CHAPTER SIX. Discussion.

This study of gelatine colour was undertaken primarily because DGI was desirous of producing from its traditional beefskin raw material a gelatin that would be more competitive with the paler pigskin and ossein gelatines with which the European and American markets were more familiar.

Gelatine manufacturers tend to be secretive as evidenced by the scarcity of publications in the field, hence there was little background on which the study could be based, other than that based on personal experience.

The problem was approached mainly from the point of view of gelatine manufacture because this was the area of expertise. The other factor was that gelatine manufacturers seem to think that their processes were unique and that their process was responsible for the uniqueness of their product. Of course, to an extent, this was true as any manufacturer trying to duplicate the gelatine from another source will know. The differences were due mainly to Bloom - viscosity relationships, ash contents and pI which were all known factors of the production processes. Hence, it was natural to suspect that differences in colour were also a function of the processing of the raw material. From experience it was also known that, to a small extent, gelatine colour was a function of the raw material used (tannery splits usually gave pale gelatine).

The occurrence of dark gelatines.

Having possibly the unique ability and experience to produce gelatine on a small scale, the first approach to the investigation of the process was to develop a technique that would allow the comparison of a number of processing variables without interference from the raw material. Gelatine manufacturers utilise waste collagenous materials, hence, they usually do not use whole hides. For this study however, most of the investigation was conducted on whole hides as it had the advantage of eliminating the raw material as a variable in a series of experiments. The result of this was that for the first time, a detailed study of the alkaline conditioning process was possible. The next factor which was of great assistance was the ability to obtain the hide of animals of known origins and age from the Irene Animal Production Institute

(IAPI) previously known as Animal and Dairy Sciences Research Institute.

The process used for the manufacture of gelatine was a traditional one, namely, lime-sulphide conditioning followed by sulphurous acid acidulation and then batchwise extraction. It was known that sulphurous acid acidulation had a marked effect on gelatine colour and if, for example, hydrochloric acid were used in its place the colour of the gelatine was very much darker. This effect was recently quantified in experiment IN0 (Cole, 1993) in which sulphuric acid acidulation was used. Sulphuric acid gave first extraction colours of 16 and overall colours of 21 compared to the sulphurous acid first extraction colour of 9.4 and overall colour of 15. Hence, in using the results of this study it must be borne in mind that the actual colours produced depended to an extent on the gelatine manufacturing process used.

Besides the sulphurous acid acidulation process it was known that alkaline peroxide bleaching of the raw material could give some additional colour improvement, however, variable decomposition of the peroxide meant that the results were variable. Alkaline peroxide bleaching of gelatine solution was also effective but the process was time dependent, hence there was a consequential quality deterioration. Activated carbon treatment of gelatine solution was also of limited effect but it added considerably to filtration difficulties. Anion exchange also, was known to have had a limited bleaching effect on dark low quality gelatine solutions but hardly any effect on the top quality paler gelatine solutions. Combination of all these processes could provide quite marked colour improvements but the cost was prohibitive and the greatest effect was on the low quality gelatines whereas the colour improvement was most desired on the naturally paler top quality gelatines.

The major finding of the investigation of the conditioning process was that variations within the process had a small to negligible effect on the colour of the resultant gelatine and animal age was shown to have been the major contributor to the colour. Furthermore, the acid conditioning process gave Type A gelatines from calf skin with colours equivalent to those from pigskin. Also there was virtually no difference in gelatine colour from calf skin whether produced by the acid or the alkaline conditioning processes, provided that the extraction pH and temperatures were the same.

The small effect on gelatine colour contributed by variations in the process was associated with darker gelatine being produced at higher levels of

extractability, in line with the most easily converted collagen yielding gelatine of the best colour. The overall colour was found to be substantially unaffected by variations within the process and no evidence was found for any effect on gelatine colour attributable to the use of sodium sulphide in conditioning.

Finally, it was found that the large variation in gelatine colour apparently related to the quality parameters of Bloom strength and viscosity was only observed with gelatines from old animal hide. Young animal's hide produced gelatines of pale and comparatively invariant colour.

The study was extended to different breeds of bovines and it was found that within the breeds available there were no statistically significant differences in gelatine colour with breed. This did not preclude the possibility that other breeds, not investigated, could give gelatines of a different colour.

Other results from the investigation.

Possibly by far the greatest importance of the study on the alkaline conditioning process was the knowledge gained of the effect of animal age. It was never suspected that lime-sulphide conditioning could have had such a small conditioning effect on old animal hide nor that the degree of conditioning was largely independent of conditioning time above 4 weeks, for older animals. Furthermore, the demonstration that the effect of sodium sulphide in conditioning was not concentration dependent above a concentration of about 1.5 g/l made considerable savings in sulphide usage possible.

Another result of the investigation was quantification of the variance in collagen (gelatine) content of hide and the resultant proximate analysis of anhydrous, ash-free, hide:

Gelatine / Collagen	59 %
Fat	4 %
Hair	9 %
Non Collagen Protein	8 %
Unknown Losses.	20 %

Gelatine colour measurement.

The second colour problem applicable to the gelatine industry as a whole was the measurement of gelatine colour. The problem, as previously explained, was due to the interference with spectrophotometric absorbance by variances in light scatter caused by variation in gelatine clarity and molecular weight.

In approaching the problem of the measurement of gelatine colour, cognisance was taken of the need for a method that was applicable to the widest possible range of gelatines. For this reason, in developing a method, production Type A and Type B gelatines were included in the assessment as well as experimental gelatines derived from horse hide, sheep skin, fish skin and chrome tanned leather as well as gelatines from other manufacturers. Regrettably only one ossein gelatine (derived from bone) was available, however, this one sample did not behave abnormally.

Firstly the problem was tackled by enzymic hydrolysis of gelatine solutions to a uniform low molecular weight and then membrane filtration to uniform clarity. The process gave an acceptable correlation ($r=0.95$, $n=35$) between instrumental and visual colour assessment provided that the initial gelatine clarity was not worse than 100 NTU on a 6.67% solution. Also it was found that there were problems when the gelatine pH was out of the effective pH range of the enzyme being used, hence for the full range of gelatines at least two enzymes were required.

In continued investigations it was found that with the same clarity limitation as above, the BYK-Gardner Tristimulus Reflectance Spectrophotometer could give as good a statistical correlation ($r = 0.96$, $n=86$) between the visual colour and the instrumental colour, without any special sample preparation. The reason for the success with the Gardner instrument was that there was an inherently large amount of light loss due to scatter, with the result that small changes in scatter due to variances in gelatine clarity and molecular weight were negligible.

The main advantage of the instrumental measurement of gelatine solution colour was its reproducibility due to the elimination of the personal errors of the visual method. As the aim was to come as close as possible to the visual colour there was neither the need nor the opportunity to remeasure all the colours of the gelatines produced in this study over the span of several

years, using the Gardner instrument.

Ancillary to the measurement of the colour of gelatine was a study of the absorbance spectra of a large number of gelatines between 150 and 700 nm. It was of interest to note that the absorbance of gelatine in the ultraviolet region between 280 and 400 nm was markedly variable, with darker gelatines exhibiting more absorbance than pale gelatines. The result of this was that after enzymic hydrolysis and filtration the 400 nm absorbance was almost as good a measure of colour as was the total area under the absorbance curve between 400 and 700 nm. Furthermore, poor clarity gelatines showed greater absorbance at 700 nm than clear gelatines. These effects of clarity variance could be reduced by moving the origin of the absorbance curve up to the 700 nm absorbance before measuring the area under the curve. This study also exhibited a spectral anomaly at 320 nm only exhibited by gelatines derived from chrome tanned leather which would point to some molecular peculiarity associated with these SPA process gelatines.

Cause of gelatine colour.

Having produced a large number of gelatines with a large variance in colour the investigation of the cause of the colour was based on the techniques available.

Past investigations using chromatography of gelatine did not lead us to have faith in that approach. The investigations of Sell & Monnier (1989), Uchiyama, Ohishi, Takahashi, Kushida, Inoue, Jugie and Horiuchi, (1991) and Kuypers, Tyler, Kurth and Horgan, (1993) lead us to realise that degradation and isolation of the chromophore was very much a dedicated team project and not within the means at our disposal.

Based on the investigations of Tanaka *et al*. (1988), Sell & Monnier (1989), Uchiyama *et al*. (1991) and later Odetti *el al* (1994), it appeared that the development of colour in gelatine could well be the result of increased degree of the Maillard cross-linking with age in which case this could be verified using spectrofluorophotometry as the Maillard "pentosidine" cross-link of Sell & Monnier (1989) was fluorescent at 335/385 nm. Furthermore, Tanaka *et al*. (1988) had shown that the Maillard cross-linking of collagen could be detected by a change in the electrophoretic mobility of the α-chain, hence, the application of SDS-PAGE could possibly yield interesting results.

Another approach that might have been applicable was the use of ELISA to detect the presence of pentosidine but it was only after the study was completed that the production of a suitable antibody by Taneda and Monnier (1994) was reported in chemical abstracts.

Fluorescence in gelatine.

No reference to gelatine fluorescence was available from the literature, hence there was nothing but collagen studies on which the investigation could be based. Collagen was not soluble, hence, it was presumed that those working in the field worked in reflectance mode rather than the transmittance mode which was possible with solutions. The reflectance mode would not, however, be as prone to concentration quenching but this mode would not be as sensitive from the quantitative point of view.

From the outset it was clear that gelatine, like collagen, did exhibit fluorescence, however the spectrum was not simply 335/385 nm "pentosidine" fluorescence but there was a second emission peak at about 410 nm. The excitation peak at 295 nm that Uchiyama *et al*. (1991), found to be due to the pyridinoline cross-link, was only observed in Type A calf skin gelatine, hence it was concluded that alkaline conditioning destroyed this cross-link. This was a most important finding and enabled a clear picture of the effect of alkaline conditioning in the gelatine manufacturing process as follows:

It was known from the research studies of Sell & Monnier (1989) and Uchiyama *et al*. (1991) that the pyridinoline cross-link remained constant with animal age but that the concentration of pentosidine cross-link increased with age. Hence, the finding that alkaline conditioning destroyed the pyridinoline but not the pentosidine cross-link explained the observation in the conditioning experiments that:

(i) liming time had a marked effect on the extractability of young animal hide low in pentosidine cross-links,
and
(ii) liming time had a comparatively limited effect on the extractability of old animal hide with a high concentration of pentosidine cross-links.

The observation that the 370/440 nm collagen fluorescence of Tanaka *et al*. (1988) was found also only in Type A calf skin gelatine indicated that the

molecule causing this fluorescence was also subject to alkaline degradation.

The second finding that was considered to be of importance was the good statistical correlation found between the 335/385 fluorescence intensity and first extraction gelatine colour and the absence of a correlation between the third extraction gelation colour and fluorescence intensity. This was interpreted as top quality, first extraction, gelatine colour being due to the Maillard-pentosidine cross-linking and lower quality gelatine colour being caused by a second unknown factor. This was confirmed experimentally by the anion exchange results presented in Chapter 4, Table 9. The multi-factor cause of gelatine colour explained the often variable response of gelatine to bleaching processes as it was probable that each process would only affect one of the two (or more) chromophores in gelatine.

The one disappointment of the investigation was the failure to throw any light on the reason for the abnormally pale colours of gelatines derived from chrome tanned leather.

Gelatine sugar interaction.

In order to verify that the Maillard reaction, as exhibited by pentosidine formation, was in fact a cause of gelatine colour, the reducing sugar experiments of Tanaka *et al*. (1988) were repeated at a physiological pH of 6 and at an alkaline pH of 9. (This latter pH was known to accelerate the aldehyde/lysine interaction). These experiments confirmed their findings by showing that the gelatine-ribose reaction rate at pH 6 was about 28 times faster than the gelatine-glucose reaction rate.

There were two important observations made from these experiments:

a. The 335/385 nm fluorescence intensity of the gelatines increased with time of reaction as did the colour of the gelatines and in the case of ribose, the reaction mixtures gelled.

b. The colour spectrum of glucose darkened gelatine was the same as the spectrum of dark gelatine, whereas the colour spectrum of ribose darkened gelatine was markedly redder than that of dark gelatine.

The first observation was expected and confirmed that gelatine darkening could certainly be caused by the Maillard cross-linking and pentosidine formation.

The second observation was interpreted as showing that *in vivo* glucose was probably the source of the five carbon atoms linking lysine and arginine to form pentosidine, even though this possibly requires the loss of 1 carbon atom. However, the fluorescence excitation and emission maxima were the same for both glucose and ribose darkened gelatine so the difference in colour could be associated possibly with a second chromophore and not the Maillard chromophore. This was not confirmed by the anion exchange bleaching experiments that followed. Furthermore, in contrasts to the finding of Sell & Monnier (1989) that a mixture of lysine, arginine and glucose did not form the fluorescent product, pentosidine, this experiment showed that with glucose and a complex protein like gelatine the Maillard reaction, with pentosidine formation, did proceed even if rather slowly.

The slowness of the Maillard reaction in solution, particularly between gelatine and glucose, was an explanation possibly of the failure to observe any effect on gelatine colour due to sulphide in conditioning. The slowness of the reaction rate would be multiplied if the steric hinderance to the Maillard reaction, (indicated by the ribose-gelatine reaction experiment to have resulted from gelation), was verified. The other explanation for the lack of a sulphide effect could be the possibility that SO_2 inhibition of the Maillard reaction was a function of the formation of a sulphite addition compound (with the aldehyde) rather than a function of the reducing properties of sulphite.

The control experiments conducted without sugars showed unequivocally that the darkening and fluorescence development were attributable to the presence of the reducing sugar in the reaction mixtures.

Pentosidine synthesis.

The pentosidine formation procedure of Sell & Monnier (1989) was modified by reducing the reaction temperature. Ambient temperature reaction of lysine, arginine and ribose favoured the formation of fluorescent products with the excitation maximum at 335 nm and an emission spectrum similar to that of gelatine (that is, with a prominent second peak at about 410 nm). At 37°C reaction favoured the 385 nm emission peak with a lesser shoulder at 410 nm.

Hence, this eliminated any suspicion that the gelatine fluorescence was not due to pentosidine because of the second peak at 410 nm, but rather it shows that there were possibly more than one isomer of pentosidine with slightly different emission spectra.

The effect of anion exchange.

It was accepted that anion exchange was useful as part of the de-ashing of gelatine solutions and the additional benefit of colour removal was demonstrated. An experiment was in progress to determine the capacity of a particular resin for colour removal hence a number of samples were available that had been anion exchanged, together with the original gelatines. The difference in the fluorescence intensities of treated and untreated gelatines were within the normal limits of experimental error, and it was concluded that the observed bleaching effect was not associated with removal of the Maillard induced chromophore.

When the anion exchange resin was regenerated with caustic soda, the very dark spent regenerant solution was examined using the spectrofluorophotometer and although the solution was mildly fluorescent, the amount of fluorescence was such as to confirm that the resin had accumulated very little of the intrinsic gelatine fluorescence. Furthermore, it was noted that the 335/385 nm fluorescence of the regenerant solution was greater at pH 6 than at pH 12 which agreed with the observation of Sell & Monnier (1989) that pentosidine fluorescence was quenched at pH 9.

An additional confirmatory experiment was undertaken to determine whether the colour produced by the Maillard reaction could be removed by anion exchange. If, as had been contended, gelatine colour was associated with a cross-link formation then the colour should not be removable by anion exchange. The experiment showed that anion exchange had a very small effect on the colour of ribose darkened gelatine solution thus confirming that the chromophore was tightly bound to the protein chain. This experiment also confirmed that because anion exchange did bleach darker low quality gelatines, the colour removed by the resin had to be due to a different chromophore from that formed by the Maillard cross-linking.

In summary:

1. Gelatine exhibited 335/385 nm pentosidine fluorescence as well as a

second emission peak at about 410 nm. The fluorescence intensity was correlated significantly to first extraction gelatine colour but not to third extraction gelatine colour.

2. Type A calfskin gelatine also exhibited weak 295/395 pyridinoline fluorescence which appeared to be destroyed by alkaline conditioning explaining the very limited time related response of old animal hide to conditioning.

3. Glucose-gelatine interaction gave colour production with a spectrum identical to that of naturally dark gelatines. Ribose-gelatine interaction produced a chromophore with a spectrum different to that of naturally dark gelatine. Hence natural pentosidine was probably the product of glucose and collagen interaction. The very slow rate of the gelatine-glucose Maillard reaction was demonstrated and it was concluded that this could be the reason for the failure to observe the Maillard reaction when it was expected.

4. The ribose-gelatine interaction at 37°C, besides exhibiting colour and pentosidine fluorescence development also exhibited gelation which clearly demonstrated the cross-linking effect of the Maillard reaction.

5. The low temperature reaction of lysine, arginine and ribose produced products with a fluorescence spectrum very similar to that of gelatine.

6. Anion exchange removed colour from low quality dark gelatine without effecting its fluorescence. It was unable to remove the colour from ribose darkened gelatine. Hence, there were at least two chromophores responsible for the colour of gelatine.

Electrophoresis.

The work of Tanaka *et al*. (1988) showed that the ribose collagen interaction caused an increase in the amount of α-chain dimer. The α-chains also appeared to migrate more slowly under the conditions of electrophoresis. Hence, it was anticipated that α-chains from dark gelatine might migrate more slowly than those from pale gelatines due to the additional size contributed by the Maillard addition products. The differences in molecular mobility observed were the previously reported differences between α-chains from Type A and Type B gelatines (Chalepakis, Tanay, and Heidemann, 1985) and also a progressive decrease in mobility of the α-chains with animal age.

The initial experiments were conducted using the methodology of Chalepakis *et al*. (1985) except for the staining where it was decided that the conventional

Stegman staining technique should be better (Miflin and Shewry, 1977). After some time it was found that staining was not good and it was realised that probably the reason for the inclusion of trichloroacetic acid (TCA) in the stain solution used by Chalepakis *et al*. (1985) was to prevent highly soluble gelatine from diffusing out of the gel during overnight staining. In order to obtain clean gels it was essential that the Coomassie blue be completely dissolved in the stain solution. Hence, as Coomassie blue was not readily soluble in TCA it was decided to try dissolving the Coomassie blue in methanol, the TCA in water and combining the two immediately prior to use. In spite of the reaction between TCA and methanol which formed an unsightly odorous oil on the surface of the polyethylene staining basins, the staining of the gels was good and the oil did not seem to adhere in any way to the polyacrylamide gel. After removing the stain solution the gel was washed in destaining solution, transferred to a clean glass plate and then transferred to a clean basin for destaining. The dirty basin was easily cleaned using methanol.

From gels 12 and 13 (Chapter 5) it was evident that the electrophoresis techniques had not been perfected however the gels were good enough to show that:

1. There was a marked difference between type A and Type B gelatines.
2. That all first extraction gelatines contained significant amounts of α-chain collagen, (hence, the most easily hydrolysed collagen was also the least coloured).
3. That all third extraction gelatines contained little or no α-chain collagen (and they were also the darkest).
4. What others seem to have failed to observe, a significant amount of a protein with a molecular weight of about 82 kD in all the gelatines.

Difference between Types A and B gelatine.

The difference between Type A and Type B gelatine was the higher mobility of the Type A α-chain material, as found by Chalepakis et al. (1985) and also the large number of discrete bands with a higher mobility (lower molecular weight) than α-chain material in Type A gelatine. Müller and Heidemann (1993) searched for and found discrete or preferred points of thermal hydrolysis of collagen.

These preferred points of hydrolysis were far less evident in Type B alkaline process gelatines than in Type A gelatines. Furthermore they found that the α, β, and gamma chain bands of Type B gelatine were far more prominent than those in Type A gelatine. This could now be attributed to the destruction of pyridinoline cross-links by liming freeing the basic collagen subunits to go into solution as Type B gelatine. In the Type A process, however, the presence of α-chains appeared to be a limited to the solution of acid-soluble collagen from young animal hide.

The amount of α-chain material in first extraction gelatines.

From the densitometer measurements it was evident that the amount of α-chain material in Type B gelatines decreased with increasing temperature of extraction or run number. However, the amount of α-chain material was surprisingly unaffected by animal age except for calf skin Type B gelatine (YSA/2) where extraction at 50°C seemed to produce almost pure α, β and gamma chain collagen. From the consistency of the amount of α-chain material in Type B gelatines extracted at 45°C it was concluded that at this temperature thermal proteolysis was negligible and protein going into solution was from new, minimally cross-linked collagen. The amount of first extraction gelatine available (after 4 to 6 weeks lime sulphide conditioning) from old animal hide was about 10 % of the total collagen which would be a measure of the collagen replacement rate in bovine hide.

Third extraction gelatines.

These gelatines evidently contained very low levels of the collagen subunits, hence it was concluded that these gelatines were truly protein with a random molecular weight profile. From the quality parameters it was evident that these gelatines could also have excellent properties like YSA/3 with a Bloom gel strength of 286 g and viscosity of 47 ms. This was a clear demonstration of the difficulty of relating electrophoretic profiles to physical quality parameters.

The 82 kD component of gelatine.

Gels 12 and 13 showed that all gelatines seemed to have had a significant amount of this component which could only be interpreted as indicating that there was a thermally unstable peptide bond some 18% down the length of the

α-chain or in the vicinity of amino acids in positions 180 or 820. It was significant that of the preferred splitting points found by Müller & Heidemann (1993), three were at amino acid number 779 of the α1 chain, one each was at No 208 and at 791 of the α1 chain and one was at No 838 (from the N terminal end) of the α2 chain.

Other known causes of colour in gelatine.

Other than the use of strong acids in the acidulation of limed hide, the only other known cause of dark colour was the presence of iron as a contaminant. The effect of iron was shown to be measurable only at about 50 ppm. Currently the iron content of edible gelatines did not exceed 50 ppm.

Based on the knowledge gained over the years it could be said that chromium was the only other inorganic contaminant which could contribute to colour and only at levels which were well above the 0 to 5 ppm currently found in edible gelatines.

The amino acid analyses on pale and dark gelatines provided by Stevens and Stevens (1992) did not show any significant change in lysine content with colour hence it was confirmed that a very small number of cross-links had a very marked effect on the properties of collagen as indicated by Dyer *et al.* (1991). The well known conversion of arginine to ornithine by alkaline treatment was also confirmed. The bonding of chromium to glutamic and aspartic acids in chrome tanning could only be seen as a small reduction in the aspartic acid content of gelatine from this source.

Finally it was worth mentioning that samples of dark and pale gelatines were given to an Australian laboratory that used Fourier Transform Infra-red analyses on a regular basis. They did observe differences in the spectra but were unable to interpret the differences in a meaningful way.

Conclusions and Recommendations.

1. Gelatine Colour.

a. On the main subject of this study it has been shown that at least in part the colour of gelatine was the result of the Maillard reaction *in vivo*. Hence, much of the colour of gelatine was a function of the age of the animal at

slaughter. As a result the prevention of this colour formation or its elimination from the gelatine did not seem to be practical.

b. Dark gelatine was a comparitavely rich source of the pentosidine cross-link and could be used for further study of this phenomenon. For example, the confirmation and location of the natural pentosidine cross-link in the helix or the telopeptide, as was done by Kuypers, Tyler, Kurth and Horgan (1994) for the Ehrlich Chromogen, would be of interest . Furthermore, the elucidation of the difference in the glucose and ribose mediated cross-links could have far-reaching significance.

c. It has been shown that there was at least a second source of colour in gelatine which was largely associated with lower quality product. This colour was not associated with fluorescence and it was removable by anion-exchange. It was felt that this would be a profitable subject for further study both from the point of view of the structure of the chromophore and its formation and also its inhibition.

d. This study was limited to gelatine produced by a liming process followed by sulphurous acid acidulation. It was known that the use of a strong acid in place of sulphurous acid had a deleterious effect on colour. Hence, the characterisation of the sulphurous acid bleached chromophore would be of interest.

e. One of the objectives of this study was to elucidate the reason for the pale colour of gelatine from chrome tanned leather produced by the SPA process (Drew, 1930). No light was shed on this phenomenon and it was felt that research in this direction would be most likely to lead to the desired gelatines of low colour.

f. There was no evidence from this study that the alkaline stage of the gelatine manufacturing process was in any way responsible for the dark colour of alkali process gelatine. In fact this study showed that alkali process Type B gelatine from calf skin (YS experiments) produced as good if not better gelatine colour than did the traditional acid process for Type A gelatine (CALF-A experiment). In fact all the evidence indicated that alkali treatment had no effect on gelatine colour and it was felt that the indications were that further studies in this area will be futile. There was however evidence from Hains (1984) that the treatment that collagen received between abattoir

and gelatine manufacture could be significant especially if processes were allowed to occur that caused the liberation of reducing sugars.

2. Gelatine colour measurement.

This study produced a commercially viable method for the instrumental and objective measurement of edible gelatine colour. It will be interesting to see whether the industry adopts the technique.

3. Hide conditioning.

The studies did much to characterise the variances in response to the alkali conditioning process, of collagen from animals of various ages . Further study of the variances in the alkali process would be valuable from the point of view of the manufacturer's ability to control the process. Currently it would appear that the traditional liming process was giving way to faster conditioning processes involving stronger alkalis.

This study showed that the apparent inverse correlation between gelatine colour and Bloom gel strength or viscosity was only valid for gelatine extracted from old animals. From young animals the colour of the gelatine was remarkably invariant.

4. Gelatine Fluorescence.

The fluorescence study established the correlation between the cross-linking of hide due to the Maillard reaction and animal age. The study also provided evidence that the source of the sugar for the Maillard reaction was in fact glucose rather than ribose, as suggested by Sell and Monnier (1989). The implications of the Maillard reaction cross-linking for gelatine extractability and quality was discussed. Further research on the correlation of extractability and fluorescence could be of great value to the manufacturer for the control of the process, in that the age of the animal at slaughter could be determined.

5. Gelatine Electrophoresis.

The electrophoresis of gelatine had been used for controlling quality in sensitive applications like photographic emulsions (Koepff, 1984). In this

study, the effects of animal age and extraction temperature were clearly shown by use of this technique. From the point of view of quality control it appeared that the technique could also be used to distinguish between Type A and Type B gelatine, however pI determination would be a simpler approach to that problem.

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