Study of the extraction kinetic of glycosaminoglycans from raw sheepskin trimmings

by

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ABSTRACT

A process for extracting glycosa Study of the Extraction Kinetic of Glycosaminoglycans from Raw Sheepskin Trimmings lycans from raw sheepskin trimmings acquired from tanneries was studied with the aim of evaluating its management. The study consists of two parts: a complete characterization of the residues and a procedure that involved bringing the trimmings into contact with an extraction liquid at pH 4 and shaking them at 150 rpm. The analysis was focused on the non-stationary phase of the process where measurements were taken, with respect to time, of the amount of glycosaminoglycans extracted, the amount of globular protein solubilized, the amount of collagen in the extract, the volume of liquid retained by the skin and the variation in the conductivity of the medium. The data were analysed by means of mathematical models that describe the behaviour of each of the variables. Finally, a mechanism of glycosaminoglycan extraction under the working conditions is proposed.

INTRODUCTION

Glycosaminoglycans (GAGs) are anionic polysaccharides composed of repeated units of disaccharides formed by a hexosamine (glucosamine or galactosamine) and an uronic acid (glucuronic acid or iduronic acid)[1]. Their structure contains both carboxylic and sulphated groups, which gives the molecule a high density of negative charge. Due to the repulsion of charges in the same chain, GAGs adopt extended structures[2] and, with the exception of hyaluronic acid, which exists as a free polymer, the others are bound covalently to a protein nucleus, thus forming proteoglycans[3].

GAGs form part of the extracellular matrix in connective tissue[4] where, due to their physico-chemical properties, they serve to retain water and the micro-ions needed for hydration and biomineralization processes in the tissues. In addition, they are able to regulate the distribution of macromolecules through steric exclusion and by acting as filters for electrically charged molecules[3]. As a result of these characteristics they have numerous applications in medicine, pharmacy, plastic surgery and cosmetic [1,5,6,7,8].

Two glycosaminoglycans are present in skin: hyaluronic acid and dermatan sulphate[9]. The amount of these polysaccharides is small and varies depending on the animal. In sheepskin, the two GAGs account for less than 0.2% of the dry weight of the skin.

In the tanning process between 17 and 30% of the animal skin is eliminated at the start of the process[10], through trimming those parts which may subsequently be a hindrance or that are not appropriate for the final product. A key feature of these trimmings is that the skin components have not been affected by chemical processes or extracted by means of physical procedures.

In a previous study[11] we studied the extraction of glycosaminoglycans from these residues with the aim of valorise the management of the process. It was shown that glycosaminoglycans can be partially extracted through a process of shaking without affecting the collagen structure of the skin. Although experimental designs have enabled the optimum working conditions to be determined, information is still lacking with regard to the extraction process over time.

It is known[11] that an acid pH and high conductivity favour the separation of skin glycosaminoglycans and that the greater the shaking the greater the yield, except when, for mechanical reasons, the bath volume is reduced. In contrast, however, research has yet to determine the point at which the greatest possible amount of GAGs can be extracted, the speed with which the equilibrium conductivity of the medium is reached, the amount of globular proteins dissolved in the medium, or the amount of liquid retained by the skin. The present research uses the optimum conditions for extracting GAGs from raw trimmings in order to study the behaviour of the process in its transitory phase, the aim being to clarify the questions posed and determine the maximum yield that can be expected from this process.

EXPERIMENTAL

Materials

The trimmings were taken from the skin of adult Spanish sheep (INPELSA, Valencia, Spain), that had been preserved by means of a salting process. The residues were cleaned, sheared by hand, cut into pieces with a surface area of approximately 1 cm^2 and then frozen at –30ºC until use.

For the determination of glycosaminoglycans we used bovine serum albumin, fraction V (SIGMA A-7906) and hyaluronic acid, sodium salt from human umbilical cord (SIGMA H-1876) being used as the standard.

The hydroxyproline analysis used L-4 hydroxyproline (FLUKA 56250) as a standard.

The purification of glycosaminoglycans used commercial-grade ethanol and ultra pure sodium chloride (PANREAC 141659).

Characterisation of the sheepskin trimmings

The first step involved characterisation of the sheepskin residues. This was done by weighing out five 15 g samples of trimmings. These were washed with enough water to remove all traces of dirt, blood and insoluble solids. The samples were then dried and weighed again to determine the percentage of foreign material.

The clean residues were sheared by hand and weighed once more. Once the percentage of wool had been determined the samples were cut into pieces with a surface area of 1 cm^2 and separated into five equal parts. One part was used for determining humidity and ash by gravimetry, the second for collagen determination using the hydroxyproline method, the third for determination of total nitrogen by means of the Kjeldahl method, the fourth for fat content using the Soxlet technique, and the fifth for the amount of glycosaminoglycans.

In order to determine total glycosaminoglycans the part set aside for this purpose was previously subjected to complete alkaline hydrolysis[12]. The skin was placed in a 0.5 M solution of sodium hydroxide at 80ºC for 2.5 h. Hydrogen peroxide was used as the coadjuvant agent. The hydrolysed product was then allowed to cool before being centrifuged at 1500 rpm for 30 min. One aliquot of the supernatant was mixed with ethanol in a ratio of 3:1 (ethanol:sample) and was left to rest for 16 h at 4ºC. At the end of this period the sample was again centrifuged at 1500 rpm for 30 min. The solid residue was placed in approximately 20 mL of 0.2 M sodium chloride solution and shaken at 500 rpm for 30 min. The sample was then centrifuged at 1500 rpm for 1 h and the supernatant was filtered and made up to 25 mL with 0.2 M sodium chloride solution. The total amount of glycosaminoglycans was analysed in this final solution.

Study of the extraction kinetics of glycosaminoglycans

The extraction kinetics of the glycosaminoglycans were studied by weighing out seventeen 20 g samples of clean, sheared, and defrosted pieces of skin. Each sample was subjected to the working conditions identified as providing the optimum extraction yield of GAGs[11]: pH was fixed at 4 using 0.05 M potassium biphthalate buffer; the bath volume was set at 500% with respect to the skin weight and was maintained constant by closing the system; working conductivity was 75 mS/cm, determined by the amount of salt present in the skin trimmings. The conductivity of each sample was regulated with 1 M sodium chloride solution. The working temperature was 25ºC.

All the samples were shaken at 150 rpm, care being taken to cover them so as to avoid any variations in the volume due to mechanical effects. After a certain time, set previously for each sample (Table II), the skin was separated from the extract by filtration through a 200 μm mesh. The final volume of the liquid obtained was then measured, and the extract was centrifuged at 1500 rpm for 30 min. Next, ethanol in a ratio of 3:1 (ethanol:sample) was added to one aliquot of the supernatant before continuing with the procedure described in the previous section for determination of total glycosaminoglycans in sheepskin residues.

Another aliquot of supernatant was used for hydroxyproline analysis and a third for the Kjeldahl nitrogen analysis.

Analytic Methods

Glycosaminoglycans were measured by using an adaptation[11] of the turbidimetric method employed by Seastone[13], Kass & Seastone[14], Tolksdorf[15] and Dorfman & Ott[16] to study hyaluronidase activity using bovine serum albumin (BSA).

Hydroxyproline analysis was carried out using the method developed by the Nordic Committee on Food Analysis[17]. To calculate the amount of collagen, 12.5% of hydroxyproline was considered[18].

Total nitrogen was determined by means of the Kjeldahl method using a Tecator Kjeltec Auto Sampler System 1035 Analyzer. Non-collagen protein was estimated by multiplying the difference between total nitrogen and collagen nitrogen by a factor of 6.25.

Fat content was determined using the Soxlet technique[19], and humidity and ash content by gravimetry.

RESULTS & DISCUSSION

Characterisation of the sheepskin trimmings

The results obtained from the characterisation of the sheepskin residues are shown in Table I. The values are expressed as a percentage in weight and represent the mean value of the

five measurements. Each column shows the percentages with respect to skin weight for the characteristics indicated by the column heading.

It can be seen from Table I that around 23% of the residue weight corresponds to foreign material (dirt, blood, manure, parasites and various insoluble material), and almost 20% is wool. These two components of the residues have to be eliminated prior to the extraction of glycosaminoglycans, and thus only 57% in weight of the trimmings can be used to this end.

Table I
Characterisation of sheepskin residues

	Percentage			
	Dirty skin	Clean skin with wool	Clean, sheared skin	Dry skin
Dirty skin	**100.00**			
Foreign material	22.75			
Clean skin	**77.25**	**100.00**		
Wool	19.72	25.52		
Sheared skin	**57.54**	**74.48**	**100.00**	
Water	30.36	39.30	52.77	
Dry skin	**27.17**	**35.17**	**47.23**	**100.00**
Non-collagen protein	9.47	12.27	16.47	34.87
Collagen	7.92	10.24	13.75	29.13
Fats	0.33	0.43	0.57	1.21
GAGs	0.04	0.06	0.07	0.16
Ash	9.41	12.18	16.36	34.64

It should be noted that the percentage of ash is very high. This is due to the salting process used to preserve the skin. Although the washing procedure used to separate the foreign material also eliminates the salt that is adhered to the surface of the residues, the salt which has passed into the skin during the preservation process remains in the structure. Several authors[1,6,7,8,11,20] have worked with saline solutions in order to favour the extraction of glycosaminoglycans, and thus the high percentage of salt in the residues proves useful for the process.

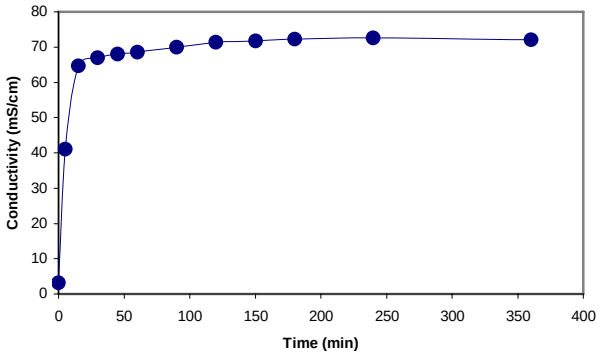

Figure 1. Variation in bath conductivity over time.

We studied the variation in the conductivity of a bath at pH = 4 (0.05 M potassium biphthalate buffer) upon adding clean, sheared pieces of skin trimmings in a ratio of 5:1 (bath:skin). The mixture was shaken at 150 rpm and in a closed system, these conditions being similar to those used in the tests of the kinetic study. The results are shown in Figure 1.

It can be seen that during the first 15 min the conductivity of the bath increases rapidly, rising above 60 mS/cm. Over the next 6 h the conductivity then reaches 72 mS/cm. This means that during the initial period of contact the conductivity of the bath reaches 80-85% of its final value, which corresponds to the concentration of salt equilibrium between the skin and the bath. According to the straight line *Conductivity vs. Concentration of NaCl* shown in Figure 2, this equilibrium concentration will be equivalent to around 0.9 M of sodium chloride. The trend of these data is only linear within the range shown.

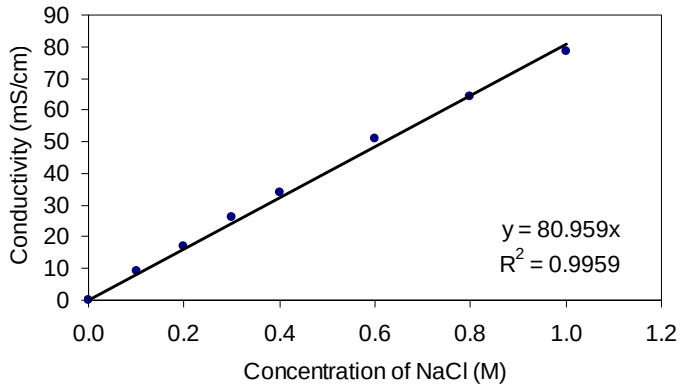

Figure 2. Conductivity of sodium chloride solutions.

Although high conductivity favours the extraction process, the subsequent separation processes are affected by the ionic strength of the medium, and therefore it is important to control this factor. To this end we studied the decrease in conductivity when subjecting the skin to several washes. Clean and sheared pieces of skin trimmings were mixed with deionised water in a ratio of 1:5 (skin:water). The liquid was replaced with a new bath every 15 min and the conductivity was measured. The results obtained are shown in Figure 3.

The conductivity of the first bath coincides with that of the first 15 min in Figure 1. This is the equilibrium value reached by the system upon initial contact. The use of subsequent baths with fresh water will reduce the conductivity of the medium to a level equivalent to a sodium chloride concentration $\leq$0.2 M, which is favourable for both extraction and the subsequent separation procedures.

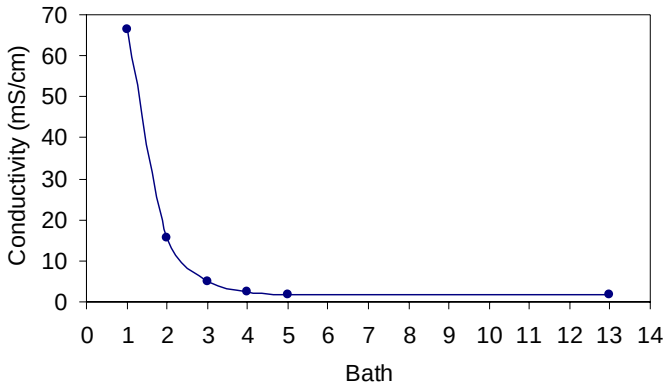

Figure 3. Conductivity of successive baths every 15 min.

Another interesting observation from Table I is the low amount of collagen present in the skin residues. Indeed, the large amount of ash and the presence of wool in the residues (including the sheared ones) cause a reduction in the collagen percentage. The percentage value of collagen protein accounts for around a half of the total protein, the remainder being composed mainly of keratin from hair and epidermis, in addition to globular proteins.

Finally, the percentage of glycosaminoglycans in the residues is 0.16% with respect to the dry weight of skin. This is the maximum reference value for the extraction procedure. The results of the kinetic study refer to dry skin weight rather than the gross weight of residues.

Study of the extraction kinetics of glycosaminoglycans

The results obtained from the tests conducted for the kinetic study of glycosaminoglycan extraction from sheepskin trimmings are shown in Table II.

Both the glycosaminoglycans and the proteins present in the extraction liquid show exponential behaviour, and asymptotically approach a maximum. It can be seen that there is practically no collagen in the bath, which means that the process does not affect the structure of the skin; only soluble proteins and polysaccharides are extracted.

The volume of the extract reduced by around 20% during the first few hours of extraction. As the experiments were conducted in a closed system this decrease must be due to part of the liquid being retained in the skin structure. As with the glycosaminoglycans and nitrogen, the retained extract tends toward a maximum per unit of skin weight, the approximate retention relationship being 1:1 (skin:extract).

Table II
Results of the kinetic study

Sample	t (h)	Volume of extract (mL)	mg GAGs / g dry skin	mg protein / g dry skin	mg collagen / g dry skin
	0	100.0			
1	4	84.0	0.324	32.15	< 0.01
2	5	82.0	0.279	35.43	< 0.01
3	6	81.0	0.366	36.96	< 0.01
4	7	80.5	0.373	42.28	< 0.01
5	8	80.0	0.404	44.86	< 0.01
6	9	80.0	0.386	47.08	< 0.01
7	10	80.0	0.670	52.49	< 0.01
8	11	80.0	0.650	53.95	< 0.01
9	13	80.0	0.595	54.77	< 0.01
10	15	80.0	0.708	47.14	< 0.01
11	17	80.0	0.856	52.03	< 0.01
12	20	80.0	0.886	65.19	< 0.01
13	24	80.0	0.904	6429	< 0.01
14	26	80.0	0.977		
15	30	80.0	1.034	69.37	< 0.01
16	40	80.0	1.203	68.22	< 0.01
17	50	80.0	1.209	70.92	< 0.01

In order to explain the behaviour of the glycosaminoglycans over time, the following mechanism is proposed. Consider the system depicted in Figure 4:

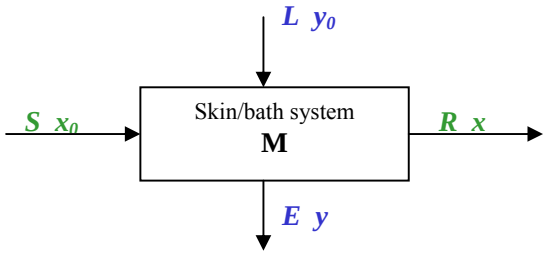

Figure 4. Skin/bath system.

Where S is the initial amount of skin, L the amount of extraction liquid that enters the system, E the amount of extract obtained after a given extraction time, and R the amount of residue obtained after extraction. The value of E will be equal to the difference between L and the liquid retained (L_R). Likewise, the value of R will be equivalent to the total amount of skin introduced into the system (S) plus the amount of liquid retained by the skin (L_R).

In terms of the fractions, x_0 is the total mass of GAGs, in mg, contained in 1 g of skin, y_0 is the initial amount of GAGs present in the initial extraction liquid, which, in this particular case, is equal to zero, y is the amount of GAGs present in the liquid after a given extraction time, and x is the residual amount of GAGs in the skin after extraction. Both the extract and the retained liquid have a concentration y, and the fraction x refers to the initial mass of skin, without considering the retention.

A general balance in the system leads to equation 1, where M is the total mass of the system.

$$M = S + L = E + R = E + S + L_R \qquad \textbf{[1]}$$

In order to work with fractions in weight, equation 1 is divided between the total mass of the system, thus giving rise to equation 2.

$$S_M + L_M = S_M + (E_M + L_{RM}) = 1 \qquad \textbf{[2]}$$

Equation 3 shows the amount of glycosaminoglycans present in the system at any point in time.

$$S_M x_0 = S_M x + L_{RM} y + E_M y \qquad \textbf{[3]}$$

The variation over time of the concentration of glycosaminoglycans in the extraction liquid depends on the speed of mass transfer between skin and liquid, and therefore it can be defined as in equation 4, where *k* is the coefficient of mass transfer, *A* is the surface area of contact between the collagen structure and the liquid, and *y'* is the mass of glycosaminoglycans with respect to the mass of liquid at equilibrium.

$$\frac{dy}{dt} = kA(y' - y) \qquad \textbf{[4]}$$

As the amount of glycosaminoglycans present in the skin is very small it can be assumed that the equilibrium value between the two phases is independent of the GAG concentration. Furthermore, given that the glycosaminoglycan molecules are located on the surface of the collagen structure of the skin, with which water can come into contact (different to the surface of the skin trimmings), it can also be assumed that in the solid/liquid interphase the equilibrium fraction (*y'*) is equal to the fraction of GAGs in the solid (*x*). Thus, there will be two resistances to the transfer of GAGs between skin and liquid:

- The mechanism by which GAGs pass from the interfibrillary surface of the skin to the extraction liquid.
- The mechanism by which GAGs are transported from the interphase to the centre of the liquid. Clearly, the greater the surface area of the skin the less resistance will be presented by the second mechanism.

In light of these considerations the value of *x* in equation 3 can be substituted by variable *y'* in equation 4. Through substitution and reordering we thus arrive at equation 5.

$$\frac{dy}{dt} = \frac{kA}{S_M}(S_M x_0 - y) \qquad \textbf{[5]}$$

Integrating equation 5 between the initial moment and a given point in time gives rise to equation 6.

$$y = S_M x_0 \left(1 - e^{-\frac{kA}{S_M} t} \right) \qquad \textbf{[6]}$$

For the data analysis equation 6 was linearised. Figure 5 shows the linear fit of the data, in accordance with equation 7.

$$\ln\left(1 - \frac{y}{S_M x_0} \right) = -\frac{kA}{S_M} t \qquad \textbf{[7]}$$

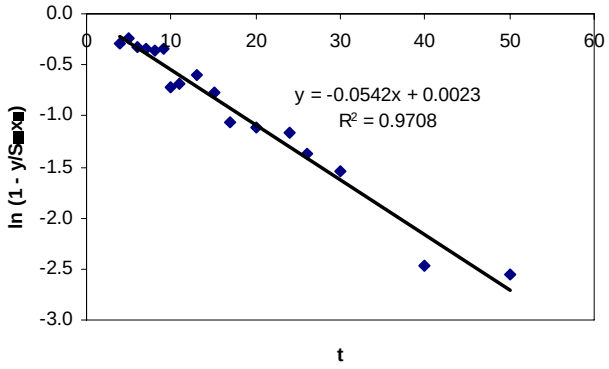

Figure 5. Fit of the linearised equation for glycosaminoglycans.

The fit of Figure 5 yields a value for *kA* of 0.009 [h^{-1} $fraction^{-1}$]. The value of the intersection with the ordinate axis is not significantly different from zero, and can therefore be considered as such. Figure 6 shows the experimental behaviour of the glycosaminoglycan fraction in the extraction liquid and the trend described by equation 6.

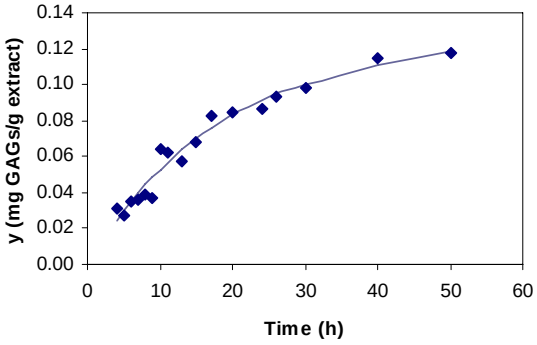

Figure 6. Fraction of GAGs in the extract.

The maximum extraction limit will be set by the value of $S_M x_0$, that is, when the fraction of GAGs in the liquid is equal to the fraction of GAGs in the solid. Figure 7 shows the values of x_0, x and y with respect to time.

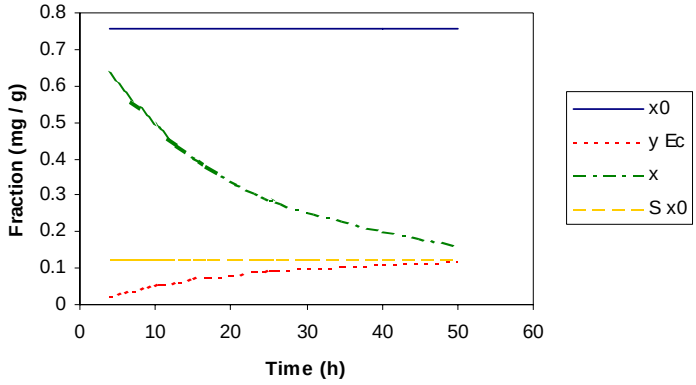

Figure 7. Fractions of GAGs in each phase.

As was pointed out initially the value of y is the fraction of glycosaminoglycans in the extraction liquid, which comprises both the extract and the retained liquid. The total mass of GAGs that may be obtained will be reduced by around 20% due to the amount of liquid that is retained in the skin. Figure 8 shows the mass of glycosaminoglycans in the skin, the extract and in the complete extraction liquid (extract plus retention). These values refer to the dry weight of skin.

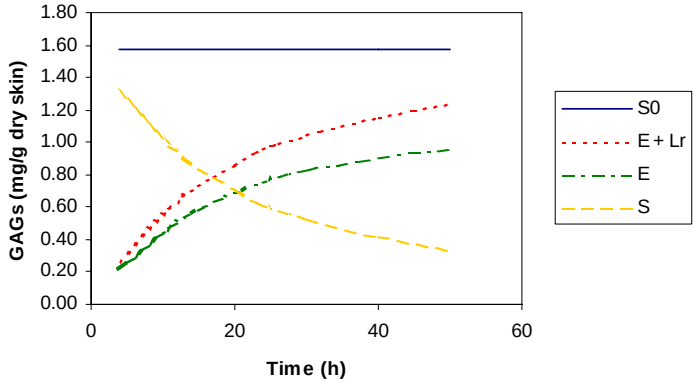

Figure 8. mg of GAGs with respect to dry skin weight in each phase.

On the basis of this analysis the amount of GAGs that can be obtained by means of shaking under the working conditions used is described by line *E* in Figure 7. The upper limit will be around 1.02 mg for each gram of dry skin. With respect to time, around 75% of the maximum possible amount will have been extracted at 24 h, this rising to almost 95% after 50 h. The increase of only 20% on the second working day must be evaluated in terms of the energy consumption it implies. The proposed kinetic model fits the obtained data well, and the value of kA will vary according to the system conditions (shaking, pH, conductivity, size of trimmings, type of skin, bath volume and temperature), increasing or reducing the extraction time.

In an analogous way, given that no collagen was detected in the extract, the extracted protein can be treated using a similar kinetic model to that proposed for the glycosaminoglycans. The maximum value in this case was determined by measuring the amount of total protein in the global extract after a three-stage crosscurrent extraction process. The mean value of three tests was 41 [mg/g fresh skin]. Figure 9 shows the behaviour of the soluble protein in the bath over time, where the value of *kA* is equal to 0.0176 [h^{-1} $fraction^{-1}$]. Equation 6 describes this behaviour mathematically.

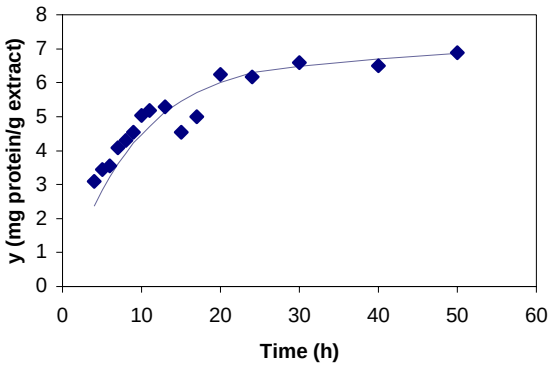

Figure 9. Fraction of soluble protein in the extract.

According to the literature[21] the amount of globular proteins present in fresh skin is around 4%. This figure is similar to the maximum value obtained in the global extract of the multistage process, and therefore it can be concluded that in the extraction process studied a large proportion of the globular proteins in the skin residues is solubilized in the medium, while the structural protein (mainly collagen and keratin) remains in the trimmings. It can thus be stated that the collagen structure of the skin is not altered during the process, and this lends support to the hypothesis proposed for the kinetic model, whereby the skin was considered as a porous solid. Indeed, under more aggressive extraction conditions, such as increased temperature or reduced pH, there will not only be a variation in the value of *kA* but also a partial hydrolysis of the collagen structure.

The variation in the extract volume due to retention in the skin can be studied in a similar way to the behaviour of the glycosaminoglycans or the total dissolved protein. With this objective we defined a retention factor (r) as the mass of liquid retained by one unit of skin mass. Mathematically:

$$r = \frac{L_R}{S} \qquad \textbf{[8]}$$

This retention will rise until reaching a maximum value. The variation of this factor over time can be described as in equation 9.

$$\frac{dr}{dt} = k_r (r_{\max} - r) \qquad \textbf{[9]}$$

The integration of equation 9 leads to a mathematical relationship similar to those used for GAGs and soluble protein (equation 10). Figure 10 shows the reduction in extraction liquid volume on the principal ordinate axis and the retention behaviour on the secondary ordinate axis, where r_{max} is equal to 1 and k_r is equal to 0.6165 [h^{-1}].

$$r = r_{\max}\left(1 - e^{-kt}\right) \qquad \textbf{[10]}$$

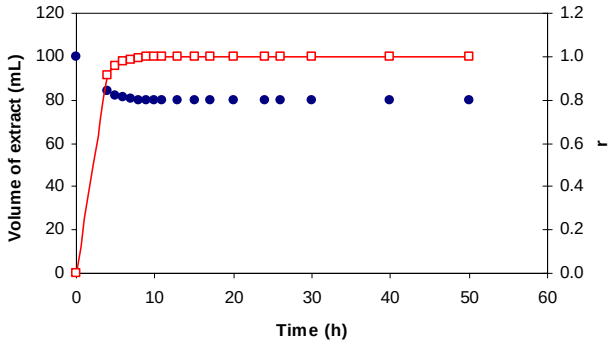

Figure 10. Volume of extract and retention factor with respect to time.

It can be seen that after 4 h of contact the skin has already retained over 90% of the maximum possible amount, and at 6 h retention reaches 97%. It can therefore be stated that in the extraction of glycosaminoglycans, the variation over time of liquid retention by the skin is of little importance as the maximum is reached within the first few hours of contact, during which time the GAGs begin to be diffused through the medium. It may be that for polysaccharide extraction to occur the extraction liquid has to penetrate completely the skin structure and, from that point on, the GAGs are diffused through the proposed mechanisms. Although the variation in volume due to retention has little effect on the extraction of GAGs it is clear that the maximum amount of retained liquid does produce a notable reduction in the final amount that can be obtained. This effect is described in Figure 8.

Finally, in order to describe the variation over time in the conductivity of the bath we proposed a kinetic model of the equivalent sodium chloride concentration, in accordance with Figure 2. Equation 11 describes the diffusion of equivalent sodium chloride from the skin/bath interphase to the centre of the liquid.

$$\frac{dC}{dt} = k_C A(C' - C) \qquad \textbf{[11]}$$

Where C is the equivalent sodium chloride concentration, C' is the equivalent concentration at equilibrium (skin/liquid interphase), k_C is the mass transfer coefficient and A the surface area of contact between the collagen structure and the liquid. Integrating equation 11 gives:

$$\frac{C'-C}{C'-C_0} = e^{-k_c At} \qquad \textbf{[12]}$$

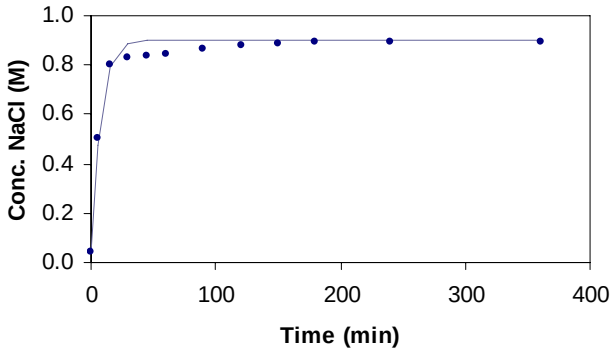

Figure 11. Equivalent sodium chloride concentration in the extraction bath.

Where C_0 is the initial equivalent sodium chloride concentration of the bath. This initial concentration is given by the 0.05 M potassium biphthalate buffer. The behaviour is shown in Figure 11. The equilibrium equivalent concentration is equal to 0.9 M of NaCl and the value of $k_C A$ is 0.142 [min^{-1}].

If we consider the linearity between the sodium chloride concentration and the bath conductivity in the range studied it can be concluded that, since the system reaches equilibrium so quickly, the variation in the conductivity of the bath over time is negligible and its effect on the extraction of glycosaminoglycans can be considered as constant.

Accepting as valid the proposed kinetic models, an analysis of the four constants obtained indicates that at the point at which the skin enters into contact with the liquid the salts contained within the collagen structure (which represent 16% with respect to fresh skin) are rapidly solubilized, and thus the system reaches a conductivity equilibrium during the first 15 min; from this point on the variation over time is negligible. At the same time, but less rapidly, the skin swells due to the pH of the bath and, consequently, the liquid comes into contact with all the collagen structure. This leads to a percentage of the bath being retained by the skin, and this increases over time until it reaches a maximum equal to the skin weight. This process takes around 6 h.

Once the skin is swollen and the liquid has reached the surface of the collagen structure the glycosaminoglycans and globular proteins begin, due to the effects of shaking and the conductivity of the medium, to diffuse to the centre of the liquid by means of the two proposed mechanisms: firstly, the passage from the interfibrillary surface to the liquid in the liquid/skin interphase, followed by the transport of the dissolved molecule from the skin to the centre of the liquid. According to the kinetic constants the globular proteins complete this process more rapidly than do the glycosaminoglycans.

A quantitative comparison of the kinetics indicates that, for example, 15.9 min are required for the conductivity of the medium to reach 90% of its maximum value, whereas the skin and bath must be in contact for 3.7 h in order for liquid retention in the skin to reach the

same percentage. Similarly, 42.5 h are required to extract 90% of the maximum possible amount of GAGs, while the same percentage of the maximum possible amount of globular proteins can be solubilized in 21.7 h; in other words, the extraction speed of glycosaminoglycans is approximately half that of globular proteins.

CONCLUSIONS

On the basis of the results obtained in the present study the following conclusions can be drawn:

- Only around 57% of the weight of sheepskin trimmings acquired from tanneries can be used for the extraction of glycosaminoglycans, the remainder being composed of foreign material and wool. These residues contain a high percentage of ash due to the salting process used to preserve them, this being useful in terms of increasing the conductivity of the bath and favouring the extraction of glycosaminoglycans. The diffusion speed of these salts in the bath is high and equilibrium is reached during the first 30 min of skin/liquid contact. If it is necessary to reduce the ionic strength of the medium in order to favour subsequent separation procedures, two 15-min washes are enough to achieve a suitable conductivity.

- The percentage of glycosaminoglycans contained in sheepskin residues is approximately 0.16% with respect to dry skin. According to the kinetic study carried out it is possible to extract a maximum amount of 1 mg of GAGs for each gram of dry skin. The remaining 0.6 mg are lost due to the equilibrium or retention of the extraction liquid by the skin.

- Of the 64% (with respect to dry weight) of protein contained in the trimmings, 29.1% corresponds to collagen and 8.5% to globular proteins. The remainder is composed of structural proteins, particularly keratin from the epidermis and wool that could not be separated by the shearing process. The extraction speed of globular proteins is approximately double that of glycosaminoglycans, and using the process studied up to 83% of the total amount contained in skin can be separated. The procedure for extracting glycosaminoglycans under the working conditions does not affect the collagen structure of the skin.

- The maximum amount of water retained by the sheepskin trimmings is equal to their weight. Part of the glycosaminoglycans and globular proteins remain trapped within this volume. The maximum retention is reached with the first 8 h of skin/liquid contact.

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