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Flavonoids of Organic Banana Peels (*Musa cavendishii*)

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Abstract: In the present work we investigated the banana peels (*Musa cavendishii*) of organic silk variety from Pachacamac (Lurín, Lima) for being waste contaminants without use that avoid the damage to the environment. The qualitative presence of polyphenols and flavonoids was determined by phytochemical marching developed in ethanol extract of banana peels. Using thin layer chromatography on an analytical and preparative scale and rapid column chromatography, it was possible to separate nine soluble fractions in methanol and five soluble fractions in double distilled water as well as their respective Rf values; and by UV-visible spectrophotometry were elucidated nine structures of methanol soluble flavones (5,7-dihydroxy-4'-methoxyflavone, 4',5,6,7-tetrahydroxyflavone, 5,7-dihydroxy-6-methoxyflavone, 4',5,7,8-tetrahydroxyflavone; 5,7,8-trihydroxyflavone, 4'5,7-trihydroxyflavone; 5,6-dihydroxy-7-methoxyflavone, 5,6,7-trihydroxy-4'-methoxyflavone and 5,7-dihydroxy-4',6-dimethoxyflavone) and two flavones that were in double distilled water soluble (5,6,7-trihydroxyflavone, 5,6,7-trihydroxy-4'-methoxyflavone); in order to contributing to the study of the components of the organic banana peels and also to the environmental health.

Keywords: Organic Banana Peels, Extracts, *Musa cavendishii*, Spectrum of Flavones, Flavones

1. Introduction

The banana is a widely spread fruit for its pleasant taste and low cost. In Peru it is produced very easily thanks to geographical and climatic conditions, both for export and for the national market, in addition to the organic production of silk banana in agricultural areas of Lima, which attracts commercial interest.

But while it is true that it can't discuss the nutritional qualities of bananas [1-3]; there is a part of the fruit known as peel that is not harvested and constitutes a waste without industrial utility, in addition to producing and increasing pollution [4]. Only in some cases and when it comes to organic production, can it be used as an integral element of the vegetable fertilizer, but on a very small scale; hence, the peel is an important part that is not used in this fruit and for both the industry and consumers, it becomes a waste that should be processed [5].

Our general research objective was to determine the presence of flavonoids and to elucidate their structures based on the analysis of the spectra obtained in each fraction thanks to the displacement reagents, the flavonoid patterns and the studies described about flavonoids [6].

2. Materials and Method

The samples were the silk banana peels corresponding to the zones of organic agricultural production in Pachacamac (Lurín, Lima), until completing the amount necessary for the production of the extract, being two kilograms per hectare.

Proximal analysis: determination of the proximal composition of the organic silk banana peels, using AOAC methods [7].

Extract: the banana peels of organic production were macerated in ethanol for 7 days, without light and with constant movement of the macerated. After this phase, the extract was filtered and dehydrated at no more than 40°C.

Phytochemical March: according to the color reactions developed [8].

Shinoda reaction: the colorless or slightly yellow alcoholic extract was placed a small piece of magnesium and concentrated HCl drops to observe the color response.

Reaction with alkalis: the aqueous extract plus alkali was mixed. The positive answer is the variation of color.

Marini Bettolo test: with a solution of $SbCl_5$ in CCl_4, the

flavonoids give characteristic colors or formation of precipitates.

Reaction with concentrated H_2SO_4: characteristic colorations.

Dimroth reagent: solution of H_3BO_3 in Ac_2O. It produces color reaction.

Reaction with aqueous or ethanol solution of $FeCl_3$: produces characteristic coloration.

Rapid column chromatography: using appropriate solvents and 60 Merck silica gel for column, from 0.063 to 0.200 mm of diameter and determination of solubility.

Analytical thin layer chromatography: for qualitative determination of the polyphenols and flavonoids present using G Merck silica gel, with fluorescence indicator at 254 nm.

Analytical thin-layer chromatography and preparative scale thin-layer chromatography: used in extracts obtained in rapid column chromatography.

Ultraviolet-visible spectroscopy: for preliminary analysis of flavonoid spectra by UV-Vis absorption in ethanol solution. The readings were made at a wavelength between 230 and 280 nm (band II) and between 310 and 560 nm (band I), using a Hewel-Packard UV-visible spectrophotometer with diode array, and flavonoid patterns.

Application of the flavonoids: the structures of the elucidated flavonoids were added to yogurt, to determine the best sample by a panel and the analysis of the results obtained were evaluated through the ranking test (α=5%).

3. Results and Discussion

The peels of *Musa cavendishii* known as organic silk banana were collected to begin the present research. Each unit of fruit had an average weight of 143,1 grams, of which 52,8 grams corresponded to the peel which was equivalent to 36,90% of the weight of fruit; and in most mature fruit, the average weight was 141,1 grams; but 32,7 grams corresponded to the peel, representing 23,18% of the product. In other cases, banana peels represented 40% of weight in fresh fruit [9]; however, in our experiments the percentage was slightly lower.

Proximal composition of organic silk banana peels (*Musa cavendishii*): the analysis of the proximal composition of the organic silk banana peels had the following results:

Table 1. *Proximal composition of organic silk banana peels (Musa cavendishii).*

Component (%)	Result
	Peels of *Musa cavendishii* (organic silk variety)
Moisture	87,84
Total protein	0,51
Fat	1,05
Raw fiber	1,19
Ash	1,21
Carbohydrates	8,20

It was found by determining the proximal composition of banana peels of Pachabale, Nendranbale and Yelakkibale varieties [10], the initial moisture content of the samples studied was from 82,6% to 88,9%; while the other constituents oscillated according to the following percentages: protein (4,6% to 7,7%), fat (5,13% to 11,26%), ash (8,9% to 12,96%) and carbohydrates (from 9,8% to 41,9%). In the variety under study, the moisture content expressed as a percentage was within the range reported by the researchers; not so the rest of the components that were below the reported ranges.

Extract: clean and chopped peels, macerated in 70°GL ethanol for 7 days were carefully filtered and kept in amber bottles. The extract was dark brown and dense. After dehydrating it at a temperature no higher than 40°C, an average yield of 8,18% was obtained. The sample was processed fresh by macerating in ethanol; and it was not previously dried the sample at 50°C and then grind it and store it in jars at 4°C [11].

Table 2. *Qualitative analysis of the extract of organic silk banana peels (Musa cavendishii).*

Reaction	Polyphenols	Results
Sample + 2 drops of gelatin	Tannins	+
Sample + 2 drops of Ninhydrin	Amino Acids	---
Sample + 2 drops of ferric chloride	Phenolic compounds	+++
Sample + 2 drops of reagent from Dragendorf	Alkaloids	---
Sample + 2 drops of Mayer Reagent	Alkaloids	---
Sample + 2 drops of NaOH 0,5%	Quinones	---
Sample + 2 drops of Lieberman's Reagent	Triterpenes and Esters	+
Sample + 4 drops of Molish reagent	Glycosides	+
Sample + metallic Mg granites + 2 drops of HCl (c)	Flavonoids	+

(---) Absence, (+) Little, (++) Regular, (+++) Fairly, (++++) Abundant.

Phytochemical March: the result of the reactions of the phytochemical march indicated that in organic silk banana peels (*Musa cavendishii*) are found:

Phenolic compounds> Flavonoids = Tannins = Glycosides = Triterpenes and esters.

The phenolic compounds have been reported in the banana [12] in greater proportion in the peel (907mg / 100g of dry matter) than in the pulp (232mg / 100g); in fresh pulp [13] was able to quantify from 30mg to 60mg per 100 grams of fresh matter; but it was observed that the content of polyphenols in the peels differed according to the variety.

The phytochemical results would depend not only on the maturity of the cultivar, geographical origin of the plant, growing season, post-harvest storage conditions, or the processing technique of the sample; but also the polarity of the solvent used, the storage time, the chemical nature of the

substance, the presence of interfering compounds as well as the particle size. Besides, there would be correlation between the mineral content of eight cultivars of Musa sp and total phenolic compounds.

Rapid column chromatography: as a previous step, the solubility was determined using one milligram of dehydrated extract and one milliliter of solvent of different polarity with the following results:

Table 3. *Determination of the solubility of the extracts of Musa cavendishii peels.*

Polarity order	Solvent	Result	Observation
Apolar	n-Hexane	Negative	---
Medium polar	Dichloromethane	Negative	---
Medium polar	Ethyl acetate	Negative	---
Polar	N-Butanol	Positive	+
Polar	Methanol	Positive	+++
Polar	Ethanol	Positive	++
Polar	Double distilled water	Positive	+++

Polar (+++), Very soluble (++), Soluble (+), Slightly soluble (---), Insoluble ().

Table 4. *Yield of dehydrated total extracts of Musa cavendishii by rapid column chromatography (RCC).*

Sample	Solvent employee	Yield (%)
Dehydrated total extracts of *Musa cavendishii*	Dichloromethane	0,18
	Methanol	30,58
	Double distilled water	23,80

According to the solubility results, dichloromethane, methanol and double distilled water were chosen for rapid column chromatography. Each fraction soluble in each solvent was dehydrated at less than 40°C and stored in amber glass bottles, hermetically sealed. The following table shows the performance obtained with the solvents used.

Analytical thin-layer chromatography: continued working with dehydrated extracts obtained from rapid column chromatography, which were soluble in methanol and double distilled water.

The methanol soluble extract was re suspended, one gram in one milliliter of ethanol, the sample was placed with the aid of a capillary on silica gel (5x20cm) and (10x20cm), with fluorescence indicator at 254 nm.

The extract components were eluted in the dichloromethane / methanol mixtures in the proportions 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, and 9:1. The best ratio that allowed the optimal separation of the components was 2:1.

In an analogous way, one gram of extract from the RCC that was soluble in double distilled water was re suspended, being seeded in silica gel (5x20 cm) and (10x20 cm), in the mixtures dichloromethane / methanol in the proportions 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1 and 9:1. The best ratio of eluents in this case was 2:1. Observing the chromatographic results with the ultraviolet light lamp, it was determined that they did not show fluorescence.

Analytical thin layer chromatography on a preparative scale: using one milliliter of ethanol and one gram of each of the two fractions soluble in methanol and soluble in double distilled water, two dilutions were prepared by plating (20x20 cm) with silica gel. The separation it was carried out with the dichloromethane / methanol mixture in the optimum proportions determined in the previous step; with the help of the UV light lamp, the following fractions were separated:

Table 5. *Fractions obtained from organic banana peels (Musa cavendishii) soluble in methanol and double distilled water from the RCC.*

Sample	Soluble in	Eluents Dichloromethane / methanol	Number of Fractions
Extract of organic silk banana peels (*Musa cavendishii*) from RCC	Methanol	2:1	C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9 = 9
	Double distilled water	2:1	D_1, D_2, D_3, D_4, D_5 = 5

The Rf values found in the fractions obtained from the soluble extracts in methanol and soluble in double distilled water obtained by RCC, are mentioned below.

Table 6. *Rf values of the fractions of the soluble extracts in methanol and soluble in double distilled water obtained by RCC.*

Rf of the fractions of the soluble extracts in methanol obtained by RCC				
C1 = 0.022988	C2 = 0.0459	C3 = 0.2447126	C4 = 0.29885	C5 = 0.3333
C6 = 0.35057	C7 = 0.52298	C8 = 0.70689	C9 = 0.74137	
Rf of the fractions of the soluble extracts in double distilled water obtained by RCC				
D1 = 0.028089	D2 = 0.410112	D3 = 0.5280	D4 = 0.6853	D5 = 0.8146

Ultraviolet-visible spectroscopy: the spectra of each one of the soluble fractions in methanol and in double distilled water containing flavonoids were determined.

Table 7. *Readings of the spectra obtained in the fractions soluble in methanol and double distilled water by the RCC of Musa cavendishii.*

Fractions of *Musa cavendishii* obtained by RCC		λ (nm)	Result
Soluble in methanol	C_1	268 320	Flavonoid
	C_2	276 325	Flavonoid
	C_3	272 316	Flavonoid
	C_4	280 328	Flavonoid
	C_5	280 315	Flavonoid
	C_6	268 326	Flavonoid
	C_7	272 305	Flavonoid
	C_8	276 317	Flavonoid

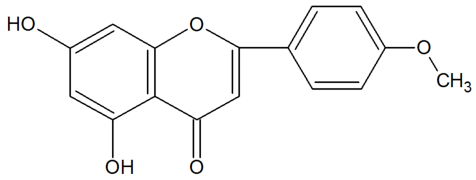
Fractions of *Musa cavendishii* obtained by RCC		λ (nm)	Result
Soluble in double distilled water	C_9	272 320	Flavonoid
	D_1	---- ----	It is not Flavonoid
	D_2	268 ----	It is not Flavonoid
	D_3	278 ----	It is not Flavonoid
	D_4	276 314	Flavonoid
	D_5	276 320	Flavonoid

In developing the readings of the fractions in the diode-array spectrophotometer, the spectra of the methanol soluble fractions were obtained. The wavelength readings of the spectra were analyzed based on the results of the displacement reagents and compared with the studies and compendium of the spectra published, proposing the following structures based on the results obtained that are mentioned below.

Proposed structures:

In the fractions of methanol soluble of *Musa cavendishii* obtained by rapid column chromatography was detected:

Fraction C_1. With Rf of 0.0229, the readings in the maximum UV-visible spectrum were:

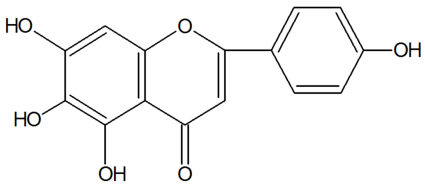


268, 320 nm

Figure 1. *5,7-dihydroxy-4'-metoxy flavone.*

According to the spectrum in ethanol, the basic skeleton corresponding to this fraction is a flavone, the structure of the Figure 1 is being proposed.

Fraction C_2. With Rf of 0.0459, the readings in the maximum UV-visible spectrum were:

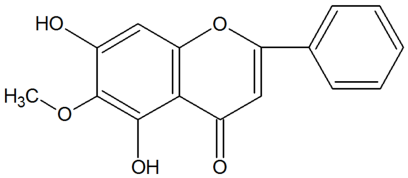


276, 325 nm

Figure 2. *4',5,6,7-tetrahydroxyflavone.*

Based on the spectrum in ethanol the basic skeleton that corresponds to this fraction is a flavone, proposing the structure of Figure 2.

Fraction C_3. With Rf of 0.2471, the readings in the maximum UV-visible spectrum were:

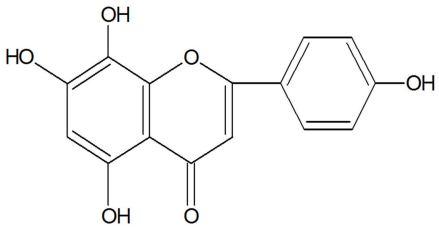


272, 316 nm

Figure 3. *5,7-dihydroxy-6-metoxyflavone.*

Based to the spectrum in ethanol, the basic skeleton corresponding to this fraction is a flavone, the structure of the Figure 3 is being proposed.

Fraction C_4. With Rf of 0.2988, the readings in the maximum UV-visible spectrum were:

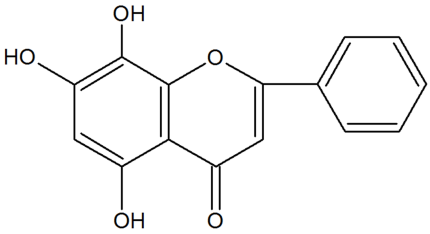


280, 328 nm

Figure 4. *4',5,7,8-tetrahydroxyflavone.*

According to the spectrum in ethanol, the basic skeleton corresponding to this fraction is a flavone, the structure of Figure 4 is being proposed.

Fraction C_5. With Rf of 0.3333 the readings in the maximum UV-visible spectrum were:

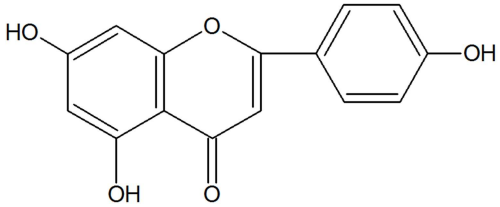


280, 315 nm

Figure 5. *5,7,8-trihydroxyflavone.*

Based on the spectrum in ethanol the basic skeleton that corresponds to this fraction is a flavone, proposing the structure of Figure 5.

Fraction C_6. With Rf of 0.35057 the readings in the maximum UV-visible spectrum were:

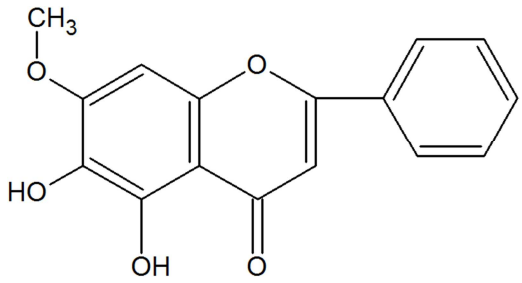


268, 326 nm

Figure 6. *4',5,7-trihydroxyflavone.*

According to the ethanol spectrum, the basic skeleton corresponding to this fraction is a flavone, the structure of Figure 6 is being proposed.

Fraction C_7. With Rf of 0.5229 the readings in the maximum UV-visible spectrum were:

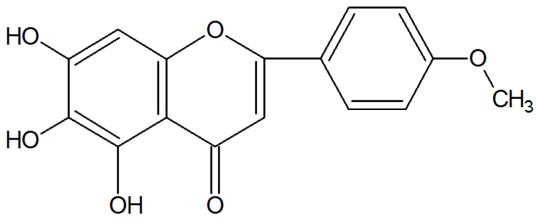


272, 305 nm

Figure 7. *5,6-dihydroxy-7-metoxyflavone.*

According to the spectrum in ethanol, the basic skeleton corresponding to this fraction is a flavone, the structure of Figure 7 is being proposed.

Fraction C_8. With Rf of 0.7068 the readings in the maximum UV-visible spectrum were:

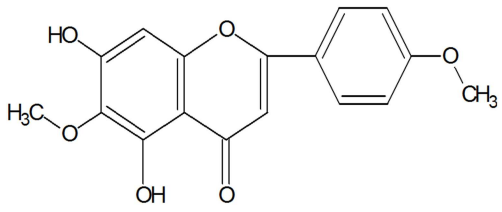


276, 317 nm

Figure 8. *5,6,7-trihydroxy-4'-metoxyflavone.*

According to the spectrum in ethanol, the basic skeleton corresponding to this fraction is a flavone, the structure of Figure 8 is being proposed.

Fraction C_9. With Rf of 0.7413 the readings in the maximum UV-visible spectrum were:



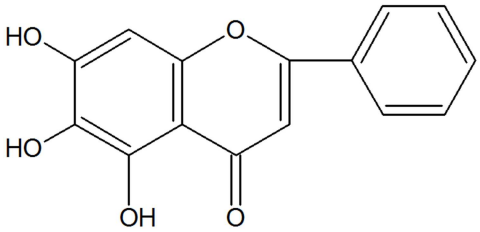
272, 320 nm

Figure 9. *5,7-dihydroxy-4',6-dimetoxyflavone*

Based to the spectrum in ethanol, the basic skeleton corresponding to this fraction is a flavone, the structure of Figure 9 is being proposed.

In fractions D_4 and D_5 of *Musa cavendishii* variety of organic silk soluble in double distilled water that came from the RCC the following results were obtained.

Fraction D_4. With Rf of 0.6853 the readings in the maximum UV-visible spectrum were:

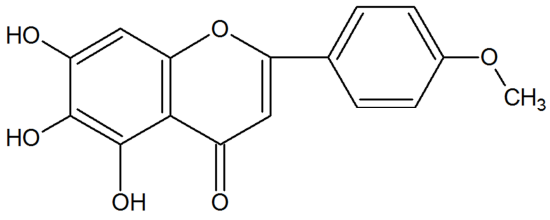


276, 314 nm

Figure 10. *5,6,7-trihydroxyflavone.*

According to the ethanol spectrum, the basic skeleton corresponding to this fraction is a flavone, the structure of Figure 10 is being proposed.

Fraction D_5. With Rf of 0.8146 the readings in the maximum UV-visible spectrum were:



276, 320 nm

Figure 11. *5,6,7-trihydroxy-4'-metoxyflavone.*

According to the ethanol spectrum, the basic skeleton corresponding to this fraction is a flavone, the structure of Figure 11 is being proposed.

In other samples [14-16], it was found a high amount of total flavonoids in solvents such as methanol, ethanol and water, achieving greater antioxidant activity in the aqueous extract; but there being no difference between the soluble extracts in methanol and ethanol. In our case, flavonoids were detected in the methanol soluble fractions and in double distilled water; but in the methanol soluble fractions all of them contained different flavonoids, whereas in the soluble in double distilled water fractions, the flavonoids were present only in D_4 and D_5 fractions.

In our research, a total of 11 flavone structures have been found in the organic silk banana peels according to the maximum UV-visible spectra readings, one of them being 5,6,7-trihydroxy-4'- methoxyflavone soluble in methanol and in double distilled water, having been detected in fraction D_5 soluble in double distilled water and in fraction C_8 in methanol soluble.

Application of flavonoids: the extract of organic banana peels was added to yogurt to select the best samples, using a trained panel.

In the ranking table with ($p < 0.05$), for (t-1 = 6) treatments and seven repetitions, the sample with 9 points was considered the best, indicating acceptance.

Additionally, in the evaluation of the samples with banana extract it was observed that the extract reduced the aroma and acidity of the yogurt, and increased its viscosity.

4. Conclusion

Banana peels (*Musa cavendishii*) of organic production possess various bioactive compounds. In the phytochemical march, abundant polyphenolic compounds were detected, but also tannins, glycosides, triterpenes and esters, and flavonoids.

The soluble extracts in methanol and double distilled water revealed a total of eleven flavone structures, therefore the banana peels constituting a potential source of flavones for the food industry. The flavones added to the yogurt modulate the aroma, acidity and viscosity of the product, with satisfactory results.

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