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Quercetin, kaempferol and apigenin in roselle (*Hibiscus sabdariffa* L.)

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Abstract

Roselle has been characterized by its intense red color due to the anthocyanins content. However, anthocyanins are mainly destabilized with sunlight and pH changes, causing a color change due to the oxidation of their double bonds or functional groups, which give them their biological activity of powerful antioxidants. The copigments, which are found within the structures, can be phenols that prevent oxidation and that are more biochemically stable because they stabilize and stop oxidation. The roselle of various colors ordered from higher to lower concentration of flavonoids are: Tecoanapa>Rosaliz>Alma Blanca>Sudan>Cotzalzin.

Keywords: flavonoids, quercetin, kaempferol, apigenin.

Introduction

The State of Guerrero is the first place in roselle (*Hibiscus sabdariffa* L.) production in the country, which is one of the main sources of revenue and additional income in the areas designed for this crop (SAGARPA, 2006).

Roselle contains flavonoids such as quercetin, kaempferol (Sawabe et al., 2005), myricetin, apigenin (Ezugwu, 2002), phenolic acids such as:protocatechuic, o-coumaric, p-coumaric, ferulic and organic acids such as: hydroxyacetic, ascorbic (Badreldin et al., 2004), citric, malic (Wong et al., 2002) hibiscus and tartaric acids (Fasoyiro et al., 2005).

The dye is extracted from the roselle calyx, which is used as a Subramanian, 2006), jelly (Badreldin et al., 2004), sauce, wine, tea, sodas, desserts, pulp, natural flavor and colorant for various foods (Tsai et al., 2002) such as: jam (Mohamed and sups (Mounigan and Bradie, 2007), fresh fruit water, ice creams, flour, liquor, cream, gelatins, seasonings, dressings, syrup and candies (D'Heureux and Badrie, 2004).

In humans, the roselle calyx extract works as: antihypertensive, antimicrobial, antibacterial, analgesic, anti-inflammatory, antipyretic (Ajay et al., 2007), antiseptic, digestive, depurative, as appetizer, as a vitamin source, sedative, diuretic (Kamali et al., 2006), anticancer, antimutagenic, antispasmodic, antiparasitic, antioxidant, stimulant (Usoh et al., 2005), emollient, restorative (Steenkamp et al., 2004), astringent, healing, lipid-lowering and emulsifying agent (Tsai et al., 2002). This biological activity has been linked to the anthocyanins content. However, the structures of anthocyanins are stabilized by copigments that can be phenols, mostly flavonols and some flavones. Therefore, the aim of this work was to determine the content of flavonols and flavones by HPLC in chalices of roselle of different colors.

Materials and Methods

The sampling was done in El Tamarindo community, municipality of San Marcos, Guerrero, Mexico.

Three samples of each plant with red, cherry, pink and white chalices were collected. The chalices were dried in an oven to remove water and then they were pulverized to continue with the analysis.

The extracts of dried chalices of roselle of the varieties: Cotzalzin, Sudan, Tecoanapa, Rosaliz and Alma Blanca (Navarro, 2004) were analyzed in order to confirm the presence of flavonoids by high performance liquid chromatography (HPLC), in particular two flavonols: quercetin and kaempferol, and a flavone: apigenin.

Initially, to 0.25 g of dried chalices were added 25 mL of 1.2 M HCl in 50% aqueous methanol. Then, the hydrolysis was done for 2 hours at 98°C under reflux, and a 50 μL aliquot was taken and acidified to 250 mL with acidified water (pH 2.5, trifluoroacetic acid). The HPLC analysis was column, 125 mm length, 4 mm internal diameter and 5μm particle size. The detection was made at 365 nm with a 1.5 mL min^{-1} flow rate, and an isocratic gradient of 35:65 methanol-water. Acidified water was used (pH 2.5, trifluoroacetic acid) according to Crozier et al. (1997) method, modified by Vargas et al. (2006), and using three reference standards. The total copigment content of each roselle variety was statistically different, according to the carried out with an Agilent Technologies 1200 chromatograph with diode array detector, hypersil ODS comparison of Tukey means $\alpha = 0.05$.

Results and Discussion

Red color roselle showed a high concentration of quercetin, kaempferol and apigenin. However, the white roselle had a higher content of quercetin and apigenin. This fact, could be associated to the synthesis of these metabolites as they are the preliminary products in the synthesis of anthocyanins. The synthesis chains are focused on anthocyanins and their concentration can increase according to the environmental conditions of each of the producing zones and the genetic content of each of the cultivars (Kong et al., 2003). These can respond to light or to the environmental stress, because for example, at the end of the rainy season it is harvested and stress is generated due to the drought. Anthocyanins and flavonoids were found in all red roselle varieties, whereas in the Sudan cultivar they were found in lower concentrations. However, flavonoids content was very similar in Tecoanapa and Rosaliz cultivars, maybe because the synthesis was divided; while in Tecoanapa, it could be inferred that it has a higher anthocyanins content in comparison to Cotzalzin, which showed a lower copigment concentration, and also the pink color limits the anthocyanin content of lower proportion than Tecoanapa.

Sudan roselle showed a lower copigment proportion, the size of its chalices is double than the other four roselles, and its intense cherry color make it special because of its high anthocyanins concentration (Ariza et al., 2014). This cultivar could be suitable to obtain natural dyes using the supercritical fluid technique. It is important to mention that each of the cultivars has its own specialty in the metabolism of the metabolites, and that each one of these can be stimulated by its genetic origin and by environmental conditions. People have chosen the Tecoanapa variety among all cultivars studied because of flavor, color and aroma. While Sudan only provides an intense red color, and the pink ones are consumed in smaller scale; however, Alma Blanca shows a high potential for biological activity of phenolic acids, flavonols and flavones, reason why they have been characterized in each one of their cultivars with beneficial health attributes.

Kaempferol is a molecule that could be the starter of the anthocyanins synthesis and other components, because of its functional groups that can be polymerized depending onthe environmental conditions and to the synthesis of receptors and stimulators (Jaakola et al., 2000: Vargas et al., 2002) that have to make the synthesis of each of the metabolites of the roselle chalices. Quercetin is a metabolite that has antioxidant activity and biological applications in humans, the synthesis of these metabolites can be because of the genetic characteristics of the plant or because the adaptation of the cultivar to conditions of deciduous forest (Sayago et al., 2007 and Usoh et al., 2005).Quercetin content in Alma Blanca cultivar may be due to its interrupted synthesis until forming anthocyanins, a synthesis deviation is generated and the quercetin synthesis increases (Jakola et al., 2002). In the Sudan cultivar it was observed abundant anthocyanins and zero quercetin, which makes the anthocyanins highly soluble, and as quercetin is absent in the calyx of this cultivar, it makes it a promising cultivar fordye extraction for the soft drink and food industries. On the other hand, Alma Blancaroselle contained high concentrations of quercetin (Jaakola et al., 2000), compared to other plant materials, this cultivar has higher concentration than other tropical plants, which can be used in traditional medicine for the treatment of diabetes, obesity and kidney stones, among others. Apigenin is a flavone that has been attributed biological activity in humans such as avoiding risks of myocardial infarction (Mohamed et al, 2006a and b; Vargo et al., 2006), and even when it is found in a lower concentration it has also been associated to the biological activity against herbivory (Crozier et al., 2000; Iwashina, 2003), as well as defense of the plant against oxidative stress (Cruz et al., 2010).

The five roselle cultivars showed their own specialty in the production of metabolites (Jeong et al., 2004), as it can be observed in Table 1. Alma Blanca and Tecoanapa had a higher concentration of total phenols analyzed by HPLC; however, the other roselle cultivars showed different metabolites of different structure, for example in Sudanit was only observed the presence of kaempferol and apigenin.

From this research it is possible to assume that depending on the roselle variety and color, they might have different uses, such as the aroma extraction, color and/or infusions of metabolites of interest for the food and industrial sectors.

Table 1. Concentration of quercetin, kaempferol y apigenin (mg kg^{-1} de dry weight)in the calyxdry of roselle variety.

Roselle varitety	Total Flavonoids	Quercetin	Kaempferol	Apigenin
			mg kg^{-1}dry weight	
Tecoanapa (red)	2102	904b	388a	810b
Rosaliz (pink)	2060	902b	355b	803c
Alma Blanca (white)	2260	1022a	359b	857a
Sudán (reda)	1120		309c	811b
Cotzalzin (pink)	793			793d

Averages in column with unequal letters are statistically different according to Tukey's mean comparison α= 0.05

Conclusion

The flavonoids from the Tecoanapa and Rosaliz roselles were in higher concentration than in the Sudan and Cotzalzin roselles; however, Alma Blanca showed higher total flavonoids concentration than Sudan and Cotzalzin. Sudan had a low concentration of kaempferol and apigenin, whereas quercetin was not detected in this cultivar.

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