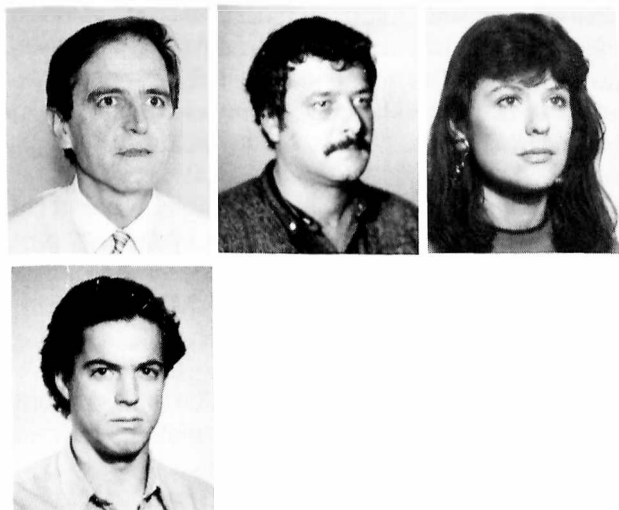


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Dechroming of shavings

Part 1. Enzymic Alkaline Treatment. Study of Variables



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Zusammenfassung

Entchromen von Chromfalzspänen.

Teil 1: Enzymatischer Aufschluß.

Untersuchung der Fakten

Die Optimierung der alkalisch-enzymatischen Hydrolyse von Chromfalzspänen ist eine wichtige Voraussetzung für die praktische Durchführung. Es geht darum, den Aufschluß so zu führen, daß ein möglichst chromfreies Hydrolysat auf der einen Seite und ein proteinfreier Chromhydroxydkuchen auf der anderen Seite anfällt. Dabei soll der Aufschluß so schonend wie möglich sein, um die bestmögliche Qualität des Hydrolysates zu erreichen bei geringstem technischen Aufwand. Einige Parameter in diesem System wurden hierbei untersucht und in der Veröffentlichung vorgestellt, die als erste einer Reihe anzusehen ist und dann auch die Nutzungsmöglichkeiten der Hydrolysate einschließt.

Summary

Dechroming of Shavings.

Part 1: Enzymic Alkaline Treatment. Study of Variables

In this report a study about experimental condition in a dechroming shavings hydrolytic process at moderate temperature involving an alkaline hydrolysis with calcium and

sodium hydroxide with and without the presence of a proteolytic enzyme is described. The percentage of solubilization of shavings is followed by total solid determination in protein hydrolysates; filtrability properties and aspect of hydrolysates is evaluated. A pilot plant trial was carried out using the developed procedure, in which a proteins mass balance through total nitrogen contents was performed. A statistical design based on a completed factorial experiment with four factors at two levels was employed and a reduced polynomial model from the experimental results was obtained.

Resumen

Descromado de rebajaduras de cuero al cromo. Parte 1. Estudio de la degradación enzimática

La optimización de la hidrólisis alcalino-enzimática de las rebajaduras de cuero al cromo es una condición importante para la realización práctica. Se trata de conducir la degradación de modo tal que pueda obtenerse, por una parte, un hidrolizado lo más exento posible de cromo y, por otra, una torta de hidróxido de cromo exenta de proteína. La degradación debe ser lo más suave posible para poder conseguir la máxima calidad posible del hidrolizado con un coste técnico lo más bajo posible. Se han estudiado algunos parámetros de este sistema y se presentan en la publicación, la cual puede ser considerada como la primera de una serie, y luego se incluyen también las posibilidades de aprovechamiento de los hidrolizados.

1. Introduction

Reference is made to the restrictions included in regulations by sanitation authorities in several countries with regard to the disposal of chromium-containing leather wastes, due to chromium leachability (mainly trimmings and shavings from the wet blue stage).

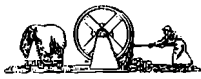
At the same time, many attempts, based on research work, are carried out aimed at demonstrating the low toxicity of chromium(III) as compared with chromium(VI). The most typical objectives involve avoiding disposal restrictions on chromium(III) or increasing the maximum authorized limits for leachate in chrome-tanned solid wastes, since such change in the regulations regarding chromium-bearing wastes is a major concern among tanners.

This issue is yet unsolved and leads to continuous discussions on chromium leachability. Meanwhile,

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thorough research work is presently underway towards developing:

- a) alternative chromium-free tanning techniques;
- b) high-exhaustion chrome tanning (minimizing chrome mobility);
- c) wet white production (chromium-free shavings); and
- d) detanning of chrome containing solid tannery wastes.

The application of these technologies, especially c) and d), depends on the severity of the restrictions concerning the discharge of chrome tanned wastes.

The purpose of our study was investigating experimental conditions so as to optimize of shavings at moderate temperature in an alkaline medium in the presence of a proteolytic enzyme to facilitate the degradation of collagen-chrome complexes and lead to the extraction of a protein hydrolysate with negligible chromium contents and of a chrome cake that would allow to recover the "metal".

2. Background

The detanning of wastes containing chromium(III) (shavings and trimmings) can be performed under different conditions:

- at 300 °C and 150 atm, in the presence of magnesium oxide¹;
- by boiling them with calcium or sodium hydroxide^{2,3};
- through hydrolyzation, at 100 °C, with sodium hydroxide, in the presence of magnesium or calcium oxide⁴;
- in an acid medium (sulphuric, p-toluen sulphonic, acetic or trichloroacetic + acetic acid)⁵;
- dechroming chrome(III) and oxidation to chrome (VI) by using hydrogen peroxide in an alkaline medium⁶;
- in a moderate alkaline medium, through a combination of a series of alkaline agents such as sodium carbonate, sodium hydroxide, magnesium oxide and calcium oxide⁷, in the presence of a proteolytic enzyme (bacterial origin).

Dechroming of shavings, when these solid wastes are considered as a secondary source rather than as wastes to be disposed, leads to the generation of two kinds of by-products:

- chrome hydroxide cake (highly basified chrome complexes) containing inorganic and organic salts, fat and proteins; a tentative application of this by-product as a chrome-tanning liquor is analyzed in detail under reference⁸;
- protein hydrolysate, which may be used for the manufacture of hydrolyzed animal foods⁸.

Hydrolysates have proved to be useful in the market and the profits that may be obtained thereof constitute a driving force for the process, if the disposal of the chrome cake can be managed economically and without generating environmental problems.

Presently, a more sound protein hydrolysate can be isolated, since more perfected techniques for alkaline hydrolysis were developed⁹. Besides, Heidemann pointed out that there is a relationship between hydrolysis intensity and the extent to which proteins are released. The time needed for hydrolysis, the alkaline conditions and the temperature are factors to be controlled.

The experimental work under reference⁷ is mainly aimed to demonstrate which is the effect of a proteolytic enzyme upon a hydrolysis process aided by a mixture of alkaline reagents. However, no studies were performed on experimental conditions related to the optimization of hydrolytic action by alkalis, mainly sodium hydroxide and its combination with calcium and magnesium oxide in the absence of an enzyme.

Much research work has been performed and abundant technological information is available regarding the alkaline conversion of collagens into protein degradation products. This has led us to think that the alkaline action upon chrome-stabilized collagen goes far beyond an adequation of the pH value to maximize enzymic digestion; that is, beyond the "holding period" mentioned under reference⁷.

It should be said that enzymes attack alkaline denatured collagen-chrome structures. In the transition from collagen to degraded products ("alkali-precursor protein hydrolysates"), peptide chains can be considered as essentially devoid of secondary structure. Therefore, peptide chains can have different configurations without any definite orientation. Consequently, it is not surprising that denatured collagen peptide segments can adapt themselves to the configuration of active centers in enzymes and be susceptible to proteolytic degradation¹⁰.

3. Experimentals

3.1 Variables

Preliminary laboratory trials have led to identify the following variables:

- a) enzyme concentration

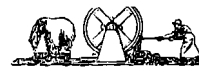
$$E_0 = 0\% \text{ enzyme} \quad E_1 = 4\% \text{ enzyme (\% based on dried shavings)}$$

- b) alkaline-enzymic treatment - hydrolysis time -

$$T_1 = \text{alkali (sodium hydroxide) 4 h} + \text{enzyme 2 h}$$

$$T_2 = \text{alkali (sodium hydroxide) 2 h} + \text{enzyme 4 h}$$

$$\text{total hydrolysis time (alkali + enzyme) was 6 h.}$$



In both cases, enzymes were added after the sodium hydroxide treatment.

When no enzymes were added, hydrolysis time was still 6 h.

The reactor vessel is stirred continuously throughout the process (see procedure under 3 below).

c) "extraction" of alkali-enzyme denatured shavings
Both the idea of using this variables and its name were taken from techniques used in the commercial manufacture of gelatin. The conditioned alkali-enzyme chrome shavings are submitted to an "extraction" or "cook" at 90 °C during 15 minutes. The "extraction" is carried out after the hydrolysis process, mentioned in b), took place. The temperature is raised from 55 to 90 °C and the reaction vessel is placed in an oven at 90 °C during 15 minutes.

d) maceration

This term is used to describe the process that takes place after hydrolysis for 6 hours and stirring, when the reactor vessel is left at a standstill, at room temperature, for 16 hours.

When this variable is combined with the "extraction" factor, maceration occurs after the temperature of the vessel is raised to 90 °C for 15 minutes.

A combination of these four variables was performed at two levels with a complete 2⁴ factorial experiment design. Factorial experiments test all possible combinations (16 trails in this design) and measure interaction among factors, as well as the main effects of single factors. In this analysis of variance, a 5% significance level was used.

Additionally, a mathematical polynomial model applicable to the experimental data was developed. The sixteen treatments were allocated at random to two sets of 8 trials.

3.2 Material and equipment

Chrome shavings were obtained from commercial tanneries and stored at 4 °C until used. Their size distribution and specifications were as follows:

>4.8 mm = 34.1%

>1.7 < 4.8 mm = 34.9%

>1.2 < 1.7 mm = 6.2%

<1.2 mm = 24.8%

Chrome content = 4.3% Cr₂O₃ dry substance (ds)

Total Kjeldahl Nitrogen (TKN) = 14.3% (ds)

Fat = 0.8% (ds)

Total ash = 16.7% (ds)

In preliminary trials, which led us to follow the procedure described below, the enzyme Alcalase (Novo

Nordisk Industry) and an enzymic product called "Recuperase" (locally manufactured by Strong Chemical S.A.) were used. The latter was employed in the factorial experience described herewith. Its activity at 50 °C (substratum casein), for different pH values, shows optimal figures ranging between 10.0 and 10.7. The appropriate temperature range is between 50 and 57 °C.

The trials were made in a thermostated bath equipped with a horizontally-movable platform with capacity for eleven 300 ml Erlenmeyer vessels.

3.3 Procedure

All the percentages mentioned are to be understood as referred to dried shavings weight (moisture free).

2% calcium hydroxide was shaken for 30 minutes, after adding 1200% of water; then, 3 g of dried shavings were added and the mix was further shaken for another 30 minutes. Afterwards, the shavings were treated with 10% sodium hydroxide during 4 hours when enzymic hydrolysis time was 2 hours and during 2 hours when enzymic hydrolysis time was 4 hours. In both cases, enzymes were added after these periods of sodium hydroxide treatment.

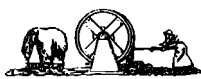
The reaction was carried out at temperatures ranging between 55 and 57 °C, while the pH values were kept under control (range: 10.3-10.7). When needed, sodium hydroxide was added.

All the samples were vacuum filtered through Whatmann #1 filtering paper, while their filtrability and the characteristics of the hydrolysates were observed. The hydrolysates residues were weighted after drying them in an oven at 70 °C during 16 hours.

Due to difficulties in assessing the amount of organic-inorganic salts produced from the shavings and reagents through control trials and in measuring their distribution between hydrolysate and cake, shavings solubility in this part 1 of our research work is shown in terms for total solids, as it also occurs under reference⁷.

It must be taken into account that the amount of inorganic agents was the same in all the trials. Interesting comparative results are obtained from an analysis of the total solids contents in the protein hydrolysates.

Total Kjeldahl Nitrogen was analyzed in some trials. This parameter is more suitable for measuring the extent of hydrolysis. Nevertheless, the total solids contents provided useful information for selecting an appropriate procedure for detanning chrome shavings at an experimental pilot-plant scale. During such



experimental work, a protein mass balance was performed (see point g below).

4. Results and discussion

4.1 Total solids contents in protein hydrolysates (% based on the weight of moisture-free shavings)

The following notation for describing the variables shown under 3.1 for the combination of the sixteen treatments was adopted:

$E_0 = 0$, $E_1 = 4\%$ enzyme (% based on dried shavings)

$T_1 = 2$ h enzymic treatment, after 4 h sodium hydroxide hydrolysis

$T_2 = 4$ h enzymic treatment, after 2 h sodium hydroxide hydrolysis

Ext. yes = extraction factor was employed

Ext. no = extraction factor was not employed

Mac. yes = maceration factor was employed

Mac. no = maceration factor was not employed.

Total solids (%)	total solids (%)
E_0-T_1 -Ext. yes-Mac. no = 86.4	E_1-T_1 -Ext. no-Mac. no = 85.7
E_0-T_2 -Ext. no-Mac. no = 81.0	E_0-T_1 -Ext. yes-Mac. no = 91.7
E_0-T_1 -Ext. no-Mac. yes = 79.2	E_1-T_2 -Ext. no-Mac. no = 83.0
E_1-T_1 -Ext. yes-Mac. no = 90.7	E_0-T_2 -Ext. yes-Mac. no = 89.6
E_1-T_1 -Ext. no-Mac. yes = 89.8	E_0-T_2 -Ext. no-Mac. yes = 84.0
E_0-T_1 -Ext. yes-Mac. yes = 89.8	E_1-T_2 -Ext. yes-Mac. no = 96.5
E_1-T_2 -Ext. no-Mac. yes = 89.3	E_1-T_1 -Ext. yes-Mac. yes = 99.8
E_0-T_2 -Ext. yes-Mac. yes = 90.0	E_1-T_2 -Ext. yes-Mac. yes = 96.1

4.2 Characteristics of protein hydrolysates

Hydrolysates resulting from treatments involving no enzyme, without extraction and maceration showed a green colour and a turbid aspect. In some trials with enzymic digestion, but without extraction or maceration, a light green colour and a translucent aspect were observed.

As a general rule, extraction and maceration variables lead to light yellowish hydrolysates, with a translucent or transparent aspect, especially when the enzymic treatment is applied. After being dried at 60–65 °C in an oven with circulating air, hydrolysates produced a light yellowish odorless and deliquescent protein. This protein is being characterized by its contents of aminoacids and by its biodigestibility. A nutritional study is presently underway in a joint effort by CITEC and the National Institute of Agricultural Technology (INTA). This studies will be the subject of a future publication.

As it was demonstrated by Heidemann⁸, the yellowish colour in hydrolysates appears as a function of time, of hydrolysis temperature and of the nature and number of alkaline agents. Our hydrolysis working conditions

were moderate as compared with Heidemann's. After his study, we adopted the measure of yellowness index¹¹. The influence of colour upon dried proteins used for animal foods is being considered (variation in chromatography patterns and modification of aminoacids profile).

4.3 Filtrability

The combination of enzymic treatments with maceration and extraction has improved filtrability properties significantly. In preliminary trials, the use of lime in a stage previous to sodium hydroxide treatment demonstrated to be advantageous for filtration purposes.

The literature shows that problems arise during the filtering operation when sodium hydroxide is used to dissolve shavings (2,3). This inconvenience can be solved when lime (2% ds) and a proteolytic enzyme (2% ds) are present.

4.4 Chrome contents in dried protein residues

In yellowish residues, the chrome contents were below 2 mg/kg (as per the sensitivity of the method applied). In greenish residues 400 mg Cr/kg was detected.

4.5 Total Kjeldahl Nitrogen content in protein residues (TKN)

Nitrogen contents ranged between 10.5 and 12.5% (percentages based on dried protein hydrolysates). Only four samples were analyzed. The nitrogen recovered from the shavings was 80%. A mass balance of proteins (through TKN contents) performed at a pilot plant is described in section g below.

4.6 Statistical analysis of the data of total solid contents

The factors mentioned in section 3.1 and their levels were the following

- a = enzyme concentration
 - low level (E_0) 0%
 - high level (E_1) 4% (% on dried shavings basis)
- b = alkaline-enzymic treatment - hydrolysis time - (see 3.1 for details)
 - low level (T_1) 2 h
 - high level (T_2) 4 h
- c = extraction at 90 °C for 15 min (see 3.1 for details)
 - "low level" means that the extraction factor was not employed;
 - "high level" means that the extraction factor was employed.
- d = maceration (see 3.1 for details)
 - "low level" means that the maceration factor was not employed;

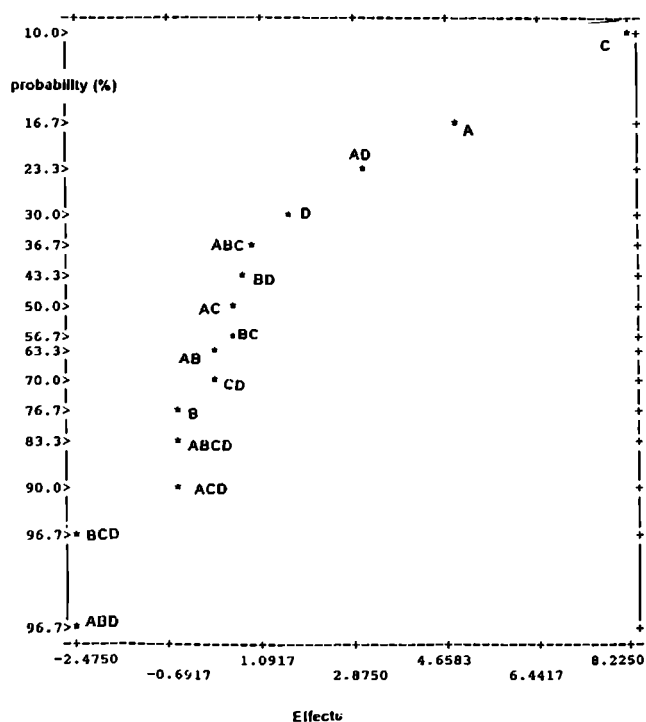


Fig. 1 Normal probability plot for effects

"high level" means that the maceration factor was employed.

In an unreplicated experiment, no direct estimate of the variance is available because real and meaningful high-order interaction occurs only occasionally.

The procedure we used to overcome this inconvenience was that developed by Daniel¹³, who suggested to graph the estimation of the main effects and of their interaction on a normal probability plot. Non-significant effects show a tendency to gather along a line (normal distribution, mean = 0 and variance σ^2), while significant effects are away from the line and their mean differs from zero. The graph can be seen in fig. 1, from which the following main effects and interactions were selected: c, a, ad, abd, bcd.

The analysis of the optimal level for each variable and their interactions for producing the maximum output (total solid contents from the hydrolysate) allowed us to select the best level for each variable.

effect a) high level (a +) effect b) low level (b-)
effect c) high level (c +) effect d) high level (d +)

The suitable reduced polynomial model for our results is:

$$Y(x_1, x_3) = \mu + A_1 \cdot X_1 + A_3 \cdot X_3 + A_{14} \cdot X_1 \cdot X_4 + A_{234} \cdot X_2 \cdot X_3 \cdot X_4 + A_{124} \cdot X_1 \cdot X_2 \cdot X_4 + e$$

Y = % total solid contents from hydrolysate (% on dried shavings basis)

μ = mean value. This mean value is considered as the one that would have been obtained if the variables

involved had been considered with their mean values; that is, as if they had no influence.

X_1, X_2, \dots = main variables ($X_1=a, X_2=b, X_3=c, X_4=d$)
 $X_1 \cdot X_2 \dots$ = interaction between or among variables.
 A_1, A_3, A_{14} = coefficients
 e = random error (uncontrollable or unknown effects), following an approximately normal distribution $[N(0, \sigma^2)]$.

The values used for the factor's low and high levels were (-1) and (+1), respectively. Details concerning design experiments may be found under references^{14,15}.

The reduced estimated model, considering the values of the effects shown in fig. 1 (coefficients are calculated by dividing effect values into 2), is as follows:

$$Y(X_1, X_3) = 88.91 + 2.45 X_1 + 4.2 X_3 + 1.55 X_1 \cdot X_4 - 1.24 X_1 \cdot X_2 \cdot X_4 - 1.21 X_2 \cdot X_3$$

The expression to be used for replacing a given X value in the reduced polynomial model, to be selected between the two levels for each factor, is the following:

$$X \sim \left(\frac{\text{low level} + \text{high level}}{2} \right) \cdot 2$$

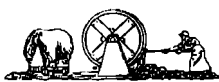
Using the above-mentioned variable selection and following the procedure described under 3.3, a pilot plant trial was performed by treating 1 kg of dried shavings. Details on this trial are described in the next section.

4.7 Characteristics and mass balance of the products obtained in the pilot plant trail

The pilot plant trial led to the following balance of reaction products: protein hydrolysate and chrome cake. Some useful parameters of both materials were analyzed and the results of such analysis are shown below. In fig. 2 a scheme of the procedure employed is represented.

Analyses of chrome shavings		
Parameter	% on dried substance (ds)	amount in 1 kg dried shavings
Total Kjeldahl Nitrogen (TKN)	15.9	
Protein (TKN x 5.51) (ref 12)	87.6	876.1 g
Chrome (as Cr)	1.8	19.0 g
Fat	0.8	
Ash	6.7	

The chrome cake was not washed during filtration. The nitrogen contents reflect the remaining hydrolyzed protein and depend on the efficiency of the filtration process (vacuum filtration was used).



1 kg moisture free shavings, treated by procedure 3.1 applying the selected factors shown in section g			
total dried residue from hydrolysates*		total dried residue of chrome cake*	
796.1 g		325.9 g	
TKN	15.2%	TKN	8.1%
Protein	666.7 g (83.8% ⁻)	Protein	145.5 g (16.6% of protein shavings)
Protein recovery	76.1%		
Cr	< 2 mg/kg	Cr	1.8% (Cr recovery = 95%)
Fat	0.04%	Fat	0.8%**
Sodium (as Na ⁺)	0.4%		
Chloride (as Cl ⁻)	0.8%		
Ash	11.5%***	Ash	38.0%

* The percentages below these headings are based on dried substance of each by-products.

** The fat contents reflect the fat in the untreated shavings.

*** This ash content reflects the alkaline treatment.

5. Conclusion

The alkaline agents considered in this study have shown a significant hydrolytic effect at moderate temperature (average total residues from protein hydrolysates from the 8 trials using no-enzymes = 86.5%) in comparison with treatment with alkaline-enzymic digestion (average total solids = 91.4%).

In the presence of proteolytic enzyme, the filtrability of the protein hydrolysates is improved and their chrome content is negligible.

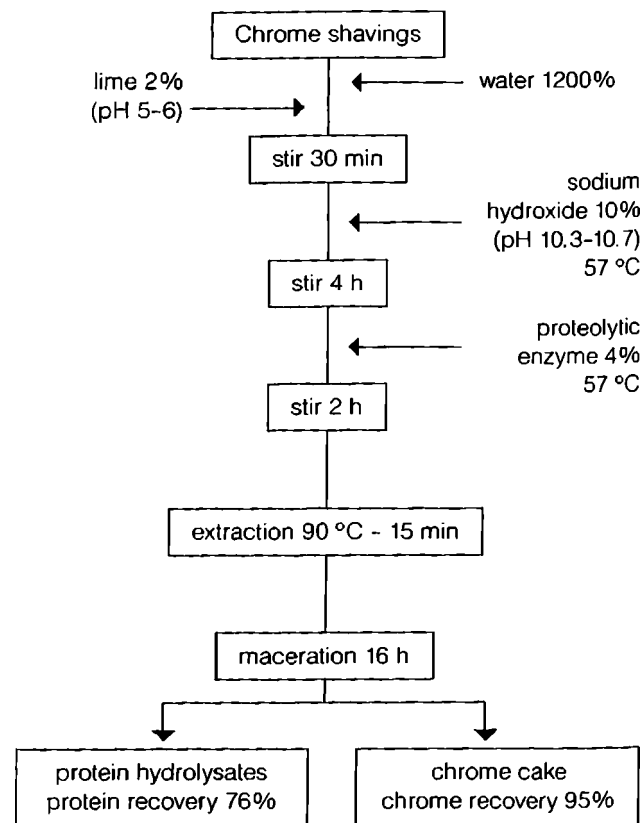
In the selected procedure, the so-called "extraction" and "maceration" factors, in combination with the alkaline-enzymic digestion, played a remarkable role in increasing the yield of protein hydrolysates.

A mathematical polynomial model could be developed through the analysis of a factorial complete design; this model can be used to evaluate the behaviour of variables within the end-level values selected.

Acknowledgement

The authors acknowledge the firm Strong Chemical S. A. by the collaboration to support this work.

Reference to brand of firm names does not constitute endorsement by The Leather Research Institute CITEC over others of a similar nature not mentioned.



* All percentages are based on dried shavings (moisture free weight)

Fig. 2 Scheme of the procedure employed in pilot plant trial based on the developed detanning process*

References

- reference 13 under⁷ of this report
- Bataille, P.; Faucher, R.; Hamel, P.; and Smith, W.E.: *Technicuir* **4** (1981), 75
- Alves, M.; Dos, Reis and Baleza, V.: *Jour. Soc. Leath. Chem.* **75** (1992), 15
- Guardini, G.: U.S. Pat. 4.483.829 (1983)
- Aronian, E.; and Urbain, J.: *Le Cuir-Proyect de Fin d'Etude Ecole Polytechnique de Montreal*, 1977
- Cot, J.; Manich, A.M.; Aramon, C.; Baucell, M.; Roura, R.; and Lacourt, G.: *Jour. Am. Leath. Chem. Ass.* **86** (1991), 141
- Taylor, M.M.; Diefendorf, E.J.; and Marmer, W.N.: *Jour. Am. Leath. Chem. Ass.* **86** (1991), 199
- Heidemann, E.; Hein, A.; Moldehn, R.; und Knölker, Ch.: *Das Leder* **42** (1991), 133
- Heidemann, E.: *Journ. Am. Leath. Chem. Ass.* **86** (1991), 331
- Veis, A.: *The Macromolecular Chemistry of Gelatin*. Academic Press - New York and London 1964.
- Heidemann, E.: private communication
- Ward, A.G.; and Courto, A.: *The Science and Technology of Gelatin*. Academic Press - New York N.Y. 1977
- Daniel, C.: *Application of Statistics to Industrial Experimentation - Wiley* 1976
- Box; Hunter and Hunter: *Statistics for Experimenters*. Wiley & sons 1978
- Mongomery: *Diseño de Análisis de Experimentos - Grupo Editorial Iberoamérica - 1991*