CITRUS: A TREASURE TROVE OF HEALTH-PROMOTING PHYTOCHEMICALS

Amit Vikram, Ram M. Uckoo, Kotamballi N. Chidambara Murthy, Bhimanagouda S. Patil, and **Guddarangavvanahally K. Jayaprakasha**
Vegetable and Fruit Improvement Center, Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843, USA

Keywords: Citrus, limonoids, flavonoids, carotenoids, furocoumarins, organic acids, phenolic acids, amines, health benefits

Contents

1. Introduction
2. Bioactive Molecules in Citrus and their Analysis
3. The Health-Promoting Properties of Citrus
4. Human Studies
5. Citrus and Food-Drug Interactions
6. Citrus in ancient Folk Medicine
7. Concluding Remarks
Glossary
Bibliography
Biographical sketch

Summary

Citrus species belong to the family Rutaceae. Their unique taste, texture and their wide range of beneficial properties have led citrus to be among the most highly-consumed fruits in the world. In recent years, *per capita* citrus consumption has increased due to accumulating evidence of the beneficial roles of certain bioactive compounds also known as phytochemicals present in citrus fruits. Besides the well-known vitamin C, citrus fruits contain a wide array of phytochemicals considered to have many health-promoting properties. Phytochemicals are secondary metabolites that are naturally synthesized by plants, and their primary role is to protect the plant from various pests and diseases. Due to various genetic, environmental and physiochemical changes, different citrus species synthesize numerous phytochemicals of varying contents and kinds. These phytochemicals are classified into different groups based on their structural scaffolds. This chapter describes the various classes of phytochemicals present in citrus fruits and their beneficial properties for human health, such as decreasing the risk of cardiovascular disease, various types of cancer, and other chronic diseases.

1. Introduction

Citrus trees are evergreen, and prefer moderate climatic conditions with optimum temperatures of 20ºC - 35ºC. They are believed to have originated in South East Asia and later spread to other continents. The plants are large shrubs reaching a height of 5 –15 m with thorny or thornless shoots and alternately arranged leaves with an entire margin. Citrus plants have solitary flowers or flowers in small corymbs; each flower is strong-scented, and 2-4 cm across, with 4-5 white petals and numerous stamens. Citrus fruit is a hesperidium, defined as a specialized berry with a leathery rind. Inside the rind, segments filled with pulp vesicles contain the citrus seeds. Due to sexual hybridization among a number of species and intraspecific hybrids, large genetic variation exists in the citrus group. The majority of the commercial citrus species are derived from hybridization and selection from chance natural mutations leading to some ambiguity in the classification of citrus species. The ambiguity could also be due to different taxonomic studies proposed by botanists and taxonomists. Before the modern system of classification, three books, the *Kee Jia Citrus Record* written in 1178 AD by Han Yanzhi, Johann Volkamer's *Nürnbergische Hesperides* published in 1708 and *Histoire Naturelle des Orangers* published in 1818 by Pierre Antoine Poiteau and Joseph Antoine Risso are some of the early notable literary compilations. These books not only provided pictorial descriptions but also laid out an important basis for identification and further taxonomic evaluations.

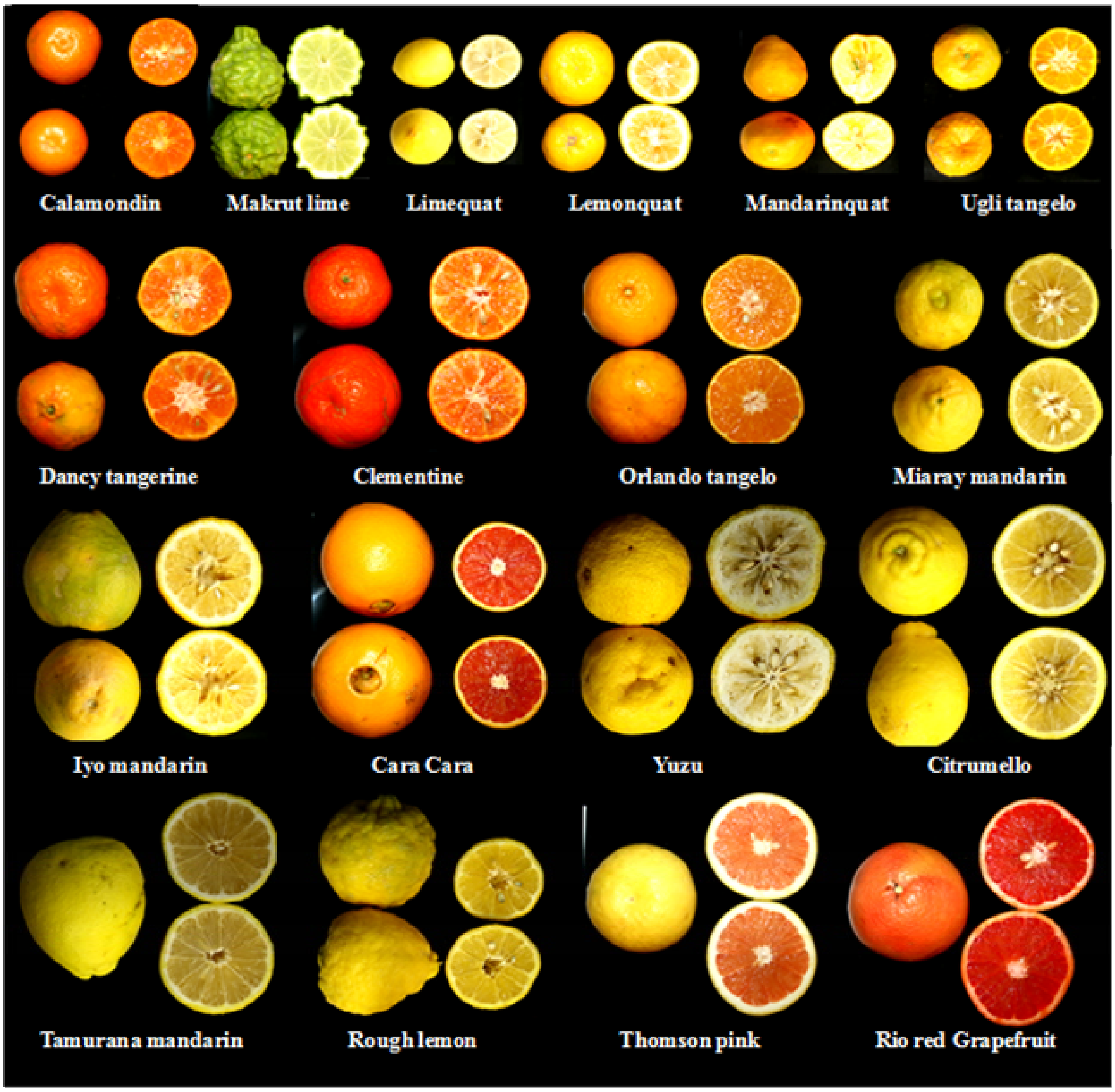

Figure 1. Citrus varieties grown in the tropical and sub-tropical regions of the world

Among the most widely-followed modern classification was given by Walter Tennyson Swingle. He classified all the citrus fruits into 16 species and their subspecies with various cultivated varieties. The edible species were included in the sub genus *Eucitrus* while the inedible species were included in the subgenus *Papeda*. Following are some of the most commonly-consumed citrus fruits (classified by Swingle) and their common names: *C. medica* (citron), *C. limon* (lemon), *C. aurantifolia* (lime), *C. limetta* (sweet lime), *C. aurantium* (sour orange), *C. sinensis* (sweet orange), *C. reticulata* (mandarin), *C. grandis* (pomelo), *C. paradisi* (grapefruit), *C. indica* (Indian wild orange), *C. tachibana* (Tachibana orange). Certain known and unique varieties of citrus fruits are presented in Figure 1. In the 1970's Tyôzaburô Tanaka produced a series of publications on citrus classifications: *Citrus fruits of Japan* 1922, *Species problem in Citrus* 1954 and *Tanaka's Cyclopedia of Edible Plants of the World* 1976. He classified citrus fruits into 4 genera: *Citrus*, *Fortunella, Poncirus,* and *Clymeia*; these genera comprised 162 species. Current classification given by D.J. Mabberley classifies the genus *Citrus* with only three edible citrus species: *Citrus medica* L. (citron), *Citrus maxima* Burm. (Merrill pomelo), and *Citrus reticulata* (Blanco mandarin). Each of these species includes several hybrids.

2. Bioactive Molecules in Citrus and Their Analysis

2.1. Vitamin C and Organic Acids

Citrus fruits are classified as acidic due to their high levels of organic acids. The relative concentrations of organic acids are specific to different species and can be used to further classify citrus fruits as acidic (limes and lemons) and less acidic or sweet (Clementine, sweet orange). Due to their effects on taste and organoleptic qualities, the organic acids have high significance for consumer acceptance, particularly in the juice processing industries. The commonly occurring organic acids in citrus fruits are ascorbic, citric, oxalic, tartaric, quinic, malic, and lactic acids (Figure 2). Among these, ascorbic and citric acid are present in higher levels than the other acids (Ranganna et al, 1983). The organic acids primarily accumulate in the vacuoles of the fruit cells and their contents increase with fruit maturity. The levels of organic acids increase during early fruit development and remain constant at maturity while the sugars increase during this period. Ascorbic acid is biosynthesized through the L-galactose pathway. The primary metabolite is D-mannose-1-phosphate, which is converted to L-ascorbate through five intermediates, GDP-D-mannose, GDP-L-galactose, L-galactose 1-phosphate, L-galactose and L-galactono-1,4-lactone (Giovannoni, 2007). Ascorbic acid rapidly degrades to de-hydro-ascorbic acid (DHAc). Ascorbic acid and DHAc cumulatively are considered as vitamin C. Oxalic acid occurs in the segment membranes, juice vesicles, and fruit rind. It accumulates in injured or diseased fruit where it chelates calcium and magnesium ions, and lowers the pH of the plant cell wall, presumably as a defensive mechanism. The low pH provides ideal conditions for the cell wall degrading enzymes to hydrolyze pectin (Ikotun, et al 1988). Tartaric acid is a sour-tasting acid that is derived from succinic acid. Lemons have higher levels (6.4 mg/g fresh wt.) of tartaric acid compared to oranges (4.1 mg/g fresh wt.) and limes (2.4 mg/g fresh wt.).

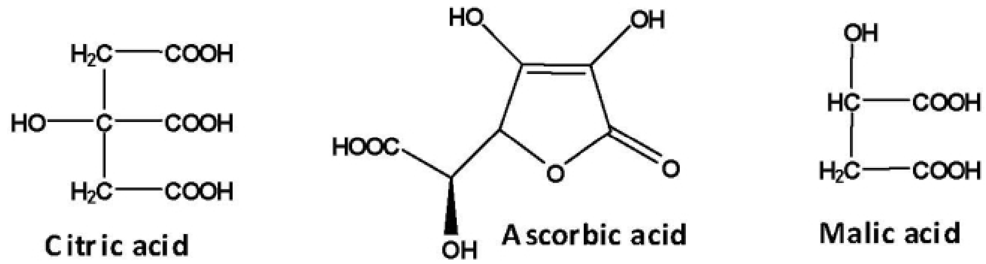

Figure 2. Structures of most common organic acids present in citrus fruits

The organic acids are highly soluble in water and can be easily extracted from citrus fruits. Certain acids such as ascorbic acid is highly susceptibility to oxidation and it requires an acidic extraction medium to prevent oxidation. Dilute (3-5%) trichloroacetic acid and meta-phosphoric acid are commonly used for extraction of organic acids. Organic acids can be analyzed by titration (expressed as titratable acidity), colorimetry, and other separation and detection techniques such as high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS). The reduced form of ascorbic acid, dehydroascorbic acid, has poor absorbance and can be converted to ascorbic acid using tris(2-carboxyethyl) phosphine hydrochloride and dithiothreitol. Ascorbic acid can be separated using a C_{18} column with a polar mobile phase such as water with phosphoric acid as a modifier at a flow rate of 1 ml/min and detected by monitoring the eluent at λ_{254} nm. Organic acids can also be separated using ion-exclusion column chromatography with phosphate buffer (pH 2.4) as mobile phase. The separation can be performed by isocratic elution at a flow rate of 0.7 -1.0 ml/min and organic acids can be detected at λ_{210} nm.

2.2. Carotenoids

Carotenoids comprise a group of compounds that are ubiquitous in the plant kingdom, primarily imparting red and yellow colors to flowers, roots, and fruits. These compounds are localized in the chloroplasts and chromoplasts and impart bright hues to the peel and juice sacs of citrus fruits. The bright orange color of mandarin, deep yellow color of sweet oranges and red color of grapefruits are due to the occurrence of different types and amounts of carotenoids. Carotenoids are synthesized through the 2-C-methyl-d-erythritol 4-phosphate (MEP) pathway in plastids, and through the mevalonate (MVA) pathway in the cytosol. Two key enzymes, 1-deoxy-d-xylulose-5-phosphate synthase and phytoene synthase, play a critical role in the biosynthesis of carotenoids. Carotenoids have important functions in photosynthesis and are precursors to hormones such as abscisic acid and strigolactone. Due to their coloration, these compounds help to attract insects for pollen distribution and seed dispersal. Lycopene and β-carotene are the two major red carotenoids present in red grapefruits. The absence of these compounds from white grapefruits results in the various hues of the juice vesicles. Other carotenoids present in mandarins and oranges are β-cryptoxanthin, lutein, luteoxanthin, neochrome, ξ-carotene, α-cryptoxanthin, C_{30} apocarotenoid β-citraurin, and violaxanthin. During fruit ripening the green chlorophyll components degrade and the red-yellow carotenoids accumulate. Consumers prefer more brightly colored citrus fruits, as they associate color with maturity. Commercial citrus packing houses induce this color change by treating the fruits with ethylene, which accelerates the degradation of chlorophylls and the accumulation of carotenoids. These compounds are also influenced by thermal processing and degrade rapidly at high temperatures. Storage of citrus juices at cool temperatures helps maintain carotenoid levels. Recent advances in processing techniques such as non-thermal high-pressure processing and electron beam irradiation have been suggested to maintain the levels of carotenoids in citrus juices.

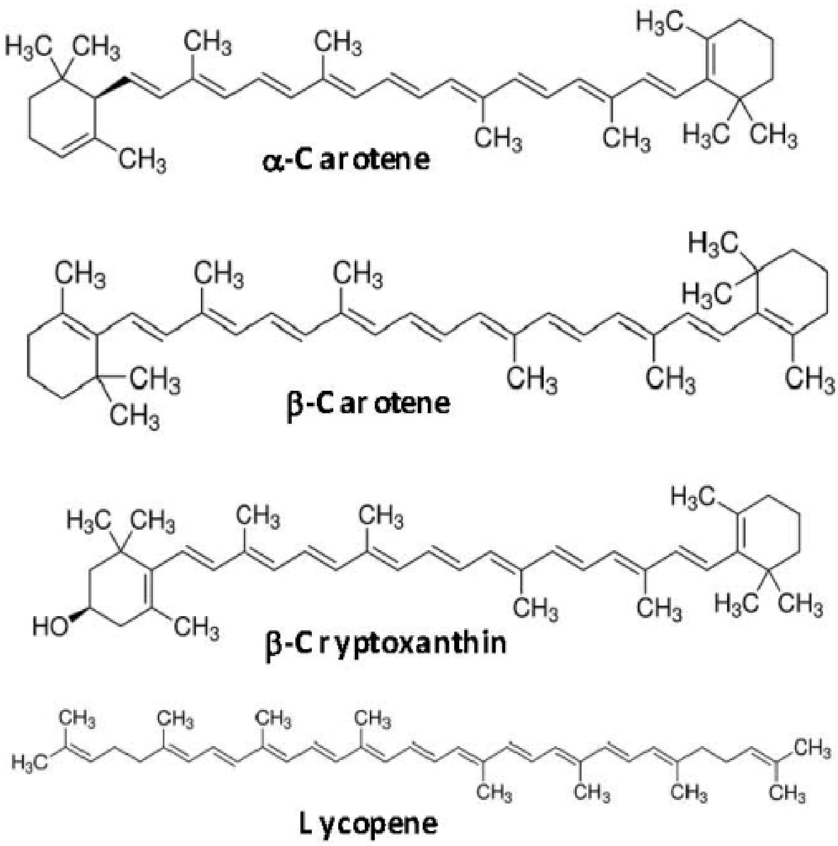

Figure 3. Structures of common carotenoids found in Citrus fruits

Carotenoids are light-sensitive and easily degrade under exposure to light. They are lipophilic compounds and can be extracted using organic solvents such as hexane, chloroform and ethyl acetate. Hexane is the most commonly used solvent due to its high affinity for carotenoids. Exposure to oxygen and elevated temperatures significantly affect the stability of carotenoids due to their oxidative degradation. Supplementing the raw material with antioxidants such as α-tocopherol and butylated hydroxyl toluene helps to reduce the degradation of carotenoids during extraction. In recent years, several advances have been made in the efficient extraction of carotenoids. Supercritical carbon dioxide extraction and enzymatic treatments such as cellulases, hemicellulases, glucoamylases and pectinases help in obtaining extracts rich in carotenoids.

Carotenoids can be quantified using several spectral techniques, including spectrophotometry, HPLC-with ultraviolet (UV)-visible detection, LC-MS, gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR). Total carotenoids can be estimated using the average molar absorption coefficient and recording the absorption at λ_{450} nm using a spectrophotometer. This technique is useful for rapid and economical estimation of total carotenoids, but it does not distinguish individual carotenoids. HPLC has been widely used for separation and quantification of carotenoids. However, separation of geometric and positional isomers common in carotenoids is challenging. Stationary phases such as C_{30} bonded silica based reversed-phase column are widely used to resolve the co-elution of these compounds. Other stationary phases for separation of carotenoids include C_{18} and C_{30}. Mass spectrometry in tandem with liquid chromatography separation provides a vital means to identify the separated compounds. The selective reaction monitoring tool in mass spectroscopy helps for structural conformation and accurate identification of carotenoids. Although GC-MS has not been extensively used for quantification of carotenoids, it could also be used for analysis of carotenoids. Most recently, LC-NMR has also been applied to analysis of carotenoids in different sample matrices. This method provides a means for unambiguous identification and structural confirmation of the carotenoids in juice samples.

2.3. Amines

Amines also known as proto-alkaloids are present in majority of citrus species, except red grapefruits. The commonly occurring amines in citrus are tyramine, octopamine, and synephrine (Figure 4). They are biosynthesized from tyrosine by tyrosine decarboxylase. These compounds were first studied in the leaves of citrus species. They are also present in other plants such as Evodia, in the family Rutaceae. In addition to monoamines, citrus fruits also contain polyamines (which contain two or more amino groups) such as putrescine, spermidine, spermine, agmatine and cadaverine. Among the commonly consumed citrus fruits, Clementine mandarins have the highest levels of synephrine (114.81 µg/ml), while oranges and lemons have moderate levels, 2-85 µg/ml of juice. Putrescine, spermidine and spermine are the predominant and most commonly occurring polyamines, and are present in free as well as conjugated form (Figure 4). They are predominantly localized in the reproductive organs where they promote somatic embryogenesis. The exogenous application of polyamines improves fruit set, yield and quality of sweet oranges. Their concentration increases throughout the development of flower buds and fruit-setting. Polyamines also play an important role in stress reduction caused by chilling injury, by balancing anion concentrations.

Their wide ranges of biological functions have led to the development of methods for analysis of amines in fruits and fruit products. Amines dissolve in polar solvents and these alkaloids can be extracted from citrus fruit juices. Water is the optimum solvent for extraction of amines and HPLC is the most common method of choice for quantification. A common method of extraction of amines is to dilute the fruit juice with water and subject the sample mixture to homogenization and centrifugation, followed by separation of the supernatant with the extracted amines. Amines can be further separated using a C_{18} stationary phase with detection at λ_{223} nm. Solvent modifiers such as phosphoric acid, formic acid, or sodium dodecyl sulfate have been used to achieve better separations of these compounds. Other methods for analysis of amines include capillary electrophoresis, column-switching cation-exchange liquid chromatography with scanning-wavelength UV and fluorescence detection, ion-pairing liquid chromatography (LC), ion-exchange chromatography, and mixed-mode reverse phase/ion-pairing LC with UV detection. Electrospray mass spectrometry has also been used for analysis of amines.

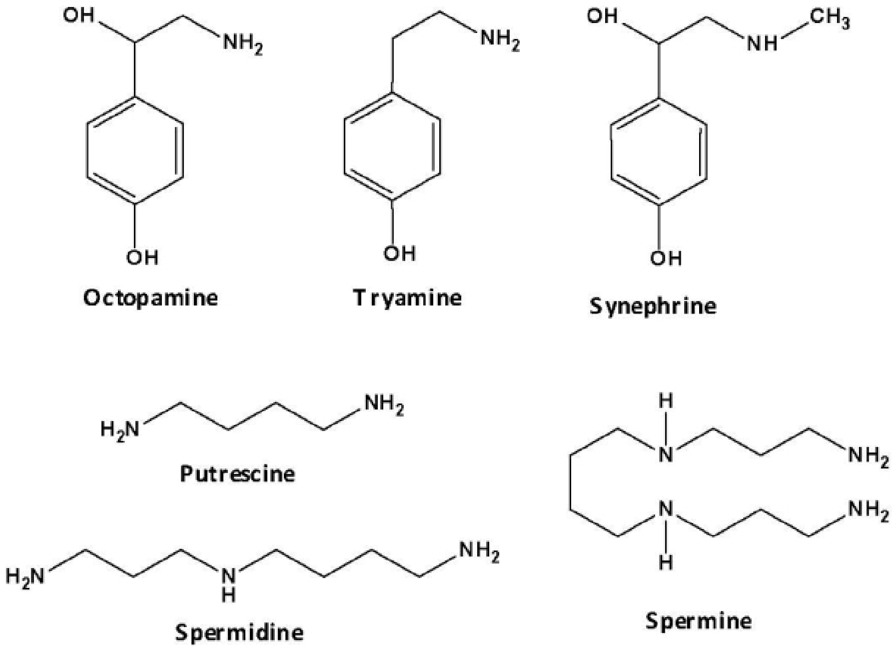

Figure 4. Structures of amines and polyamines reported in most citrus fruits.

2.4. Flavonoids

Flavonoids are a group of polyphenolic compounds. The major and most common chemical structures are shown in Figure 5. The compounds were considered vital nutrient and referred as Vitamin P; however subsequent research showed that they are non-essential leading to their current nomenclature as non-essential health-promoting compounds. The word flavonoid is derived from the Latin word ‘flavus’, which means yellow. Flavonoids are ubiquitous in nature and are present in several fruits and vegetables. Approximately 4000 flavonoids have been reported from the plant kingdom. The flavonoids are broadly classified into flavone, flavonol, flavanone and flavanonol based on structural variations. The basic structure of flavanone consists of two aromatic rings (designated as A and B), connected through a dihydropyrone ring. They occur as glycosyl derivatives with sugar moieties such as D-glucose and L-rhamnose. These sugar moieties are usually *O*-glycosides that are typically bound to the aglycone hydroxyl group at C-7 or C-3. Flavonoids are synthesized through the phenylpropanoid and MVA pathways and occur as aglycones, glucosides and methylated forms (polymethoxyflavones). Citrus fruits primarily contain flavanones, with naringin and hesperidin being the predominant forms, which selectively occur in different species of citrus. Flavonoids play an important role in the flavor of citrus juice. The unique bitter tangy taste of grapefruits is due to the presence of naringin. Oranges and mandarins contain neutral-tasting hesperidin, which has the sugar rutinose (6-*O*-α-L-rhamnosyl-D-glucose). Other glycosides such as neo-hesperidin, narirutin, eriocitrin, neo-eriocitrin, didymin and poncirin occur in lower quantities, ranging from <0.1 mg – 6 mg/ 100g fresh juice weight.

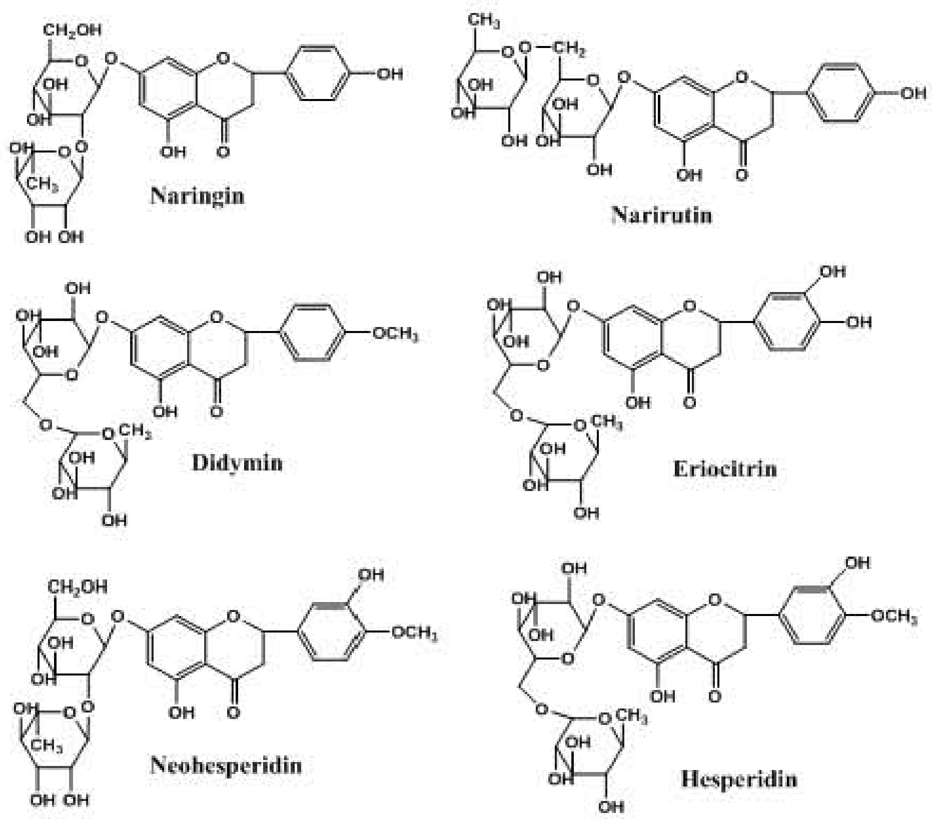

Figure 5. Structures of most commonly found flavonone glycosides in citrus.

Citrus flavonoids also occur in methylated form containing two or more methoxyl groups, known as polymethoxyflavones (PMFs) (Figure 6). Approximately 25 PMFs have been reported from various citrus species (Uckoo, et al, 2012). Tangeretin (5,6,7,8,4′-pentamethoxy flavone), heptamethoxy flavone (3,5,6,7,8,3',4'-heptamethoxyl flavone), nobiletin (5,6,7,8,3,4'-hexamethoxy flavone), tetramethoxy flavone (5,6,7,4'-tetramethoxy flavone) and sinensitin (5,6,7,3',4'-pentamethoxy flavone) are the predominant PMFs and vary in concentration from species to species. PMFs are synthesized through the methoxylation of flavone or flavanone aglycones. They are present in both the vegetative and reproductive parts, such as leaves and fruits including peel and juice.

-

-

-

Bibliography

1. Ranganna, S.; Govindarajan, V.; Ramana, K.; Kefford, J., (1983) Citrus fruits—Varieties, chemistry, technology, and quality evaluation. Part II. Chemistry, technology, and quality evaluation. A. Chemistry. *Critical Reviews in Food Science and Nutrition*, 18, 313-386. [A comprehensive discussion on citrus quality and chemistry.]

2. Giovannoni, J. J., (2007) Completing a pathway to plant vitamin C synthesis. *Proceedings of the National Academy of Sciences,* 104, 9109-9110.[A comprehensive review of vitamin C biosynthetic pathway in plants.]

3. Ikotun, T.; Faboya, O.; Umoh, S. O., (1988) Oxalic acid content of healthy and infected citrus fruits in Nigeria. *Food / Nahrung*, 32, 335-341. [This paper describes the increase in oxalic content in infected fruits.]

4. Uckoo R. M.; Jayaprakasha, G. K.; Bhimanagouda, S. P., (2012) Chromatographic Techniques for the Separation of Polymethoxyflavones from Citrus. In *Emerging Trends in Dietary Components for Preventing and Combating Disease*, American Chemical Society; Vol. 1093, pp 3-15. [A comprehensive review describing purification methods of polymethoxyflavones from citrus.]

5. Banthorpe, D. V.; Charlwood, B. V.; Francis, M. J. O., (1972) Biosynthesis of monoterpenes. *Chem. Rev*., 72, 115-155. [A comprehensive discussion on monoterpene biosynthesis.]

6. Lucker, J.; El Tamer, M. K.; Schwab, W.; Verstappen, F. W. A.; van der Plas, L. H. W.; Bouwmeester, H. J.; Verhoeven, H. A., (2002) Monoterpene biosynthesis in lemon (Citrus limon). *Eu. J. Biochem*., 269, 3160-3171. [This paper identified the genes encoding monoterpene synthases in lemon.]

7. Attaway, J. A.; Pieringer, A. P.; Barabas, L. J., (1966) The origin of citrus flavor components-I : The analysis of citrus leaf oils using gas-liquid chromatography, thin-layer chromatography, and mass spectrometry. *Phytochemistry*, 5, 141-151.[This presents an analysis of steam ditilled citrus leaf oils.]

8. Rodríguez, A.; Alquézar, B.; Peña, L., (2013) Fruit aromas in mature fleshy fruits as signals of readiness for predation and seed dispersal. *New Phytologist,* 197, 36-48.[A comprehensive discussion on the role of plant volatile oils in fruit dispersion.]

9. Jinhee, K.; Jayaprakasha, G. K.; Amit, V.; Bhimanagouda, S. P., (2012) Cancer Chemopreventive Properties of Citrus Limonoids. In Emerging Trends in Dietary Components for Preventing and Combating Disease, *American Chemical Society*; Vol. 1093, pp 37-50, references cited therein. [A comprehensive discussion on anti-cancer properties of citrus limonoids.]

10. Jayaprakasha, G. K.; Patil, B. S.; Bhat, N. G. (2006) A process for isolation of limonin glucoside and Isoobacunoic acid glucoside from citrus seeds. USA patent Application # 60/74437. Application # 60/744372, 4th April 2006.[Patented method describing two step isolation of limonoids.]

11. Shen, Y.; Sun, Y.; Qiao, L.; Chen, J.; Liu, D.; Ye, X., (2013) Effect of UV-C treatments on phenolic compounds and antioxidant capacity of minimally processed Satsuma mandarin during refrigerated storage. *Postharvest Biol. Technol.*, 76, 50-57.[This paper describes a method to preserve postharvest quality of citrus fruits.]

12. Hertog, M. G.; Feskens, E. J.; Kromhout, D.; Hollman, P.; Katan, M., (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *The Lancet*, 342, 1007-1011.[This study reports the benefit of flavonoids rich diet in prevention of coronary heart disease in elderly people between the age group of 65-84 years.]

13. Poulose, S.; Harris, E.; Patil, B., (2005) Citrus limonoids induce apoptosis in human neuroblastoma cells and have radical scavenging activity. *J Nutr,* 135, 870 - 877. [This paper presents apoptosis induction as a contributing factor for anti-cancer activity of citrus limonoids.]

14. Vikram A, Jesudhasan PR, Jayaprakasha GK, Pillai SD, Patil BS., (2010) Grapefruit bioactive limonoids modulate *E. coli* O157:H7 TTSS and biofilm. Int. J. Food Microbiol. 140, 109-116. [This paper describes the anti-quorum sensing activity of citrus limonoids.]

15. Poulose, S.; Harris, E.; Patil, B., (2006) Antiproliferative effects of citrus limonoids against human neuroblastoma and colonic adenocarcinoma cells. *Nut Cancer*, 56, 103 - 112. [Study explains the role of citrus limonoids in inhibition of proliferation of colon and neuroblastoma cells].

16. Tian, Q.; Miller, E. M.; Ahmad, H.; Tang, L.; Patil, B. S., (2001) Differential inhibition of human cancer cell proliferation by citrus limonoids. *Nut Cancer,* 40, 180-184. [The study explains the role of major limonoids found in *Citrus reticulata* Blanco on proliferation of different human cancer cells such as leukemia (HL-60), ovary (SKOV-3), cervix (HeLa), stomach (NCI-SNU-1), liver (Hep G2), and breast (MCF-7)].

17. Gutherie, N.; Morley, K.; Hasegawa, S.; Manners, G. D.; Vandenberg, T., (2000) Inhibition of human breast cancer cells by citrus limonoids. In *Citrus limonoids functional chemicals in agriculture and foods*, Berhow, M.; Hasegawa, S.; Manners, G., Eds. American chemical society: Washington DC; pp 164-174. [The study attempts to understand how some of the major limonoids of citrus fruits are useful in inhibiting proliferation of both estrogen dependent ad independent breast cancer cells].

18. Kim, J.; Jayaprakasha, G. K.; Patil, B. S., (2013) Limonoids and their anti-proliferative and anti-aromatase properties in human breast cancer cells. *Food & function,* 4, 258-265. [This paper describes the anti-aromatase activity as contributory mechanism for anti-proliferative activity of citrus limonoids].

19. El-Readi, M. Z.; Hamdan, D.; Farrag, N.; El-Shazly, A.; Wink, M., (2010) Inhibition of P-glycoprotein activity by limonin and other secondary metabolites from Citrus species in human colon and leukaemia cell lines. *Eur. J. Pharmacol*., 626, 139-145. [P-glycoprotein inhibitory activity of citrus limonoids is described.]

20. Vanamala, J.; Leonardi, T.; Patil, B. S.; Taddeo, S. S.; Murphy, M. E.; Pike, L. M.; Chapkin, R. S.; Lupton, J. R.; Turner, N. D., (2006) Suppression of colon carcinogenesis by bioactive compounds in grapefruit. *Carcinogenesis*, 27, 1257-1265. [This paper describes anti-proliferative effect of citrus limonoids in mice model.]

21. Lam, L. K. T.; Li, Y.; Hasegawa, S., (1989) Effects of citrus limonoids on glutathione S-transferase activity in mice. *J. Agric. Food Chem*., 37, 878-880. [The article explains how the limonoids from cross helps in activation of phase-II metabolizing enzymes in mice. These enzymes are responsible for removal of toxins and other metabolites known to cause cancer].

22. Kelly, C.; Jewell, C.; O'Brien, N. M., (2003) The effect of dietary supplementation with the citrus limonoids, limonin and nomilin on xenobiotic-metabolizing enzymes in the liver and small intestine of the rat. *Nutr. Res*., 23, 681-690. [The paper describes the in vivo induction of glutathione-S-transferase by citrus limonoids.]

23. Tanaka, T.; Maeda, M.; Kohno, H.; Murakami, M.; Kagami, S.; Miyake, M.; Wada, K., (2001) Inhibition of azoxymethane-induced colon carcinogenesis in male F344 rats by the citrus limonoids obacunone and limonin. *Carcinogenesis*, 22, 193-198.[This paper describes in vivo anti-proliferative activity of citrus limonoids.]

24. Murthy, K. N. C.; Jayaprakasha, G. K.; Kumar, V.; Rathore, K. S.; Patil, B. S., (2011a) Citrus limonin and its glucoside inhibit colon adenocarcinoma cell proliferation through apoptosis. *J. Agric. Food Chem*., 59, 2314-2323 [This paper presents apoptosis induction as a contributing factor for anti-proliferative activity of citrus limonoids.]

25. Jayaprakasha, G. K.; Jadegoud, Y.; Nagana Gowda, G. A.; Patil, B. S., (2010) Bioactive compounds from sour orange inhibit colon cancer cell proliferation and induce cell cycle arrest. *Journal of Agricultural and Food Chemistry*, 58, 180-186. [This paper presents apoptosis induction as a contributing factor for anti-proliferative activity of citrus limonoids.]

26. Kim, J.; Jayaprakasha, G. K.; Vikram, A.; Patil, B. S., (2012) Methyl nomilinate from citrus can modulate cell cycle regulators to induce cytotoxicity in human colon cancer (SW480) cells in vitro. *Toxicology in Vitro*, 26, 1216-1223, PMID: 22728232. [This study explain how one of the compounds from citrus namely, methyl nomilinate can alter cell cycle and induce programmed cell death in human colon cancer cell lines].

27. Lam, L.; Hasegawa, S.; Bergstrom, C.; Lam, S.; Kenney, P., (2000) Limonin and nomilin inhibitory effects on chemical-induced tumorigenesis. *ACS Publications*; Vol. 758, p 185-200. [This describes anti-proliferative activity of citrus limonoids.]

28. Murthy, K. N. C.; Jayaprakasha, G. K.; Patil, B. S., (2011b) Obacunone and obacunone glucoside inhibit human colon cancer (SW- 480) cells by the induction of apoptosis, *Food and Chemical Toxicology*, 49, 1616–1625. [This paper presents apoptosis induction as a contributing factor for anti-cancer activity of citrus limonoids.]

29. Jayaprakasha, G. K.; Murthy, K. N. C.; Uckoo, R. M.; Patil, B. S., (2013) Chemical composition of volatile oil from *Citrus limettioides* and their inhibition of colon cancer cell proliferation. *Industrial Crops and Products*, 45, 200-207. [This describes anti-proliferative activity of citrus volatile oils.]

30. Murthy, K. N. C.; Jayaprakasha, G. K.; Patil, B. S., (2012) D-limonene rich volatile oil from blood oranges inhibits angiogenesis, metastasis and cell death in human colon cancer cells. *Life Sciences*, 91, 429-439, PMID: 22935404. [This explains the benefit of volatile oils from blood orange in prevention of colon cancer through multiple mechanisms].

31. Borradaile, N.; Carroll, K.; Kurowska, E., (1999) Regulation of HepG2 cell apolipoprotein B metabolism by the citrus flavanones hesperetin and naringenin. *Lipids*, 34, 591-598. [The study shows how citrus flavonoids namely, hesperetin and naringenin can help in regulation of liver cancer cells growth through metabolism of a lipoprotein].

32. Patil, J. R.; Jayaprakasha, G. K.; Murthy, K. N. C.; Chetti, M. B.; Patil, B. S., (2010) Characterization of *Citrus aurantifolia* bioactive compounds and their inhibition of human pancreatic cancer cells through apoptosis. *Microchem*. J., 94, 108-117. [The study shows how different phytochemicals in lemon will help in inhibition of pancreatic cancer cells].

33. Jayaprakasha, G. K.; Murthy, K. N. C.; Demarais, R.; Patil, B. S., (2012) Inhibition of Prostate Cancer (LNCaP) Cell Proliferation by Volatile Components from Nagami Kumquats. *Planta Medica*, 78, 974-980. [Results of this study show chemical composition of volatile principles from Kumquat and how these volatiles can inhibit prostate cancer cells].

34. Miller, E.; Gonzales-Sanders, A.; Couvillon, A.; Binnie, W.; Hasegawa, S.; Lam, L., (1994) Citrus limonoids as inhibitors of oral carcinogenesis. *Food Technol*. 48, 110-114. [This study explains how the terpenoids of citrus inhibit initiation of tumor in oral cavity]

35. Lam, L.; Hasegawa, S., (1989) Inhibition of benzo[a]pyrene-induced forestomach neoplasia in mice by citrus limonoids. *Nutr Cancer,* 12, 43 - 37. [Study showed how citrus bioactive compounds can inhibit chemically induced forestomach cancer].

Biographical Sketches

Amit Vikram is Postdoctoral Research Associate at University of Pittsburgh. He earned his PhD from Vegetable and Fruit Improvement Center, Texas A&M University. His research is focused on the role of phytochemicals and natural products in shaping enteric pathogen infections and microbiome. He has published more than 25 papers and book chapters on the role of phytochemicals and microbiome. He discovered that phytochemicals from edible plants like citrus can signal to microbes, telling them to switch their gene expression pattern, often times beneficial for human health. He also discovered that certain microbes produce small molecules to regulate their pathogenicity that can be utilized for benefit of mankind. Dr. Vikram received several awards for his research.

Ram M. Uckoo is a graduate from the Marathwada Agricultural University, India (2002). He received Master's degree (2006) in plant and soil science from Texas A&M University Kingsville, and PhD (2011) in Horticultural sciences from Texas A&M University, TX. Dr. Uckoo worked as Post-doctoral research Associate at the Vegetable and Fruit Improvement Center. Dr. Uckoo's research interests include studying phytochemicals in fruits and vegetables. His basic research includes studying the factors influencing the levels of the phytochemicals though pre and post-harvest factors such as cultivation practices, processing and storage. He has also developed innovative and advanced analytical and isolation methods of phytochemicals and has published several research papers in this field. He is an active member of the American Society of Horticultural Science and the Crop Science Society of America.

Kotamballi N. Chidambara Murthy is working as Principal Scientist and Head of translational research at MS Ramaiah Medical College and Hospitals, Bangalore, India. Dr. Murthy is pharmacist by training (MS in Pharmacy) and PhD in Biotechnology. He has over eight years of research experience along with one year of experience in teaching and pharmaceutical industry. He completed postdoctoral fellow at Texas A&M University. His research is focused on understanding the role of naturally derived molecules on prevention and treatment of chronic diseases. At present, he mainly involved on pre-clinical, clinical and molecular studies using *in vitro* models. Along with his research, he also trained undergraduates, postgraduate and faculty of medicine in molecular and cell biology research. Dr. Murthy has published over 75 original research papers with over 2000 citations. He edited two books published form American Chemical Society and Horticulture Society, contributed 15 national and international book chapters, authored 10 popular articles, presented more than 60 papers at National and International scientific meetings and delivered over 45 invited lectures including invited and plenary talks. He has filed US, European and Indian patents in the area of nutraceuticals and pharmaceuticals. He is recipient of several meritorious awards and fellowships from Indian as well as International organizations, which includes recently awarded Dr. SS Mishra Memorial award for 2013 by National Academy of Medical Sciences, for his scientific achievement. He is part of several research and scientific advisory committees of various academic institutions and industries.

Bhimu Patil is the Professor and Director of Vegetable and Fruit Improvement Center. His research focus is on multidisciplinary "Foods for Health" led to publish more than 135 peer reviewed research papers and received 15 awards. Drs. Patil and Yves Desjardin initiated an international symposium "FAVHealth" symposium in 2005, and hosted in Houston 2007. He has chaired or co-chaired 25 symposium. He was invited speaker, including plenary speaker, for his scientific research and educational excellence by several countries including China, South Korea, Brazil, Indonesia, Spain, Sweden, France, India, Canada, Portugal, and different states in the USA. He has developed two multi-disciplinary and multi state first-of-its kind course, "Science of Foods for Health" and "Phytochemicals in Fruits and Vegetables to Improve Human Health".

G.K. Jayaprakasha, has 25 years of research and 3 years of industrial experience focused on isolation of Natural Products from Fruits, Vegetables, Spices and Medicinal plants as well as semi-synthesis of bioactive lead natural products and evaluation of biological properties focused towards cancer prevention *in vitro* models. His research involves bioassay directed discovery, purification and chemical characterization of natural compounds using preliminary *in vitro* assays such as anticancer, antimicrobial and antioxidant properties. Isolated and characterized more than 80 novel, rare and bioactive compounds from Citrus, Pomegranate, Carrots, Cinnamon, Lichen, Turmeric and *Garcinia*. He filed 24 patents in US, Europe and India. He also published more than 140 research papers, reviews, book chapters and 130 presentations in national and international meetings. In addition, he co-edited three ACS symposium series books. Based on his research accomplishments, he has been admitted as "Fellow of Royal Society of Chemistry" (FRSC), Royal Society of Chemistry, Cambridge, England, Fellow of Agricultural Food Chemistry, American chemical society, Washington, and Fellow of Indian chemist, India.