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Citrus

Health Benefits and Production Technology

Edited by Muhammad Sajid and Amanullah

CITRUS - HEALTH BENEFITS AND PRODUCTION TECHNOLOGY

Edited by **Muhammad Sajid** and **Amanullah**

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Notice

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Meet the editors

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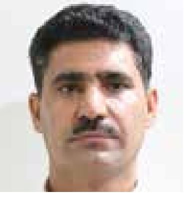

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Contents

Preface

Citrus is one of the world's major fruit crops, with global availability and popularity contributing to human diets. Characterized by the distinct aroma and delicious taste, citrus fruits have been recognized as an important food and an integral part of our daily diet, playing key roles in supplying energy and nutrients and in health promotion. The growth of the citrus industry, including rapid development of the processing technology of frozen concentrated orange juice, has greatly expanded with international trade and steadily increased consumption of citrus fruits and their products during the past several decades. About 30% of citrus fruits are processed to obtain various products, mainly juice.

Citrus fruits are the highest-value fruit crop in terms of international trade. There are two main markets for citrus fruit: the fresh fruit market and the processed citrus fruits market (mainly orange juice). Total production and consumption of citrus fruit has grown strongly since the 1980s. Current annual worldwide citrus production is estimated at over 70 million tons, with more than half of this being oranges. The rise in citrus production is mainly due to the increase in cultivation areas, improvements in transportation and packaging, rising incomes, and consumer preference for healthy foods. Citrus fruit growth and quality are dependent on climatic conditions, in addition to soil type, water availability, cultural practices, and nutrient supply.

Citrus comprises seven chapters that offer complete up-to-date information regarding citrus fruit biology, biotechnology, and quality. The book describes the basic and applied scientific information that serves to help students, researchers, marketers, nutritionists, etc. It also briefly explains the fruit morphology, anatomy, physiology and biochemistry, growth phases, maturity standards, grades, and physical and mechanical characteristics of citrus trees. This book also provides the foundation for understanding the growth, harvest, and post-harvest aspects of citrus fruits. Insect pests and diseases, irrigation, nutrition, and rootstocks are also addressed in this book.

We are thankful to all the authors who contributed their valuable chapters to this book. We are also extremely grateful to Jasna Božić of IntechOpen for helping us to publish the book in an excellent form in the shortest possible time. We owe our sincere thanks and lasting gratitude to our families whose consistent encouragement and love have been a tremendous impetus for the completion of this book.

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Section 1

Citrus Nutrition

Chapter 1

Citrus and Health

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Additional information is available at the end of the chapter

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Abstract

Citrus has been proposed as an interesting ingredient in the elaboration of food products as soft drinks due to its distinctive aroma and high nutritive value. It is a rich source of nutrients that contains higher amounts of vitamin C, citric acid, minerals, and flavonoids, especially flavanones and flavones (reaching values of 400–600 mg/L) and in lesser amounts flavonols and hydroxycinnamic acids. Citrus flavonoids decrease capillary permeability and are beneficial in the treatment of vascular diseases. Scientific studies suggest that the ingestion of food products based on citrus fruits improves the blood lipid profile, reduces oxidative stress, prevents atherogenic modifications of LDL and platelet aggregation, as well as contributes to the improvement of HDL levels. Other benefits attributed to citrus are antiaging, anticancer, neuroprotective, and antidiabetic. The present revision tries to empathize the most relevant studies regarding citrus and health.

Keywords: citrus, obesity, neurodegeneration, diabetes, cancer, cardiovascular diseases

1. Introduction

There are numerous evidences supporting the crucial influence of diet in the prevention of diseases related to oxidative and inflammatory processes. Citrus are one of the most important foods included in a healthy lifestyle, due to their composition in bioactive compounds. The biological activity of citrus bioactive compounds is mainly their free radical scavenging property, increasing the antioxidant activity which closely related to disease prevention.

Healthy properties of citrus have been linked to its high vitamin content C and flavonoids, mainly attributed to its antioxidant capacity. Citrus are considered adjuvant in the prevention of cardiovascular diseases and metabolic diseases such as obesity, diabetes mellitus or dyslipidemia, as well as certain types of cancer. In citrus (particularly lemon), more than 60 individual flavonoids have been identified.

2. Citrus and their role in different pathologies

The health benefits described with the consumption of these fruits are related to their complete profile on nutrients, including simple sugars, fiber, potassium, high contents of vitamin C and phytochemicals as flavonoids, particularly flavanones that may act synergistically. They are low in fat and proteins, ranging from 0.1 to 0.3 g and from 0.69 to 0.94 g/100 g fresh weight, respectively. Citrus are particularly rich in vitamin C (ascorbic acid), providing amounts in the range of 23–83 g/100 g fresh weight. Considering that the Recommended Dietary Allowance (RDA) is set at 75–80 mg and a medium-sized orange or grapefruit contains from 50 to 70 mg ascorbic acid, it is easy to provide the necessary quantities with these fruits in a daily dietary pattern [1].

Micronutrients are secondary metabolites synthesized in the plant as a defense mechanism against pathogens, parasites, or to protect from UV radiation. We find two main groups in citrus fruits: terpenes and flavanones.

Terpenes are present in the essential aromatic oil produced by cells in the flavedo, and the main compounds are limonene and citral (mixture of isomers geranial and neral) (**Figure 1**). These volatile substances contribute to the flavor of citrics; similar to the protection effect against biotic stress in plants, they have shown antimicrobial activities interesting for food preservation and medicinal purposes [2, 3].

Besides, citrus fruits are especially rich in the flavanones hesperetin, naringenin, and eriodictyol [4]. Flavanones have the characteristic 15-carbon backbone ring structure common in the flavonoids (C6—C3—C6), consisting of two aromatic rings linked by three carbon atoms in an oxygenated structure as pirane derivative [5]. In particular, flavanones have a further degree of oxidation, with a ketone group at position C-4 in C-ring.

These compounds are mainly found glycosylated, with a disaccharide linked by glycosidic bond; common positions are the hydroxyl groups of C3 and C7. The free form (aglycone) can render different flavanones, depending on the position and type of sugar linked. In this sense, grapefruit is abundant in narirutin and naringin, which are both heterosides from the aglycone naringenin, but the glucose moiety is different (rutinoside or neohesperoside, respectively). Orange is rich in hesperidin, that is the glycoside of hesperetin, while lemon is rich in eriocitrin that contains the aglycone eriodictyol [6].

Flavanones are not uniformly distributed in the fruit but are more abundant in the albedo. Because this part is discarded in juice processing, the level of flavanones is lower in citrus juices than in the whole fresh fruit [7]. In fact, levels of in orange fruit range between 35 and 147 mg/100 g of total flavanones and 44 and 106 mg/100 g of naringin and narirutin

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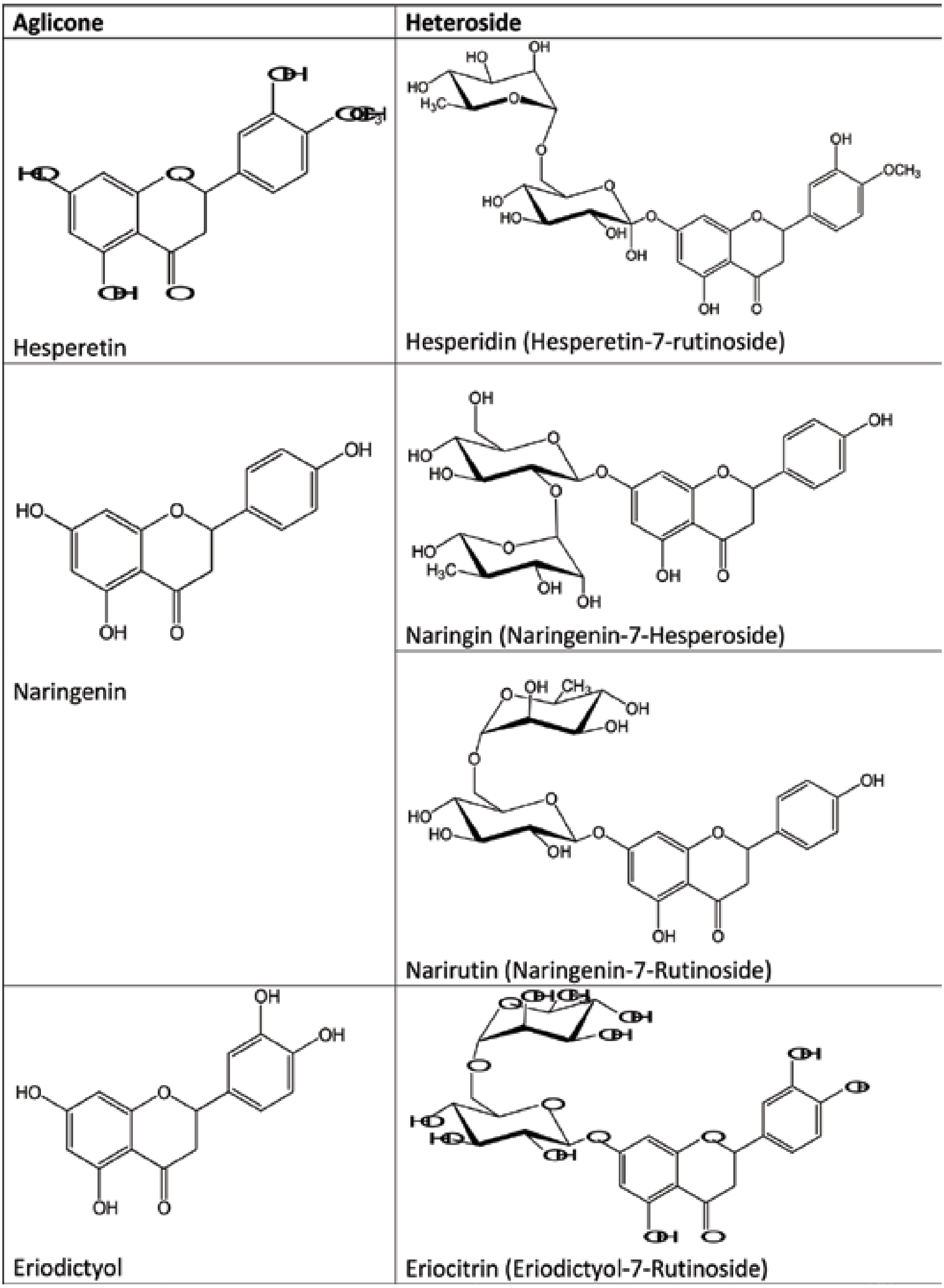

Figure 1. Chemical structures of the main terpenes present in citrus essential oil.

in grapefruit [8]. By contrast, orange and grapefruit juices showed values between 10 and 80 mg/100 g of hesperidin and narirutin [9] and naringenin [10]. Therefore, the pattern of consumption of these fruits greatly affects the flavanone total intake and further biological activities. Moreover, the sugar moiety modifies the in vivo pharmacokinetic properties of the compound. Aglycones are more easily absorbed than their heterosides counterparts, as glycosides are more hydrophilic and need active transport by proteins in gastrointestinal lumen and/or hydrolysis by gut microflora in order to be absorbed [11].

2.1. Citrus and cancer

Flavonoids are major compounds in citrus, and have been investigated since more than 20 years ago. Dietary flavonoids have showed to be able to exert chemopreventive or anticancer capacity [12]. Anticancer capacity of citrus flavonoids takes place through three main mechanisms: defense against DNA injury, inhibition of tumor growth, and inhibition of cell proliferation [13].

The best anticancer compound must exert the most possible inhibition of tumor growth or to able to destroy cancer cells, but origins minimum adverse health collateral effects [14]. Flavonoids are natural and considered innocuous and great compounds for the treatment of cancerous processes [15, 16]. The synthetic molecules that can be used for the treatment of cancer are extremely noxious, and can be able to destroy healthy cells. Due to the safe long-term consumption of flavonoids, and their innate biological activity, flavonoids can be considered as good applicants regarding cancer treatment. In fact, scientific literature has revealed cytotoxic effects of citrus flavonoids on cancer cells, with slightest adverse health effects.

That fact has led the research in order to implement flavonoid-based cancer treatments [17]. As other polyphenols, the presence of aromatic rings in flavonoids leads to pro- and antioxidant capacity that can be useful for chemotherapies [18]. Cancerous cells show an increment on oxidative stress, which leads to the possibility to be attacked by a substance that improves reactive oxygen species level as flavonoids do [19, 20]. As defined by Pacifico et al. [21], pro- or antioxidant capacity of citrus flavonoids is dependent on the concentration, type of cell, and culture condition (*in vitro* models).

Flavonoids exert DNA protection by their ability to absorb ultraviolet light. Some experiments on a UV-irradiated model of plasmidic DNA indicate protecting capacity of naringenin and rutin against UV-induced damage of DNA [22]. Indeed, naringin plays an important role in regulating antioxidative capacity by increasing superoxide dismutase and catalase activities and by upregulating the gene expressions of superoxide dismutase, catalase, and glutathione peroxidase in cholesterol-rich diet-fed rabbits [23].

Apart from UV protection, flavonoids can also diminish tumor promotion at the beginning of carcinogenesis by the intensification of the detoxification processes. In particular, citrus flavonoids inhibit ornithine decarboxylase induction of skin tumor promotion, activating protein kinase C [24, 25]. Miller et al. [26] studied the inhibition of oral carcinogenesis by citrus flavonoids in hamsters and the antineoplastic activity, concluding that hesperetin, neohesperetin, tangeretin, and nobiletin were ineffective, while naringin and naringenin gave good results.

Citrus flavonoids can inhibit invasion, by rat malignant cells, in cardiac and hepatic tissue of syngenetic rats [27]. Hydroxycinnamates, glycosylated flavonoids, and the polymethoxylated flavones have shown inhibitory activity on several tumoral cell line proliferations [13]. Other studies showed eriocitrin and its aglycone, eriodictyol, as potent inhibitors of lipoxygenases, which are involved in the biosynthesis of various bioregulators that are closely related to the pathogenesis of several diseases such as allergy and atherosclerosis and cancer [28]. Hesperidin in different citrus juices also showed antiproliferative activity [29], reporting lemon in particular potent antiproliferative activities on HepG2 human liver-cancer cell in a dose-dependent manner [30].

Also, the positive effect of vitamin C in reducing the incidence of stomach cancer has been studied, being most probably due to the inhibitory action in the generation of nitrous compounds by interrupting the reaction between nitrites and amine groups [31], although it has recently shown that this effect may be due to a cytotoxic effect of vitamin C on human gastric cancer cell line AGS [32]. Consistent protective effect of vitamin C has also been found in lung and colorectal cancer [33].

One stretched revision done by Turati [34] reported a diminution on cancers of the digestive tract and larynx regarding high intake of citrus. That effect was found to be due to the content on vitamin C, flavanones, and other compounds with antioxidant, antimutagenic, and antiproliferative properties [35]. Subjects consuming more than one portion of citrus fruit per week showed OR between 0·42 and 0·82 for oral cavity and pharyngeal cancer, esophageal cancer, stomach cancer, colorectal cancer, and laryngeal cancer. However, despite the good results obtained, no correlation was found for other neoplasms, including cancers of breast, ovary, endometrium, prostate, or kidney [35].

The most recent and huge research about citrus and cancer was published in 2016. An adaptive meta-analysis of cohort studies revealed that regular dietary intake of citrus prevents the development of gastric cancer, particularly cardia gastric cancer [36].

2.2. Citrus and cardiovascular diseases

Cardiovascular diseases (CVD) are one of the main causes of illness and death in Western countries, and cardiovascular drugs are the most commonly used medications. There are two types of factors involved in the development of CVD. Some factor can be modified, like life style, diet, environment, or smoking. Other cannot be modified: genetic factors, gender, history, or age. Atherosclerotic plaque formation is the most common phenomenon involved in CVD [37].

Consumption of citrus is inversely associated with incidence of CVD, due to the presence of bioactive compounds like flavonoids. Current research has focused on diet containing bioactive compounds, as an alternative to pharmaceutical medication. It can be concluded from the analysis of multiple studies that as the mean consumption of flavonoids increases, mortality due to CVD decreases. Epidemiological evidence of clinical and preclinical studies suggest that flavanones present in the citrus fruits positively influence cardiac and metabolic parameters, preventing CVD [38].

A study performed on approximately 70.000 women highlighted an inverse correlation between the intake of flavanones and the risk of suffering a cerebral ischemia, which is significantly different when contemplate women who consume high levels of flavanones [39]. Another recent meta-analysis study of three randomized clinical trials, including 233 patients, demonstrated a correlation between flavanones intake and a reduction in blood pressure [40].

Another recognized cardiovascular risk factor is metabolic syndrome, characterized by altered glucose metabolism, elevated blood pressure, dyslipidemia, and obesity. In 2016, a study on 10,000 subjects demonstrated an inverse association between polyphenols and metabolic syndrome, which was particularly significant in individuals with the highest intake of polyphenols [41].

Several studies carried out so far support a preventive role of citrus fruits on the main risk factors of CVD, such as hypertension, dyslipidemia, overweight, and hyperglycemia. Among CVDs, the effect of flavonoids on stroke is not clear. Mursu et al. [42] studied the association between intake of flavonoid and risk of stroke and mortality caused by stroke and concluded that a greater intake of flavonoids decreases the chances of ischemic stroke as well as mortality caused by CVD.

Chronic inflammation is caused by the excessive production of chemokines and cytokines. Cytokines and chemokines act as regulatory proteins under normal physiological conditions, but their excessive production disrupts the gradient balance and more reactive oxygen species (ROS) are produced. It has been shown that the grape flavonoids control chronic inflammation by reducing ROS level and by modulating pathways of inflammation. As flavonoids are natural compounds, they can target multiple steps in the inflammation pathway as compared to monotargeted synthetic anti-inflammatory drugs [43].

Atherosclerosis, characterized by the plaque formation in arteries, is one of the major factors contributing to incidence of stroke and myocardial infarction. It is caused by high level of lipoprotein and cholesterol in plasma [37]. High intake of citrus flavonoids reduces several risk factors for development of atherosclerosis including: high tolerance to glucose, maintaining good body mass index, and lowering blood pressure [44].

In another study, patients with metabolic syndrome had reduced cholesterol and ApoB due to the intake of a supplement of hesperidin for 3 weeks [45]. Furthermore, in a 2012 clinical study performed in our laboratory on patients with metabolic syndrome diagnosed, after 4 or 6 months drinking a citrus fruit juice, the glycemic profile was unchanged but the lipid profile improved, as observed by decrease in the cholesterol, LDL-C, and C-reactive protein [46].

Naringenin plays an important role to overcome the metabolic problem that is connected to dyslipidemia and resistance to insulin. It was shown to prevent atherosclerosis development in mice fed a high fat diet. Naringenin treatment attenuated the adverse effects caused by hyperinsulinemia and hyperlipidemia which was induced by western style diet. In mice that were fed a western diet, hyperlipidemia led to development of atherosclerosis in the aortic sinus evidenced by the development of plaque is that increased 10 times as compared to chow-fed animals. Naringenin treatment decreased the incidence of atherosclerosis by 70% [44].

A clinical study with 500 mg of naringin plus 800 mg of hesperidin did not show a significant improvement in the lipid profile in patients with moderate hypercholesterolemia. This study suggests that citrus flavonoids have no effect on LDL-C in humans, at least not when consumed in a capsule format [47]. A plausible explication of this results could be the inter-individuals variability of pharmacokinetic parameters. Despite preclinical results are clearer, further clinical studies need to be performed.

2.3. Citrus and diabetes

Diabetes is a chronic disease in which metabolic alterations of multiple etiologies characterized by chronic hyperglycemia and disorders in the metabolism of carbohydrates, fats, and proteins occur. These alterations are the result of defects in the secretion of insulin, in the action itself or in both. The long-term manifestation of insulin results in damage and dysfunction of various organs like nerves, kidneys, eyes, blood vessels, and heart. People living with diabetes have a higher risk of morbidity and mortality than the general population [48].

Diabetes is an important public health problem, one of four priority noncommunicable diseases (NCDs) targeted for action by world leaders. Both the number of cases and the prevalence of diabetes have been steadily increasing over the past few decades [49].

A recent report on diabetes by the World Health Organization estimates that 422 million cases in 2014 [49], and an expected number of nearly 650 million subjects in 2040 was estimated [48]. This dramatic rise is largely due to type 2 diabetes (T2D).

The treatment of diabetes consists of pharmacological, dietary, and lifestyle measures. Many trials have effectively tested different lifestyle and pharmacological intervention methods both in terms of prevention and treatment [50].

The use of plants with antidiabetic properties is widely known and described in the scientific literature. A lot of studies have reported that either plant parts or extracts of plant parts possess antidiabetic properties. This antidiabetic activity of plants is due to the presence of phytochemicals which are termed as flavonoids. In this way, several studies reported antidiabetic activities of flavonoids [51, 52].

Citrus fruits are one of the most consumed fruits mainly as fresh or raw materials for juices. Additionally, citrus fruits can also be used in the food, beverage, cosmetic, and pharmaceutical industries [53].

Citrus fruits show several bioactivities of vital importance to human health, like antioxidative and anti-inflammatory activity, cardiovascular protective effects, antidiabetic activity, among others. Citrus species contain a number of secondary metabolites, such as flavonoids, alkaloids, coumarins, limonoids, carotenoids, phenol acids, and essential oils [53]. Of all of them, flavonoids (especially flavanone, flavanonol, and methoxylated flavones) are more active compared to other secondary metabolites in citrus for their remarkable various bioactivities. There are a lot of studies where have been widely reported on plentiful bioactivities from flavonoids.

Flavonoids, a group of natural substances with variable phenolic structures, are well known for their beneficial effects on health. Flavonoids are now considered as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal, and cosmetic applications [54]. Flavonoids are distinct based on structural characteristics in the following six subclasses: flavonols, flavones, isoflavones, flavanones, anthocyanins, and flavanols (catechins and proanthocyanidins) [6]. In *Citrus* genus, flavanones comprise approximately 95% of the total flavonoids, and these foods are the main source of flavanones [6].

Citrus flavanones are glycosylated in vegetables. The same aglycone can be combined with several glycosides to give different flavanones; for example, the most representative flavanones in grapefruit are narirutin and naringin, those in orange fruit are hesperidin and narirutin, and that in lemon is eriocitrin [6]. Naringin, naringenin, nobiletin, narirutin, and hesperidin are the most important flavonoids thus far isolated from citrus fruits [35].

There has been a substantial body of evidence suggesting that oxidative stress is a key mechanism in pathogenesis of diabetes. Flavanones and flavanones-rich botanical extracts have been a subject of great interest for scientific research. Citrus flavanones like naringin and hesperidin exert a variety of biological activities such as antioxidant, anti-inflammatory, antihyperglycemic, antiapoptotic, etc. Naringin and hesperidin along with their respective aglycones, naringenin, and hesperetin have been shown to attenuate diabetes and its related complications [55]. In this way, Ashafaq et al. [56] demonstrated that hesperidin treatment significantly attenuated the altered levels of oxidative stress and neurotoxicity biomarkers. Their results demonstrate that hesperidin exhibits potent antioxidant and neuroprotective effects on the brain tissue against the diabetic oxidative damage in STZ-induced rodent model.

Iskuender et al. observed that after administration of hesperidin and quercetin in STZ-induced diabetic rats, glucose levels increased and liver and kidney damage markers decreased significantly [57]. In the same way, Akiyama et al. [50] demonstrated that hesperidin normalizes blood glucose by altering the activity of glucose-regulating enzymes, and lowering serum and liver lipid levels in STZ-induced marginal type 1 diabetic rats without any body weight loss due to STZ injection. Thus, hesperidin showed both hypoglycemic and hypolipidemic effects.

In a study, Gupta et al. [58] demonstrate the dipeptidyl peprtidase-4 (DPP-4) inhibition activity of citrus bioflavonoid nutraceuticals as compared to known gliptins (oral antidiabetic agents). The naringin and hesperidin compounds have the best individual activity in comparison to that of the gliptins. Natural gliptin-like alternatives may make these supplements a promising group of natural products for use in improving blood glucose levels in prediabetes and early stages of type 2 diabetes.

The hypoglycemic effect of naringin and naringenin is very well documented in animal and cell studies. So, naringin (30 mg/kg) and vitamin C (50 mg/kg) cotreatment ameliorated streptozotocin-induced diabetes in rats by improving insulin concentration and prevented oxidative stress [59]. Naringenin supplementation (0.2 g/kg of diet) improved glucose intolerance and insulin resistance in a model of high-fat-diet-fed mice [59]. More research is needed to determine the mechanism by which naringenin has hypoglycemic effect. So far, some authors have suggested the following: that is mediated via uptake of glucose in the skeletal muscle [60]; increased activities of hexokinase [61]; decreased production and expression of IL-1b, IL-6, and MCP-1 [62].

Rutin is another flavonoid present in citrus fruits to which many biological activities have been attributed, among them having antihyperglycemic properties. In 2017, Ghorbani [63] in a review discussed the antihyperglycemic property of rutin. Proposed mechanisms for this effect include a decrease of carbohydrates absorption from the small intestine, inhibition of tissue gluconeogenesis, an increase of tissue glucose uptake, stimulation of insulin secretion from beta cells, and protecting Langerhans islet against degeneration. Rutin also decreases the formation of sorbitol, reactive oxygen species, advanced glycation end product precursors, and inflammatory cytokines.

In conclusion, it can be affirmed that flavonoids are useful in the prevention and treatment of diabetes, especially in diabetes type 2, as Xu et al. [64] affirm the meta-analysis of prospective cohort studies carried out in 2018. Now, more studies are needed to elucidate the mechanism or mechanisms by which they carry out this antidiabetic activity.

2.4. Citrus and neurodegenerative diseases

Neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's disease represent rapidly growing causes of disability and death, which have profound economic and social implications; nonetheless, only few effective disease-modifying therapies are available for these diseases [65, 66].

Citrus flavonoids exert little adverse effect and have low or no cytotoxicity to healthy, normal cells. The main citrus flavonoids can also traverse the blood-brain barrier; hence, they are promising candidates for intervention in neurodegeneration and as constituents in brain foods [67].

Assessment of cognitive performance in middle-aged individuals has indicated that consumption of different polyphenols such as catechins, flavonols, and hydroxybenzoic acids is strongly associated with language and verbal memory. Hydroxycinnamates, phenolic acids, and phenolic alcohol are also capable of inducing neuroprotective effects in the same way as flavonoids [68].

Naringenin and hesperidin are abundant polyphenols in citrus fruits and have been shown to have protective effects in Huntington's disease due to their mechanism of nitric acid against 3-nitropropionic acid, which presents neurotoxicity in experimental models with rats [69].

5-Hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone (HHMF) from the *Citrus* genus and nobiletin, the most abundant polymethoxyflavone in orange peel extract are compounds that enhance neuronal survival and exerted prosurvival action in PC12 cells [70].

Ushikubo et al. [71] demonstrated that 3,3′,4′,5,5′-pentahydroxyflavone prevents Aβ fibril formation and that lowering fibril formation decreases Aβ-induced cell death in rat hippocampal neuronal cells. In another study, ursolic acid, *p*-coumaric acid, and gallic acid extracted from *Corni fructus* plant were shown to attenuate apoptotic features such as morphological nuclear changes, DNA fragmentation, and cell blebbing induced by Aβ peptide in PC12 cells [72].

The citrus flavanones hesperidin, hesperetin, and neohesperidin are known to exhibit antioxidant activities and could traverse the blood-brain barrier [73]. These authors showed that hesperetin, hesperidin, and neohesperidin inhibited the decrease of cell viability (MTT reduction), prevented membrane damage (LDH release), scavenged ROS formation, increased catalase activity, and attenuated the elevation of intracellular free Ca^{2+}, the decrease of mitochondrial membrane potential and the increase of caspase-3 activity in H_2O_2-induced PC12 cells. Meanwhile, hesperidin and hesperetin attenuated decreases of glutathione peroxidase and glutathione reductase activities and decreased DNA damage in H_2O_2-induced PC12 cells. These results first demonstrate that the citrus flavanones, such as hesperidin, hesperetin, and neohesperidin, even at physiological concentrations, have neuroprotective effects against H_2O_2-induced cytotoxicity in PC12 cells. These dietary antioxidants are potential candidates for use in the intervention for neurodegenerative diseases.

Antunes et al. [74] demonstrated that hesperidin (50 mg/kg) treatment was effective in preventing memory impairment in the Morris water maze test, as well as depressive-like behavior in the tail suspension test. Hesperidin attenuated the 6-OHDA-induced reduction in glutathione peroxidase and catalase activity, total reactive antioxidant potential, and the dopamine and its metabolite levels in the striatum of aged mice. This study demonstrated a protective effect of hesperidin on the neurotoxicity induced by 6-OHDA in aged mice, indicating that it could be useful as a therapy for the treatment of PD.

Chakraborty et al. [75] showed that hesperidin completely inhibits the amyloid fibril formation which is further supported by atomic force microscopy. Hesperidin exhibited moderate ABTS(+) radical scavenging assay but strong hydroxyl radical scavenging ability, as evident from DNA nicking assay.

3. Conclusions

The Mediterranean diet, considered a good example of a prudent and healthy diet, has undergone important changes in recent years. Factors such as urbanization, pollution, economic development, excessive working hours, and the adoption of inadequate lifestyles cause the population to be exposed to environmental and nutritional factors associated with the onset and progression of diseases related to aging. In this sense, citrus fruits are an important source of bioactive compounds, powerful antioxidants whose health benefits have been scientifically demonstrated in several studies for their protective role against oxidative damage. For this reason, the regular consumption of citrus fruits should be promoted as part of a varied and balanced diet. The absence of sufficient scientific evidence and validated tests to reliably measure the antioxidant activity in vivo of the bioactive compounds present in citrus justifies the need of interventional studies in humans for the correct determination of bioactive properties of citrus and their bioactive compounds.

Conflict of interest

Authors declare that they do not have conflict of interest.

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Chapter 2

Citrus: An Ancient Fruits of Promise for Health Benefits

Waseem Ahmed and Rafia Azmat

Additional information is available at the end of the chapter

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Abstract

Citrus is a group of fruit species, comprise an impressive list of additional vital nutrients, quite heterogeneous in many aspects and ancient times used to prevent and cure different diseases of the human body. It has a range of bioactive chemicals which is suitable for balanced diet and health. Citrus is one of the most important fruit in the world for health-related elements. Some species of the citrus act as a source of potential antioxidant showed prevention against heart diseases, anticancer, inflammation, antiviral, antibacterial and antifungal activity. Citrus fruit contained a higher level of flavonoids, terpenes, phytonutrients and range of phenolic compounds, vitamins C and carotenoids. These biochemicals are present in fruit rag, juice, seed and peel. The biocompounds present in citrus depend upon production, species to species. The aims and objectives of this chapter are to highlight the primary bioactive compounds in citrus and their role in controlling of diseases of a human.

Keywords: grapefruit, health related elements, bioactive compounds

1. Introduction

Citrus fruits are outstanding immune-enhancing source of vitamin C, belong to the family of Rutaceae, grown worldwide, including the members of Sweet oranges, Mandarins, Limes, Grapefruits, Lemons, and Citrons, etc. in which sweet oranges contribute almost 70% [1, 2]. Citrus fruits, being a perennial and tropical crop, subjected to significant seasonal variations of the climate changes during its growth and maturity periods [1]. Citrus fruits cultivated in more than 64 countries throughout the world [2] where annual production reaches 105.4 million tons among the fruit crops [3]. The main fruits crop in Pakistan is citrus which covers a prevalent area of cultivation [4] and acquired 12th position all over the world in citrus production with a landmark of 199,000 acres followed by overall yearly production of 2.36

million tonnes [2]. There four and five group of citrus fruits which include grapefruit, pummelo, sweet oranges, lemons and mandarins [3]. These citrus fruits are the precious resource of phytochemicals which are beneficial for the human body as vital bioactive medicines [5]. The last two to three decades, there has been a growing consciousness of diet and irrelevant diet causing different diseases in the human body [3]. Depending on the geographical area, growing season and harvesting time, these environmental conditions could be more or less limiting factors for bioactive compounds synthesis, accumulation, and formation [5]. Research programs initiated to improve the quality of citrus fruit with sound management practices [4, 5]. However, there appear to be slightly limited data available on the seasonal accumulation of bioactive compounds during their growing seasons [6]. Citrus has a unique value of essential nutrients, and these nutrients protect against several chronic diseases of the human body [5, 6]. It is utmost significant fruitlet of the biosphere after grapes and has marvelous economic, social and cultural impact on the society [4]. Bioactive compounds are the higher class of secondary plant metabolites which contained 9000 structures but popular and famous in citrus juice and its parts (rag, peel, seeds) [7]. Flavonoids and phenolic compounds derived from 2-phenylchromane commonly found in many vegetables, fruits, and especially citrus possess flavonoids, polyphenols, and antioxidants in a massive amount in different parts such as a peel, rags, juices and seeds [8]. Citrus has potential health benefits like antimicrobial, anti-inflammatory, antiviral and anticancer [9]. The world trend emphasis in natural bioactive compounds in plants and these compounds remain well known for their essential role in human health [10]. The maturity had a relationship with these bioactive compounds, and immature and early harvested fruits showed lower concentrations of these essential compounds [10, 11]. Maturity is a critical factor for responsible of its quality changes during growth and developmental stages of citrus health and full mature fruit was harvested [8, 9]. The abiotic stress involve the effect of the various quality parameters of citrus fruits [9, 10]. Citrus juice contains a lower amount of cholesterol that helps for diabetes patients [11]. Recently, the physical and chemical changes of pomegranate fruit have reported which showed that composition of minerals vary markedly among the three ripening stages [10]. The effects of maturity stages on change of bioactive compounds of berries were reported in [9].

2. Nature and occurrence of phytochemicals in citrus

Phytochemicals are naturally present in citrus juices, grouped into diverse chemicals and play a role in physiological functions and metabolic change of human body [18]. It comprises different phenolic compounds including the saponins, sulfides, phytosterols, carotenoids, monoterpenes, and inhibitors protease [10]. Phytochemicals play a vital role in mediating the plants against environmental stress through proactive compounds. These compounds act as signaling fascinating molecules provide protection and resistance against pathogenic diseases. In citrus, a wide range of phytochemicals and its distribution reported in its various parts [8]. These chemicals are helpful in different oxidative stress involved in the balanced and coordinate system to improve the human body. They have linked with a different range of enzymes and created a signal pathway with different organs and maintained the human metabolites [10, 11].

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2.1. Citrus nutritional functioning of phytochemicals

Fruit and vegetables are the best natural resource of phytochemicals which play a vital function in the prevention of infectious diseases [12]. These phytochemicals disclose the promising health effects in acting as antioxidants, controlling blood sugar and blood pressure [5]. Citrus phytochemicals also exhibit the antibacterial, antiviral, antifungal, anti-carcinogenic, antithrombotic or anti-inflammatory properties followed by cholesterol-lowering assets [11]. These active compounds are phenols, carotenoids, phytoestrogen and sulfides having antioxidative potential, and marked as health promotor due to their broad spectrum in the human body [10–12]. The purpose of perspective and analysis of extraction of essential compounds of citrus as presented in **Figure 1**.

2.2. Citrus phytochemical classes and its distribution in various parts

2.2.1. Flavonoids

Flavonoids are leading miscellaneous assembly of phytonutrients to present virtually almost in all fruits and vegetable with more than 6000 types [13]. The flavonoids basic structure of comprises of a frame of diphenyl propane, having two aromatic benzene (ring A and B) allied through a three carbon chain which forms a closed pyran ring using benzene A ring [15]. Consequently, their structure is also denoted as C_6-C_3-C_6 [16]. They are classified as flavanones, flavones, and flavonols, in which more than 60 individual flavonoids identifies in citrus now [14]. They are present in the form of the glycoside or aglycone, especially in citrus juices as glycosyl derivatives (flavonoid glycosides, FGs) which showed potential health benefits for human body [3]. Glycoside forms consist of two types of di-glycosides, L-rhamnosyl-glucosyl derivatives, which are classified as neohesperidosides and rutinosides, connected through 1,2 or 1,6 inter glycosidic bond, respectively. It had excellent potential to control

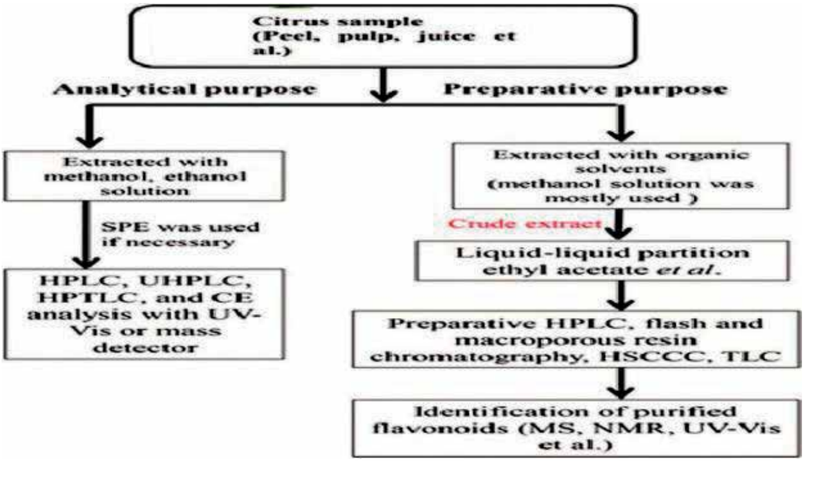

Figure 1. The schematic flow of analytical techniques for isolation of health-related elements.

many chronic diseases and suppressed the infectious in the body [13, 14]. The classification of flavonoids showed in **Figure 2**. The exaction methods and bioactivity of significant citrus compounds are shown in **Figure 3**.

2.2.2. Glucosinolates

The significant phytochemicals group is sulfur compounds which are present in higher quantity in seeds and peel of citrus, converted into isothiocyanates and showed properties of anti-infective followed to improve mucus. They also help in cancer control in the human body [14, 15].

2.2.3. Essential oil

Mostly citrus species have a rich source of aromatic compounds; have more than 400 compounds with volatile and nonvolatile compounds [16, 17]. Citrus peel is a rich source of essential oil and used as more than thousands of medicine, these compounds used in various cosmetics, the pharmacy-related industry. Moreover, 10 kg of citrus peel produced an essential oil of 1 ml, showed the properties of antispasmodic and antimicrobial activity [14].

2.3. Mucilage

Mucilage is present in seed, peel and rag of citrus species; it is a fiber-like and forms a gel-like structure which mixed with water [17]. The seeds of citrus have psyllium, with improving the digestives system, improving the functioning of the intestines and facilitating the elimination of cholesterol [18].

2.3.1. Tannins

The grapefruit, lemon and lime are rich source of tannins compounds, they also have the ability to stop diarrhea and reduce bleeding and controls other excessive secretions of body [19].

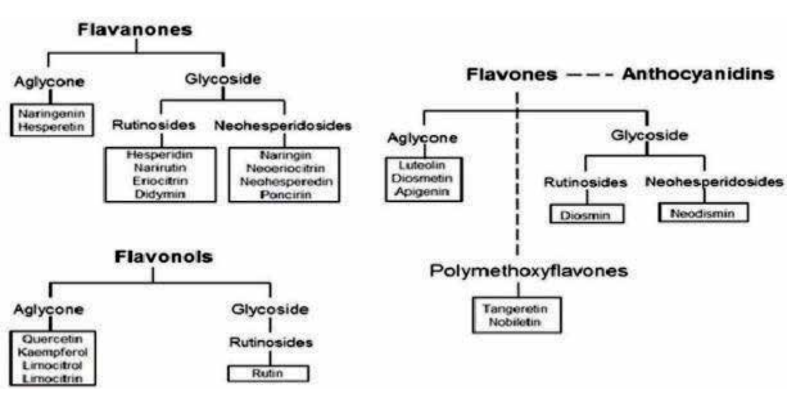

Figure 2. The major classification of health-related elements in citrus.

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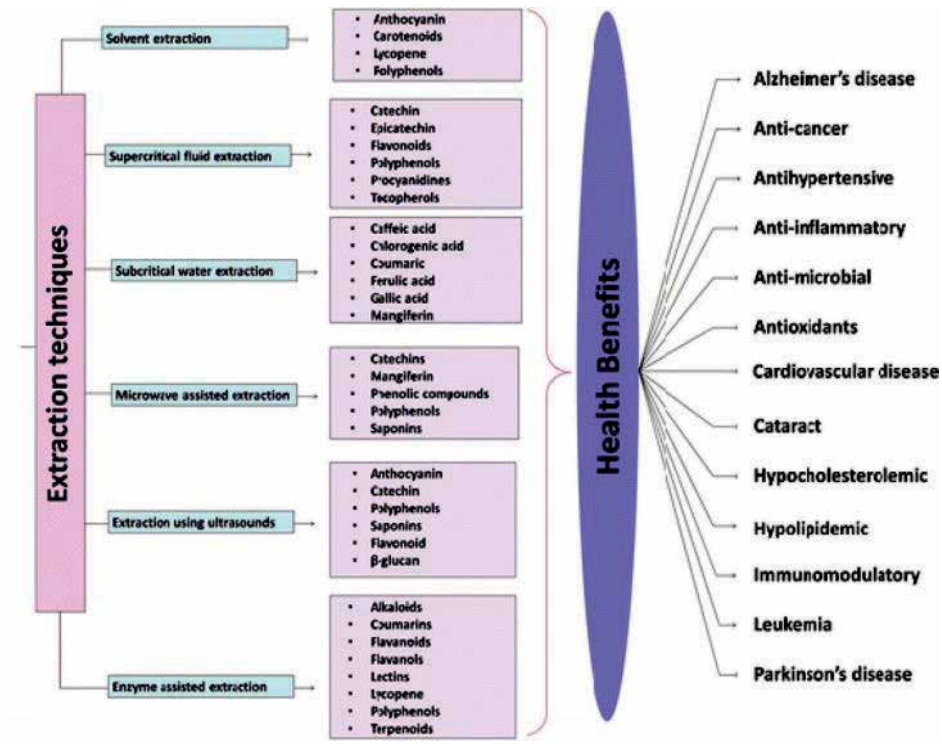

Figure 3. Various extraction methods of bioactive compounds in citrus and their impact on health.

2.4. Role of phytonutrients in the human body

Naturally, the citrus pulp comprised of 85–90% water and more than 300 compounds [13]. The composition of citrus fruits varies with the nature of species, cultivar, agro-climatic conditions, cultivation practices and rootstocks [12]. The role of citrus bioactive compounds is ancient and traditionally used in the early twentieth century [13]. The citrus flavonoids were found to help in capillary fragility. A detailed literature search indicates a positive correlation between consumption of citrus fruits and reduced incidence of ischemic stroke [14]. Flavonoids, terpenoids, carotenoids, and coumarins as primary and, secondary metabolites accumulated in edible as well as inedible parts of citrus fruit tissues [14]. Monoterpenes are the principal components of essential oil and contribute to aroma and flavor of citrus. Many of these monoterpenes are believed to exhibit health beneficial properties owing to their antioxidant and anti-cancer activities [20]. However, the recent enthusiasm about health beneficial properties of citrus centered on the flavonoids and the limonoids. The dietary fiber, carotenoids, saponins, protease, indole, and isoflavones are found in the citrus fruit. While the juice of citrus categorized through their distinctive odor, attractive perception and accepted as an essential diet and act as an essential part of natural nutrition, display central characters in providing energy and nutrients for health advancement. Citrus well recognized due to its low protein and fats, supply sucrose, glucose, and fructose in the form of carbohydrates [13].

Citrus is a good resource of nutritional integrity, which linked with lowering cholesterol and prevention of gastrointestinal diseases. Citrus juice is the excellent resource of Vitamin C followed by B vitamins including thiamin, pyridoxine, niacin, riboflavin, pantothenic acid, and folate [15]. The citrus fruits help in reducing the risk of many chronic diseases through significant phytochemicals like carotenoids, flavonoids, and limonoids [21].

2.5. Citrus phytonutrient role in the human body

The citrus fruit contained a massive amount of minerals and vitamins. In sweet oranges, the range of phytonutrients more as compares to limes, similar to the higher range of phytonutrient found in grapefruit and lemons. The naturally occurring folate and water-soluble vitamins in citrus can act as a coenzyme and involve in many biological process methylation, repair of DNA cell division growth and development of new cells. Usually, citrus is consumed in the form of the fresh juice while the vital phytonutrients showed in **Table 1**.

2.6. The major carotenoids in citrus fruits

The primary role of carotenoids pigment compounds to protect the various diseases of the human body and control-health-related elements; sweet orange, mandarin's and grapefruits are a rich source of carotenoids. Lutein and zeaxanthin are rich sources of citrus which are suitable for eyes and immunes system of the body. A vibrant source of carotenoids present in

Sr. no	Minerals	Health promoting elements in citrus
1	Copper	Copper is trace element for essential in health
2.	Calcium	It is main part in bones, teeth and major role in metabolism
3.	Manganese	It involves in metal enzymatic activity and fat metabolism
4.	Magnesium	Muscles contraction
5.	Selenium	Antioxidants role in body process
6.	Potassium	Role in fluid system and assists in nervous system
7.	Phosphorus	It is involves in DNA and part of energy distributions
8.	Sodium	Its balance the human body and nerves and muscles functions
9.	Zinc	Blood circulation and metabolism in body
10.	Vitamin B1 (thiamin)	Heart, brain, nervous system, cofactor in gastrointestinal, muscular functions
11.	Vitamin B2 (riboflavin)	Reduction reactions and coenzyme in oxidation
12.	Vitamin B3 (niacin)	System metabolism, maintained circuitry system
13.	Vitamin B6 (piridoxina)	Its balance the body, fluid, metabolism, hemoglobin
14.	Vitamin B9 (folic acid)	It play a role in an acid, nucleic acid and metabolism balance
15	Vitamin C (ascorbic acid)	Formation of connective tissues, collagen, absorption of iron and properties of antioxidants

Table 1. General table of phytonutrients in citrus.

Cara navel oranges and mandarin fruits. The comparison of significant carotenoids present in citrus fruits shown in **Table 2**.

2.7. Flavonoids (flavones, flavonols and flavanols or catechins)

Flavonoids are polyphenol compounds that occur in plant-based food, more than 400 flavonoids have been indefinite in fruit and vegetables, but the significant flavonoid sources are citrus fruits [14, 15]. Flavonoids are usually present as glycosides; Citrus juice showed mostly biological activity whereas its antioxidants potential activity is more important. The activity based on tumor control, heart diseases, daily intake of flavonoids is a significant balance of disease in the human body [13]. The citrus considered as affluent fruit springs for sinensetin (peel oils and juice), total polymethoxylated flavones (mandarins and oranges peel oils), and tangeretins Limes juice has been used to control cholera which contains luteolin and tangeretin. The comparison of different flavonoids found in citrus species shown in **Table 3**.

2.8. Phytosterols in citrus

The citrus sterols used in different medicine while citrus juice has permanently linked to cancer prevention and reduction of cholesterol. The significant phytosterols present in grapefruit and oranges, and are helpful in stomach cancer, colon, rectal. The phytosterols are also involved in weight loss. The citrus peel is a precious resource of volatile compounds with a range of 95–98%. The significant phytosterols present in different citrus species shown in **Table 4**.

2.9. Role of phytochemicals of citrus in different diseases of the human body

2.9.1. Cancer

The scientific community engaged in the role of citrus flavonoids, phenolic compounds in the prevention of cancer disease and its use in the drug. Mostly cancer is caused by improper

Citrus species						
Carotenoids mg/110 g	**Grapefruit**	**Oranges**	**Lemon**	**Oranges valencia**	**Tangerine**	**Mandarin**
Auroxanthin	—	0.23	—	—	—	—
Cis β-carotene	—	—	—	—	—	11
α-Carotene	1	19–20		—	1	12–20
Cryptoxanthin	3.3	—	—	—	—	10-20
β-Cryptoxanthin	150	—	—	—	—	—
α-Cryptoxanthin	Present	—	—	—	—	—
Lutein	9.5	27		20–35	106	20–50
Luteoxan	Present	—	—	—	—	—
Lycopene	1	—	—	3–4	—	—

Table 2. Major group of carotenoids found in citrus.

Flavonoids ug/110 g	Grapefruit	Oranges	Lemon	Oranges valencia	Tangerine	Mandarin
Apigenin	—	—	—	0–0.3	—	—
Acacetin	—	—	—	—	—	—
Hexamethoflavone	—	—	—	0.3	—	—
Polymethoxylated flavones	—	0.2	—	—	—	0.65–0.72
Myricetin	—	0.0–0.05	—	—	0.1	—
Quercetin	0.49–0.70	0.57	—	—	—	—
Sinensetin	—	—	—	—	—	20–70
Tangeretin	0–120	—	—	—	190	0–180
Violaxanthin	—	—	—		—	12

Table 3. Major group of flavonoids found in citrus.

Phytosterols ug/110 g	Grapefruit	Oranges	Lemon	Oranges valencia	Tangerine	Mandarin
Auraptene	0.14–0.17	0.13	—	—	—	—
Coumarin	0.26	0.54	—	—	—	—
Meranzin	0.16–0.25	0.55	—	—	—	—

Table 4. Phytosterols in citrus distribution.

diet intake [6]. The colon cancer is a serious issue caused by the imbalances and wrong uses of diet. Mostly 90% cases of colon cancer have reported in the world due to diet. The tyrosine modulator as citrus flavonoids is useful in cancer treatments. Several studies reported that in treatments of different cancers lines the juice of citrus showed an antiproliferative. The role of flavonoids (nobiletin, hesperetin, tangeretin and neohesperetin) is tumor controlling activity in the human body. The citrus peel oil like d-limonene showed an anticancerous activity, especially peel oil of citrus effective in skin cancer control. It is like a sheet in the tumor cell, and oil application suppresses mostly cell [15].

2.9.2. Oxidative damage, cardiovascular and coronary heart diseases (CVD and CHD)

The cardiovascular diseases stand as real problems of this world with many deaths has been reported yearly while the drug resistance is more prominent in the world. The save and sound methods required for the isolation of citrus new bioactive compounds in juice, peel rag, and seeds. This area of investigation required more attention of the scientists for the development of new natural methods of isolation of active compounds from citrus fruits like lemons, grapefruits, sweet oranges, involves in treatments of hypertension. The chronic, hemorrhoids and leg cancer, the 6, 8-di-*C*-glucosyldiosmetin and Vicenin-2 suppressive of blood adhesion molecules are mostly found in citrus. The different citrus extracts were significant control of hemorrhages. The principal role of flavonoids chrysin, luteolin, 7-hydroxyflavone on induced on the umbilical vein, the lipoprotein. The body of human cell endothelial cells oxidized LDL and stimulated the more intracellular production of ROS. These bioactive compounds managed under the control of LDL. The bioactive compounds are better for hypercholesterolemia and atherosclerosis control.

2.9.3. Role of citrus juice on lipid metabolism and obesity control

Polyphenols from citrus fruits have been evaluated in prevention and treatment of obesity. The role of nobiletin, flavones, and hesperidin in hepatic mechanisms are extraordinary. The lemon and sweet orange have a vast range of bioactive compounds with reported a 60–70% control of liver diseases [13]. The grapefruit juices have individual enzymes which control the obesity of human body. The adipose tissue has a significant role in energy storage, but it performed several functions in human tissues to joined with endocrine organ due to paracrine secreted called adipokines [15]. The tissues involved in the signal pathway of endocrine glands. The grapefruit juice contained enzymes called P-45 and range of different protein which burn human fats [14]. The lemon has many bioactive compounds, and the juice of lemon possesses more than 200 compounds which involve regulators of the human body [14]. Many clinical studies approve the citrus juice is beneficial for control higher cholesterol and major lipid problem of the human body [6, 7]. In a world mostly death has been reported by the obesity while the health drinks with full of nutrients shown significant role in the reduction of fats in the human body [17].

2.9.4. Anti-microbial activity

Citrus fruit is a rich resource of flavonoids with many physiological properties involved in controlling antiviral activity and anti-microbial activity. Hesperidin and quercetin involve in control of herpes virus, parainfluenza and polioviruses [15]. The naringin metabolites are rich source of natural antimicrobials activity against the positive and negative bacteria [4]. The sweet oranges and grapefruit is a rich source of phytochemicals, suitable for microbial control.

2.9.5. Role of citrus nutrigenomics

The dietary phytochemicals showed a gene expression in many processes of the human body. The gene and nutritional approaches have linked with health. The bioactive compounds can normalize the transcriptome gene expression. The different studies have shown that activation of gene and modulating the targeted molecules. The study showed a lower density lipoprotein receptor expression by citrus flavonoids. The citrus flavonoids and carotenoids showed a different genes expression COX-2, NFkB and cytochrome P450. The citrus fruits have a range of biological activity for maintaining the health of different organs [22, 23].

2.9.6. Activity against other diseases

Citrus has a precious resource of soluble and insoluble fiber with several benefits in preserving and removal of toxic effects in the body [13]. Fiber improves the gastric adsorption in the small intestine like the gastric emptying, reduces the energy absorption process, the bile duct and liver maintaining [14]. Fiber and pectin of citrus in the small intestine of human recover the weight of villus and depth of crypt depth [15]. Cell proliferation of an intestinal cell, brush border membrane enzymes and the short-chain fatty acid production in the cecum stimulated by pectin are followed by an increase in the plasma level. It is also a factor for the illegal growth of mucosal. In the gastric tract, the cell promotes the efficiency of the track. All these changes are closely related to the modification of proteins with energy linked [15, 16].

3. Conclusions

Citrus is a significant source of bioactive compounds; the bioactive compounds are suitable for controlling different human diseases. The bioactive compounds have saved and healthy effects on diseases. The Citrus fruits and their components have a rich source of flavonoids, carotenoids, and bioactive compounds. There is a need for the development of awareness and uses of such compounds in life for saving the life threating diseases by using the bioactive compounds. The world always focused on the proper uses and consumption of citrus fruits and juices in our daily life. This chapter discloses the important compounds found in citrus that are highly required by the human body and their use play a significant impact on the human life for diseases controls.

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Conflict of interest

The author of this chapter declares that there is no conflict of interest among the author of this project.

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Chapter 3

Exploitative Beneficial Effects of Citrus Fruits

Idoko Alexander

Additional information is available at the end of the chapter

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Abstract

Citrus fruits trees have come to gain a worldwide recognition for their suiting refreshing juice, nutritious value and numerous health benefits and maintenances. Their applied health therapeutic uses have been exploited in the treatment of several health challenges as antitumor, anti-inflammatory anticancer, antiviral, antimicrobial activities, against cardiovascular diseases and macular degeneration. Lime (*Citrus aurantifolia*) juice has been shown to effectively serve as hypolipidemic, possesses the ability to interact with orthodox medicines. Obviously, citrus fruits' abilities on the exploited benefits are not far from their rich bioactive compounds and phytochemical such as minerals, vitamins, flavonoids and carotenoids. These phytochemicals may act as antioxidants, boosting the action of protective enzymes in the liver, reverse lipid peroxidation of genetic material and improve immune system. A close look at this chapter includes introduction, history and description, structures and biochemistry of phytochemicals, metabolism of phytochemicals and bioactive compounds and beneficial effects of citrus fruits.

Keywords: citrus, phytochemical, antioxidants, orthodox medicine, health benefits

1. Introduction

Citrus fruits have a worldwide spread, grown across the globe and are well-appreciated for their refreshing juice and health benefits [1]. They are fruits bearing trees, which are members of the rutaceae family. Citrus fruits have five main species according to [2], which include *Citrus sinensis* (sweet orange fruits tree), which have about 70% in the majority of the citrus family, *Citrus aurantifolia* (the lime fruits tree), *Citrus reticulata* (the tangerine fruits tree) *Citrus limonum* (the lemon fruits tree) and *Citrus vitis* (the grape fruits tree).

1.1. History and description

1.1.1. History

The origin of citrus fruits is not very clear. The trees flourish well in tropical and subtropical climates. They were thought to originate in Southeast Asia. Arab traders brought lime trees back from their journey to Asia and introduced them into Egypt and Northern Africa around the tenth century [3]. Researchers assert that Mexico, Florida, Brazil and California in America are where we currently find the largest orange orchards in the world and citrus fruits were transported by the Spaniards [4]. Citrus fruit like many other fruits and vegetables, were reported to have been brought to the Americans by Christopher Columbus, when he made his second voyage in the sixteenth century to the New World in 1493, and have been since then grown in Florida [5]. Citrus was highly appreciated such that in 1849, there was a great demand of lemon that people were willing to pay up to $1 per lemon, a price that would still be considered costly today and was extremely expensive at that time [6]. George [6] reported that the introduction of limes to the United States began in the sixteenth century when Spanish Explorers brought the West Indies lime to the Florida Keys, beginning the advent of Key limes. However, in the following century, Spanish missionaries attempted to plant lime trees in California, but the climate did not support their growth. In great demand by the miners and explorers during the California Gold Rush as a fruit that was known to prevent scurvy, limes began to be imported from Tahiti and Mexico at this time in the mid-nineteenth century. Today, Brazil, Mexico and the United States are among the leading commercial producers of limes [7].

1.1.2. Description

Citrus fruit trees are greenish and of different sizes and height, according to the species. Lime fruit trees are small in height with multiple and spiny branches and smaller green leaves [8]. Citrus fruit trees produce fruits of various sizes forms and shapes such as oblong and round shapes. The fruit is covered or protected against damage by a rough bright green or yellow color epicarp. This epicarp is composed of glands which contain essential oils, responsible for the peculiar citrus fragrance. The epicarp also houses a white, thick and spongy mesocarp which together with the epicarp forms the pericarp or peel of the fruit. Inside the fruit is the cavity which is divided into separate segments or juice sacs containing seeds or without seeds for the seedless variety. The seed is covered by a thick radical film or endocarp [9]. This inner part is rich in soluble sugars, ascorbic acid, pectin, fibers, different organic acids and potassium salt that give the fruit its characteristic citrine flavor [9]. According to the working list of all plant species, citrus species hybridize easily and that new hybrids are continuously developed by cross pollination to obtain desired qualities such as seedless, juicy and fresh taste fruits [10].

According to [11, 12], the working list of all plant species, the taxonomy of citrus plants follow the order; **Kingdom**: Plantae; **Subkingdom**: Tracheobionta; **Superdivision**: Spermatophyta; **Division**: Magnoliophyta; **Class**: Magnoliopsida; **Subclass**: Rosidae; **Order**: Sapindales; **Family**: Rutaceae; **Genus**: *Citrus.* However, citrus species/types have numerous common names depending on the country and language. Ali [10] highlighted the following common names for some species;

Citrus aurantiifolia: **Arabic**: laimon helo; **Chinese**: lai meng; **English**: Egyptian lime, Indian lime, Key lime, lime, Mexican lime, sour lime, lime; **French**: citron vert, citronnier gallet, lime

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acid, limettier, limettier des Antilles, limettier mexicain; **German**: Limette, Limettenbaum, Limone, saure Limette; **India**: kagzi nimboo, kagzi nimbu; **Italian**: lima; **Portuguese**: limão-galego, limão-tahiti; **Spanish**: limón agrio, limón ceutí, lima, lima mejicana, limero [10–13]. However, in Nigeria, Lime fruits are locally identified as follows: in Idoma, it is called Alemu Ogwuchekwo; in Igbo, it is called Oloma-oyinbo; in Hausa, it is called Lemun tsami or Babban lemu; in Yoruba, it is called Osan ghanhin-ghanhin; and in Igala, it is called Alemu inale [13].

1.2. Structures and biochemistry of phytochemicals

Phytochemicals are the numerous chemicals present in plants. They are primarily produced by the plants to serve the purpose of defense to insects and microbial attack. They are thus, called plants secondary metabolites. The following are some citrus phytochemicals.

1.2.1. Citrus flavonoids

Flavonoids are a group of extensively large class of plant phytochemical of over 5000 hydroxylated polyphenol compounds, which abound in fruits, vegetables, legumes and tea [14]. Flavonoids are divided into a major subclass of 12 based on differences in chemical structures [15]. Flavonoids that are of dietary importance include flavones, flavonols, flavanones, anthocyanidins, flavan-3-ols and isoflavones [16]. Citrus has been identified to have the following class of flavonoids; flavonols, flavans, flavones, flavanones and anthocyanins. Anthocyanins are included as citrus flavonoids because it has been isolated in blood oranges [17].

Citrus flavonols, flavones and flavanones (**Figures 1–3**) abound largely. Most flavonoids exist in their glycosylated forms or aglynol and aglycone forms. Glycosylated forms of flavonoids (**Figures 4–6**) include, naringenin, maringin, rutin and hesperidin.

The glycosylated forms have been classified into two types, these are; the neohesperidosides and the rutinosides [18].

Neohesperidosides, naringin, neohesperidin and neoeriocitrin are said to have a bitter taste [19]. While rutinosides, hesperidin, narirutin and didymin, have been found to be tasteless and

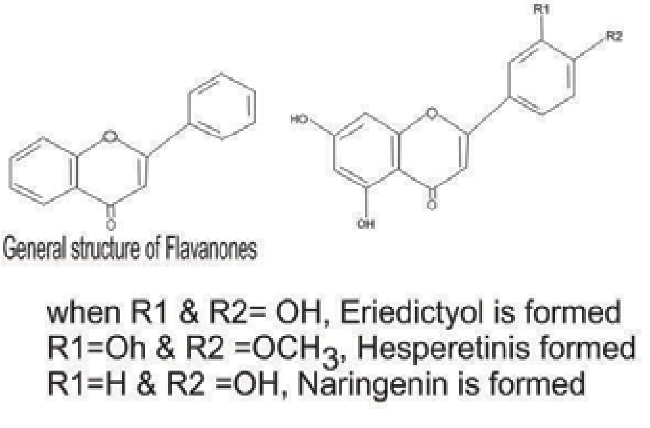

Figure 1. Structures of flavanones and derivatives.

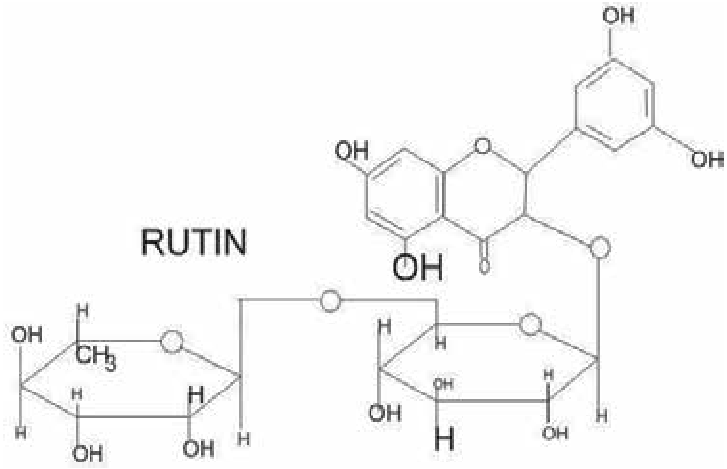

Figure 2. Structures of flavonols and derivatives.

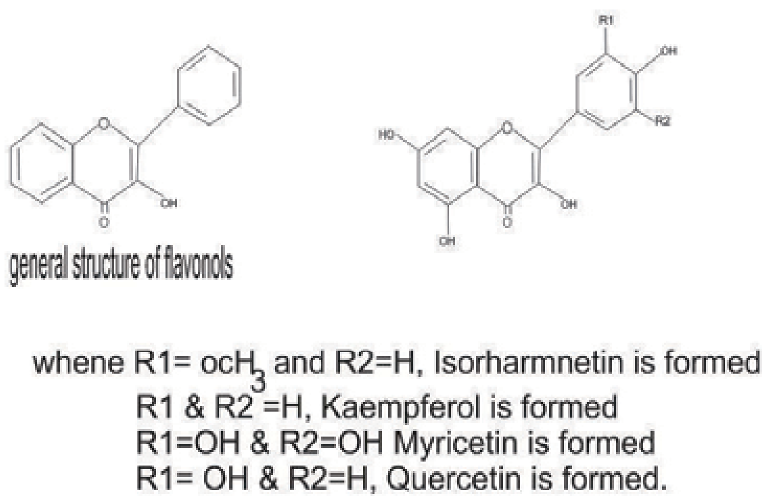

Figure 3. Structures of flavones and derivatives.

have a disaccharide residue e.g. rutinose (ramnosyl-a-1,6 glucose). Most flavanones are usually found in diglycoside forms, which confer the typical taste to Citrus fruits [19]. Using UV, IR, FABMS, 1H NMR, and 13C NMR analyses, [20] isolated two glyvones (C-glucosylflavones) from the peel of lemon fruit (*Citrus Limon* BURM. f.), and identified 6,8-di-C-b-glycosyldiosmin and 6-C-b-glycosyldiosmin. The compositions of the seed and peel of citrus fruits are not always the same. The lemon seed contains eriocitrin and hesperidin and the peel contains neoeriocitrin, naringin and neohesperidin [21]. The concentration of the glycosylated.

Flavanone in peel and seed varies. In peels, the concentrations of neoeriocitrin and naringin are similar while, in seed, the concentration of eriocitrin is reported to be 40 times higher than the concentration of naringin [22].

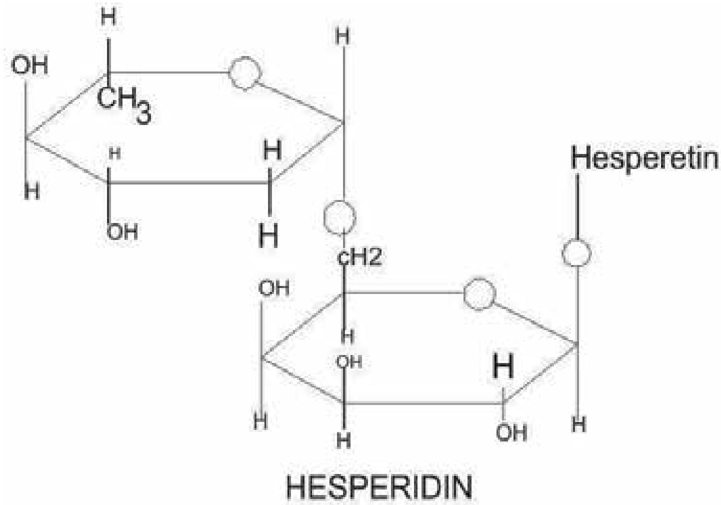

Figure 4. Structure of rutin, a glycosylated flavonoid.

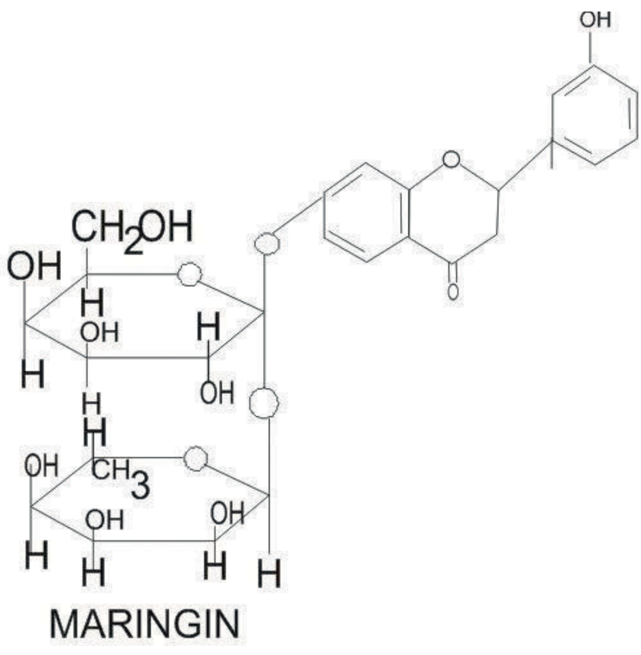

Figure 5. Structure of hesperidin, a glycosylated flavonoid.

Neohesperidin, naringin and neoeriocitrin are extracted from peel in great amounts [23]. It has been reported that bitter orange is a marvelous source of neohesperidin and naringin which are very significant in the industry for the production of sweeteners [19]. Generally, most citrus fruits are said to possess little quantity of glycosylated naringin [24].

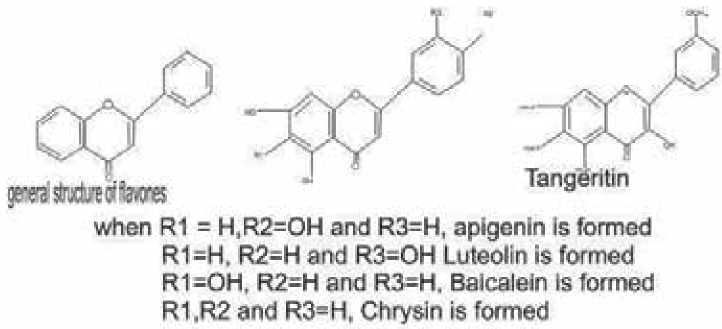

Figure 6. Structure of naringin, a glycosylated flavonoid.

It is reported that naringin is found in lemon peel and seed, in mandarin seed and absent in the juices [25]. Mouly et al. [26] found that glycosylated flavanones, responsible for bitterness, cannot be in sweet orange juice, thus their presence will mean the fruit is aldulterated or spoit.

1.2.2. Citrus carotenoids

It has been reported that pink grapefruit has a higher content of carotene than other citrus fruits such as tangerines and oranges, which contain high levels of other carotenoids, including lutein, zeaxanthin, cryptoxanthin that have significant anti-oxidant activity [27].

Carotenoids are hydrocarbon of the class of carotene and their oxygenated derivatives, the xanthophyls. Carotenoids are composed of eight isoprenoid units linked in a reversed isoprenoid units at the center of the molecule, making the two central methyl groups to have 1,5-position relationship, and are the pigments responsible for the colors of many plants [28]. **Figure 7** shows structures of some selected (including lutein, zeaxanthin, lineal, epoxy carotenoid, lycopene and β-carotene) carotenoids. There are more than 800 carotenoids and their derivatives identified and isolated and are divided into two main groups, called carotenes and xanthophylls. Carotenes are composed of hydrocarbon structure and xanthophylls that contain oxygen atoms in their structure [29]. The pink grapefruit also has been found to be very rich in the red pigment, lycopene, with a potent anti-tumor activity [30]. They serve as light harvesting complexes in photosynthesis [1]. Carotenoids (β-carotene and lycopene clarified in the carotenes) are known to be responsible for the orange-red colors found in orange, tomatoes and carrots fruits as well as the yellow colors of many flowers [31] and in xanthophylls, lutein in spinach and broccoli and β-cryptoxanthin in Satsuma mandarin are well-known [29]. Yokayama and White [32] reported that the flavedo of the fruit of the trigeneric hybrid, Sinton citrangequat contains new carotenoid ketones (apocarotenones) pigments that are unique in the carotenoid series in that they contain the terminal methyl ketone group in the side chain responsible for the rich red color of the flavedo. Carotenoids in plants are a very important component of photosynthesis and prevent disastrous photo oxidation [31]. They isolated and characterized these methyl ketone carotenoids with nonaeneone and decaeneone chromophores to include; sintaxanthin, citranaxanthin, 3-OH-sintaxanthin, reticulataxanthin

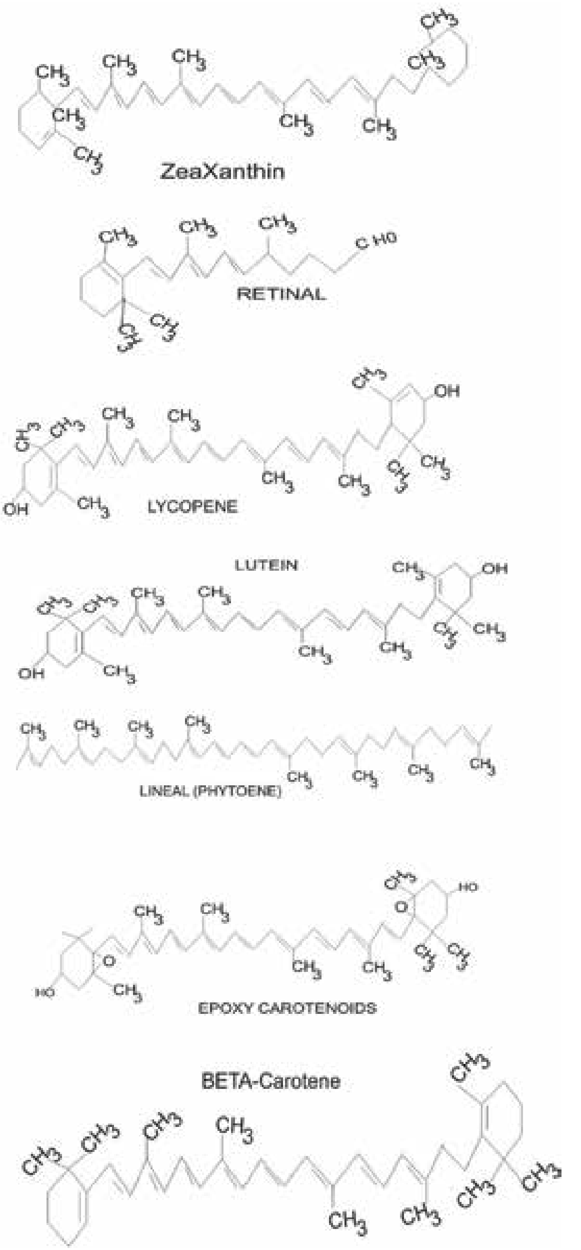

Figure 7. Structures of citrus carotenoids.

and an in-chain hydroxyl group 8′-OH-7′8′-dihydrocitranaxanthin methyl ketone carotenoid [32]. For the first time, other carbonyl carotenoids consisting of β-apo-10′-carotenal, β-apo-8′carotenal, β-citraurin, Neurosporene, γ-carotene, β-carotene and probably 3-OH-β-apo-10′-carotenal. β-Zeacarotene were detected and isolated from citrus in minor amounts [32].

1.2.3. Citrus limonoids

In citrus, there are more than 30 limonoids with limonin and nomilin being the most identified. Limonoids are compounds which have high concentration in grapefruit (*C. vitis*) and orange juice (*C. sinensis*), which are partly associated with the bitter taste in citrus [33]. Shin and Masaki [34] reported the potentials of some limonoids to include antifeedant activity against insects, suggesting that one of the biological functions of limonoids in plants is pest control and that citrus limonoids are unique for many species and varieties of citrus, which make them excellent taxonomic markers.

1.2.4. Citrus terpene

Citrus terpenes are clear, colorless and liquid cyclic hydrocarbons called monoterpenes or D-limonene and are produced as oil from the rind of citrus through the process of distillation [35]. Terpenes are not derived from isoprene rather they are found as isopentenyl pyrophosphate in nature. Isopentenyl pyrophosphate is derived in a series of complex metabolic reaction steps in the mevalonate pathway [36]. Limonene is a terpene, which get its name from lemons and is the main constituent extracted from the citrus fruit rind. Limonene preparations in the laboratory have been reported to employ Diels-Alder reaction, an addition reaction which involves the joining of two isoprene molecule without librating nothing [37]. Limonene is reported to be the source of citrus flavor and fragrance—whether in desserts as a food-grade chemical or in cleansers—or to produce other flavors and fragrances via the use of chemical reactions [37]. Limonene is a terpene which is relatively stable and can be distilled without decomposition. However, it is reported to crack at elevated temperatures to form isoprene [38]. Limonene reacts with sulfur by dehydrogenation to produce p-cymene and limonene oxide, carveol and carvone are formed as oxidation products when exposed to moist air to form carveol, carvone, and limonene oxide [39]. With sulfur, it undergoes dehydrogenation to p-cymene [40].

1.2.5. Citrus alkaloids

Ref. [41] developed a method for the analysis of adrenergic amines in tangerine juice. The method is sensitive, fast, simple and reproducible when using Cogent Diamond Hydride HPLC column and an Agilent MS TOF instrument. The alkaloids analyzed were tyramine, N-methyltyramine and synephrine (**Figure 8**).

1.3. Metabolism of phytochemicals and bioactive compounds

The retinoids (vitamin A and retinal), are a very important metabolites of carotenoids in mammals, including humans and monkeys [42, 43]. The report on the synthesis of vitamin A from β-carotene showed that it could be formed by central or eccentric cleavage of β-carotene [30].

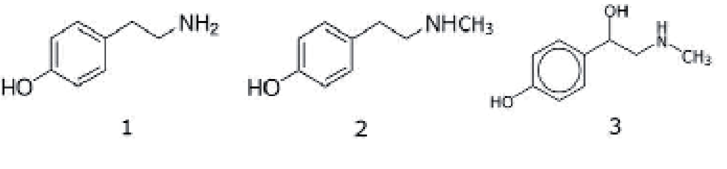

Figure 8. Structures found in citrus alkaloids (1 = tyramine; 2 = N-methyltyramine, 3 = synephrine).

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It was demonstrated that the enzyme 15–15[1] β-carotenoid dioxygenase in the intestine and liver, convert α-carotene, β-carotene and β-cryptoxanthin to vitamin A (retinal) [30]. However, [28] reported that such in vivo formation of retinal is homeastatically controlled, so that the conversion to retinol is limited in individuals with adequate vitamin A. Currently, there is an increase production of carotenoids by biotechnology due to its demand in industry, added as colorants to many manufactured food drinks, fruit juice and animal feeds either in the form of natural extracts or as pure compounds manufactured by chemical synthesis [30].

The importance of dietary citrus flavonoids becomes appreciated only when they are absorbed and become available to target tissues within the body. In the intestine and liver, absorption and metabolism of flavonoids is rapidly carried out. In the liver phase II reaction, flavonoids metabolized to intermediate metabolites and transported in the bloodstream and excreted as urine [44]. It appears that the biological activities of flavonoid metabolites are different from their parent compounds [45] and these metabolites (xenobiotics) must first be modified in the mucosa of intenstine and then in the liver [46]. Enzymatic transformation of flavonoids by the gut microbial enzymes of the large intestine is done through deglycosylation, ring fission, dehydroxylation, demethylation into metabolites that can then be absorbed or excreted [46, 47]. Different metabolites are produced after transformation but production depends on the diverse activity of the colon bacteria, resulting from an individual's dietary intake of flavonoids rich diet [47, 48]. The bioavailability of flavonoids in the system increases the beneficial exploits of the nutrients which in turn depend on the composition of the colon bacteria [49]. When polyphenols are administered orally, only small quantities of these compounds appear in systemic circulation because of very high levels of uridine diphospho (UDP)-glucuronosyltransferases and sulfotransferases in the small intestine and liver, thus resulting in very low oral bioavailability [50]. Quercetin was originally assumed to be absorbed from the small intestine following cleavage of the β-glucoside linkage by colonic microflora [51].

1.3.1. Metabolism of minerals and vitamins

Metabolism is a dual process involving catabolism (breaking down or oxidation) and anabolism (biosynthesis). It a biochemical process which makes energy available to an organism following the conversion of ingested food. Catabolism involves hydrolyses, digestion, absorption and excretion of ingested food. Most vitamins and mineral are absorbed by the intestinal cells of the body. Magnesium is absorbed by the intestinal cells through a specific carrier system; zinc is absorbed mainly in the duodenum, dietary Mn is normally absorbed in the small intestine, however, iron inhibits the absorption of Mn. Metallothionein is the transport protein that facilitates copper absorption mainly in the duodenum. Iron in the ferous form is soluble and readily absorbed in the stomach and duodenum [52]. About 90% of K^+ is absorbed from the gastrointestinal tract. Sodium is readily absorbed in the gastrointestinal tract. Phosphate absorption takes place at the jejunum. However, calcitriol promotes phosphate uptake along with calcium. By an energy dependent active process, calcium is mostly absorbed in the duodenum [53].

1.3.2. Biosynthesis of phytochemical in citrus plant

Generally, the biosynthesis of phytochemicals in citrus and other plants has been reported to be organ, cell or development specific in almost all higher plant species [54]. The pathways, and genes involved in their synthesis are most tightly regulated and may be linked to environmental, seasonal or external triggers. Cellular sites of synthesis are compartmentalized in the plant cell, with the majority of pathways being at least partially active in the cytoplasm [54]. There are evidences that compounds such as alkaloids, quinolizidines, caffeine and some terpenes are synthesized in the chloroplast [55–57]. Most often, phytochemicals are detected throughout the plant, however, they are initially synthesized in single organ such as roots, fruits or leaves and later transported by the phloem or xylem tissues around the plant or via symplastic or apoplastic transport and stored in a number of different tissues [54]. The site of storage often depends on the polarity of the compounds, with hydrophilic compounds such as alkaloids, glucosinolates and tannins being stored in vacuoles or idioblasts, while lipophilic compounds such as the terpene-based essential oils are stored in trichomes, glandular hairs, resin ducts, thylakoid membranes or on the cuticle [56]. The storage of some compounds such as alkaloids, flavonoids, cyanogenic glycosides, coumarins that are present in the plant and serve defense purpose are in the epidermis [56–60]. Shin and Masaki [34] reported that nomilin, a limonoid is biosynthesized from acetate through the terpenoid biosynthetic pathway in the phloem region of stems and then transported to the leaves, fruit tissues, peels, and seeds where it is further metabolized to other limonoids. The citrus limonoid aglycones are then glucosidated by limonoid UDP-ᴅ-glucose transferase in maturing fruit tissues and seeds. These limonoid glucosides are accumulated in such high concentrations that they are one of major secondary metabolites in citrus fruit tissues [34].

The enzymatic biosynthesis of terpenes is understood to be divided into four enzyme catalyzed steps to include; (1) biosynthesis of two precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) [36]. This is done through two different pathways; (2) repetitive addition of the precursors to form a series of homologs of prenyl diphosphate, which are the immediate precursors of the different classes of terpenes; (3) enlargement of the terpenes backbones by the activity of specific synthases and (4) secondary enzymatic modification of these backbones resulting in the functional properties and family diversity [61]. The two pathways involved in the synthesis of IPP and DMAPP are called the acetate-mevalonate pathway, located in the cytoplasm and non-mevalonate pathway located in the plastids of the cell [36, 61]. The acetate-mevalonate pathway is reported to be involved in the synthesis of sesquiterpenes and sterols while the non-mevalonate pathway is responsible for the synthesis of monoterpenes, diterpenes, tetraterpenes and polyterpenes [35]. Marco et al. [35] also reported that terpene synthases enzymes need geranyl diphosphate, farnesyl diphosphate and geranylgeranyl diphosphate as substrates for the production of different terpenes. Monoterpenes are derived from geranyl diphosphate, sesquiterpenes from farnesyl diphosphate, and diterpenes from geranylgeranyl diphosphate by the action of terpene synthases or cyclases [35]. Biosynthesis of limonene involves a cyclization of a neryl carbon from geranyl pyrophosphate [62].

Masaya [29] reviewed and reported the biochemical pathway of the first committed step in carotenoids biosynthesis in plant as demonstrated by [63, 64] to involve a head-to-head condensation of two molecules of a 20 caborn molecule of geranylgeranyl pyrophosphate (GGPP) to form a colorless 40 carbon molecule of phytoene catalyzed by phytoene synthase (PSY). Carotenoid biosynthesis and its regulation have been studied in tomato fruit during fruit ripening and development [65]. Carotenoid concentration and composition are influenced by growing conditions and fruit maturity. They also differ among geographical origins [66]. Kato et al. [66] reported their investigation on the relationship between carotenoid accumulation and the gene responsible for the expression of carotenoid biosynthetic during fruit maturation in three citrus varieties, Satsuma mandarin (*Citrus unshiu* Marc.), Valencia orange (*Citrus sinensis* Osbeck), and Lisbon lemon (*Citrus limon* Burm.f.). After successful cloning of the genes, in the flavedo of Lisbon lemon and Satsuma mandarin, massive accumulation of phytoene was observed with a decrease in the transcript level for *CitPDS* and concluded that the carotenoid accumulation during citrus fruit maturation was highly regulated by the coordination of the expression among carotenoid biosynthetic genes [66]. Massive accumulations of carotenoids have been shown to occurred concomitantly with the degradation of chlorophyll during citrus fruit development in mandarin varieties, such as Satsuma mandarin [67]. β-Cryptoxanthin accumulated predominantly in the flavedo and juice sacs in mature fruit [68].

1.4. Beneficial effects of citrus fruits

1.4.1. Citrus phytochemicals and bioactive compounds in disease prevention

The anti-oxidant activities of carotenoids are said to be associated with a lower incidence of age-related macular degeneration, which happen to be the leading cause of blindness in human after the age 65 [28]. The role of citrus carotenoids in disease prevention and in human health management cannot be over emphasized. Carotene plays an essential role as sources of vitamin A. The most active role is protection against serious disorders such as cancer, heart diseases and degenerative eye diseases. An inference can be deduced from an epidemiological data provided that diets which are rich in carotenoids containing fruits are associated with pronounced decreased risks for a variety of degenerative diseases [31]. Similarly reports from several epidemiological studies have shown decrease in cataract onset with high blood content of carotenoids [69]. It was reported that the combination of vitamin C and β-cryptoxanthin intakes might provide benefit to bone health in post-menopausal Japanese female subjects [70]. The stimulating ability of limonoids on the enzyme glutathione S-transferase (GST) to inhibit tumor is reported [33]. Glutathione S-transferase is a detoxifying enzyme that catalyzes the reaction of glutathione with dangerous electrophiles to form less toxic and more importantly water soluble compounds that can be easily excreted from the body [71]. Craig and Okwu [33, 72] reported that orange and lemon oil contain substantial amounts of GST that also possesses anti-cancer activity. The potentials of citrus pulp and the albedo (the white of the orange) are extensively being studied to be rich in glucarates and in preventing breast cancer and to lower the risk and symptoms of premenstrual syndrome [33]. Flavonoids have reported to poccess strong inherent ability to modify the body’s reaction to allergens, viruses and carcinogens as they have demonstrated effective anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity [73].

1.4.2. Domestic and industrial benefits of citrus

D-Limonene has been reported to be used as botanical insecticide [74] and in the production of the organic herbicide “Avenger” [75]. It is an important additive to cleaning products in the preparation of hand cleansers to give a lemon-orange fragrance. The ability of citrus oil byproduct of orange juice manufacture produced from a renewable source as an organic solvent in dissolving oils is also known, as it has been used for the removal of oil from machine parts. Limonene is reported to be used as a paint stripper, as a constituent of some paints and used as an alternative fragrance to turpentine [75]. Limonene uses as a solvent in some model airplane glues and commercial air fresheners is documented. It was also shown that Philatelists used air propellants containing limonene to remove self-adhesive postage stamp from envelope paper [75].

1.4.3. Antimicrobial potentials of citrus

D-limonene has been reported to be used by researchers in preparing tissues for histopathological analysis as a less toxic substitute for the chemical, xylene when clearing dehydrated specimens [76, 77]. The often used clearing agents are liquids which are miscible with alcohols such as ethanol or isopropanol and with melted paraffin wax, in which specimens are embedded to facilitate cutting of thin sections for microscopy [78]. The use of D-limonene in traditional medicine has been reported to manage heartburn, gallstones and gastroesophageal reflux disease. However, high quality and robust clinical research is yet to support such claim [79]. The antibiotic effects of citrus have been shown from a research carried out in several villages in West Africa where cholera epidemics had occurred, the inclusion of lime juice during the main meal of the day showed that lime juice has protective effects against the contraction of cholera. This gave birth to the use of lime juice as a sauce eaten with rice and was also found to have a strong protective effect against cholera [80, 81]. The antibacterial and antioxidant potential of the essential oil of *Citrus aurantifolia* leaf was reported by [82]. Results shows the essential oil in *Citrus aurantifolia* leaf exhibited pronounced activity against Gram-positive and Gram-negative bacteria and their activity was quite comparable with the standard antibiotics such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin screened under similar conditions [82]. Another antibacterial study in Malaysia, involving five different Malaysian citrus varieties using *Citrus aurantifolia*, *Citrus reticulata*, *Citrus microcarpa*, *Citrus limon* and *Citrus sinensis* against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* was carried out by [83] to evaluate the antibacterial potentials of these five species. Results showed that the methanol extract of the five varieties of citrus exerted no inhibition at 5 and 10 mg/ml. The methanol extract of *Citrus microcarpa*, *Citrus reticulata* and *Citrus sinensis* at 20 mg/ml showed better inhibition compare to *Citrus aurantifolia* and *Citrus limon* against *Staphylococcus aureus* and *Escherichia coli* [83].

1.4.4. Citrus effects on immunity

Owing to the antioxidant ability citrus vitamin C, its function in boosting a strong immune system has been reported [84]. The potentials of lemon, *Citrus medica* L. (citrus), and *Cydonia oblonga* as immunomodulators and antiallergic substances were investigated on an in vitro human mast cells, IL-8 and TNF-α with results which showed reduction of degranulation of basophil cells and inhibition of IL-8 and TNF-α of human mast cells [85].

1.4.5. Anti-rheumatoid arthritis and cardiovascular effects of citrus

A human study documented in the Annals of the Rheumatic with more than 20,000 subjects showed that subject who maintained high consumption of citrus food rich in vitamin C, had protection against inflammatory polyarthritis, a form of rheumatoid arthritis involving two or more joints. Subjects who consumed the lowest amounts of vitamin C-rich foods were more than three times more likely to develop arthritis than those who consumed the highest amounts [86].

An experimental study of the effects of *Citrus aurantifolia* on cardiovascular parameters was carried out on Spargue Dawely rats by checking the anti-hypertensive effect on three experimental hypertensive models. The models include cadmium induced hypertensive model, glucose induced hypertensive model, Egg feed diet induced hypertensive model, and normotensive model. Result obtained after 0.75 mg oral administration of *Citrus aurantifolia* methanol extract revealed a significantly ($p < 0.01$) reduced blood pressure parameters of the test groups compared to control groups [87]. The diastolic blood pressure of healthy middle-aged, normal-weight men was reported to be reduced after consuming orange juice for 4 weeks [88]. A study on the protective effect of the ethanolic extracts of Otroj, *Citrus medica* (EEOT) against isoproterenol (ISO)-induced cardiotoxicity was evaluated in rats. Results obtained from histopathological examination and myocardial biochemical assay demonstrated cardioprotective potential of EEOT [89]. It was reported recently that citrus fruits offer protection against cardiovascular diseases by reducing levels of homocysteine [90]. Homocysteine is a toxic agent for the vascular wall and, when plasma levels rise above normal, there is an increased risk of cardiovascular disease. It was reported that a low dietary intake of citrus folate contributes to the decrease of plasma folate and the raising of plasma homocysteine levels [91]. A recent study from the juice of freshly squeezed oranges, with high intakes of vitamin C (500 mg/day) showed that a rise in the levels of oxidized LDL was prevented, even in the presence of a high-saturated fat diet [92].

1.4.6. Anticancer potentials of citrus

In a laboratory test carried on human cells and animal studies, limonoids from different species and category of citrus fruits, including lemons and limes, have been reported to posses' anticancer ability against cancers of the mouth, skin, lung, breast, stomach and colon [3]. Do-Hoon et al. [93] reported that the numerous phytochemical contents in citrus including terpernoids, alkaloids, flavonoids, limonoids, and coumarins are found to be associated with a reduced risk of gastric cancer, breast cancer, lung tumorigenesis, colonic tumorigenesis, hepatocarcinogenesis, and hematopoietic malignancies [94, 95]. The flavedo extract of Ougan (*Citrus reticulata cv. Suavissima*) was found to exhibit potential anti-tumor effects by its inhibitory effect on epithelial-to-mesenchymal transition and interfering with the canonical TGF-β1-SMAD-Snail/Slug axis [96]. Purified bioactive compounds isolated from seeds and peels of *Citrus aurantifolia* have been reported to have inhibiting and suppressing effects on pancreatic cancer and colon cancer cells respectively [97]. *Citrus aurantifolia* potentials as anticancer were reported to be due to apoptosis-mediated proliferation inhibition of human colon cancer cells by volatile principles [98]. Human colon cancer has been reported to have 78% inhibition and induction of apoptosis confirmed by isolated volatile oil of *Citrus aurantifolia* fruit [99]. Effects of volatile oils from fresh *Citrus limon* fruit peels have been shown to possess a genotoxic effects on human lymphocytes by measurement of mitotic and blast indexes [100].

1.4.7. Citrus hypoglycemia and antidiabetic effect

The hypoglycemic potential of citrus flavonoids including hesperidin, naringin, neohesperidin, and nobiletin, were reported to significantly inhibit amylase-catalyzed starch digestion, where naringin and neohesperidin specifically inhibited amylose digestion, hesperidin and nobiletin inhibited both amylose and amylopectin digestion. Results showed the potential of citrus flavonoids in preventing the progression of hyperglycemia, partly by binding to starch, increasing hepatic glycolysis and the glycogen concentration, and lowering hepatic gluconeogenesis [101]. Also, the dietary hesperidin, was reported to have exhibited antidiabetic activities, partly by lowering hepatic gluconeogenesis or improving insulin sensitivity in diabetic animals [102]. Annadurai et al. [103] demonstrated in a study the antihyperglycemic and antioxidant effects of a flavanone, naringenin, in streptozotocin-nicotinamide-induced experimental diabetic rats and showed that naringenin conferred protection against experimental diabetes through its antihyperglycemic and anti-oxidant properties in streptozotocin-nicotinamide-induced diabetic rats. In another study, it was shown that in vivo chronic treatment of diabetic rats with naringenin could prevent the functional changes in vascular reactivity in diabetic rats through a NO-dependent and prostaglandin-independent pathway [104]. Another study evaluated the antihyperglycemic activity of *Citrus limetta* fruit peel in streptozotocin-induced diabetic rats and the results showed that hexane extract exerted significant hypoglycemic activity and the activity of extract was comparable to that of standard drug [105].

1.4.8. Citrus effect on body weight

Asnaashari et al. [106] investigated and reported that essential oil from *Citrus aurantifolia* prevents ketotifen (an antihistaminic drug that causes weight-gain) induced weight-gain in mice. Groups treated with *Citrus aurantifolia* essential oil showed decrease in body weight and food consumption, possibly through promoting anorexia which might have played a role in weight loss. The results reveal the potential of *Citrus aurantifolia* essential oil in weight loss and could be useful in treatment of drug-induced obesity and related diseases. Similarly, the effect of *Citrus aurantifolia* (fresh lime fruit juice) and honey on lipid profile fed different concentrations of cholesterol enriched diet, using rat model were investigated. During the experiment, groups were administered with lime alone, honey alone and mixture of lime and honey. Administration of lime alone resulted in significant decrease ($p < 0.05$) of LDL, TAG and TC and a significant increase ($p < 0.05$) in HDL and a corresponding weight loss compared to other groups [13].

1.4.9. Citrus effect on hypolipidemia

The effects of Lime Juice and Honey on Lipid Profile of Cholesterol Enriched Diet Fed Rat Model were investigated by [13]. The research investigated the effects of lime juice and honey on lipid profile of albino Wistar rats fed varying concentrations of cholesterol enriched diet. Results obtained showed that fresh undiluted lime juice, honey and mixture of lime juice and honey possess anti-inflammatory ability in preventing hypercholesterolemia, with effect greater in administration of fresh lime juice alone than in mixture of lime juice and honey. Another study using *Citrus medica* cv Diamante peel extract, showed a lowered plasma cholesterol and triglycerides in mice [107]. Demonty et al. [108] reported that tangeretin and nobiletin, with the optimal molecular structure, may lower blood cholesterol and triacylglycerol concentrations, whereas other citrus flavonoids without a fully methoxylated A-ring such as hesperidin and naringin may have virtually no or only weak lipid-lowering effects in humans. The effect of *Citrus aurantifolia* peel essential oil was studied on serum triglyceride and cholesterols in thirty Wistar rats of five groups. The results of experimental groups treated with peel essential oil in 50 and 100 μl/kg doses demonstrated a significant reduction in triglyceride, cholesterol, and LDL ($p < 0.01$) [109]. In a study of a high-fat fed $Ldlr^{-/-}$ mice, the addition of nobiletin resulted in a dramatic reduction in both hepatic and intestinal triacylglycerol accumulation, attenuation of very low-density lipoprotein(LDL)-triacylglycerol secretion and normalization of insulin sensitivity [110].

1.5. Citrus mineral, nutrients and vitamin contents

Citrus is loaded with appreciable mineral, nutrients and vitamins, especially the antioxidant vitamins contents. The nutritional content of carbohydrate, protein and fats in citrus fruits were reported to varied from 4.60 to 8.50, 5.80 to 7.90 and 2.50 to 9.50 g, respectively [111]. Katrine [112] reported some mineral value of citrus fruits as follows; calcium in citrus fruits ranges between 20 and 30 mg calcium/100 g and the iron content of citrus ranges from 0.2 to 0.4 mg/100 g. Obviously, citrus is generally not a good source of iron, however, iron is concomitantly released from other source of food owing to the high level of vitamin C content in citrus and citrus juices and therefore maintaining iron status [113, 114]. Consumption of orange juice or citrus foods with iron containing foods has been recommended by nutritionists for optimum iron absorption [115]. Low iron status has been reported to be one of the major deficiency challenges in Australia, particularly for adolescent girls and young women [112]. Citrus was reported to have a magnesium value of ranges between 8 and 11 mg/100 g, phosphorus value from 16 to 24 mg/100 g, a very low sodium content between 0 and 2 mg/100 g, a very low zinc content ranging from 0.1 to 0.2 g/100 g in citrus, copper value of citrus also very low to be between 0.03 and 0.05 mg/100 g, manganese value in citrus to be 0.01–0.03 mg/100 g, the content of the antioxidant element, selenium, ranges from 0.4 to 1.4 mg/100 g in citrus and the value of potassium in citrus fruits ranges between 120 and 145 mg/100 g potassium [116]. It is reported that fruits currently provide about 10% of potassium in the Australian diet daily [112].

The nutrients and non-nutrients contents of citrus fruits and juices products are wide spread. An assessment carried out in Australia by [112] on the composition of oranges, lemons, mandarins and grapefruit in relation to other common fruits and the composition of orange juice in comparison to soft drinks and sports drinks shows that the carbohydrate (sugar) content of citrus fruits ranges from 1.8 g/100 g for lemons to 4.8 g/100 g for grapefruit and about 8 g/100 g for oranges and mandarin. The values of carbohydrate in citrus and many other fruits assessed show a low glycemic index [117]. Protein content of citrus fruits ranges from 0.6 g/100 g for lemon to about 1 g/100 g for other citrus and generally, protein is low for all fruits assessed, ranging from 0.3 to 1.7 g /100 g [118]. While citrus fruits assessed for dietary fiber, ranged from 0.6 g/100 g (grapefruit) to 2.5 g/100 g (lemons) [112].

Assessment of citrus fruits vitamins reveals that citrus fruits have vitamin A value from 2 to 20 μg and vitamin A retinol equivalents of 10–130 μg betacarotene [112]. Citrus fruits vitamin C content ranges from 36 to 52 mg/100 g. Essentially, fruits are not known to be a rich source of vitamin E, a fat-soluble vitamin. However, the US data base, states that the vitamin E content of citrus is about 0.25 mg/100 g. Fruits are generally not a major contributor to the B vitamins, other than folate [118]. For vitamin B, citrus fruits content of thiamin range from 0.03 to 0.11 mg thiamin/100 g, riboflavin content in citrus is between 0.02 and 0.03 mg/100 g, niacin content in citrus ranges from 0.3 to 0.6 mg, vitamin B6 values in citrus was assessed to be between 0.04 and 0.08 mg and citrus seem to be a rich source of folate with the value ranging from 11 mg/100 g in lemons to 30 mg/100 g in oranges [112, 118]. Folate anticancer and protective effects against heart disease and spinal tube defects and its role in maintaining mental function have been reported [119, 120]. It was reported that a glass of orange juice of 225 ml provides about 75 mcg of folic acid [121].

2. Conclusions

Numerous therapeutic properties have been attributed to citrus fruits, like anticancer, anti-viral, anti-tumor, anti-inflammatory activities, and effects on capillary fragility as well as an ability to inhabit platelet aggregation. It is therefore established that the exploitative benefits of this plant are not unconnected to the active biochemical substances present in the plant in abundance. These bioactive substances (vitamins, phytochemicals, minerals and other nutrients) may act as antioxidants, which stimulate the immune systems; induce protective enzymes in the liver or block the damage of the genetic materials. From the review, it may be concluded that fresh citrus fruits juice offer better advantage thus, the best way to exploit citrus, especially the fruits parts is using it freshly **Table 1**.

	Orange	Grapefruit	Tangerine
Weight (g)	131	236	84
Energy (kcal)	62	78	37
Fiber content (g)	3.1	2.5	1.7
Ascorbic acid (mg)	70	79	26
Folate (mcg)	40	24	17
Potassium (mg)	237	350	132

Source: [122].

Table 1. Nutritional facts about citrus fruit.

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Section 2

Citrus Production

Citrus Industry in Brazil with Emphasis on Tropical Areas

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Additional information is available at the end of the chapter

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Abstract

This chapter is a review on citrus-producing areas in Brazil with emphasis on the tropical zone. Considering the degrees of latitude, the citrus industry can be classified into four major regions: (1) South, represented by the State of Rio Grande de Sul with temperate climate; (2) São Paulo, Minas Gerais and Paraná States in the central part of the country, where the major citrus-processing industries are established under subtropical conditions; (3) Amazon basin, the northern part of Brazil around the Equator; and (4) Northeast, the typical tropical region. For each region, data are presented as to the geographical position, climate and soils, area harvested production volume and yield. A strong climatic influence on fruit quality can be observed. There is a tendency for fruits to be smaller in size, but with a longer maturation and life periods on the trees, as they are produced at increasing distances from the equator.

Keywords: climate, latitude, scion and rootstock cultivars, fruit quality

1. Introduction

The history of the citrus industry in Brazil is intimately linked to its own history. Sweet orange seeds were introduced by the Portuguese jesuits 30 or 40 years after the discovery of Brazil (1500), in the States of Bahia and São Paulo. Due to favorable ecological conditions, the trees (as seedling) produced quite well. The activity remained unknown until the nineteenth century when, during the colonial period, the fruits of the 'Bahia' ('Washington Navel') orange, originated in the Bahia State, were recognized by the Portugal reign as being larger and juicier than those produced in that country. More important fact, however occurred after its introduction in California it was recognized as 'more important as the gold extracted from the soils of the Golden State' and considered as responsible for the development of the citriculture in the five continents. Nevertheless, only in the 1930s, the citriculture began to be implanted commercially in States of São Paulo, Rio de Janeiro and Bahia, with greater growth rate in the states of the Southeast. This chapter is a review on the Brazilian citriculture focusing the four main citrus poles (**Figure 1**) with their respective producing states and geographical locations, climate, harvested area, production and yield.

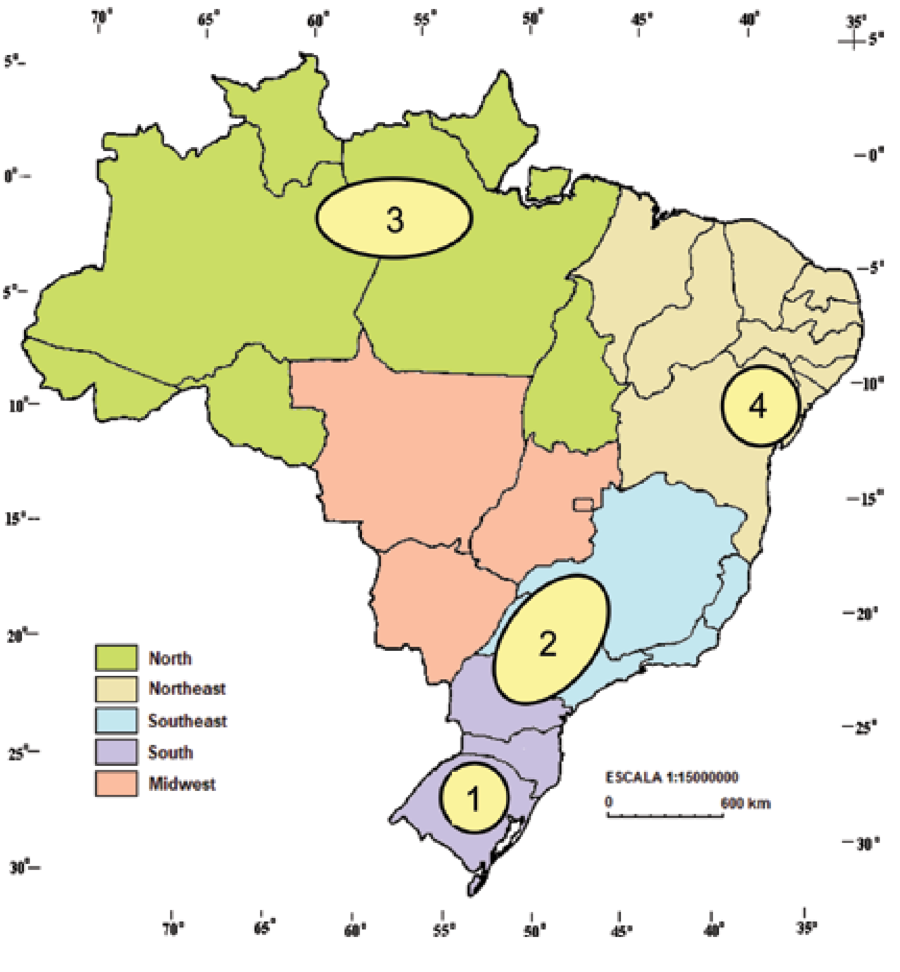

Figure 1. Map of Brazil with the physiographic regions and the main citrus poles. (1) South, represented by the State of Rio Grande de Sul (temperate climate), (2) São Paulo, Minas Gerais and Paraná States (central part of the country), (3) Amazon basin, represented by the States of Amazonas and Pará (equator region) and (4) Northeast, represented by the States of Bahia and Sergipe (typical tropical region).

2. Main citrus poles

There are no climatic limitations for citrus growing in Brazil. Irrigation is not necessary, except in the semiarid areas of the Northeast, where the rainfall is below 700 mm and in the south where frosts can occur. The altitude varies from 20 to 500 m. Rainfall varies from 1,000 to 1,800 mm, during the winter in the Northeast (March–August) and in the summer in the Southeast (September–March). In Rio Grande do Sul, the rainfall is almost monthly. The relative humidity is higher in the Northeast, where in the winter it almost reaches 100%, with the annual average being between 75 and 80%. The annual average temperature varies from 19°C in the South to 25°C in the Northeast. Independent of the area, flowering occurs in September, one or more times depending on the distance to equator. The farther from the equator, smaller are the fruits but they stay on the trees longer. The soils of the citrus-growing areas are sandy/loam, deep, well drained, but with poor fertility especially in phosphorous. Except the shallow soils of some areas, like the cocoa-growing area in Bahia, the humid Amazonian area or the loamy areas of the States of Paraná and São Paulo, where the coffee and the sugarcane are cultivated, there is an immense area which is available to the citrus industry in Brazil. In an analysis on the Brazilian territory (8.5 millions square meters), it would be possible to adopt a classification of the citriculture on four main citrus poles which are described as follows.

2.1. Citrus belt in the south region

The citrus production in the South of the country is represented by the State of Rio Grande do Sul, which is achieved in 2016, 553,372 tonnes, being the largest concentrations of sweet orange (71.5%) and mandarin (25.4%) [8].

There are 35 microregions in the State of Rio Grande do Sul, 34 of them produce citrus (**Figure 2**). The regions that most stand out are: Montenegro, Frederico Westphalen and Erechim. The microregion of Montenegro has its production concentrated in orange (45.8%) and mandarin (47.0%) and only 7.2% in lemon. The most important counties are Montenegro, Harmonia, Pareci Novo, Tupandi and São José do Hortêncio. From these, Montenegro highlights the production of mandarin. The second most important microregion is Frederico Westphalen, whose participation in the production was 91.9% of orange, 7.1% of mandarin and 1.0% of lemon. From the 27 remaining countries that compound the microregion, only 3 deserve special mention: Liberato Salzano, Planalto and Alpestre. The microregion Erechim comes next and it is composed of 30 counties, the most important ones being Aratiba, Itatiba do Sul and Mariano Moro.

2.1.1. Climatic characterization

In the State of Rio Grande do Sul, the latitudes varies from 27°14′56″ S in Alpestre to 30°53′27″ S in Santana do Livramento do Sul near to Uruguay. Longitudes varies from 53°02′06″ to 55°31′58″ W in the same municipalities. Annual media temperature varies from 19.8 to 18.4°C and the rainfall from 1,892 to 1,467 mm in the same municipalities. The climate of the State of Rio Grande do Sul is humid subtropical (or temperate). It is constituted by four reasonably

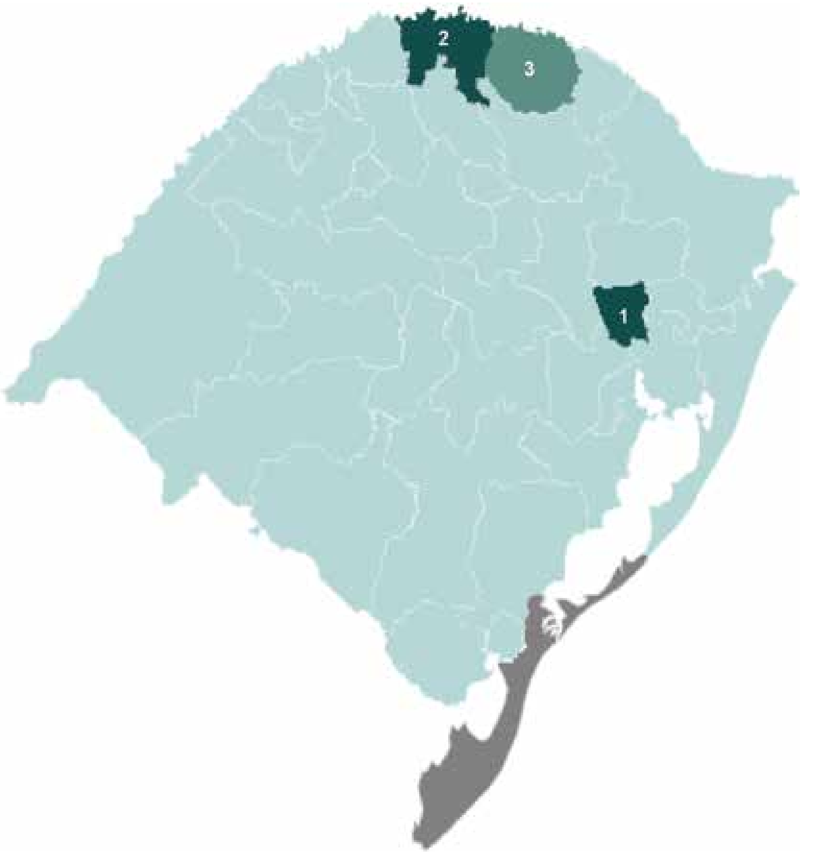

Figure 2. Concentration of the citrus production in Rio Grande do Sul in the principal microregions. Source: [8]. Obs.: Dark color means data not available.

well-defined seasons, with moderately cold winters and hot summers (mild in the higher parts), which are separated by intermediate seasons of approximately 3 months of duration and rains well distributed along the year. Due to its latitudinal situation (inserted in the context of the average latitude), Rio Grande do Sul presents peculiar features different from the climate of the rest of Brazil. The temperatures of the state, in diverse regions, are among the lowest ones of the Brazilian winters, reaching 6°C in cities like Bom Jesus, São José dos Ausentes and Vacaria, where frequent frosts and occasional snowfall happen, and where it is not recommended in the planting of citrus. There are still the altimetric differences, with special feature for Serra do Sudeste and Serra do Nordeste, which is not recommended for planting of citrus because of the high frequency of frosts. During autumn and winter, the state is also liable to the summer phenomenon, which consists of a succession of days with not normal high temperatures for the season. Different from the other states of Brazil, the occurrence of heavy frosts is relatively strong in the whole state demanding the usage of rootstock tolerant to cold.

2.1.2. Production characterization

The citriculture of the State of Rio Grande do Sul comprehends an almost complete chain, involving around 20,000 farmers, more than 100 nurserymen, producers of various inputs, beneficiators of fruit, industries of concentrated and ready to drink juices and of others byproducts of the fruit, wholesalers, marketers, retailers and around 11 millions of consumers. The annual production of orange is 396,000 tonnes (24,000 ha), of mandarin is 141,000 tonnes (11,000 ha) and of acid limes and true lemons is 17,000 tonnes (1,400 ha), being the state, respectively, the sixth, the fourth and the sixth greatest national producer of these fruits [8]. Even so, Rio Grande do Sul imports from others states, especially from Paraná and São Paulo, and from others countries, principally from Spain and Uruguay, almost 50% of the citrus which consume fruit and juice. The vast majority of the citrus growers is family-based, being the average planted area with citrus beneath two hectares per property. The business citriculture is conducted by less than a hundred producers located mainly in the regions of Vale do Caí and Campanha Gaúcha, with the cultivated area of 3–300 ha per property. The associativism is very practiced in Rio Grande do Sul, notably in Vale do Caí, wherewith the small citrus growers seek to overcome their limitations of production, mostly in the processing and in the marketing of the fruit. The principal poles of production are found in Vale do Caí, Campanha Gaúcha and in the northwest region of the state. The citriculture of the Vale do Caí exists for three generations, standing out, nowadays, for the production of mandarins. In the northwest region, the citriculture is much more recent and its expansion was supported by Emater – RS, it concerns small orchards where the orange tree Valência is primarily cultivated and good part of the fruits is destined to the industrial process. In the Campanha Gaúcha region, the production pole of seedless citrus is found. It was initiated in 1998 with the support of Embrapa Clima Temperado, where it is cultivated approximately 2500 hectares and the production is marketed in the principal supermarket network of the state and in others parts of the country. For cultivars, the region of the Vale do Caí detaches in the production of Montenegrina (principal), Caí and Pareci mandarins; the northwest region in orange tree Valência (principal) and Folha Murcha; and the Campanha Gaúcha in navel orange tree Navelina, Lane Late and Cara Cara (**Figure 3**), orange tree Salustiana, mandarin tree Okitsu and hybrids Ortanique and Nadorcott (**Figure 4**) and other varieties (**Figure 5**). The Trifoliata is the principal rootstock used, highlighting itself by the longevity of the plants, the tolerance to various diseases and to induce the high quality of the fruits. The system of conventional production is used in the great majority of citric properties. However, there are more than one hundred of organics products and practically the same number using the principles of integrated production. Among the main limitations of the culture, the phytosanitary nature ones are bounced. According to the producers, the black spot disease is outstandingly the biggest problem of the region of the Vale do Caí, followed by the citrus canker and by the brown spot of alternate. These two last diseases have been controlled especially by the usage of tolerant cultivars. The black pint and the brown spot of alternate do not exist up to the moment in the region of the Campanha Gaúcha, where the citric canker is the major limiting factor. These disease is endemic in the larger part of Rio Grande do Sul and it causes great losses notably in the rainy season. The handling of the disease has been conducted by means of spraying copper-based products and specific cultural practices to reduce source of inoculum. The Huanglongbing (HLB) has not been found in Rio Grande do Sul yet, according to the annual lifting accomplished by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) in partnership the Embrapa Clima Temperado. However, the vector insect exists in some regions. Although there are around 10 juice and citrus byproducts industries, the production is directed mainly to the market of fresh fruits, prioritizing the state demand.

Figure 3. 'Cara Cara' (navel) sweet orange in Rio Grande do Sul, the first producing Brazilian state for fresh consumption. Source: Roberto Pedroso.

Figure 4. Harvesting of 'Nadorcott' mandarin in Rio Grande do Sul. Source: Roberto Pedroso.

Figure 5. 'Nova' tangelo and 'Meyer' lemon in Rio Grande do Sul. Source: Roberto Pedroso.

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2.2. Citrus belt in São Paulo, Minas Gerais and Paraná states

The production of citrus in the principal citrus pole of the country encompasses the States of Minas Gerais, São Paulo and Paraná. The production from São Paulo, the most expressive one, is distributed in sweet orange, lemon and mandarin, with volumes of 12.8 million, 875,000 and 345,000 tonnes in 2016, respectively, in order of importance. In the State of Minas Gerais, the citrus production achieved 1,258,767 tonnes being 80% of orange and 13% of mandarin and 7% of lemon. The State of Paraná produced 922,422 tonnes of citrus—80% of orange and 20% of mandarin [8]. There are 63 microregions in the State of São Paulo, and from these, 25 microregions are the most important in the citrus production (**Figure 6**). Of these 25, the highest concentrations are in 7 microregions, in order of importance: Bauru, Avaré, São João da Boa Vista, Araraquara, São José do Rio Preto, Jaboticabal and Itapetininga. Lower concentrations occur in 18 microregions: Barretos, Botucatu, Mogi Mirim, Pirassununga, Itapeva, Novo Horizonte, Jales, Ourinhos, Catanduva, Limeira, Rio Claro, São Carlos, Franca, Lins, Fernandópolis, Jaú, Piracicaba and Sorocaba. Although Minas Gerais owns 66 microregions, just 2 of them stand out in the production of citrus: Frutal and Uberlândia. Both of them are part of the Triângulo Mineiro and in 2016 they produced about 414,000 and 259,000 tonnes, respectively. In the microregion of Frutal, the most important counties are Comendador Gomes and Frutal, while in the microregion of Uberlândia the counties that most highlight are Prata, Uberlândia and Monte Alegre de Minas. There are 39 microregions in Paraná, of which only 2 do not produce citrus. The two most important microregions in the citrus production are Paranavaí and Cerro Azul. Both microregions produce citrus, but Paranavaí presents the greatest volume in the orange production (99.4%) and Cerro Azul calls attention in the mandarin production (92.4%). The microregion of Paranavaí owns 29 counties, of which 9 do not produce any kind of citrus and the 3 that more stand out are Paranavaí, Guairaçá and Alto Paraná. In the microregion of Cerro Azul, two counties highlight, Cerro Azul and Doutor Ulysses, both of them concentrate their productions to the mandarin fruit.

2.2.1. Climatic characterization

Citrus trees are cultivated in São Paulo State often under mountain subtropical climate, that is, Cwa according to Köppen's classification. Considerable areas are in Cfa climate, and minor cultivation is carried under Aw and Cfb climates, respectively, on the coast and in the highlands. Considering Cwa as the prevalent condition, climate is characterized with hot, rainy summers, and dry, relatively cold winters. Two main climate types for citrus cultivation could be described: (i) mean annual air temperature higher than 17°C and annual water deficit of 0–60 mm and (ii) mean annual air temperature higher than 17°C and annual water deficit higher than 60 mm [16]. Minimal air temperatures are in the range of 8–10°C, and maximum can surpass 40°C. Annual rainfall ranges from 1,000 to 2,000 mm, often 1,400–1,800 mm, with distribution concentrated from November to March. Altitude ranges from 400 to 1000 m, but 550–750 m is prevalent. Citrus areas are free of severe frosts in São Paulo, even though it is regularly observed in the South of the State and in Paraná. Prolonged drought is frequent, especially on the North of São Paulo and in Minas Gerais State, as drought intensity decreases with the latitude. In recent years, heat stress associated to drought was reported in the main citrus areas in September–October, which is the period of the main blossom and fruit set.

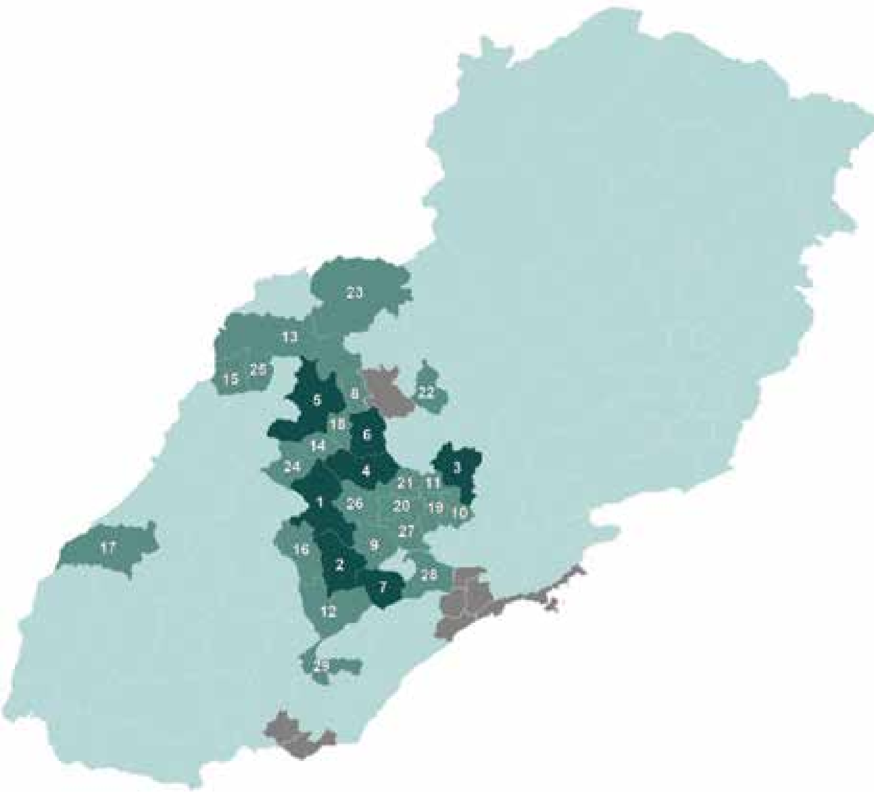

Figure 6. Concentration of the citrus production in the states of Minas Gerais, São Paulo and Paraná, concerning the principal microregions. Source: [8]. Obs.: Dark color means data not available.

2.2.2. Main producing areas

The citrus belt comprises the Northwest of Paraná State (23°04′ S–52°27′ S); the Triângulo de Minas Gerais region (19°18′ S–48°55′ S) and São Paulo State (21°49′ S–49°12′ S), which is divided in the following areas (as percentage of the total citrus area in this state): North (22%), Northwest (11%), Center (29%), South (20%) and Southwest (18%) [16].

2.2.3. Scion and rootstocks varieties

São Paulo, Minas Gerais and Paraná had about 430,000, 38,000 and 25,000 ha of sweet orange groves in 2016–2018, respectively [5, 6]. The main varieties are, in decreasing order, Pera (mid-season), Valencia (late), Hamlin (early), Natal and Folha Murcha (both late) and the early season varieties of Valencia Americana, Westin and Rubi, although the former four comprise more than 80% of the total trees. Some other varieties including navels and acidless oranges are cultivated in smaller areas. Persian lime and lemons are also cultivated mainly in São Paulo (39,000 ha in 2018), and mandarins, largely Ponkan mandarin and Murcott tangor, are important for all states (12,000; 8,000 and 10,000 ha for São Paulo, Minas Gerais and Paraná, respectively). Sweet oranges are produced mainly for juice processing, and the citrus belt represents more than 85%

of the Brazilian production. This is the most important orange production area in the world (34%) resulting in 56% of the juice produced and 76% of the marketed in the world [11]. Almost 97% of the juice is exported, while mandarins are for fresh fruit in the internal market, and limes and lemons are for fresh fruit and few processing, and exportation of fresh fruit too. Rangpur lime was the most used rootstock until the 2000s, as a result of its tolerance to both citrus tristeza virus (CTV) and drought, high and early yield, and great vigor and graft compatibility in the nursery. However, it is sensitive to citrus sudden death (CSD), blight, citrus nematode and gummosis of *Phytophthora* spp., and induces low juice quality, therefore after 2000, it has been increasingly replaced by the Swingle citrumelo. This rootstock is tolerant to all mentioned diseases. Despite being sensitive to drought, it induces high production of high quality juice [13]. Sunki mandarin is currently the third most used rootstock, especially for Pera since this scion in addition to Murcott and some selections of lemons are graft-incompatible with Swingle citrumelo. Cleopatra mandarin, trifoliate orange and Flying Dragon are used in a smaller amount. The Tropical selection of Sunki mandarin and a few citrandarins are been tested and used in increasing areas being considered promising rootstocks for the orchard diversification.

2.2.4. Other information

The citrus industry in São Paulo, Minas Gerais (**Figure 7**) and Paraná (**Figure 8**) employs more than 200,000 people and contributes with US$ 6.5 billion annually. Although about 6,000 farms cultivate oranges, 88% of the growers have less than 50,000 trees, while 12% of farms with more than 100,000 trees correspond to 77% of the total trees (194 millions) [6]. Therefore, the citrus cultivation in the citrus belt is nowadays a highly intensive, technological entrepreneurial activity. However, harvesting and fruit transportation reaches almost 50 of the production cost (**Figure 9**). Nursery stocks have been grown in insect-proof screen houses since 2003, and about 10 million grafted trees are produced annually in pots filled with potting media. Orchards use currently an average of 484 trees/ha, but new groves increased tree density to 656 trees/ha in average. About a third of the area is currently irrigated, and major cultivated area corresponds to trees from 5 to 15 years old. Citrus diseases and pests are major limiting factors to the citrus industry of the three states that substantially increase the

Figure 7. Ponkan mandarin orchard in Minas Gerais. Source: Eduardo Girardi.

Figure 8. Planting of citrus in Paraná. Source: Eduardo Girardi.

Figure 9. Harvesting of sweet orange in São Paulo, the first citrus-producing state in Brazil. Source: Eduardo Girardi.

production costs. Huanglongbing (HLB) is the most devastating one, and the average incidence in São Paulo and Triângulo de Minas Gerais was about 17% in 2017 [7]. The smaller the farm, the higher the incidence, because HLB management essentially depends on the eradication of symptomatic trees and on the control of the vector, the Asian citrus psyllid, in addition to control measures on inoculum sources outside the farm. As a result, management is more efficient if taken by all growers in an area wide approach. Other important phytossanitary problems include black spot, citrus canker, leprosis virus, citrus variegated chlorosis, citrus sudden death, post bloom fruit drop (*Colletotrichum acutatum* and *C. gloeosporioides*), *Alternaria* brown spot of mandarins, fruit flies, mites and scales.

2.3. Citrus belt in the north region (Amazon basin)

Considering the enormous area of Northern Brazil, citriculture is poorly exploited in this region, with the States of Pará and Amazonas showing the highest productions. From 270,370 tonnes of sweet orange, 53,806 tonnes of acid lime and 4,722 tonnes of mandarin, the State of Pará is responsible for 70.8% of sweet orange, 73.9% of acid lime and 20.9% of mandarin, while in the State of Amazonas these values are 14.9, 4.2 and 6.7%, respectively. Cultivated area comprises only 19,515 ha with the following distribution: 15,876 ha of sweet oranges, 3,054 ha of lemons/limes and 585 ha of mandarins. Average yield in these states is 14.1 t/ha indicating that the regional yield is about 54.6% of the national average (25.8 tonnes/ha).

2.3.1. Concentration of the production

All states in the North of Brazil produce citrus. However, Pará and Amazonas are highlighted once contribute with 70.6 and 13.0%, respectively, of the regional production, and these states rank in seventh and thirteenth position among Brazilian citrus-producing states. There are 22 microregions in the State of Pará, but citrus is cultivated in 17 of them. In this state, the two main citrus-producing areas are Guamá and Santarém. The former has the major concentration of citrus crops (sweet orange, lime/lemon and mandarin) (**Figure 10**). In the Guamá area, the greatest producer is the municipality of Capitão Poço, most notably with oranges, while in the Santarém area the production of lemon/lime is more important in the municipalities of Monte Alegre and Alenquer. The State of Amazonas has 13 microregions, and citrus production is mainly sweet orange cultivated in the Rio Preto da Eva microregion in the municipality with the same name.

2.3.2. Climatic characterization

The North region consists of the largest part of the Amazon Basin and it is characterized by low altitudes between 0 and 200 m. The climate is tropical equatorial with predominance of the type Af in the States of Amazonas and Acre, and of the type Am in the States of Pará,

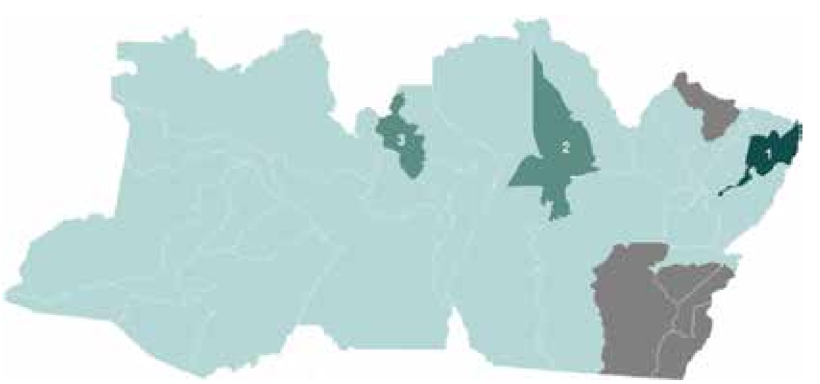

Figure 10. Concentration of the citrus production in the States of Amazonas and Pará, concerning the principal microregion. Source: [8]. Obs.: Dark color means data not available.

Amapá, Roraima and Rondônia. Only the State of Tocantins presents the climate type Aw. Atmospheric circulation systems, in the intertropical convergence zone, are responsible for the climate variability and for the rains in the state of the Amazon Basin. The average annual precipitation exceeds 2,000 mm, as until 3,000 mm in the estuary of the Amazonas River in Belém, and 2,400 mm in the innermost region of the Amazon Basin, in Manaus. In the direction of Roraima, East of Pará, there is less rainfall, with the annual total in the order from 1,500 to 1,700 mm. The rainy season in the greatest part of the region comprehends the period from December to May. During the rainiest months, March and April, precipitations of up to 400 mm monthly are reached. The 'dry season' from June to November still shows precipitations of 60–120 mm per month. Unlike, in the State of Roraima, due to the influence of the climatic conditions from the North hemisphere, the maximum rainfall indices occur in the period from April to September, with a longer dry season between October and March. Concerning the temperatures, the predominant climate is hot, with average annual temperatures varying from 22 to 28°C, average temperature of the coldest month of 18°C and maximum of 42°C in the hottest months. The temperatures are high in most of the region with low thermal amplitude except in some locals of higher altitude in Roraima and in Acre. In Rondônia, due to the entrance of cold air masses from the Atlantic Ocean, passing by the State of Mato Grosso, temperatures are reduced causing the phenomenon of 'coldness' for short periods of 5–6 days.

2.3.3. Production characterization

2.3.3.1. Citriculture in State of Pará

Considered as the largest citrus pole in the equatorial zone (Amazon basin), the citriculture of Pará is represented by the municipality of Capitão Poço in an area of 11,000 of hectares [4]. The fruit production, made by at least 1,000 growers, is destined for other states including for juice processing plants. It is reference as organic orange producer, being 70% of family farming, and the municipality restarted its certification process [9]. Different as compared to other producers in Brazil, the harvesting season occurs from September to December with a minor harvest in March and April. As in the Northeast region, the combination scion/rootstock cultivated is 'Pera' sweet orange × 'Rangpur' lime and the yield of orange and acid lime is very low, around 15 tonnes per hectare per year.

2.3.3.2. Citriculture in State of Amazonas

Oranges: in the orchards of the North region, there is a predominance of the 'Pera' sweet orange variety (**Figure 11**), even though some farmers produce the 'Valencia' in small scale. In spite of the good productivity and of the uniformity in fruit size of the 'Valencia', in the States of Amazonas and Roraima, there is a consensus among the producers in refusing this variety claiming that there is no market space for it. In smaller proportions, there are orchards with the 'Folha Murcha' variety. **Mandarins**: 'Murcott' and BRS Piemonte tangors and 'Mexerica do Rio' Mediterranean mandarin are the most commons in the North region. 'Tahiti' acid lime, especially the IAC-5 and 'Quebra Galho' clones, were initially used, and later substituted by the 'CNPMF 2001' clone. Although there are appropriate conditions, the productivity of mandarin is low. **Rootstocks**: since the period of the introduction of the citriculture in the

Figure 11. Big 'Pera' sweet orange tree in Amazonas. Source: Luciano Souza.

North region, the rough lemon, the 'Cleopatra' mandarin and the 'Rangpur' lime were used as rootstocks, being the last one the predominant in the regional orchards nowadays. In smaller proportion, we still may find 'Cleopatra' mandarin orchards formed 15 years ago. 'Rough' lemon, in small scale, has been used by some growers as rootstock. 'Swingle' citrumelo has just been used grafted with the 'Tahiti' acid lime. As a characteristic of equatorial conditions,

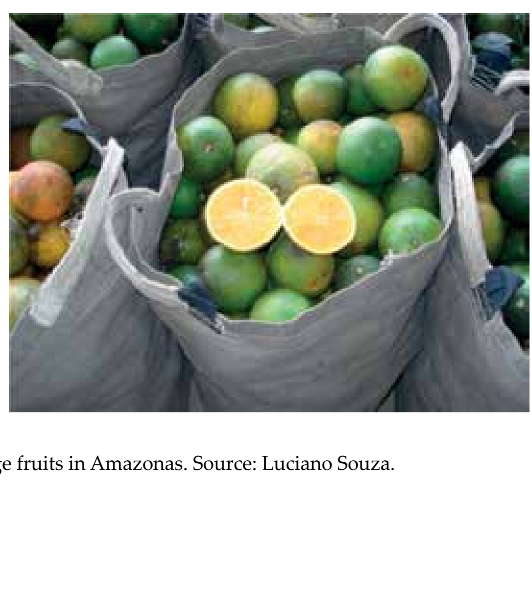

Figure 12. 'Pera' sweet orange fruits in Amazonas. Source: Luciano Souza.

the 'Pera' sweet orange in the Amazon region presents greenish fruits even in the maturation period, although it presents the relation Brix/acidity and pulp coloration adequate to the consumption (**Figure 12**).

2.4. Citrus belt in the northeast region

Sweet orange, lime and mandarin fruits are being produced in the states of the Northeast region, however the States of Alagoas, Pernambuco and Piauí do not produce mandarin. From the nine Northeastern States, Bahia and Sergipe stand out, with 66.2 and 26.1% of the regional production, and second place in the national production. From 1,744,673 tonnes of sweet orange, 169,123 tonnes of acid lime and 34,247 tonnes of mandarin, the State of Bahia is responsible for 64.8% of sweet orange, 88.1% of acid lime and 30.0% of mandarin, while in the State of Sergipe these values are 28.0, 4.9 and 30.3%, respectively. In these states, the citriculture occupies an area of 127,517 ha as follows: sweet orange 118,473 ha, acid lime 7,769 ha and just 1,275 ha with mandarin. The yield average is very low, just 14 tonnes/ha, representing almost half of the national average.

2.4.1. Climatic characterization

The Northeast region is located between 2 and 18° South latitude and 35° and 50° West longitude. The climate along the sea coast is hot and humid (tropical), with annual temperature average varying between 20 and 28°C and rainfall between 300 and 2,000 mm. The sunshine time varies from 2,300 per year in the humid areas up to 3,000 in the semiarid areas. The largest area in the Northeast is under semiarid conditions ('Polígono das Secas')—less than 750 mm of rain per year), The region comprises nine states: Maranhão, Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Sergipe and Bahia, that occupies 18.2% of the national territory. Analyzing the regions and its ecological diversity, in relation to the citrus trees, it is possible divide them in three grand zones: (1) sea coast (Coastal Tablelands) represented by the municipality of Cruz das Almas (BA); (2) area of altitude, represented by the municipality of Morro do Chapéu—Chapada Diamantina (BA), over 1,000 m of altitude and (3) semiarid zone, represented by the municipality of Petrolina (PE).

2.4.2. Production in traditional areas

The sea coast (Coastal Tablelands) is located along the sea, near the main capitals. The relative humidity is high and rainfalls around 1,000 mm per year but very concentrated during the summer time (December–March). Under these conditions, it predominates the sweet orange group represented almost exclusively by 'Pera' sweet orange (**Figure 13**), which fruit quality is typical in the tropical areas: larger fruits, juicier, less colored and less acid than those produced under subtropical conditions. More recently, the 'monocitriculture' of 'Pera' sweet orange × 'Rangpur' lime rootstock (almost 100% of the orchards) has been broken by the use of 'Tahiti' acid lime, unfortunately on the same rootstock. The fruit production destination is divided between the fresh fruit market and for processing (frozen concentrated juice). For a long time, Embrapa is stimulating the scion and rootstock diversification recommending as early varieties: 'Rubi', 'Westin' and 'Salustiana'; midseason: 'Pineapple', 'Pera' and 'Sincorá'; late: 'Natal', 'Valencia' and 'Folha Murcha' (Curled Leaf) [1], as well the following rootstocks: 'Indio', 'Riverside' and

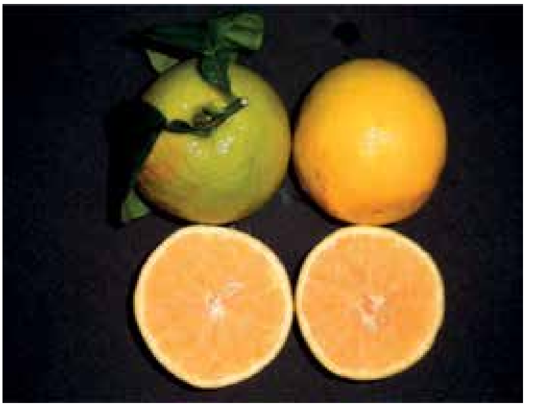

Figure 13. 'Pera CNPMF D-6' sweet orange in Bahia—The most popular variety in Brazil. Source: Orlando Passos.

'San Diego' citrandarins (from USDA), 'Sunki Tropical' mandarin and 'Santa Cruz Rangpur' lime. News rootstock hybrids are being released by the Embrapa Citrus Genetic Improvement Program [15] due to traditional areas that represent the Northeastern citriculture, the analyses on fruit productions will be concentrated in the States of Bahia and Sergipe. In Bahia, there are 32 microregions but only 4 can be considered as citrus producer: Alagoinhas, Santo Antonio de Jesus, Ribeira do Pombal and Entre Rios (**Figure 14**). From these, the first presents the largest citrus concentration represented by the municipalities of Rio Real, Inhambupe and Alagoinhas. In the Santo Antonio de Jesus microregion, sweet orange, acid and sweet lime and mandarin fruits are produced in the municipalities of Cruz das Almas, Sapeaçu, Muritiba, Governador Mangabeira and Cabaceiras do Paraguaçu as the most important. In Cruz das Almas, the largest participation comes from acid lime designated to the exporting market. Ribeira do Pombal microregion is concentrated just on sweet orange production and is located in a climatic transition zone which rainfall is less than 1,000 mm. The most important municipality is Itapicuru and its neighbors where exist the most appropriate conditions in the State for the citrus expansion, due to the existence of Tucano aquifer. In Entre Rios microregion, sweet orange production is concentrated in the municipalities of Esplanada and Jandaíra. In Sergipe State, there are 13 microregions being the most important, in a descending order, Boquim, Estância and Agreste de Lagarto. The Boquim microregion is most important in the production of sweet orange, mandarin and limes mainly in the municipalities of Itabaianinha, Cristinápolis, Salgado, Boquim, Arauá, Umbaúba and Tomar do Geru. In the microregion Estância, similarly, the citrus production is concentrated in the same groups. The main municipality producers are Santa Luzia do Itanhy, Estância and Indiaroba. Finally, Agreste de Lagarto microregion is predominated with the sweet orange and mandarin, mainly in the municipalities of Lagarto and Riachão do Dantas.

2.4.3. Production in potential areas

2.4.3.1. Altitude zone

It is located in the States of Bahia, Pernambuco, Paraíba and Ceará with milder climate, low temperatures in the winter (July is the coldest month), and insufficient rainfall for the culture

Figure 14. Concentration of the citrus production in the states of Bahia and Sergipe, considering their microregions. Source: [8]. Obs.: Dark color means data not available.

necessity, what requires complementary irrigation. Inside this ecosystem is the Chapada Diamantina tableland, whose altitude varies between 1,000 and 1,400 m. In this zone, the table fruits should be prioritized, preferably the mandarins without despising the seedless navel oranges ('Bahia' sweet orange). Among these fruits, some stand out: 'Cara Cara' ('Bahia' of red pulp), 'Baianinha' (litle Navel) and 'Lima' (no acidity). In the mandarins group, beyond the traditional 'Ponkan' and 'Murcott', special attention should be given to the tangelo mandarin tree 'Page' (seedless fruit, in isolated plantation), to the tangor mandarin tree 'Piemonte' (**Figure 15**) and to the BRS Salibe Murcott (fruits with few seeds).

2.4.3.2. Semiarid zone

The Brazilian semiarid is an ecoregion defined from the isoieta of 800 mm. The climate can be classified, according to Köepen, as type Bswh, which corresponds to a very hot semi-arid region. The annual rainfall index is 571.5 mm with concentration from December to March [2]. The average annual temperature is 26.4°C, with average minimum of 20.6°C, and with average maximums of 31.7°C. The daily thermal amplitude is around 10°C, monthly of 5–10°C and annually from 1 to 5°C; very strong insolation (annual average of 2,800 h/year);

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Figure 15. 'Piemonte' mandarin-tangor in Bahia—A new variety for fresh consumption. Source: Orlando Passos.

low relative humidity (annual average around 50% per year); and high evapotranspiration (average of 2,000 mm/year) [10, 14]. In areas of hot or tropical climate, like in the Northeast region, the amplitude is smaller, what implies in the fruit production of less coloring, not just inside but also outside. However, the contents of soluble solids (°Brix) are higher and present low acidity, resulting in sweeter fruits, but with the relation Brix/acidity unfavorable. It is worth pointing out that in hotter climates, like in the Northeastern semiarid, grapefruits and 'Tahiti' acid lime present a thin peel and a very colorful pulp, besides a great productivity, when compared to fruits produced in others regions of the country. It is important to accentuate that in citrus cultivation under high temperatures, the period between flowering and maturation is reduced, what enables anticipation of the harvesting in relation to the others producing areas. Although having a potential for grapefruit, lemons and acid limes, there are small areas producing citrus in the São Francisco Valley, specifically with 'Tahiti' acid lime (**Figure 16**) [12]. In 2016, the results were 204 ha of planted area with the yield of 26.2 t/ha, in the following counties: Juazeiro, Casa Nova, Sobradinho and Curaçá [8]. In this region, due to its production characteristics, it is recommended rootstocks that determine reduced size to the canopy, drought tolerance and fruits of good quality, like rootstocks hybrids obtained by Embrapa Citrus Breeding Program in crosses with 'Trifoliate' orange, 'Swingle' citrumelo and 'Troyer' and 'Argentina' citranges, among others. The citrandarins 'Indio', 'Riverside' and 'San Diego', obtained by USDA Citrus Breeding Program and recommended by Embrapa Mandioca e Fruticultura, are hybrids of 'Sunki' mandarin with trifoliate orange, and have been highlighting in the Northeastern citrus scenario, because of their citrus foot-rot tolerance and production of good quality fruits. In researches with citrus fulfilled by Embrapa Mandioca e Fruticultura in partnership with Embrapa Semiárido, in Petrolina-PE and in Juazeiro-BA, it was verified that the grapefruit and the 'Tahiti' acid lime behave well. The Flame grapefruit (**Figure 17**) present thin peel and deep flesh color and

Figure 16. 'Tahiti' acid lime in Bahia—In Ascension in the northeastern region. Source: Nilton Sanches.

fair balance between Brix/acidity, what are considered outstanding characteristics [12]. Some sweet orange varieties have great potential under semiarid conditions, as the clones C-21, D-9, D-12 and D-25 of Pera, and Rubi, Westin, Salustiana, Natal CNPMF-112 and Valencia Tuxpan, besides 'Page' and 'Piemonte' mandarin hybrids [3]. Examining the different climatic situations, it is possible to point out as competitive advantages of the Northeast region: (1) multiplicity of climates and soils and area availability; (2) geographic privileged localization in relation to the main markets (Economic European Community and United States of America) in comparison to the others citrus fruit producers regions in the country; (3) non-occurrence of bacterial diseases, like the HLB (huanglongbing, ex-greening) and the citrus canker and others like leprosis (not in endemic form) and the black spot, which are causing serious losses to the Brazilian southwest citriculture, mainly in the States of São Paulo, Minas Gerais and Paraná.

Figure 17. 'Flame' grapefruit in the São Francisco Valley—An option for the regional citriculture. Source: Orlando Passos.

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Citrus Water Use

J. Teunis Vahrmeijer and Nicolette J. Taylor

Additional information is available at the end of the chapter

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Abstract

Citrus is grown in semi-arid regions or subtropical regions in large parts of the world, where rainfall is seasonal and irrigation a necessity. Water is a vitally important element in all ecosystems and as agriculture is the largest user of fresh water resources, it needs to be efficient in the use of water. This is particularly true for the citrus industry, as it has a significant irrigation requirement. Good irrigation scheduling practices rely on accurate estimates of plant water-use (transpiration) for different climatic regions, citrus varieties, tree and canopy size, and choice of rootstock. This usually requires the use of a model, where a thorough understanding of the regulation of transpiration will improve the estimation capabilities of such a model. Results from our study (Quantifying citrus water use and water stress at tree and orchard scale, Water Research Commission Project K5/2275//4) showed that transpiration (T) follows diurnal and seasonal trends and is influenced by stomatal conductance (g_s) and leaf water potentials (Ψl). Good correlations between T and temperature, vapour pressure deficit (VPD) and solar radiation (SR) were found, indicating the importance of the environment in supplying the energy to drive transpiration. There was also a good relationship between canopy size and T, with larger canopies having higher T.

Keywords: transpiration, vapour pressure deficit, leaf water potential, stomatal conductance

1. Introduction

Citrus is an ancient crop, with the oldest known reference to be found in Sanskrit literature (pre-800 BC), where citron and lemon are referred to as *jambhila* in the book, *White Yahir-venda*. Twenty seven varieties of mandarins are described in Chü lu (1179 AD), one of the oldest known monographs of citrus [1]. Citrus trees are perennial evergreen plants that were probably cultivated in south-east Asia for the first time [2], from where it was introduced into North

Africa and Spain. Sweet oranges were brought to Europe by Portuguese seafarers and then spread via sea mariners and settlers to the rest of the world [1].

Citrus do not grow well in humid tropical rainforests and most likely evolved in low latitude forests, as a substory species in drier monsoon regions and became widely adapted to semi-arid regions [2]. Remnants of these earlier attributes that are still evident in some of the varieties are [3]:

i. Vegetative growth can readily assume dominance over reproductive development.

ii. Excessive foliar development, which can be up to 25% of the fresh tree mass.

iii. High stomatal density and low hydraulic conductivity as a result of a shallow suberized root system with only vestigial root hairs. This often results in potential transpirational losses exceeding the water uptake capacity of the root system.

Citrus orchards require irrigation, in most parts of the world, to avert lower yields and lower return on investments. With agriculture being the largest user of fresh water resources, climate change and competition for this already scarce resource from a number of end-users, emphasise the need to improve water use productivity and water use efficiencies [4] in citrus production. Reliable estimates of citrus water use are, therefore, essential to provide effective advice to both established and emerging commercial farmers on irrigation methods and scheduling.

Several reports on citrus water use exist. A prominent feature of these reports is the broad range of water use rates given, even scientific literature is full of contradicting values for water use of citrus trees. This large variation in reported values is not completely unexpected, due to the different measurement techniques used under a wide range of conditions, which includes [5–11]: different orchard characteristics and management practices, tree and canopy size, cultivars, rootstocks, climatic conditions under which the trees are grown, irrigation methods and available soil water content. At orchard level, water use is influenced by the change in citrus orchard management practices, such as the introduction of high density plantings, different pruning techniques and various micro-irrigation systems.

1.1. Citrus rootstocks and root development

Citrus has a well-defined taproot, however, its identity is often lost during the process of replanting or poor nursery practices [12]. The taproot is supplemented by lateral roots that branch and re-branch irregularly to form a dense mat in the soil surface layers. For mature citrus trees the greatest mass of fibrous roots occurs in the top 0.4 m of the soil profile, with structural roots extending to at least 1.5 m [3]. The extent of the root system is, however, dependent on soil physical properties, cultivar and rootstock [2]. Carrizo citrange and Swingle citrumelo are examples of rootstocks with few fibrous roots below 0.7 m and less lateral development [13] that are well suited for high-density, intensively managed plantings [14]. Root distribution, measured as fibrous root length density (FRLD), was determined for 'Hamlin' orange trees grown on Swingle citrumelo and on Carrizo citrange [15]. Results showed that Swingle citrumelo developed significantly higher FRLD in the top 0.15 m of the soil profile than trees on Carrizo citrange. Conversely, at a soil depth between 0.15 m and 0.75 Carrizo citrange had a greater FRLD than trees on Swingle citrange [15]. FRLD distribution increase in two modes. Firstly a dense root mat developed just below the soil surface with few roots deeper than 0.5 m at a distance of 1.5 m from the tree trunk. When these roots are well established, a second region of roots develop below 0.3 m from the soil surface (trees aged between 5 and 10 years). By the time the canopy reaches full hedgerow dimensions (trees aged between 10 and 15 years), the bimodality of the root system has fully developed [15].

One of the numerous factors influencing citrus water use include rootstocks that differ in root quantity distribution and/or efficiencies in water uptake and transport. Xylem vessel size is related to root hydraulic conductance, which affects water uptake and transport, which in turn influences the leaf transpiration rate [16]. Results from a study on the hydraulic conductivity of four rootstocks [17] showed that rough lemon and Carrizo citrange had the highest, whereas sour orange and Cleopatra mandarin had the lowest root conductivity and thus the lowest uptake and transport of water in the tree. During the last 30 years a major shift in rootstock and cultivars with better disease, drought and salinity resistance and dwarfing capabilities has taken place. For example in South Africa, 56% of the citrus trees were grafted on rough lemon and only 10% on Troyer and Carrizo citrange in 1986. In 2004 the use of rough lemon decreased to 12% and Troyer and Carrizo citrange increased to 45% [18]. This has implications for the water use of orchards, with less vigorous rootstocks having lower hydraulic conductances.

1.2. Water potentials, stomatal conductance and climate

Stomatal conductance (g_s) regulates transpiration and photosynthesis and therefore impacts directly on water use and is sensitive to environmental factors such as light, CO_2, plant water status, vapour pressure deficit (VPD) and temperature [3]. Leaf age, canopy size and tree age were found to influence stomatal conductances. New leaves on 15 year-old citrus trees have a greater stomatal conductance than old leaves. However, this was not true for smaller trees, where the stomatal conductance for the old and new leaves was similar due to the smaller trees having rough, well ventilated canopies, with a more exposed position that tightly couples them to the atmosphere [19]. Stomata in citrus leaves require only low light levels to open fully [19]. Even shaded leaves transpire, with their transpiration (T) rates being lower than sunlit leaves due to lower temperatures and thus a lower saturated water vapour pressure [20]. Stomatal conductances were observed to decline rapidly when midday leaf water potentials (Ψl) went below −1.0 MPa for 30 month old Pera orange trees [21], while the closure of stomata occurred at a midday Ψl lower than −2.2 MPa for Washington Navels [22]. Syvertsen [23] found that stomatal closure occurs over a relative narrow range of Ψl's within each age class of leaves, with stomatal closure occurring at −1.6 MPa for young leaves and for mature leaves (3–6 months old) at −3.6 MPa. Sinclair and Allen [24] also noted stable maximum rates of T regardless of environmental conditions, suggesting strong stomatal control over T.

2. Materials and methods

2.1. Description of orchards

Citrus orchards near Citrusdal (32°27′15.82″S, 18°58′44.84″E) in the Western Cape Province (winter rainfall region) of South Africa and close to Letsitele (23°48′21.48″S, 30°28′6.62″E) in the Limpopo Province (summer rainfall) of South Africa were used for measurements of T and water relations. The orchards in Citrusdal were drip irrigated, with two drip lines per tree row using pressure compensating emitters spaced 0.8 m apart with a discharge of 1.8 L h^{-1}. In Letsitele the orchards were irrigated with one 30 L h^{-1} microsprinkler per tree. Details are provided in the relevant sections.

2.2. Weather data

Hourly and daily weather data were obtained from the Campbell Scientific Automatic Weather Station (AWS) on the respective farms, which was installed over a short vegetated surface according to standard conditions specified in FAO 56 [25]. Irrigated orchards (2–3 m in height) were found within 10 m west, 60 m north, 30 m east and 50 m south of the AWS. The AWS was situated approximately 620 m from the 13-year old 'Midknight' Valencias and 2 km from the 5-year old 'Midknight' Valencias. For the 13 year-old 'Afourer' mandarin orchard, weather data was collected from a second Campbell Scientific AWS that was installed approximately 2 km from the orchard. Weather data for the 23 year-old 'Midknight' Valencia in the summer rainfall region (Letsitele) was obtained from QMS Laboratories™. The weather data was used to estimate the potential evapotranspiration (ET_o), using the Penman-Monteith method according to the FAO 56 procedure [25].

2.3. Root development

Profile pits were dug and soil/root samples were taken at three depths (0.2, 0.4 and 0.6 m) for a 5 year-old 'Midknight' Valencia orchard and at four depths (0.1, 0.2, 0.4 and 0.6 m) for a 13 year-old 'Bahianinha' Navel orchard. Samples were taken within the tree row close to the tree trunk, midway between the tree trunk and the canopy edge and at the canopy edge. Another set of samples were taken perpendicular to the tree row, between the tree rows close to the tree trunk, midway between the tree trunk and the canopy edge and at the canopy edge. Root samples were taken by gently tapping a metal cylinder of known volume into the soil. The edges of the metal cylinder were sharpened to facilitate the cutting of the roots. Samples were sealed in a plastic bag and transported to the laboratory. The sample was placed on a 1 mm sieve and the soil was removed by gently washing the roots under running water. The washed roots were collected and excess water was removed by placing the roots on absorbing paper. The fresh root mass of each sampling point was determined, after which the roots were then dried at 60°C and the dry mass recorded.

2.4. Water potential and stomatal conductance measurements

Leaf water potential was determined using a Scholander pressure chamber (PMS Instrument Company, Albany, USA) on three sunlit and three shaded leaves of three trees in the 'McLean' Valencia orchard (**Table 1**). Stomatal conductance was determined using an AP4 porometer (Delta-T Devices, Ltd., Cambridge, United Kingdom). Stomatal conductance was determined on three 'McLean' Valencia trees (**Table 1**), with three leaves on the east side of the tree, three leaves on the west and three shaded leaves on the inside of the canopy measured per tree. Both Ψl and gs measurements were made hourly, with Ψl measurements starting before sunrise until sunset. Due to the occurrence of dew on the leaves early in the morning, gs could

	'Mclean' Valencia
Age (years)	5
Rootstock	Swingle/Carrizo
Tree spacing (m × m)	3.0 × 5.0
Canopy cover	0.35
Orchard - LAI ($m^2\ m^{-2}$)	3.28
Canopy dimensions:	
Height (m)	2.53
Rainfall region	Winter
	Citrusdal, South Africa

Table 1. Details of 'McLean' Valencia orchard used for water potential and stomatal conductance measurements.

only commence once the leaf surface was dry. The hourly values of the Ψl and gs measurements were combined separately and the average for the tree was calculated.

2.5. Tree water use measurements

Tree water use was determined using the heat ratio method [26, 27] of the heat pulse velocity (HPV) technique. Four trees were selected per trial (**Table 2**) and four probe sets were installed per tree. The probe set installation depths varied according to trunk size, but were in general in the order of 10, 25, 35 and 50 mm. Each probe set consisted of a central 60 mm long and 1.8 mm thick stainless steel heater probe and two Type-T (copper-constantan) thermocouples installed 5 mm above and 5 mm below the heater probe. The probe sets were placed in the tree sapwood area via three vertically aligned and parallel holes drilled with the help

	'Midknight' Valencia			'Afourer' Mandarin
Age (years)	17	9	23	13
Rootstock	Troyer/Carrizo	Troyer/Carrizo	Carrizo	Swingle
Tree spacing (m × m)	2.5 × 5.0	3.0 × 4.8	7.0 × 3.0	2.0 × 5.0
Canopy cover	0.83	0.54	0.74	0.81
*Orchard LAI ($m^2\ m^{-2}$)	5.63	4.51	2.54	5.65
**Canopy dimensions:				
Height (m)	4.92	3.38	4.30	5.01
Rainfall region	Winter	Winter	Summer	Winter
	Citrusdal	Citrusdal	Letsitele	Citrusdal

*LAI Leaf area index is the average of 5 measurements; **Average of four selected individual trees.

Table 2. Orchard details of experimental site for tree water use trial sites in South Africa.

of a drill guide strapped to the trees. Petroleum jelly was used to ease probe insertion and maintain thermal contact between the probe and wood tissue [28]. Individual thermocouples were wired to an AM16/32B multiplexer (Campbell Scientific Inc., Logan, UT, USA). Heat pulse velocities were calculated at hourly intervals on a CR1000 logger (Campbell Scientific Inc., Logan, UT, USA). Heat pulse velocity was corrected using the wounding correction equations and a measured wound width for each orchard. The sap flux density and final sap flow volumes were calculated according to [28, 29]. The area represented by each probe was determined. The heat pulse velocity from each probe was then multiplied by the specific area represented by the probe, which yielded the volumetric sap flow per hour.

3. Results and discussions

3.1. Root distribution

The root distribution of a 6 year-old 'Midknight' Valencia tree and that of a 13 year-old 'Bahianinha' Navels are given in **Figure 1**. The soil of the 6 year-old 'Midknight' Valencias can be classified as a clay soil [30]. Field measurements and observations revealed that the

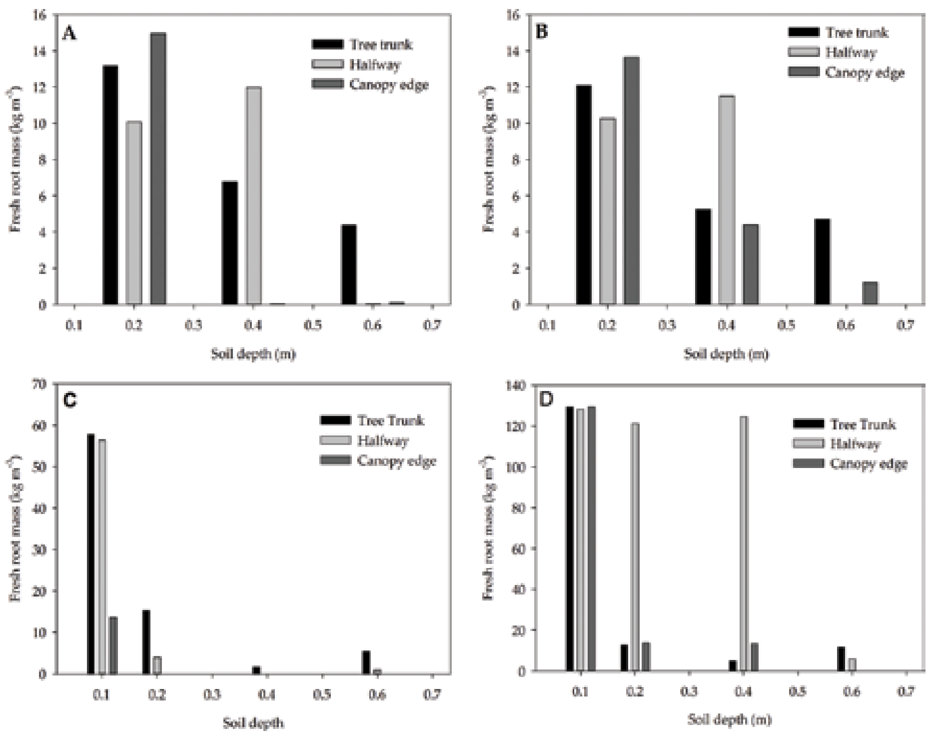

Figure 1. Root distribution (kg m^{-3}) between the rows (A) and within the row (B) in a 6 year-old 'Midknight' Valencia orchard grafted on Carrizo citrange rootstock. Root distribution (kg m^{-3}) between the rows (C) and within the row (D) in a 13 year-old 'Bahianinha' navel orchard grafted on Carrizo citrange rootstock.

roots of the 6 year-old 'Midknight' Valencias were thinner than the roots of the 13 year-old 'Bahianinha' navels. A typical bimodal distribution of the roots was also evident, with most of the roots (>60%) within the top 0.2 m of the soil surface and a less dense root mass at 0.4 m (**Figure 1A** and **B**). Ninety five percent of the roots were in the top 0.4 m. For the samples taken close to the tree trunk and at the canopy edge, the root mass decreased with soil depth within the tree row and between the tree rows. More roots were found at the 0.4 m sample depth, halfway between the tree trunk and canopy edge than at the 0.2 m sample depth (**Figure 1A** and **B**).

The soils of the 13 year-old 'Bahianinha' navel orchard consisted of coarse sand (0.5–2.0 mm) with no stones. Most of the roots (61%) were found within the top 0.1 m of the soil and more than 80% in the top 0.2 m, forming a dense mat under the drippers within the tree row. Substantially fewer roots were found within the work row. Roots were also in general thicker (2.7 mm) with larger structural roots at a depth of 0.4 m (**Figure 1C** and **D**) and substantially more than for the 6 year-old 'Midknight' Valencias (**Figure 1A** and **B**). This distribution was not unexpected, as citrus fibrous root length, in well-drained sandy soils, is a function of tree size, soil depth, distance from the trunk and rootstock [15]. The fibrous roots of young trees (canopy volume < 5 m^3) develop just below the soil surface, with more than 85% of the roots within 0.5 m of the soil surface. As the canopy volume (5–15 m^3) increases the fibrous roots close to the soil surface grow laterally towards the dripline of the tree, that extends to 2 m between tree rows and to a depth of 0.9 m, with a further increase in canopy volume (>15 m^3) [15]. The distribution and density of roots impacts water and nutrient uptake of the trees and could influence the maximum T rate based on the rate at which water can be supplied to the leaves.

3.2. Climate as a driving force for citrus water use

It is custom to use VPD to describe evaporation from a leaf or soil surface, because it can be expected that the humidity at the leaf or soil surface is less than 1.0 and the vapour concentration is less than the saturation concentration. However, evaporating surfaces of most leaves have a humidity close to 1.0 and it can generally be assumed that the vapour concentration at the leaf surface equals the saturation vapour concentration if the air and surface temperatures are the same. For more detail on the discussion on the relation between liquid- and gas-phase of water in biological systems, the reader is referred to [31].

For this discussion of T, water potentials in a 'McLean' Valencia orchard were either calculated or measured to illustrate the direction of water flow. Thus, evaporation from a leaf (T) and soil surface is driven by a water potential gradient between the atmosphere and the leaf or soil surface. In **Figure 2** the water potential at the soil surface (−0.4 MPa), leaves (−1.68 MPa) and atmosphere (−176 MPa) was calculated for a 'McLean' Valencia orchard, in a winter rainfall region, for a summer's day (February) and a winter's day (July). Because of the steep water potential gradient between the leaves and atmosphere, citrus trees regulate the opening and closing of the stomata to prevent excessive water loss [32, 33]. An interesting observation is that although the atmospheric demand was substantially higher during summer (−176 MPa) than winter (−130 MPa) the measured Ψl's did not differ substantially (**Figure 3**). This is

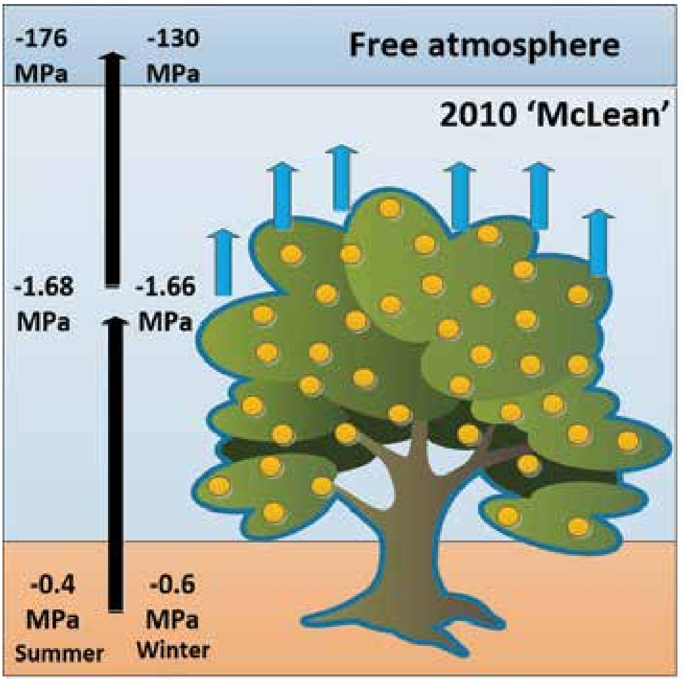

Figure 2. Measured water potentials for a 'McLean' Valencia orchard in a winter rainfall region (Citrusdal, South Africa).

probably due to strong stomatal control of T rate and therefore Ψl [34, 35], which is typical behaviour for an isohydric plant [36].

Leaf water potentials and g_s measured for a 'McLean' Valencia orchard are at their highest early in the morning and decreased during the day, with the Ψl reaching a minimum just after midday (**Figure 3**).

In conceptualising the inter-dependence between Ψl and g_s (**Figure 3**), at daybreak when the sun rises and the leaves are exposed to radiation from the sun, the stoma open [19, 20] and

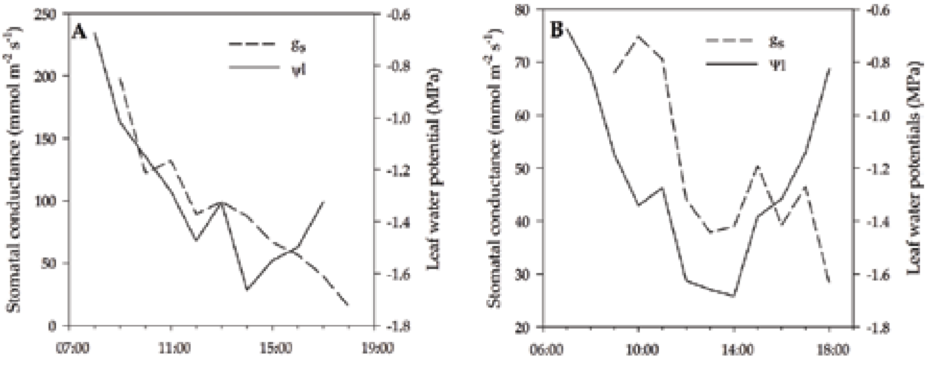

Figure 3. Measured stomatal conductance's (g_s) and leaf water potentials (Ψl) for a 5 year-old 'McLean' Valencia orchard during the (A) winter and (B) summer season.

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water vapour moves through the stoma (stomatal conductance). As the plant loses water (T) the Ψl decreases relative to the soil, which establishes the gradient for water movement within the plant or sap flow. As water loss cannot exceed the rate of uptake, the plant must have some control over the resistance to water loss and this is achieved by regulating stomatal conductance [37]. Therefore as VPD increases during the day, stomata start to close to prevent a decline in Ψl which would cause cavitation and a loss of xylem integrity. Water continues to move into the tree, due to the water potential difference between the soil and the tree (roots, stem and leaves) causing the Ψl to increase in the afternoon. Water movement from the soil into the tree will cease when the water potential in the roots equals the soil water potential, which generally occurs in the early hours of the morning.

The important role stomata play in controlling 0l is clearly illustrated in the differences between the winter and summer measurements in **Figure 4**. A much lower stomatal conductance in summer resulted in very similar minimum Ψl's in summer and winter, which reflects a much bigger vapour pressure gradient out the leaf at this time due to a hotter and drier atmosphere.

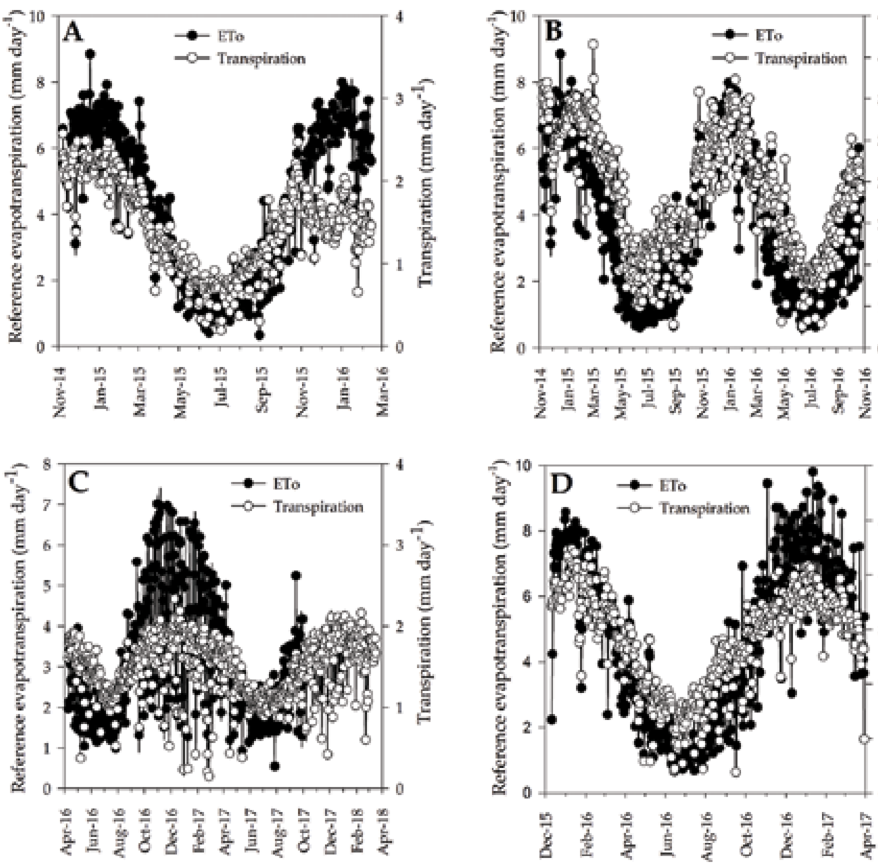

Figure 4. Daily transpiration (T) and reference evapotranspiration (ETo) for (A) a 17 year-old 'Midknight' Valencia, (B) a 9 year-old 'Midknight' Valencia in a winter rainfall region, (C) for a 23 year-old 'Midknight' Valencia in a summer rainfall region and (D) 13 year-old 'Afourer' Mandarin in a winter rainfall region.

Results from multi-seasonal T measurements (**Figure 4**), 717 days for a 17 year-old, 677 days for a 9 year-old, 707 days for a 23 year-old 'Midknight' Valencia orchard and 475 days for a 13 year-old 'Afourer' mandarin orchard, show large day-to-day variation in T. A clear seasonal trend is also evident with higher T rates recorded during the summer and lower T rates recorded during the winter season for all measuring sites. Daily T closely follows ETo, indicating that the prevailing climatic conditions influence T, as expected. Transpiration deviated from the ETo trend for the 17 year-old 'Midknight' Valencia (**Figure 4A**), because the orchard was heavily pruned. The pruning reduced the LAI of the measuring trees by 30% and T by 52% compared to the corresponding period of the previous season. This clearly indicates that reducing canopy volume during periods of drought can be used as a technique to reduce tree water use. For the 23 year-old 'Midknight' Valencia orchard (**Figure 4C**) the daily T did not follow ETo as distinctly during the mid-summer period (November 2016–March 2017) compared to the orchards in the winter rainfall region, due to a severe drought that resulted in the implementation of irrigation restrictions [38].

The daily ETo for the 17 and the 9 year-old 'Midknight' orchards (**Figure 4A**, **B**) ranged between 0.34 and 8.85 mm day^{-1}. For the 23 year-old 'Midknight' Valencia (**Figure 4C**) in the summer rainfall region ETo ranged between 0.54 and 7.01 mm day^{-1}, while for the 13 year-old 'Afourer' Mandarin in a winter rainfall region the ETo ranged between 0.66 and 9.81 mm day^{-1} for the measurement period. A summary of the T measurements for the different orchards is given in **Table 3**. Transpiration ranged from 0.3–3.7 mm day^{-1} in the 17 year-old (**Figure 4A**), 0.1–2.1 mm day^{-1} in the 9 year-old (**Figure 4B**), 0.15–2.33 mm day^{-1} in the 23 year-old 'Midknight' Valencia orchard (**Figure 4C**) and 0.4–4.5 mm day^{-1} for the 13 year-old 'Afourer' mandarin orchard (**Figure 4D**) over the measurement period (**Table 3**). For the 'Midknight' orchards, the lowest daily T measurement (0.10 mm day^{-1}) was recorded in the 9 year-old 'Midknight' orchard, and the highest

	Transpiration				
	Total	**Maximum**	**Minimum**	**Average**	**Measuring period**
	mm (L)	**mm day^{-1} (L day^{-1})**			**days**
'Midknight' Valencia					
Winter—17 year-old	1295	3.70	0.30	1.80	717
	(19417)	(54.8)	(4.0)	(27.0)	
Winter—9 year-old	684	2.10	0.10	1.10	677
	(10256)	(31.0)	(1.6)	(16.0)	
Summer—23 year-old	1037	2.30	0.15	1.46	707
	(21767)	(49.0)	(3.1)	(30.7)	
'Afourer' mandarin					
Winter—13 year-old	1325	4.5	0.4	2.8	475
	(13248)	(44.9)	(38.1)	(27.8)	

Values in brackets indicate litres.

Table 3. 'Midknight' Valencia water use in a winter and summer rainfall region and 'Afourer' Mandarin in a winter rainfall region.

(3.70 mm day^{-1}) in the 17 year-old orchard (**Table 3**). The highest daily T (4.5 mm day^{-1}) was recorded in the 13 year-old 'Afourer' mandarin orchard. The higher T values for the 17 year-old 'Midknight' orchard can be attributed to the larger canopy of the 17 year-old 'Midknights' compared to the 9 year-old Midknight orchard as demonstrated by the LAI and canopy cover (**Table 2**). In general the average daily T reflected the canopy cover. The impact of canopy size on tree water use can be substantial, with the average daily T of the 17 year-old 'Midknight' being 39% higher than the average daily T of the 9 year-old 'Midknight' orchard (**Table 3**). However, the impact of the drought in the summer rainfall region (23 year-old 'Midknight') resulted in lower T measurements, due to the reduction in irrigation [38] and therefore the average daily T measured for this orchard was lower.

It is evident from these results that tree water use is also influenced by specie. The LAI, canopy cover and canopy dimensions of the 17 year-old 'Midknight' orchard are comparable to the

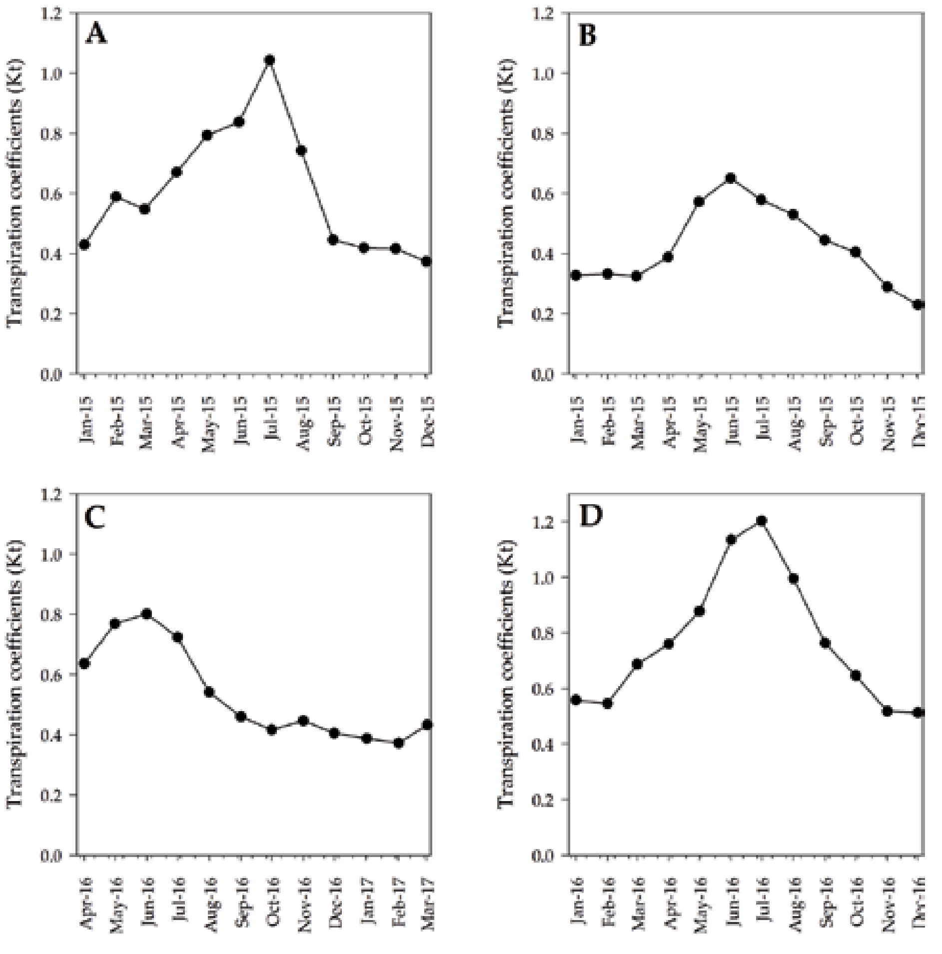

Figure 5. Transpiration coefficients for (A) a 17 year-old 'Midknight' Valencia, (B) a 9 year-old 'Midknight' Valencia in a winter rainfall region, (C) for a 23 year-old 'Midknight' Valencia in a summer rainfall region and (D) a 13 year-old 'Afourer' Mandarin in a winter rainfall region.

13 year-old ‘Afourer’ orchard (**Table 2**). But, the multi seasonal daily T average (**Table 3**) for the 13 year-old ‘Afourer’ orchard (2.8 mm day^{-1}) is 36% higher than the 17 year-old ‘Midknight’ orchard (1.80 mm day^{-1}). This difference in tree water use between the two citrus species may be attributed to leaf anatomical and morphological differences [3].

Monthly transpiration coefficient (Kt = T/ETo) values were calculated for the different orchards for a full season (**Figure 5**). The Kt values varied substantially for the different ‘Midknight’ Valencia orchards (**Figure 5A–C**) and ranged from 0.37–1.04 (17 year-old ‘Midknight’ Valencia), 0.22–0.65 (9 year-old ‘Midknight’ Valencia) and from 0.37–0.80 for the 23 year-old ‘Midknight’ Valencia in the summer rainfall region. For the ‘Afourer’ mandarin orchard, the monthly Kt values ranged from 0.49–1.2 (**Figure 5D**). Average monthly Kt values showed a similar trend between all orchards, with the maximum values calculated for the winter and the lowest for the summer seasons. The proportional relationship between water use to canopy size was also evident, with the 17 year-old ‘Midknight’ Valencia and ‘Afourer’ mandarin orchard (**Figure 5A**, **D**) having the largest canopy cover (**Table 2**) and generally the highest Kt values for the same month. The orchard with the lowest canopy cover (9 year-old ‘Midknight’ Valencia) also have the smallest Kt values (**Figure 5D**) when compared for the same month.

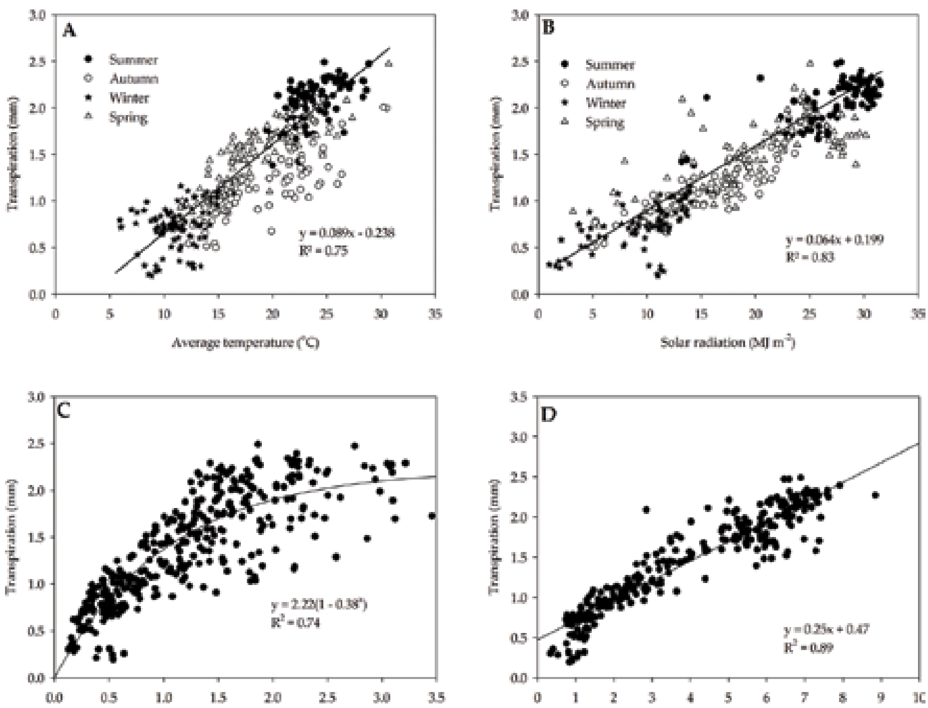

Figure 6. Relationship between transpiration (mm) and (A) average temperature (°C), (B) solar radiation (MJ m^{-2}), (C) average vapour pressure deficit (VPD, kPa) and (D) reference evapotranspiration (ETo, mm) for the 9 year-old ‘Midknight’ Valencia in a winter rainfall region.

A typical response of citrus to average daily temperature, solar radiation, average daily VPD and ETo is given in **Figure 6**. The response of transpiration to increases in temperature (**Figure 6A**), solar radiation (**Figure 6B**) and ETo (**Figure 6D**) was linear in the 9 year-old 'Midknight' Valencia orchard, with a clear seasonal trend is evident. The lower daily average temperatures and solar radiation in winter that corresponds with the lower T values are grouped together at the bottom of the graph and the higher daily average temperatures and solar radiation in summer that corresponds with higher T values are grouped at the top of the graph. The T in the transition seasons (autumn and spring), which represents a wider range of daily average temperatures and solar radiation, fills the region between the winter and summer values. From the relationship between VPD and T it seems if the upper limit of T was influenced by VPD. Thus, in the mornings, when VPD is still low, a linear relation between solar radiation and T exist, however, as the day progresses the VPD increases and becomes the limiting factor for T, which overrides the influence of radiation. Transpiration seems to reach a maximum once VPD had exceeded 3.0 kPa.

4. Conclusion

Results from the research showed that transpiration (T) followed diurnal and seasonal trends and was influenced by stomatal conductance (g_s) and leaf water potentials (Ψl). Good correlations between T and temperature, vapour pressure deficit (VPD) and solar radiation (SR) were found, indicating the importance of the environment in supplying the energy to drive transpiration. A good relationship between canopy size and T, with larger canopies having higher T was evident and should be taken into account with the planning of irrigation infrastructures (convey to field and on-field delivery) and irrigation scheduling. Especially the cumulative effect over a season must be considered when planting new orchards. Provision in the allocation of water should be made for when newly planted orchards with smaller canopies, which use substantially less water, develop mature canopies.

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Conflict of interest

Both authors have no conflict of interest in conducting this research.

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Flowering and Fruiting Haploid and Doubled Haploid Pummelos

Masaki Yahata and Hisato Kunitake

Additional information is available at the end of the chapter

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Abstract

Haploid and doubled haploid (DH) plants are of great value for genetic analyses and premeditated breeding. This is especially true for woody species, which are generally characterized by a long reproductive cycle, a high degree of heterozygosity, a large plant size, and self-incompatibility. In *Citrus* and related genera, some haploid and DH plants have been produced by techniques such as anther culture, interploid hybridization, and the pollination of irradiated pollen. However, there are few reports of the characteristics of haploid and DH plants' flowers, fruits, or reproductive potential. We selected a haploid progeny among small seed-derived seedlings obtained from 'Banpeiyu' pummelo [*C. maxima* (Burm.) Merr.], and we produced the DH plant of this haploid using colchicine-treated axillary shoot buds. Both this haploid pummelo and the DH pummelo showed normal growth and produced many flowers and fruit. In this chapter, we describe about the morphological characteristics and the reproductive potential of the haploid pummelo and the DH pummelo.

Keywords: first division restitution (FDR), homozygosity, reproductive function, unreduced gamete

1. Introduction

Haploid and doubled haploid (DH) plants are of great value for genetic analyses and developmental studies, as well as for premeditated plant breeding [1–5]. Technologies using DH plants also enhance the effectiveness of the selection of desired recombinants, especially when quantitative traits are evaluated [6]. This is the case for fruits, which are generally characterized by a long reproductive cycle, a high degree of heterozygosity, a large plant size, and self-incompatibility. In *Citrus* and related genera, triploid somatic hybrids can be obtained through the fusion of haploid protoplasts [7, 8] although one of the method for producing seedless cultivars is the use of triploids [9–12].

Several haploid induction methods such as *in vitro* androgenesis induced by anther culture, *in vitro* and *in situ* gynogenesis induced by pollination with irradiated pollen, and followed by the application of new anti-microtubule herbicides for chromosome doubling, have been described in the literature [1, 13, 14].

In *Citrus* and related genera, haploid seedlings were first obtained by the application of γ-rays in natsudaidai (*C. natsudaidai* Hayata) [15]. Esen and Soost [16] described a haploid embryo obtained from an immature seed of clementine mandarin (*C. clementina* hort. ex Tanaka). Since then, haploid plants have been produced by anther culture [17–20], interploid hybridization [21–23] and the pollination of irradiated pollen [24–28]. However, these haploids were very weak and grew more slowly than the original diploid plants. To date, the flowering haploids are only a haploid of clementine mandarin by gynogenesis *in situ*, induced by irradiated pollen [26], and the flowering and fruiting of haploids have rarely been reported. The available information on the reproduction of haploids is also quite limited.

Reports regarding DH plants' production are very few in number. DH plants have been induced only by anther culture in clementine mandarin [19] and sweet orange [*C. sinensis* (L.) Osbeck] [20, 29], and by the pollination of irradiated pollen in Clementine mandarin [26]. Detailed information regarding the morphological characteristics and the reproductive potential of the DH plants in *Citrus* and related genera have not yet been reported.

Our research group selected a haploid progeny among small seed-derived seedlings obtained from the 'Banpeiyu' pummelo, and we produced the DH plant by using colchicine-treated axillary shoot buds of the haploid pummelo. Both the haploid and DH plants continue to grow normally, and they flowered and fruited. In this chapter, we present the morphological characteristics and the reproductive potential in the haploid pummelo [30–33] and the DH pummelo [34, 35].

2. Production of the haploid pummelo and DH pummelos

We selected a haploid (2n = x = 9) from among small seed-derived seedlings obtained from the cross between 'Banpeiyu' pummelo and 'Ruby Red' grapefruit (*C. paradisi* Macfad.) (**Figure 1A**–**C**) [22]. The haploid was confirmed to be derived from female gamete of 'Banpeiyu' pummelo by molecular biological techniques: isozyme (**Figure 1D**), random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) analyses. This haploid pummelo also showed dwarf growth behavior and rosette morphology, similar to that of the haploids obtained from other methods [17, 21, 25]. However, it grew very well while maintaining the haploidy when it was grafted onto trifoliate orange [*Poncirus trifoliata* (L.) Raf.]. The tree growth habit of the haploid pummelo showed intermediate between upright and spreading (**Figure 2A**). Seven years after germination, the haploid pummelo had many flowers for the first time (**Figure 2B**) [30]. Three years after achieving reproductive growth, the haploid pummelo bore fruits for the first time (**Figure 2C**) [33].

Chromosome doubling of the haploid pummelo was achieved with colchicine treatment of axillary shoot buds of the haploid [34]. Many shoots with cytochimeras (X + 2X and 2X + 4X)

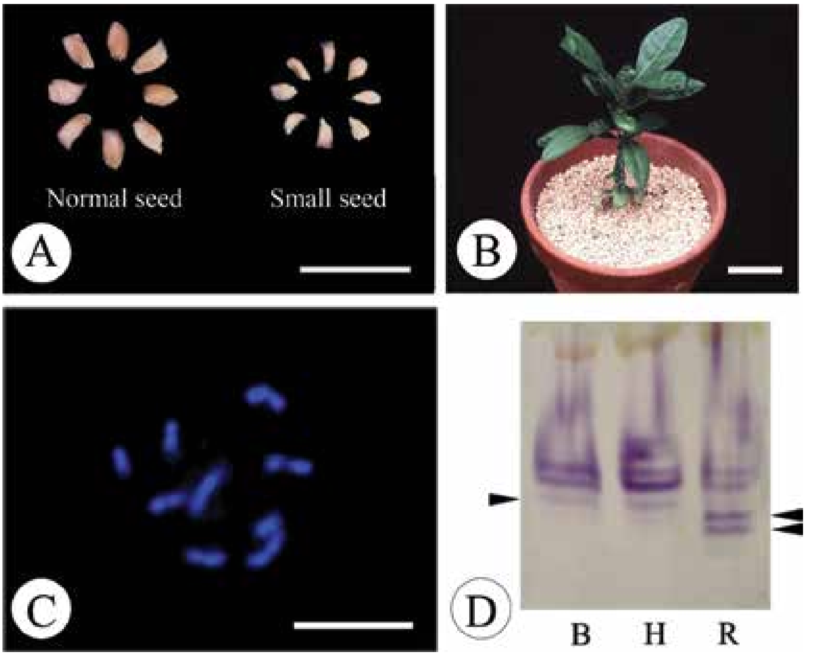

Figure 1. Production of the haploid pummelo [22, 30]. A: Normal (left) and small (right) seeds obtained from the cross between 'Banpeiyu' pummelo and 'Ruby Red' grapefruit. Bar = 5 cm. B: Initial growth of haploid plant, one year after grafting. Bar = 3 cm. C: The chromosomes of young leaf cells (2n = x = 9) Bar = 10 μm. D: Zymogram patterns of shikimate dehydrogenase (SADH) in 'Banpeiyu' pummelo (B), the haploid (H), and 'Ruby Red' grapefruit (R).

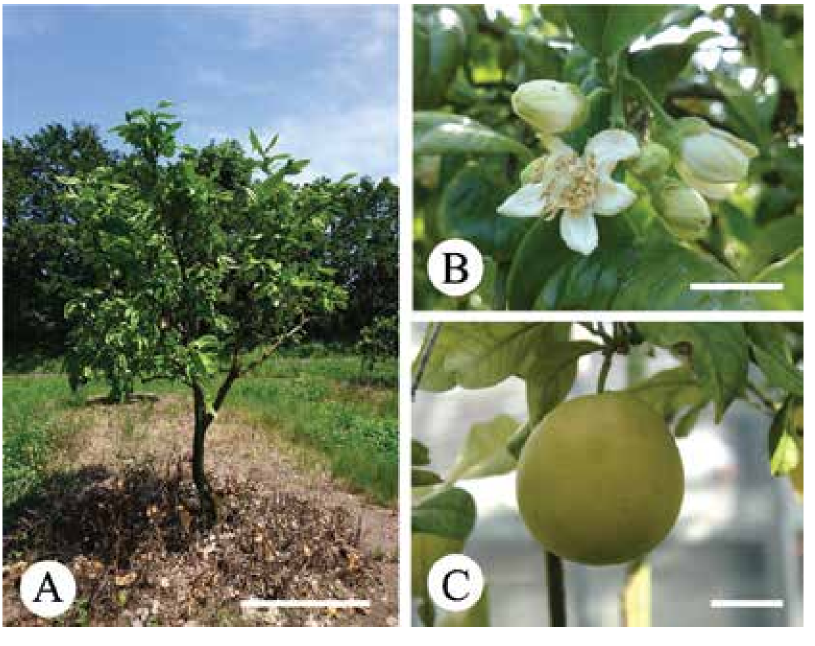

Figure 2. The haploid from among small seed-derived seedlings obtained from the cross between 'Banpeiyu' pummelo and 'ruby red' grapefruit [22, 33]. A: 10-year-old haploid tree. Bar = 30 cm. B: Flowers. Bar = 3 cm. C: Fruit. Bar = 5 cm.

arose from the colchicine-treated axillary buds. When cytochimeric buds of 2X + 4X were top-grafted onto trifoliate orange, a complete diploid shoot with 18 chromosomes was obtained from the cytochimera (**Figure 3A**, **B**). This DH pummelo produced thorns, and it showed vigorous growth compared to the original haploid pummelo. The tree growth habit of the DH pummelo showed spreading similar to that of 'Banpeiyu' pummelo (**Figure 4A**). The DH pummelo also produced many flowers and fruit for the first time at 5 years after the top-grafting onto trifoliate orange (**Figure 4B**, **C**) [35]. Moreover, thorns of the DH pummelo disappeared with advancing age.

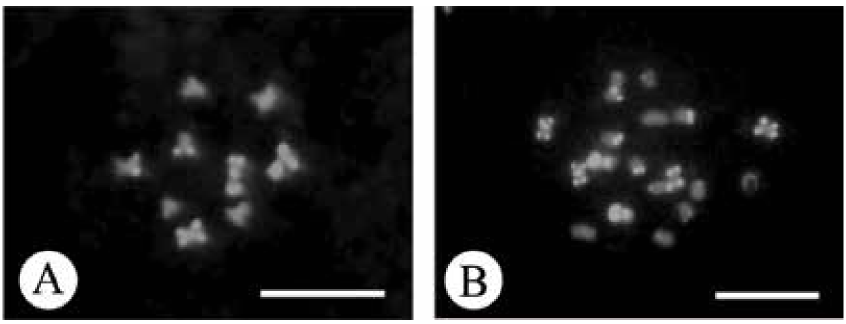

Figure 3. Photographs of the chromosomes with chromomycin A_3 banding patterns of the haploid (1A + 1B + 1C + 2D + 4E) and the DH pummelo (2A + 2B + 2C + 4D + 8E) [34, 52]. A = two telomeric bands and one proximal band, B = one telomeric and one proximal band, C = two telomeric bands, D = one telomeric band, E = no band. Bars = 10 μm.

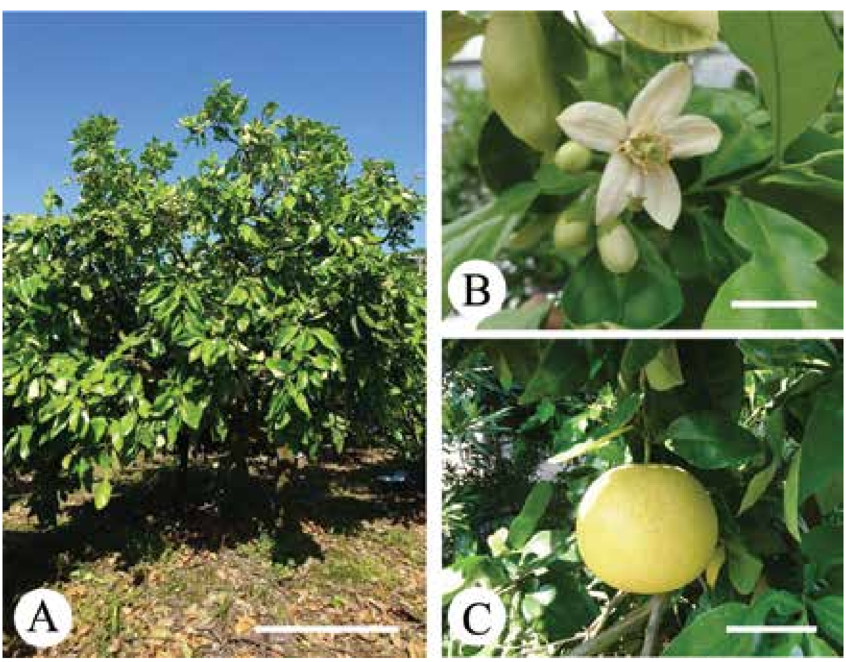

Figure 4. The DH induced by colchicine-treated axillary shoot buds of a haploid plant from 'Banpeiyu' pummelo [35]. A: 15-year-old DH tree. Bar = 100 cm. B: Flowers. Bar = 3 cm. C: Fruit. Bar = 10 cm.

3. Morphological characterization of the haploid and DH pummelos

The leaves of haploids of fruit crops tend to be smaller than those of diploid plants [2, 36, 37]. Haploids of trifoliate orange, mandarin, tangor and tangelo also show rosette morphology with small leaves in *Citrus* and related genera [17, 21, 25]. Although the flowering of haploids has rarely been reported for fruit crops, the morphology of haploid flowers has been reported in peach (*Prunus persica* Batsch) and clementine mandarin. These haploids had smaller flowers than the original diploids, and they shed very few pollen grains [26, 36–38]. In peach haploids, fertile pollen grains were observed [37, 38]. Among fruit crops, the fruiting of haploids have been observed only in peaches [37, 38]. Hesse [38] reported that two genotypes of haploid peaches showed very small fruit compared to the original diploid plants. Pooler and Scorza [37] found that five out of seven genotypes of haploid peach had fruits that were smaller than those of the original diploid cultivar, whereas the other two genotypes produced large fruits with fertile seeds.

Our group [30, 32, 33, 35] observed that the haploid pummelo had small, narrow, and lightness leaves compared to those of 'Banpeiyu' pummelo (**Figure 5A**). The guard cell size of the haploid was also significantly smaller than that of 'Banpeiyu' pummelo. The haploid formed raceme inflorescence (**Figure 2B**). The flowers of the haploid were approximately half the size of those of 'Banpeiyu' pummelo (**Figure 5B**). In addition, the haploid had a significantly reduced number of stamens and ovules compared to those of 'Banpeiyu' pummelo. In the flowers of the haploid, moreover, abnormalities such as the adhesion of pistils and stamens were rarely observed. Regarding the morphology of the pollen grains, most of pollen grains of 'Banpeiyu' pummelo were elliptical in shape (**Figure 6A**), whereas the shape of the pollen grains of the haploid showed severely depressed morphology; these pollen grains were thus presumed to be sterile, although a few normally shaped pollen grains from the haploid were also observed (**Figure 6B**). The average size of the pollen grains from the haploid was smaller than that of the grains from 'Banpeiyu' pummelo. While the 'Banpeiyu' pummelo showed a 97.5% acetocarmine-stainability rate, the haploid rate was only 14.1% (**Figure 6D**, **E**), and the haploid had slightly fertile pollen grains. The fruit weight of the 'Banpeiyu' pummelo was approx. 1800 g, whereas that of the haploid pummelo was only approx. 200 g, or about 11% that of 'Banpeiyu' pummelo (**Figure 5C**). The number of seeds per fruit obtained from 'Banpeiyu' pummelo was approx. 100, whereas the haploid had no seeds. Whereas the 'Banpeiyu' pummelo showed low parthenocarpy and rarely produced seedless fruits, the development of the haploid's fruit might be caused by parthenocarpy. We are planning detailed studies of the expression of parthenocarpy in the haploid pummelo.

Details of the morphology of DH plants have rarely been reported for fruit crops, although DH plants of several species have been produced, e.g., kiwifruit, apple, banana, sweet cherry, peach, and Japanese pear [1, 2]. Several DH plants of apple were produced by *in vitro* androgenesis and *in situ* parthenogenesis, and their morphology and reproductive potential have been reported [39–41]. Those studies showed that most of the DH apple lines had smaller leaves, flowers and fruit than the original diploid cultivars, and some of these DH lines also showed aberrant morphology of flowers.

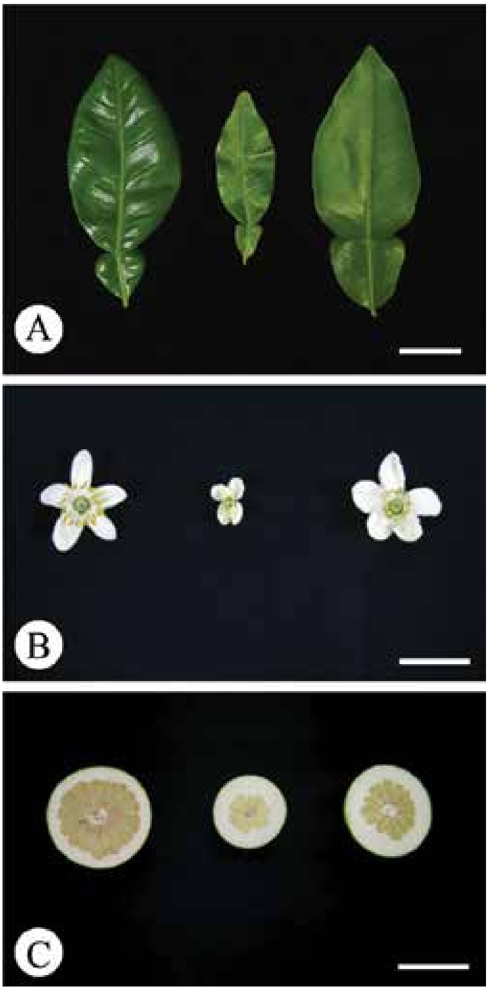

Figure 5. The morphological characteristics of leaves (A, Bar = 10 cm), flowers (B, Bar = 3 cm) and fruit (C, Bar = 10 cm) in 'Banpeiyu' (left), the haploid (center) and the DH (right) pummelo [35].

The sizes of the leaves and guard cells of the DH pummelo were almost equal to those of the 'Banpeiyu' pummelo (**Figure 5A**). The inflorescence of the DH plant was also raceme (**Figure 4B**). The flower organs of the DH showed normal morphology. The DH plant' flowers were larger than those of the haploid, and no difference in flower size was observed compared to those of the 'Banpeiyu' pummelo (**Figure 5B**). However, the DH had a reduced number of locules and ovules per ovary (approx. half) compared to that of the 'Banpeiyu' pummelo. The pollen fertility of the DH (an acetocarmine-stainability rate of ca. 85.0%) was a bit lower than that of 'Banpeiyu' pummelo (**Figure 6C**, **F**). The fruit size of the DH was approx. 900 g, which was approx. Half that of 'Banpeiyu' pummelo (**Figure 5C**). The number of seeds per fruit obtained from the DH plant was significantly less than that of the 'Banpeiyu' pummelo at approx. 60. Moreover, there was no difference among the haploid, the DH and the 'Banpeiyu' pummelos in terms of Brix and the titratable acidity of the fruit juice [35].

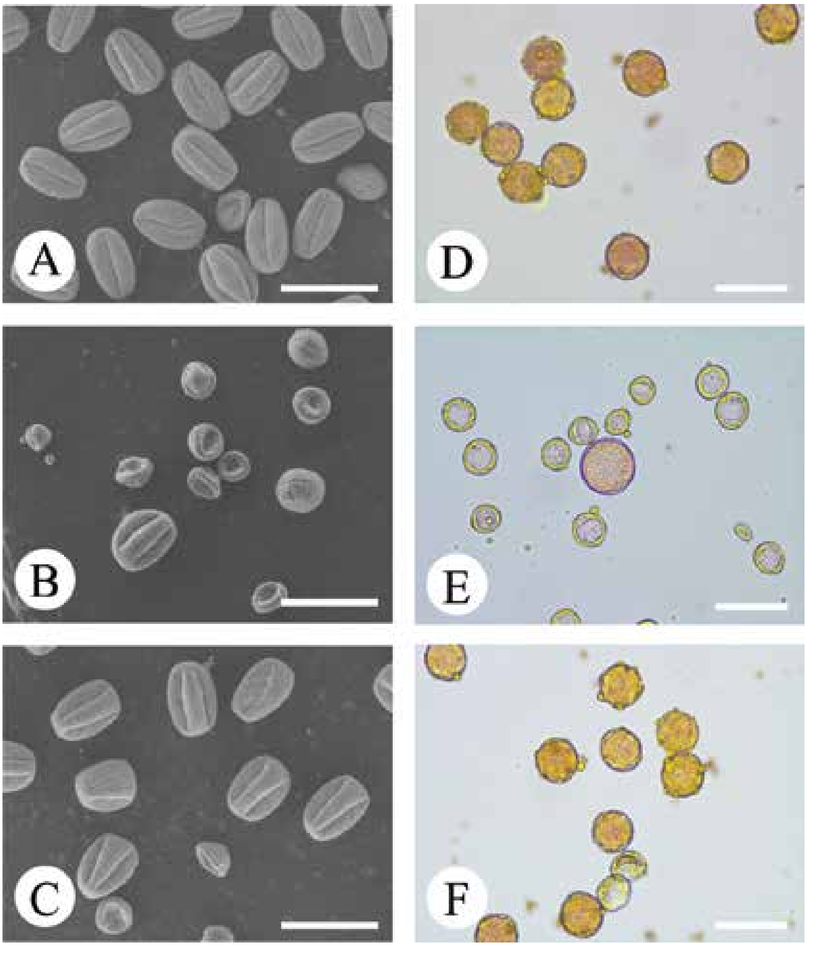

Figure 6. Micrographs of scanning electron (A-C) and stainability by 1% acetocarmine (D-F) in pollen grains of 'Banpeiyu' (A, D), the haploid (B, E) and the DH (C, F) pummelo. Bars = 30 μm.

4. Evaluation of the reproductive potential of male and female gametes in the haploid and DH pummelos by cross pollination

We carried out crosses with some diploid cultivars in order to evaluate the reproductive potential of the haploid and DH pummelos [31, 35]. When the haploid was the seed parent, no fruit set followed the pollination of the haploid with the pollen of diploid cultivars, because all flowers dropped within a month after pollination despite the crossing to the inflorescence with leaves. In the crosses with the haploid as pollen parents, conversely, fruits were set and some developed seeds were obtained. The developed seeds obtained from these crosses germinated almost normally, and their seedlings grew vigorously and developed large wing leaves, which is typical of the haploid (**Figure 7A**). The ploidy level of these seedlings was diploid with 18 chromosomes (**Figure 7B**). This result reveals that fertilization occurred between the normal eggs of diploid cultivars and pollen grains with nine chromosomes from the haploid.

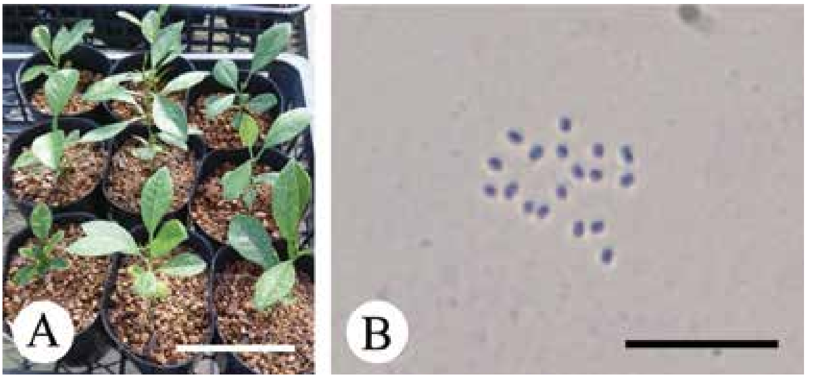

Figure 7. The seedlings obtained from the crosses between the diploid cultivars and the haploid (A, Bar = 10 cm) and the metaphase chromosomes in a root tip cell in one of the seedlings (B, 2n = 2x = 18, Bar = 20 μm).

In the reciprocal crosses between the DH and diploid cultivars, in contrast, when the DH was used as the seed and/or pollen parent, fruit and developed seeds were obtained compared to those of the haploid. Most of these developed seeds showed normal germination, and all of the seedlings examined were diploid [35]. In apple, it was difficult to use the DH lines as breeding materials because most of them had low and/or no reproductive potential, and no or only a few progeny were obtained in their cross combinations [39–41]. Our DH pummelo has no problem in term of the reproductive potential of female and male gametes.

4.1. Cause of the sterility of female gametes in the haploid pummelo

No fruit set followed the pollination of the haploid with the pollen of diploid cultivars in the reciprocal crosses between the haploid and some diploid cultivars. In *Citrus* species, the formation of embryo sacs is incomplete at the flowering stage, and the sacs remain at the two- or four-nucleate stage until the mature embryo sacs are formed at 3 or 4 days after flowering (DAF) [42]. We used the paraffin-sectioning method to observe the process of female gamete formation [32]. The formation of the embryo-sac mother cell (EMC) was detailed in the ovules at 1/4 of the size of flower buds (SOFB) of the 'Banpeiyu' pummelo (**Figure 8A**, **B**). Subsequently, the initiation of meiosis and tetrad formation were observed at 1/3 SOFB and 2/5 SOFB, respectively (**Figure 8A**, **B**). Approx. 20% of the ovules contained EMCs or further developed embryo sacs. The, embryo sacs then developed rapidly at the flowering stage (**Figure 8A**, **B**), and embryo sacs at the two-nucleate stage were observed at 3/4 SOFB. Eight-nucleate mature embryo sacs were formed in the flowers at 2 DAF (**Figure 8A**, **B**), at a frequency of approx. 25%.

In the haploid pummelo, in contrast, no EMCs were formed throughout flower bud development, and no embryo sac was formed in the flowers at 2 DAF (**Figure 9A**, **B**). We concluded that the lack of EMC formation was responsible for the complete sterility in the haploid pummelo. Regarding the morphology of the inner and outer integuments of the ovules, moreover, that of the haploid showed abnormalities such as detached growth of the integuments from the nucellar tissue, and the formation of a void between the inner and outer integuments (**Figure 10A**–**C**) [32]. These morphological abnormalities of the ovules has also been observed in the haploid plant of the clementine mandarin [26].

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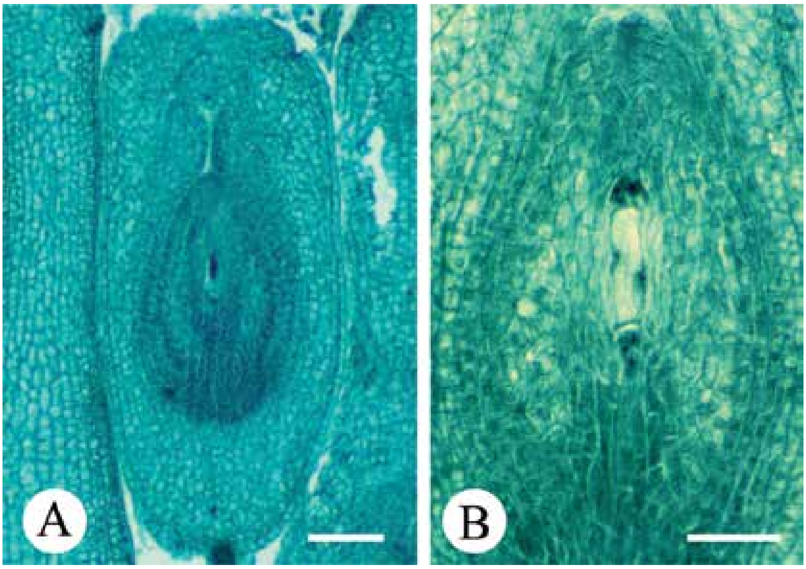

Figure 8. Ovule morphology and embryo sac development in 'Banpeiyu' pummelo [32]. A: Ovule morphology of 'Banpeiyu' pummelo at 2 days after flowering. Bar = 100 μm. B: Eight-nucleate embryo sac in at 2 days after flowering. Bar = 50 μm.

4.2. Formative mechanism of fertile pollen grains in the haploid pummelo

We observed the process of male gamete formation by the squash method [32]. The male meiosis of the 'Banpeiyu' pummelo occurred normally (**Figure 11**). In the first meiotic division at prophase I, duplicated chromatin condensed (**Figure 11A**), and condensed chromosomes were

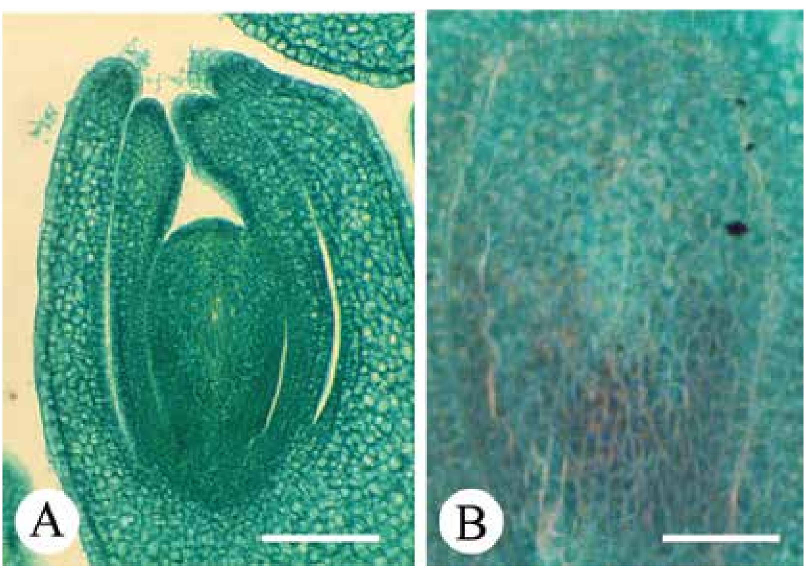

Figure 9. Ovule morphology and embryo sac development in the haploid pummelo [32]. A: Ovule morphology at 3/4 size of flower bud. Bar = 100 μm. B: No embryo sac at 2 days after flowering. Bar = 50 μm.

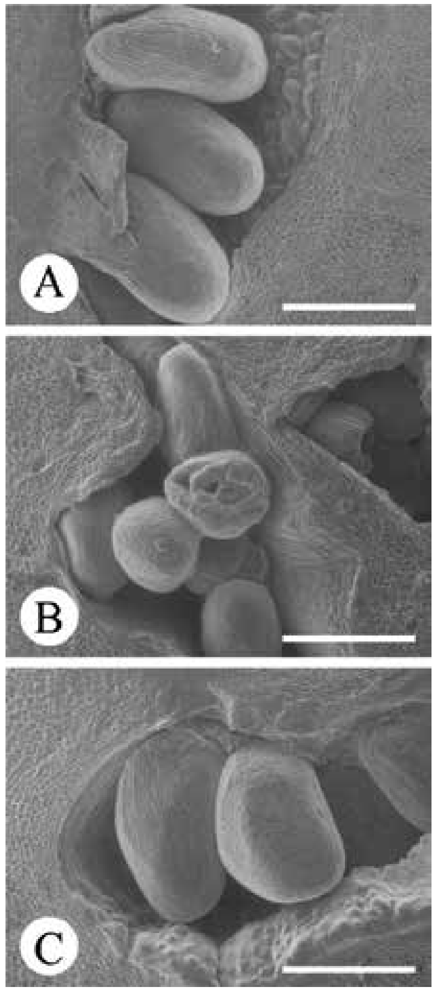

Figure 10. Scanning electron micrographs of ovule morphology in 'Banpeiyu' (A), the haploid (B) and the DH (C) pummelo. Bars = 500 μm.

visible. At metaphase I, homologous chromosomes aligned at the equatorial plate, and nine bivalents were observed (**Figure 11B**). The bivalents separated into univalents and migrated towards each pole at anaphase I (**Figure 11C**, **D**). In the second division, the chromosomes aligned at the equatorial plate at metaphase II (**Figure 11E**), and the chromatids migrated towards each pole separated at anaphase II (**Figure 11F**, **G**). Consequently, the 'Banpeiyu' pummelo predominantly produced normal tetrads (99.2%) with four microspores of equal size (**Figure 11H**).

In the haploid pummelo, meiotic division also occurred twice in the pollen mother cell (PMC), but abnormalities were observed in most dividing cells (**Figure 12**). Although nine univalents aligned on the equatorial plate at metaphase I (**Figure 12A**, **B**), they migrated unequally to each pole (**Figure 12C**, **D**). In the second division, their chromatids also migrated separately to each pole (**Figure 12E–G**). Another type of abnormal division was also observed in some meiocytes (**Figure 13**), in which all of the univalent chromosomes remained near the equatorial plate without distributing to either pole at anaphase I (**Figure 13A**, **B**). In addition, the nine

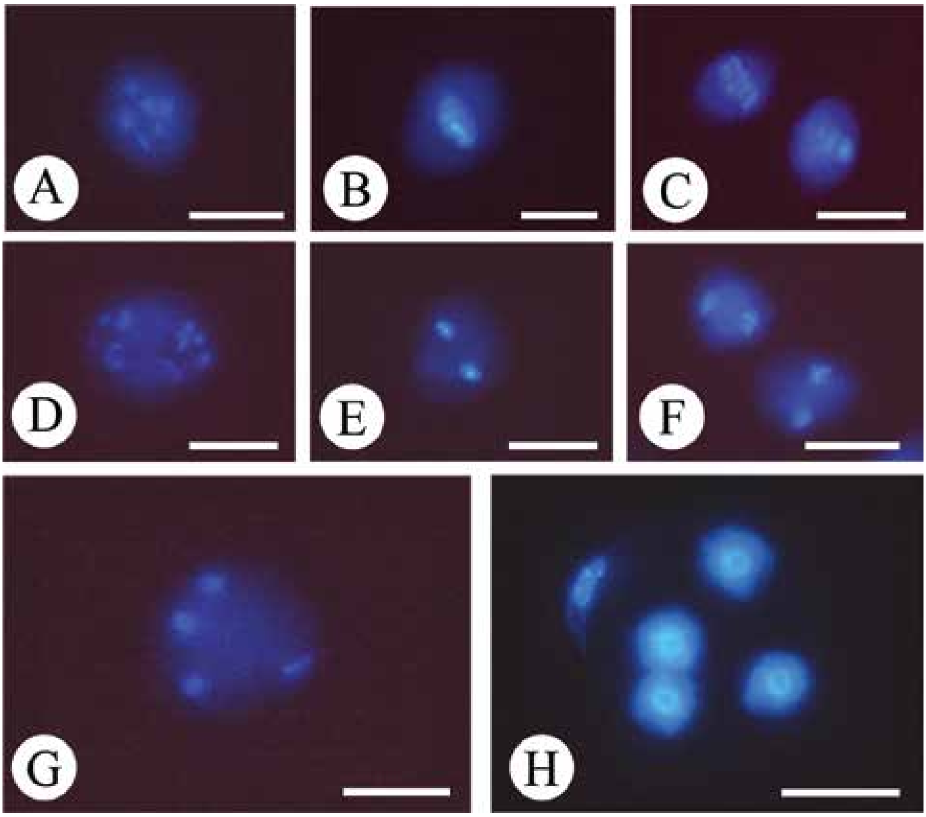

Figure 11. Meiotic stages in 'Banpeiyu' pummelo [32]. A: Prophase I, B: Metaphase I, C: Anaphase I, D: Telophase I and prophase II, E: Metaphase II, F: Anaphase II, G: Telophase II, H: Tetrad stage. Bars = 10 μm.

univalents that remained on the equatorial plate showed mitotic division to segregate each set of chromosomes in the directions of opposite poles during the second meiosis (**Figure 13C**). Consequently, microspore types from monads to hexads were observed in the tetrad stage of the haploid (**Figure 12H**, **I**). Notably, the dyads appeared at a high frequency (24.7%) and produced two microspores of equal size (**Figure 12I**).

Some species can form fertile gametes in haploid plants [36–38, 43, 44]. For fertile gamete formation to occur in a haploid plant, the complete set of the haploid genome (i.e., all chromosomes in the meiocyte) should migrate to the same pole during meiosis I. The probability of the occurrence of such an event in the pummelo haploid is theoretically $(1/2)^9 = 0.2\%$. However, the pollen fertility of the haploid was 14.1%, which was higher than the expected fertility rate. Meiotic nuclear restitution has been identified as a causal factor of this phenomenon [45].

In the haploid plant of *Capsicum annuum* L., Yan et al. [44] found laggards in many meiocytes of the first division at meiosis of the PMC, which resulted in first division restitution (FDR) at meiosis that led to the restitution of pollen fertility in the haploid. They also reported that the microspores formed by FDR were dyads. In the haploid pummelo, although two successive divisions occurred in the PMC (as occurs in normal meiosis), we observed the following abnormalities in some meiocytes: all of the univalent chromosomes remained

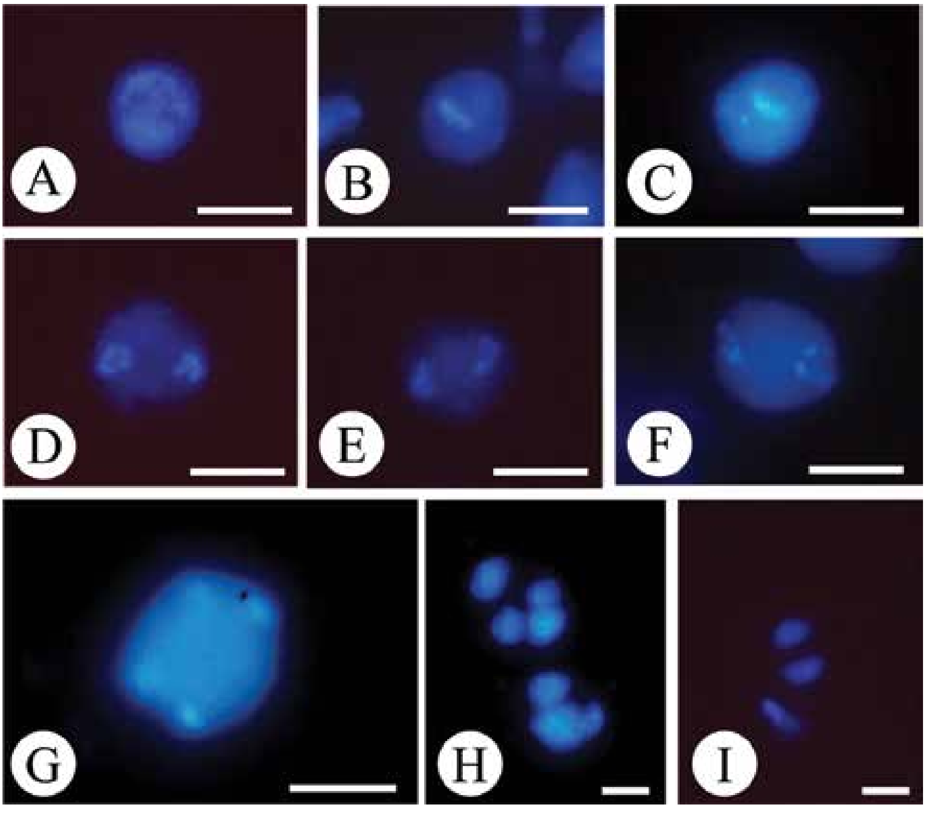

Figure 12. Meiotic stages in the haploid pummelo [32]. A: Prophase I, B: Metaphase I, C: Anaphase I, D: Telophase I and prophase II, E: Metaphase II, F: Anaphase II, G: Telophase II, H, I: Tetrad stage [H: Tetrad (upper) and triad (lower), I: Dyad]. Bars = 10 μm.

near the equatorial plate without distributing towards either pole at anaphase I, and nine univalents on the equatorial plate showed normal mitotic division to segregate each set of chromosomes in the direction of opposite poles during the second meiosis. Moreover, many dyads were formed at the tetrad stage. This observation indicates that the fertile pollen grains in the haploid pummelo were of dyad derivation, as was reported in the haploid plant of *C. annuum*.

Since the dyads were formed through the arrest of the first meiotic division, it can be speculated that meiotic nuclear restitution such as FDR took place in the haploid pummelo. By using single pollen genotyping, Honsho et al. [46] demonstrated that unreduced 2n pollen grains of 'Nishiuchi Konatsu' hyuganatsu (*Citrus tamurana* hort. ex Tanaka) had heterozygosity transmission exceeding 50% in all six alleles, and fitness tests indicated that the FDR map function better fitted the heterozygosity transmission observed rather than the second division restitution (SDR) function. We concluded that the formation of fertile pollen grains in the haploid pummelo was due to abnormalities in the first meiotic division such as FDR.

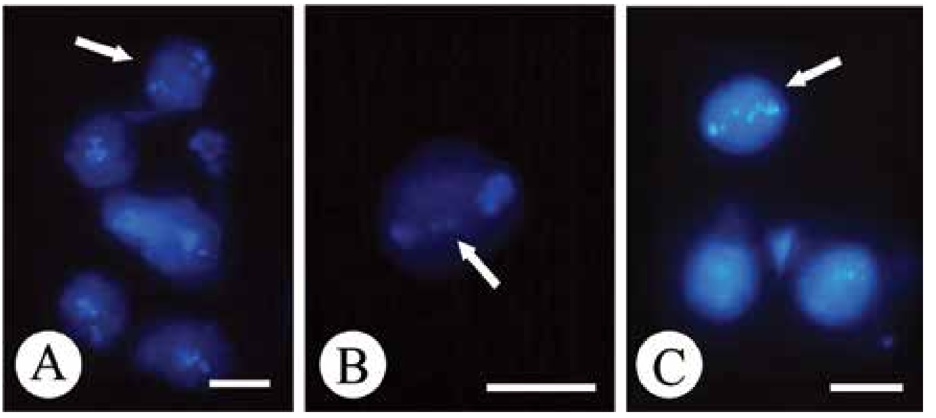

Figure 13. Abnormalities of the meiotic stage of the haploid pummelo [32]. A, B: Chromosomes remained in the equatorial plane, C: Nine bivalents aligned in the equatorial plane. Bars = 10 μm. Arrows indicate the abnormal cell.

5. Conclusion and prospects

Our studies of the morphological characteristics and reproductive potential of haploid [30–33] and DH pummelos [34, 35] are summarized in this chapter. The haploid pummelo showed morphology similar to that of the haploids of other fruit crops. When the haploid was the seed parent, there was no fruit set in any of the cross-combinations. However, when diploid cultivars were pollinated with pollen of the haploid, fruits were set and many developed seeds were obtained. We examined the process of meiosis in both gametes in the haploid pummelo, and our findings revealed that the lack of EMC formation was responsible for the complete sterility of the female gamete and that unreduced gamete formation by FDR caused partial fertility of the male gamete. The DH pummelo showed morphology similar to that of 'Banpeiyu' pummelo, and it had significantly large leaves, flowers and fruit compared to those of the original haploid pummelo. The DH pummelo also showed higher pollen fertility and a larger number of seeds than the haploid. In the reciprocal crosses with some diploid cultivars, the DH plant produced many developed seeds as both seed and pollen parents. These seeds germinated normally and developed into diploid plants.

Haploid and DH plants provide beneficial information regarding the location of major genes and quantitative trait loci (QTLs) for agronomically important traits, and they have been used for genome sequencing in some fruit crops such as apple, peach and pear [13, 14]. In *Citrus*, a rough draft of the genome was completed using the haploid clementine mandarin and the DH sweet orange [3–5]. This genomic information has been applied in the development of DNA markers, genetic analyses, and the production of new cultivars [47–49]. Chang et al. [50] reported that they constructed the detailed genetic linkage maps based on RAPD and SSR markers for 'Fina Sodea' clementine and Byungkyul (*C. platymamma*), using the information of whole-genome sequencing.

Figure 14. Variant shoot with spindly and variegated leaves arose from the haploid plant of pummelo. Arrows indicate the variant shoot. Bar = 5 cm.

Our research group also obtained some haploid plants by means of interploid hybridization and the pollination of irradiated pollen [51] in 'Banpeiyu' pummelo. These haploid pummelos showed vigorous growth (like the haploid pummelo introduced in this chapter), and flowering and fruiting lines among them were also observed. Bud mutation with spindly and variegated leaves arose from one of these haploids (**Figure 14**). We are now conducting studies on self-incompatibility, mutagenesis by ion-beam irradiation, and genetic analyses of mutants using these haploid and DH pummelos and their mutants. Our haploid and DH plants can also be used in various research fields such as plant breeding, mutant isolation, transformation, cytogenetic analyses, linkage maps, and the genome sequencing of *Citrus* and related genera.

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Chapter 7

Antioxidant Capacity and Food Pathogenic Bacteria Inhibition of *Citrus limetta* and *Citrus reticulata*

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Additional information is available at the end of the chapter

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Abstract

In this study, phenolic compounds in the juice, seed and bagasse of *C. limetta* and *C. reticulata* cultivated in Mexico at two ripening stages were determined, and their antioxidant capacities were evaluated using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), 2,2′-azin-bis-(3-etilbenzotiazolin-6-sulfonic acid) (ABTS) and oxygen radical absorption capacity test (ORAC) methods, as well as their antibacterial growth inhibition. We found that bagasse had the highest total phenol content and the highest total flavonoid content. The dominant flavonoid, hesperidin, was observed to be the highest in bagasse. Ascorbic acid was analyzed and *C. limetta* juice and *C. reticulata* bagasse had the highest contents. Antioxidant capacity showed variations in both, *C. limetta* and *C. reticulata*, juices which had the highest ABTS value; *C. limetta* juice and *C. reticulata* bagasse had the highest DPPH value; *C. limetta* juice and *C. reticulata* bagasse had the highest ORAC value. *C. limetta* and *C. reticulata* extracts showed the bactericidal effect at the range of 4–40 mg/mL, assayed against *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella enterica* and *Staphylococcus aureus*. Overall, ripeness increased total phenol content (TPC), total flavonoid content (TFC), hesperidin content, antioxidant capacity and bactericidal effect. These results may provide useful information for future utilization of *C. limetta* and *C. reticulata*.

Keywords: citrus, phenolic compounds, antioxidant, bactericidal, ripeness

1. Introduction

At present, there is a growing interest in the effective, economic and innocuous obtaining of extracts with antioxidant and antibiotic properties from natural matrices [1], which opens a field of investigation in the extraction fields, purification and characterization of plant extracts obtained from secondary products [2]. Given a large number of bioactive compounds present in plant extracts, several mechanisms of action may act simultaneously. The two main antibacterial effects that have been identified are (i) the opening of pores in the bacterial membrane at concentrations below the minimum inhibitory concentration (MIC), which leads to the leakage of intracellular components in the period initial contact, without causing a decrease in the viability of the bacteria. This effect can be reversed inappropriate cultivation conditions only when there have been short periods of exposure to the bactericidal agent. When the bacteria are exposed to concentrations above the MIC, the second effect occurs, (ii) causing irreversible damage to the membrane, due to the modification of the carboxyl groups of the fatty acids of the membrane or by reaction with the polysaccharides of the cell wall. This effect can also be seen when bacteria are exposed to MIC for an extended period, affecting cell viability [3]. Citrus species such as *C. aurantifolia*, *C. sinensis*, *C. paradisi*, *C. reticulata* and *C. limetta* are widely cultivated in Mexico and other parts of the world; some secondary metabolites of these fruits have shown bactericidal activity, helping to combat some infections in humans [4–6]; they are also useful as antivirals (herpes, influenza, etc.) [6], antifungal [7] and antibacterial [8, 9]. Similarly, these bioactive molecules can form the structural basis for developing new antibiotics, more effective, less expensive and with fewer collateral effects [6, 10]. A group of polyphenols' with microbicide properties present in citrus fruits are flavonoids [6]. In particular, phenolic and flavonoid compounds [11] have attracted the attention of the scientific community due to a well-established connection between the consumption of flavonoids and the prevention of sufferings [12]. These compounds have shown the antibacterial effect at diluted concentrations, with the rupture of the membrane show the mechanism of action [13]. Altering the macromolecular structure of bacterial membranes affected the carboxylic groups of the fatty acid in the membrane [3]. The almost daily obtaining of new evidence of these compounds interfering with several virulence factors in pathogenic bacteria, including enzymes, toxins and signal receptors opens the possibility of using them in anti-infection therapies or even the development of new drugs [5]. Several recent publications report the regular presence of antibacterial activity of the isolated flavonoids [14]; with MIC for heperidine against *E. coli* CCTCC AB94014 de 800 μg/mL and against *S. aureus* CCTCC AB9105 of 200 μg/mL [15]; for naringine against *E. coli* ATCC 35218 of 4 μg/mL, against *P. aeruginosa* ATCC 10145 de 2 μg/mL and against *S. aureus* ATCC 25923 of 16 μg/mL; for nobiletine against *E. coli* CCTCC AB94014 of 1600 μg/mL and against *S. aureus* CCTCC AB9105 of 1600 μg/mL [15]; for quercitine against *E. coli* ATCC 35218 de 4 μg/mL, against *P. aeruginosa* ATCC 10145 de 4 μg/mL and against *S. aureus* ATCC 25923 de 2 μg/mL; for tangeretine against *E. coli* CCTCC AB94014 of 1600 μg/mL and against *S. aureus* CCTCC AB9105 of 1600 μg/mL [15]; of gallic acid against *E. coli* ATCC 35218 of 4 μg/mL, against *P. aeruginosa* ATCC 10145 of 2 μg/mL, against *S. aureus* ATCC 25923 of 16 μg/mL [6], which are similar to bacterial inhibition of grape seed extracts with MIC against *S. aureus* ATCC 25923 of 1.25 mg/mL, against *L. monocytogenes* ZM58 > 10 mg/mL, against *E. coli* O157: H7 of 2.5 mg/mL [16].

	IM	Diameter (cm)	High (cm)	Weight (g)	Bagasse (%)	Juice (%)	Seed (%)
C. limetta	17.7	5.83 ± 0.31[a]	5.73 ± 0.32[a]	92.33 ± 9.06[a]	53.76 ± 3.50[a,b]	39.51 ± 6.15[a,b]	1.93 ± 0.37[a]
	9.9	5.03 ± 0.46[a]	4.97 ± 0.35[a]	64.79 ± 10.81[a]	60.49 ± 6.15[b]	35.02 ± 0.24[a]	1.18 ± 0.64[a]
C. reticulata	9.5	6.23 ± 0.21[a]	5.13 ± 0.21[a]	109.54 ± 17.22[a]	26.76 ± 7.26[a]	52.57 ± 0.26[b]	2.83 ± 0.75[a]
	6.3	6.07 ± 0.40[a]	5.00 ± 0.36[a]	107.32 ± 7.81[a]	31.83 ± 2.53[a,b]	49.46 ± 0.23[a,b]	2.06 ± 0.49[a]

Each value is the average of three replications ± deviation standard. The different superscripts indicate significant differences between samples ($p < 0.05$).

Table 1. Characterization of the used fruits.

This has boosted the development of commercial citrus extracts, with a good bactericidal performance and MIC of 20–80 ppm against *S. enterica* CECT 4300 [3]. Still, some citrus compounds do not inhibit certain bacteria such as *L. monocytogenes* 01/155 and 99/287, *P. aeruginosa* ATCC27853, *S. aureus* ATCC29213, and *E. coli* O157:H7, such as those who showed no inhibition at concentrations up to 0.25 mM of Naringin [17]. Currently, research on citrus-derived antioxidant compounds has neglected the study of low-crop species, such as *C. limetta* and *C. reticulata*, which also have compounds with potential bactericidal effect [11]. The extracts of *C. limetta* and *C. reticulata* have shown good antioxidant capacity in tests with the 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH), with values of 261 ± 9 μM Trolox equivalent (TE) in juice of *C. limetta* [11], 210 ± 37.0 mg/mL in seed of *C. reticulata* [18], 48.8 ± 0.91 mg vitamin C equivalent (VCE)/100 ml in *C. reticulata* juice [19], de 9.10 ± 0.68 a 19.75 ± 0.87 μM TE/g, in pulp of *C. reticulata* [20] and 29.04 ± 1.20 to 50.46 ± 3.57 μM TE/g, in shell of *C. reticulata* [21]. By the method with acid 2,2′-azin-bis-(3-etil-benzotiazolin-6-sulfonic acid) (ABTS) have been found 1446 ± 30 μM TE in *C. limetta* juice [11], 59.3 ± 0.23 mg VCE/100 ml in *C. reticulata* juice [19], of 22.92 ± 0.32 to 34.28 ± 1.12 μM TE /g in *C. reticulata* pulp [20]; and of 65.62 ± 1.43 to 108.60 ± 0.24 μM TE/g in *C. reticulata* shell [21]. Currently, research on citrus-derived antioxidant compounds has neglected the study of low-crop species, such as *C. limetta* and *C. reticulata* which also have compounds with potential bactericidal effect [11]. The extracts of *C. limetta* and *C. reticulata* have shown good antioxidant capacity in test with the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), with values of 261 ± 9 μM Trolox equivalent (TE) in juice of *C. limetta* [11], 210 ± 37.0 mg/mL *C. reticulata* seed [18], 48.8 ± 0.91 mg vitamin C equivalent (VCE)/100 ml in juice of *C. reticulata* [19], de 9.10 ± 0.68 to 19.75 ± 0.87 μM TE/g, in pulp of *C. reticulata* [20] and 29.04 ± 1.20 to 50.46 ± 3.57 μM TE/g, in *C. reticulata* shell [21]. With the method of 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), it has been found 1446 ± 30 μM TE in C. limetta juice [11], 59.3 ± 0.23 mg VCE/100 ml in C. reticulata juice [19], de 22.92 ± 0.32 a 34.28 ± 1.12 μM TE/g in C. reticulata pulp [20] and de 65.62 ± 1.43 a 108.60 ± 0.24 μM TE/g in C. reticulata shell [21]. Moreover, these extracts have shown antioxidant capacity of oxygen radicals in the oxygen radical absorption capacity test (ORAC), with values of 126.90 ± 1.96–13.22 ± 231.29 μM TE/g in *C. reticulata* pulp [20] and of 395.66 ± 14.42 to 834.37 ± 16.98 μM TE/g in *C. reticulata* shell [21].

Consequently, citrus fruits are of great importance due to the interest of finding new sources of antibacterial and antioxidant compounds. However, no work has been done in bacterial inhibition and antioxidant capacity of citrus fruits to different stages of maturation [22]. Therefore, in this research, it was determined the bactericidal activity and antioxidant capacity of bagasse, juice and seeds of two citrus species cultivated in Mexico, *C. limetta* and *C. reticulata*, in two stages of maturity (**Table 1**). The results were compared with those found in the literature, in order to provide innovative information for future applications of these citrus species.

2. Methodology

2.1. Obtaining the extracts

The fruits of *C. limetta and C. reticulata* were acquired in the city of Morelia, Michoacán, from different regions of the same state. To obtain the extract, the juice, the shells and the seeds were separately dried in the oven at a temperature of 40°C for 48 h and then ground. The materials were washed with acetone (Meyer, México) in proportion 1:1 (g of material: mL of acetone) in a flask. The mixture was stirred for 30 min at 200 rpm, at room temperature and then filtered to vacuum. The operation was repeated with hexane (J.T. Baker, México) in proportion 1:4 (g of material: mL of hexane). For the extraction of phenolic and flavonoid compounds, 4 mL of methanol (J.T. Baker, México) was used for each gram of material, stirring the mixture for 30 min at 200 rpm [23] at room temperature. The mixture is vacuum-filtered and the extracts obtained are stored in refrigeration at 4°C until posters test.

Total phenol content (TPC) was tested using the reagent of Folin-Ciocalteau (Hycel, Mexico) with a standard of Gallic acid (Golden Bell, Mexico), and absorbance was measured at 750 nm [24]. An aliquot of 0.1 mL of the methanol extract is diluted with 0.4 ml of distilled water; then, the solution was mixed with 2.25 mL of Folin-Ciocalteau reagent to 10% and 2.25 mL of solution (Golden Bell, Mexico) (20 g/mL of distilled water) of sodium carbonate.

Absorbance was measured after 2 h of incubation at 750 nm compared to a methanol target (J.T. Baker, Mexico), using a UV/Vis spectrophotometer (Jenway, Model 7305). The TPC was expressed in mg equivalents of Gallic acid (GAE) by g of dry matter (DM) of bagasse and seeds and in mg GAE/mL for the juices. The total flavonoid content (TFC) was analyzed with a modification of the spectrophotometric method described by Abeysinghe et al. [12]. A 1.5 mL of methanol was added to 0.1 ml of the diluted extract, to then add 0.1 mL of solution 6.8 g/50 mL of distilled water of aluminum chloride (Golden Bell, Mexico), and the resulting solution was diluted with distilled water to a final volume of 5 mL. The mixture was stirred, it was left to rest for 30 min and its absorbance was measured at 510 nm. The TFC was calculated using a calibration curve of quercetin (Aldrich, USA) and the results were expressed in mg equivalent of quercetin (QE) per grams of dry matter (DM) of bagasse and seeds and in mg QE/mL for the juices. The quantitative analysis of hesperidin and ascorbic acid was performed in a high-performance liquid chromatography (HPLC) Varian LC920 equipped with a column C18 Varian, 25 cm × 4.6 mm I.D. and a diode array detector. The TFC was calculated using a calibration curve of quercetin (Aldrich, USA) and the results were expressed in mg equivalent of quercetin (QE) per grams of dry matter (DM) of bagasse and seeds and in mg QE/mL for juices. The quantitative analysis of hesperidin and ascorbic acid was performed in a high-performance liquid chromatography (HPLC) Varian LC920 equipped with a column C18 Varian, 25 cm × 4.6 mm I.D. and a diode array detector. Mobile phases of KH_2PO_4 20 mM, phosphoric acid at 0.1%v, and 1%v methanol for the determination of ascorbic acid were used; 33%v methanol and 67%v water to determine hesperidin. Absorbances were measured at 215 nm and 283 nm, respectively. The samples were analyzed in duplicate, and the calibration curves were constructed from the average areas of the peaks. The contents of hesperidin (HD) and ascorbic acid (AA) expressed in mg/g DM for bagasse and seeds and in mg/mL for juices.

2.2. Determination of minimum inhibitory and bactericidal concentrations

The evaluation of the minimum inhibitory concentration (MIC) and minimum concentration bactericidal (MBC) It was carried out by microdilution of the extract in culture medium [25], in concentrations of 2–40 mg/μL. A 5 mL of bacterial suspension was placed in culture medium (cetrimide broth for *P. aeruginosa*, Muller-Hinton broth for the rest of the bacteria) in a sterile microplate of 96 wells. The volume was completed with the dilutions of the extract in the culture broth to obtain the test concentrations. Control wells were prepared with culture broth, diluted extract and bacterial suspension separately. The microplate was incubated for 24 h at 37°C. The MIC was the lowest concentration where no viability was observed in the well after 24 h [16]. To determine the MBC, 20 μL were taken from wells where no growth was observed and transferred to a plate with solid agar (*Pseudomonas* agar for *P. aeruginosa*, Muller-Hinton agar for the other bacteria) and incubated for 24 h at 37°C. MBC was the lowest concentration of colony-forming units (CFU) growth after the incubation period. Positive controls were wells with bacterial suspension in breeding stock. The negative controls were wells with culture broth and with the dilution of the extract. All the determinations of MIC and MBC were repeated in triplicate.

2.3. Test organisms

Escherichia coli ATCC 15597, *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella enterica* ATCC 14028 and *Staphylococcus aureus* ATCC 6538 were used as test organisms.

2.4. Antioxidant capacity

The antioxidant capacity was determined by testing the oxidation inhibition of the acid radicals 2,2′-Azino-bis-(3-etilbenzotiazolin-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH), as well as the absorption capacity of oxygen radicals (ORAC).

The capacity of the extracts was measured to inhibit the DPPH radical by placing in a microplate 10 μL of the extract solution to the given concentration and added 140 μL of the DPPH radical adjusted to an absorbance of 0.70. The reaction was allowed to elapse for 30 min and the absorbance of the solution was read at 518 nm. The percentage of inhibition was calculated and the results were expressed in μmol equivalents of Trolox per milligram of a sample (μmol TE/mg), using a standard curve of this antioxidant. The tests were carried out in triplicate [26].

The determination of the inhibition of the radical ABTS was carried out by adding in a well of microplate 5 μL of extract to the appropriate dilution of test and 245 μL of radical ABTS solution adjusted to an absorbance of 0.70. It was allowed to react for 5 min and the absorbance was measured at 754 nm. The percentage of inhibition was calculated and the results were expressed in μmol TE/mg using a standard curve of Trolox [1]. Tests were carried out in triplicate.

The reaction mix for the ORAC test was prepared with 150 μL de fluorescein 10 μM, 25 μL of Trolox standard (standard curve of 6.25–200 μM), 25 μL of phosphate buffers (75 μM, pH 7.4) like control and 25 μL of extract; the reaction begin with the addition of AAPH (2,2′-Azobis(2-amidinopropane) dihydrochloride, 240 mM). The fluorescein drop was evaluated every 90 s during an hour and a half, at an excitation wavelength of 485 nm and a wavelength of 520 nm with a microplate reader FLUOstar Omega (BMG Labtech Inc., USA). The ORAC values were calculated using a linear regression equation of a standard Trolox curve [27]. The results were expressed as equivalent μmol of Trolox per milligram (μmol TE/mg).

2.5. Analysis of statistics

There were three replicas and at least three independent experiments. The data were presented as mean ± deviation standard (SD). STATGRAPHICS Centurion XVI.I version was used for the statistical analysis. (Statpoint Technologies, Inc., Warrenton, VA, USA). Differences between groups were detected by ANOVA and Tukey multiple comparison tests; *p* values less than 0.05 were considered as statistically significant.

3. Results and discussion

3.1. Effect of maturity on the contents of hesperidin, ascorbic acid, TPC and TFC

The highest values of TPC were found in bagasse, followed by juice and finally in the seed, for both fruits regardless of the level of maturity (**Figure 1**). Changes in the maturation in the TPC showed similar trends in pulp, juice and seed. It was determined an increase of 13.3% 14.4% at the TPC of bagasse for the maturity of 20.38 ± 0.97 to 23.09 2.57 ± and 34.95 ± 0.11 to 39.97 ± 1.25 mg GAE/g DW) for *C. limetta* and *C. reticulata*, respectively. The TPC juice content increased 16.62% and 0.90% during maturation (of 10.98 ± 0.14 to 12.80 ± 1.20 and 17.15 ± 0.42 to 17.30 ± 1.14 mg GAE/g DW). The content TPC in seed increased 2.59 ± 0.95 to 5.63 ± 0.19 and of 6.85 ± 0.95 to 7.46 ± 0.95 mg GAE/g DW, which did not represent a significant difference to $p > 0.05$. It is possible that the variations are due to differences in culture, origin, growth conditions and the same extraction process. Phenolic compounds are secondary metabolites that have mainly been correlated with antioxidant activity in several fruits, vegetables and grains [12]. The presence of a high TPC in pulp and juice *C. limetta* and *C. reticulata* confirms the nutritional value of these fruits and the presence of phenols in the seeds makes them an alternative source for later uses.

The highest values of TFC were found in bagasse, followed by juice and finally in the seed, for both fruits regardless of the level of maturity (**Figure 2**). Changes in the maturation in the TFC showed similar trends in pulp, juice and seed. The higher content of TFC was found in bagasse for both fruits. It measured an increase of 7.11% and 2.23% in the TFC of bagasse during ripening of 1579.03 ± 12.85 to 1691.33 ± 22.83 and of 1892.63 ± 6.89 to 1934.89 ± 7.58 μg QE/g DW) for

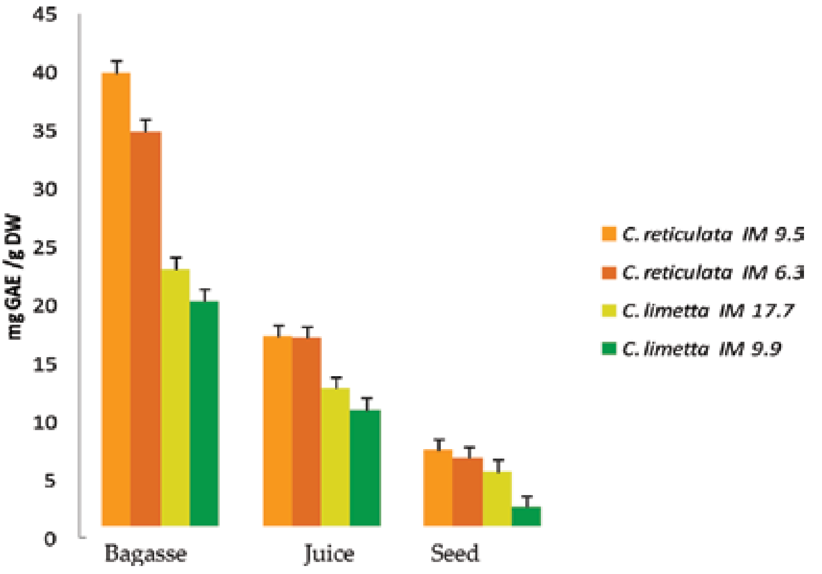

Figure 1. Content of total phenols (TPC) en *C. limetta* y *C. reticulata.*

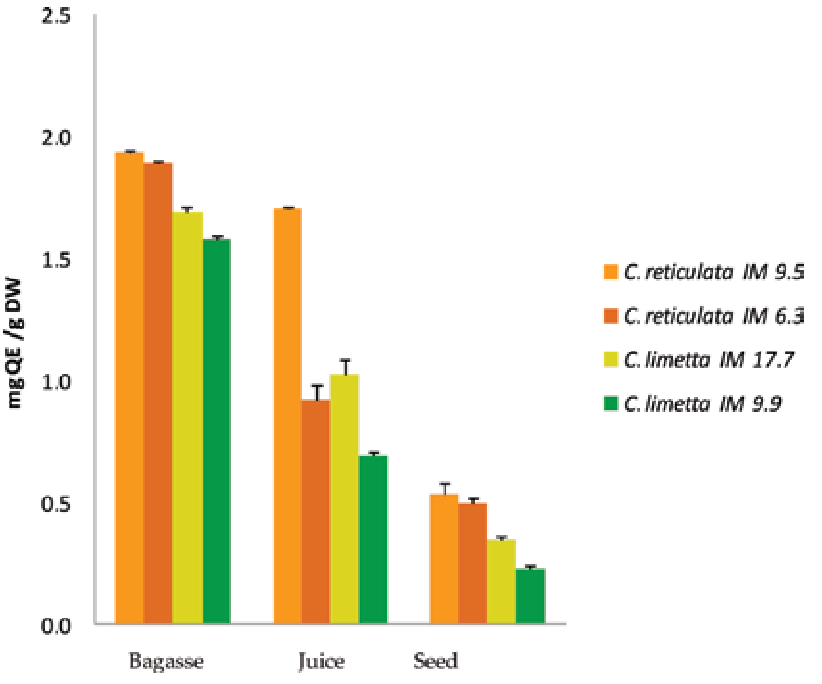

Figure 2. Content of total flavonoids (TFC) in *C. limetta* y *C. reticulata.*

C. limetta and *C. reticulata*, respectively. The content of total flavonoids in juice increased 48.33% and 84.62% during maturation (of 694.70 ± 15.19 to 1030.44 ± 59.58 and from 1709.78 ± 6.23 to 1892.63 ± 6.89 µg QE/ g DW). The TFC in seed increased from 230.74 ± 14.56 to 344.76 ± 15.59

and from 499.65 ± 18.61 to 535.15 ± 40.96 μg QE/g DW, which did not represent a significant difference to $p > 0.05$. It is possible that the variations are due to differences in culture, origin, growth conditions and the same extraction process. Phenolic compounds show flavonoids may inhibit radical free and catch reactive oxygen species (ROS) and therefore provide an effective means to prevent and treat ailments promoted by free radicals [28]. The presence of high TFC in *C. limetta* and *C. reticulata* defines them as a significant source of antioxidants with potential prophylactic applications and in the development of functional foods.

Hesperidin content was determined by an analysis of HPLC-DAD. The presence of hesperidin was obviously different between bagasse, juice and seed and was influenced by maturation (**Figure 3**). In general, the highest value of hesperidin was registered in bagasse, followed by juice and seed. However, the variation was larger in *C. limetta* during maturation. Large amounts of hesperidin were identified not only in bagasse but also in juice and seed, which is consistent with levels reported previously for *C. reticulata* [28–30]. The concentration of hesperidin in bagasse *C. limetta* and *C. reticulata* increasing during ripening of 397.37 ± 20.01 to 617.21 ± 70.73 and 966.49 ± 14.68 to 978.89 ± 43.46 μg/g DW, respectively. Similarly, it increased the amount of hesperidin in the juice of 129.45 ± 24.81 to 444.97 ± 109.57 and 200.69 ± 22.01 to 255.54 ± 37.21 μg/g DW, respectively. Hesperidin is accumulated in seeds of *C. limetta* and *C. reticulata* during maturation, and their quantities increased 90.25 and 14.46%, respectively.

The highest content of Ascorbic acid was found in bagasse, followed by juice and finally the seeds regardless of maturity for both fruits (**Figure 4**). The content of ascorbic acid in the pulp of *C. limetta* and *C. reticulata* decreased slightly during the maturation of 3.13 ± 0.41 to

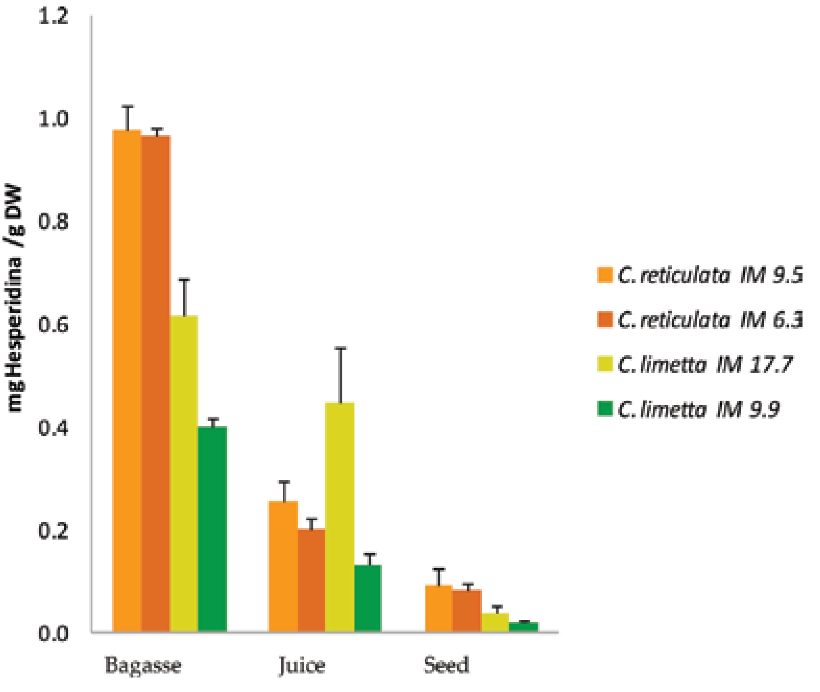

Figure 3. Contents of hesperidin in *C. limetta* y *C. reticulata*.

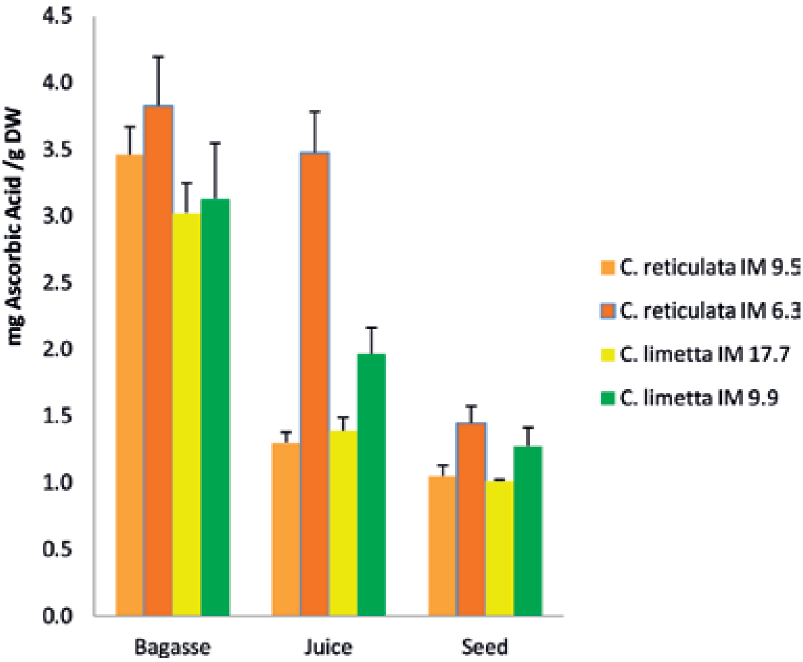

Figure 4. Ascorbic acid content in *C. limetta* y *C. reticulata*.

3.02 ± 0.22 and 3.83 ± 0.37 to 3.46 ± 0.20 mg/g DW for *C. limetta* and *C. reticulata*, respectively. During maturation, the content of ascorbic acid in juice decreased 1.96 ± 0.20 to 1.39 ± 0.10 and 3.47 ± 0.31 to 1.30 ± 0.08 mg/g DW. Ascorbic acid decreased from the seeds of *C. limetta* and *C. reticulata* with maturation, and their number was reduced by a 20.80 and 27.31%, respectively. How the ascorbic acid is an essential nutrient to prevent scurvy, cancer, cardiovascular and nervous system diseases [31], they seem to be a beneficial and important source of this compound. The concentrations of metabolites found in the juices of *C. reticulata* are consistent with those reported previously [19, 32, 33]; also the amounts found in seed [18].

3.2. Antioxidant capacity

The results of this study showed, in general, similar trends in the inhibition of radical capacity for pulp, juice and seed and increased with maturation, as shown in **Table 2**. In the ABTS test, the highest values were found in juice, of 72.71 ± 1.72 to 82.19 ± 6.39 μmol TE/g DW from *C. limetta* and of 61.48 ± 1.40 to 62.00 ± 5.47 μmol TE/g DW of *C. reticulata*, being higher than the data reported in the literature [11, 19]; the inhibitory activity of bagasse with ABTS for *C. limetta* increased during maturation, while it came down to the bagasse of *C. reticulata*, and their values agreed with previously reported data [20, 21]. ABTS of seed value did not change significantly with maturation and ranged from 6.06 ± 0.21 to 13.30 ± 1.07 μmol TE/g DW for *C. limetta* and 16.73 ± 1.39 to 42.82 ± 1.67 μmol TE/g DW for *C. reticulata*. The DPPH of juice *C. limetta* is consistent with that reported by Barreca et al. [11]. The seeds of *C. reticulata* showed good DPPH [18], but below that obtained for juice, as expected [19]; the highest DPPH antioxidant capability found in this study were for bagasse of *C. reticulata* (7.56 ± 0.88 μmol

	IM	ABTS	DPPH	ORAC
C. limetta				
Bagasse	17.7	47.34 ± 2.84^{c,d,e}	1.64 ± 0.27^{a,b}	14.82 ± 0.33^{d}
	9.9	25.26 ± 2.21^{a,b,c}	3.31 ± 0.11^{a,b}	12.51 ± 0.17^{c}
Juice	17.7	82.19 ± 6.39^{f}	5.61 ± 0.62^{a,b}	11.97 ± 0.33^{c}
	9.9	72.71 ± 1.72^{e,f}	3.78 ± 1.06^{a,b}	11.33 ± 0.04^{c}
Seed	17.7	13.30 ± 1.07^{a,b}	0.61 ± 0.24^{a}	1.69 ± 0.01^{a}
	9.9	6.06 ± 0.21^{a}	0.13 ± 0.02^{a}	0.79 ± 0.04^{a}
C. reticulata				
Bagasse	9.5	36.04 ± 2.27^{b,c,d}	7.56 ± 0.88^{b}	22.56 ± 0.28^{f}
	6.3	59.67 ± 5.31^{d,e,f}	3.65 ± 0.37^{a,b}	16.97 ± 0.13^{e}
Juice	9.5	62.00 ± 5.47^{d,e,f}	2.72 ± 0.86^{a,b}	22.56 ± 0.36^{f}
	6.3	61.48 ± 1.40^{d,e,f}	3.50 ± 1.27^{a,b}	22.07 ± 0.29^{f}
Seed	9.5	42.82 ± 1.67^{b,c,d,e}	2.08 ± 0.13^{a,b}	3.73 ± 0.12^{b}
	6.3	16.73 ± 1.39^{a,b}	1.60 ± 0.60^{a,b}	2.26 ± 0.05^{a,b}

Each value is the average of three replications ± standard deviation. The different superscripts indicate significant differences between samples ($p < 0.05$).

Table 2. Antioxidant capacity of *C. limetta* y *C. reticulata*, μmol TE/g DW ± SD.

TE/g DW), which is higher than the values reported in previous studies [20, 21]. Although the DPPH values of seeds were lower than those of juice and pulp, this inhibiting activity DPPH implies a potential application as a valuable source of antioxidants. In general, values of DPPH showed similar trends for pulp, juice and seeds during maturation, with the exception of *C. limetta* and of *C. reticulata* juice.

ORAC testing revealed that the pulp and juice of *C. reticulata* had ORAC values significantly higher than pulp and juice of *C. limetta*, probably due to the higher levels of phenolic compounds and flavonoids in *C. reticulata* [11, 20, 21] which can lead to further investigation of this fruit. The ORAC values of seeds did not change significantly during maturation and were 0.79 ± 0.04 to 1.69 ± 0.01 μmol TE/g DW for *C. limetta* and 2.26 ± 0.05 to 3.73 ± 0.12 μmol TE/g DW for *C. reticulata*. A comparative evaluation indicates that the seeds of *C. limetta* and *C. reticulata* may be an attractive source of antioxidants for future applications. The results suggest that both *C. limetta* as *C. reticulata* possess a remarkable inhibitory activity of radical and therefore a significant antioxidant capacity.

3.3. Bacterial inhibition

Table 3 shows testing of antibacterial susceptibility of extracts against some Gram-positive and Gram-negative bacterial strains. Observed growth inhibition varied from one organism

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		Gram positive				Gram negative					
		Listeria monocytogenes		*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Salmonella enterica*	
	IM	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
C. limetta											
Bagasse	17.7	18	22	31	40	13	22	3	4	13	22
	9.9	9	13	31	40	13	22	3	4	9	13
Juice	17.7	40	>40	>40	>40	36	40	31	40	31	40
	9.9	40	>40	>40	>40	>40	>40	36	40	36	40
Seed	17.7	>40	>40	>40	>40	>40	>40	36	40	36	40
	9.9	>40	>40	>40	>40	>40	>40	36	40	40	>40
C. reticulata											
Bagasse	9.5	22	31	40	>40	31	40	3	4	18	22
	6.3	22	31	40	>40	31	40	9	13	18	22
Juice	9.5	27	31	40	>40	31	40	13	22	27	31
	6.3	31	40	40	>40	36	40	31	40	31	40
Seed	9.5	>40	>40	>40	>40	>40	>40	36	40	40	>40
		>40	>40	>40	>40	>40	>40	>40	>40	>40	>40

MIC, Minimum inhibitory concentration; MBC, minimum bactericidal concentration. Each value is the mean of three replicates with standard deviation <10%.

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts, mg/mL.

to another and a summary to another. Gram-positive strains showed values higher than the Gram-negative strains MIC and MBC. Extracts that showed greater bactericide effect were those obtained from bagasse for both fruits, with up to 3 mg/mL and MIC MBC 4 mg/mL (**Table 3**), which is consistent with the reported values of for essential oil of *C. sinensis* [34], this effect may be due to the increase in phenolic compounds and flavonoids during the ripening of fruit. *S. aureus* was the microorganism tested that showed greater resistance to the extracts, with MIC above 31 mg/mL and MBC greater than 40 mg/mL for the majority of tests. *L. monocytogenes* had MIC from 18 mg/mL and MBC from 13 mg/mL. *E. coli* showed MIC from 13 mg/mL and MBC from 22 mg/mL. *P. aeruginosa* recorded MIC from 3 mg/mL and MBC from 4 mg/mL. *S. enterica* recorded MIC from 9 mg/mL and MBC from 13 mg/mL. Extracts of *C. reticulata* showed one inhibitory effect greater than the *C. limetta*, but still below that reported for extracts of green tea [16]. Lower bacterial inhibition was in seed for both fruits extracts, with MIC values higher to 36 mg/mL, but still below those obtained with extracts from grape seed [16]. Even so, extracts showed better antibacterial compounds isolated citruses such as quercetin, gallic acid [6] and naringin [17]; still better than the performance shown by some commercial fruit extracts [3].

4. Conclusions

We investigated the content and antioxidant activity of the phenolic compounds and flavonoids from two Mexican varieties of *C. reticulata* and *C. limetta*. Bagasse, juice and seeds of *C. reticulata* and *C. limetta* contain phenolic compounds and flavonoids. Its content increases as the maturity increases. The extracts show microbicide effect on microorganisms of study under in vitro conditions. The effect increases when rising the ripening of the fruit. Extracts of *C. reticulata* have a higher bactericide effect than those obtained *C. limetta*, test microorganisms. As a result, the inhibition is directly related to the content of phenolic compounds and flavonoids. The extracts show antioxidative effect in vitro tests. The effect increases when rising the ripening of the fruit *C. reticulata* has antioxidant capacity and content of secondary metabolites greater than *C. limetta*. Inhibition of oxidation is directly related to the content of phenolic compounds and flavonoids. Our findings suggest that *C. reticulata* and *C. limetta*, especially its bagasse, are good sources of antioxidant and antibacterial compounds. The results of citrus species analyzed in this study can motivate more widely used them in the pharmaceutical and food products.

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Conflict of interest

I confirm there are no conflicts of interest.

Nomenclature

AAPH	2,2′-azo-bis-(2-aminopropane)-dihydrochloride)
ABTS	2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA	analysis of variance
ATCC	American Type Culture Collection
CCTCC	China Center for Type Culture Collection

DPPH	2,2-diphenyl-1-picrylhydrazyl-hydrate
DW	dry weight
GAE	gallic acid equivalents
HPLC	high performance liquid chromatography
MBC	minimum bactericidal concentration, mg mL^{-1}
MIC	minimum inhibitory concentration, mg mL^{-1}
ORAC	oxygen radical absorption capacity, μmol TE/g DW
QE	quercetin equivalents
SD	standard deviation
TE	Trolox equivalents
TFC	total flavonoid content, mg GAE g DW^{-1}
TPC	total phenol content, mg QE g DW^{-1}
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
UFC	colony forming units

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Citrus is one of the world's major fruit crops, with global availability and popularity contributing to human diets. Citrus fruits are the highest-value fruit crop in terms of international trade. Current annual worldwide citrus production is estimated at over 70 million tons, with more than half of this being oranges. The rise in citrus production is mainly due to the increase in cultivation areas, improvements in transportation and packaging, rising incomes, and consumer preference for healthy foods. Citrus fruit growth and quality are dependent on climatic conditions, in addition to soil type, water availability, cultural practices, and nutrient supply. The book briefly explains the fruit morphology, anatomy, physiology and biochemistry, growth phases, maturity standards, grades, and physical and mechanical characteristics of citrus trees. It also provides the foundation for understanding the growth, harvest, and post-harvest aspects of citrus fruits. Insect pests and diseases, irrigation, nutrition, and rootstocks are also addressed in this book.

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