

Studies on the utilization of *Pinus pinaster* bark

Part 2: Kinetics and yields of alkaline extractions

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Summary. The influence of several variables (temperature, time, alkali type and concentration) on the yield of polyphenol from alkaline extractions is studied, using *Pinus pinaster* bark. For this purpose, the gravimetric Stiasny method and a spectrophotometric procedure based on molybdate ions are used. The influence of time on the extraction process was evaluated, and the results were correlated by semiempirical equations.

Introduction

In a previous paper, the composition of fractions which were extracted from pine bark through sequential extraction with solvents of increasing polarity was studied. On the basis of type and amount of solubilized compounds, a framework of its profit was proposed, which begins with an alkaline extraction of bark. In addition to phenolic acids, all compounds extracted with nonpolar and polar organic solvents and with water can be found in the extract. Flavonoid oligomers and polymers, waxes and suberin degradation products, uronic acids, and polysaccharides occur in amounts that depend on treatment conditions. These extracts, which constitute a substantial fraction of the raw material, can be utilized for the production of wood adhesives (Hall 1960; Anderson 1974, 1977; Hemingway 1978; Weissman 1980; Ayla 1981, 1982).

A detailed literature study showed that some variables such as pine species, growing conditions and site of the trees, type and concentration of alkali, temperature, particle size distribution and contact time affect the type and amount of products extracted from pine bark (Chang 1955; Pearl 1975; Hemingway 1976; Labosky 1977; Dix 1983).

The authors focused their attention on the influence of time on the yield of formaldehyde condensable polyphenols from *Pinus pinaster* bark through alkaline extractions performed under mild conditions. The time effect is very important in any industrial application of the proposed process. Most authors who study pine bark extraction use one time only, within 20 min and 72 h (Hall 1960; Fang 1975).

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The application of a mathematic model for the interpretation of the experimental results of extraction has been considered. The literature consulted refers to two kinds of equations: those which accept intraparticle diffusion as a controlling step based on Fick's law (Yang 1958; Krasuk 1967; Plachco 1970, 1972a, 1972b, 1973; Cahela 1983) and others which take into consideration a semiempirical model proposed by Othmer (1955, 1959) who uses the Hagen-Poiseuille equation. The model derived from Fick's law needs very restrictive hypotheses. They have been applied to extraction problems where only one product is obtained, of known diffusivity and molecular weight, in the absence of chemical reactions. These limitations make the model inapplicable here, because in the bark extraction, ionizable polyphenols with a large scatter of molecular weights are obtained (Hemingway 1977). The Othmer model, which proposes a potential interrelationship between non extracted fraction and time, was applied to our results.

Material and methods

The sampling and bark conditioning were performed as described part I. A systematic study of all variables which influence pine bark extractions is not possible. Therefore, some variables were fixed or neglected.

The variables not considered here are those which depend on origin, age and growing conditions of the trees. Considered variables are temperatures: 30–95 °C, alkali concentration: 0–2 g/100 g water, treatment time: 0–450 min and alkali type: Na₂CO₃ or NaOH. Fixed variables are solid:liquid ratio 1:10, stirring speed: 250 rpm, maximum particle size: 1 mm. The particle size distribution (referring to a representative sample) was the following:

Size, mm	1–0.75	0.75–0.50	0.50–0.30	0.30–0.12	0.12–0
Sample weight, %	22.9	16.2	14.1	17.8	29.0

The extractions were performed in a reactor with a stirrer with a 600 ml volume.

The gravimetric analysis of formaldehyde-condensable polyphenols (Stiasny polyphenols) was carried out with extracts obtained under several conditions using the procedure proposed by Wissing (1955). This method is extensively used, but it presents some inconveniences when applied to systematic experiments, because it is laborious and slow and requires the use of comparatively large amounts of solution. These characteristics make it inadequate for our purposes. In order to solve these problems, we took great interest in developing a spectrophotometric method to determine polyphenols in the extracts with accuracy and simplicity.

In the literature four spectrophotometric procedures were found that could be adapted to our purpose, based on the following chromogenic agents: vanillin, Rochelle salt, ammonium heptamolybdate and sodium molybdate (Roux 1951; Burns 1971; Dalby 1978; Pizzi 1980). The comparison among these methods was based on the range of the Lambert-Beer law observance and on the constancy of slope (*m*) obtained from the absorbance/concentration diagram which corresponds to extracts obtained from different bark samples treated under the same condi-

tions. The method using sodium molybdate was chosen because it provides an easier experimental procedure, better reproducibility and a greater range of Lambert-Beer law observance. The experimental procedure originally suggested by Pizzi (1980) is currently being developed.

Preparation of the solution containing chromogenic reagent buffer

In a 500 ml Erlenmeyer containing 243 g water, 1,025 g sodium acetate, 0.75 g acetic acid and 5 g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ were successively added with stirring. The reagent was prepared every 4 hours. The pH changes during the addition of the alkaline extracts could be hardly noticed because high degrees of solution were applied.

Experimental procedure

The alkaline extracts were filtered from the extraction medium, placed in a test tube which was sealed, air cooled for 20 min and diluted. Five ml each of the solution were placed in 30 ml test tubes and 10 ml of the chromogenic reagent-buffer solution were added to each one, with stirring. The average transmittance was measured at 400 nm, 20 min after mixing, using a Coleman MAS 50 spectrophotometer equipped with 2.5 cm cylindric cuvettes. The blank was treated like the samples; the polyphenol solutions were replaced by distilled water. The gravimetric determination of formaldehyde condensable polyphenols in the initial extracts makes it possible to calculate the concentration of compounds in the samples, applying a spectrophotometric analysis.

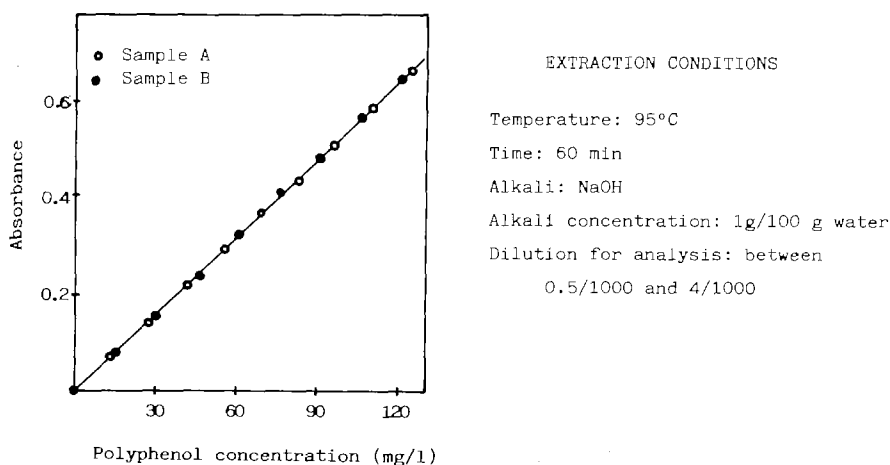


Fig. 1. Relationship between absorbance and concentration of polyphenols using the molybdate method. Data relative to extracts obtained from two bark samples of different origin, treated under the same experimental conditions

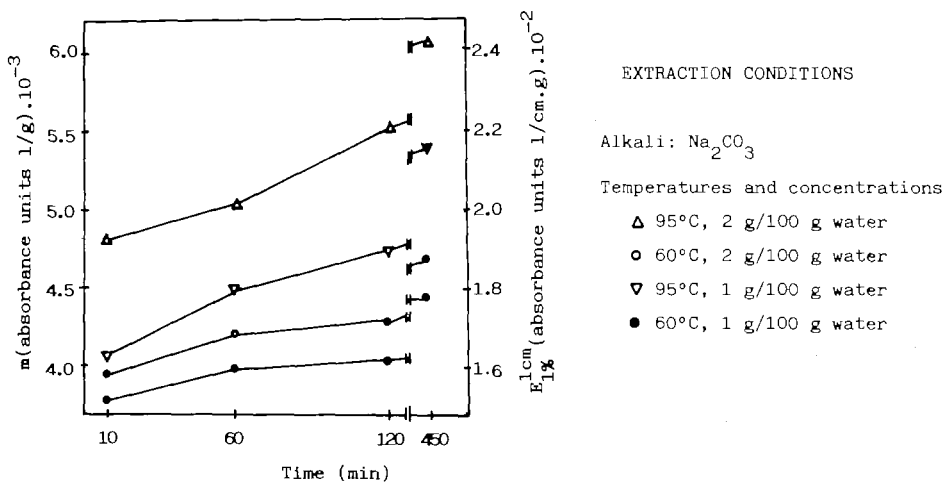


Fig. 2. Relationship between experimental conditions, average of m and $E_{1\%}^{1\text{cm}}$ in extractions using Na_2CO_3

Table 1. Formaldehyde-condensable polyphenol yields from *Pinus pinaster* bark samples with different origin. Data represent NSP (g polyphenols/100 g initial oven-dry bark). Alkali: Na_2CO_3

Temperature °C	Time min	Na_2CO_3 -concentration			
		2g/100 g H_2O		1 g/100 g H_2O	
		Run 1	Run 2	Run 1	Run 2
95	10	16.1	14.3	17.1	18.3
	60	15.9	17.8	24.3	23.1
	120	17.4	17.1	17.0	19.8
	450	20.3	23.5	22.0	23.9
60	10	14.4	15.8	13.3	12.5
	60	17.9	18.3	15.5	14.4
	120	18.3	18.1	15.6	15.5
	450	18.1	18.0	16.5	15.8

The extraction conditions (temperature, concentration, type of alkali and contact time) affect the chromogenic capacity of the extracts, which produces variations in m or $E_{1\%}^{1\text{cm}}$ values. This involves much experimental work in order to obtain calibration data for all conditions studied.

The Lambert-Beer law observance in the spectrophotometric molybdate method is excellent in the 0–0.7 A interval (roughly 20–100 T%), with average correlation coefficients (r^2) of 0.9996 and 0.9997 for the extractions, using Na_2CO_3 and NaOH . As an example Fig. 1 presents the absorbance/concentration obtained from two bark samples of different origin, treated under representative conditions. The deviations between values of the slopes (m) obtained from different bark samples treated under the same conditions gave a measure for the reproducibility

Table 2. Formaldehyde-condensable polyphenol yields from *Pinus pinaster* bark samples with different origin. Data expressed as NSP (g polyphenols/100 g initial oven-dry bark). Alkali: NaOH

Temperature °C	Time min	NaOH concentration									
		2 g/100 g H ₂ O		1 g/100 g H ₂ O		0.5 g/100 g H ₂ O		0.25 g/100 g H ₂ O		0 g/100 g H ₂ O	
		Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
95	10			25.0	26.3	29.2	33.0	17.6	17.0		
	60			28.8	31.7	31.2	30.5	18.9	19.8		
	120			30.5	34.4	32.1	31.2	21.9	19.7	4.4	4.3
	450			43.4	42.4	37.4	39.7	22.5	20.9	4.0	4.4
60	10			22.0	23.4	28.2	21.9	14.5	14.4		
	60			30.7	34.8	30.4	29.4	15.3	14.9		
	120			32.4	27.6	29.4	29.8	17.2	15.5		
	450			30.7	31.2	34.4	35.5	18.8	16.7		
30	10			19.2	16.9						
	60			19.0	19.0						
	120	18.0	18.6	19.5	20.0						
	450	20.2	19.6	22.5	24.8						

of the method. Under the conditions of Tables 1 and 2, duplicate experiments were carried out. The deviation values for extracts, obtained under the same conditions, gave satisfactory results: 0.3–3.6% for Na₂CO₃ extractions (average 1.5%) and 0–2.7% for NaOH extractions (average 0.9%). The reproducibility is poor for extractions performed with water (95 °C) and NaOH (2 g/100 g water, 30 °C), which is due to the tendency of these extracts to form suspensions during cooling.

Figures 2 and 3 show the dependency of m and $E_{1\%}^{1\text{cm}}$ (parameters which measure the chromogenic capacity of extracts) on the extraction conditions. The fact that $E_{1\%}^{1\text{cm}}$ varies (although slightly) with the experimental conditions, reveals the existence of a chemical reaction. Figures 2 and 3, which provide all necessary information for spectrophotometric analysis, show that an increase in time involves an increase in m . This could be due to a reaction between polyflavonoids and the molecular oxygen of the air, which was already reported by Fang (1975) and Wissing (1955). An oxidation by oxygen of both A and B rings of the flavonoids to the corresponding quinones has been already proposed by Hemingway (1978). These structures could justify the well known development of carbonyl absorption bands in the IR spectra of polyphenols obtained by alkaline extraction of bark (Fang 1975).

Results and discussion

Alkaline extractions of *Pinus pinaster* bark were carried out under the conditions of Tables 1 and 2. Under these conditions two bark samples of different origin

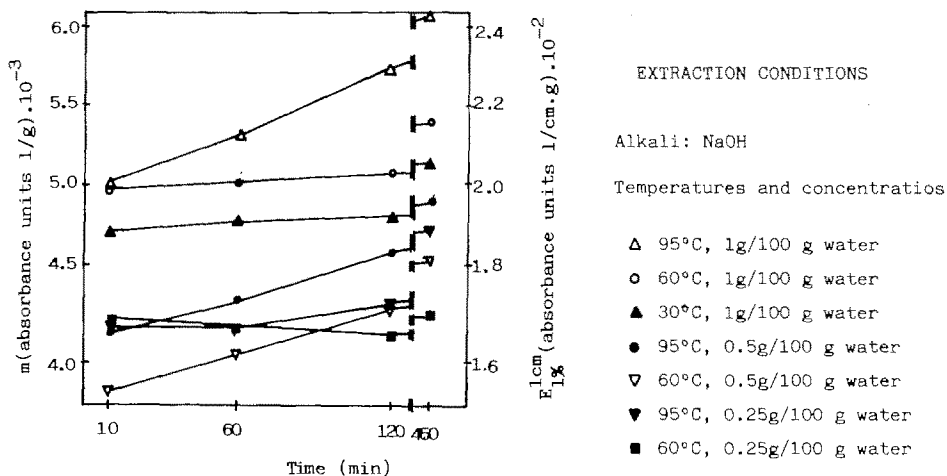


Fig. 3. Relationship between experimental conditions, average of m and $E_{1\%}^{1cm}$ in extractions using NaOH

were treated. At the end of the extractions, two samples were: The first one (1 ml of extracts) was submitted to different degrees of dilution in order to cover the range of the Lambert-Beer law observance in the spectrophotometric analysis, while the second one (15–50 ml) was used to determine gravimetric polyphenols. The results of gravimetric analysis are shown in Tables 1 and 2. The yields of solubilized polyphenols are given as “Numbers of Stiasny polyphenols” (NSP), defined as the weight in grams of HCOH-condensable polyphenols solubilized from 100 g of oven-dry bark.

It should be noted that the amount of polyphenols solubilized is significantly different from the amount in the extracts separated by filtration, because the raffinate retains a fraction of the solution, more than 50% of the wet solid. Nevertheless, the polyphenols which remain in the filtration cake could be recovered by washing. The experimental results from Tables 1 and 2 show that the fraction of alkali-soluble polyphenols varies very much with the sample selected, even under the same treatment conditions. This has been reported for other pine species by Labosky (1977).

The aqueous extraction is an effective method for separating polyphenols from the bark of some pine species (extracted fractions of more than 20% have been reported by Dix 1983), but in the present case the average NSP is only 4%, which is too low for practical purposes. However, the addition of small amounts of alkali modifies the extraction results, causing an increase in the solubilized sample amount and an increase of the ratio of solubilized polyphenols to the total solubilized sample weight.

An increase in the alkali concentration does not entail a systematic increase of the previous ratio, which shows a maximum. The most favourable alkali concentration depends on the pine species studied. Values of 0.5–1% for NaOH concentration to reach optimum values for the above ratio have been reported by Dix (1983). Our results show that the use of NaOH is more favourable than Na_2CO_3 .

Table 3. Values of parameters a and b

Temperature °C	Alkali type and concentration	a	b
95	2 g Na ₂ CO ₃ /100 g H ₂ O	0.1221	-0.3608
	1 g Na ₂ CO ₃ /100 g H ₂ O	0.1360	-0.1840
	1 g NaOH/100 g H ₂ O	0.2128	-0.4717
	0.5 g NaOH/100 g H ₂ O	0.1949	-0.4234
	0.25 g NaOH/100 g H ₂ O	0.1020	-0.4932
60	2 g Na ₂ CO ₃ /100 g H ₂ O	0.0805	-0.2641
	1 g Na ₂ CO ₃ /100 g H ₂ O	0.0696	-0.2745
	1 g NaOH/100 g H ₂ O	0.1774	-0.5692
	0.5 g NaOH/100 g H ₂ O	0.2438	-0.7266
	0.25 g NaOH/100 g H ₂ O	0.0799	-0.2961
30	1 g NaOH/100 g H ₂ O	0.1120	-0.2961

The extractions with 2 g Na₂CO₃/100 g H₂O (60 and 95 °C) and with 1 g Na₂CO₃/100 g H₂O at 60 °C for 10–450 min, gave NSP in the range of 14.3–23.9%. Similar values are obtained with 0.25 g NaOH/100 g H₂O and 95 °C: 17.0–22.9%. When the Na₂CO₃ concentration is increased from 1 to 2 g/100 g water, no appreciable advantage can be noticed. For 95 °C, approximately the same effect is obtained by NaOH with concentrations four times smaller. The amount of polyphenols extracted with 1 g Na₂CO₃/100 g H₂O and 60 °C (12.5–16.5%) is smaller than the previous ones and is in an order similar to that obtained with 0.25 g NaOH/100 g H₂O at the same temperature (14.4–18.5%). When extractions are performed at 60 °C, the weight ratio of NaOH : Na₂CO₃ = 1 : 4 is roughly maintained to get the same NSP.

In the extractions using 0.5–1 g NaOH/100 g H₂O at 60 and 95 °C, NSP's between 22.6 and 43.4% are obtained, which are better than those obtained under other conditions. The differences seem to depend more on the studied samples than on experimental conditions, although the 40%-limit is only passed under strongest conditions. The extractions carried out at 30 °C with 1 and 2 g NaOH/100 g H₂O, gave NSP's of approx. 20%, markedly less than with same alkali concentrations at 60 °C.

The experimental results in Table 1 allow a first evaluation of the time effect on the polyphenol yields, with bark samples of different origin; the results are very much influenced by this. With the calibration data in Figs. 2 and 3, a major study on the influence of the experimental conditions on the extraction kinetics is possible, using the extracts from the same extraction and eliminating undesired effects of the sample origin.

For this purpose, suspension samples were drawn and filtered from the extractive, with a volume great enough to give 1–2 ml of extract, after 1, 2, 4, 6, 10, 15, 30, 60, 90, 120 and 450 min. The spectrophotometric analysis was performed as previously described. The absorbances measured divided by the corresponding m-values (see Figs. 2 or 3) give the polyphenol concentrations. Duplicate experi-

ments were carried out under the operational conditions studied here. By this method, the solid/liquid ratio does not significantly vary. In order to make an easier comparison between the results obtained, the extraction percentage (EP) was used, which is as follows:

$$EP = \frac{NSP_t}{NSP_{450}} 100,$$

where NSP_t and NSP_{450} are the numbers of Stiasny polyphenols measured after t and 450 min respectively. The fraction of polyphenols extracted after 450 min is considered as the greatest amount which can be solubilized under the conditions studied here. This seems to be correct when taking into account the small variations of EP when going from 120 to 450 min (0–6.4%). The results obtained are shown in Figs. 4 and 5, where each point represents the average of two measurements. From these figures it can be seen that the shape of all curves is very similar: the extraction rate (measured by the slope of the curves) is high at the beginning, because 50–65% of the total amount is extracted in the first minute, and it decreases notably after 8–15 min. The extraction is faster in the experiments carried out at both temperatures and with high alkali concentrations. Under the same conditions, NaOH presents a more favourable kinetic than Na_2CO_3 .

Considering the curves in Figs. 4 and 5, 15 min were chosen as contact time because:

1. After 15 min the slope of the curves notably diminishes, especially in extractions with 1 and 2 g NaOH/100 g H_2O at 60 and 95 °C, giving the maximum polyphenol yields.

2. In the extractions where substantial amounts of samples are solubilized, at this time high EP's are reached (80–85% for Na_2CO_3 and about 90% for NaOH, at temperatures above 30 °C and concentrations of 0.5 or 1 g/100 g H_2O).

3. Using extraction times somewhat shorter, high EP's are obtained also.

The differences between the EP determined by a duplicate are usually within $\pm 5\%$ of the mean value. The greatest deviations occur at 1 min, probably due to variations in the humectation time.

The EP data were correlated with the equation proposed by Othmer for oil seed extraction. From the Hagen-Poiseuille equation, Othmer developed the following semiempirical equation:

$$C_s = a \cdot t^b,$$

where:

C_s = concentration of residual solute in the solid phase (g solute/g inert solid)

t = time (min), and

a, b = parameters depending on the physical properties of the extracts.

The values of C_s are easily calculated from the corresponding EP (in Figs. 4 and 5) and the NSP_{450} .

The above equation predicts a linear interrelationship between $\log C_s$ and $\log t$. The results obtained in the alkaline extraction for bark show this behaviour in the 1–20 min interval, as is shown in Fig. 6 for three typical cases. Figure 7 makes it

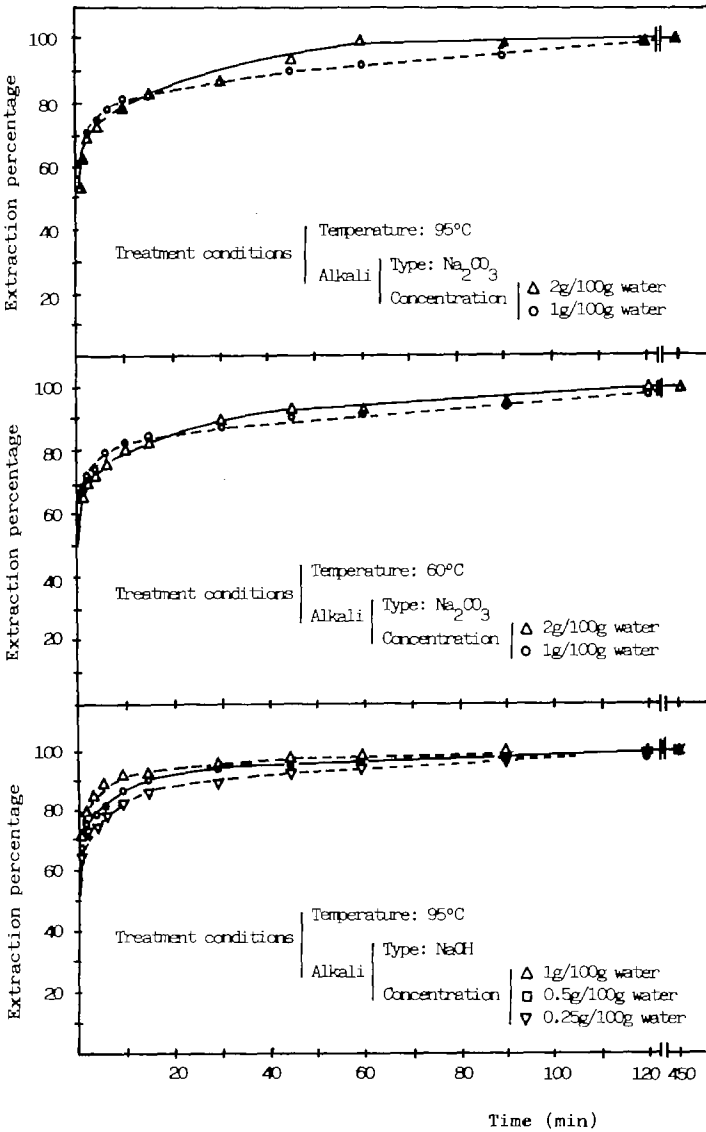


Fig. 4. Relationship between extraction percentage and time under various treatment conditions

possible to compare the values of experimental EP with those obtained from the above equation. The parameters a and b were calculated from a linear correlation of $\log C_s$ against $\log t$ in the interval of 1–15 min. The values used for NSP were the means of value given in Table 1 for extractions at 450 min. The results obtained for parameters a and b under the experimental conditions considered here are shown in Table 3. Although the extraction lasted for 450 min, the 1–15 min interval is the most important, because it includes the period where the most substantial part of the extraction occurs.

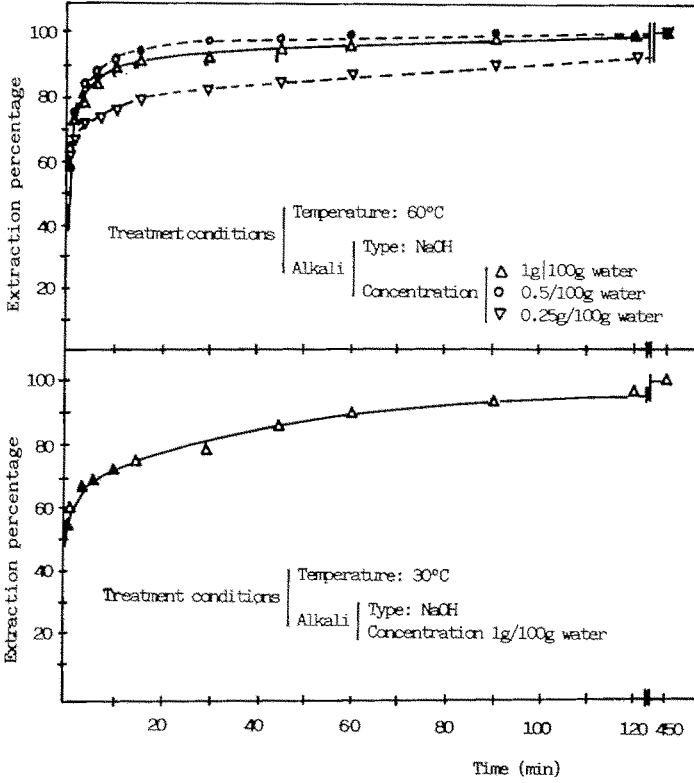


Fig. 5. Relationship between extraction percentage and time under various treatment conditions

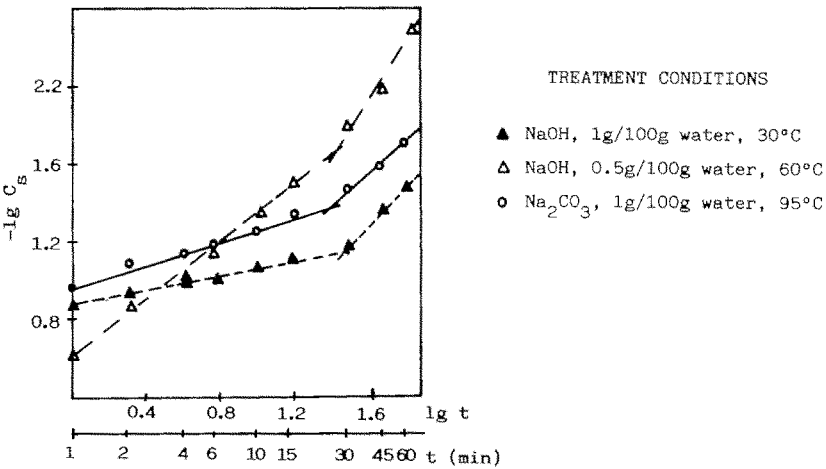


Fig. 6. Relationship between $-lg C_s$ with t and $lg t$ in some of the cases

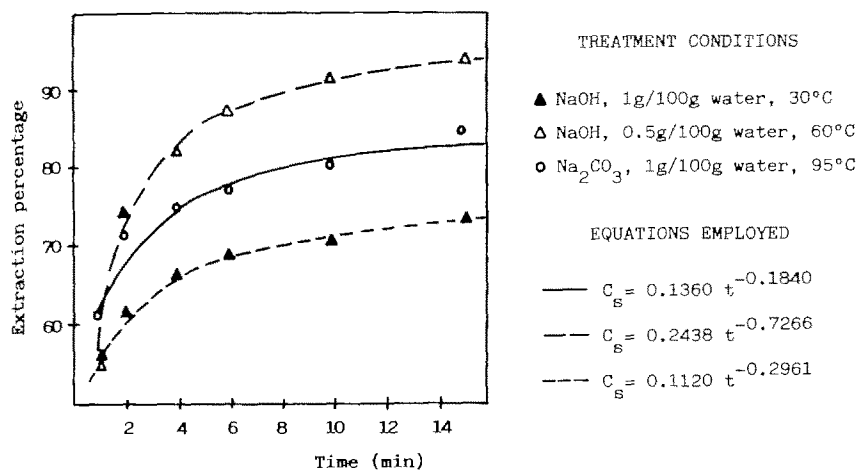


Fig. 7. Experimental and calculated data of extraction percentage at different times in some of the cases

Conclusions

The amount of formaldehyde-condensable polyphenols solubilized from *Pinus pinaster* bark by alkaline solutions was studied under various experimental conditions. The solubilized fraction depends on treatment conditions and sample origin. Results of NSP from 12.5 to 43.4% were found. At 60 and 95 °C and the same amount of solubilized polyphenols, the extractions with Na₂CO₃ need four times more concentration of alkali than extraction with NaOH.

Results of the gravimetric analysis have been used to obtain calibration data for a spectrophotometric procedure, based on the use of molybdate ions which permits a quick analysis of polyphenols. This method was applied for studying the effect of time on the extraction process. The data obtained with all extraction conditions show that the extraction proceeds very fast at the beginning (more than half of the polyphenols were extracted in 1 min). The rate markedly decreases after 8–15 min. The results of EP were correlated with the time by means of semi-empirical equations proposed in the literature for other extraction processes. The agreement between experimental and calculated data is satisfactory for the 1–15 min interval. This is the most important period, because it allows EP's up to 90%.

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