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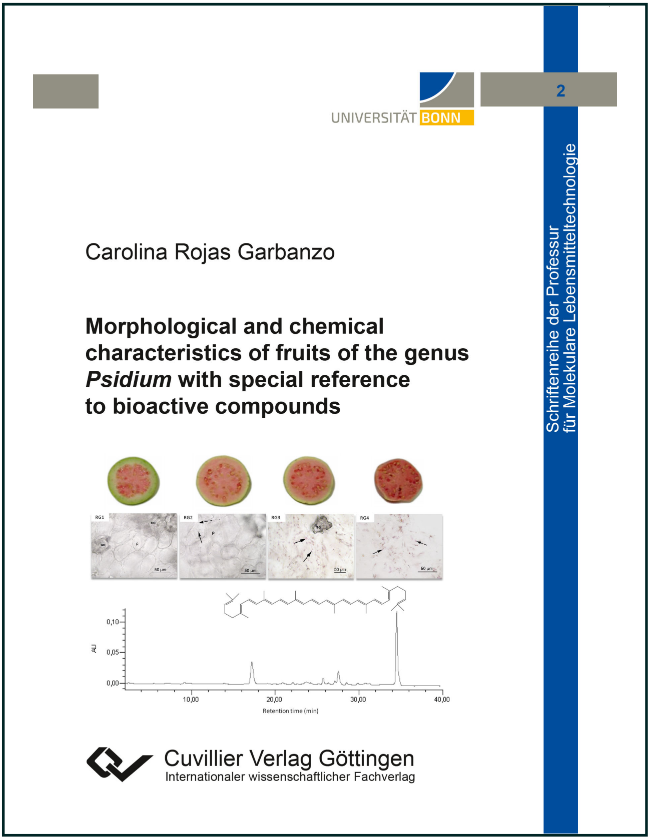

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Morphological and chemical characteristics of fruits of the genus *Psidium* with special reference to bioactive compounds

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1. INTRODUCTION

Herbs, fruits, and vegetables play an important role in preventing and treating different diseases such as cancer, cardiovascular and neurological diseases, cataracts, and macular degeneration (da Silva Oliveira *et al.*, 2010: Dembitsky *et al.,* 2011). Individuals eating five or more servings of fruits and vegetables have approximately half the risk of suffering from some types of cancer, especially those that develop in the gastrointestinal tract (Borges *et al.,* 2014; de Souza Sant´Ana, 2011; Dembitsky *et al.,* 2011). These positive effects on human health are attributed to bioactive compounds such as vitamins, dietary fiber, and secondary metabolites, e.g., polyphenols, carotenoids, sterols, glucosinolates, and saponins (Dembitsky *et al.,* 2011).

Numerous beneficial properties have been reported for tropical and subtropical fruits that are mainly consumed in the country of origin. However, many tropical fruits such as mango, white and pink guava, and papaya are nowadays commonly consumed in North America and Europe (Dembitsky *et al.,* 2011). Chemical and biological studies of tropical and subtropical fruits have been done, but they are scarce compared with studies of fruits from the temperate regions. Research focused on fruits of the tropical and subtropical region have shown that they can be considered functional food since they contain many phytochemicals (Dembitsky *et al.,* 2011). Among tropical fruits, those of the genus *Psidium* have gained increasing attention in recent years and some attempts have been made to identify phytochemicals such as carotenoids, polyphenols, and triterpenoids (Ribeiro *et al*., 2014; Chang *et al.,* 2013; Flores *et al.,* 2013; Flores *et al.,* 2015; Gordon *et al*., 2011: Matsuzaki *et al.,* 2010, Mercadante *et al.,* 1999; Padula & Rodríguez-Amaya, 1986, Setiawan *et al.,* 2001; Wilberg & Rodríguez-Amaya, 1995). Nevertheless, a detailed phytochemical characterization of these fruits is still currently lacking.

1.1 The genus *Psidium*

The genus *Psidium* is native to Central America and nowadays it is grown throughout the tropical and subtropical regions (Cuadrado-Silva *et al.,* 2016; Flores *et al.,* 2013). This genus belongs to the Myrtaceae family and comprises more than 150 species. The most widely cultivated edible fruits include the pink guava (*P. guajava* L.), the Costa Rican guava (*P. friedrichsthalianum* Nied.), the strawberry guava (*P. cattleianum* Sabine), and the Brazilian guava (*P. guineense* Sw.) (Mani *et al*., 2011). They are very important fruit crops in countries such as Costa Rica, Mexico, Brazil, Pakistan, Thailand, China, and India. In 2014, India was considered the largest guava producer worldwide, followed by China and Thailand (FAOSTAT, 2016).

Among these fruits, *P. guajava* and *P. friedrichsthalianum* (**Figure 1**) are the most common ones in Latin America (Cuadrado-Silva *et al.,* 2016; Flores *et al.,* 2013; Flores *et al.,* 2015). The fruits of both plants are suitable for fresh consumption and some typical preparations are juice, ice cream and jellies. Their processing has gained attention due to their high nutritional value, availability at a moderate price, and a pleasant aroma (Mitra *et al.,* 2012). The plants of these fruits have been used as a traditional medicine in countries such as Mexico, Central America, Brazil, Taiwan, Japan, China, and Korea (Chang *et al.,* 2013; Díaz-de-Cerio *et al.,* 2015; Matsuzaki *et* *al.,* 2010). The positive effects on health reported for extracts obtained from these plants are antibacterial, anti-inflammatory, cicatrizant, antidiabetic, anti-allergic, and anti-carcinogenic, among many others. These medicinal properties are attributed then to the content of phytochemicals such as vitamin C, lycopene, quercetin, quercitrin, myricetin, and ellagic acid in both species (Chang *et al.,* 2013; Cheng *et al*., 2009; Eidenberger *et al.,* 2013; Flores *et al*., 2013; Vasconcelos *et al.,* 2017).

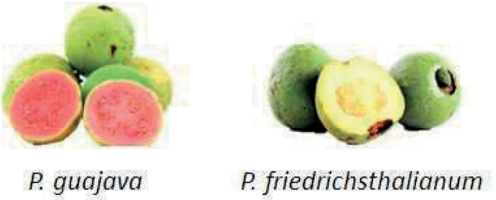


Figure 1. Ripe fruits of *Psidium guajava* L. and *Psidium friedrichsthalianum* Nied.

Research on health effects of *Psidium* fruits has been focused on polyphenol-containing leaf extracts of *P. guajava*, e.g., their effect on dipeptidyl-peptidase IV (DP-IV), a key enzyme for blood glucose homoeostasis (Eidenberger *et al.,* 2013), and the impact of quercetin from an aqueous guava leaf extract on the improvement of hypoglycemia diabetes (Cheng *et al.,* 2009). Moreover, the antimicrobial activity of an extract from guava leaves has been evaluated against *Salmonella enteritidis* and *Bacillus cereus* (Arima & Danno, 2002). The authors attributed the antimicrobial activity to the presence of quercetin. Also, the activity of two phenylethanoid glycosides isolated from seeds of pink guava has been tested *in vitro* against Ehrlich ascites carcinoma cells and leukemia, and positive effects were reported (Salib & Michael, 2004). In the case of *P. friedrichsthalianum*, a phenolic extract obtained from the fruit was evaluated against chronic obstructive pulmonary disease. The ethyl extract showed high antioxidant and anti-inflammatory activities, a decrase in the release of interleukin-8 and matrix in the expression of metalloproteinase-1 was observed (Flores *et al.,* 2013). Nevertheless, an association of the bioactivity to specific phyytochemicals has not been reported.

1.2 Biologically active compounds

Fruits have been recognized as a good source of bioactive compounds. Many positive effects on human health have been attributed to the action of compounds such as carotenoids, polyphenols, triterpenoids, and vitamin C (Rivera & Canela-Garayoa, 2012; Silva & Lopes, 2014). Hence, consumption of fruits has been encouraged due to the growing recognition of their nutritional and therapeutic values (Rufino *et al.,* 2010). Among the bioactive compounds contained in fruits, carotenoids, polyphenols and triterpenoids are the focus of this study.

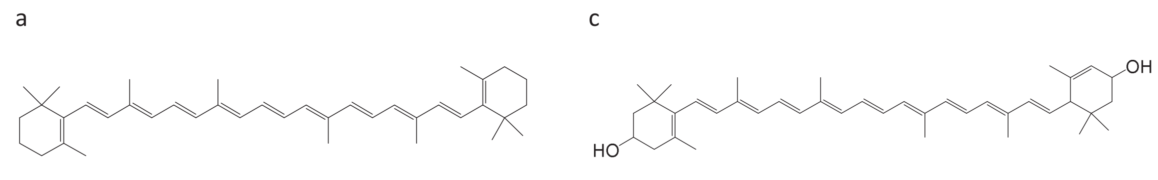
1.2.1 Carotenoids

Carotenoids have become of interest because of their association with the prevention of atherosclerosis, the maintenance of immune functions, and the risk reduction for age-related macular degeneration, cataract, cancer, and cardiovascular and degenerative diseases (Britton & Khachik, 2009). Some carotenoids have provitamin A activity as well. They are transformed into vitamin A, which can prevent night blindness, susceptibility to infection, rough, scaly skin, and retarded tooth and bone development (Fernández-García *et al.,* 2012). This is particularly important because vitamin A deficiency is today a worldwide public health issue that affects at least 122 countries (WHO, 2009).

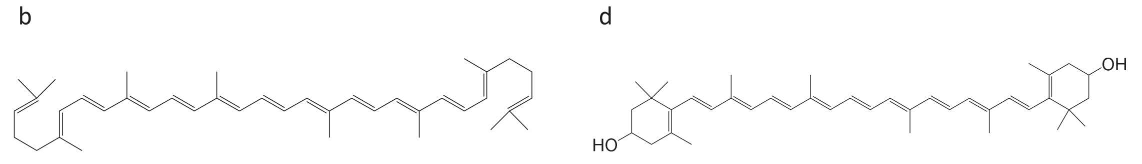
The preventive role of carotenoids against diseases has been attributed to their strong antioxidant properties. They can capture free radicals, e.g., atoms or groups of atoms with an unshared electron, because of their conjugated double bond system (Rodríguez-Amaya, 1996). Carotenoids with nine or more conjugated double bonds provide the highest protection (Rodríguez-Amaya, 1996). Known vegetables and fruits containing carotenoids are carrots, tomatoes, peppers, pink guava, mangoes, and papaya (González *et al*., 2011; Schweiggert *et al.,* 2012a).

These compounds are widely distributed in plants and contribute to the yellow, orange, and red color in vegetables and fruits. They are exclusively synthesized *de novo* by plants, algae, fungi, and bacteria. They also exist in green plants where their color is masked by chlorophyll. In most cases, biosynthesis of food related carotenoids arises from eight isoprene units that are joined together resulting generally in molecules that consist of 40 carbon atoms. Thus, carotenoids are counted among tetraterpenes under the wide class of secondary plant metabolites (Belitz *et al.,* 2008). The different carotenoids derive from the basic C_{40}-structure during the biosynthetic process. Modifications occurring through hydrogenation, dehydrogenation, double-bond migration, isomerization, introduction of oxygen functions or cyclization of the carbon skeleton, generate the different carotenoids. Cyclization may occur on one or on both ends of the molecule (Rodríguez-Amaya, 2001). In addition, modifications involving chain elongation or degradation may occur (Fraser & Bramley, 2004).

Carotenoids can be separated into two main groups, carotenes and xanthophylls (**Figure 2**). Carotenes are pure polyene carbohydrates, e.g., α- or β-carotene and lycopene, whereas xanthophylls, e.g., lutein and zeaxanthin, are formed by the introduction of hydroxy, epoxy or oxygen functions (Berlitz *et al.,* 2008). These compounds are synthesized in all types of plastids; among them, chromoplasts have the highest capacity for carotenoid accumulation (Li & Yuan, 2013). Depending on the carotenoid-bearing fine structural elements in the final state of chromoplast development, they are classified as globulous, tubulous, membraneous, and crystallous (Sitte *et al.,* 1980). The elucidation of the ultrastructure of carotenoids contained within chromoplasts has been pursued due to the influence in stability and bioavailability of these compounds (Schweiggert *et al.,* 2012a; Vásquez-Caicedo *et al.,* 2005). It has been demonstrated that carotenoids present in chromoplasts of the globular type are absorbed better than carotenoids contained in chromoplasts of the crystal type (Schweiggert *et al.,* 2012a). However, carotenoids with globular and tubular structure are more prone to *trans-cis* isomerization than those of crystal structure and undergo, therefore, easily through oxidative mechanisms (Vásquez-Caicedo *et al.,* 2005).



β-Carotene Lutein



Lycopene Zeaxanthin

Figure 2. Examples of carotenoid structures. a and b: carotenes, c and d: xanthophylls.

Carotenoids have previously been investigated in *P. guajava* and correlated to the pink color of the flesh of the fruit. Research on the profile of carotenoids in *P. friedrichsthalianum* has not been performed perhaps due to the lack of color in its pulp. According to previous studies, the main carotenoid in ripe pink guava is lycopene, followed by β-carotene and traces of other pigments (González *et al.,* 2011; Mercadante *et al.,* 1999; Padula & Rodríguez-Amaya, 1986; Setiawan *et al*., 2001; Wilberg & Rodríguez-Amaya, 1995). The carotenoid profile of pink guava from Brazil included the xanthophylls β-cryptoxanthin, rubixanthin, cryptoflavin, lutein, neochrome, zeinoxanthin, 5,6,5'6'-diepoxy-β-carotene, and 5,8-epoxy-3,3',4-trihydroxy-β-carotene, and the carotenes all-*trans*-lycopene, phytofluene, all-*trans*-β-carotene, 9-*cis*-β-carotene, 13-*cis*-β-carotene, 15-*cis*-β-carotene, γ-carotene, ζ-carotene, all-*trans*-lycopene, 9-*cis*-lycopene, 13-*cis*-lycopene, and 15-*cis*-lycopene (Mercadante *et al.,* 1999; Padula & Rodríguez-Amaya, 1986).

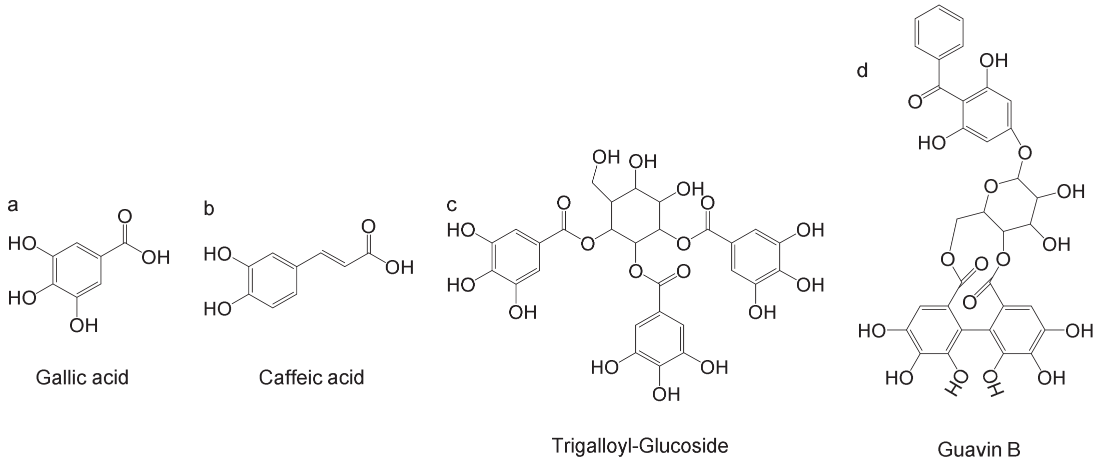
Quantification of the main carotenoids of pink guava has also been performed. According to González *et al.* (2011), the content of lycopene ranged between 18.25 and 28.07 µg/g fw, whereas the content of lutein varied from 0.03 to 0.11 µg/g fw. These contents were reported for three varieties of pink guava grown in Colombia. In the case of pink guava from Brazil, lycopene content ranged from 6.20 to 53.40 µg/g fw, whereas the content of β-carotene varied between 3.70 and 11.90 µg/g fw (Chandrika *et al.,* 2009; da Silva Oliveira *et al,* 2010; Setiawan *et al,* 2001; Wilberg & Rodríguez-Amaya, 1995). Based on these reports, ripe pink guava can be regarded as a high source of carotenoids with provitamin A activity as the levels of β-carotene surpass 2 mg/100 g, the limit set Britton and Khachik (2009) to classify high sources of carotenoids.

Provitamin A carotenoids are precursor of retinol, the active form of vitamin A. The most abundant dietary carotenoids that convert into vitamin A are β-carotene, α-carotene and β-cryptoxanthin. Among them, β-carotene is the most important vitamin A precursor mainly because of its prevalence in plant foods consumed by humans. Each provitamin A carotenoid has a bioactivity, that is, the ability of the carotenoids to generate one or two molecules of vitamin A, or to act as an intermediate in the synthesis of carotenoids with provitamin A activity. The relative bioactivity reported for all-*trans*-β-carotene is 100%; it results in two molecules of vitamin A for each β-carotene molecule (Rodríguez-Amaya, 1996). Lycopene, an acyclic carotenoid with 11 linearly arranged conjugated double bonds, lacks the β-ionone ring structure present in β-carotene, and is therefore devoid of provitamin A activity. However, due to its structure, lycopene has an exceptionally high oxygen-radical quencher capacity compared with other carotenoids (Britton & Khachik, 2009).

1.2.2 Polyphenols

Polyphenols are widely found in fruits, vegetables, nuts, and seeds. Known food containing high amounts of polyphenols, among others are cocoa, apples, tea, berries, coffee, wine, and onions (Motilva *et al.,* 2013). Polyphenols and their metabolites have the ability to modulate gene expression, epigenetic regulation, cell signaling, inflammation, antioxidant function, detoxification, and immune functions (Andarwulan *et al.,* 2012).

Polyphenols are characterized by a chemical structure with at least one aromatic ring with one or more hydroxyl groups. They range from simple aromatic ring structures such as phenolic acids to complex condensed tannins (**Figure 3**) (Motilva *et al.,* 2013). There are many groups of polyphenols; they can be classified as flavonoids and non-flavonoids. Flavonols, anthocyanidins, flavones, flavanone, flavanonols, isoflavones, flavanols, condensed tannins, complex tannins, and hydrolysable tannins comprise the first group, whereas the non-flavonoids consist of simple phenolic acids, phenyl alcohols, stilbenes, chalcones, gallotannins, and lignans (Motilva *et al.,* 2013).



Gallic acid

Caffeic acid

Trigalloyl-Glucoside

Guavin B

Figure 3. Examples of simple and complex phenolic compounds. a and b: Phenolic acids, c: hydrolysable tannin, d: complex tannin.