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Phenolic Compounds in the Pulp, Pericarp and Seed of Litchi (*litchi chinensis sonn.*) in different Stages of Maturation

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Abstract

Numerous studies exist regarding the antioxidant contents of fruits, including the so-called exotic fruits. Mexico has a wide range of fruits and vegetables and even has cultivars that originate from other regions. One example is the *Litchi chinensis* Sonn, which is consumed fresh because it ripens quickly and is difficult to handle and conserve. Since there are few reports regarding lychees harvested in Mexico, in the present work, the physicochemical and antioxidant properties of the pulp, seed and skin of the litchi were determined, and a drink is proposed to preserve litchi bioactives. Higher values of phenolic compounds were obtained in the seed and pericarp (3549 and 2075mg EAG/100g wb, respectively) with antioxidant activities of 252.58 and 239.9μMol TE/g wb, respectively. In the liquor, the phenolic compounds and antioxidant capacity decreased by only 18 and 12%, respectively, during storage (17 weeks).

Keywords: Litchi; Pulp; Pericarp; Seed; Phenolic compounds; Antioxidant Capacity

Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are constantly generated in vivo in physiological reactions and overproduce in pathological conditions, which produces oxidative stress [1]. The amount of damage caused is controlled by antioxidant substances produced in the body itself or acquired in the diet [2]. The use of antioxidants of natural origin is currently proposed, which are found in spices, fruits and vegetables and mainly include vitamins, carotenoids, anthocyanins and phenolic compounds [3]. The antioxidant activity is determined by the chemical structure [4]. Certain research has found and identified phenolic compounds in the litchi shell, pulp and seed that have anti-carcinogenic, anti-inflammatory and cardio-protective effects and are capable of eliminating free radicals and preventing chronic degenerative diseases [5].

Mexico has a wide range of fruits and vegetables, some of which are considered exotic and have been proven to have antioxidant properties [6] such as the litchi (*Litchi Chinensis* Sonn.). The litchi is a species in the family Sapindaceae and is a fruit of subtropical climates such as southern China and northern Vietnam [7]. It is a bittersweet fruit with high phenolic and vitamin C contents that give it antioxidant properties [8]. The litchi is commonly consumed fresh due to the difficulty in handling and transporting it and because it ripens quickly. Using traditional methods, this fruit is used to produce liqueurs, drinks with fresh fruit, fermented beverages, liquors and fruit juice. In industry, this fruit is mainly sold in canned syrup or as dried fruit [9]. In the past ten years, the number of litchi plantations in Mexico has significantly increased, and the annual production is greater than 18 thousand tons, with Veracruz State as the major producer at 9 thousand tons [10,11]. Because there are few reports regarding litchis of Mexican origin, in the present work the amount of phenolic compounds and total antioxidant activity present in this fruit was determined, and a beverage that conserves the bioactive compounds present in this fruit is proposed.

Materials and Methods

Collection and Transport

Samples of *Litchi chinensis* were collected in the Misantla region of Veracruz State, Mexico. Due to the rapid deterioration of the pericarp coloration under ripening conditions (the physiological state required for analysis in the laboratory) the transportation of the samples was carried out under refrigerated conditions (4 °C) before analysis was performed.

Raw Materials

The fruits were collected at different ripening stages and selected considering their physical characteristics, fruit size and pericarp color according to the [12] standards and the CDMX supply center (CEDA) classifications [13], the latter because they are national sources and conduct trade with the fruit merchants in Mexico.

Sample preparation

The pericarp was separated from the fruit manually; then, the sample was reduced in size. The pulp or aril of the dehusked fruits was ground until a homogeneous sample was obtained, and the seed was crushed in a mortar.

Physicochemical parameters

The soluble solids (°Bx) in the pulp juice were determined as established by using an Abbe refractometer [14]. The pH was measured using a pH 21HANNA potentiometer according to Official Mexican Standard [15]. The density (g/ml) and the percentage of acidity (g malic acid/100g) in the juice of the pulp were measured by the methods of the standards [16,17], respectively.

Extract preparations. The extracts were prepared using three solvents: 100% methanol, methanol/HCl (2%), and 96% ethanol. Samples of 2g per 10ml solvent were mixed and kept for 24h in the dark at room temperature, then the extracts were filtered with Whatman No 45 paper and stored in amber glass jars.

The total phenolic compounds (TPC) were quantified with the Folin-Ciocalteu reagent described by [18], with some modifications. A sample of 100µL of extract was mixed with 100µl of Folin-Ciocalteu reagent and allowed to react for 3 minutes at room temperature. Then, 2ml of sodium carbonate (7.5%) and distilled water were added for a total of 5ml. The mixture was stored in the dark for one hour, then the absorbance at 725nm was measured; the results were expressed in milligram equivalents of gallic acid (GAE) per 100g of fresh sample (wb).

The same extract was used to determine the antioxidant activity by DPPH radical scavenging [19]. Acidified methanol was not used as the extraction solvent because HCl creates interference that results in overestimated values of antioxidant capacity [20]. A 100-µL aliquot of the extract and 2.9ml of DPPH 0.1mM solution were mixed and stored in the dark at room temperature for 30 minutes, then the absorbance was measured at 517 nm and recorded as A1. The capacity to reduce DPPH radicals was determined by the change in color due to the reaction with the extract. A blank was prepared with 2.9ml of the DPPH solution and 100 µL of the solvent used in the extract, and the absorbance of the blank was recorded as A. The percent inhibition was calculated using Equation 1 and was expressed as equivalent µMol TROLOX (TE) per g of fresh sample

$$\%\text{Inhibition.} = (⟦A\text{-}A⟧\_1/A)\times 100 \quad (1)$$

The calibration curve of Trolox concentration vs. percentage of inhibition was measured (Sánchez-Moreno, 2002). To obtain the IC 50 value, decimal dilutions of the extract or sample from 10% to 100% were reacted with the DPPH solution, obtaining the absorbance values. The percentage of inhibition was calculated for each dilution, and the concentration required to inhibit 50% of the initial concentration of DPPH was calculated [21].

Analysis of phenolic compounds by UV-VIS Spectrophotometry. The extracts used were the same as above and were prepared for TPC determination using 100% methanol HPLC grade. After the extracts were filtered with Whatman No 45 paper, they were baked in a rotary evaporator under vacuum at 35 °C until dry to concentrate the samples. The samples were then were recovered with 3ml of HPLC grade methanol and stored in amber bottles at -15 °C. The absorption spectra were obtained with the methodology proposed by Camont et al., [22] with certain modifications. Standard solutions were prepared with water to obtain the absorbance maxima on a scale of 0 - 0.5, then the absorption in the range of 230 to 320nm was measured. HPLC grade methanol was used as a solvent to adjust the concentrations of gallic acid, catechin and epicatechin to achieve absorption maxima within the desired scale.

Preparation of litchi liquor: A liqueur was formed from the litchi pulp (ground aril) by a traditional method: 500g of the pulp and 500ml of ethyl alcohol 96 °GL were macerated for a month in the dark at room temperature. Then, the mixture was filtered, and a crystalline solution was obtained. A sucrose syrup was prepared at 50 °Bx and mixed with 250ml of the litchi ethanolic solution and 250ml of water. This mixture was poured in bottles to 90% capacity that were stored at room temperature in the dark. The shelf life was studied by measuring pH, °Bx and total phenol values over 17 weeks, and the antioxidant capacity at the beginning and the end (weeks 0 and 17). All the liquor samples were produced in triplicate, prepared at the same time, and packed in the same day.

Chemicals

Gallic acid (>98.0%), Sodium carbonate anhydrous (7.5%), catechin (>98%), epicatechin (>90%), 2,2-Diphenyl-1-picrylhydrazyl, Folin & Ciocalteu's reagent, Potassium permanganate, Sodium hydroxide (0.1N), Methanol grade HPLC (>99.9%). (Sigma-Aldrich, St. Louis MO, USA).

Results and Discussion

The fruits were selected by the color of their pericarp and their caliber (measure of the maximum equatorial diameter) according to the classifications of the CEDA (Supply Center of CDMX) [13] and were grouped into 3 categories (first, second and third), while according to the [12] an "Extra" Category, Category I and Category II were designated as shown in Figure 1.

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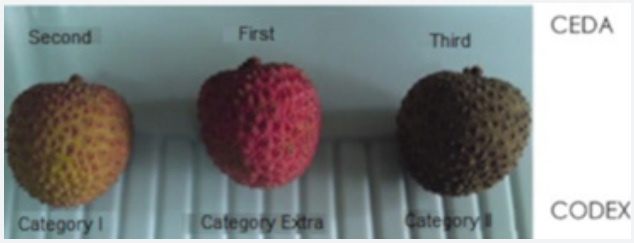

Figure 1: Classifications of litchis according to CEDA [12] and [13].

The fruits in the first category are distributed among supermarkets and luxury restaurants, while the second category includes fruits widely distributed between local markets and street markets; this category is the best known among the general public due to its low price and accessibility, while the third category is considered waste. For this reason, the first and second categories were chosen for analysis in this work and coincide with the "Extra" and "I" categories according to the CODEX STAN. Category II marks a fruit of acceptable quality that due to size and coloration does not fall into the higher categories.

Physicochemical parameters

Table 1: Physicochemical parameters for the pulp, TPC and AA of litchis (aril (pulp), pericarp and seed) in the categories Extra and I.

Sample		Category Extra	Category I
Aril	°Bx	18.2±0.6	17.6±0.7
	pH	4.3±0.1	4.5±0.2
	Acidity (malic acid (g/100g)	0.35±0.07	0.34±0.09
	Density	1.2571±0.058	1.1406±0.045
	Ethanol	---	---

As shown in Table 1, no significant differences were found between the two categories for the soluble solid values (°Bx), which indicates that these categories have similar phenological states (maturations) [23]. Rivera et al., [24] reported that litchi maturation presents an increase in pH and soluble solids, indicating that changes in non-climacteric fruits occur as a natural part of metabolic changes during maturation that give the lychee pulp its characteristic sweet taste. Azevedo et al., [25] reported values of 17.1 ± 1.0 °Bx for litchis cultivated in Brazil. Other authors have reported similar values for soluble solids (15-20 °Bx) in lychees from China, India, Taiwan, Hawaii and Australia [26,27]. No significant differences were found between the pH of the two categories. The pH values ranged between 4.3-7.5; pH values in this range stabilize certain phenolic compounds such as anthocyanins [28]. The density of the fruits in the Extra Category was greater than that for Category I, presenting a significant difference that is attributed to different stages of maturity [29]. However, no significant difference was found in the °Bx values, so other compounds present in the pulp may contribute to the higher density of the fruits in the Extra Category30. No significant difference was found between the acidity values of these categories (Table 1).

Azevedo et al., [25], found acidity values of 0.7 ± 0.1 g citric acid/100g in litchis harvested in Brazil. Islam et al., [30] analyzed the chemical compositions of six varieties of this fruit, reporting acid values in the range of 0.26-0.36% citric acid.

For the Mexican cultivar "Brewster", the total soluble solid content is between 19.7 and 21.3 °Bx, and the acidity is between 0.3 and 0.5% malic acid [31]. The differences in the data reported are attributed to the origin of the fruit tested [11]. One of the determining factors that divides the categories is the color of the pericarp. The anthocyanins present in the pericarp react with oxygen or humidity in the environment, changing their characteristic pink color brown and resulting in colorless compounds [32]. Therefore, according to the physicochemical parameters obtained, the difference in the pericarp coloration is independent of the phenological state of the fruit. Classification methods do not take into account the values of these physicochemical parameters.

Total phenolic compounds (TPC)

The phenolic compound contents and antioxidant activities present in the aril, pericarp and seed are presented in Table 2.

Table 2: TPC and AA of litchi (aril (pulp), pericarp and seed).

		Category Extra		Category I	
	Solvent ↓	**TPC** (mg GAE/100 g wb)	**AA** (µMol eq. TROLOX/g wb)	**TPC** (mg GAE/100 g wb)	**AA** (µMol eq. TROLOX/g wb)
Aril (pulp)	Methanol	65.34±5.83	4.31±0.42	47.44±3.08	1.4±0.03
	MetOH-HCl	**129.28±6.41**	----	**80.04±4.65**	----
	Ethanol	66.18±6.19	3.73±0.32	---	----
Pericarp	Methanol	1497.58±153.42	135.6±12.04	803.52±67.15	59.9±4.01
	MetOH-HCl	**2075.49±180.2**	----	**1179.39±89.43**	----
	Ethanol	----	----	---	
Seed	Methanol	1868.22±104.62	252.58±24.1	1793.29±152.05	239.88±13.93
	MetOH-HCl	**3549.69±267**		**3307 ±287**	----
	Ethanol	---		---	----

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The methanol in the acid medium was the solvent that the extracted the highest amounts of phenolic compounds for all the samples. Significant differences were found between the two categories for the aril extracts prepared in both methanol and methanol-HCl, with higher values measured in the Extra Category because the fruit in this Category receives adequate management and therefore retains higher quality [33]. In both categories, the TPC contents of the aril extracts were higher when methanol-HCl 2% (v/v) was used because the acid helps to break the cells down, thus increasing the extraction of the compounds [34]; in addition, a low pH helps to conserve the compounds once extracted, preventing them from polymerizing or carrying out reactions that decrease the amounts of certain compounds in the extract [35]. For Category I, no significant difference was observed between values of TPC in the aril when methanol and ethanol were used, since both solvents have affinities for the types of phenolic compounds present in the litchi. The amount of TPC in the aril of the litchis grown in Mexico was greater than the reported value of those grown in India (31.2mg GAE /100g wb) [36] and lower than those of Thailand and France with reported values of 391.2 and 222.3mg GAE/100g wb, respectively [37,38]. Su et al., [34] mentioned that the types and amounts of bound phenolic compounds that are released in the litchi pulp are influenced by the acid hydrolysis method in addition to its origin. Pesis et al., [39] reported that in the same cultivate, the chemical composition of the litchi fruits could vary due to the influences of climatic conditions. Valle et al., [40] suggested the establishment of quality standards for litchi fruits that are specific to each production region.

A significant difference was observed between the TPC values of the pericarp extracts of the two categories, and the value was higher for the Extra Category when the methanol-HCl medium was used. The differences in the phenolic compound contents could be due to post-harvest management and storage [29]. The litchi is considered a non-climacteric fruit, so once it is cut from the tree, it undergoes minimal changes in maturation, which makes it sensitive to changes in temperature, light, humidity, etc. [41]. Additionally, the anthocyanin concentration increases with ripening to its optimal condition, which contributes to variations in the phenolic compound content [42]. The phenolic compound content is directly related to the coloring of the litchi pericarp [43]. The pigments that give the fruit a red color have been identified as the anthocyanins cyanidin-3-rutinoside, cyanidin-3-glucoside (main) and malvidin-3-acetylglucoside [44]. The Extra Category fruits had an intense and bright red color characteristic of the fruit and presented higher contents of phenolic compounds. The Category I fruits had red coloration of lower intensity with brown areas and spots on the pericarp that are due to the gradual loss of anthocyanins. In unsuitable storage conditions and when in contact with atmospheric oxygen, various enzymatic reactions occur within the tissue of the pericarp such as an increase in the activity of polyphenol oxidase that generate the loss of color and likewise loss of moisture [45]. The Mexican litchi had lower TPC values in its pericarp (2075mg GAE /100g db) than the fruit grown in China (10040, 12135, 12500 mg GAE/100g db) according to data reported by Wang et al., [46], Zhou et al., [21] & Ruenroengklin et al., [28] respectively but had values greater than that harvested in India (1790 mg GAE/ 100 g db) [47]; these results may be due to the origin of the fruit or the method used for the extraction. When compared to another fruit such as the Vitis vinifera grape var. Cabernet Sauvignon, which characteristically has a high TPC content, the Litchi pericarp presented a value close to that of the grape pericarp (2771mg GAE/100g db) reported by Gonzalez et al., [48].

There were no significant differences in the TPC contents of the two categories in the seed samples (Table 2). Different seeds contain compounds that are toxic to humans and other animal species, which is a defense mechanism to ensure the propagation of the plant. In the case of the litchi, it has not been reported that the seed contains toxic compounds [46]. However, the seed is not consumed on a daily basis but are used in traditional medicine in their region of origin. The high phenolic compound content confirms their medicinal use since certain compounds have been proven to have various anti-inflammatory, analgesic and even antipyretic effects [49].

The Mexican litchi seed had a TPC value higher than that of seed from India with 2460mg GAE/g db [47] and lower than that of seed from China with 15655mg GAE / 100g db [21]. The phenol content reported for grape seed (Vitis vinifera, Cabernet Sauvignon variety), 5753mg GAE/100g db [48], is higher than that obtained for the litchi seed, 3549.69mg GAE/100g db, both of Mexican origin; however, the TPC of the litchi seed is greater than those of other fruits, so the use of litchi seed could be recommended for polyphenolic extracts that are used in food or pharmaceutical supplement applications.

Antioxidant activity

The results of the antioxidant capacity are presented in Table 2, where higher values were observed in the Extra category when methanol was used as solvent. The antioxidant activity of the aril of the litchi (4.3 µMol TE/g wb) was greater than that of other commonly used fruits considered exotic such as guava (Psidium guajava L.) with 2.3 µMol TE/g wb and passion fruit (Passiflora edulis S.) with 1.5 µMol TE/g wb [50]. The value of AA obtained for the aril was close to that of blackberry (Rubus glaucus B.) with 5.5 µMol TE/g wb [51], which is a fruit that has been considered to have great antioxidant properties. The differences could be due to the types and concentrations of phenolic compounds that are present in litchis (3,4-dihydroxybenzoic acid, catechin, vanillic acid, caffeic acid, syringic acid, epicatechin, 4-methylcatechol, ferulic acid, rutin and quercetin) [34]. The litchi reportedly contains a vitamin C content of 27.6mg/100g of edible fruit [52], while the guava has a content of 268.7mg/100g of edible

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fruit [53]. This difference indicates that the vitamin C in the guava greatly contributes to its antioxidant capacity, while in the litchi the antioxidant activity is due to the presence of phenolic compounds; for the guava a phenolic content of 40mg GAE/100g db is reported [50].

The value of the antioxidant activity of the pericarp was higher in the Extra Category (Table 2), which may be due to its retention of its organoleptic characteristics such as its red color [45]. No significant differences were found between the AA of the seed samples of the two categories. A Pearson correlation coefficient of 0.9669 was obtained by relating the AA and TPC of the fruit, considering the aril, pericarp and seed, which indicates a linear correlation.

Table 3: Total antioxidant capacity expressed as.

Simple	IC50 DPPH (µg/mL)	
Aril	137.45	±5.49
Seed	49.93	±4.49
Pericarp	118.41	±11.56
BHT*	131.64	±1.25
Vitamin C*	105.38	±1.25

*Date taken from [21]

Table 3 shows the antioxidant capacity expressed as IC50 for the three parts of the fruit and the values of the standards of antioxidant compounds such as BHT and vitamin C reported by Zhou et al., [21]. The value of IC50 for the aril was higher than the values of BHT and vitamin C, which indicates that a higher concentration of the aril extract is needed to reduce the initial DPPH at 50% (the higher the IC50 value, the lower the antioxidant capacity). The pericarp and the seed presented higher antioxidant activities than BHT, while that of the seed was greater than the vitamin C. The litchi contains antioxidants mainly in the fruit and the flower [54]. Certain research has found and identified phenolic compounds in the litchi shell, pulp and seed that have anti-carcinogenic, anti-inflammatory and cardio-protective effects and are capable of eliminating free radicals and preventing chronic degenerative diseases [55] (Table 3).

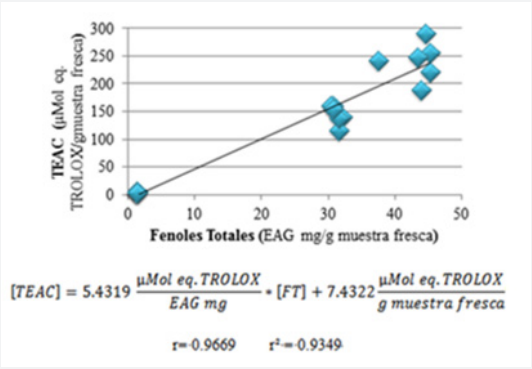

Figure 2: Linear relationship between the total phenolic compounds and the TEAC for the complete litchi fruit.

By relating the phenolic compounds against the antioxidant capacity of the fruit both in the aril and pericarp seed by means of a linear regression; a Pearson correlation coefficient of 0.9669 is obtained, which is a high value that indicates a high correlation. Also, the coefficient of determination provides a value of 0.9349 indicates a good fit of the data towards the linear model (Figure 2), which represents that there is a directly proportional relationship between the phenolic compounds and the antioxidant capacity, this same relationship is shown in the study conducted by Rodríguez et al., [50] in four different fruits.

Phenolic compounds by ultraviolet-visible spectrophotometry (UV-Vis)

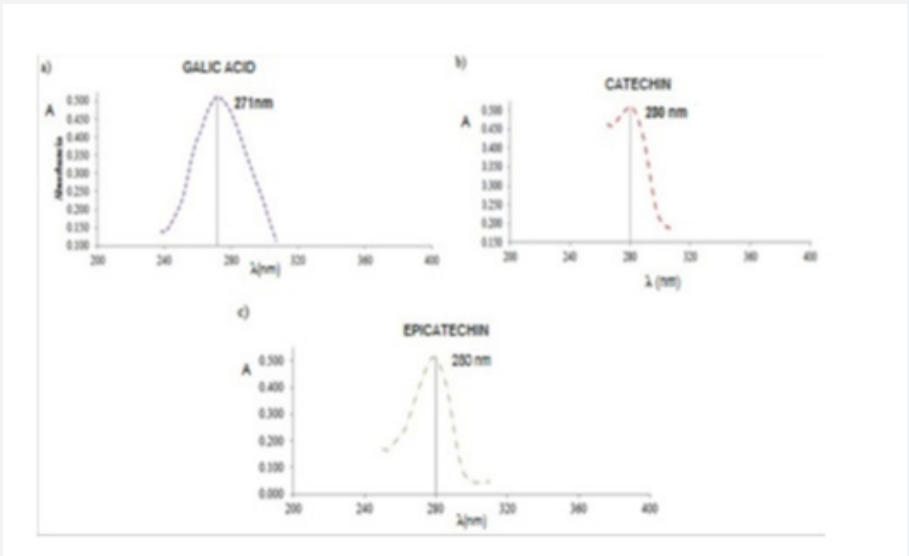

Figure 3: UV-Vis absorption spectra of pure compounds of a) gallic acid b) catechin c) epicatechin

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Figure 3 shows the absorption spectra of the standards, and Figure 4 shows an example of the absorption spectra overlap of the standards and the aril extract. The maximum absorbance of the sample was similar to the maximum absorbance of gallic acid, which could indicate that this compound is present in the aril. In the literature, several flavonoids and anthocyanidins have been identified in litchi pericarp, aril and seed, one of which is gallic acid [38].

The absorption maxima of catechin and epicatechin occur at approximately 280nm. In the sample, the maximum absorption occurred at approximately 280nm, indicating that these compounds could be present, but a hypochromic effect could be present in the sample that shifts the absorption spectra to lower frequencies due to the solvent, substituents or other compounds seen in the spectrum of Figure 4.

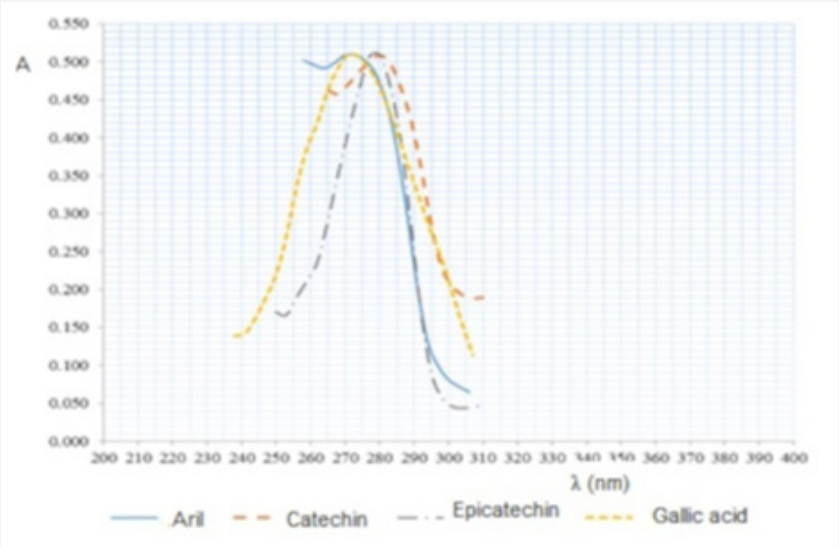

Figure 4: Comparison of the UV-Vis absorption spectra of the aril with the different standards A: absorbance.

The absorption band presented in the pericarp and the seed coincide with epicatechin and catechin.

The presence of gallic acid, catechin and epicatechin could be inferred for the three parts of the fruit. However, a complete identification of these compounds must be performed using methodologies such as HPLC.

Litchi liquor shelf life

During the shelf life the °Brix and pH values as a function of time (17 weeks) indicated that these liquor parameters were stable during this period. The phenolic compound content decreased 18% during 17 weeks of storage from 234.84 ± 32.18mg GAE/mL (week 0) to a final value of 190.83 ± 30mg GAE/ ml (week 17). This decrease may be due to the effects of fluctuations in temperature and light during storage that could degrade phenolic compounds [28]; likewise, slight changes in pH could cause certain compounds to undergo polymerization [56]. One way to prevent degradation and / or polymerization of phenols is the addition of a pH regulator and an acidulant to avoid changes in pH, and in this way, the phenolic compounds that are sensitive to pH close to 7 will not polymerize by the action of the OH- ions that are present in the solution [57].

The total antioxidant capacity was calculated as TEAC, and the values at only week 0 and week 17 were taken. When performing the statistical analysis, significant differences were found between the values in week 0 and week 17 with values of 83.78±2.99 and 73.16, respectively, that correspond to a decrease of 12.6% during the storage time.

Conclusion

The physicochemical parameters (°Bx, % acidity, density and pH) showed that the Extra Category and Category I of litchis present similar phenological states, which indicates that the coloration of the pericarp is independent of these values. The phenolic compound contents and the total antioxidant capacities were higher in the pulp and pericarp of the Extra category; there were no significant differences between the seed of the two categories. The phenolic compounds and the antioxidant capacity, expressed as TEAC, in the fruit have a linear relationship. By UV-VIS analysis, the presence of gallic acid, catechin and epicatechin could be inferred in all three parts of the fruit.

In the elaborated liquor, the °Brix and the pH remained constant during 17 weeks of testing while the phenolic compounds and the antioxidant capacity suffered slight decreases of 18 and 12.6%, respectively.

The litchi is a fruit with great potential as a functional food because it has a high phenolic compound content and antioxidant capacity according to in vitro tests, which suggests that it is a valuable raw material for use as an additive in various

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food products focused on health. The litchi pericarp and seed represent important sources of bioactive compounds but are not edible, so additional processes are required to extract these compounds for application as additives in functional foods or as part of food supplements.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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