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CHANGES IN BIOACTIVE COMPOUNDS IN FRUITS OF *Eriobotrya japonica* GROWN IN THREE DIFFERENT LOCATIONS IN NORTHEASTERN PERU

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ABSTRACT

Recently, there has been a growing interest in bioactive compounds metabolized by plants, which are an important nutritional source for the human diet and are found in almost all vegetables and fruits. The objective was to evaluate the changes of bioactive compounds during the ripening of loquat *(Eriobotrya japonica)* fruit. For this purpose, fruits were collected at three different stages of ripening from three different production sites in the Amazon region, located in northeastern Peru. Color, total phenolic content (Folin-Ciocalteu method), antioxidant activity (DPPH free radical method) and total flavonoids (colorimetric assay) were determined for all samples. Data were subjected to analysis of variance and means were compared by Tukey's test ($p \leq 0.05$). Color and bioactive compounds depend on the stage of ripening and, to a lesser extent, on the origin of the fruit. Ripe fruits have a higher content of phenolic compounds and flavonoids (up to five times higher) that can be used in the food and pharmaceutical industry.

Key words: antioxidant, maturity, loquat, origin.

INTRODUCTION

Bioactive compounds, mainly composed of secondary plant metabolites such as polyphenols, are antioxidant compounds capable of eliminating free radicals in the human body (Hemmat y Hikal, 2017). The fruits are considered an important source of antioxidants that reduce oxidative stress, enhance immunity and reduce the incidence of disease (Mazumdar *et al.*, 2019).

The loquat (*Eriobotrya japonica* Lindl.), a fruit of Asian origin, is cultivated in many parts of the world. It belongs to the Rosaceae family and its flowering period is from April to June (Ahumada *et al.*, 2017; Hemmat y Hikal, 2017). It develops in tropical to temperate conditions. Its nutritional value has been widely demonstrated, so it can be consumed fresh and has a high potential for industrialization. (Sagar *et al.*, 2020). Ahumada *et al.* (2017) y Dhiman *et al.* (2021) mentioned that in addition to the fruit, different parts of the plant such as leaves and flowers have been studied, reporting a

high content of phenols and triterpenes. The fruit has sugars, organic acids, flavonoids, vitamins, phenolic acids and carotenoids (Alos *et al.*, 2019; Cañete *et al.*, 2015), as well as a low toxicity due to its high antioxidant capacity (Delfanian *et al.*, 2016). Its extracts can be used in the pharmaceutical and food industry.

One of the problems faced by fruit growers is the uniformity of fruit maturity. In this regard, it has been shown that, during ripening, the content of phenolic compounds can be reduced by up to 60% (Ahumada *et al.*, 2017). Therefore, a deeper understanding of the changes in these compounds during fruit ripening is needed to standardize harvest timing and uniformity. For Liu *et al.* (2016), it is necessary to study the content of polyphenols, antioxidants and other similar compounds at different stages of ripening (Kim *et al.*, 2019). Therefore, the objective of this study was to evaluate the variations of color, antioxidant activity, flavonoids and polyphenols in loquat fruit at different stages of maturity.

MATERIALS AND METHODS

Plant material

The fruits of *E. japonica* were harvested during April 2021 at three stages of maturity, in plantations located in three different districts: Camporredondo (6° 12′ 50″ S, 78° 19′ 6.96″ W), Pisuquia (6° 30′ 42.01″ S, 78° 4′ 30″ W) and Ocallí (6° 14′ 6″ S, 78° 16′ 0.84″ W), belonging to the province of Luya, Amazonas region, Peru. Ripening stage was determined by fruit color, coded with numbers: (03) yellowish coloration and sparse hairiness; (04) orange coloration and sparse hairiness; and (05) intense orange coloration and minimal hairiness where it reaches its maximum size (Figure 1).

At each site, for each stage of ripening and in triplicate, 1 kg of fruit was collected. They were transported to the Agroindustrial Technology Laboratory of the Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, where the pulp was manually separated from the peel. It was then stored under frozen conditions (-18 °C) until further analysis.

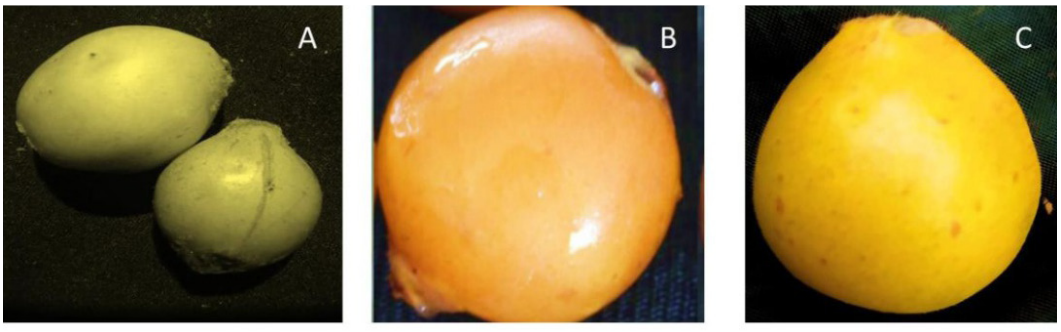

Figure 1. Ripening stages of loquat (*Eriobotrya japonica* Lindl.) fruits collected from plantations in three different districts for the study. A: (03); B: (04); C: (05).

Determination of color index

From each sample, 20 uniform fruits that did not show lesions or damage were selected. In three zones around the equatorial plane of the fruit, measurements were made with a colorimeter (Chroma Meter, CR-400, Japan). Color coordinates were captured on the Cie L*a*b* scale, following the procedure described by Alos *et al.* (2019) using the Hunter a*/b* ratio, which is negative for green fruit, around 0 for yellow fruit at color break and positive for orange fruit.

Determination of total polyphenol content

Total polyphenols were determined by the Folin-Ciocalteu spectrophotometric method established by Singleton y Rossi (1965). For this purpose, the fruit pulp was conditioned in accordance with Seon *et al.* (2020). 100 µL of the sample was prepared and diluted with water to 3 mL, then 0.5 mL Folin-Ciocalteu reagent was added, followed by 2 mL of a 20 % sodium carbonate solution. The absorbance was measured at 650 nm in a UV-VIS spectrophotometer (Unico, S2100, USA). A gallic acid calibration curve was performed with different increasing concentrations of gallic acid between 0 and 16 ppm from a concentrated solution of 100 mg L^{-1}. Total polyphenol content was expressed in terms of mg gallic acid equivalents (GAE) per 100 g of fresh weight material.

Evaluation of the percentage of inhibition (antioxidant activity)

The antioxidant activity of loquat fruit pulp was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay established by Brand-Williams *et al.* (1995) and described by Hadjipieri *et al.* (2020). For this, initially a DPPH solution was prepared in 100 mL ethanol (0.1 mM), 50 µL of sample extract was taken and mixed with 950 µL of ethanol. Subsequently, 2 mL of DPPH radical solution was added, made up to 4 mL with ethanol and allowed to stand for 30 min in the dark. In addition, a "blank" solution was prepared with the same procedure substituting ethanol for the sample. The absorbance was determined spectrophotometrically at 517 nm. The results were expressed as percentage of inhibition (%) by the difference in absorbance of the blank solution and the pulp, with respect to the blank solution.

Determination of total flavonoid content

The determination of flavonoids was performed according to the method employed by Akbulut *et al.* (2017), where 1 mL of sample that was previously diluted with 4 mL of distilled water, mixed with 300 µL of a 5 % $NaNO_2$ solution and 300 µL $AlCl_3$ (10 %), and allowed to rest for 5 min. Subsequently, 2 mL of NaOH solution (1M) was added and diluted with distilled water until 10 mL of total solution was obtained. The absorbance was measured using a spectrophotometer (Unico, S2100, USA) at a length of 510 nm; in addition, a calibration curve was performed with different routine concentrations. Total flavonoid content was expressed on a rutin equivalent (RE) basis as mg RE g^{-1} of fresh weight material.

Data analysis

Results were expressed as mean ± standard deviation and were processed by one-way analysis of variance (ANOVA). The Tukey test was used in each place of origin to demonstrate possible statistical differences with a significance level of $p < 0.05$ using Minitab 19 Statistical Software.

RESULTS AND DISCUSSION

A decreasing trend was observed in the L* and b* coordinates. In contrast, a* showed an increase as the fruit ripened. It is known that color is related to ripening due to the accumulation of pigmentation and the variation in sugar and acid content in the fruit; (Samaniego *et al*., 2020); as mentioned, lightness varied between 40.09 and 61.3, similar to that reported by Hadjipieri *et al*. (2017) for loquat (52.12–74.32). The tendency to decrease brightness, according to Samaniego *et al*. (2020), expresses the intensity of fruit color as the fruit ripens. The chromatic coordinate a* ranged from 1.3 to 13.2, and b* ranged from 9.2 to 29.1 with a certain tendency to decrease in the three states (Table 1), which are values approximating those obtained in loquat in the study of Hadjipieri *et al*. (2017). These coordinates indicate the variation of red or yellow color in the fruit, related to the accumulation of anthocyanins (Samaniego *et al*., 2020).

Table 1. Color characteristics (L, a* and b*; a*/b*) of loquat (*Eriobotrya japonica* Lindl.) fruits at different ripening stages.

Origin	E	L*	a*	b*	Color index
Camporredondo	3	58.848±3.577 a	4.277±2.151 c	27.706±3.987 a	0.161±0.083 b
	4	58.811±3.429 a	6.778±1.859 b	28.475±4.208 a	0.245±0.087 b
	5	41.619±3.622 b	13.130±1.313 a	11.802±4.084 b	1.219±0.361 a
Ocallí	3	58.808±4.312 a	1.303±1.115 c	28.167±4.368 a	0.060±0.051 b
	4	59.228±3.749 a	3.691±0.949 b	25.894±3.754 b	0.147±0.047 b
	5	41.837±1.689 b	11.814±0.788 a	9.277±3.935 c	1.440±0.473 a
Pisuquia	3	59.925±2.016 b	2.098±1.199 c	28.508±2.798 a	0.074±0.044 b
	4	61.325±2.708 a	4.035±1.462 b	29.146±3.693 a	0.142±0.061 b
	5	40.091±3.293 c	13.233±1.649 a	9.680±3.154 b	1.453±0.296 a

L*: brightness; a*: red or green content; b*: yellow or blue content. For each ripening stage, different lowercase letters indicate significantly different means among genotypes (Tukey; $p \leq 0.05$).

The Hunter ratio in the study did not report negative values at the ripening stages, showing an increase as the fruit ripened, with values ranging from 0.06 to 1.45. The color index reported values close to zero, which usually present pale yellow colors; the highest values reported (orange colors in the case of loquat) are also associated with ethylene production during ripening (Hadjipieri *et al*., 2017) (Figura 1). The maximum color index obtained in loquat was 1.45, which, according to Alos *et al*. (2019) y Hadjipieri *et al*. (2017) may be due to the accumulation of carotenoids by fruit development. This variation contradicts the theory that determines this fruit as non-

climacteric, which according to Alos *et al*. (2017; 2019) indicates a ripening pattern due to color changes by ethylene production and respiration rate, demonstrating that loquat presents a climacteric ripening.

As for the variation in total polyphenol content, the values ranged from 39.2 to 150.9 mg GAE 100 g^{-1}, with the fruit from Ocallí being higher. The variation of the content in the three locations may be due to genetic, environmental and postharvest conditions of loquat fruit (Delfanian *et al*., 2016; Dhiman *et al*., 2021). The results obtained are similar to those reported by Ahumada *et al.* (2017) per 100 g of sample (66 to 96 mg GAE); in addition, a tendency to increase as the fruit ripens is observed, since there is an adequate fit of the data line between polyphenols and ripening stage with an R2 greater than 0. 77, which are related to the concentration of certain phenols that appear during ripening (Hadjipieri *et al*., 2020), an inverse relationship with the content of total soluble solids in the form of free sugars and a greater primary metabolism that generates the necessary substrate for the biosynthesis of phenolic compounds (Dhiman *et al*., 2021; Kim *et al*., 2019).

The polyphenol content in loquat (Figure 2) was found to be below that reported in comparison with native berries from the Amazon region reported by Rojas-Ocampo *et al.* (2021), which reached a maximum of 4.87 mg GAE 100 g^{-1}; and by Grández-Yoplac *et al.* (2021) who obtained 12.61 mg GAE 100 g^{-1} in the drying of blackberry. Similarly, the content found in the ripening stages of loquat was low in comparison to extracts of guava, blueberry and aguaymanto calyx fruits as reported by (Chauca-Aguilar y Chávez-Quintana, 2020).

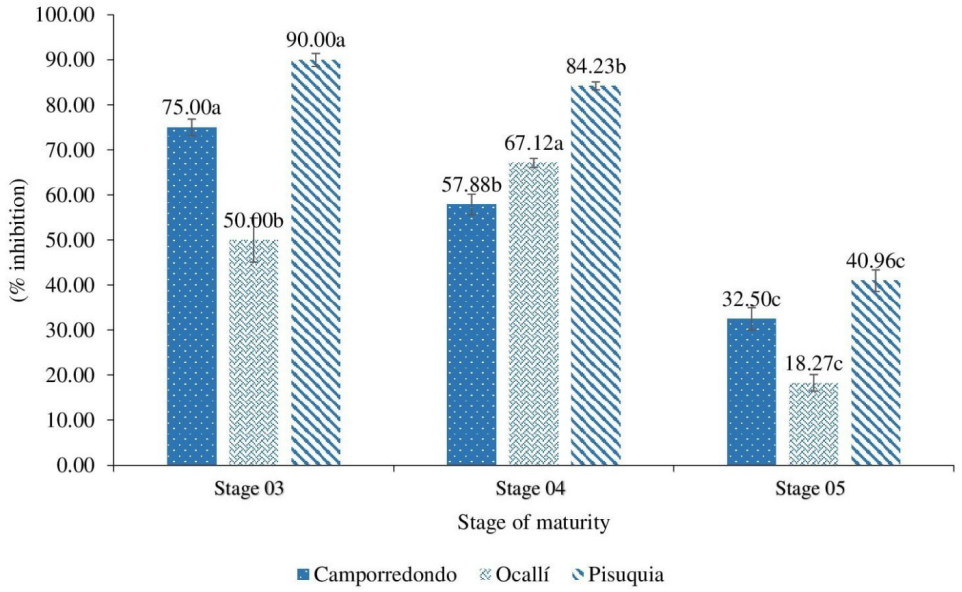

Figure 2. Changes in total polyphenol content at different ripening stages of loquat (*Eriobotrya japonica* Lindl.) fruits from three origins in the Amazon region. For each ripening stage, different lowercase letters indicate significantly different means among genotypes (Tukey; $p \leq 0.05$).

The percentage of inhibition decreased from 90 % to 18.27 % as the fruit ripened, although in the Ocalli fruit at stage 4 an atypical increase was reported that generated a low linear fit to the data ($R^2 = 0.4$). On the other hand, Pisuquia fruit showed higher antioxidant activity by DPPH assay compared to the rest at the three ripening stages. In general, it was observed that there was a linear fit with a R^2 greater than 0.83 in two districts (Figure 3). The variation in loquat antioxidant activity is due to the higher phytochemical content of oxidative substances in the early stages of ripening, which tends to decrease during ripening, as shown in the results of the study (Hadjipieri *et al*., 2020; Hou *et al*., 2021).

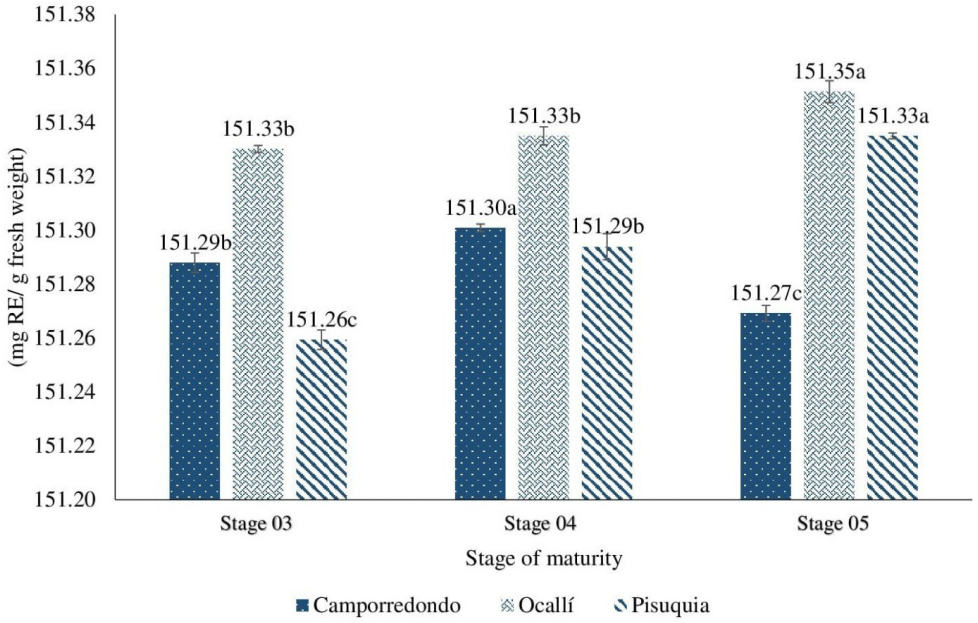

Figure 3. Changes in antioxidant activity (percentage of inhibition) at different ripening stages of loquat fruits (*Eriobotrya japonica* Lindl.) three provenances from the Amazon region. For each ripening stage, different lowercase letters indicate significantly different means among genotypes (Tukey; $p \leq 0.05$).

Differences in percent inhibition by provenance, according to Sagar *et al*. (2020), are attributed to antioxidant activity in loquat due to cultivar contrasts, environmental effects, and genetic background influencing fruit antioxidant accumulation. Likewise, the linear fit of the data among the variables (Figure 3) is related to that determined by Ahumada *et al*. (2017), who report a significant decrease in antioxidants associated with fruit ripening. Furthermore, loquat and other fruits grown in the Amazon region with high antioxidant activity, as reported by Chauca-Aguilar y Chávez-Quintana (2020), Grández-Yoplac *et al.* (2021) y Rojas-Ocampo *et al*. (2021), are a source of bioactive compounds of plant origin, which in turn are influenced by genetic factors, environmental conditions and the state of ripening of the fruit (Chauca-Aguilar y Chávez-Quintana, 2020).

The flavonoid content (Figure 4) was higher than 150 mg RE g^{-1} sample. As the fruit passes through the ripening stages considered, the flavonoid content increased, thus showing that this fruit exhibits an important content of the nutrient that helps to inhibit oxidative action *in vivo*, demonstrating in turn its capacity to eliminate free radicals (Dhiman *et al*., 2021; Seon *et al*., 2020). It was also demonstrated that loquat has a high degree of accumulation of flavonoids (Akbulut *et al*., 2017), with a higher content in the fruits from Ocallí.

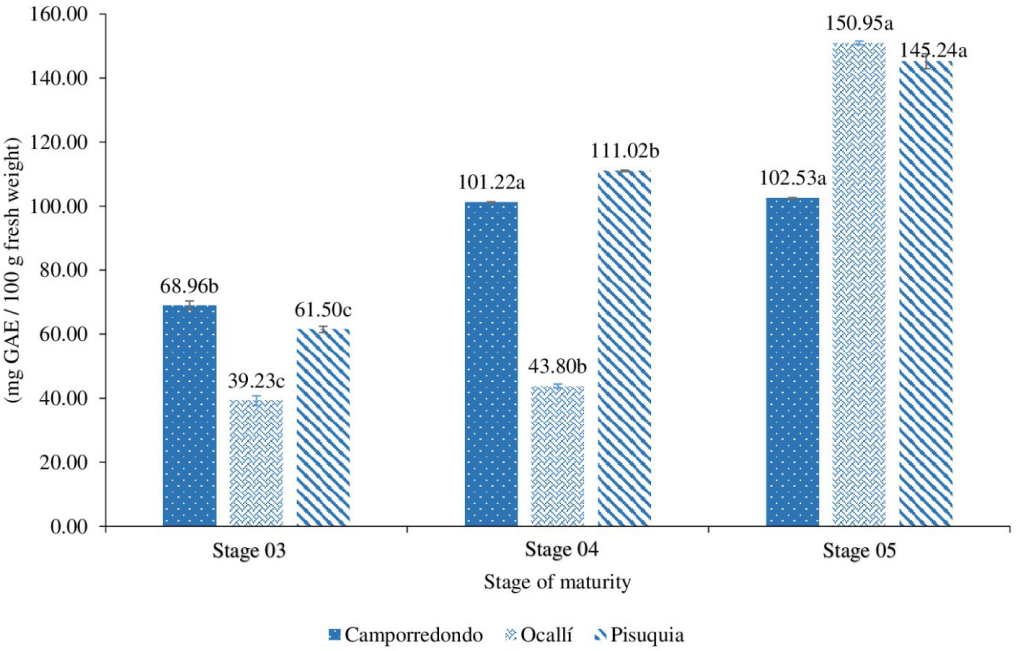

Figure 4. Changes in flavonoid content at different ripening stages of loquat fruits (*Eriobotrya japonica* Lindl.), three origins from the Amazon region. For each ripening stage, different lowercase letters indicate significantly different means among genotypes (Tukey; $p \leq 0.05$).

The data showed a linear trend of ripening stage with flavonoid content, obtaining R^2 values greater than 0.9 in the districts of Ocallí and Pisuquia, with the exception of the fruit from Camporredondo, which showed atypical behavior. From this, it can be deduced that loquat, in similarity with other fruits, presents a relationship between the level of flavonoids and the state of ripening as obtained by Grigio *et al.* (2021) y Neves *et al.* (2015), who agree in indicating that the more advanced the ripening, the higher the flavonoid content. Depending on the place of cultivation and stage of ripening, the content of flavonoids tends to increase (Samaniego *et al*., 2020). The differences can be explained by the presence of enzymes responsible for flavonoid synthesis that influence their variation during fruit ripening (Hou *et al*., 2021). Likewise, the increase of flavonoids during the ripening of loquat shows a similar behavior to camu-camu,

where Grigio *et al*. (2021) reported that the flavonoid content in the ripe fruit presents the highest level, followed by the semi-ripe and unripe fruit.

CONCLUSIONS

The color of loquat fruits (*Eriobotrya japonica* Lindl.) depends basically on the stage of maturity, the place of production does not have a considerable influence. As loquat fruit increases in maturity, the content of phenolic compounds and flavonoids increases; however, the antioxidant activity of the extract decreases. The ripening stage is essential to obtain loquat fruits with a high content of bioactive compounds for food and industrial purposes.

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