Naringin contentin during physiological stages of citrus fruits

| Citrus fruit | Unripe stage | Semi ripe stage | Fully ripe stage | Reference |
|--------------------------------|---------------------|------------------------|-------------------------|------------------|
| Assam lemon (mg aglycone/100g) | - | - | 0.18 | [72] |
| Grape fruit (mg/100 g FW) | 12102 | - | 2195 | [66] |
| Tangerine (mg/l) | 181 | 154 | 140 | [68] |
| Kinnow (mg/g DW) | 0.68 | 1.21 | 0.54 | [65] |
| Mandarin ($\mu\text{g/g}$) | 15.31 | 3.03 | 4.31 | [70] |
| Bitter Orange (%) | 13.29 | 15.03 | 19.29 | [69] |
| Pamelo ($\mu\text{g/ml}$) | 40.27 | 7.56 | 0.53 | [53] |
| Chinotto (mg/l) | 19.95 | - | 6.08 | [71] |

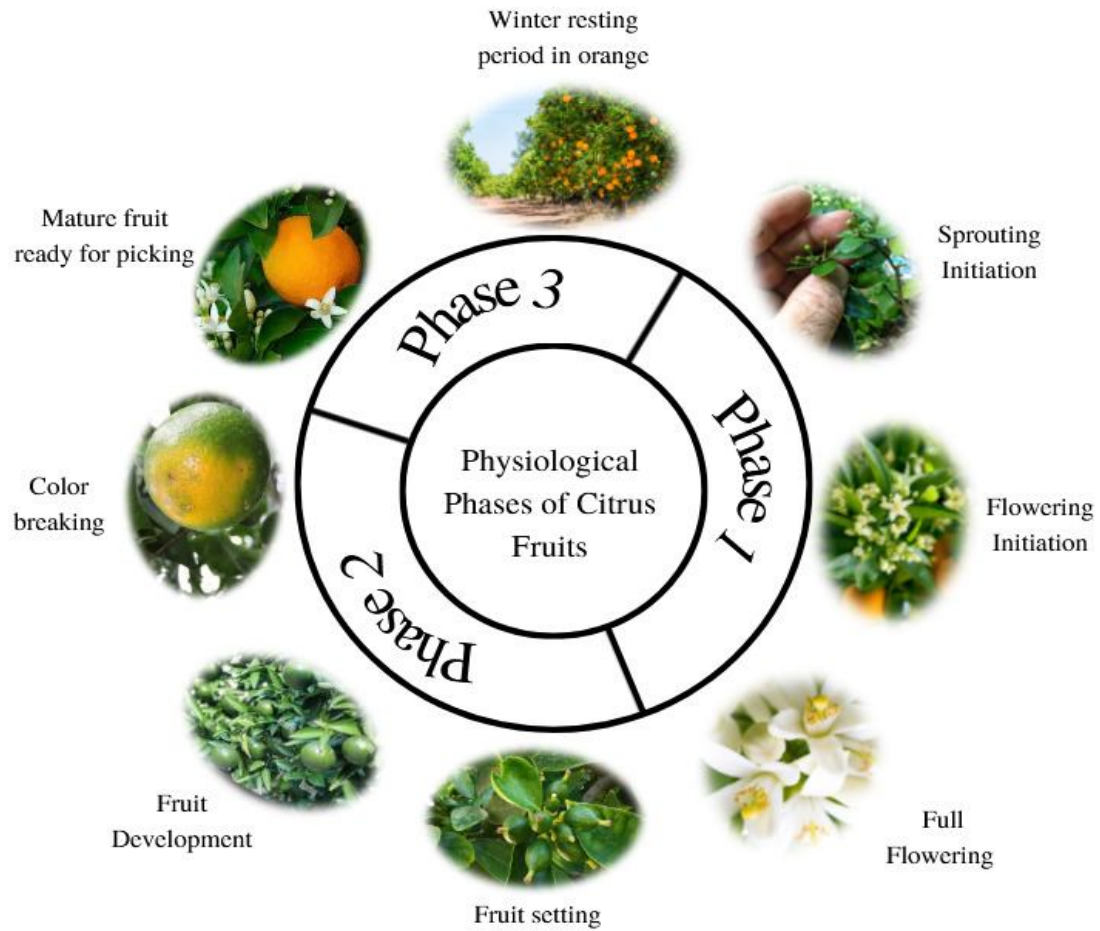


Fig. 1. Physiological phases of citrus fruits during growth, development and maturation