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B. De Castro[a]; M. A. Ferreira[a]; R. T. Markus[b]; A. Wyler[b]
[a] Departamento de Química Faculdade de Ciěncias do Porto, Porto, Portugal [b] Jerusalem College of Technology, Jerusalem, Israel

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INFLUENCE OF PROCESSING TEMPERATURE AND PRESSURE ON THE STABILITY OF THE STRUCTURE OF HOT-PRESSED GROUND LEATHER: *PLEATHER*

B. DE CASTRO* and M. A. FERREIRA

Departamento de Química
Faculdade de Ciências do Porto
4150 Porto, Portugal

R. T. MARKUS and A. WYLER

Jerusalem College of Technology
P.O.B. 16031, Jerusalem 91160, Israel

ABSTRACT

The increased degree of transformation of leather to a material with thermoplastic properties is shown to increase this material's solubility in both dimethylsulfoxide and in a buffer similar to those used to dissolve high molecular weight proteins. This increase in solubility is controlled by the variable values of the processing parameters, temperature, and pressure, and is interpreted to suggest that this transformation of leather must be accompanied by the destruction of its collagen superstructure into fragments with a much smaller molecular weight. This model is further supported by molecular weight distributions as determined by size exclusion chromatography and by electrophoresis. The results of the investigation at the molecular level are corroborated by scanning electron microscopy of *pleather* samples.

INTRODUCTION

A novel method was recently developed to transform collagenous material to a new material that exhibits a thermoplastic behavior—*pleather*, a hot-pressed leather–fiber composite [1]. Thermoplastic leather is a new material, the properties of which can be modulated by adequate control of processing temperature and pressure, and of processing time [2–4]. Thermoplastic leather products may be made from hide and leather scrap, which are significantly less expensive than production-quality leather and are price competitive with common plastics. Thus the process allows for the valorization of solid waste from the tanning industry, providing an incentive to alleviate environmental problems associated with the disposal of such wastes.

In previous publications we presented a model for the internal structure of *pleather* [2] and discussed its structure as a function of the processing parameters temperature, pressure, and time [3]. In the course of these studies we observed that the aforementioned transformation takes place without amino acid degradation. This implies that the transformation must be associated with a breakdown of the collagen superstructure of leather.

Leather has an extended network of collagen fibers that make it insoluble in almost all solvents [5]. However, as its transformation to *pleather* must be accompanied by the breakdown of the collagen superstructure, it is to be expected that the new material must be soluble in certain solvents, namely those that are able to solubilize proteins of high molecular weight.

This paper concerns the determination (a) of the solubility of several samples of *pleather*, (b) of the molecular weight distribution of the soluble fragments that are the result of the transformation to *pleather*, (c) of correlations that can be established between these quantities and the applied processing variables, and (d) of the insight these data provide on the molecular structure of *pleather*. Furthermore, as *pleather* can be partially or completely solubilized in certain solvents, the determination of molecular weight distribution of the soluble components of *pleather*, both by size exclusion chromatography and by electrophoresis, provides further proof of the destruction of the original collagen superstructure that must be associated with the transformation to *pleather*. It also helps to characterize the extent of fragmentation associated with this transformation, and that for fully transformed *pleather* this results in a set of fragments with an almost continuous distribution of molecular weight in the range of 20 to 300 kDa. The conclusions drawn from both characterization methods and from the proposed model are further corroborated by scanning electron microscopy data gathered from samples of unprocessed leather and of *pleather* produced from this leather.

MATERIALS AND METHODS

Leather Processing

Hot-pressed leather (*pleather*) was obtained from leather comminuted in a Wiley mill and subsequently heated in a hot press, using a process described in Ref. 1. The raw materials for these experiments were: (A) mixed tanned bovine leather (vegetable tanned with a subsequent light chrome tanning); (B) normal chrome

tanned leather; and (C) glutaraldehyde tanned sheepskin (upper split; ca. 2.5% of glutaraldehyde based on weight of drained pickled skin). Moisture contents have been measured by weight loss of samples that were processed or dried at temperatures higher than 100°C, and again before solubility measurements. The equilibrium moisture contents of the materials were approximately 14–16%.

Reagents

Urea, tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), sodium dodecyl sulfate (SDS), *threo*-1,4-dimercapto-2,3-butanediol, β-mercaptoethanol, dimethylsulfoxide [$(CH_3)_2SO$], potassium dihydrogen phosphate, and disodium hydrogen phosphate were from Merck (grade pro analysi) and used without additional purification. Aqueous solutions were prepared with deionized/double distilled water (resistivity greater than 4×10^6 ohm·cm). A Sigma molecular weight calibration kit MW-GF-200 (12–200 kDa; Blue Dextran) was used.

Sample Preparation

Samples of natural and processed leather were ground in a blender or in a mortar, washed with ethanol, and dried overnight at 60°C. The resulting powder (20 mg) was taken up in 2.0 cm^3 of the appropriate solvent [either $(CH_3)_2SO$ or a buffer of urea 4 M, Tris-HCl 20 mM, SDS 2%, pH 7.2; future reference to this buffer will be made as SB]. This mixture was subsequently placed in an ultrasonic bath for 18 hours. After centrifugation (at 10,000 rpm for 15 minutes) the supernatant was removed, collected, and applied as such to the chromatographic column (see below).

Solubility Measurements

The solids remaining after centrifugation were suspended in deionized water (1.5 cm^3), the suspension was centrifuged for 5 minutes, and the supernatant was removed and discarded. This process was repeated with ethanol, and the remaining solid was then dried by lyophilization and weighted. The solubility was calculated as the ratio of weight loss (initial weight less weight of collected solid) to initial weight. The results for two independent determinations are reported on a percent basis.

Size Exclusion Chromatography

A Pharmacia Ultropac TSK G 3000 SW (7.5 × 300 mm) column with a fractionating range of 1–300 kDa and protected with a guard-column Ultropac TSK SWP (7.5 × 75 mm) was used in a Philips PU 4100 HPLC that used a PU 4110 UV/Vis detector equipped with a 8-μL flow cell and with a Rheodyne 7125 injection valve equipped with a 20-μL loop). Elution was performed with a solution of urea 4 M, Na_2HPO_4 0.013 M, KH_2PO_4 0.011 M, pH 7.2. Calibration was performed with the protein molecular weight calibration kit MW-GF-200; Blue Dextran was used as a standard in the determination of retention time for the collagen fragments with the highest molecular weight.

Electrophoresis

Samples of *pleather* were separated in a Sepharose 4B (90 × 1.6 cm) column with a fractionation range of 6×10^4 to 2×10^7 Da, using the elution buffer described above and with a flow rate of 11 cm^3/h. The absorption of standards (MW-GF-200 kit) and samples were read at 280 nm in a UV detector Gilson 112. Sample fractions of 15 minutes were collected in a LKB FRAC 100. Collected fractions were used in SDS-Page electrophoresis after being extensively dialyzed against deionized water and lyophilized. Electrophoretic separation of the chromatographed collagen fragments was performed by an adaptation of the Laemmli method [6, 7] in a system of gradient gel of 7.5–15% acrylamide. The gels were stained using a modification of the silver stain method of Henkeshaven and Dernick [8].

Scanning Electron Microscopy (SEM)

A Jeol JSM-35C was used for SEM observation of *pleather* samples coated by sputtering with a 200-Å thick gold layer.

RESULTS AND DISCUSSION

Solubility Tests

The amino acid composition of mixed tanned leather samples before and after processing is very similar (Table 1), thus indicating that no significant amino acid degradation took place during processing.

Extensive solubility tests were performed with alcohols, ketones, and with both saturated and unsaturated hydrocarbons. Both ground and processed leather were almost insoluble in all these organic solvents. Ground leather is slightly soluble in dimethylsulfoxide, but the solubility of processed leather, *pleather*, is significantly higher, although dependent on processing variables (temperature, pressure, and time). A buffer, usually used for solubilization of proteins of high molecular weight, was successfully tried, and the solubility in this solvent is slightly higher than in $(CH_3)_2SO$. A reasonable linear correlation between solubility in $(CH_3)_2SO$ and in sample buffer is observed $\{S[(CH_3)_2SO] = 1.032 \times S[SB] - 5.62;\ R = 0.98\}$.

Solubility Dependence on Processing Conditions

The samples were grouped in four different sets, named A through D, according to the original tanning process. In all sets the index 0 refers to ground unprocessed leather. The solubility of the different samples and the processing conditions used are reported in Table 2. In Fig. 1 the solubility of *pleather* is represented as a function of processing temperature for several samples. Each set of points represents almost identical conditions for the other operational variables: pressure and time.

TABLE 1. Amino Acid Composition of Leather and Some *Pleather* Samples[a]

Amino acids	Total amino acid concentration, g/100 g		
	Leather	*Pleather* I	*Pleather* II
Aspartic acid	1.9	2.9	2.7
Threonine	0.9	0.9	0.8
Serine	1.5	1.6	1.5
Glutamic acid	4.7	4.9	4.5
Proline	4.9	5.0	4.8
Hydroxyproline	4.7	4.9	4.7
Glycine	10.6	11.1	10.7
Alanine	4.2	4.3	4.2
Valine	1.3	1.5	1.2
Methionine	0.3	0.4	0.3
Isoleucine	0.7	0.7	0.7
Leucine	1.5	1.4	1.3
Tyrosine	0.3	0.3	0.3
Phenylalanine	0.9	1.0	1.0
Histidine	0.4	0.4	0.3
Lysine	1.7	1.6	1.5
Hydroxylysine	0.6	0.7	0.7
Arginine	3.3	3.5	3.5
Ammonia	0.4	0.5	0.5

[a]The starting material for all samples was mixed tanned bovine ground leather; *pleather* I was processed at 140°C and 300 atm; *pleather* II was processed at 160°C and 760 atm.

Classification of Samples

Series A. In this series (samples A1–A12), mixed tanned bovine leather with a water content of about 12% was used as raw material for *pleather*. It can be clearly seen that by raising the processing temperature, the solubility of *pleather* increases (Fig. 1). The processing temperature appears to be the most important processing variable in controlling solubility. Samples A1–A5 (curve α_1) were obtained from untreated leather that was processed for ca. 8 minutes at 600 atm, whereas samples A9–A10 (50 atm) and A11 (100 atm) were processed at lower pressures (curve α_2). The effect of the processing pressure on solubility is of less importance than that of processing temperature, as can be concluded by analyzing the solubility of samples A9–A10. Nevertheless, there is a small increase in solubility with raised processing pressure (Fig. 1).

An additional remark must be made on the effect of prolonged treatment at high pressure and temperature. A sample of mixed tanned leather heated at 160°C for 30 minutes at pressures of 760 atm (A12) results in a material that is completely soluble in both $(CH_3)_2SO$ and in the buffer used.

TABLE 2. Solubility As a Function of Processing Temperature and Pressure for Different Samples of Ground Leather and Their Hot-Pressed Products (*pleather*)[a]

Sample	*P*, atm	*T*, °C	*t*, min	S1[b]	S2[c]	Sample	*P*, atm	*T*, °C	*t*, min	S1[b]	S2[c]
A0	–	–	–	46	54	B5	760	170	6	41	20
A1	600	76	5	49	48	B6	760	180	6	81	81
A2	600	85	7	55	54						
A3	600	105	8	59	57	C0	–	–	–	43	39
A4	600	120	6	69	64	C1	200	160	6	88	84
A5	600	150	8	91	81	C2	750	160	6	97	92
A6[d]	600	120	6	52	57						
A7[d]	600	155	10	55	53	D0	–	–	–	20	18
A8[d]	600	162	8	87	83	D1	50	110	6	23	17
A9	50	110	9	61	58	D2	50	120	6	21	17
A10	50	155	9	80	79	D3	50	130	6	23	17
A11	100	105	8	62	61	D4	50	155	6	22	19
A12	760	160	30	100	99	D5	600	110	6	23	19
						D6	600	120	6	22	17
B0	–	–	–	19	14	D7	600	130	6	23	17
B1	760	130	6	23	18	D8	600	160	6	24	18
B2	760	140	6	19	14	D9	600	170	6	48	44
B3	760	150	6	23	13	D10	600	155	12	23	18
B4	760	160	6	29	16	D11	3000	160	6	24	16

[a]Starting material for the different series: A, mixed tanned bovine leather; B, chrome tanned bovine leather; C, same as B, but after chromium removal; D, glutaraldehyde tanned sheepskin. The index 0 indicates unprocessed leather.

[b]S1 represents sample solubility in *sample buffer* (see text for details of its composition).

[c]S2 represents sample solubility in *DMSO*.

[d]These samples were predried previous to being converted into *pleather*.

Series B. The raw material used in this series (samples B1–B6) was chromium-tanned bovine leather that was processed at 760 atm for 6 minutes. The initial solubility of the ground leather is lower than that observed for mixed tanned leather. This must be attributed to a higher degree of crosslinking in chrome-tanned leather [9]; here, too, an increased solubility with processing temperature is observed (Fig. 1; curve β).

Series C. In this series (samples C1–C3) the same batch of chromium-tanned bovine leather as that used in series B was investigated, but chromium was removed prior to processing by means of organic chelating agents (oxalic, tartaric, or citric acid). Their solubilities (Table 2) are greater than those of *pleather* samples obtained from leather with no chromium removal and processed at the same conditions. The results obtained at different pressures also lend support to the assertion that the processing pressure is not a determining factor in the solubility of processed leather.

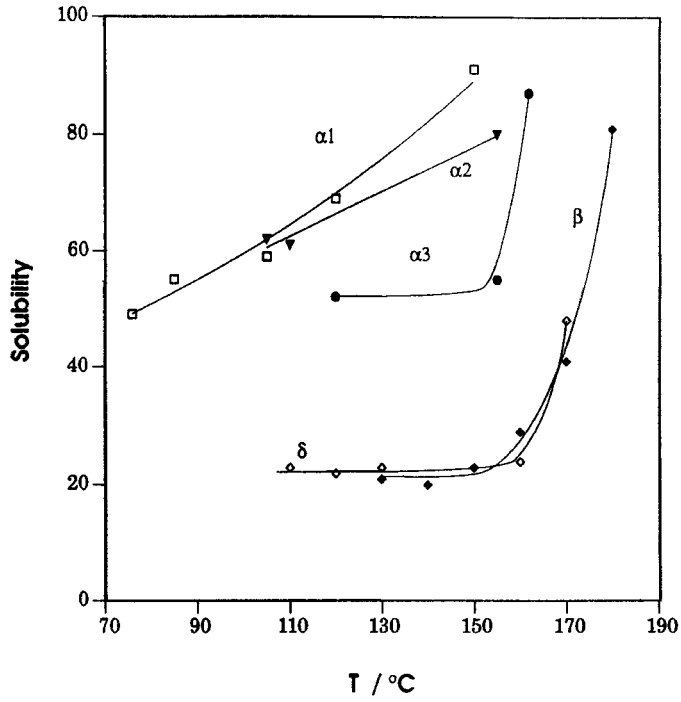

FIG. 1. Dependence of the solubility of the samples studied on the processing temperature. Curve set α refers to mixed tanned bovine leather: α_1 processed at 600 atm; α_2 processed at 50 and 100 atm; α_3 processed at 600 atm after predrying. Curve β is for chrome tanned bovine leather and curve δ for glutaraldehyde tanned sheepskin.

Series D. Pleather samples in this series (samples D1–D11) were obtained by processing sheepskin glutaraldehyde-tanned leather, with about the same water content as bovine leather, at 50 and 600 atm for 6 minutes (except for sample D10, 12 minutes) and at several processing temperatures. The most striking result is the low initial solubility of ground sheepskin and the sharp increase at $\approx 165°C$, and the apparent insensitivity of these samples of *pleather* on the processing conditions up to that temperature (Fig. 1, curve δ). Sample D11 was processed at 160°C and 3000 atm for 6 minutes, and even under these harsh conditions, but with processing temperature below the critical temperature of transformation, its solubility is virtually identical to that of the original sheepskin.

Influence of Water Content of Raw Materials on Solubility of *Pleather*

For samples A6–A8 (curve α_3) in series A the raw material was dried prior to being processed. It was observed that predried samples have smaller solubilities than those of normal samples when made at processing temperatures below a "critical" processing temperature ($\approx 150°C$). However, the solubilities of samples processed at temperatures above this "critical" processing temperature shows a marked increase. The existence of such a "critical" processing temperature ($\approx 160°C$) can also be inferred for series B.

Molecular Weight Distribution in *Pleather* as Determined by Size Exclusion Chromatography

Molecular weight estimation of collagen fragments that are soluble in the buffer used, SB, was made using the curve calibration method [10, 11]. Here, ln (molecular weight) is plotted against elution time (T_e). In Figs. 2 and 3 are shown HPLC chromatograms of several samples solubilized in SB. A remark must be made on the molecular weight range of the column. Since it has no separation capacity above 300 kDa, the band with a retention time of less than 14 minutes will include all soluble fragments with molecular weights larger than ca. 300 kDa. We note that the chromatograms were obtained under identical operational conditions and that their area must be proportional to the total concentration of soluble mate-

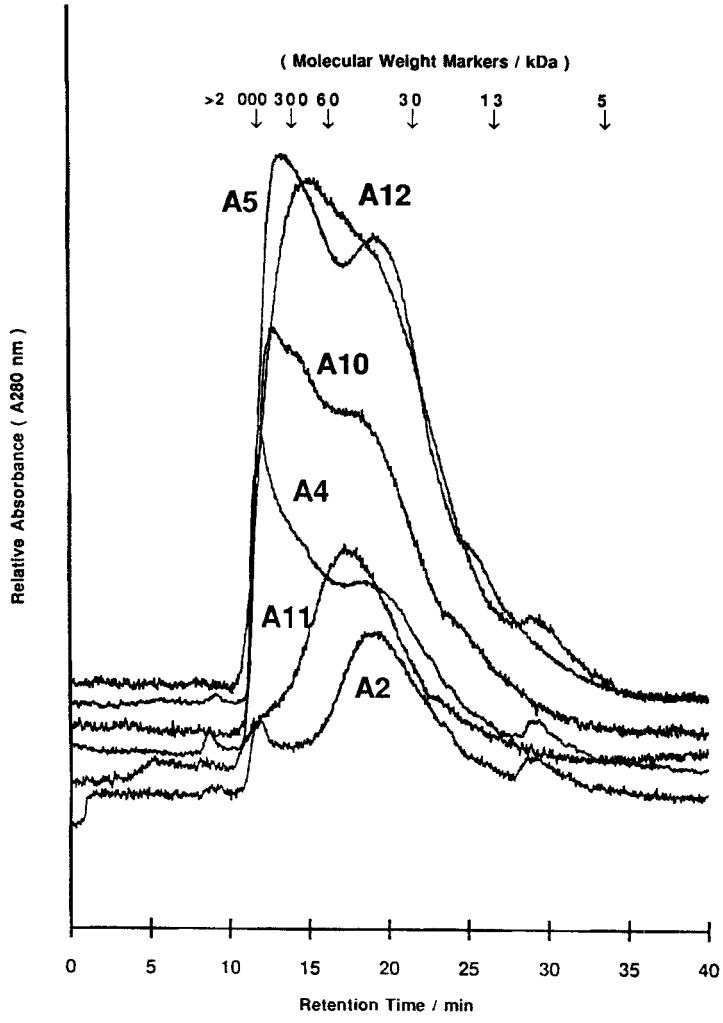

FIG. 2. HPLC chromatograms of samples of series A solubilized in SB (see text for details).

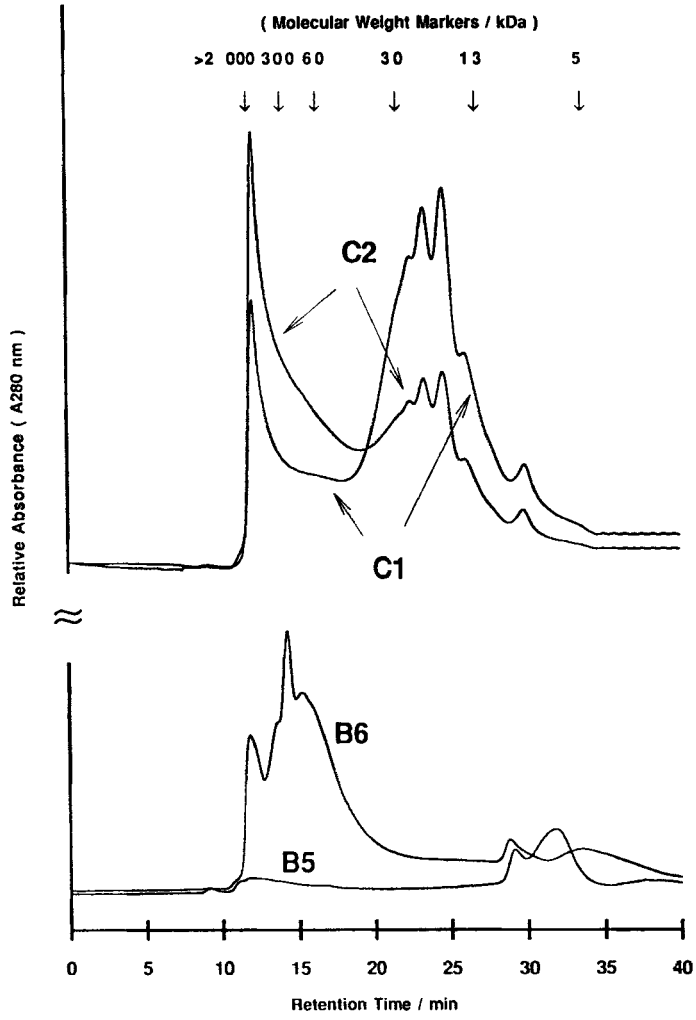

FIG. 3. HPLC chromatograms of samples of series B and C solubilized in SB (see text for details).

rial in the processed leather samples. In fact, a plot of the solubility of *pleather* samples vs the chromatogram total area yields a straight line with a final R value of 0.94, thus supporting the assumption that the solubility is in fact proportional to the amount of low molecular weight fragments in the samples.

Series A. The solubility of unprocessed leather results mainly from fragments with low molecular weight, in the 30–60 kDa range, although a small contribution from fragments of very low molecular weight ($\approx$ 10 kDa) and of high molecular weight ($\approx$300 KDa) is also noticeable. Samples A2, A4, A5, A10, A11, and A12 show that the increased solubility with the processing temperature is reflected mainly in an increase in the area of the bands at $\approx$2000–300 kDa and at 60 and 20 kDa.

Finally, the chromatogram of a sample that was processed for a long time and under very harsh conditions (A12) reveals that all fragments have a molecular weight of less than 2000 kDa and that a continuous distribution between this value and 20 kDa is observed. These results prove conclusively that the transformation from leather into *pleather* does indeed break up the collagen fibers into much shorter fragments, and that the extent of transformation can be judged by the average molecular weight of the resulting fragments. Also, the fragments in the range 20–60 kDa must be responsible for the almost continuous material, as seen in SEM observations [3, 4].

Series B. Here the solubility of ground leather is much smaller than that of mixed tanned bovine leather, thus suggesting that chrome-tanned leather fragments have a higher molecular weight due to the more effective crosslinking process of this tanning agent. Upon processing, there is similarly a decrease of the molecular weight of the collagen fragments. The resulting fragments have molecular weight in the range 50–500 kDa, although a small fraction at higher values is also observed. However, the result is that fragmentation of the collagen superstructure takes place to a much lesser extent than with mixed tanned leather.

Series C. As described above, this series comprise samples of chrome-tanned bovine leather from which chromium was removed with hot ($\approx 60°C$) aqueous solutions of chelating agents. This treatment is reflected in the solubility and in the molecular weight distribution of the samples. Partial hydrolysis is to be expected from this treatment and, not only must the resulting leather samples have a larger amount of low molecular weight fragments, but also some of the crosslinking induced by chromium binding to the carboxylic groups of collagen must be lost due to partial metal extraction. From observation of Fig. 2 it is clear that two main bands can be discerned: one with high molecular weight fragments (60–2000 kDa) and other in the 10–50 kDa range. Again, the area of the chromatograms is larger than that observed for series B. This is in accordance with the partial hydrolysis/chromium removal, induced by the treatment without chelating solutions.

Series D. The very low solubility of both untreated and processed sheepskin glutaraldehyde tanned leather precluded attainment of solutions to be used in size exclusion chromatography.

Electrophoresis of Selected Samples of *Pleather*

SDS-PAGE electrophoresis of solutions of samples A5, A12, C1, and C2 in SB was performed using a system of gradient gel of 7.5–15% bis-acrylamide. The resulting electrophoresis plates do not show sharp bands as observed with macromolecules of well-defined molecular weight, but instead a continuous spread between 25 kDa and ca 60 kDa was observed, indicative of an almost "continuous" variation of molecular weight collagen fragments in this range.

Molecular Weight Distribution and Scanning Electron Microscopy Observation of *Pleather*

Chromatography and electrophoresis data support our hypothesis of a breakdown of the collagen superstructure in leather when heated at high pressure. An analysis of the molecular weight distribution of the resulting fragments shows that

harsher processing conditions (higher temperature and pressure) produce a more extensive degradation of collagen. Not only is the leather fiber superstructure destroyed, but the collagen fibers are also broken down into smaller fragments.

It is interesting to relate the events that take place at the molecular level to those observed by scanning electron microscopy: sample A0 shows that the normal fibrous structure of unprocessed leather with fiber bundles of 5 μm diameter is composed of primitive fibers of 0.5–1 μm (Fig. 4). On the other extreme, an SEM picture taken at a magnification of 10,000 of sample A12 (Fig. 5) shows the existence of continuous material only.

For *pleather* produced at intermediate processing conditions, the solubility tests show that the solubility is larger than with leather but smaller than for fully transformed material. Also, the molecular weight distribution of the solubilized components in this material shows a significant amount of high molecular weight fragments. SEM pictures of these samples show a structure composed of a continuous material in which a fibrous material is embedded (Fig. 6).

CONCLUDING REMARKS

Solubility and molecular weight distribution data in conjunction with amino acid analysis show that the transformation of *leather* → *pleather* is accompanied by a destruction of the collagen network and that no amino acid pyrolysis takes place. This view is confirmed by SEM microstructure determination that reveals the destruction of leather fibers and the formation of a continuous material [2–4].

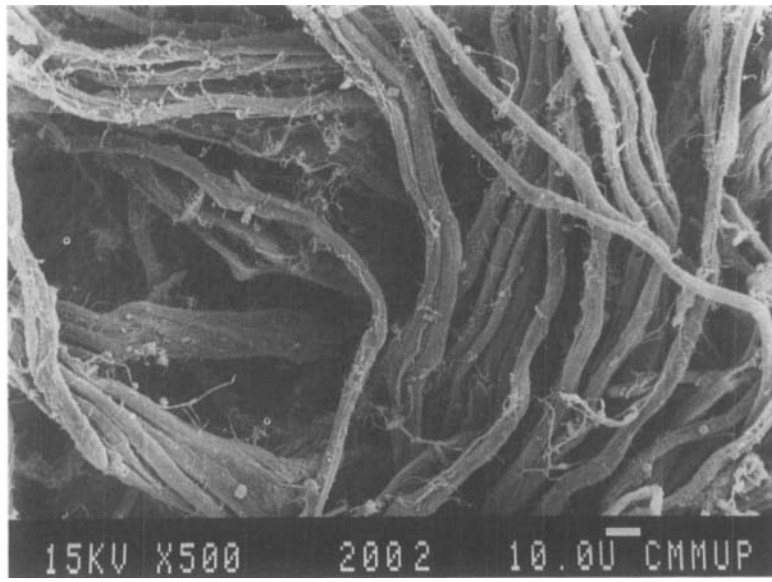

FIG. 4. SEM micrograph of unprocessed, mixed tanned bovine hide: fibers (diameter: 5–10 μm), primitive fibers (diameter: 0.1–0.5 μm), and fibrils (diameter: 0.1 μm); magnification = 500×.

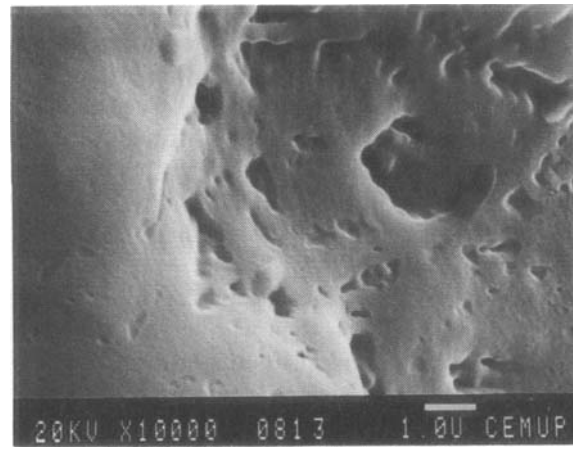

FIG. 5. SEM micrograph for the final stage of transition from leather fibers to continuous material, *pleather*, processed at $P = 760$ atm and $T = 160$ °C. The original fibrous character disappeared.

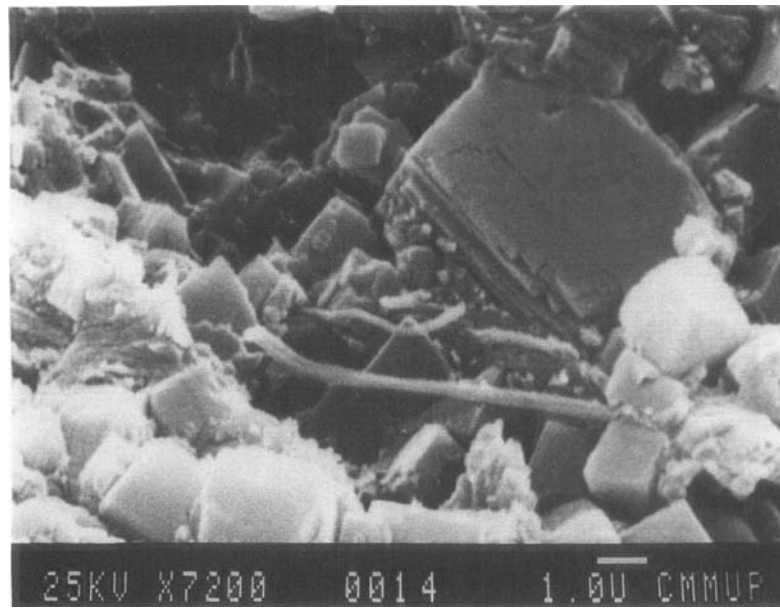

FIG. 6. SEM micrograph showing successive phases in the transformation from leather to the continuous material, *pleather*; fibers, flaky material, cubes, and continuous material.

The existence of a critical processing temperature for extensive transformation of the properties of leather was incurred from the results of both the analysis of the solubility and of the molecular weight distribution dependence on processing conditions. There is a good correlation of solubility with the transformations induced in leather which are due to destruction of the collagen superstructure. This critical temperature is highly dependent on the origin of the leather and on the tanning process. The influence of the processing pressure is much less marked, and above 200 atm has almost no influence on the solubility.

Furthermore, predrying bovine leather raises the temperature of transformation, implying the transformation *leather* → *pleather* takes place at higher temperatures in the absence of water, thus suggesting that water must play an important role in the mechanism for collagen cleavage. This is a plausible result, as the transformation must occur mainly through hydrolytic collagen cleavage, and high temperatures promote hydrolysis.

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