



Received on 20 June 2022; received in revised form, 16 August 2022; accepted 30 August 2022; published 01 March 2023

INSIGHTS INTO THE ROLES OF ANTIOXIDANTS IN CITRUS FRUITS

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Keywords:

Antioxidants, Phenolic acids, Flavonoids, Coumarins, Polyphenols, Terpenoids, Citrus fruits maturation, Amino acids

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ABSTRACT: Citrus fruits are known for their nutritiousness. Role of vitamins and minerals play a major role in citrus fruits during plant-pathogen interactions. These fruits are the supreme fruit crops which contain flavonoids and phenolic compounds. These molecules benefit individuals who are all consuming and play an important role in fruit ripening and development. Mainly these molecules act as antioxidants to eliminate free radicals formed during fruit development. Primary metabolism is the same in citrus as it estimates the flow of carbon in fruits. In this review, we discuss a few antioxidant molecules that have an additional role in the fruit development. These molecules are vitamins, phenolic compounds and terpenoids, mostly except vitamins; all the compounds are found in an actual form where vitamins A, C, E are available in the form of beta Carotenes, ascorbic acid or ascorbate and tocopherols in plants. Phenolic acids and coumarins are rich in citrus fruits and are powerful due to their hydroxyl groups. Limonoids are a terpenoid molecule mostly seen in the seeds, peel and juices of citrus fruit. The antioxidant production is associated with its genes that will directly mediate the production of those molecules, or it will be involved in the pathway of molecular biosynthesis. Transcriptomics studies revealed the function of these genes, and the understanding helps in creating better breeds, for example, up-regulation of *CitPSY* expression produces fruit with aesthetic texture, *CsMYB96* is a gene that aids for phenolic production, directly controlling pathogens and preventing plants from drought.

INTRODUCTION: Citrus crops are the world's supreme and widely cultivated crop around the globe¹. Citrus crops belong to the Rutaceae family, genus Citrus L. It is widely cultivated in tropical areas.

All over the globe, customers ratify citrus because of its flavors, colour, aroma and medicinal properties. Citrus **Fig. 1** is rich in vitamin C and has now become a predominant nutritive fruit daily. As the fruit develops, the locule provides sustenance¹.

Antioxidants maintain cell structure, eliminate free radicals, inhibit lipid peroxidation and prevent oxidative damage. Citrus **Fig. 2** contains vitamins A, C and E, minerals, flavonoids, phenolic acids, limonoids, coumarins, amino acids and compounds².

	DOI: 10.13040/IJPSR.0975-8232.14(3).1098-07
	This article can be accessed online on www.ijpsr.com
DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.14(3).1098-07	

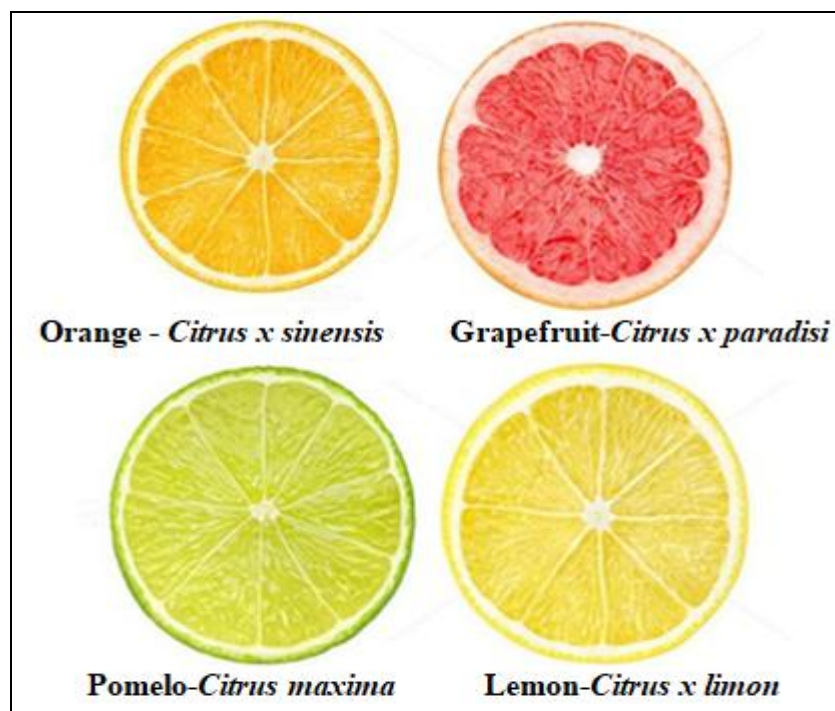


FIG. 1: CITRUS FRUITS



FIG. 2: JUICY CITRUS

Role of Antioxidants: Antioxidants play a major role in citrus fruits and in fruit development and maturation. The antioxidant components are minerals, vitamins, phenolic compounds and terpenoids. Developmental changes happen through antioxidant enzymes. In recent literature, more than 160 antioxidants have been studied².

Vitamins and Antioxidant Activities during Fruit Development in Citrus: Basically, citrus fruits contain around six vitamins. Among six, vitamin A, C and E are assessed for an antioxidant role as mentioned in **Table 1**. Vitamin A is a kind of fat-soluble vitamin⁵¹ that is available in the form of retinol, retinoic acid and beta-carotene. It's mostly present in animal and plant food diets,

whereas in plants it is only available in beta Carotenoids³. It is a red-orange pigment found in fruits and vegetables that is majorly responsible for pigment formation in fruit development, including oranges and tangerines⁵². Carotenoids are responsible for the aesthetic and attractive appearance of the fruit, whereas it is widely present in the edible parts that have become a high dietary source for animals and humans⁴. Naturally, beta-carotenoids are generally present in all fruits, but they will remain unrevealed due to the presence of a green pigment called chlorophyll; as fruit starts to ripen, the chlorophyll starts to disappear and reveal the colour of carotenoids [β -carotene (β , β -carotene), lutein (β ,t-carotene-3,3'-diol), neoxanthin (5',6'- epoxy-6,7-didehydro-5, 6, 5', 6'- tetrahydro- β , β -cotene-3,5,3'- triol) and violaxanthin (5, 6, 5',6'-diepoxy-5, 6, 5', 6'- tetrahydro - β , β - carotene - 3, 3'-diol)⁵. Simultaneously new carotenoids were synthesized during the ripening of fruit, which in turn changed fruits from its yellow colour to red-orange colour. But in contrast, these beta-carotenoids start to react with fatty acids in a process called esterification to produce colour in fruit which is more stable than the non-esterified formation. Based on the double bond conjunction, it increases the colour intensity, higher the interaction directly proportional to the absorption maxima (λ_{max}). Antioxidant properties of vitamin A react with free radicals^{6, 7}.

Its content is encrusted oranges (*Citrus sinensis* L. Osbeck) is 1.28mg/kg and 0.75mg/kg in tangerine (*Citrus x tangerina*)¹. The carotenoid biosynthesis is highly regulated by phytoene synthase (*CitPSY*), phytoene desaturase (*CitPDS*), zeta-carotene (car) desaturase (*CitZDS*), carotenoid isomerase (*CitCRTISO*), lycopene beta-cyclase (*CitLCYb*), beta-ring hydroxylase (*CitHYb*), zeaxanthin (zea) epoxidase (*CitZEP*), and lycopene epsilon-cyclase (*CitLCYe*) genes in *Satsuma mandarin*. The peel colour change was associated with *CitLCYe* transcripts getting suppressed, increasing the transcript of *CitLCYb*⁸.

Accumulation of carotenoids is directly related to the increase in the juice sacs of citrus fruits which is related to the environment stimuli, where carotenoids content can be induced when the plant is exposed to blue light⁹ and undisturbed when exposed to red light, where the *CitPSY* expression is upregulated¹⁰. Vitamin C-also called ascorbic acid or ascorbate, antiscorbutic vitamin, water-soluble vitamin that humans cannot be able to synthesize¹¹ has antioxidants role. This vitamin is rich in citrus and also found in fleshy and peeled fruits¹². This vitamin is necessary for the growth and development of collagen formation¹³. It is a radical scavenger, which can beneficially react with reactive oxygen species (ROS) and decreases sulfur radicals¹.

Fruit ripening involves ROS metabolism taking place in many subcellular organelles like mitochondria, peroxisomes and chloroplast, ascorbate helps the fruit ripen by removal of free radicals produced. Ascorbate is generally present in most of the fruits that are produced by many biosynthesis pathways, crucially by L-galactose pathway⁶⁰ were L-galactono-1,4-lactone (GalL) as mentioned in **Fig. 3**, is oxidised to form ascorbate as in final step of the pathway catalysed by L-galactono-1,4-lactone dehydrogenase (GalLDH). Studies shows that gene expression of the particular enzyme remains unchanged during the ripening. Stable and elevated levels of ascorbate reduced the collateral cellular damage that was caused by a ripening-associated oxidative burst in addition to ROS-related cellular damage. Ascorbate removes free radicals by a key pathway called ascorbate glutathione cycle⁵⁸ as mentioned in **Fig. 4**. In this cycle ascorbate peroxidase detoxifies hydrogen

peroxide by oxidizing ascorbate. Thus, it shows antioxidants to eliminate free radicals produced during many metabolism processes and formed during many biotic and abiotic stress conditions¹⁴. Ascorbic acid content is highly affected by light, lower incidence of light or shade-grown plants will result in less ASA content in fruit¹⁵. Three groups of genes regulate ascorbic acid (ASA) accumulation, biosynthetic genes (CitVTC1, CitVTC2, CitVTC4, and CitGLDH), GSH-producing genes (CitGR and CitchGR), regeneration genes (CitMDAR1, CitMDAR2, and CitDHAR) where contributed towards the increasing accumulation of ASA content in few citrus varieties. The blue light treatment upregulating these genes will directly help in the ASA content in the fruit sac¹⁶. The ASA biosynthesis pathway and its regulation is not fully understood, in which degradation and recycling genes are involved in the higher concentration of ASA. The accumulation involves 15 genes, with 5 degradation genes and 5 recycling genes¹⁷.

The expression is associated with GMP, GGP and GPP, MDHAR3, and DHAR1. L-galactose pathway genes and the myo-inositol pathway genes are correlated with the accumulation of ASA, where both pathways are synergic during the beginning of the ripening. At the same time, the L-galactose predominates at both the beginning and end¹⁸. Vitamin E-(another) fat-soluble compound that restrains tocopherols. This helps regulate and synthesize fruit development in oranges and lemon. Though the function of vitamin E in citrus fruit development is not well studied, its main role is to act as an antioxidant. It is a lipophilic antioxidant compound widely found in the seeds of oil seeds⁵⁹, which the human system cannot synthesize, and photosynthetic plants produce it. It is available in two groups as (α -, β -, γ -, δ -tocopherols and α -, β -, γ -, δ -tocotrienols, respectively), which are characterized by the number of methyl groups on the ring structure¹⁹. It is encrusted in orange (*Citrus sinensis* L. Osbeck), tangerine (*Citrus x tangerina*), and lemon (*Citrus limon*) and can reach 4.56 mg/kg, 4.52 mg/kg and 10.30 mg/kg¹. This protects cell damage which is caused by free radicals against lipid peroxidation. In plants, α -tocopherols help in cell signaling, protect cell membranes, and act as a free radical scavenger.

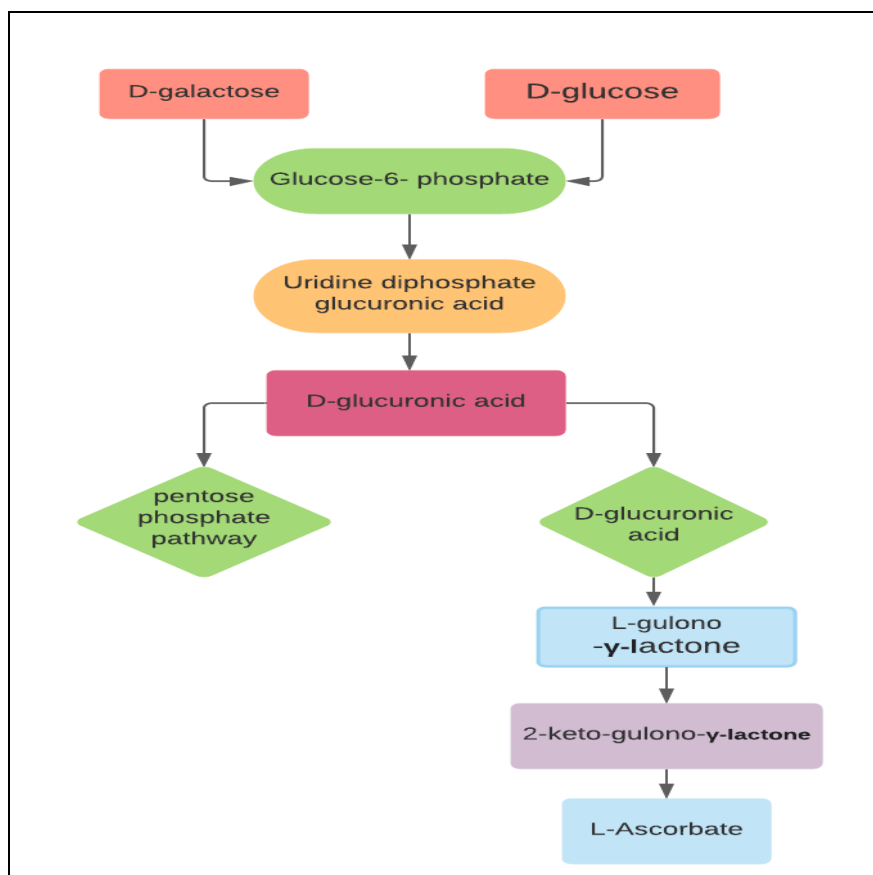


FIG. 3: L-GALACTOSE PATHWAY WERE L-GALACTANO1, 4-LACTONE (GALL)(VITAMIN C BIOSYNTHESIS PATHWAY)

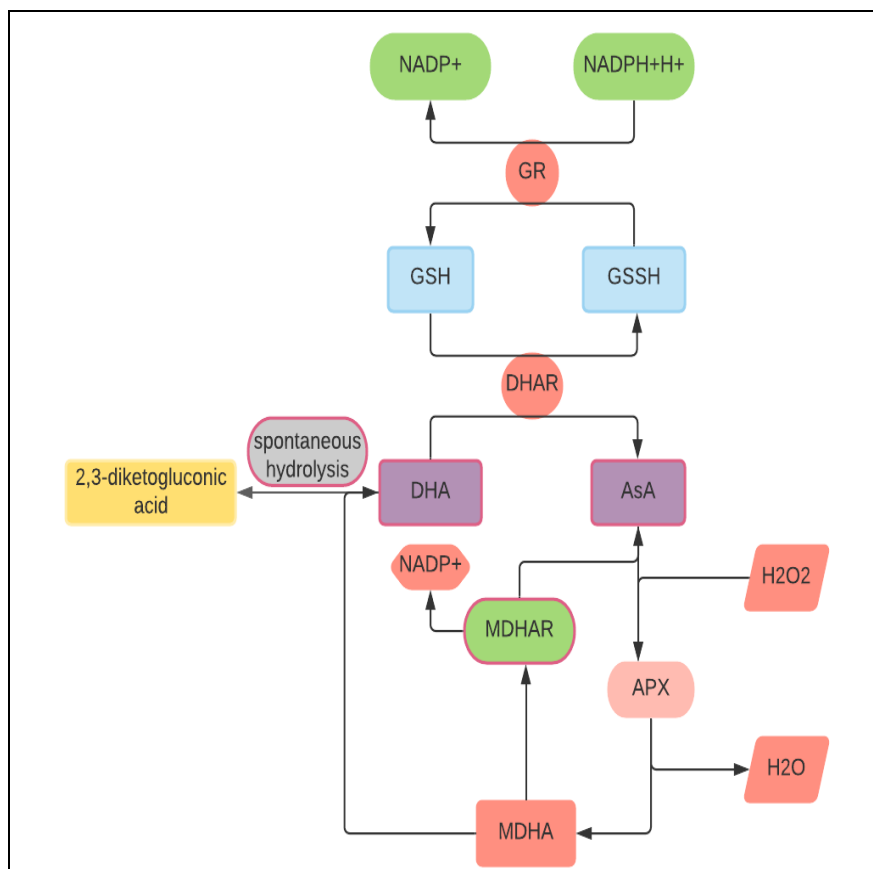


FIG. 4: ASCORBATE - GLUTATHIONE CYCLE

TABLE 1: ANTIOXIDANT VITAMINS AND THEIR ROLES

Compound name	Chemical name	Water/ fat soluble	Antioxidant activity	References
Vitamin A	Retinol	Fat	Reacts, Free radicals, Peroxyl radicals	3, 6
Vitamin C	Ascorbate	Water	Scavenge radicals, Decreases sulfur radicals	11, 12
Vitamin E	Tocopherol	Fat	Free radicals, reduction of ferrous ion to ferric ion, Minimises catalyses, Free radical against lipid peroxidation	19

Phenolic Compounds and Antioxidant Activities: Phenolics act as an antioxidant in many ways. It also acts as pro-oxidants by chelating and maintaining or intensifying catalytic activity¹². Polyphenols consist of bioactive compounds such as hydroxybenzoic acids, proanthocyanins, flavonoids, anthocyanins, stilbenes and lignans²⁰. Phenolic compounds exhibit antioxidants property that is formed of one or more aromatic rings attached to the hydroxyl group and phenolic ring; its antioxidants property is dependent on this²¹. Among multiple phenolic compounds, flavonoids and phenolic acid are essential dietary phenolic compounds²².

These potent antioxidants are predominant in the reduction of lipid oxidation²³. Thus, the role of phenolic compounds is to exhibit antioxidants to remove free radicals and act as a natural preservative that prevents the fruit quality at the time of pre and post-harvesting²⁴. The structural alignment of the molecule makes it as strong antioxidants molecule, based on the amount are hydrogen molecules adjacent to the hydroxyl group attached to its ring structure, double bond interaction between the functional group(-C=O), and benzene ring of some of the flavonoids²⁵ make these molecules makes it highly antioxidants²⁶. Studies show a positive correlation between the phenolic level and the antioxidant level at multiple stages of fruit maturation²⁷. Due to its nature as a hydrogen donor, it acts as a free radical scavenging molecule. The total phenolic and flavonoid compounds are directly proportional to the antioxidant activity, which shows a linear correlation²⁸; studies analyzing correlation coefficient (R-values), suggest that these phenolic acid and flavonoids are highly antioxidant molecules²⁹.

Flavonoids: Citrus fruits are a rich source of flavonoids. Two main antioxidants in citrus flavonoids are hesperidin and naringin³⁰ mentioned

in **Table 2**. Naringin signifies and increases the immune system and intensifies catalytic activity (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx). Naringin is a flavone glycoside mainly seen in pomelo and oranges. This helps in the development of fruit, bitterness and enzymatic activities. Level of naringin in pomelo (*Citrus grandis L. Osbeck*) may range to 10.80mg³⁰.

Hesperidin is a flavanone glycoside seen in citrus fruits⁵⁴ for growth and development and also helps during harvest time and maturation. It is majorly found in lemons and oranges. Hesperidin content in encrusted orange (*Citrus sinensis L. Osbeck*), lemon (*Citrus limon*), and pomelo (*Citrus grandis L. Osbeck*) is 10 mg, 20 mg, and 3.37 mg/100g³¹, mentioned in **Table 2**. The flavonoid metabolism will be highly induced during drought conditions⁵⁷ and the flavonoid metabolism is mediated by CYTOCHROME P450s, the largest gene family in the plant kingdom³².

Phenolic Acids: These are high in citrus⁵⁶ and have dissimilar scavenging radicals. This shows strong antioxidant properties due to hydroxyl groups^{12, 1}, mentioned in **Table 2**, which correlates between antioxidant activities and phenolic acids. In citrus fruits, CsMYB96 promotes biosynthesis of phenolic acid synthesis⁵⁵; its expression will be higher during the pathogenic infection, thus, it helps in defense mechanisms, and it binds with transcription factors responsible for Sialic acid synthesis³³.

Coumarins: Coumarins fall under the type of phenolic compounds rich in citrus fruits. Coumarins synthesis from the phenylalanine metabolic pathway leads to furocoumarins through citrus and persian synthesis³⁴. This has powerful antioxidant properties due to the hydroxyl groups mentioned in **Table 2**. Coumarins are necessary for the development of edible parts in fruit formation³⁵.

BAHD acyltransferases and aromatic prenyltransferases are said to have a role in the biosynthesis of paclitaxel and meroterpene; later

studies have shown that these have a role in the synthesis of many aromatic compounds like Coumarins³⁶.

TABLE 2: PHENOLIC COMPOUNDS, MOLECULAR FORMULA AND THEIR ACTIVITY

Phenolic compounds	Molecular formula	Antioxidant activity	Reference
Flavonoids- Naringin	C ₂₇ H ₃₂ O ₁₄	Inhibits oxidant enzymes	30
Flavonoids- Hesperidin	C ₂₈ H ₃₄ O ₁₅	Scavenges (ROS) reactive oxygen species	31
Phenolic acid	C ₆ H ₆ O	Scavenging radicals Hydroxyl groups	33
Coumarins	C ₉ H ₆ O ₂	Phenolic hydroxyl group in compounds, Decrease of free radical production	34, 35

Terpenoids in Citrus Fruit and Antioxidants Activities during Fruit Development:

In terpenoids, the activities of limonoids and carotenoids are described³⁷, as mentioned in **Table 3**. Limonoids and carotenoids are the major terpenoids that help in fruit growth and maturation³⁸. During the development, limonoids and carotenoids content escalates during April, culminates in September, and dwindles after October when it reaches a steady point. So, the juice sacs and tissues proliferate first and then subsides. CioSC gene encodes a crucial enzyme called oxidosqualene cyclase (OSC) helps in the biosynthesis of precursors of triterpene, where its presence is directly proportional to bioaccumulation of limonoid³⁹.

Limonoids and Carotenoids: Limonoids are highly oxygenated and tetracyclic in secondary metabolic activities. More than 13 limonoid glycosides have been found in fruits⁴⁰ glucosides were resolved in pomelos. These limonoids are highly present in the flavedo region of the citrus³⁸. Mostly found in seeds, fruits and peel tissues of citrus. This also has free radical scavenging activity⁴⁰, as in **Table 3**. In Citrus limonoids (CLs)

formation of β-D-glucosides catalyses glucosyltransferase which reduces concentration of aglycones in citrus fruit development and juices. The natural debittering enzyme is coded by the limonoid glucosyltransferase (LGT) gene⁴¹. The overexpression of LGT helps in the cultivation of less bitter citrus fruits. Cytochrome P450s (CYT450s) and transcription factor MYB are crucial factors promoting limonoid biosynthesis. MYB transcription factor CiMYB42 has a crucial role in limonoid synthesis. The expression of CiMYB42 increases the limonoids content in some plants, and in some citrus fruits, it is mediated by CiOSC expression. RNAi-based CiMYB42 deleted plant shows fewer limonoids, whereas the overexpression in orange shows higher than the limonoid⁴².

Carotenoids are tetraterpenoids, a rich source of citrus known for their decoction and maturation³⁸. They protect cell membranes from oxidative damage, certify signals between receptors and cells, and maintain cell function^{38, 43}. The contribution of carotenoids leads to antioxidant capacity as mentioned in **Table 3**, with pulp coloration in citrus⁴⁴.

TABLE 3: TERPENOIDS AND THEIR ACTIVITY

Terpenoids	Molecular formula	Antioxidant activity	Reference
Limonoids	C ₂₆ H ₃₀ O ₈	Induces cell death and free radicals, Different variables of antioxidants	38, 39
Carotenoids	C ₄₀ H ₆₄	Causing Free radicals	38, 43

Variation of Antioxidant Components in Citrus and Their Capacity: Citrus plant extraction is controlled by chemical composition and antioxidant content.

However, the peroxidation system of antioxidants of various citrus species is associated with polyphenol content¹². Polyphenols may be dominant in antioxidant components in citrus⁵³.

The amount or capacity of antioxidants found in citrus was coordinated with ascorbic acid level and with the flavanone glycosides presence⁴⁴.

Citrus Fruit Development and Conditions:

Fruit Development- Divided into Three Stages: Division of cells, Expansion of cells, and maturation of fruit^{12, 45}. At the first stage, adequate fruit growth happens: the peel, albedo, thickens cell

division. At this stage, sacs juice grows out (into the locule). In the second stage, sudden fruit development happens due to cell expansion. In the third stage, the volume intensifies, diminishes, and colour changes externally and internally, and sugar and acid levels for utilization and harvesting^{12, 45, 46}.

Secondary metabolite changes occur, giving the fruit an eccentric aroma and relish. The fruit quality is insistent and determined by patron choice. Fruit development completion is controlled by the cultivar, which is ready for a process of 4-6 months, such as Valencia oranges (*Citrus sinensis*),⁴⁷ are processed for 11–13 months after flowering. Climatic conditions, fruit escalates and decreases the time for maturation by 40%⁴⁵. Two major fruit quality heritability in the pulp are sugar and acid level. An organic acid is correlated with pulp acidity in citrate, which assembles at the fruit

development of the second stage, fruit juice, and cell prompts⁴⁸. This accumulates for a week and reaches a peak when the volume of fruit is 50%; acid diminishes as fruit matures. In some varieties, sugar accumulates in the early development of fruit, but major accumulation happens during the third stage, and the content of acid reduces.

Especially compound is sucrose, which proliferates and increases glucose or fructose. Climatic conditions have a major dependence on fruit development and maturation⁴⁹. In hot climatic conditions, this enlarges and accelerates the heat hours, and acid diminishes⁵⁰. However, not only fruit accumulation, the development of colour doesn't happen fully; it may remain green in some extreme cases. So, the expected outcome of climatic conditions in winter temperatures with shorter-cold night times is seen.

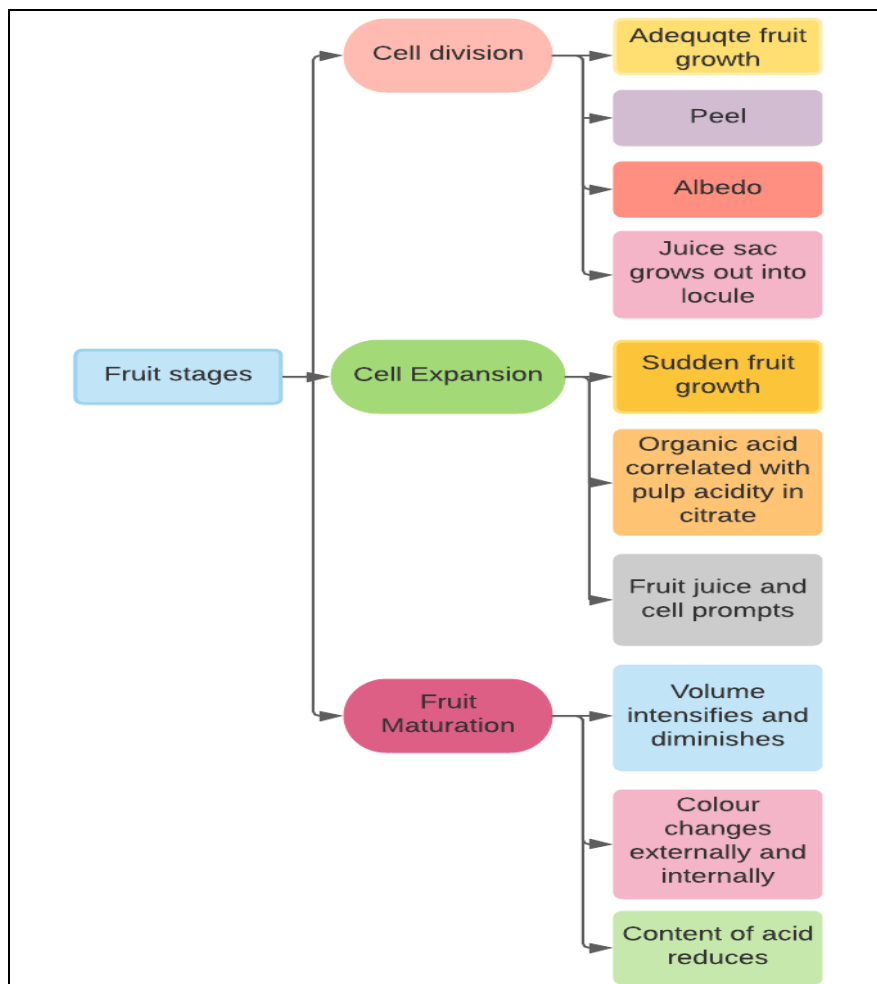


FIG. 5: STAGES OF FRUIT DEVELOPMENT

CONCLUSION: Traditional medicines have been used in citrus plants for over 1000 years. So, a high

rise of antioxidants may be destructive to the state of health, but recent literature has proved that the

utilization of fruits is favourable to dietary conditions. Instant evolution in spotting the innovation of bioactive compounds of plants is seen. This engages awareness and considerations in the world. Along with secondary metabolism, primary metabolites carbohydrates, esters, amino acids, terpenoids, alcohols, and polymer compounds impart powerful components to taste the fruit, aroma, and the value of nutrition. Variation of fruit is seen in structure, size, and initiation as secondary and primary metabolites. The juice sacs and pulp in citrus- are eccentric among fruits. But, the sugars, amino acids and organic acids are brought together at a metabolic rate.

In his review, we encapsulate movement, metabolism, and accumulation. Understanding the role of these vitamins and other studied molecules in the role of fruit ripening and development helps to develop better harvesting and cultivation of citrus fruits. Knowledge about these genes and their function of these genes are helpful in citrus fruit cultivation with values. Today genetic engineering technologies have developed, where they use these genes carrying multiple functions which transfer one particular character from one plant to another where it lacks.

ACKNOWLEDGEMENT: I express my earnest and deep sense of gratitude to my project guide, Dr. P. Senthil Kumar, Department of Genetic Engineering, SRM Institute of Science and Technology, who has always been moral support and guided me with his valuable ideas.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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How to cite this article:

Jayam JR, Rajendran AK, Suganthi M and Palanisamy S: Insights into the roles of antioxidants in citrus fruits. *Int J Pharm Sci & Res* 2023; 14(3): 1098-07. doi: 10.13040/IJPSR.0975-8232.14(3).1098-07.

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