EXTRACTION AND CHARACTERISATION OF QUEBRACHO (SCHINOPSIS SP.) TANNINS

EXTRACCION Y CARACTERIZACION DE TANINOS DE QUEBRACHO (SCHINOPSIS SP.)

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SUMMARY

Vegetables tannins are organic substances; when considering the great number of different tannins, it is possible to state certain properties which are common to all of them such as: a high molecular weight, causing colloidal solutions in which the particles of tannins are present in all forms of dispersions; a great number of free hydroxy phenolic groups, which are mainly responsible for the solubility of tannins in water; with few exceptions, all tannins are amorphous and no crystalline; a different degree of acidity resultant from the polarity of the hydroxy phenolic groups which in some cases is enhanced by free carboxyl groups; tannins formed by esterification of carboxyl groups usually exhibit a higher acidity than those made by condensation or polymerisation of components free from carboxyl groups; formation of intense precipitates in the presence of soluble iron (III) salts, etc.

This last property of the tannins, that is the capacity of forming chelates with the hexahydrated iron (III) ion, could be employed efficiently to modify the kinetic of oxidation of iron and steel substrata. In this paper, the extraction, the purification, the concentration and the characterisation of tannins obtained from quebracho (Schinopsis sp.) were performed, particularly with the objective of knowing some inherent properties to the reaction between the tannins and the iron.

Keywords: *tannins, quebracho, extraction, purification, concentration, characterisation, iron- tannins reaction.*

INTRODUCTION

The generic term tannins involves a group of compounds of high molecular complexity, widely distributed in the vegetable kingdom and with common characteristics. It is useful the employment of the word tannins in plural in view of the difficulty to define with precision their chemical composition.

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In the last years, the chromatographic study of the tannins has permitted to establish the heterogeneity of the different extracts and also to determine the presence of small quantities of phenols, which generate by polymerisation different and complex compounds.

The classification of the vegetable tannins was evolving according to the state of the knowledge over these compounds. At first, since the chemical structure was unknown (only some products of hydrolysis or those obtained from destructive distillation were identified), the cause of their formation was taken into account. Afterwards, the behaviour against certain chemicals was studied and finally the composition was considered **[1-4]**.

Freudenberg classified the tannins according to the structure, in pyrogall tannins and catechol ones. In general, the vegetables supply extracts of mixed base.

The pyrogall tannins, which are also called hydrolyzable ones, are weakly polymerised and are soluble in water. They have a polyester structure and consequently hydrolyse with facility by the action of the acids in a sugar, a polyalcohol and a phenol carboxyl acid. Their bencenic nuclei are bonded through atoms of oxygen forming large complexes.

On the other hand, the catechol tannins are strongly polymerised and the bencenic group is bonded to the carbon atoms of the chain. They have low solubility in water at laboratory temperature; treated with acid solutions produce a progressive polymerisation to form amorphous tannins called flavatannins or red tannins. They are in consequence derived by condensation of flavanoid units, being also formed in a long time postmorten metabolic process **[5,6]**.

In spite of their heterogeneity, the vegetable tannins present a series of properties in common. Particularly in 0.4 % aqueous solutions, they produce an intense coloration with iron (III) salts: blue the pyrogall tannins and green the catechol ones. These tannins, in more concentrated solutions, generate an abundant blue black precipitate. This property of the tannins, that is the capacity of forming complex chelates with the iron (III) ion, could be employed to modify the kinetic of oxidation of iron and of steel substrata **[1,7]** . The aim of this paper was the optimisation of the operative conditions for the tannins extraction from the heartwood of quebracho (Schinopsis sp.) and later the determination of their properties, particularly those connected with the reaction of extracted tannins and the hexahydrated iron (III) ion.

EXPERIMENTAL AND RESULTS

1. Preparation of raw material. The sample of quebracho (Schinopsis Lorentzii, Province of Formosa, Argentina) was extracted from a live and healthy tree, discarding all those parts that did not satisfy the before mentioned conditions. Heartwood was only selected since the tannins find almost exclusively in that zone of the tree **[8]**. Observations by means of optical and electron microscopes as well as an histochemistry test were performed; the results indicate the following anatomical characteristics: diffuse-porous wood, solitary pores, simple perforation plates and scanty vasicentric paratracheal parenchyma. The presence of tannins in the heartwood is characterised by secretory radial canals (tanniniferous tubes) and by thickwalled cells caused by tannins impregnation, Fig. 1.

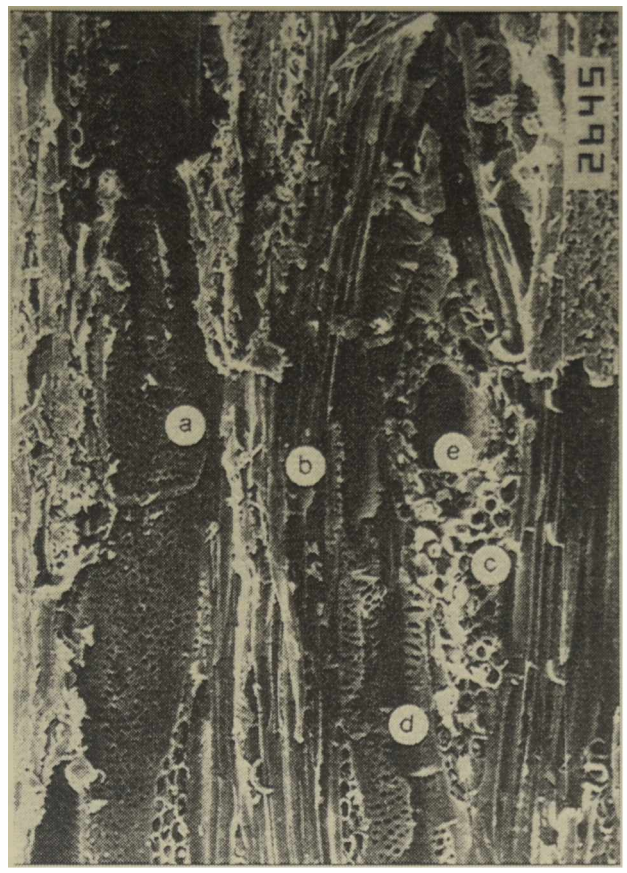

Fig. 1.- Quebracho wood, Schinopsis sp.: longitudinal tangential cross-section (SEM, 200 x). a) Vessel; b) fibres; c) heterogeneous rays; d) parenchyma cells and d) radial secretory canals.

However, other plants possess important quantities of tannins in blades, bark, etc. **[9,10]**.

The sample was cutted in parallel manner and also transversally to the fibrovascular bunches. Afterwards, it was dried in stove at 50 °C until 4-6 % humidity. The determination was performed in a glass dish over 10 g at 100-105 °C up to constant weight (approximately 10 hours).

Later, it was crushed in a hammers mill ; this mill has an axis that turns at great speed, mutually binding to a disk with articulate hammers in the form of cross. The rotor moves in the interior of a chassis supplied with a feeding hopper. Finally, the sample was sieved in 30 mesh.

2. Extraction of the tannins. This is fundamentally an osmotic process through the cellular membranes and was carried out in a high speed stirring disperser. The vertical vessel has a double jacket to permit the heating and to control the temperature. The following factors were taken into account:

2.1. Distilled water / heartwood ratio. The selected ratio was 3 / 1 in weight, since it permits to cover completely the sawdust of the quebracho wood. Major quantity of distilled water would present the inconvenient of diluting excessively the liquor, besides not to get to improve significantly the efficiency of the extraction.

2.2. Number of extractions. This variable influenced meaningfully the quantity of tannins extracted from the quebracho wood (Table I).

Table I

Influence of the number of extractions

	First extraction	Washing						
		1	2	3	4	5	6	Total
Tannins on wood, % in weight	8.9	5.6	2.9	1.8	1.3	1.1	0.8	22.4

Note: Humidity of the quebracho wood, 6.2 %. The content of tannins was determined by applying the method of Lowenthal.

In the first extraction, approximately the half of the water remained retained in the sawdust and the other half corresponded to the liquor. In this experiment, two subsequent washes were performed; in these cases the water employed was the 50 % of the former quantity.

2.3. Temperature. This variable influenced significantly the composition of the extract (Table II). The results indicate that the quantity of tannins extracted and the tannins/no tannins ratio increased with the temperature, reaching the major efficiency at 70-75 °C and 75-80 °C; first rank was selected to avoid the oxidation of the extract. The two final washes were performed at the same temperature.

Table II

Influence of the extraction water temperature

Temperature	Tannins, %	No tannins, %
30-35	57	80
40-45	68	82
50-55	79	85
60-65	88	90
70-75	100	100
75-80	100	100
80-85	91	100

Note :The results correspond to the extract composition at 70-75°C. The content of tannins was determined by applying the method of Lowenthal and the no tannins by difference between the soluble components and the level of tannins.

2.4. Time. To get a high yielding extraction of the tannins without overpassing the quoted temperature, the sawdust was let in macerating with distilled water for 3 hours at laboratory temperature and later 1 hour, with strong agitation, at 70-75 °C. The two subsequent washes were also carried out for 1 hour, in the before mentioned conditions.

The liquor presented a marked turbidity: the concentration was approximately 3° Bé (60 g solid extract/1000 ml liquor) and the pH 5.2. The wood and extract compositions are shown in Table III.

Table III

Composition, % in weight*

	Wood	Extract powder	Dry extract (by calculus)
Tannins**	22-23	60-62	72-74
No tannins***	2-3	6-7	7-8
Insoluble Components	67-70****	15-16	18-19
Humidity	6-7	15-18	----

* Six extractions were made (water/sawdust, 3/1 ratio in weight, 70-75 °C
** Method of Lowenthal
*** It was calculated by difference between the soluble components content and the level of tannins
**** Fibres of wood are included

3. Purification of the liquor. In the composition of the vegetable extracts, the following compounds are conventionally distinguished: tannins, no tannins, water insoluble substances and water **[11]**.

The no tannins are usually glucids, organic acids, phenols, salts, etc. while the water insoluble components (generally they are in suspension or forming sediments) are tannins of high degree of polymerisation (flavatannins or condensed tannins), incapable to keep dispersed due to the effect of remaining components and of hydrolysis products of pyrogall tannins, which are also water insoluble.

A series of dissolved substances like gums, resins, etc. and other of inert type influence the formation of precipitates and sediments which are difficult to separate by filtration due to their characteristics. The solubility of these substances increases with the temperature and also as the concentration does it.

To eliminate these gumresins and the water insoluble components from the liquor, which remained in the liquid in colloidal manner due to their small particle size, the original extract was cooled at 0-2 °C for 24 hours and afterwards centrifuged. The extract was separated in two defined layers, a superior which is clear and other inferior, whose turbidity increased from overhead toward down and that practically contained almost all the water

insoluble substances and the gumresins. These solids were finally washed several times with distilled water to recuperate the tannins retained in them.

4. Concentration of the liquor. To avoid the serious problems derived from the employment of elevated temperatures, which generates losses of tannins and produce darkness of the extract, a system of vacuum concentration (approximately 550- 600 mm Hg) was employed at a temperature inferior to 40 °C. The selected container is made from glass to avoid chemical reactions with the acids present in the liquor. The final extract had a 18-20 °Bé concentration (410- 450 g/1000 ml liquor) and also a clear aspect, that is turbidity free, with a pH remaining between 4.5 and 5.0. For practical effects, acid fermentation was not observed at laboratory conditions which allow to infer that the sugars content in the experimental liquor was reduced.

5. Quantitative determination of tannins: method of Lowenthal. It is based on fact that the tannins are oxidised in solution by potassium permanganate in presence of indigo carmine, which is employed as indicator of the final point **[12,13]**. Taking as reference the titration of a sample of tannic acid, the volume of potassium permanganate of known normality, consumed by the extract of quebracho, was assessed. Since the tannins in the liquor are accompanied by other reducing agents, the tannins were eliminated by coagulation with gelatine.

Afterwards, the content of the remaining reducing substances were evaluated and by difference of both determinations the content of tannins in every solution was calculated. The trials were performed in triplicate, taking the precaution of to carry out them in the same conditions that in the liquor, particularly in relation with addition, dilution and agitation time of chemicals. The results are indicated in Tables I/IV.

6. Characterisation of the tannins solutions. The quebracho wood is very hard and dense (approximately 1.3 $g.cm^{-3}$). Connected with the density of the purified extracts, the values ranged between 1.52 and 1.54 $g.cm^{-3}$, at an humidity of 6 % in weight.

Furthermore, the difference between pyrogall and catechol tannins present in the extracts was qualitatively established; an analytic solution of approximately 0.4 % tannins was employed. This solution, previously acidified with drops of acetic acid, showed abundant precipitation when bromine water is incorporated. This evidenced the presence of catechol tannins; on the other hand, with the addition of a lead acetate solution to the acidified liquor the precipitation was not meaningful, which indicated that the content of pyrogall tannins in the experimental extracts is not quantitatively significant.

7. Oxidation of the quebracho liquor. Chemical composition of the vegetable tannins points out that are condensed phenolic substances and consequently, susceptible to be oxidised **[12,13]**. The oxidation can be initiated in the same raw material, during the process of extraction and also in the liquor concentration. Numerous factors act as catalyst of this process, such as air, light, temperature, pH, etc.

Table IV

Oxidation of the quebracho liquor:
light and air exposure for 30 days, at laboratory temperature

pH		Tannins on dry solid extract			No tannins on dry solid extract			Insoluble components on dry solid extract		
Initial	Final	Wi	Wf	Δ	Wi	Wf	Δ	Wi	Wf	Δ
5.02	4.84	14.624	14.164	-2.16	0.731	0.569	-0.76	5.931	6.580	+3.05
5.73	5.41	15.975	14.929	-4.91	1.278	1.725	+2.10	4.047	4.914	+4.07
6.97	6.39	17.732	16.678	-4.96	1.419	2.093	+3.17	2.105	2.991	+4.17
8.03	7.21	17.783	16.134	-7.74	2.134	3.044	+4.27	1.388	2.639	+5.87
9.14	8.17	17.942	15.777	-10.17	2.871	4.089	+5.72	0.472	1.862	+6.53

Note: Initial pH was adjusted with 0.1 N Na H0 solution
Wi and Wf are, respectively, the initial and final weights in g/100 ml liquor
Δ is the percent variation, in weight on dry solid extract

In this particular case, it was studied the oxidation of the quebracho extract at different values of pH and a concentration of about 10 ° Bé, after 3 hours exposition to light and laboratory ambient.

The analysis of the results shown in Table IV permits to conclude that :

- The water solubility of the quebracho extract, which depends on tannins and no tannins content, increased as the pH increased in the range studied.

- The oxidation originated a considerable loss of tannins, which was more meaningful to higher values of pH.

- The decrease of tannic substances during the oxidation is attributable to their transformation in no tannins and insoluble compounds.

- The oxidation drove to the acidification of the liquor.

8. Iron/tannins reaction

In laboratory tests, several acidified tannins solutions were brushapplied on steel plates with an insufficient mechanical preparation to eliminate all the products of corrosion. Visual and microscopic observations allowed to establish the formation of a blue black layer due to the reaction of the tannins with the residual iron oxides and that furthermore no immediate reaction over the steel exempt from superficial oxides was registered. Consequently, the decision of studying some aspects of the reaction tannins/iron was taken **[1,7]**; the trial was carried out with concentrated extracts and employing different iron (III) salts solutions, at pH placed between 2 and 6. The formation of an abundant blue black precipitate was observed; from a quantitative point of view, the precipitate increased when acidity was reduced. The reaction between the hexahydrated iron (III) ions and the hydroxy phenolic groups gives insoluble chelates and liberates $[H_30]^+$ ions, which explains the major yield of the reaction as the pH increased; pH must be lower than 6 to avoid oxidation reactions (Table IV). A sodium acetate solution was employed to regulate the hydrogenionic concentration between the cited values.

Similar trials were carried out in parallel with iron (II) salts solutions instead of iron (III) ones showed also the formation of a blue black precipitate. Although the divalent cations do not form insoluble compounds with the hydroxy phenolic groups of the tannins, the iron (II) ions are rapidly oxidised to iron (III) ions, specially if the reaction is carried out under conditions in that there is access of oxygen to permit the oxidation and to reduced pH. The reaction of oxidation of the iron (II) ions takes place according to the following way:

$$4\ Fe^{2+} + 2\ H_20 + 0_2 \rightarrow 4\ Fe^{3+} + 4\ 0H^-$$

Considering that the totality of the extract solids are from tannic nature, increasing quantities of the quoted iron (III) salts were added to the solutions, at every pH studied and working under intense stirring, until reaching a maximum mass of the blue black precipitate,

which was determined after centrifuging, washing with distilled water and drying in stove at 50 °C.

The blue black chelates obtained at laboratory temperature showed an iron content, according to atomic absorption determinations, between 2.0 and 2.5 % in weight. The iron tannates are highly water insoluble and in consequence their redissolution is practically impossible; furthermore, they showed a colloidal aspect, that is particles extremely fine, which does not permit the total retention in the filter paper during the step of washing. As a result of that, the separation of precipitates was carried out by centrifuging.

9. Infrared spectrophotometry. The characterisation of the tannins was also performed working in the infrared spectrum, on account of which it was necessary to prepare pills of approximately 0.3 - 0.5 mm in diameter, with a content of 0.1 % of extract or else of tannic acid employed as reference; potassium bromide was used as dispersing agent.

The spectra did not show marked differences between the tested extracts, but demonstrated the presence of polyphenols due to bonds of hydrogen between the hydroxyl ions: maximum of absorption in the 3400-3200 cm^{-1} band were observed in all cases.

Regarding samples of iron tannates prepared at laboratory according to before mentioned conditions, the spectra confirmed a reduced absorption at 3400-3200 cm^{-1} indicating that the formation of iron tannates involves the chelation with the hydroxyl groups of the quebracho tannins structure. Figure 2 show the infrared spectrum corresponding to iron (III) tannates.

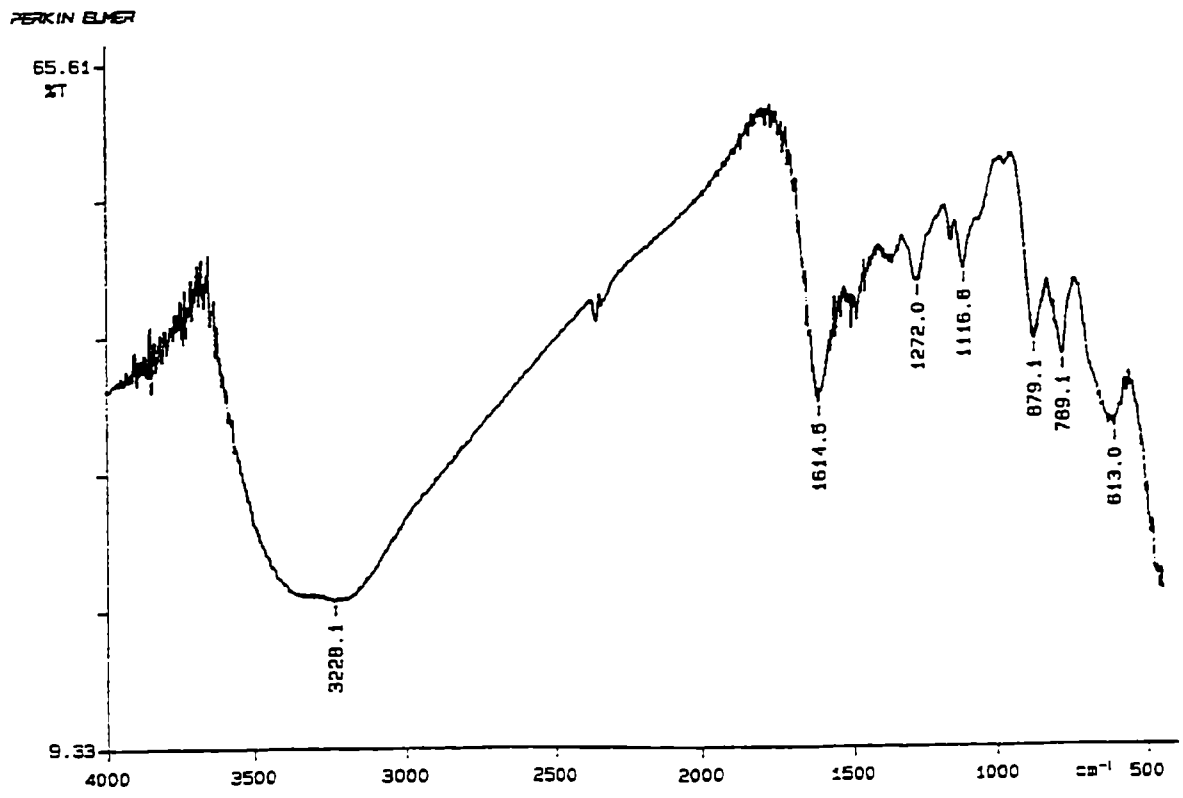

Fig. 2.- Infrared spectrum of iron tannates: the reduced absorption in 3400-3200 cm^{-1} band corresponds to the remaining hydroxy phenolic groups of the original tannins

Future works

In the last years, tannins chemistry has shown a high development and many and different compounds and their corresponding structures have been identified. In spite of the those progresses, the condensed tannins are widely distributed among the plants and, as a consequence, it is very important to carry out extensive researches on this subject and particularly, from corrosion and anticorrosion points of view, the conditions affecting the tannins/iron reactions and the properties of these compounds.

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