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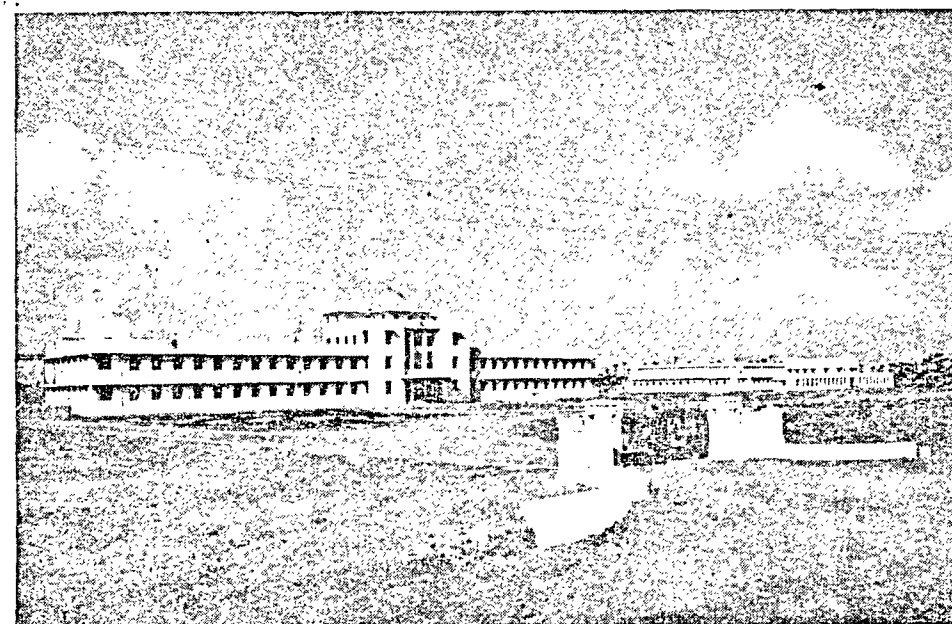
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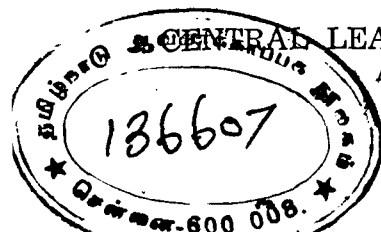
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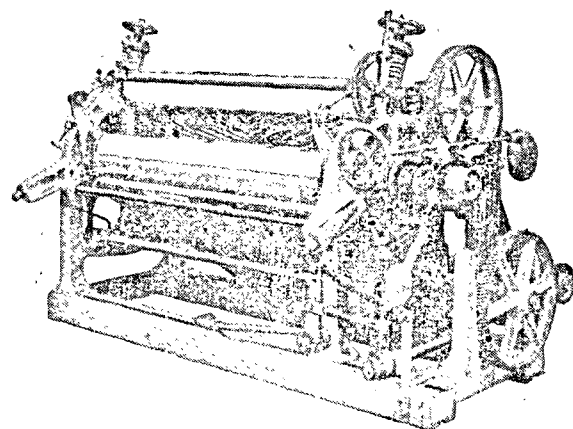
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STUDIES ON PROTEASES AND AMYLASES AS UNHAIRING AGENTS FOR SKINS AND HIDES WITH PARTICULAR REFERENCE TO REACTIONS OF SKIN MUCOIDS WITH AMYLASES

By

325

S. M. BOSE & W. MADHAVA KRISHNA

SUMMARY

(1) Samples of latex from different plants were examined for proteolytic activity and the amylase activity of several samples of germinated cereals was estimated.

(2) Making use of the latex of madar plants (*Calotropis gigantea*) and the water extract from germinated "ragi" (*Eleusine coracana*) which were found to be rich sources of protease and amylase respectively, two new processes of enzymic unhairing were developed.

(3) The seasonal variation of the proteolytic activity and certain constituents of madar latex was studied.

(4) The effect of certain antiseptics and preservatives on the activity of madar latex protease and "ragi" amylase was investigated.

(5) The optimum conditions of the hydrolysis of skin mucoids by α -amylase of "ragi" were investigated with particular reference to the effects of pH, temperature, enzyme and substrate concentrations, period of hydrolysis and addition of certain salts. The effect of pH on the hydrolysis of skin mucoids by total amylase and β -amylase of "ragi" was also studied and the results obtained were discussed.

(6) The storage property of the proteolytic activity of madar latex was investigated with reference to the effects of storage temperature, pH of the latex and the addition of certain reducing agents. The keeping quality of the samples of protease isolated from madar latex by different methods was also studied and the results obtained were discussed.

It is well known that proteolytic enzymes are used for unhairing and bating of skins and hides for the manufacture of leather. Proteolytic enzymes are the chief components of commercial bates which are widely used for modern leather manufacture. It has also been reported¹ that unhairing by

means of certain amylolytic enzymes is quite practicable. Proteolytic as well as amylolytic enzymes are known to be distributed widely in plant, animal and microbial kingdom. With a view to find out very potent sources of proteolytic and amylolytic enzymes that could be used as unhairing agents for leather manufacture, it was thought desirable to examine several sources of these enzymes.

In a previous communication,² it has been reported that the process of enzymic unhairing by means of proteolytic or amylolytic enzyme depends on the hydrolysis and removal of skin mucoids. It has also been shown² that the mucoids are hydrolysed by amylolytic enzymes with the liberation of reducing sugars. Cordon,³ while working with enzymic unhairing, has observed that sugar is produced by the action of certain amylase preparations on skins and hides. Skin mucoids exhibit both protein and carbohydrate functions. The total carbohydrate content of skin mucoids has been reported⁴ to be of the order of about 20 per cent. No free reducing sugars are, however, present, in them. The acid hydrolysis of mucoids has been reported⁵ to liberate equimolecular amounts of glucose and galactose. In the present paper, the results obtained on the reactions of skin mucoids with amylases under different conditions have been recorded.

Experimental

Examination of different sources for proteolytic and amylolytic activity: The samples of latex of certain plants like papaya (*Carica papaya*), fig (*Ficus carica*), madar (*calotropis gigantea*), banyan (*Ficus bengalensis*) and prickly pear (*Opuntia dillenii*) were collected fresh in the early hours of the morning and analysed for the proteolytic activity by the modified phenol reagent method.⁶ For this, a suitable volume of the enzyme solution was added to 12.5 ml. of 5 per cent egg-albumin (E. M.) solution of desired pH and the mixture was allowed to digest at 45°C for 30

minutes and the amount of tyrosine liberated during proteolysis was estimated.⁶ The results are presented in Table I.

TABLE I
Examination of different plant latex samples for proteolytic activity

Name of sample.	No. of samples.	pH of substrate.	Proteolytic activity (Mgms. tyrosine liberated per .5 ml. digestion mixture).	
			Average Value	(Range)
Papaya Latex:	3	5.5	1.35	(1.03 — 1.60)
Madar Latex:	7	4.0	1.64	(0.91 — 2.68)
Fig Latex:				
from stem	2	6.5	0.91	(0.76 — 1.05)
from fruit and leaves	2	6.5	1.23	(1.17 — 1.28)
Prickly Pear Latex:*				
Banyan Latex:*				

* No proteolytic activity.

The results indicate that all the samples examined, the madar latex possesses the highest proteolytic activity. It is evident that the activity of the latex varies from sample to sample. It also appears that the activity is somewhat dependent on certain environmental factors such as the season, age of plant etc. The presence of ascorbic acid, cysteine and glutathione in madar latex was reported by Basu and Nath⁷ who also observed the activating influence of these constituents on its proteolytic activity. It was therefore thought interesting to study the seasonal variation of the proteolytic activity and the contents of these constituents of madar latex. For this purpose, fresh latex was collected on a particular day in each month from a plant selected within the compound of the Institute and its proteolytic activity and other constituents were estimated. The ascorbic acid content was estimated by the modified titrimetric method of Bessey⁸ after treatment with metaphosphoric acid solution as

adopted by Hochberg *et al.*⁹ The free cysteine content was estimated by the colorimetric method of Mirsky and Anson¹⁰ making use of a Klett-Summerson photoelectric colorimeter with red filter No. 66. For this, the latex was triturated in a glass-mortar with acid-washed sand and 20 per cent trichloroacetic acid and centrifuged. The residue was repeatedly extracted in the same manner and the combined centrifugates were made up to a volume and a suitable aliquot was used for the estimation of cysteine. Another aliquot was used for the estimation of total—SH groups in the latex by the iodimetric titration method of Kuhnau.¹¹ Necessary corrections for the ascorbic acid content and for the—SH groups due to the free cysteine content of the latex were made in the iodine titre value. The remaining titre value was expressed in terms of glutathione. Thus the approximate concentration of glutathione in madar latex was estimated. The results obtained are presented in Table II.

TABLE II
Seasonal variation of proteolytic activity of madar latex

Month.	Proteolytic activity.
January	.. 2.57
February	.. 1.93
March	.. 1.10
April	.. 1.15
May	.. 1.40
June	.. 1.62
July	.. 1.40
August	.. 2.05
September	.. 2.48
October	.. 2.33
November	.. 2.53
December	.. 2.61

The results indicate that the proteolytic activity and the ascorbic acid, cysteine and glutathione contents of madar

latex vary from season to season. The ascorbic acid content of madar latex has been found to vary from 60 to 88 mgms. for 100 ml. latex, cysteine content from 80 to 100 mgms. and glutathione content from 30 to 58 mgms. No correlation, however, has been found between the contents of these constituents and the proteolytic activity of the latex. The activity appears to increase to some extent in winter and to decrease in summer.

It is well known that amylases develop on germination of certain cereals like "barley" (*Hordeum vulgare*), "ragi" (*Eleusine coracana*) and "jowar" (*Sorghum vulgare*). Hence the amylase activity of different samples of germinated cereals was estimated. For this, the grains were washed well in water and steeped in running water for about 24 hours. They were then spread well over a wet cloth covering wet sand and allowed to germinate for about three days. After germination, the grains were crushed into a fine paste and extracted with the requisite volume of water. The extract was analysed for the amylase activity. For this, 5 ml. of 2 per cent solution of soluble starch (B.D.H.) buffered at the desired pH were mixed with 2 ml. of enzyme extract and hydrolysed at 45°C for 30 minutes at the end of which the amount of reducing sugars produced was estimated by following the official method of A.O.A.C.¹² for determining the diastatic activity. A blank experiment was always run in an identical manner using boiled enzyme solution. The results were expressed as milligrams of maltose liberated from 7 ml. digestion mixture and are presented in Table III.

TABLE III
Estimation of amylase activity of germinated cereals

Enzyme extract.	No. of samples.	pH of substrate.	Amylase activity Mgms. of maltose per 7 ml. digestion mixture.
Average value. (Range).			
Germinated "barley":	2	5.0	55.57 (54.33 — 56.81)
Germinated "ragi":	3	4.6	46.57 (40.31 — 52.18)
Germinated "jowar":	2	4.5	39.99 (38.61 — 41.38)

The results indicate that the enzyme extract from germinated "barley" has a higher amylase activity than that from germinated "ragi" or "jowar". But, since the cost of barley in India is rather high and also as it is not abundantly available, further experiments were carried out with "ragi" amylase only.

Development of enzymic unhairing processes: Making use of the latex of madar plants, which was found to be very rich in proteases, a new process of enzymic unhairing was developed for unhairing skins and hides within 24 hours. Making use of the enzyme extract from germinated "ragi" which was found to be a rich source of amylases, another new process of enzymic unhairing was developed for unhairing skins and hides within three days. It was also shown that the new enzymic unhairing processes can be applied for the manufacture of different types of leather. As these two enzymic unhairing processes have been patented,^{13, 14} the technical details of the processes are not furnished in the present paper.

Effect of certain antiseptics and preservatives on the activity of madar latex protease and "ragi" amylase:

In the two enzymic unhairing processes as mentioned above, skins or hides were kept immersed in the enzyme solution for one to three days. To prevent any bacterial action during this period, the use of some antiseptic or preservative was attempted. In order to ascertain whether these agents will themselves have any adverse effect on the activity of the enzyme, the following experiment was performed.

5 ml. lots of enzyme solution were mixed with 1 ml. of the agent to be tested and the mixture was stored at room temperature (24°—31°C) for a period of 24 hours and the activity of the mixture was tested as mentioned before. As control, 5 ml. of enzyme solution mixed with 1 ml. of

distilled water were also stored similarly. The results obtained are presented in Table IV.

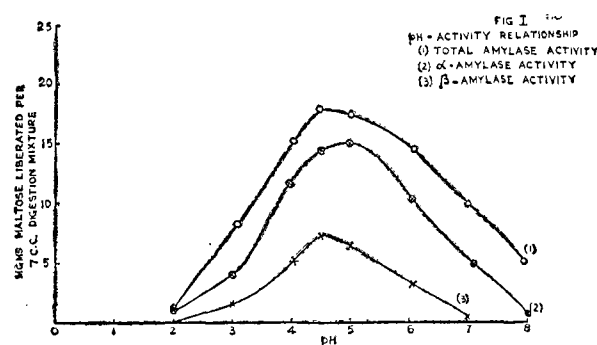
TABLE IV
Effect of antiseptics and preservatives on enzyme activity.

Reagent added.	Proteolytic activity of madar latex.	Amylolytic activity of "ragi" extract.
Water (control)	.. 2.01	42.19
Boric acid (2% solution)	.. 1.90	21.86
Thymol (2% solution)	.. 2.04	5.22
Iodine (0.01 N solution)	.. 0	0
Formalin (2% solution)	.. 0.84	16.25
Merthiolate (1:10,000)	.. 2.01	42.19
Toluene	.. 2.01	42.19
Chloroform	.. 0.32	42.00
Sodium benzoate (2% solution)	.. 1.13	36.31
P-nitro phenol (2% solution)	.. 0	0
Phenol (2% solution)	.. 0	0

Preparation of skin mucoids and "ragi" amylases: With a view to studying the reactions of skin mucoids with "ragi" amylases, the mucoids were isolated^{15, 2} from a goat-skin which was obtained fresh from the slaughter-house. From the water extract from germinated "ragi", the total amylases were isolated by precipitation with ten volumes of pure acetone per one volume of the extract. The solvent and the water extract were cooled to about -8°C and 2°C respectively and the extract was added drop by drop into the solvent under constant agitation. The precipitate was recovered by centrifuging and dried under vacuum over P₂O₅ and stored in the frigidaire.

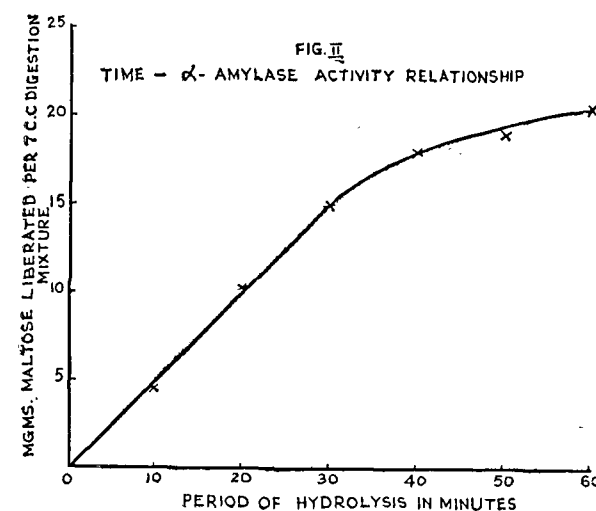
The α and β -amylases were separated by the procedure Ohlsson¹⁶ as modified by Acharya,¹⁷ and Chandrasekhara and Swaminathan.¹⁸ The β -amylase was inactivated by adjusting the pH of the enzyme solution to 6.5 and then keeping it at 70°C for 45 minutes. The α -amylase was inactivated by adjusting the pH of the enzyme solution to 3.6 and then keeping it at 0-4°C for 30 minutes. Thus the effects of α -amylase or β -amylase alone on the hydrolysis of mucoids were studied.

Effect of pH on the hydrolysis of mucoids by total amylases, α -amylase and β -amylase: 5 ml. portion of 5 per cent suspension of mucoids buffered at different pH with universal buffer were digested with 2 ml. of a 4 per cent enzyme solution at 45°C for 30 minutes at the end of which the amount of reducing sugars produced was estimated¹² as mentioned before. This set of experiments were repeated with α -amylase or β -amylase alone. The results obtained are presented in Figure I.

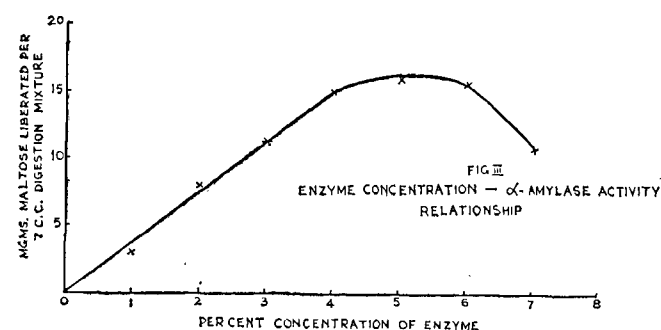


Effect of time on the hydrolysis of mucoids by α -amylase: 5 ml. aliquots of a 5 per cent suspension of mucoids buffered at pH 5.0 with universal buffer were digested with 2 ml. aliquots of a 4 per cent enzyme solution at 45°C and at regular intervals, the samples were analysed¹² for the

amount of reducing sugars produced. The results are presented in Figure II.

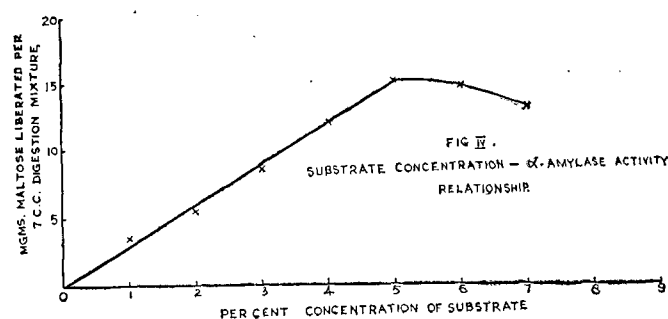


Effect of enzyme concentration on the hydrolysis of mucoids by α -amylase: 5 ml. portions of a 5 per cent suspension of mucoids buffered at pH 5.0 with universal buffer were digested with 2 ml. portions of enzyme solutions of different concentrations at 45°C for 30 minutes at the end of which the amount of reducing sugars produced was estimated.¹² The results are presented in Figure III.

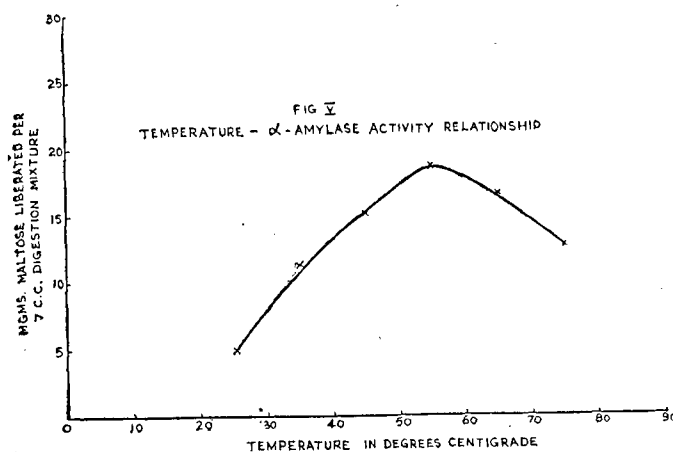


Effect of substrate concentration on the hydrolysis of mucoids by α -amylase: 5 ml. portions of mucoid suspension

of different concentrations, buffered at pH 5.0 with universal buffer were digested with 2 ml. portions of 4 per cent enzyme solution at 45°C for 30 minutes at the end of which the amount of reducing sugars was estimated.¹² The results are presented in Figure IV.



Effect of temperature on the hydrolysis of mucoids by α -amylase: 5 ml. portions of 5 per cent suspension of mucoids, buffered at pH 5.0 with universal buffer were digested with 2 ml. portions of 4 per cent enzyme solution



for 30 minutes at different temperatures and the amount of reducing sugars produced was estimated.¹² The results are presented in Figure V.

Effect of certain salts on the hydrolysis of mucoids by α -amylase: 2 ml. portions of 4 per cent enzyme solution were mixed with required amounts of certain salt solutions so that the concentration of the salt in the reaction mixture was 0.05 per cent. The enzyme-salt solutions were incubated at 37°C for one hour and then added to 5 ml. portions of 5 per cent suspension of mucoids buffered at pH 5.0. The mixtures were digested at 45°C for 30 minutes at the end of which the amount of reducing sugars produced was estimated.¹² The results are presented in Table V.

TABLE V
Effect of salts on the hydrolysis of mucoids by α -amylase

Salt added.	Mgms. maltose liberated per 7 ml. digestion mixture.
Control	.. 15.01
NaCl	.. 16.03
KCl	.. 15.59
NH ₄ Cl	.. 15.86
CaCl ₂	.. 15.40
MgCl ₂	.. 15.94
BaCl ₂	.. 15.68

Effect of storage on the proteolytic activity of madar latex: During the course of the investigation on enzymic unhairing by the use of madar latex, it was observed that the latex on storage gradually loses its proteolytic activity. In actual practice, however, fresh latex may not always be available for use in commercial tanneries. It was, therefore, thought desirable to investigate under various conditions the keeping quality of the proteolytic activity of madar latex. The latex was freshly collected, centrifuged and the centrifugate was equally distributed into a number of glass-stoppered bottles (100 ml.). The bottles were stored

in incubators of different temperatures, viz., $37^{\circ}\text{C} \pm 0.1$, $50^{\circ}\text{C} \pm 0.1$, $60^{\circ}\text{C} \pm 0.1$. One bottle was stored in the refrigerator (about 4°C) and another bottle in a cool incubator at $25^{\circ}\text{C} \pm 0.1$. For comparison, the original latex was also stored at $37^{\circ}\text{C} \pm 0.1$ under identical conditions. At regular intervals, required amounts of the samples were withdrawn and analysed⁶ for the proteolytic activity. The percent loss in proteolytic activity was calculated in each case. The results are presented in Table VI.

TABLE VI

Keeping quality of the proteolytic activity of madar latex when stored at different temperatures.

Period of storage days.	Percent loss in Proteolytic Activity					
	Original latex 37°C.	Latex centrifugate 37°C.	Latex centrifugate 4°C.	Latex centrifugate 25°C.	Latex centrifugate 50°C.	Latex centrifugate 60°C.
1	13.7	12.2	0	7.8	77.0	90.2
2	21.6	17.6	5.9	14.7	91.1	—
3	27.4	24.5	9.8	20.1	—	—
4	34.3	31.4	14.2	26.5	—	—
5	40.7	37.3	20.6	28.9	—	—
6	46.1	47.1	26.5	32.3	—	—
7	49.0	51.5	31.4	36.3	—	—
15	66.7	69.6	37.3	42.2	—	—
30	84.3	82.3	49.0	56.4	—	—
45	91.1	90.2	65.1	71.6	—	—
60	97.0	97.0	77.5	85.8	—	—
75	—	—	88.2	97.6	—	—
90	—	—	93.6	—	—	—

Effect of pH on the keeping quality of madar latex: The natural pH of the latex was usually found to be in the neighbourhood of 5.0. It was thought interesting to study whether the alteration of pH would enhance the keeping quality of the proteolytic activity of the latex. Equal portions of the fresh latex centrifugate were adjusted to different pH values and buffered with McIlvaine's phosphate-citric acid buffer or universal buffer. They were stored in glass-stoppered bottles at a temperature of $37^{\circ}\text{C} \pm 0.1$. At regular intervals, the samples were withdrawn, and analysed⁶ for the proteolytic activity. The percent loss in proteolytic activity was calculated in each case. The results are presented in Table VII.

TABLE VII

Keeping quality of the proteolytic activity of madar latex when stored at 37°C under different pH conditions.

Period of storage days.	Per cent loss in Proteolytic activity.			
	Latex centrifugate pH 3.0.	Latex centrifugate pH 5.05 (Natural pH).	Latex centrifugate pH 7.0.	Latex centrifugate pH 9.0.
3	60.8	27.5	11.8	48.7
7	85.1	47.7	27.1	65.4
15	100	66.4	47.2	86.6
30	—	85.6	58.1	—
45	—	89.6	70.4	—
60	—	—	80.7	—
75	—	—	86.6	—
90	—	—	93.0	—

Keeping quality of the protease isolated from madar latex by different methods: The protease can be precipitated or dehydrated from madar latex by different methods, viz:

Precipitation methods

Acetone precipitation: The latex centrifugate the pH of which was adjusted at 6.0 was cooled to 2°C and added drop by drop under constant stirring to 2.5 volumes of pure redistilled acetone which was cooled to about -8°C. The precipitated enzyme was quickly removed by centrifugation and dried over CaCl₂ under vacuum.

Alcohol precipitation: The procedure followed was essentially the same as in acetone precipitation except that 4 volumes of pure redistilled alcohol were used for precipitation of the enzyme.

Ammonium sulphate precipitation: To the latex centrifugate the pH of which was adjusted at 6.0 was added solid ammonium sulphate till the solution was fully saturated. The resulting precipitate was removed by centrifugation, dialysed and dried over CaCl₂ under vacuum.

Dehydration methods

Freezing and drying under vacuum: The latex centrifugate was allowed to freeze as a thin layer in a shallow basin surrounded by freezing mixture and then quickly dried under high vacuum in a vacuum desiccator which was also surrounded by freezing mixture.

Vacuum distillation: The latex centrifugate was concentrated to a small volume by distilling off the water under high vacuum at a temperature not exceeding 35°C. and was finally dried over CaCl₂ in a vacuum desiccator.

Hot air blowing: The latex centrifugate was spread in a thin layer in a dish and dried by blowing warm air (40°C ± 1°C.) in a hot air blowing drier.

The keeping quality of the above-mentioned samples of isolated enzymes and also a purified protease which was pre-

pared¹⁹ from madar latex was studied. The samples were stored at 37°C ± 0.1° in glass-stoppered bottles, under identical conditions. At regular intervals, necessary amounts of the samples were withdrawn, dissolved in water and analysed⁶ for the proteolytic activity. The percent loss in proteolytic activity was calculated in each case. The results are presented in Table VIII.

TABLE VIII

Keeping quality at 37°C of the protease precipitated or dehydrated from madar latex by different methods.

Period of storage days.	Per cent loss in Proteolytic Activity						
	Acetone* precipitated. (a)	Alcohol* precipitated. (b)	Ammonium* sulphate precipitated. (c)	Freezing & drying under vacuum.	Vacuum distillation.	Warm air drying.	Purified* enzyme (d)
7	0	53.2	7.4	5.9	4.7	15.5	43.1
15	6.4	81.4	27.4	19.1	21.6	33.1	80.6
30	15.4	100	52.1	31.9	45.8	69.7	100
60	31.9	—	83.2	59.3	80.5	100	—
90	51.6	—	—	80.4	—	—	—
120	63.8	—	—	—	—	—	—
150	84.6	—	—	—	—	—	—
180	91.5	—	—	—	—	—	—
210	—	—	—	—	—	—	—

* Specific Activity of (a)—1.16

* Specific Activity of (b)—0.31

* Specific Activity of (c)—1.08

* Specific Activity of (d)—118.18

Effect of Certain reducing agents on the keeping quality of the protease in madar latex: With a view to stabilising the proteolytic activity of madar latex, the effects of addition of certain reducing agents were compared. Four lots of 20 ml. each of latex centrifugate were kept in glass-stoppered bottles. To the second, third and fourth bottles, 20 mgms. of sodium hydrosulphite, metabisulphite and sulphide were added respectively, the first one being kept as control. All the four bottles were stored at $37^{\circ}\text{C} \pm 0.1$ and the proteolytic activity of each sample was determined⁶ at regular intervals. The per cent loss in activity was calculated. The results are presented in Table IX.

TABLE IX
Effect of reducing agents on the stabilization of proteolytic activity of madar latex.

Period of Storage days.	Percent loss in Proteolytic activity			
	Control.	Sodium hydrosulphite.	Sodium metabisulphite.	Sodium sulphide.
7	48.1	6.0	8.3	11.6
15	58.8	19.4	25.9	21.7
30	80.5	45.4	49.5	47.7
45	85.6	66.2	72.2	69.9
60	—	92.6	95.4	96.3
75	—	—	—	—

Discussion

As shown in Tables I and III, the madar latex and germinated "ragi" are rich sources of proteolytic and amylolytic enzymes respectively. It may be seen from Table IV that there is no inhibition of the protease or amylase activity when toluene or merthiolate is used as a preservative. The same trend of results were obtained in the practical

dehairing processes also. The use of antiseptics like phenol, p-nitrophenol, iodine has been found to completely inhibit the protease or amylase activity. Although, thymol does not inhibit the protease activity, it inhibits the amylase activity. On the other hand, chloroform does not inhibit the amylase activity, but inhibits the protease activity.

It may be seen from Fig. I that the optimum pH for the hydrolysis of skin mucoids by the total amylase of "ragi" is 4.5 whereas the pH optima for α -amylase and β -amylase are 5.0 and 4.5 respectively. Although both α and β -amylases are capable of hydrolysing mucoids with the production of reducing sugars, the α -amylase activity has been found to be comparatively higher than the β -amylase activity. The activity of total amylase has been found to be the highest as compared to α or β -amylase activity.

Fig. II shows that up to a period of 30 minutes, the digestion of mucoids by the α -amylase at 45°C bears a linear relationship. Figs. III and IV indicate that the optimum concentration of α -amylase required for hydrolysis of 5 ml. of 5 per cent suspension of mucoids is 2 ml. of 4 per cent enzyme solution. It may also be seen from Fig. V that the maximum hydrolysis of mucoids by α -amylase takes place at 55°C , when the period of digestion is 30 minutes. Table V shows that the addition of certain salts, e.g., NaCl, MgCl_2 to the α -amylase preparation increases the enzyme activity only very slightly. Sodium chloride, however, has been reported²⁰ to increase the activity of animal amylase appreciably.

It may be seen from Table VI that the keeping quality of the proteolytic activity of madar latex stored at 4°C is significantly higher than that of the other samples stored at higher temperatures. Temperatures of 50°C and above have been found to inactivate the enzyme at a very rapid rate. Table VII shows that the storage property of the madar

latex protease is higher at the neutral pH than at the natural pH of about 5.0 of the latex. Highly acidic or highly alkaline pH appear to inactivate the protease at a very rapid rate. These observations indicate the need for adjusting the pH of the madar latex at 7.0 as quickly as possible after the collection of the latex.

Table VIII shows that the keeping quality of the madar latex protease prepared by the acetone precipitation method is higher than that of the samples prepared by the other methods. It has been particularly noted that the protease prepared by the alcohol precipitation method loses its activity at a very fast rate. The purified enzyme¹⁹ which was 196-fold more active than the original latex has also been found to lose its activity at a very rapid rate when stored at 37°C.

It may be seen from Table IX that the addition of reducing agents like sodium hydrosulphite to fresh madar latex retards to some extent the loss of its proteolytic activity. For example, only 6 per cent loss of the activity occurs in the case of the sodium hydrosulphite treated sample whereas the untreated control sample has been found to lose 48 per cent of its activity when stored at 37°C for 7 days. Balls²¹ also observed that the keeping quality of the papaya latex protease when activated by the addition of sodium sulphide was greater than that of the untreated sample. In a previous communication,¹⁹ it has been shown that the activity of the madar latex protease depends more or less on the essential -SH group of the enzyme molecule. It, therefore, appears that the gradual auto-oxidation of the active -SH group in the protease on storage may be at least partly responsible for the loss of the proteolytic activity. Possibly, the role of sodium hydrosulphite being a strong reducing agent is to retard this auto-oxidation. Balls *et al*²² showed that in the case of papaya latex, the proteolytic activity was apparently destroyed through an oxidative process.

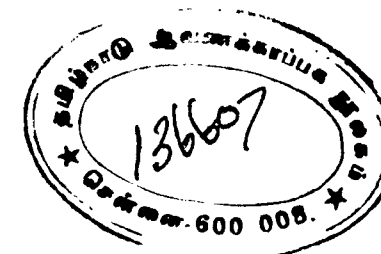
Acknowledgement

Our thanks are due to Dr. Y. Nayudamma for his kind permission to publish these results.

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SWELLING AND SHRINKAGE TEMPERATURE OF TANNED COLLAGEN*

B. N. GHOSH & S. GHOSH **

SUMMARY

From a consideration of the shrinkage phenomenon in the light of Flory's theory of the viscosity of high polymers, a relation has been obtained between the shrinkage temperature and isoelectric swelling of leathers having different degrees of crosslinking. Experimental data on the swelling and shrinkage temperature of formaldehyde and chrome tanned leathers of different degree of tanning have been shown to be in keeping with the above relation.

Ewald¹ observed in 1919 that collagen fibres when heated in contact with water contract sharply at a certain temperature, known as the shrinkage temperature. Since then the subject of the shrinkage temperature of hide and differently tanned leathers has been studied by a number of workers.² The phenomenon involves a disorganisation of the structure as has been shown from the change in the physical properties on shrinkage, for example, in the X-ray diffraction pattern.³ In the molecular model, this has been pictured as a breakdown of the stable parallel orientation of molecular chains in the fibres due to the destruction of the cohesive bonds between the fibres, and the subsequent coiling up to the molecules. The shrinkage temperature should, therefore, decrease with the decrease in cohesion between molecules and increase with the increase in cohesion, and is thus very naturally related to the swelling characteristics of the material, since swelling also depends on the cohesive forces of the material.⁴ Although this has been recognised

*Forms part of D.Phil., thesis, 1955 of S. G. submitted to the Calcutta University.

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qualitatively, no attempt has hitherto been made to correlate these two properties (e.g. shrinkage and swelling) quantitatively on a theoretical basis. In the present work a quantitative relation between the shrinkage and swelling has been arrived at.

It may be mentioned that a kind of order-disorder transformation takes place also in rubberlike substances and is known as the second order transition. A treatment of this phenomenon has been put forward by Boyer and Spencer⁵ on the basis of Flory's treatment of the viscosity of polymers,⁶ and the phenomenon of shrinkage may also be treated in a similar manner.

Thus it may be assumed that during shrinkage, the units of collagen chain responsible for shrinking, are freed from each other due to the increase in kinetic energy caused by the rise of temperature. The isolated chains then coil up. The chains should thus be able to move past one another, against the force of cohesion which prevented such a movement. The force of cohesion may, therefore, be supposed to put forth a kind of viscous force against the free movement of a chain. The chain can move only when the viscosity has been lowered, by the rise of temperature, to a value when the chains can assume their most probable configuration. For a series of compounds with increasing degree of cross-linking, therefore, because of the reinforcing effect of the cross-links, a higher temperature will be required to lower the internal viscosity to the point where shrinkage occurs. The problem, thus, reduces to the finding out of the conditions of isoviscous state for such systems.

Flory (*loc. cit.*) has shown that it is possible the viscosity of concentrated solutions of polyesters can be represented by the following formula over a range of temperature,

$$\log \eta = A + B (W_2)^{\frac{1}{2}} + E/RT$$

where A and B are constants, W_2 is the weight fraction of

the polymer in solution and E is the activation energy for viscous flow. If the internal viscosity is constant at the temperature of shrinking for a system containing various amounts of solvent (because of the varying degree of cross-linking), we get for the isoviscous state of such a system:

$$(W_2)^{\frac{1}{2}} = D - E/BT_s$$

where D is a constant. Thus there should be a linear relationship between the square root of the weight fraction of the protein and the reciprocal of the shrinkage temperature.

This has been tested by us by determining the shrinkage temperature of (1) formaldehyde tanned hide containing different amounts of combined formaldehyde and of (2) chrome tanned hide containing different amounts of combined chrome.

Experimental: The samples of chrome-tanned leather were prepared from fully tanned leather by soaking 5 gms. portions in N/10 solutions of boric, adipic, phosphoric, lactic, tartaric and oxalic acids. The acids (except boric) stripped off chromium from the leather, the amount stripped off increasing in the series from adipic to oxalic. The pieces were taken out after 7 days, washed thoroughly till free from electrolytes and their isoelectric swelling determined.

The formaldehyde tanned pieces were prepared by tanning 5 gm. portions of cow-hide from adjacent portions of the same piece in different concentrations of the formaldehyde in phosphate buffer at pH 7.8. The pieces were then washed thoroughly and their isoelectric swelling determined as in the previous case.

The shrinkage temperature of the samples were determined in the usual manner. The isoelectric swelling and shrinkage temperature of the different samples have been compared in the following tables.

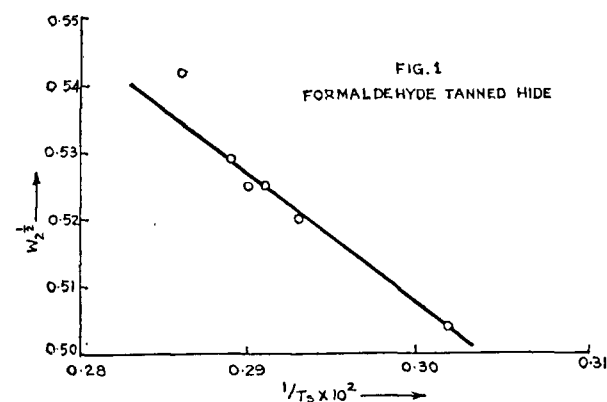
TABLE I
Swelling and shrinkage temperature of formaldehyde tanned leathers.

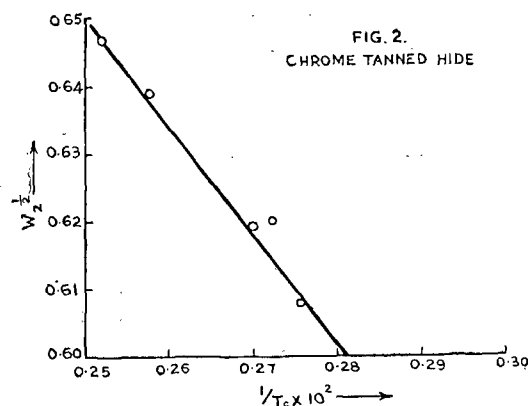
	% HCHO used in tanning.	Ts. Shrink. temp. °C.	Swollen wt. as % of dry wt.	$W_2^{1/2}$	$1/T_s \times 10^3$
1.	0	58	394	.504	.302
2.	.25	68	370	.520	.293
3.	.5	70	363	.525	.291
4.	1	72	364	.525	.290
5.	5	73	357	.529	.289
6.	10	77	340	.542	.285

TABLE II
Swelling and shrinkage temperature of stripped chrome tanned leathers.

	Acid employed for stripping.	Ts. Shrink. Temp. °C	Swollen wt. as % of dry wt.	$W_2^{1/2}$	$1/T_s \times 10^3$
1.	Boric	124	259	.647	.252
2.	Adipic	115	245	.639	.258
3.	Phosphoric	94	260	.620	.272
4.	Lactic	97	261	.619	.270
5.	Tartaric	90	271	.607	.275
6.	Oxalic	61	268	.611	.299

The values of the square root of the weight fraction of the tanned material, obtained directly from the values of swelling, have been plotted against the reciprocal of the shrinkage temperature converted to absolute scale in figs. 1 and 2.





The straight lines obtained show that the relationship deduced above between shrinkage temperature and swelling of related substances holds good fairly well. It thus appears that shrinkage can occur only when the kinetic units in the individual molecules are freed from the field of the other units which can move past one another, resulting in a shortening of the length of the molecule. Further, the above treatment suggests that the shrinkage temperature is affected by the degree of cross-linking in so far as the latter determines also the swelling of the material.

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TECHNICAL SEMINAR

The correlation between some physical properties of chrome retan upper leathers*

N. RAMANATHAN

As a first step towards the formulation of the inter-relationships between the various physical properties of chrome retan leathers, the correlations between some of the mechanical properties of the leathers were arrived at by considering two of the properties at a time. The samples consisted of both light and heavy leathers. The former were retanned with 10% extracts of Cutch, Quebracho, Myrab, Wattle and Gambier while the heavy leathers were given the following treatments after chrome tannage :

- (a) Cutch retannage and soap fatliquored,
- (b) Cutch retannage and fatliquored with sulphated Pongam oil and free Pongam oil,
- (c) Cutch retannage and hand stuffed,
- (d) Cutch retannage and double bath fatliquored,
- (e) Quebracho retannage and hand stuffed,
- (f) Fatliquored,
- (g) Hand stuffed.

The chrome tanned leathers as at (f) and (g) were included for comparison.

The samples for the various physical tests were cut out from a block 11" × 9" situated at a distance of 2" from the

* Summary of the Seminar talk delivered on 3rd December, 1957.

backbone line and 4" from the butt-end, the breadth of the piece being parallel to the backbone line. The test pieces were conditioned for more than a week in an atmosphere maintained at 80°F. and 65% relative humidity. A Scott Tester — Model J — was used for tensile strength, elongation, tongue tear and double hole stitch tear tests. The stiffness factor was determined by using a Tinius Olsen Stiffness Tester (6 inch-pound capacity). The span and the maximum bending moment were adjusted to $\frac{1}{2}$ " and 0.15 inch-pound respectively.

In testing different types of tannages like chrome-vegetable and chrome alone or leathers of different weight ranges such as those designated 'light' and 'heavy', the t-test was applied to the small number of observations associated with each type in order to determine the feasibility of combining the different tannages or the different weight ranges for purposes of correlation. For the extent of the correlation, the correlation coefficients were calculated considering two properties at a time. Further, two sets of correlation coefficients were obtained, one for the group, "all chrome retan leathers," the other for the group, "all heavy leathers."

The results of the investigation show that the correlations between any two properties are in general better for the "all heavy leathers" group, probably because of the inclusion of chrome leathers and the fact that chrome is the first tannage in all the remaining cases. The tongue tear and double hole stitch tear tests between themselves show a fair correlation. The tongue tear test correlates fairly well with elongation probably because during the tongue tear test a certain amount of elongation takes place. The stiffness factor which has been considered for correlation with other properties for the first time, shows in general a poor correlation. This is no doubt due to the process of bending itself, wherein an elongation and contraction take place simultaneously on the convex and concave sides. The mechanism

of bending in leather must necessarily be complex because of the unhomogeneous fibrous nature of the material and needs a detailed investigation. The tensile strength and elongation correlate poorly contrary to general expectation; the probable reason for this is the complicated weave pattern. The finding that the correlations obtained are worthy of note in spite of the various retannages and the other treatments shows that the first tannage, at least when it is chrome, decides to a large extent the mechanical properties of chrome retan leathers.

CLASSIFIED ABSTRACTS

RAW MATERIALS

COLLECTION AND PRESERVATION OF HIDES AND SKINS IN SWEDEN. *B. Ivarsson and H. Fredholm (Leder 1956, 7, 79-82 (1956) via J. Appl. Chem, 7, 6, i-515 (1957).—Recent Swedish investigations show that brining of skins is preferable to salting in regard to leather quality. The time between flaying and curing should be short. "Salt spots" are due to presence of Ca and Mg; these may be derived from the salt or water. Skins cooled rapidly after flaying seldom show veininess.*

PROCESSES PRIOR TO TANNING

LIMING OF ANIMAL SKIN VI. EFFECTS OF ORGANIC NITROGEN COMPOUNDS IN ACCELERATING UNHAIRING OF ANIMAL SKIN BY LIME LIQUOR I. AMINES. *H. Toyoda and A. Fukami Rep. Tokyo Chem. industr. Res. Inst. 51, 429-436 (1956) Via J. Appl. Chem, 7, 6, i-515 (1957).—Primary and sec. aliphatic amines were found to have an accelerating effect, the most active being diethylamine. tert. aliphatic, and aromatic, amines had no such effects. The introduction of acetyl or phenyl groups into the amine mol. reduces or destroys their accelerating effects (31 references) (From English summary).*

THE EFFECT OF FREEZE-DRYING ON THE SOLUBLE PROTEINS OF BOVINE HIDE. *D. R. Cooper. J.S.L.T.C., 41, II, 387 (1957).—The influence of drying technique on the rehydration of bovine hide shows that the method which should cause least denaturation of the soluble proteins (i.e. freeze-drying) does, in fact, affect the solubility of some components present in the ground substance of hide. Isolation of the salt-soluble constituents of the ground substance shows that these are essentially serum proteins, other non-collagenous proteins and mucopolysaccharides, with a very small percentage of collagenous proteins. Freeze-drying affects the solubility of the non-collagenous proteins other than serum proteins and by this means reduces the rehydration of hide. More drastic drying with heat will denature the serum proteins as well. Methods for dissolving these denatured proteins have been studied.*

VEGETABLE TANNING

BREAKDOWN OF HYDROLYZABLE TANNINS, AND ITS EFFECT IN PRACTICE. *E. Komarek (Leder 1956, 7, 148-153): via J. Appl. Chem. 7, 6, i-515 (1957) : The breakdown of hydrolyzable tannins by moulds can be prevented by adding suitable syntans of the tannin solution. In this way, tannin losses and the deposition of ellagic acid upon the leather can be prevented.*

EFFECT OF SULPHITING ON SOLUBILITY OF VEGETABLE TANNINS. *D. G. Roux (Leder 1956, 7, 111-112) Via J. Appl. Chem. 7, 6, i-515 (1957): Comparison of paper chromatograms of sulphited and unsulphited quebracho extract shows that a reduction in particle size is not caused by sulphiting.*

CHANGES IN THE MECHANICAL PROPERTIES OF COLLAGEN FIBRES DURING VEGETABLE TANNAGE. *R. G. Mitton and F.R. Morgan, J.S.L.T.C., 41, II, 381 (1957).—Single fibres of acetone-dehydrated collagen have been examined for extensibility through a complete vegetable tannage. In the pre-tanning processes, the extensibility changes were rapid and relatively small, and equilibrium was reached quickly when the load required to give a standard extension of the fibre was taken as a measure of equilibrium. Tannage in 40°Bk. mimosa liquor resulted in a steady decrease in extensibility over about six days, and a further tannage with 140°Bk. mimosa gave another steady decrease in extensibility over two days. Two days hot-pitting at 40° C in 140°Bk. liquor was sufficient to complete the tannage; the extensibility increased during this time. The dried, tanned fibre had an extensibility comparable with that of the raw dry material. After tannage, prolonged soaking in solutions of sulphated cod oil served to increase the extensibility slightly. This result is consistent with the view that the oil does not penetrate the structure of the fibrils, but merely increases the lubrication between them.*

TANNINS AND POLYPHENOLS OF SCHINOPSIS (QUEBRACHO) SPP. THEIR GENESIS AND INTER-RELATIONSHIP. *H. G. C. King and T. White, J.S.L.T.C. 41, 11,368 (1957).—Quebracho tannin extract is made from the heartwoods of *schinopsis balansae* and *schinopsis lorentzii*. Chemical examination of the commercial extract disclosed the nature of some of the polyphenols in the extract and gave evidence of their structural inter-relationship. This has now been reinforced by examination of laboratory*

prepared extracts of the leaves, twigs, bark, sapwood and heartwood of each of the above species and a reasonable number of the polyphenols present in these tissues have been identified. Consideration of the over-all results in the light of knowledge of other tannin-producing members of the *Anacardiaceae* suggests that the Quebracho trees synthesise only "hydrolysable" tannins in the leaves and that these tannins constitute the polyphenolic raw material which is ultimately converted to the typical "condensed" tannins of the Quebracho heartwoods. Catechin, and leucoanthocyanins which appear in the sapwood, also participate in the final stages of conversion.

CHROME TANNING

DETERMINATION OF CONSTITUTION OF SULPHITO-CHROME COMPLEXES. H. Erdmann, (*Leder* 1956, 7, 198-206) *Via J. Appl. Chem.* 7, 6, i-515 (1957).—Sulphito-chrome complexes may occur in liquors prepared by reduction with SO_2 or NaHSO_3 by addition of Na_2SO_3 as masking agent; by neutralization of leather with Na_2SO_3 ; by the use of $\text{Na}_2\text{S}_2\text{O}_3$ in any of the previous process at pH 3. The constitution of the complexes, and their condensation on raising basicity, is determined by conductimetric titration.

CHANGES IN STRENGTH OF CHROME LEATHER ON RETANNAGE. B. Zorn, (*Leder*, 1956, 7, 193-197) *Via J. Appl. Chem.* 7, 6, i-515 (1957).—Chrome-tanned goat skins and ox hides were retanned with (1) a dicyanodiamide resin (2) syntan, or (3) chestnut or mimosa extract. The breaking and tearing strengths of the retanned leathers were determined; in making comparisons, absolute values, not those based on unit cross-sectional area, should be considered as the thickness of the leathers (but not the collagen content) changes on retannage.

FATTY SPUE ON CHROME LEATHER. R. G. Mitton and K. G. A. Pankhurst (*Leder*, 1956, 7, 169-172) *Via J. Appl. Chem.* 7, 6, i-515 (1957).—At least two kinds of fatty spue exist which are distinguished by their different behaviour in regard to temp and R. H. of storage. One type consists principally of triglyceride, and the other of fatty acid.

OTHER TANNAGES

AMERICAN SYNTANS TO-DAY. Philip S. Chem. *The Lea. Manfr.* 74, 7, 37 (1957).—To supplement the list of American syntans published by the author in his book "Syntans and Newer Methods

of Tanning", in order to revise the old list due to the many changes that took place in syntan industry the author has furnished the names of the syntans and analytical data supplied by the manufacturers for the benefit of tanning industry.

K. S. R.

MODERN WHITE AND GLOVING LEATHERS. F. Immen-dorfer and A. Bauer (*Leder*, 1956, 7, 210-212) *Via J. Appl. Chem.*, 7, 6, i-515 (1957).—Tanning with a basic aluminium chloride yields a better white leather than the historical process of alum tawing. Satisfactory white leathers may be obtained by a combination process comprising (1) light, buffered formaldehyde pretannage, followed by (2) tannage with a sulphochlorinated hydrocarbon and (3) after acidifying, a final treatment with basic aluminium salt.

NEW AND REPLACEMENT TANNING MATERIALS. Dr. W. Schweisheimer, *Australian Lea. J.*, 60, 1, 148 (1957).—A few hints on the properties of the amino resins as tanning agents are given. Resin suitable for tanning the following properties: (1) The resin must be in the monomeric form. (2) Must be water soluble and tolerate the salt solutions (3) Polymerisation of the resins should take place at low temperatures. (4) The catalytic factor for polymerisation should not damage the grain. (5) A reasonable sheef life desirable. Though the costly melamimine resins produce better leathers than urea resins, a combination of both gives interesting possibilities. The properties of the leathers can be considerably improved by a combination of these resins and mineral tanning agents and the amount of resin can be reduced. The resin formaldehyde alum tannage can be advantageously used in splits and other thin leathers, since alum gives fullness and thickness to the leather. The resin tanning agents are most popular as retanning agents for chrome leather, which will impart an overall improvement for the leathers because of their filling properties. The resin tannage produces a white leather and gives a fine nap for suedes, etc.

V. V. S.

PROCESSES AFTER TANNING

THE APPLICATION OF NON-THERMOPLASTIC BINDERS IN THE RESIN FINISH OF LEATHER. Dr. K. Schubert. *Lea. & Shoes*, 133, 20, 33 (1957).—Resin finishing practised in all

larger tanneries of the world offers a variety of advantages and disadvantages for the leather producer and user. The thermoplastic nature of resin binders causes difficulties in shoe production under the influence of heat, producing dull and blind spots. The natural binders in finishing like gums and cellulose derivatives which dissolve relatively easily in water cannot be fixed water-tight when used in greater portions. The author while dealing with disadvantages caused by the usage of the thermoplastic and natural resin finishes, discusses some new methods to overcome such difficulties. By condensation of natural products with synthetic compounds, considerable improvements have been effected. Specific properties indicating the advantages as compared to thermoplastic binders are obtained.

V. V. S.

THEORIES

AMINO-ACID SEQUENCES OF COLLAGEN. I. FORMATION OF PROLINE AND HYDROXYPROLINE. W. Grassmann, K. Haunig, H. Endres and A. Riedel (*Hoppe-Seyl. Z.*, 1956, 306, 123-131) via *Appl. Chem.* 7, 8, ii-199 (1957).—Procollagen is degraded with crystalline trypsin and a number of peptides, some of them long chains, are isolated from the degradation products. Electrophoretic and chromatographic examination of the peptides indicates that they are homogeneous. Proline and hydroxyproline peptides are in general deficient in diamino-acids and dicarboxylic acids while proline and hydroxyproline-deficient peptides appear to be rich in such acids. A peptide consisting of 42-44 amino-acids, of which approximately 3 are proline and hydroxyproline, is investigated and it is shown that neither of the amino-acids belongs to the C-terminal or N-terminal parts of the chain. The two acids, together with glycine and certain other amino-acids, belong to the central part of the chain whose structure has not yet been elucidated.

BYE-PRODUCTS

THE SPONTANEOUS IGNITION OF WOOL. I. THE CAUSES OF SPONTANEOUS FIRES IN NEW ZEALAND WOOL. I. K. Walker and H. M. Williamson. *J. Appl. Chem.* 7, 8, 468 (1957).—An investigation was made on the causes of heating and spontaneous ignition of baled wool in the New Zealand wool

trade. It is shown that temperatures high enough to cause combustion could not be promoted solely by exothermic wetting, bacterial rotting, or by friction during 'dumping'. Direct reaction with oxygen at temperatures below 60° can cause temperature rises up to the ignition point in some samples of pie wool. Results of analyses of representative pie wools are presented. It is concluded that the majority of (and perhaps all) spontaneous fires in raw wool are caused by pie wool. Adequate scouring of the pie wool removes the combustion hazard.

FABRICATION

LEATHER GRAINOMETER. W. Schweshemen, *L.T.R.* 124, 3714, 441 (1957).—The quality of the footwear upper leather is graded by using the test instrument called graino-meter developed by the Research and Navy Clothing Supply Office in Brooklyn, New York. This measures "break" or "pipeyners" which can be indicated by the number of wrinkles formed at the vamp of shoe in walking, replacing the old method of testing it by experienced leather experts. A new tentative appearance standards have been set up to navy calf skin leather. The method of using this is described. The samples free from mechanical damage are introduced in the instrument which depresses the leather and causes wrinkles to be formed. These wrinkles may then be converted with the aid of the self-illuminating lens. The break shall be classified as Fine 19 or more wrinkles, Good, 14 to 18, Fair 8 to 13 and coarse 7 or less wrinkles.

K. S. R.

HYDROEXTRACTION CUTS COSTS, SAVES SPACE. L. T. R. 125, 3719, 105 (1957).—Thomas Broadbent and Sons Ltd. Huddersfield have been manufacturing Broadbent hydro-extractor for a considerable time. This type of extractor gives an even moisture content throughout the extracted goods in the various drying and sammying processes employed in the industry without damaging even the most delicate skins. It saves space; reduces the amount of capital expenditure for new constructions; cuts costs stepping up production. The machinery is economical both to operate and maintain.

K. S. R.

TESTING

IODIMETRIC DETERMINATION OF SILICON. R. M. Veltsman (*All-Union Sci. Rest. Inst. of Alloys*). *Zavod. Lab.* 1957, 23 (2) 153-157—*via. Anal. Abst.* 4, 9, 2925 (1957).—Reduction of the yellow complex of molybdate and silicic acid with KI yields iodine which, after extraction with isobutyl alcohol, can be determined colorimetrically or by titration. With samples of titanium dioxide, silicon carbide, tungstic oxide and concentrates, the material is fused with Na_2CO_3 and a portion of the aq. soln. of the melt is just acidified with dil. H_2SO_4 . With steel, 1 g is dissolved in 40 ml of dil. H_2SO_4 (1: 8) and the soln. is heated with 3 g of $(\text{NH}_4)_2\text{S}_2\text{O}_8$ and 20 to 30 ml. of water until the Fe is oxidised and the excess of oxidising agent is decomposed. Ethanol (25 ml) is added and the heating is continued until Cr is completely reduced. The soln. is diluted to 100 ml. and 10 ml. is mixed with 1 g of H_3BO_3 and treated with 20% NaOH soln. until a ppt. of basic salts forms. Dil. H_2SO_4 (1: 4) is then added to dissolve the salts, leaving an excess of 0.2 ml. The slightly acid soln. is treated with 3 ml. of 10% $(\text{NH}_4)_2\text{MoO}_4$ soln. and set aside for 5 min. The yellow soln. is mixed with 2 ml. of satd. NaH_2PO_4 soln. and 5 ml. of 10% citric acid soln. The flask is stoppered and set aside in the dark for 15 min. The soln. is then diluted with water to a vol. of 100 to 150 ml. and titrated with 0.004 N $\text{Na}_2\text{S}_2\text{O}_3$ in the presence of starch. One ml = 0.24 mg. of SiO_2 . A colorimetric method is also described.

THE DETERMINATION OF ALKALI SALTS OF ORGANIC ACIDS BY RESIDUAL NON-AQUEOUS TITRATION. M. I. Blake (*School of Pharmacy, North Dakota Agric. Coll. Fargo, N.D., U.S.A.*) *J. Amer. Pharm. Ass. Sci. Ed.*, 1957, 46 (3) 163-166. *via. Anal. Abst.*, 4, 9, 3012 (1957).—The U.S.P. XV method of assay of the alkali-metal salts of organic acids is compared with a method in which back-titration in glacial acetic acid is used. The non-aqueous back-titration is rapid and accurate and is applicable to a variety of salts. It is particularly suitable for tartrates, which are poorly soluble in glacial acetic acid and are not readily determined by direct titration. *Procedure*—Dissolve the sample (1 milli equiv.) in a mixture of glacial acetic acid (30 ml.) and 0.1 N HClO_4 in glacial acetic acid (25 ml) and back-titrate with 0.1 N Na acetate in glacial acetic acid, either potentiometrically or with methyl violet as indicator to a visual end-point (first permanent blue showing no trace of green).

THE DETECTION OF FLAVONES AND THEIR DECOMPOSITION PRODUCTS WITH STABILISED DIAZONIUM SALTS BY MEANS OF SPOT REACTIONS. R. Neu (*Firma Dr. Willmar Schwabe G.m.b.H. Karlsruhe, Germany*). *Mikrochim. Acta*, 1957 (2) 196-214—*via. Anal. Abst.*, 4, 9, 3026 (1957).—Tests have been devised for the detection of microgram quantities of di- and tri-hydroxybenzenes and of aromatic hydroxy acids. The tests consist of spot reactions on filter-paper with stabilised diazonium salts to give characteristic dyes. Limits of detection and colours of the dyes in both daylight and u.v. have been studied with 28 flavones.

SEPARATION OF AMINO ACIDS BY PAPER ELECTROPHORESIS. STUDIES ON THE MIGRATION DISTANCE OF AMINO ACIDS. Kiyoshi Sakamoto and Kazuko Takeoka (*Junior College, Kagoshima Prefectural Univ. Yamashita-cho, Kagoshima*). *J. Agric. Chem. Soc. Japan*, 1956 30 (8), 463-469—*via. Anal. Abst.* 4, 9, 3072 (1957).—The paper electrophoresis of various amino acids was studied at various pH values near the iso-electric point, and a new method of expressing the mobility is proposed. Each amino acid migrates, under given conditions of the composition of the soln. potential and current, towards a definite point, at a rate that decreases with time. This point is shifted towards the anode with increase in current. The rate of migration does not increase linearly with potential, but the mutual distance between spots of any two amino acids is proportional to the applied potential. When the distance of the spots of asparagine and glycine is taken for the standard, its ratio to that between the spots of glycine and a given amino acid remains constant, irrespective of the potential, current, time for electrolysis, length of the paper and the position of the initial spot.

GENERAL

INVESTIGATIONS OF BEHAVIOUR OF AUXILIARY MATERIALS IN LEATHER MANUFACTURE, BY MEANS OF RADIOACTIVE SUBSTANCES. I. EFFECT OF SURFACE ACTIVE AGENTS. R. Heyden, J. Plapper and K. Sauerwein (*Leder*, 1956 7, 100-107) *via J. Appl. Chem.*, 7, 6, i-516 (1957).—By employing wetting, dispersing and emulsifying agents containing ^{35}S , it was found that they were attached to the hide substance during leather processing. Small amounts are attached to the skin surface, but increasing amounts produce penetration.

NEATSFOOT OIL. *H. Lange (Leder 7, 122-126, 1956 via J. Appl. Chem., 7, 6, i-508 (1957)).*—Commercial neatsfoot oil is made from the fat glands between the foot bones and from the marrow of the metatarsal bones. Originally, the former source only was used; today such oil is extracted only for watch lubrication. The influence of manufacturing conditions on oil quality is discussed. Specifications and methods of test are reviewed. It is considered chemically impossible by analysis to guarantee a neatsfoot oil sample to be free from adulteration by vegetable oils.

SEPARATION OF CASTOR OIL PRODUCTS BY THE METHOD OF PANZER-NIEBUHR. *O. Dietsche and H. Lubbe Leder 7, 119-122 (1956) via J. Appl. Chem., 7, 6, i-508 (1957).*—The separation of the emulsifying from the emulsified constituents of an oil emulsion by the Panzer-Niebuhr method depends upon the solubility of the former in dilute ethyl alcohol, and of the latter in light petroleum. In the case of castor oil products, the oil is insoluble in light petroleum; a 1 : 1 mixture of this and ethyl ether may be used for the separation.

PATENTS

PREPARATION OF ANIMAL SKINS FOR TANNING. *Osterreichische Stickstoffwerke A. G. (B. P. 751, 141, 2.4.54. Austria 3.4.53) via J. Appl. Chem., 7, 7, ii-88 (1957).*—Unhaired animal skins are treated with thioglycollic acid (5 %) and/or water-sol. salt thereof, to break down non-skeletal proteins while preserving skin substance.

DRYING OF HIDES OR SKINS. *British United Shoe Machinery Co., Ltd. (B. P. 750, 416, 9.6.53) via J. Appl. Chem., 7, 7, ii-88 (1957).*—A hide or skin is rapidly dried without shrinkage by placing it on a sintered metal or porous ceramic sheet and covering it with a sheet of impermeable material such as rubber. Vacuum is then applied beneath the porous material by a vacuum pump, the sinter being supported by crosspieces which are notched to allow even distribution of the vacuum. The rubber sheet is pressed to the skin, and a hot air floor or i.r. lamp used to warm the skin through the rubber.

POROUS SHEET MATERIAL. *Wingfoot Corp. (B.P. 749, 489, 31.12.53. U.S., 24.9.53) via J. Appl. Chem., 7, 7, ii-88 (1957).*—Porous fibrous sheet material, useful as artificial leather and having the porosity and permeability to water vapour of real leather is

made by crushing a gelled mass of a frothed natural or synthetic rubber latex having fibrous filler (cotton fibres 30% of wt. of latex) three-dimensionally dispersed therein, and curing the crushed latex.

WATER-VAPOUR-PERMEABLE COMPOSITE LEATHER-LIKE SHEETS. *E. I. du Pont De Nemours & Co. (B.P. 751, 117 10.12.53. U.S., 12.12.53) via Appl. Chem., 7, 7, ii-88 (1957).*—Water-vapour-permeable sheet material (artificial leather of superior "breathing" properties and ability to transpire water vapour) is made by (a) forming an initial sheet from a mat of unwoven liquid swellable fibres (*I*) (cotton, wool, rayon, polyacrylonitrile, and especially a nylon) and a film of an initially thermoplastic polymeric binder (*II*) having a softening temp. below the softening temp. of *I* (polyethylene) (b) hot pressing the initial sheet at temp. above the softening temp. of *II* and below that of *I* to form an essentially impermeable composite sheet (c) soaking at a temp. between the softening temp. of *II* and *I* in a liquid which swells the fibres (in water at 95-100°), (d) cooling and drying (at room temp. or at temp. at least 30-40° below the softening temp. of *II*). Preferably the initial sheet is constituted by 20-70 % of fibres (comprises equal vol. of *I* and *II*). For optimum strength, tear strength, flexibility and flex life of the product the fibres should have a length of ≤ 0.5 (0.5-1.5) in.

DYEING WOOL WITH COMPLEX HEAVY METAL COMPOUNDS OF DYESTUFFS. *J. R. Geigy A. G. (B.P. 750, 648, 1.3.54. Switz 6.3.53) Via J. Appl. Chem., 7, 7, i-95 (1957).*—The dyeing of wool fibres, yarns, fabrics etc. (if desired mixed with other textile fibres, especially those similar to wool, particularly synthetic materials such as nylon or Perlon with complex heavy metal compounds of monazo or azomethine dyestuffs of the type 2 dyestuff mol.: 1 heavy metal atom of atomic no. 23-28 and containing no solubilizing group is effected to produce (rapidly, in 10-30 min.) even and level dye: of good penetration by commencing the dyeing in a hot dye liquor of pH ≤ 7.5 and performing the dyeing while the pH of the liquor is lowered, by the use in the dye liquor of ammonium salts (sulphate) or of esters of alcohols which are saponified under the dyeing conditions. A buffering mixture of ammonium salts of inorg. acids and ammonia may be used to adjust the initial pH value of the goods and liquor.

NOTES & NEWS

DETERMINING AMOUNT OF LIGNOSULFONATES IN TANNING MATERIAL.

The U.S. National Bureau of Standards in Co-operation with American Leather Chemists' Association, has recently developed a method of determining the amount of lignosulfonates present in tanning materials. Precipitating tanning mixtures with cinchonine provides a convenient means of determining the amount of lignosulfonate adulterants present. To form a precipitate a cinchonine reagent is added to a sample solution and heated to a temperature of 70° C. The precipitate is filtered and dried for a period of three hours and weighed to ascertain the amount of lignosulfonate present in the sample solution. [L.T.R. 125, 3723, 257 (1957)].

LEATHER CRAFT IN BYGONE VEHICLES

The reopened Transport Museum in High Street, Hull, after 16 years offered some of the many uses to which leather was put in bygone times. The most obvious use of leather is in the seating and upholstery which are found still in excellent condition after their usage from 100 to 200 years. Some of the private carriages have splendid hammercloths and fittings of tooled leather and some of the early handsome cabs—each demonstrating in one form or another the dependence on leather craft for comfort and adornment. Mention is made of the exhibits depicting early cycling and motoring days when the leather was widely used. [G. Bernard-wood, L.T.R. 125, 3725, 328 (1957)]

THAT UNIQUE PRODUCT—LEATHER

A leather water pipe made from chrome combed leather with a longitudinal spliced joint, cement with cellulose was used for the private water supply to the home of Mr. H. Raistrick of M/s. S. Raistrick & Sons Ltd., N. Keighley, York. It was replaced by the same methods using that unique product—"Leather", after giving 14 years sterling service. The leather pipe has now been placed in the small museum of leather articles at Milton Park,

where it stands, joint and leather still in excellent condition apart from the point of failure caused by being cramped to the iron pipe. [L.T.R. 125, 3735, 328 (1957)]

INDIAN HIDE AND SKIN OUTPUT DROPS.

Bovine hide and skin production in India approximated 19 million pieces, somewhat lower than the annual average of 21.2 million. Goat and sheep skin production, estimated at 36.5 million skins in 1956, also was smaller, compared with the annual average of 38.3 million skins. Despite the sizable hide production the quality is not very desirable as 87 per cent of hides were from fallen animals, but in skins this total is only 20 per cent. The Indian tanning industry in 1956 consisted of 24 large-scale organized tanneries with an installed capacity of 3,304,000 hides of vegetable tanning; 13 of these also produce chrome leather with an installed capacity of 1,679,000 hides. Actual production in the tanneries is substantially below installed capacity. In addition to the large-scale tanneries, about 500 small-scale tanneries produce vegetable-tanned leather known to the trade as East India kips and East India skins. Output is estimated at 8.9 million pieces and 20.22 million pieces, respectively. Some 250 small-scale chrome tanneries produce about 1.5 million chrome-tanned hides, and a large number of village tanneries highly dispersed throughout the country process about 8.9 million hides and 3.4 million skins for the production of vegetable tanned leather. [Foreign Commerce Weekly Reports. U.S. Consulate-General, Madras, Through Tanner, 12, 6, 194 (1957).]

LEATHER USED IN MAKING PERSONAL CONSUMPTION GOODS.

More industrialized countries use leather for a variety of purposes, but in India its use is confined primarily to the production of "personal consumption goods", such as country pattern shoes principally sandals, western type shoes, different types of buckets for drawing water from wells, industrial and agricultural leather straps, suit-cases, bags, harness and saddlery, ropes and laces, and for repair purposes. For these uses, approximately 16.5 million hides are tanned annually, of which about 62 per cent is used for footwear. Twelve large-scale leather-footwear factories are in operation with an annual capacity of 5,975,000 pairs of western-type of footwear. Production by these factories was estimated at

5,540,000 pairs in 1955 of which 3,243,000 were western-type shoes and 2,303,000 were country pattern shoes—principally sandals. [*Foreign Commerce Weekly Reports, U.S. Consulate General, Madras, Through Tanner, 12, 6, 194 (1957)]*

SMALLER FOOTWEAR INDUSTRIES PRODUCE MORE SANDALS.

Annual production of small-scale and cottage industries is estimated at 77·83 million pairs—12·13 million pairs western type and 65·70 million pairs country pattern. A small-scale shop is a productive unit with a capital investment of 500,000 rupees or less, employing less than 50 workers and using electric power; or less than 100 workers if working without electric power. A cottage type is a productive unit with a capital investment 10,000 rupees or less employing 10 or less workers but depending primarily on family members for labour force. The unit is generally located in the homes of the artisan and may or may not use electric power. These industries are spread all over the country but the important centres are at Kanpur, Agra, Calcutta and Bombay. Footwear imports were curtailed in the past few years to conserve foreign exchange; thus requirements of the domestic market were met by the local industry. At the same time, exports of footwear doubled. [*Foreign Commerce Weekly Reports, U.S. Consulate General, Madras, Through Tanner 12, 6, 194 (1957).*]

C. L. R. I. NEWS

Dr. Y. Nayudamma, Assistant Director-in-charge, Central Leather Institute, attended the Conference of Directors of the National Laboratories held on 16th and 17th November 1957 at New Delhi.

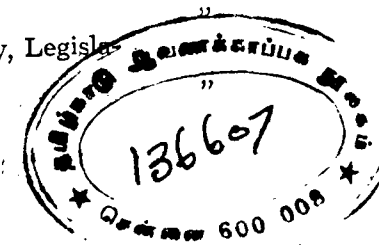
Dr. S. K. Barat, Senior Scientific Officer, left on tour to visit Forest Research Institute, Dehra Dun. He will also visit the Indian Agricultural Research Institute, New Delhi, Messrs. Wood Products Co. Ltd., Barielly and T.D.E. Laboratories and some tanneries in Kanpur.

Dr. E. C. Mathew, Junior Scientific Officer, has been appointed Hony. Reader, Leather Technology, University of Madras.

Practical Demonstration No. 9 on the Manufacture of Fancy Leathers was successfully concluded on the 25th November 1957.

VISITORS

Name	Date of visit
Mr. N. Chandappa, Indo-Swiss Synthetic Gem Mfg. Co. Ltd., Mettupalayam	20-11-1957
Miss Mary Douglas, IFYE, Indiana, U.S.A.	21-11-1957
Mr. J. Rama Rao, Indukurpet, Nellore.	21-11-1957
Members of the Commonwealth Parliamentary Delegation :	
The Hon. Mr. Asa H. Pritchard, Speaker and Joint President, Bahamas.	26-11-1957
Mr. Ajodha Singh, M.L.C., British Guiana	"
Mr. Jean Paul Deschatelets, M.P., Canada	"
Mr. Irvin William Studer, M.P., Canada	"
Mr. Frederic Davis Shaw, M.P., Canada	"
Mr. Alexander M. Nicholson, M.P., Canada	"
The Hon. Mr. J. Darling, Speaker, Saskatchewan	"
Mr. Richard A. Donahoe, Attorney-General, Nova Scotia	"
Mr. Casely-Hayford, Ghana	"
Mr. Kobina Orleans Thompson, M.P., Ghana	"
Mr. Joseph Dawson Wirako, M.P., Ghana	"
The Hon. Dr. Glendon Logan, Minister of Local Government & Housing, Jamaica	"
Chief T. T. Solaru, Nigeria Federal	"
Mr. Jaja A. Wachuku, Nigeria Federal	"
Mr. Charles Aniweta Abangwu, Dy. Speaker, Eastern Nigeria	"
Alhaji Othman Ladan, N. Nigeria	"
Dr. W. Mahabir, Trinidad	"
Mr. R. V. Vanderfelt, OBE., Secretary, United Kingdom	"
Mr. Abdul Karim, M.P., Pakistan	"
Mr. G. M. Alagarswamy, Assistant Secretary, Legislature Department, Madras	"



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