

Extraction of Tannin by *Acacia mearnsii* with Supercritical Fluids

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ABSTRACT

Studies were carried out on solvent and supercritical extraction to obtain natural tannins. The results showed that the best co-solvent to extract tannin with CO₂ supercritical was water with a concentration of 5.0% and the best trap rinse solvent was methanol.

Key words: Co-solvents, tannin, CO₂ supercritical

INTRODUCTION

Tannins are polyphenolic compounds present in plants, foods and beverages (Makkar and Becker, 1998) which are soluble in water and polar organic solvents (Haslam, 1996). Tannin of *Acacia mearnsii* finds application in the natural tannage of leather, as alternative procedure instead of leather chromium tannage. The traditional tannage process, with chromium, is highly pollutant. Brazil produces about 40,000 ton/year of the tannins which represents 90% of the world extracted from *Acacia mearnsii* (Deixheimer, 1998). The process used to extract tannins is the hydrosolubilization. Because this process operates with temperatures around 100°C, the extraction process motives a hydro cracking of sugar and others organic compounds with a darkening of the final product. We studied the supercritical extraction process as alternative procedure to obtain the natural raw material to leather tannage (Readel, 2001). In

which supercritical carbon dioxide and polar or non-polar co-solvents were used as solvents. The advantages of supercritical extraction process with regard to hydrosolubilization and solvent extraction are low extraction temperature, short extraction time and absence organic solvent concentration in the extract. Another objective was to compare the different extraction technology (Taylor, 1996).

MATERIAL AND METHODS

Natural Material

The raw material, black acacia bark, was provided by EXTRATOS BRASIL. A part of bark was milled with cutting mill (TECNAL - Willye TE 650) with 2.0 mm average particle diameter. In the solvent extraction with Soxhlet apparatus, dry natural material was employed. The dryer used

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was a BIOMATIC Equipment. The tannin was dried for 10 days at 36°C.

Solvent Extraction Process

Samples (50 g of dark acacia bark) were extracted with 500 mL of solvent for 24 h. The extracted solution was concentrated on a SAVANT Lyofilizator.

Supercritical Extraction Process

A Hewlett Packard 7680 extraction module was used to perform all the experiments. This system consisted of a nozzle/trap assembly that acted as a controllable variable restrictor, allowing an instantaneous depressurization of the supercritical fluid as well as the decoupling of flow and

pressure. The material to be extracted was loaded into a self-sealing extraction cell of 7.0 mL thick-walled stainless steel thimble. Supercritical fluid extracts were deposited in an internal trap rinsed off into a vial with 1 mL of hexane and methanol. Samples (0.4 g of bark) were extracted with supercritical carbon dioxide according to the described procedure, where different experiments to optimize the extraction conditions were done. The operation temperature and pressure ranges tested were 40 to 80°C and 150 to 200 bar, respectively. All other variables were kept constant: CO₂ flow, 2.0 mL/min; and extraction time, 30 minutes. The pressure and temperature conditions tested in experiments are shown in Table 1.

Table 1 - Pressure and temperature experimental condition

T (°C)	P (bar)	T (°C)	P (bar)
40	200	40	250
50	205	60	204
60	150	80	155
60	182	60	253
70	201	80	202
80	155	80	253

Analysis

The identification of tannins was made by thin layer chromatography (TLC). The analyses were run with standard compounds, catequin and tannic acid, using aluminum cromatofolios. Plates were developed using ethyl acetate:acetic acid (20:4) as the mobile phase. After drying, tannic acid was viewing using FeCl₃ (Sharma et al., 1998).

The quantification of tannins was made by spectrophotometry (BECKMANN Model Life Science). Standard solutions were formulated by Folin Denis reagent and the calibration curve was developed at 725 nm (Seigler et al., 1986).

RESULTS AND DISCUSSION

Solvent Extraction Process

Three organic solvents were tested to obtain the tannin with Soxhlet Apparatus: ethanol, dimethyl ether, and n-hexane. The experimental results, using TLC showed that n-hexane and dimethyl ether solubilized only non-polar organic compounds presented in acacia bark, while the ethanol extracted the tannin compounds. The results for ethanol extract can be seen in Fig. 1.

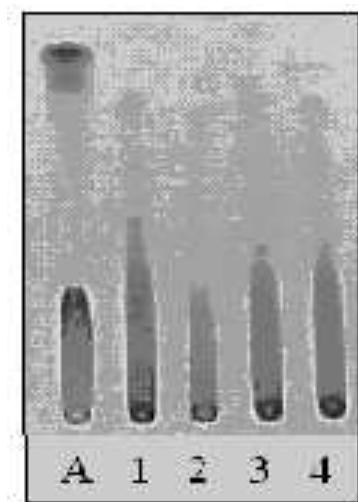


Figure 1 - TLC ethanol extract from acacia bark (1-dry and milled; 2-dry and entire; 3-natural and milled; 4-natural and entire; A-tannic acid standard)

With regard to quantification of tannins by ethanol, using spectrophotometry analysis, the results are written in Table 2. Sample 1 presented

higher tannins concentration, but a milled and dry bark was not feasible in the industrial process.

Supercritical Extraction Process

The supercritical carbon dioxide extraction did not present good results. As alternative on selectivity and quantity augment of extracts obtained was co-solvents addition (Taylor, 1996). Four solvents as co-solvents were tried in the process: acetone,

methanol, ethanol and water. The co-solvents concentration employed in the experiments to select the best tannins extraction was 5.0 % with regard to carbon dioxide quantity. Fig. 2 showed the concentration of the tannins, percent tannins in acacia bark (p/p), obtained with co-solvents extractions.

Table 2 - Concentration of the tannins extracted by Soxhlet Apparatus (1 - dry and milled; 2-dry and entire; 3 - natural and milled; 4 - natural and entire)

Acacia bark sample	% tannins in solvent extract (p/p)	% tannins in acacia bark (p/p)
1	60	23,7
2	68	21,6
3	37	4,8
4	54	6,4

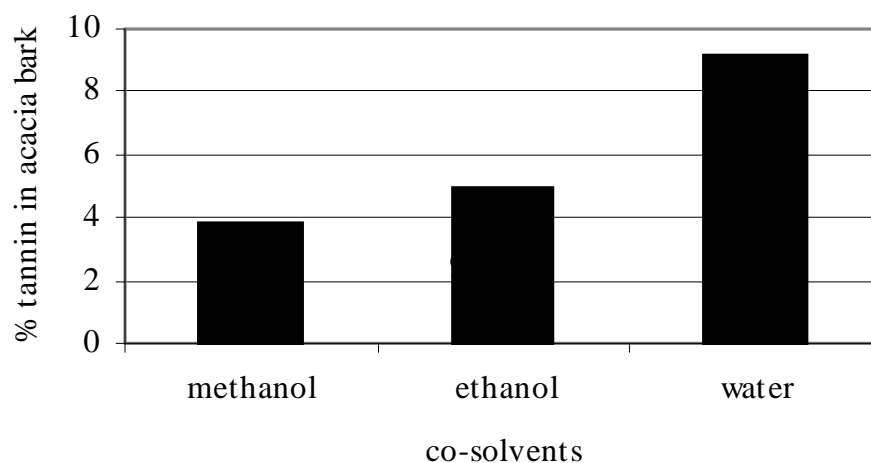


Figure 2 - % tannins in acacia bark (p/p) extracted with co-solvents (60°C, 200 bar and 2.0 mL CO₂/min)

Table 3 - Yield of tannins extracted by supercritical process (% p/p) using water and ethanol as co-solvent and CO₂ as solvent

Trap Solvent	Co-solvent	Bark Mass (g)	T (°C)	P (bar)	Yield (% p/p)
methanol	ethanol	0,0538	60	250	9,5
methanol	water	0,0282	60	250	13,5
n-hexane	water	0,0106	60	250	6,0
methanol	water	0,0200	60	200	25,0
n-hexane	water	0,0092	60	150	1,0
methanol	water	0,0120	60	150	0,5
n-hexane	water	0,0100	40	200	0,5
methanol	water	0,0100	40	200	0,5
n-hexane	water	0,0100	50	200	6,5
methanol	water	0,0015	50	200	0,5



Figure 3 - TLC supercritical extract from acacia bark (1-sample; A-tannic acid standard)

To define the best temperature and pressure condition, after selecting water as the best co-solvent, the extraction experiments were carried with 2.0 mL/min during 30 min. Ten experiments were realized changing temperature and pressure conditions, respectively (40 to 60° C; and 150 to 200 bar). The results are presented in Table 3.

Results showed T = 60°C and P = 200 bar as the best conditions to extract tannins. Figure 3 showed the presence of tannic acid in the supercritical extract.

Results indicated the possibility of changing the traditional process (hydrosolubilization) that resulted approximated 20 % of tannins from *Acacia mearnsii* (Graebin, 2000).

The solvent laboratory experiments with Soxhlet Apparatus were important in this study to demonstrate the poor tannin extraction with non-polar solvents. The complexity of natural tannins molecules demand experiment test to choose the solvent, therefore it was tried solvents with different polarity.

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RESUMO

Neste trabalho foram utilizados dois processos de extração de tanino vegetal: extração a quente em aparelho Soxhlet e extração com CO₂ supercrítico. Os resultados mostraram que o melhor co-solvente para extração de taninos com CO₂ supercrítico foi a água na concentração de 5% e o melhor solvente para lavagem do trap foi o metanol.

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