CHAPTER TWO. The Occurrence of Dark Coloured Gelatine.

INTRODUCTION.

The aim of this study was to determine the principal factors influencing the production of dark gelatine colour.

From Davis Gelatine Industries (DGI) production statistics it was known that paler gelatines were produced at the start of each day's production. This gelatine was extracted at the lowest temperature (45-50°C) and also had the best Bloom gel strength and viscosity as determined by British Standards Institution (1975) (BS) methods. By increasing extraction temperature more gelatine could be extracted from the raw material, however, the Bloom gel strength, viscosity and colour deteriorated as shown in Table 1.

Table 1. *The variation in gelatine colour with Bloom gel strength and viscosity.*

Bloom[2] (g)	Colour (DGI[1])	Clarity (DGI[1])	Viscosity[2] (ms @ 60°C)
269	7.5	11.0	35.0
247	9.0	9.2	32.4
220	11.0	10.0	31.1
182	12.6	9.9	27.0
145	13.3	10.0	22.2
66	14.8	10.8	18.9

Bloom gel strength / colour correlation coefficient r = -0.95

[1] Colour and clarity as determined by the DGI methods.

[2] As determined by BS 757 (1975) methods.

The best gelatine colour of 7.5 was variable between 6 and 9 and the reason for the variation was not well defined. In the laboratory "splits" (pg 2) from the tannery gave the best coloured gelatine often in the range 4 to 4.8 and the reason was thought to be associated with the tannery pretreatment of the material. However, although the tannery process was well known the colour

effect could not be reproduced using the whole hide. Also, from experience, even a colour of 4 was inferior in comparison with pigskin gelatine where colours of ≤ 3.2 were not uncommon.

Based on the observation that the best bovine hide gelatine colours appeared to come from tannery splits, there was a feeling that gelatine colour must be associated, at least in part, with the alkali conditioning process. Many attempts to prove this theory had failed or the experimental results had been misinterpreted because of deficiencies in the "twinned" experiment method. In this method face pieces or masks were divided into halves and the difference between the gelatines from the two halves could be attributed to differences in the process. A critical evaluation of the results from two different twinned experiments suggested that they could not be compared as the raw materials were not identical.

In leather manufacture, the leathers from different parts of the same hide were different - thin from the belly, thick from the butt, and of intermediate thickness from other parts. However, from the gelatine manufacturer's point of view this may not be as important. Hence, a series of experiments was conducted in which the same weight of hide from faces (the normal hide used for gelatine manufacture) and the rest of the hide were processed in parallel (by identical methods) to ascertain what differences may exist in yield, extractability and gelatine properties. This study showed that hide from the same animal was in fact a uniform (invariant) source of gelatine.(Experiments GF, GR, SF, SR)

Based on this finding the following studies were undertaken:

1. The conditioning process was investigated to determine whether liming time was a factor in gelatine colour and whether variances in the concentration of sodium sulphide had an effect on gelatine colour.

2. The hide structure was investigated by processing hide in a tannery. The flesh, corium split and grain layers were recovered and converted into gelatine.

3. To investigate the correlation between animal senescence and gelatine colour, animals of various ages and breeds were examined to determine the effects of these factors.

Heavy metals as a source of colour.

Gelatine, being a protein, was an amphoteric polyvalent polymer with the ability to complex polyvalent ions. Tanning made use of the ability to complex polyvalent cations as the complexes resisted microbiological degradation. It was possible therefore, that complexed cations could be responsible for the colour of gelatine. It was known that gelatine formed complexes with aluminium, zirconium and titanium and these complexes were used in the production of colourless leathers. Calcium was also thought to form a complex with gelatine which modified its gelling properties but there was no evidence for calcium forming a coloured complex (Cole, 1980).

Table 2. *The effect of iron contamination on gelatine colour.*

Iron added. ppm.	Davis Colour.
20	4.4
70	5.2
220	8.5
520	13.0
720	14.5

From Williams (1957).

Known coloured complexes formed by collagen or gelatine and cations were those formed with chromium and iron. The Cr(IV) complex used for the production of chrome tanned leather imparts a pale blue-green colour to collagen or gelatine which in gelatine was just visible at 200 ppm. As production gelatines had chromium contents well below 10 ppm and as the colour imparted by chromium was so different to the normal colour of gelatine it was decided that further investigation of this cation was not warranted. Iron on the other hand was known to impart red-brown stains to leather and colour to gelatine. From a study at DGI (Williams, 1957), it was known that low levels of iron contamination (50 ppm) had a negligible effect on gelatine colour. Higher levels of contamination could have a marked effect as shown in Table 2.

Linear regression analysis of the data in Table 2 gave the following result:

DGI Colour = 4.5 + 0.015 x Fe ppm. r = 0.988.

Because of the potential significance of iron contamination to this study it was decided to reinvestigate the effect of iron contamination on gelatine and to analyse different coloured gelatines from old and young animals for iron contamination.

The effect of colour on amino acid analysis.

It has been mentioned elsewhere that gelatine from chrome tanned leather was of an exceptionally pale colour. Heidemann (1982) had shown that in tanning, chromium was complexed by the glutamic and aspartic acid side chains of collagen. When gelatine was made from chrome tanned leather, the collagen was extracted at relatively high temperatures (80° to 100°C), and at approximately pH 9. The gelatine contained less than 10 ppm chromium, and the original (approximately 3%) chromium in the leather remained as an insoluble residue after extraction. From this it appeared that gelatine from chrome tanned leather could be low in glutamic and or aspartic acid residues. Furthermore, as this study progressed it became evident that there was an inverse correlation between animal age and hide extractability and a correlation between animal age and colour. Hence, it seemed probable that there could be a correlation between cross-links and gelatine colour. As lysine seemed to be involved with the formation of most if not all collagen cross-links (Dyer, Blackledge, Katz, Hull, Adkisson, Thorpe, Lyons and Baynes, 1991) it seemed reasonable to try and show a correlation between colour and amino acid analysis of gelatine. This prompted the submission of appropriate samples of gelatine to a consultant for amino acid analysis.

METHODS.

Lime slaking and conditioning liquor preparation.

The required quantities of quick lime (CaO), usually 640 g, and commercial sodium sulphide (60%) flakes (sulphide), were combined in a 5 l beaker. Two point six litres of water was added and the mixture was stirred with a rod until hot (approx. 60°C) and gelatinous. The mixture was then allowed to stand for about 1 h before being transferred to a 40 l plastic bin and diluted with water to 20 kg. After standing for at least 1 h, 10 ml aliquots were pipetted from the supernatant for sulphide determination using the Leather Industries Research Institute (LIRI) method for sulphide determination.

Preparation of hide for conditioning.

In this study, salted bovine hide was the starting material. In the few cases where green (fresh/untreated) hide was received it was salted by tumbling with 50% (w/w) coarse salt for at least 18 hr, after which it was drained for about 72 hr. Salted hide was stored in a sealed plastic bag to prevent drying and at ambient temperature, until required for conditioning. In only one instance, where the difference between green face pieces and the rest of the hide was being investigated, was refrigerated green hide put into process immediately upon receipt.

Two stainless steel tumblers were available for processing hide. These consisted of cylindrical vessels of about 200 l capacity which were rotated mechanically about their diameter at about 15 revolutions per minute. Each vessel had a door in its side which was used for adding hide and chemicals. The door could be closed with a perforated stainless steel plate which allowed drainage as the drum rotated. The perforated plate could be covered with a sheet of rubber if the drum was to be used without drainage. Furthermore, the axles of the drum were hollow allowing for the introduction of water or chemicals during rotation. In addition, the drum was fitted with internal "raisers" which ensured mixing during rotation.

Whole salted hides were cut into approximately 100 x 100 mm pieces which were then placed into a tumbler. The door was closed with a perforated plate and the hide was tumbled for 15 to 30 min to remove loose salt and to mix the pieces thoroughly. The hide was then weighed into aliquots, sealed in plastic bags and stored at ambient temperature until required. Before starting an experiment with salted hide the hide was washed as described later. The hide was then removed from the tumbler, allowed to drain for at least an hour, weighed and then placed into prepared conditioning liquor.

Hide washing.

Hide was placed in the tumbler. The door was closed. The tumbler rotation was then started as was the continuous flow of water. Washing (by decantation) was continued overnight (16 hr) unless otherwise stated.

Hide conditioning.

The quantities of hide and conditioning liquors used are stated in the experimental detail in the ADDENDA. The hide was placed in conditioning liquor in a constant temperature room (22°C) for the required conditioning period of 1 to 10 weeks. During this period the bins were kept covered with a polythene sheet to minimise losses due to evaporation. Three times a week (on Mondays, Wednesdays and Fridays) the bins were agitated by hand which was protected by an elbow-length PVC glove. The temperature of one or two bins was measured, using an electronic digital thermometer and recorded. The average liquor temperature for the conditioning period was used as the conditioning temperature. At the end of the conditioning period the bin was weighed for the determination of losses due to evaporation. A sample of the spent conditioning liquor was taken for analysis and then the contents of a bin were tipped into a stainless steel tumbler for washing. The washed hide was then transferred to a clean 40 l polyethylene bin for acidulation.

Acidulation.

After washing the conditioned hide was covered with 5 x 20 l lots of 0.1M sulphurous acid solution of at least 8 hr duration each. Acidulation usually lasted for 4 days which gave complete acid penetration of the hide. On the 5th day, the hide was washed with tap water for one hour using a hose placed in the bin. The hide was then soaked in the bin full of water for approximately 20 hr before the commencement of extraction. From experience, this process gave an extraction pH of about 3 and if the extractability of the hide was reasonable then the ash content of the final dry gelatine was approximately 1% W/W.

Extraction.

The soak water from the final acidulation step was sampled for pH determination before being discarded. The hide was packed into 5 l glass beakers which were filled to the 4.5 l mark with hot tap water and then placed in a thermostatically controlled waterbath set to the required extraction temperature (45°C etc.). During the extraction period the beakers were stirred gently by hand using a stirring rod. The temperature was noted using a digital thermometer with stainless steel probe. Towards the end of the extraction time fat was carefully skimmed (using a stainless steel ladle) and the volume was

recorded. At the end of the extraction time the liquor and skins were separated using a colander. The volume of liquor from each extraction was measured before commencing liquor processing. The residual skin was returned to the beakers for a second and third extraction, and finally placed in a stainless steel pot for boiling.

At the start of this series of experiments the boiled liquor was separated from the unextractable residue comprising epidermis, fat, hair, bone etc. The liquor volume was measured and a sample filtered through filter paper (Whatman 541) for the determination of gelatine concentration. This allowed the calculation of the amount of gelatine available in the raw material. Later it was found that on most occasions (if boiled for long enough) the boiled liquor could be filtered to a good clarity using Whatman GF/A paper in a Büchner funnel. If this liquor sample had a concentration of greater than 4 % gelatine then it could also used for colour determination by the normal DGI method, (after dilution). Thus, on many occasions, the colour of all the gelatine was available, making it possible to calculate the overall colours.

LIQUOR PROCESSING.

Resumé.

After the separation of the extraction liquor from the residual solids, the extraction liquor volume was determined. A sample (100 ml) was taken for pH determination. After filtration of the liquor, a further (100 ml) sample was used for the gravimetric determination of gelatine concentration.

After filtration, liquors were either concentrated immediately by vacuum evaporation or they were stored in a refrigerator (5°C) overnight and then processed. (After storage gelatine solutions were warmed in a 45°C bath before evaporation). After evaporation to approximately 10 % gelatine the solution was refiltered, the pH was adjusted and the gelatine solution was then set in a refrigerator. The set gel was cut into slices which were dried to about 10 % moisture content in a current of air. The dry gelatine was then ground to a powder for analysis.

Evaporation.

The evaporator was a purpose built single effect, steam heated, rising film

glass evaporator connected to a water vacuum pump and a condenser (cooled by mains water). The evaporator had a capacity of 2 to 3 l condensate per hour (depending on the temperature of the cooling water) and was run at a maximum liquor recirculation temperature of 42°C.

Heavy liquor filtration.

After evaporation the liquor was refiltered by the heavy liquor procedure described below. In the few instances where the amount of gelatine extracted was low and filtration through paper pulp would have led to unacceptable losses, the heavy liquor was vacuum filtered through Whatman GF/A paper in a Büchner funnel.

Laboratory filtration procedure:
EQUIPMENT.
40 l plastic open top container (bin).
Sheets of cotton linters paper pulp 680 x 800 mm, ex Carlson Ford U.K. (Rosenmeyer.W.H & Co. Joubert Park, Johannesburg).
Water Vacuum Pump.
Pressure hose to connect vacuum pump to Büchner flask.

For light (approx. 4%) liquor filtration:

Büchner Flask - 10 l size.
Porcelain Büchner Funnel - 270 mm ID.
Rubber bung to attach the flask to the funnel.

For heavy (approx. 12%) Liquor Filtration:

Büchner Flask - 2 l size.
Porcelain/plastic Büchner funnel - 130 to 160 mm ID.
Rubber bung to attach the funnel to the flask.

PROCEDURE.

1. Sheets of paper pulp were torn into approximately 100 mm pieces and disperse in approximately 20 l water in a plastic bin. After soaking for about 15 min the paper pulp was rubbed between the palms of the hands and stirred into the water until all resemblance to the original sheets was destroyed.

2. A Büchner funnel was attached to a flask. The funnel was filled with a maximum amount of dispersed pulp. The vacuum supply was attached and as the water was sucked out of the pulp, hand pressure was applied to the pulp such that it formed a pad of uniform thickness. Once most of the water had been removed from the pad, the pulp was compressed as far as was possible using the fist or a porcelain pestle while continuing with the suction. After compression a light liquor filter pad was at least 50 mm thick and a heavy liquor pad at least 30 mm thick. The pad was washed with cold water until there was no loose pulp in the filtrate.

3. Immediately before starting filtration, the filter was washed with sufficient hot water (60°C) such that the surface of the funnel was hot to the touch. The surface of the pad was sucked dry and then liquor filtration was commenced. The water displaced from the filter was wasted and the filtered liquor was collected.

4. If concentration was to be determined on the filtrate then the liquor was divided into 3 lots such that the first two lots were used for "washing" the filter pad and equipment and only the third lot was sampled for concentration determination.

Heavy liquor SO_2 & pH adjustment.

In order to ensure compliance with the requirements of the National Specification for edible gelatine heavy liquors were treated with 5% H_2O_2 to an approximate excess of 30ppm as indicated by Merck (Merck (Pty) Ltd. Midrand) test strips. The liquors were then treated with 5% NH_3 solution to give a pH of 5 to 5.5 as indicated by Merck test strips.

Heavy liquor drying.

The heavy liquor in a glass beaker was gelled in a refrigerator (5°C). The gelled liquor was cut into slices which were placed on trays. The trays were dried in a constant current of ambient air in a purpose built drying tunnel. The dry sheets were ground to a powder in a Waring Blender. The physical properties of the ground gelatines were determined by BS 757 (1975) methods as well as in house methods for colour and clarity.

ANALYSES.

Unless otherwise stated the analytical methods used on gelatine were those of the British Standards Association (1975).

Spent conditioning liquor analysis.

The sample of each spent conditioning liquor was vacuum filtered through Whatman GF/A paper. Duplicate aliquots of the filtered liquor were pipetted into tared silica crucibles and dried at 105°C overnight. After cooling in a desiccator and weighing the crucibles were placed in a 550°C muffle furnace for 24 hr before cooling and reweighing to give the total solids and ash contents of the conditioning liquor. Duplicate aliquots were also pipetted for the determination of the final sulphide concentration using the LIRI ferricyanide method.

LIRI method for sulphide analysis.

This method, obtained from the Leather Industries Research Institute (Rhodes University, Grahamstown, RSA) was intended for the determination of sulphide in alkaline solutions. The sulphide was oxidised to sulphur by titration with standard potassium ferricyanide solution in the presence of a ferrous dimethylglyoxime complex as indicator. Sulphite was known to interfere and was removed by precipitation with barium chloride. Thiosulphate was known not to interfere under the conditions of the determination.

REAGENTS.

Potassium ferricyanide 0.05N: 16.4625 g/l (Analitical Reagent grade, dried at 105°C for 2 hr). (Solution kept in the dark was stable for at least 30 days).

Buffer: 200 g NH_4Cl
200 ml ammonia (S.G. 0.88) per litre.

Barium chloride solution: 12.5 g/l. (10 ml precipitates 0.3 g sodium sulphite).

Indicator: 10 ml 0.6% $FeSO_4$.
50 ml 1% dimethylglyoxime in ethanol.
0.5 ml conc. H_2SO_4.

PROCEDURE.

1. The sample was filtered through glass wool if it contained suspended solids.
2. To a 250 ml stoppered flask was add 20 ml buffer and 20 ml barium chloride solution.
3. To the flask was added a suitable aliquot (equivalent to about 0.04 g Na_2S) of alkaline sulphide solution, by pipette. The flask was stoppered, swirled, and allowed to stand for one minute.
4. Indicator solution (1 ml) was added and the sulphide was titrated with standard ferricyanide solution until the pink colour was converted to a green colour which persisted for 15-30 seconds.

1 ml 0.05N ferricyanide = 0.00195 g Na_2S.

Note. If it was necessary to standardise the potassium ferricyanide solution this could be accomplished by treating an aliquot (20 ml) with 2 g KI, 10 ml 30% sulphuric acid and 10 ml 20% zinc sulphate and titrating the liberated iodine with standard sodium thiosulphate solution using starch indicator.

Light liquor concentration determination.

The liquor was warmed to 40°C. Duplicate 10 ml aliquots were pipetted into weighed stainless steel dishes. The dishes were dried at 105°C for 48 hr. After cooling in a desiccator the dishes were weighed and the gelatine concentration (% w/v) at 12.5% (m/m) moisture content was calculated by multiplying the weight of dry gelatine by a factor of 11.4286 in order to express the result as commercial gelatine rather than anhydrous gelatine.

Determination of gelatine colour (DGI Method).

EQUIPMENT:
Fluorescent table lamp.
Sheet of white filter pulp.
1 matched pair of 100 ml Nessler Tubes.
3 x 100 ml measuring cylinders.
Waterbath. Thermostatically controlled at ca. 45°C.
Beaker of distilled water at ca. 45°C.

METHOD.

Gelatine colour standard (7.5g) was weighed into a Bloom gel strength bottle and dissolved as for the Bloom gel strength determination. This standard solution was measured using a measuring cylinder (60 ml) and diluted to 100 ml with warm distilled water. The dilute standard solution was transferred to the first Nessler tube, taking care to avoid the formation of bubbles, and ensuring a homogeneous mixture.

Melted Bloom gel strength sample (60 ml) of an unknown gelatine in another measuring cylinder was diluted to 100 ml with warm water and transfer to the second Nessler tube.

The Nessler tubes were compared down their length against white paper illuminated by the fluorescent light. Solution was poured from the darker tube into the appropriate measuring cylinder until a colour match was achieved.

If the colour value of the Standard was C_1 and the colour value of the unknown was C_2 then:

$$C_2 = C_1 \times (\text{Volume of Standard}) / (\text{Volume of Unknown})$$

Determination of gelatine clarity (DGI Method).

EQUIPMENT.

Nephelometer and glass cuvette. (ICM Turbidimeter. ICM. 163 S.W. Freeman, Hillsboro. OR 97123. USA)
40 NTU (National Turbidity Units) standard for the Nephelometer.

METHOD.

The Nephelometer was set to the to 0 to 100 scale. The 40 NTU standard supplied with the instrument was inserted into the sample compartment and the instrument was set to read 40 using the sensitivity adjustment. The Nephelometer cuvette was filled with the clarity standard gelatine solution (6.67%). Cleanliness of the cuvette was ensured by wiping with a paper tissue. The cuvette was then inserted into the Nephelometer cuvette holder. If the reading given by the standard was correct then the determination of the

clarity of the melted 6.67% Bloom gel strength samples was undertaken. The results were reported in NTU units (0 to 100) which could then be scaled to Davis Gelatine units (14.0 to 2.5):

$$\text{DGI Clarity} = 13.9 - 0.133 \times \text{NTU} \quad \ldots\ldots\ldots\ldots\ldots\ldots 1$$

Processing of analytical results:

In calculations using a gel strength, the square root ($\sqrt{}$) of the gel strength was used. The reason for this was that gel strengths were known not follow the laws of simple proportions but the $\sqrt{}$ (gel strength) was known to be closely proportional to concentration over a wide range of gel strengths, Veis (1964a), Jones (1977).

1). Overall quality.

Results were expressed as required by the methods, however for most extractions "overalls" were calculated. This procedure calculated the value of the parameter as if all the gelatine from a particular raw material had been composited as a single product. In order to do this the contribution of each gelatine from the raw material was taken into consideration according to the amount extracted. For example:

$$\Sigma\ (\text{Colour} \times \text{Mass}) \div \Sigma\ \text{Mass} = \text{Overall Colour}.$$

2). Corrected Bloom gel strength value and viscosity.

The reason for this calculation was that gelatine concentration had a marked influence on the particular values. Hence to simplify comparisons, the value at 12.5 % non-protein components in the starting gelatine, was calculated to give the "corrected" value (e.g. gel strength). (Non-protein components of gelatine were moisture and ash.)

$$\text{Corrected Gel Strength} = ((\sqrt{\text{Gel Str.}}) \times 87.5 \div \text{concentration})^2.$$
$$\text{Corrected Viscosity} = \text{Viscosity} \times 87.5 \div \text{concentration}.$$

3). Yield corrections.

Firstly as concentrations were expressed as gelatine containing 12.5% moisture it was necessary to apply a factor of 0.875 to obtain the yield on an anhydrous basis. Thereafter a factor of 0.95 was applied because in various trials it had been found impossible to recover more than 95% of the gelatine

indicated by volume and concentration determinations.

Iron analysis.

The method used was developed by Eastoe and Eastoe (1951) of The British Gelatine and Glue Research Association (BGGRA).

Silica crucibles were prepared by boiling in 32% HCl and then standing in the solution overnight. After rinsing with distilled water several times and drying, the crucibles were heated in a muffle furnace at 550°C for 1 hr, cooled and weighed.

Test Solution.

Duplicate 5 g samples of gelatine were weighed into crucibles. The gelatine was ashed in a muffle furnace at 550°C, overnight. The ash was cooled in a desiccator, weighed and then moistened with a few drops of distilled water. The ash was then digested with 5 ml cHCl on a hot plate until dryness was just achieved. Hydrochloric acid (N - 10 ml) was then added and the residue in the crucible was dissolved with gentle warming. The solution was transferred into a 50 ml volumetric flask through a Whatman 541 filter paper which was washed 3 times with distilled water. The flasks were then made up to the mark with distilled water.

Colour development:

10 ml test solution.
2 ml 10% hydroxylamine hydrochloride solution.
2 ml 2M sodium acetate solution.
2 ml 0.25% O-phenanthroline solution.

These were mixed in a 25 ml volumetric flask, which was then made up to the mark with distilled water. After 10 min the absorbances were read using a Jenway (Jenway Ltd. Dunmow, Essex. UK.) Colorimeter with No 3 filter (490 nm) and water as the blank.

100 ppm Fe-III solution:

Standard 1000 ppm Fe solution (5 ml) (ex SaarChem (Pty) Ltd. Krugersdorp. RSA.) was pipetted into a 50 ml volumetric flask. Potassium permanganate solution (N/50 - 4 drops) was added to impart a permanent pink colour. The solution was then made up to the mark with distilled water to give a 100 ppm solution of Iron-III.

Standard curve.

Suitable aliquots of 100 ppm Fe solution were used to prepare 50 ml solutions containing 0, 50, 100, 200 and 300 μg Fe, equivalent to 0, 10, 20, 40 and 60 ppm Fe in 5 g of gelatine.

Aliquots (10 ml) of these solutions were used for colour development as above. A standard curve was generated from which the iron contents of the unknowns could be read. Average results for the duplicate samples were reported.

Effect of iron on gelatine colour.

Four gram samples of a pale gelatine 155/1 were weighed into Bloom gel strength bottles. Various amounts of Fe solution (0, 0.8, 1.6, 2.4, 3.2, and 4 ml of 100 ppm Fe) were added to different samples. The samples were then diluted to 100 g with distilled water. After soaking the samples were dissolved in a 45°C waterbath. The colour of the solutions was then determined by comparison to 100 g if a 4% w/w solution of the colour standard with an ascribed colour value of 8, in 100 ml Nessler tubes. Three ml aliquots of these solutions were scanned using the Jenway Colorimeter in 1 cm plastic cells. The remainder of the gelatine solutions were used for pH measurement.

Amino acid analysis.

Stevens and Stevens (1992) (Applied Science and Technology, 169 Havannah St, Bathurst, NSW 2795. Australia.) were experienced in the amino acid (AA) analysis of gelatine and had worked for Davis Gelatine Australia on a number of occasions. Hence it was agreed to make use of their services as long as there were no prescriptions as to what had to be divulged about the

samples prior to analysis. It was decided to submit 6 samples as a preliminary trial as follows:

One pale gelatine from chrome tanned leather. Labelled "A".

Two pale gelatines from a young animal. YSA/1 and YSA/3. Labelled "B" and "C".

One pale duplicate sample. YSA/3. Labelled "D".

Two dark gelatines from an old animal. WT3/1 & WT3/3. Labelled "E" & "F".

Prior to the commencement of the investigation they were advised that the variation in amino acid analysis with gelatine colour was of importance as was the possible detection of minor peaks that could be attributable to cross-link residues.

Details of the methods used were not made available, however they advised that the sample preparation involved hydrolysis to reduce the protein to amino acids and the amino acid separation involved "Picotag precolumn derivatisation". The hydrolysis procedure caused destruction of methionine and cystine. Furthermore as histidine was a minor constituent with its peak close to glycine its quantisation was problematical.

The amino acid data was received in two lots. The first lot (Report "Interim Report on South African Gelatin Samples. Amino acid and crosslink analysis." dated 11.6.92) was on samples B to F and is designated B1 to F1 in ADDENDUM 13. This data did not contain results for the ornithine content of the gelatines.

The second lot of data (Report c:\wp51\gfwi\aacole dated 1 July 1992) contained duplicate results from duplicate hydrolyses of the gelatines. This data is designated A2 and A3 to F2 and F3 in ADDENDUM 13. Sample D3 was lost and the aspartic acid result for sample C2 was obviously in error.

The results received (Addendum C13) were entered in a Quattro Pro (Version 5. Borland International, Inc. 1800 Green Hills Road, Scotts Valley, CA 95067-0001, USA) spreadsheet. The means and relative standard deviations (% RSD) for each amino acid were calculated. The % RSD was the standard deviation as a percentage of the mean.

1. "Experimental Error" (EE) used the values for samples C and D only to give a % RSD. due to experimental error only, as the samples were identical.

2. "All" included all data. Thus if ALL-RSD was substantially larger than the EE-RSD it would indicate an effect due to the samples A, B, E & F.

3. "All - A" (All minus A) included all data except that for the chrome gelatines of sample A. Thus if All - A RSD was less than the ALL-RSD it would indicate an effect due to Chrome gelatine.

The data in ADDENDUM 14 was calculated from the data in ADDENDUM 13 by:

1. Correcting for the variable protein content of the samples due to variable moisture and ash contents. Each AA value was divided by the % protein and multiplied by 100. This gave the % (AA) by mass.

2. The results from 1 above were converted to moles amino acid by dividing mass of each amino acid by its molecular weight and multiplying by 100. This gave the number of moles of amino acid per 100 g protein. This value was then converted to moles of each amino acid per 100 moles.

Due to the obvious error in the aspartic acid value of gelatine C2 in ADDENDUM 13 this value was replaced by the average molar numbers of the aspartic acid values of samples C1, C3, D1 and D2, i.e. the value of 4.07 was used.

ADDENDUM 15 is a compressed form of ADDENDUM 14 in which the molar percentages for each sample were averaged. The mean and % RSD data were copied from the values in ADDENDUM 14. Table 18 was copied from ADDENDUM 15.

EXPERIMENTAL CODES.

Each series of experiments was given a code which was a mnemonic of the main variable/s for the series. For example:

CT = conditioning time.
G or S = green or salted hide.
ST = sulphide usage and time variables.
WT = winter temperature experiments.
3Y = Three year-old animal.

EXPERIMENT GR. Face Pieces versus the rest of the hide.
(GF, GR, SF, SR).

This experiment was designed to determine whether there were any significant differences between the hide of the head and the hide of the rest of the animal. The Animal and Dairy Sciences Research Institute (ADSRI) abattoir was asked to provide the mask of an animal and a piece of the hide of an equal mass in a plastic bag. This was done with 6 animals. The hide was stored in the abattoir cold room overnight. The next day the samples were checked for equal weights of mask and the rest. Three of the masks were salted in a tumbler (SF) and the corresponding "rest" pieces were salted in a second tumbler (SR). The 3 green face pieces (GF) and rest pieces (GR) were placed in lime/sulphide conditioning liquor immediately. For the detailed data on this experiment see Addendum C1.

EXPERIMENT CT. - The effect of conditioning time.

This experiment was designed to determine the effect of conditioning time on hide extractability, yield, and gelatine properties. A large salted hide was required to conduct the number of experiments envisaged and it was obtained from a hide merchant. The supplier could only advise that the animal was a Brahman and it had been reared on a "feed lot". From this it was surmised that the animal was approximately 18 months old at slaughter. This size of hide allowed conditioning experiments to be carried out for 1 to 6 weeks while keeping all other variables constant. There was a small amount of hide left over for examining the effect of replacing the conditioning liquors weekly for three weeks. For detailed data on this experiment see Addendum C2.

EXPERIMENT CTO. - Old animal hide and conditioning time.

This experiment was designed as a replicate of experiment CT to examine the effect of conditioning time on the hide of an old animal. A salted hide from a 13 year-old animal was provided by ADSRI. This hide was divided into four equal parts (within 5 days of slaughter) which were conditioned for 2, 4, 7 and 10 weeks. For detailed data on this experiment see Addendum C3.

EXPERIMENT ST1. - The effect of time and sulphide concentration on the conditioning of the hide of an old animal.

Sodium sulphide was considered to be a "sharpener" or an accelerator of the conditioning process. In the past, work had been done using the "twinned experiment" technique which had often lead to confusing results due to the variation in the raw material used between the pairs of each trial. In experiments CT and CTO it had been shown that conditioning time did not appear to have had a significant effect on gelatine colour. Hence, a statistically designed factorial experiment Montgomery (1985) was executed to evaluate the effect of varying both time and sodium sulphide concentration using the F statistic. F values were based on (variance of the means) / (mean variance due to a treatment). It was required that the experiments be conducted in random order, so, for random permutations of 9 numbers reference was made to Cochran and Cox (1957) from which it was found that the experiments could be started in a convenient random order #3, #1, #8, #5, #9, #4, #2, #6, #7 as shown in Table 3.

A Quattro Pro (*loc. sit*) spread sheet was used to calculate the F ratios for the variables of interest (Bloom gel strength value, yield, extractability etc.) using the formulas provided by Montgomery (1985) and Freund and Williams (1964a).

Table 3. *Factorial design of experiment ST.*

Time in Weeks	2	4	6
Sulphide Conc. in g/l			
1.5	#1(1-2)	#2(1-4)	#3(1-6)
2.2	#4(2-2)	#5(2-4)	#6(2-6)
2.9	#7(3-6)	#8(2-4)	#9(3-6)

Code #7(3-6) denoted:
Experiment No. 7;
Sulphide Concentration No. 3 (or 2.9 g/l);
Time = 6 weeks.

Details of this experiment are given in ADDENDUM C4. The raw material for this experiment was from 2 salted hides provided by ADSRI. Both animals were 12

years old at slaughter. 9 x 3 kg lots were obtained from the first hide to which was added 9 x 2.7 kg from the second. Random punchings were taken from each of the 9 lots for duplicate moisture and ash determinations.

Due to the poor extractability resulting from 2 weeks conditioning the "Boil" of Experiment #1 was conducted in 2 parts. After 15 minutes of boiling the liquor was separated from the hide to give the fourth liquor. After an additional 7 hours of boiling the final boil liquor was separated from the scutch residue to give the fifth liquor ST1-2/5.

EXPERIMENT ST2 (or WT). The effect of temperature and sulphide concentration on conditioning.

In experiment ST1 it was found that variation in sulphide concentration did not vary the degree of extractability of hide. This finding was at variance with the accepted DGI practices of decades and it was necessary to determine whether sulphide had any effect on conditioning at all. The fact that it was a depilatory was important from the point of view of production but it could be that the cost of the benefit was not justified.

Sodium sulphide was a reducing agent and the initial Maillard reaction was oxidative hence the role of sulphide could simply be one of inhibiting the Maillard cross-linking and darkening during the alkaline conditioning process by reducing the availability of oxygen to the system. To investigate the reduction theory it was decided to conduct a conditioning in lime only under nitrogen to reduce the oxygen tension of the conditioning system and determine whether this would duplicate the effect of sulphide.

To verify the lack of conditioning effect due to sulphide concentration it was decided to determine the effect of a 6:1 variance in sulphide concentration at winter conditioning temperatures which was far greater than the 2.5:1 variance normally used in production to "compensate" for reduced conditioning temperatures of winter.

Details of this series of experiments are in ADDENDUM C5. They were conducted on the salted hide of a single 12 year-old animal provided by ADSRI and the hide was divided into 5 x 4.0 kg lots.

For Experiment WT1 the lime slurry was made using water that had been boiled

and then cooled in full, sealed plastic bottles to prevent oxygen absorption. The hide was placed in a glass carboy. The neck was sealed with a rubber bung which was wired to prevent loosening. The tap at the bottom of the carboy was also wired and after adding the lime to the hide. It was used to evacuate the air above the lime using a water vacuum pump for 1 hr. The vacuum was then replaced with nitrogen from a cylinder. On the first day the evacuation and nitrogen flushing was repeated once and then again on day 2, day 3, day 6 and day 13.

During conditioning it was noted that the carboy surface temperature was always 2° to 3°C higher than the conditioning liquors in the bins. This could have been due to the elevation of the carboy relative to the heaters used to control the temperature of the room and could have caused a slight enhancement in the degree of conditioning.

In summary:
WT1 was a pure lime anaerobic conditioning.
WT2 was a pure lime aerobic conditioning.
WT3 was a control conditioning with a normal 1.8 g/l Na_2S at 22°C.
WT4 was a conditioning with 1.8 g/l Na_2S at 12°C.
WT5 was a conditioning with 11.1 g/l Na_2S at 12°C.

EXPERIMENT YS. The effects of animal age - 10 month old animal salted hide from ADSRI.

As experiments CTO, ST1 and ST2 had been conducted on old animals' hide it was decided to do a comparative experiment on young animal's hide. Hence, a suitable large hide was obtained from ADSRI.

Details of this experiment are in ADDENDUM C6. As the animal was so young it was decided to condition with a nominal 2 and 6 g/l Na_2S, for 2 weeks, (YSA & YSB) and 3 weeks, (YSC & YSD). A four weeks conditioning with no sulphide, (YSE) was also conducted to confirm the effect of sodium sulphide on conditioning.

EXPERIMENT KTO. Gelatine quality from various layers of the salted hide from a 12 year-old animal.

It was often maintained that the best gelatine colours were obtained from "splits" raw material. To investigate this perception it was necessary to

split a hide as in tanning into the flesh layer, the corium or "split" layer and the grain layer. The help of a tannery with equipment for splitting a hide after pretreatment was obtained. In a previous trial (Cole, 1989) with similar aims, a hide provided by the tanner was used. The extractabilities indicated that the skin was from a young animal so the colours of the gelatines recovered were pale but the experiment yielded the following information:

Flesh split yielded gelatine of darkest colour. (8.5 to 12.0)
Middle split yielded gelatine of palest colour. (4.0 to 5.2)
Epidermis split (grain) yielded gelatine of intermediate colour. (5.2 to 5.0)
The whole hide yielded gelatine with colour 5.2 to 5.0.

Furthermore, from this experiment it was concluded that all portions of the hide should receive similar conditioning treatments and if an old animal was used perhaps the colour differences would be more pronounced. Hence a salted hide from a 12 year-old animal was obtained from ADSRI.

The tannery operations were conducted by Kwiktan, Delporton, Krugersdorp as follows:
Tumbler washed overnight to rehydrate the hide.
Treated with 3% w/w (60%) Na_2S + 3% $Ca(OH)_2$ in a 100% float:
Drummed 3 hr and then every 30 min. for approx. 69 hr.
Washed by decantation with continuous water flow for 10 min.
The hide was then cut into halves down the backbone and one half was split into flesh, middle and grain splits.

The details of this series of experiments are recorded in ADDENDUM C7. The 4 lots, whole hide, and flesh, middle and grain splits, could not be treated simultaneously, so parts KTO3 and KTO4 (middle and grain splits) were placed in lime only for 7 days and then the sulphide was added in order to obtain substantially the same conditioning on all four parts. Conditioning procedures were otherwise normal.

As there was no hair on the hide during conditioning this experiment afforded the possibility of determining how much colour the conditioning liquor removed from the hide during conditioning. When the spent conditioning liquor samples were filtered, a portion of the liquor was used for the determination of its absorbance (colour) using the Colorimeter with the No 2 (470 nm) filter and water as the blank. Furthermore, as there was no hair, the sulphide

determination, performed two hours after the addition of sulphide solution to the limed hide mixtures KT03 and KT04, gave good estimates of the initial sulphide concentration.

EXPERIMENT CALF-A. Type A gelatine from calf skin.

It was well known that pigskin gelatine is made from animals that were slaughtered at about 6 months of age. From this study it had become clear that young animals gave paler gelatines than old animals. Hence there was a need to determine the quality of gelatine that could be produced from calf skin by the acid process. Reich, Walther, and Stather (1962a,b) had covered the "acid process" in detail, however, they did not concern themselves with the colour of the gelatines produced. It was of interest to note that they concluded that the "acid conditioning process" was in fact no more than an acid treatment to equilibrate the skin to the required acid extraction pH and to quote "with the acid process there is no possibility to compensate for age-related differences in the stability of skin collagen as is the case with the alkaline conditioning process".

In order to show whether there was any difference in colour between calf skin Type A gelatine and pigskin gelatine, three salted calf skins were obtained from a merchant. The skins had Friesland black and white colouring and from their size it was evident that they were from calves of less than six months of age. One skin still had the umbilical cord attached indicating that it was from an animal of between one week and one month of age. The skins were stored in a sealed plastic bag for seven days to equilibrate the moisture content. The skins were very hairy and so to make liquor drainage efficient and to maximize yield and recovery it was decided that they should be subjected to a very quick tannery dehairing process the details of which are given in Addendum C8. This was followed by acidulation with 0.1N sulphuric acid overnight using the method of Reich *et al.* (1962a). The hide was then washed with tap water (pH 7.5 to 8) by upflow, in a static washer for 2.5 hours and soaked in 20 l water until the next day to give an extraction pH of close to 4.0. Extraction and liquor handling were normal, except that after filtration it was decided to raise the pH of the light liquors by passing a portion of the liquor through a mixed bed ion-exchange column and then mixing this with untreated liquor to obtain a liquor pH of approximately 5 before evaporation. From experience it was known that paper pulp filtration could have a marked

effect on gelatine liquor pH after mixed bed ion exchange which was presumably due to the ion exchange effects of cellulose. It was concluded that this was the reason for the relatively high pHs of the final gelatines. In the case of the third extraction too much liquor was deionised hence the need to add sulphuric acid to the heavy liquor after evaporation.

In order to determine the isoionic point of the gelatines produced the ion-exchange method described by Veis (1964b) was used. A 4.5 cm diameter glass column was charged with 400 ml of Rohm & Haas (ACIX, Germiston) mixed bed MB3 ion exchange resin. After warming with 2 bed volumes of warm distilled water the column was used to treat 600 ml of 1% (w/v) gelatine solution at a flow rate of about 5 bed volumes per hour. The last 50 ml of eluate was collected and the pH determined. This pH was taken as the isoionic pH of the gelatine.

EXPERIMENT 3Y & 6Y. The effect of 3 & 6 years old animal's hide on conditioning response and gelatine quality.

It was decided that it was necessary to include animals of ages between 18 and 144 months to complete the data on the effect of animal age on the response to conditioning and resultant variances in gelatine quality. Furthermore it would be important to know whether breed would affect the results significantly. Hence, salted hides from Friesland animals of 40 and 78 months of age were obtained from ADSRI for inclusion in the study.

This experiment was also used to confirm the previous findings with regard to the role of sodium sulphide in lime-sulphide conditioning. The 4 week conditionings 3Y4 and 6Y4 were split into two parts, part A being conditioned with 2 g/l sodium sulphide and part B with 4 g/l sodium sulphide. The six week conditionings (3Y6 and 6Y6) were similarly split into the A part with 2 g/l sodium sulphide and the B part with no sodium sulphide. Details of this series of experiments are in ADDENDUM C9.

EXPERIMENT 5Y. Effect of age and breed on conditioning response and gelatine quality.

The hide from a 58 month old Chianina cow was available from ADSRI and was included in the study. Details of this series of experiments are in ADDENDUM C10.

EXPERIMENT INO. A 12 year-old Inguni cow's hide was made available by ADSRI for inclusion in the study. Details of this experiment are in ADDENDUM C11.

RESULTS and DISCUSSION.

EXPERIMENT GR.
(Green face pieces v/s the rest of the hide.)

In the past the procedure of "twinning" hide had been found to give two halves which responded very similarly to processing. In the procedure each piece of hide was halved as equally as possible using the same approach as in tanning where a hide could be "sided" by halving down the backbone to give two halves which would be expected to behave similarly in processing. To the tanner however different parts of the hide were not identical. For example the belly area was thin and the haunch area was thick. The face or mask was of very variable thickness and was never used by the tanner, nor were the irregular pieces covering the legs and tails. It was considered that if hide from two closely similar areas from "twinning" behaved similarly towards processing then perhaps the whole hide might be considered a uniform piece of raw material as far as gelatine manufacture was concerned. This experiment was designed to test the theory that the hide of a single animal was a uniform raw material from the point of view of gelatine manufacture. As a further consideration, it was known that the salting of hide caused it to exude an amount of serum equivalent to some 15 % of the weight of the raw hide (see Introduction). This exudate would contain salt soluble proteins (Na, Phillips and Freire, 1989) and as a result salted hide could be different in response to green hide, hence both types of material were investigated.

From a comparison of the detailed data in ADDENDUM C1 on green hide - GR and GF - the difference in 45°C extractability of 6% might seem "significant" but in light of the virtually identical yields and identical gelatine properties of corrected Bloom, viscosity, and colour the difference in extractability was considered to be due to random variation.

In the salted hide comparison, SR and SF, the differences in extractabilities and yield were negligible but there was an apparently significant difference in the corrected Bloom gel strength values of 12 g on the first run gelatines. However, at 280 g Bloom gel strength the standard deviation of the

determination was known to be of the order of 4 g, hence, the significance of a Bloom gel strength difference of 12 g was minimal. From the detailed data in ADDENDUM C1 and from experience of the errors inherent in the estimation of extractability, yield and quality parameters, it was concluded that there were no significant differences in the response of either green or salted hide to processing or to the quality of the gelatine produced. Hence, it was accepted that the hide of a single animal was a uniform raw material from the point of view of gelatine manufacture. However, in using this finding it was decided that in all cases the hide would firstly be reduced to small pieces which would then be randomised by tumbling before the hide was divided between the parts of an experiment. Finally, it was considered that the experiments which followed, especially experiment ST, completely vindicated the assumption that the hide of a single animal was a uniform raw material.

EXPERIMENT CT.

In the absence of evidence to the contrary it was presumed that the conditioning process must play a role in determining the colour of gelatine produced. Conditioning for 1 to 6 weeks was considered to be adequate to show the expected effects on colour and detailed data on extractability and yield would be an added benefit.

From the detailed results in ADDENDUM C2 extracted into Table 4 below it was evident that the colour of gelatine was largely invariant with respect to the time of conditioning. Furthermore, the small change in gelatine colour with extraction temperature was totally at variance with experience and as a result it had to be concluded that gelatine colour was almost entirely a function of animal age. Finally, the observation that part CT7 gave the same gelatine colours as parts CT1 to CT6, even after weekly changes in conditioning liquor, was a very strong confirmation that "conditioning" played no direct role as far as gelatine colour was concerned.

Table 4. *The effect of hide conditioning time on the (DGI) colours of the extracted gelatines.*

Exp. No	Conditioning. Time in Weeks	GELATINE COLOUR				
		1st Extract	2nd Extract	3rd Extract	4th Extract	Overall
CT1	ONE	5.6	5.6	5.2	6.8	6.5
CT3	THREE	5.2	5.6	6.4	7.2	6.2
CT7	THREE	5.6	5.6	6.4	-	-
CT5	FIVE	6.0	6.4	6.4	8.0	6.4

- Not available.

This experiment was of great value because it illustrated the many effects of conditioning time:

The drop in the sulphide content of the conditioning liquor of 1.8 g/l down to 1.1 g/l after 2 weeks and 0.8 g/l after 6 weeks was thought to be significant. The exact role of sulphide during conditioning was the subject of speculation, however, the Maillard reaction was known to be associated with oxidation so it was considered that reducing conditions during conditioning should be significant and this was investigated in experiments ST and WT.

The change in conditioning liquor volatile solids (VS) (dissolved organic matter) with conditioning time from 0.8% after 2 weeks to 1.4% after 5 weeks was also of interest as it could indicate loss of "collagen contaminants" from the hide. However, as the increase in VS was accompanied by a small drop in yield from 27% to 26% the change in conditioning liquor volatile solids could be due to losses of collagen. However, the similarity in VS between CT5 and CT6 with a further loss of yield indicated that in fact loss of collagen into conditioning liquor was not the cause of VS but rather it was most probably (see experiments 3Y and 6Y) due to the dissolution of hair caused by the presence of sulphide. From this it followed that the hair content of the hide was about 288 g or 5% and also that these "hair burn" contaminants of conditioning liquor had no effect on the colour of the final gelatine.

From the point of view of the proximate analysis of hide it was

noteworthy that the gelatine yield on dry hide substance averaged 79% which could be taken as an estimate of the collagen content of the "hide substance".

EXPERIMENT CTO.

To confirm the indications in the author's thesis (Cole, 1986) that animal age was a cause of gelatine colour experiment CT was repeated using the hide of a 13 year-old animal. The detailed data is recorded in Addendum C3.

This data showed the normal darkening in gelatine colour as extraction temperature increased and confirmed the tentative conclusion that animal age was a most important contributor to gelatine colour. Furthermore, with this experiment it was observed that the first extraction gelatine colour increased with conditioning time as did extractability but most importantly the overall colour, that is the colour expected from combining all the gelatine extracted from the hide, was substantially constant and independent of conditioning time. This indicated that the most easily converted collagen, which was presumably the most recently produced collagen, yielded the best gelatine colour but in a relatively small quantity and as the extractability was increased by conditioning so the older collagen was caused to dissolve yielding darker gelatine.

The conditioning liquor volatile solids (VS) in this instance reached a maximum of 358 g indicating 8% hair plus alkali soluble organics on the sample weight. This represented some 22% of the anhydrous hide substance.

The average anhydrous gelatine yield was only 54% and in this instance the fat recovered during extraction was between 5 and 20 ml from approximately 1450g of anhydrous hide substance.

EXPERIMENT ST1.

Experiment ST1 was designed to investigate the effect of sodium sulphide on conditioning. There were no clear indications of the effect of sodium sulphide. It was thought to have had a "sharpening" effect on conditioning as it clearly improved extractability when compared to conditioning with lime only. In removing the hair from the hide it also facilitated processing. Finally if the Maillard reaction was in any way responsible for the colour of

gelatine then Na_2S could possibly play a role in preventing *post mortem* colour development. The detailed results are in Addendum C4.

The design of the experiment allowed for statistical assessment of the effect of time and sodium sulphide concentration on gelatine colour and the other attributes/parameters. A Quattro Pro spreadsheet was developed to perform the necessary calculations as shown in Tables 5 and 6.

Table 5. *Analysis of variance - Two factor factorial design. First extraction colour.*

Na_2S g/l	TIME IN WEEKS			SUM	MEAN	VAR.	MEAN VARIANCE	
	2	4	6					
1.5	6.4	10.0	10.7	27.1	9.0	5.3		
2.2	11.4	8.9	11.4	31.7	10.6	2.1	2.8	
2.9	10.0	9.4	11.4	30.8	10.3	1.1	$F_{SULPHIDE}$ = 0.7	
					10.0	0.7	(0.7 X 3 / 2.8)	
SUM	27.8	28.3	33.5	89.6				
MEAN	9.3	9.4	11.2		10.0	1.11		
VAR.	6.7	0.3	0.2					
MEAN VAR.		2.4					F_{TIME} = 0.4 (1.11 X 3 / 2.4)	

Table 6. *Analysis of variance - Two factor factorial design. Overall colour*

Na_2S g/l	TIME IN WEEKS			SUM	MEAN	VAR.	MEAN VARIANCE	
	2	4	6					
1.5	18.1	14.2	13.1	45.4	15.1	6.9		
2.2	14.6	15.0	15.8	45.4	15.1	0.4	2.9	
2.9	14.7	14.3	14.6	45.5	15.2	1.4	$F_{SULPHIDE}$ = 0.0	
					15.1	0.0	(0.0 X 3 / 2.9)	
SUM	47.4	43.5	45.4	136.3				
MEAN	15.8	14.5	15.1		15.1	0.42		
VAR.	4.0	0.2	3.2					
MEAN VAR.		2.5					F_{TIME} = 0.52 (0.42 X 3 / 2.5)	

From the low values of $F_{0.05\ (2.2)} < 19$ (Freund and Williams, 1964b) in both tables it was concluded that there was no statistically significant correlation between conditioning time, or sulphide concentration and first extraction colour or overall colour.

Comparison of the colours of ST1-2/4 and ST1-2/5 (17.8 and 22.8) boil gelatines in ADDENDUM C4, showed the deterioration in colour with the progress of extraction as was normally experienced in production. Hence, it appeared that the production of dark gelatines at the end of the production cycle was a consequence of the hide from old animals being part of the raw material mixture.

EXPERIMENT ST2 / WT.
(Old animal and the effects of sulphide and temperature).

It was accepted that the Maillard reaction was an oxidation reaction and that SO_2 could inhibit colour formation (Monnier, Sell, Miyata and Nagaraj, 1990). Hence, it was proposed that sulphide in conditioning might also have had an effect on colour due to its reducing properties. This experiment was designed to compare conditioning with lime only under nitrogen against aerobic lime only and lime plus sulphide. As there was material available it was decided to investigate the effect of temperature as well, by conditioning with two levels of sulphide under ambient winter conditions. Based on the previous findings that old animal hide yielded the darkest gelatine it was considered that a hide from an old animal would best exhibit any effect due to the treatments, so the hide of a 12 year-old animal from ADSRI was used. Details of the results are in ADDENDUM C5.

Comparing the overall colours of the WT1 (15.7), WT2 (12.7) and WT3 (14.3) gelatines it appeared that liming with lime under nitrogen (WT1), gave the worst overall colour. The same observation was made with respect to first extraction colours!

Comparing WT2 and WT3 the conditioning enhancing effect of sodium sulphide was evident from the increase in the first extraction proportion of WT2 (aerobic lime only) from 4.1% to 10.4% in WT3. The effect of sulphide on colour appeared to be one of darkening as shown by the increase in overall colour from 12.7 with lime only to 14.3 with lime-sulphide conditioning. However, as

this was only just larger than the 1.5 units accepted as the error of visual determinations of colour, it would need confirmatory data before it could be accepted as significant. Nevertheless, it could be concluded that sodium sulphide in conditioning had no beneficial effect on gelatine colour.

Comparing WT3 conditioned at 22°C and WT4 conditioned at 12°C, the effect of temperature on conditioning could be clearly seen from the drop in first run extractability from 10% to 4% and an increase in gelatine recovered in the boil from 49% to 71%. Comparison of the extractability data of WT3 and WT4 with ST1-2 and ST1-4 (Experiment ST - ADDENDUM C4) shows that they were very similar. As both experiments were conducted on "old" animal hide it could also be proposed that a 10°C drop in conditioning temperature was approximately equivalent to a 2 week drop in conditioning time at 22°C.

The effect of the 10°C drop in temperature on overall colour was apparently also one of darkening. This was the first indication that conditioning parameters could have had an effect on the colour of the gelatine but the indication was not unequivocal because the poor clarity of the boil liquor would probably have lead to an overestimation of the colour of 71% of the gelatine in the boil from the low temperature conditioning.

Comparing WT4 and WT5 it was apparent that the 6 fold increase in sulphide concentration in WT5 had only a marginal effect on extractability and no significant effect on colour which confirmed the findings in experiment ST1.

If the overall colour result of 15.7 in WT1 was taken with the overall colour results of 16.3 and 16.6 in WT4 and WT5 it appeared that possibly the low overall colour results in WT2 and WT3 could be considered due to random variations.

The WT series of experiments was particularly important in showing that high levels of dissolved organic matter (volatile solids) in conditioning liquor had not had a deleterious effect on gelatine colour. Furthermore, if it was accepted that there was no solubilization of hair in the absence of sulphide then this experiment indicated that liming with sulphide could solubilise some 13.5% of the hide substance but lime only, solubilised only 7% of the hide substance. Hence, by subtraction, some 6.5% of hide substance was hair, and 7% was alkali soluble non-collagen organic matter.

EXPERIMENT YS.

(Young animal - effect of sodium sulphide)

Most of the previous trials had been conducted on the hide of old animals in order to ensure that any treatment effects on colour would be evident. This experiment was designed to demonstrate the best possible colour obtainable from young bovine hide and from the one part in which no sodium sulphide was used it was hoped to obtain a measure of the conditioning enhancing effect of this additive. From experience it was known that the conditioning time required by the hide of such a young animal was very much less than that for older animals, hence conditioning was carried out for 2 and 3 weeks with lime and sulphide and for 4 weeks without sulphide. The detailed results are in ADDENDUM C6.

The observations made during this experiment were:

1. The effect on extractability of increasing the sulphide concentration, from 1.6 to 5.7 g/l (YSA & YSC v/s YSB & YSD), was 4.5 %. The size of this effect was such as to make it impossible to say whether the change in extractability was due to the treatment or to experimental error. It was noted that there was no effect on gelatine colour.

2. The young animal hide gave gelatines with colours almost as good those expected from Type A pigskin gelatines. In a later investigation gelatine YSB/1 was measured as having an absorbance area of 4.41, whereas the best result on American pigskin gelatines was an absorbance area of 4.57, hence, it would appear that the best colour from Type B calf skin would be equal to that of the best Type A pigskin.

3. Four weeks of lime only (part YSE) gave a 45°C extractability of 30%. Part YSA which received 2 weeks of lime sulphide conditioning had an extractability of 35%. Both parts had the same proportion recovered in the residue boil. Hence, it would seem that the presence of sulphide during conditioning had an effect equivalent to about 2 weeks of lime only at 22°C.

This experiment confirmed that the effect of sulphide was not proportional to concentration so it could be proposed that the effect was probably one of

inhibiting the primary Maillard reaction which would be promoted by the low pH of liming and would result in cross linking of the collagen by any available aldose. The lack of effect on gelatine colour would mean that the sulphide did not inhibit the formation of coloured byproducts of the Maillard cross-linking that had occurred prior to liming. From data to be reported under the fluorescence study it could be deduced that the amount of colour that would be generated by the Maillard reaction during 4 weeks of liming at 22°C would probably not be noticeable especially if the aldose available was mainly glucose as will be seen from the discussion of the gelatine/glucose interaction (*loc cit*).

EXPERIMENT KTO.

(Tannery treatment of an old animal hide).

Details of this experiment are shown in Addendum C7. The experiment was designed to investigate the colours of the gelatines that were recovered from different layers of the same hide. In order to maximise the effects on colour the experiment was conducted on the salted hide of a 12 year-old animal from ADSRI.

It had long been the experience at DGI that good quality dry splits gave gelatine of superior colour to that obtained from whole hide. Also those gelatine manufacturers that produced almost entirely from tannery wet limed splits, had a gelatine colour advantage over those who used the whole hide. However, whether this observation could be attributable to the use of corium only needed to be substantiated. The results obtained are shown in Tables 7 and 8.

Table 7. *Gelatine colours from various layers of the same hide.*

RAW Material	Overall Colour	First Extract Colour	Second Extract Colour	Third Extract Colour	Boil Extract Colour
Flesh	--	8.4	10.0	16.0	NM[a]
Middle	12.3	6.4	6.0	6.4	16.0
Grain	12.6	6.0	6.0	6.8	16.7
Whole Hide	11.6	8.0	7.2	9.4	13.3

-- Not available.

[a] Not measurable.

Table 8. *Gelatine Clarity from various layers of the same hide.*

Raw Material	Overall Clarity	First Extract Clarity	Second Extract Clarity	Third Extract Clarity	Boil Extract Clarity
Flesh	--	11.1	7.0	3.5	NM[a]
Middle	14.0	12.5	12.5	11.1	15.4
Grain	10.1	12.5	12.5	11.1	9.0
Whole Hide	12.4	11.8	11.8	9.0	14.3

-- Not available.

[a] Not measurable.

From Tables 7 and 8, it was concluded that the flesh associated with the hide made the biggest contribution to the colour of the extracted gelatine as well as being responsible for the worst clarity. The poor colour of gelatine from the flesh split was in agreement with a previous experiment (not reported) on hide of unknown origins.

The colour of the first extract gelatine from the whole hide may appear dark but the expected colour, calculated from the weighted contributions from the three layers, of 7.8, was in good agreement with the 8.0 measured colour of this gelatine.

It was of particular interest to note that the grain (epidermis) split and the middle split gave gelatines of very similar colour and clarity and that this colour was markedly better than that of the whole hide. The fact that the middle split gelatine colour was not as good as could be expected from pigskin was probably due to the age of the animal.

With lime-sulphide conditioning of hairy hide, the conditioning liquor normally becomes very dark in colour and it was a matter of conjecture whether this darkening was due to "hair burn" by the sulphide or whether it was due to the removal of colour from the collagen. In this experiment all the hair had been removed by the tannery pretreatment, hence, the very low variance in the absorbance data recorded on the filtered conditioning liquors indicated that lime-sulphide conditioning did not remove significant amounts of coloured substances from the hide.

Finally, the high extractability of the flesh split was interesting as it indicated that this layer contained the most recently formed collagen. However, the dark colour was at variance with the finding so far, namely, that the younger the animal the paler the gelatine.

EXPERIMENT CALF-A.

(Type A gelatine from calf skin).

The detailed results of this experiment are in ADDENDUM C8.
Due to the dehairing step used, the proximate composition of the salted hides was found to be moisture 44.8%, ash 17.0%, hair 17.6%, gelatine 16.8%, fat 0.7%, acid solubles (collagen) 0.8% and an unknown balance of 2.3%(Table 10).

It should be noted that the gelatine yield of 44% on an anhydrous, ash free basis was markedly low. More normal yields were in the range 56% to 78%. Furthermore, the residue after the boil was abnormally high in spite of the raw material being calf skin. This residue could easily account for the 2.3% (175g) of the original material that was otherwise unaccounted for.

Based on the isoionic points of the gelatines produced it was concluded that the short dehairing process had had a negligible effect on the acid amides of the collagen. Also the drop in pI with extraction temperature was in line with the results of Toda (1986).

The Type A calf skin gelatines had an undoubtedly good colour but the three run overall colour (4.1) was no better than that recorded for Type B gelatine from 10 month old animal skin of 3.4 for experiment YSD above.

EXPERIMENT 3Y & 6Y.

(3 and 6 year-old Friesland animal's hide).

This experiment was designed to show the effect of Friesland breed and animal age on gelatine colour as well as to act as a confirmatory experiment on the role of sulphide in lime-sulphide conditioning. Detailed data is in ADDENDUM C9.

Gelatine Colour.

From the point of view of gelatine colour the 3 year-old animal (3Y) gave very

similar gelatine colours irrespective of conditioning time, with overall colours close to 5 which was even better than the overall colour of about 6 obtained in experiment CT on 18 month old animal, above. This appeared to indicate that breed does in fact play a role in gelatine colour.

The six year-old animal hide gave rather erratic first extraction colours with the best colour being associated with the shortest conditioning time and lowest extractability. In this experiment the overall colours were about 7.5 which was markedly darker than the overall colour produced from the 3 year-old Friesland.

Role of Sodium Sulphide.

Comparison of the **4A and **4B parts of these experiments confirmed that doubling the sodium sulphide concentration during conditioning had virtually no effect on gelatine extractability and colour. The 3Y6 and 6Y6, B parts which were conditioned for six weeks with no sulphide, again gave gelatines of the same colour as was produced in the A parts with 2 g/l of sulphide in the conditioning liquor. This confirmed that sulphide had no effect on gelatine colour.

The effect of sulphide on conditioning was shown by both experiments (3Y & 6Y). The first extraction proportions after 6 weeks with lime only were intermediate between 2 and 4 weeks with sulphide. This, combined with the result given by experiment YS, indicated that the presence of sulphide during conditioning was equivalent to about 3 weeks of extra time at 22°C without sulphide. Hence, the previous conclusions with regard to the role of sulphide in conditioning, namely, that it inhibited the Maillard cross-linking promoted by the alkaline conditions, were unaffected.

EXPERIMENTS 5Y and INO.
(Five year-old Chianina hide and a 12 year-old Inguni hide)

The details of these experiments are recorded in ADDENDUM C10 and ADDENDUM C11. The main point of interest of these experiments was that they were conducted on a Chianina hide and an Inguni hide. The results obtained were in no way unusual, hence, it was concluded that breed had not had a significant effect on conditioning response or gelatine colour.

THE EFFECT OF ANIMAL AGE AND PROCESSING ON GELATINE COLOUR.

The detailed colour data extracted from ADDENDA C1 to C11 is presented in ADDENDUM C12. From this table it might appear that there was no systematic change in first extraction gelatine colour with conditioning time or with extractability, hence there was no justification for separating the data using conditioning time. For this reason the average colours produced from a single hide, as shown in Table 9, were used to evaluate whether there was a statistical correlation between animal age and gelatine colour.

The first extraction colours were subjected to polynomial regression analysis against animal age. The first and second order correlation coefficients (0.892 and 0.898) were very similar hence there was no reason to use the second order regression equation and the linear coefficients were:
B(0) = 4.117093
B(1) = 0.0366585.
The correlation coefficient (r) of 0.89 for 8 degrees of freedom was significant at the highest (0.0005) level of probability, however the fact that r was not closer to 1 indicated that there was variance in the data due to other factors.

Table 9. *Gelatine colour response to animal age.*

EXPERIMENT No	ANIMAL AGE MONTHS	1ST EXTRACT COLOUR	OVERALL COLOUR
CALF-A	3	4	
YS	10	3.3	4
CT	18	5.5	6.3
3Y	40	6.3	5.8
5Y	58	6.1	7.6
6Y	78	6.9	7.6
CTO	152	8.4	15.1
ST1	144	10	15.1
ST2	144	11.4	15.1
INOE	143	9.4	14.9
KTO	144	8	11.6

In the case of Experiment CTO, from inspection, it appeared that one of the "other" factors causing darker gelatine could be an increase in extractability due to the increase in conditioning time. In the case of the other experiments either the animal was too young to show the effect, or the changes in conditioning times were insufficiently varied to demonstrate the effect. There was additional evidence that the gelatine was darker as the degree of extraction increases in the ST1-2/4 and ST1-2/5 (ADDENDUM C4) boil data.

In the case of the correlation between average overall colour and animal age data, the second order polynomial regression correlation coefficient was 0.966 and the equation coefficients were:
B(0) = 4.68104
B(1) = 0.0221079
B(2) = 0.00030458
This correlation coefficient indicates that the other factors affecting overall colour were probably quite small and possibly limited to random or experimental error.

Also the data in Table 9 indicated that there was no marked effect on gelatine colour attributable to breed differences.

THE EFFECT OF ANIMAL AGE ON GELATINE EXTRACTABILITY.

The significance of the extractability data in Table 10 below, lies in the understanding it provides of the reasons behind the variable response of raw material to a conditioning process. The data was obtained using a single conditioning process controlled at 22°C, and a single extraction regime, hence the variables were minimal and the differences in extractability could be attributed to the differences in animal age and conditioning time.

When the data in Table 10 was submitted to 3 way linear regression analysis, however, the correlation coefficient between extractability and conditioning time was only 0.27 (26 degrees of freedom) which was only significant with a probability of 0.1, whereas, the correlation between extractability and age was 0.645 which was significant at the 0.0005 level of probability. Hence, statistically, animal age was the most important factor when it came to extractability as a result of lime/sulphide conditioning.

Table 10. *The changes in the 45°C extractability of hide due to conditioning time and animal age.*

		CONDITIONING TIME IN WEEKS.								
EXP. No.	ANIMAL AGE MONTHS	1	2	3	4	5	6	7	8	10
YS	10	-	35.3	45.9	-	-	-	-	-	-
CT	18	3.3	11.3	21.2	22.5	35.0	39.5	-	-	-
3Y	40	-	9.6	-	28.3		36.1	-	36.9	-
5Y	58	-	7.5	-	20.0	-	21.4	-	-	-
6Y	78	-	6.6	-	15.3	-	21.4	-	-	-
CTO	152	-	4.4	-	8.4	-	-	10.9	-	10
ST1	144	-	3.6	-	9.6	-	11.5	-	-	-
ST2	144	-	-	-	10.4	-	-	-	-	-
INOE	143	-	-	-	11.4	-	-	-	-	-
KTO	144	-	-	-	10.5	-	-	-	-	-

- Not applicable.
Lime-sulphide conditioning using 2 g/l Na_2S at 22°C.

From an inspection of the data in Table 10, however, it could be seen that conditioning time was a major factor in determining gelatine extractability for animals under the age of about 5 years but for old animals conditioning time beyond about 4 weeks at 22°C had a negligible effect on extractability.

THE COMPOSITION OF HIDE.

Due to the possible variation in the amount of hide substance in any sample, gelatine yields etc. were expressed in terms of anhydrous ash-free hide substance for comparative purposes.

Due to the single acidulation solution used in the production of Type A calf skin gelatine it was practical to estimate the losses due to acidulation as acid soluble collagen by determining the amount of organic matter in the solution as volatile solids (at 550°C). Furthermore, it was apparent that the 2.1 kg of residue after extraction could easily have been responsible for the 2.3% of the raw material not accounted for.

Table 11. *Composition of calf skin ex the acid process.*

ATTRIBUTE	% of Raw Material	% of Anhydrous Ash-free Hide
Moisture	44.8	-
Ash	17.0	-
Hair	17.6	44.5
Gelatine	16.8	44.0
Fat	0.7	1.8
Acid Soluble Collagen	0.8	2.1
Extraction Residue. (by difference)	2.3	6.0

Data from Addendum C7.
- Not applicable

In the case of experiment YS it was evident that unaccounted losses were considerably higher than with the calf skin used for acid processing, in spite of the extraction residues being only about 300 g. The reason for this was thought to be due to losses of collagen as eucollagen (Balian and Bowes, 1977) into the large amounts of sulphurous acid solution used in acidulation. (The

term eucollagen was used to distinguish acid soluble collagen from alkali treated hide, from acid soluble collagen from untreated (calf) hide).

The hair content of the alkali processed calf hide (YS) was determined by the difference between the organic content of spent conditioning liquor where no sulphide was used (YSE) and where a high level of sulphide was used (YSB). The large difference in this attribute between the two lots of calf skin was noteworthy and this was obviously a factor in the lower gelatine yield indicated in Table 11. However, in general the data in Tables 11 and 12 was in good agreement with the findings of Bowes, Elliot and Moss (1958).

Table 12. *Composition of calf skin ex the alkaline process.*

ATTRIBUTE	% of Raw Material	% of Hide Substance
Moisture.	61.0	-
Ash.	0.2	-
Hide Substance.	38.8	-
Gelatine.	21.3	54.9
Hair.	1.2	3.0
Lime solubles.	2.8	7.3
Fat.	2.0	5.2
Unaccounted / Losses.	11.2	29.6

- Not applicable.

The data in Table 13 on the composition of hide used in alkali conditioning was extracted from the data in the addenda. The gross composition of hide was limited to the major constituents, namely moisture and ash with the residue being "hide substance".

Table 13. *Average crude composition of adult bovine hide.*

TYPE	Green Hide	Washed Hide	Salted Hide
Number of Samples.	1	5	6
Moisture %	65.5	59 - 69	38 - 48
Ash %	0.6	0.2 - 0.6	14 - 17
Hide Substance % (Anhydrous Ash Free).	33.9	30 - 40	38 - 44

The average composition of bovine (anhydrous, ash-free) organic hide substance is given in Table 14. The variability of the amount of hair in hide substance has been mentioned above. The large variability of gelatine yield (or collagen content) from 40% to 80 % was also noteworthy. The highest yield (80%) was given by an 18 month old Brahman and the lowest (42%) by 6 year-old Friesland. It was also observed that with the high yield of the Brahman went a very low fat recovery from the extraction liquors.

A part of the 21% of the hide unaccounted for could be losses due to eucollagen dissolved during the sulphurous acid acidulation process. Another part would be the "scutch" or insoluble residue remaining at the end of extraction. A further part of the losses could be material passing through the tumbler screens during washing, however, unless the material was grossly over conditioned, this loss was very small.

The "lime soluble material" determined from those experiments where no sulphide had been used (WT1, WT2, YSE, 3Y6B and 6Y6B) was assumed to be a measure of the albumins, globulins and products of the destruction of glucoseaminoglycans (GAGs) and elastin components of skin (Bowes and Elliot, 1958; Haines, 1984). Where sulphide was used this value was much higher and therefore included much of the products of keratin destruction as well. The residue after spent conditioning liquor filtration was always black, hence, part of the hair including the melanin pigments of hair were not solubilised by sulphide.

Table 14. *The constituents of anhydrous ash-free bovine hide.*

ATTRIBUTE	MEAN	STANDARD DEVIATION	NUMBER OF SAMPLES	RANGE
Gelatine %	58.7	11.1	40	42.4 - 82.7
Fat %	3.8	2.0	6	Nil - 6.7
Lime/Sulphide Solubles % = A.	16.4	3.8	6	11.2 - 21.6
Lime Solubles % = B.	7.35	2.15	6	4.3 - 9.8
A - B = Hair %	9.0	-	-	-
Unaccounted / Losses %.	21.1	-	-	-

GELATINE IRON CONTENT.

Linear regression was performed on the calibration data giving:

Fe ppm = 2.82 + 113.9 x Absorbance. r = 0.997.

Slope error = ± 13 hence Fe error = ± 7 ppm.

The error of 7 ppm was considered satisfactory for quality control purposes of estimating gelatine iron contents in the range of 10 to 50 ppm.

Table 15. *Gelatine iron content and colour.*

Sample	Absorbance	Fe ppm.	Corrected ppm Fe	Colour Value.
40 ppm Instrument Control	0.309	40		
Reagent Blank.	0.014	4.4	0	
Gelatine 155/1	0.187	24	20	6.4
Gelatine YSA/1	0.159	21	17	3.6
Gelatine YSA/3	0.165	22	18	4.8
Gelatine WT3/1	0.301	37	34	10.7
Gelatine WT3/3	0.220	28	24	16.0

Blank = Distilled water.

Table 16. *The effect of added iron on the colour of gelatine 155/1.*

Fe added. ppm.	Fe content. ppm.	Colour Value.	pH
0	20*	6.4	5.63
20	40	6.8	5.46
40	60	7.6	5.33
60	80	8.0	5.21
80	100	8.0	5.13
100	120	8.9	5.03

* From Table 15.

Linear regression analysis of colour and iron content from Table 16. gave the following result:

Colour Value = 6.0 + 0.0236 x ppm Fe. r = 0.975.

From this one could conclude that each ppm Fe contributed 0.02 units to the colour value of the gelatine in the range 0 to 120 ppm Fe. However, as can be noted from Table 15, most gelatines had an iron content well below 50 ppm. Hence, the contribution of iron to the normal colour of gelatine would be less than one unit. However, due to the subjective nature of the colour determination the error of the method was generally accepted as 1.5 units, hence the contribution of iron to gelatine colour could be negligible when iron contents were less than 50 ppm.

Finally, it was possible that pH could have played a part in the observed changes in colour with iron content in Table 16. However, the pHs of 5.0 to 5.6 were in the normal range and the differences were considered too small to be significant.

The gelatines chosen above were:

155/1 a normal production gelatine with a colour value of 6.4.
YSA/- Pale gelatines from a 10 month old animal skin.
WT3/- Dark gelatines from a 144 month old animal skin.

From Table 15, it was evident that there was no correlation between gelatine colour and iron content and that iron content alone was certainly not the cause of gelatine colour.

GELATINE AMINO ACID ANALYSIS.

The results of the amino acid analyses in ADDENDUM 13 had quite large standard deviations. It was evident that by removing the variables due to moisture and ash content the standard deviations were markedly reduced as shown in Table 17.

The standard deviation due to analytical errors was calculated from the standard deviation on analysis of the same gelatine, namely samples C and D. Thus any increase in standard deviation, when all the data was taken into consideration could possibly be attributed to variation due to the different samples. The results in Table 17 where "%-RSD" changes exceeded 1% were marked with a *. Similarly differences in "%-RSD" between all samples and all samples without A (chrome tanned leather gelatine) could be interpreted as due to chrome gelatine.

From the Experimental Error %RSDs (EE-RSD) it was concluded that all the values for methionine and cystine should be ignored and (as stated by Stevens and Stevens, 1992) the data for histidine was very unreliable.

Comparing EE-RSD and ALL-RSD the largest discrepancy was in the ornithine data. This result was in agreement with the progressive alkaline conversion of arginine to ornithine, with the liberation of urea (Veis, 1964c). In sample A (gelatine from tanned leather waste), the collagen had received tannery treatment plus extraction at 70° to 90°C at pH 9. Samples B and C had been limed for 2 weeks. From the ornithine contents these treatments seem to have been similar in effect. In samples E and F the collagen had been limed for 4 weeks (*loc cit*) and the ornithine content of the gelatine was double that of samples B and C. As was expected this phenomenon also lead to a marked change in the arginine %-RSDs.

Table 17. *The molar % amino acid content of gelatines A to F.*

	SAMPLE						EXPERI-MENTAL ERROR % RSD OF SAMPLES C-D	MEAN OF ALL SAMPLES	% RSD OF ALL SAMPLES		MEAN WITHOUT SAMPLE A	% RSD WITHOUT SAMPLE A	
AMINO ACID	A	B	C	D	E	F							
ASP	3.9	4.5	4.5	4.4	4.1	4.3	2.6	4.3	5.2	*	4.4	4.3	*
GLU	6.4	6.7	6.7	6.6	6.6	6.5	3.6	6.6	3.5		6.6	3.4	
HOPRO	10.1	9.8	10.7	10.1	10.6	10.2	3.0	10.3	3.3		10.3	3.5	
SER	3.2	3.3	3.5	3.5	3.4	3.4	2.4	3.4	3.0		3.4	2.6	
GLY	32.6	32.8	33.2	33.0	33.4	32.9	2.6	33.0	2.5		33.1	2.7	
HIS	0.2	0.2	0.2	0.3	0.2	0.2	123.3	0.2	130.1		0.2	134.8	
ARG	6.4	5.9	5.8	5.6	5.8	5.6	5.9	5.8	7.1	*	5.7	6.6	
METSO1	0.0	0.0	0.0	0.0	0.1	0.1	er	0.0	267.3		0.0	247.5	
METSO2	0.0	0.0	0.0	0.0	0.1	0.0	er	0.0	387.3		0.0	360.6	
THR	1.3	1.6	1.6	1.6	1.6	1.6	5.5	1.5	6.7	*	1.6	4.4	*
ALA	11.4	10.9	10.6	10.6	10.5	10.6	0.8	10.7	2.9	*	10.6	1.7	*
PRO	13.5	13.3	12.9	13.1	12.9	13.4	3.3	13.1	3.8		13.1	3.9	
TYR	0.0	0.2	0.2	0.4	0.1	0.1	50.7	0.2	82.4		0.2	68.5	
VAL	2.1	2.1	1.9	2.0	2.0	2.0	2.2	2.0	3.7	*	2.0	3.5	
MET	0.0	0.0	0.0	0.0	0.0	0.1	er	0.0	276.3		0.0	256.0	
CYS	0.0	0.0	0.0	0.0	0.0	0.0	er	0.0	er		0.0	er	
ILE	1.2	1.2	1.2	1.3	1.2	1.3	6.1	1.2	4.4	*	1.2	4.7	
LEU	2.4	2.4	2.2	2.3	2.3	2.4	5.3	2.3	5.6		2.3	6.0	
HOLYS1	0.8	0.8	0.7	0.7	0.8	0.7	10.8	0.8	9.1		0.8	9.7	
HOLYS2	0.2	0.2	0.2	0.2	0.2	0.2	19.6	0.2	18.9		0.2	18.5	
PHE	1.2	1.3	1.2	1.3	1.1	1.2	5.9	1.2	5.2		1.2	5.6	
LYS	3.0	2.7	2.7	2.9	2.7	2.9	5.8	2.8	4.5		2.8	4.2	
ORN	0.1	0.2	0.1	0.2	0.4	0.4	9.9	0.2	51.4	*	0.3	44.0	*
TOTAL	100	100	100	100	100	100	-	-	-		-	-	

- Not applicable. er = Error result of calculation..

Comparison of the ALL-RSD and the RSD-A lead to the conclusion that aspartic acid, threonine with an hydroxyl group in the side chain and possibly alanine with no functional groups in the side chain could be involved in the chrome tanning process. It was important to note that glutamic acid did not appear to be involved with the binding of chromium.

Hence, when in addition the hide from an animal was cut into 100 x 100 mm pieces and these were randomised by tumbling, this allowed several experiments to be conducted without interference from the effects from variance in raw material.

2. Alkaline conditioning variables had no significant effect on the overall colour of the gelatine extracted. In particular, the inclusion of sulphide in the conditioning liquor could not be shown to have had any effect on gelatine colour.

3. Possibly the most significant conclusion to be drawn from this study was that the apparent inverse correlation between gelatine colour and the other quality parameters of Bloom gel strength and viscosity was only valid when the gelatine was extracted from old animals. When gelatine was extracted from young animal's hide the colour of the product was comparatively invariant.

4. When the extractability of old animal hide was increased by long liming time, the colour of the first extract (45°C) gelatine darkened with liming time. This effect was only demonstrated once but it could account for some of the random variation in first extraction gelatine colours.

5. The correlation between gelatine overall colour and animal age was 0.97 with 8 degrees of freedom which besides being significant at the 0.9995 level of probability was also high enough to indicate that other factors contributing to the colour were hardly significant.

6. From the data there was no indication that breed played a significant role in determining gelatine colour.

7. Of importance to the manufacturer was the extractability data showing that as animal age increased so the extractability decreased to the extent that there was little advantage to be gained from increasing the conditioning time with lime and sodium sulphide beyond four weeks at 22°C. The demonstration that the effect of sodium sulphide in conditioning was equivalent to an extra 3 weeks in lime only, was also of economic significance. Finally, from experiment WT it was concluded that a drop of 10°C in liming temperature caused a drop in

extractability which could be compensated for by approximately 2 weeks extra conditioning time.

8. The average proximate analysis of anhydrous hide substance was collagen/gelatine 58.7%, fat 3.8%, lime soluble substances 7.4% and hair 9%.

Although it was recognised that iron contamination could contribute to the colour of gelatine, this study showed that at normal levels of iron contamination (<50 ppm) this source of colour was small and possibly negligible.

It was hoped that there would be a correlation between amino acid analysis (particularly lysine) and gelatine colour. The only correlations found were the well known correlations between arginine, ornithine and liming time. Also, gelatine from chrome tanned leather showed a slight depletion of the aspartic acid content, but how this was related to its pale colour was not evident.

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ADDENDA.

ADDENDUM C1. The effect of position of the hide on the animal.

EXPERIMENT FR

Green Face pieces v/s Green Rest of the hide.

Raw Material and Conditioning.

Raw Material **Green Hide from IAPI.**

Moisture Content	65.45
Ash Content.	0.58
Sample GF	10.05 kg
Sample GR	10.00 kg

Conditioning.
Conditioning Liquor: CaO 640 g
Na_2S 60% 120 g
Water to 20 kg.

Sample No	GF	GR	
Cond: Time (Days)	27	27	
Cond: Temperature (°C)	23.0	23.0 ±1.2	
Init: Sulphide (g Na_2S/l)	2.29	2.35	
Final Sulphide (g Na_2S/l)	1.35	1.50	
Final Liquor Total Solids (%)	4.24	3.52	Error <0.02%
Final Liquor Volatile Solids (%)	3.19	2.52	Error <0.02%
Final Liquor Organic Solids (g)	210	200	
Ex-Lime Wash for 16hrs			
Limed Mass (kg)	17.55	16.95	
Swelling (%)	175	170	
Acidulation 5 coats of H_2SO_3 soln. (days)	3	3	
Wash 1hr.			
Soak in fresh water ± 22hr.			
Wt: for Extraction (kg)	22.0	22.35	
Soak Water (pH)	1.98	1.92	

ADDENDUM C1. Continued...

Raw Material **Salted Hide from IAPI.**

Moisture Content	48.09 %
Ash Content	14.10 %
Sample SF	4.75 kg
Sample SR	4.70 kg

Conditioning.

Conditioning Liquor: CaO 640 g
Na_2S 60% 120 g
Water to 20 kg.

Sample No	SF	SR	
Cond: Time (Days)	29	29	
Cond: Temperature (°C)	23.0	23.0 ±1.0	
Init. Sulphide (g Na_2S/l)	2.98	2.93	
Final Sulphide (g Na_2S/l)	1.43	1.34	
Final Liquor Total Solids (%)	5.63	6.27	Error <0.01%
Final Liquor Volatile Solids (%)	1.94	2.07	Error <0.01%
Final Liquor Organic Solids (g)	388	414	
Ex-Lime Wash for 16hrs			
Limed Mass (kg)	10.05	10.70	
Swelling (%)	12	228	
Acidulation 5 coats of H_2SO_3 soln.. (days)	3	3	
Wash 1hr.			
Soak in fresh water ± 22hr.			
Wt: for Extraction (kg)	12.3	12.65	
Soak Water (pH)	2.07	2.05	

ADDENDUM C1. Continued...

Extraction & Quality Data. Experiment GF.

Extraction.

Run No	1	2	3
Time hrs	5	5	7
Temperature (°C)	45	50	Boil
Liquor Volume (l)	21.68	7.39	6.27
Liquor pH	2.88	3.14	
Liquor Concentration (%w/v)	5.15	7.46	5.57
Scutch (g)			310
Gelatine (g)	1116.5	551.3	349.2
Gelatine % Proportion	55.4	27.3	17.3
Total Gelatine Recovered (%)			2017
Total Gelatine Yield (%)			20.07
Anhydrous Gelatine Recovered (g) (f 0.875)			1674.9
Anhydrous Gelatine Corrected (g) (f 0.95)			1676.6
Anhydrous Gelatine Yield on Raw Material (%)			49.1

Gelatine Quality.

Run No	1	2
Bloom	285	304
Colour (DGI)	4.8	6.4
Clarity (DGI)	8.5	9.0
pH	5.2	5.8
Moisture (%)	10.54	8.87
Ash (%)	1.14	0.72
SO_2 (ppm)	80	56
Viscosity (ms @ 60°C)	55.5	65.7
Corrected* Bloom	274	284
Corrected* Viscosity	55	64

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C1. Continued...

Extraction & Quality Data. Experiment GR.

Extraction.

Run No	1	2	3
Time hrs	5	5	7
Temperature (°C)	45	50	Boil
Liquor Volume (l)	21.43	8.42	8.01
Liquor pH	2.76	3.04	
Liquor Concentration (%w/v)	5.74	6.69	2.48
Scutch (g)			40
Gelatine (g)	1230.1	563.3	198.6
Gelatine % Proportion	61.7	28.3	10.0
Total Gelatine Recovered (g)			1992.0
Total Gelatine Yield (%)			19.9
Anhydrous Gelatine Recovered (g) (f 0.875)			1743.0
Anhydrous Gelatine Corrected (g) (f 0.95)			1655.9
Anhydrous Gelatine Yield on Raw Material (%)			48.7

Gelatine Quality.

Run No	1	2
Bloom	286	301
Colour (DGI)	4.8	5.2
Clarity (DGI)	10.0	12.0
pH	5.0	5.7
Moisture (%)	11.08	8.57
Ash (%)	1.00	0.62
SO_2 (ppm)	56	112
Viscosity (ms @ 60°C)	56.7	54.5
Corrected* Bloom	283	279
Corrected* Viscosity	56	53

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C1. Continued...

Extraction & Quality Data. Experiment SF.

Extraction.

Run No	1	2	3
Time hrs	5	5	7
Temperature (°C)	45	50	Boil
Liquor Volume (l)	13.78	7.18	7.07
Liquor pH	3.02	3.26	
Liquor Concentration (%w/v)	5.04	5.14	3.03
Scutch (g)			160
Gelatine (g)	694.5	369.1	214.2
Gelatine % Proportion	54.3	28.9	16.8

Total Gelatine Recovered (g)	1227.8
Total Gelatine Yield (%)	26.9
Anhydrous Gelatine Recovered (g) (f 0.875)	1118.1
Anhydrous Gelatine Corrected (g) (f 0.95)	1062.2
Anhydrous Gelatine Yield on Raw Material (%)	59.1

Gelatine Quality.

Run No	1	2
Bloom	292	278
Colour (DGI)	6.4	8.5
Clarity (DGI)	10.5	4.5
pH	5.3	4.5
Moisture (%)	11.30	11.54
Ash (%)	0.78	0.75
SO_2 (ppm)	152	112
Viscosity (ms @ 60°C)	52.1	48.1
Corrected* Bloom	289	277
Corrected* Viscosity	52	48

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C1. Continued...

Extraction & Quality Data. Experiment SR.

Extraction.

Run No	1	2	3
Time hrs	5	5	7
Temperature (°C)	45	50	Boil
Liquor Volume (l)	13.95	7.28	7.16
Liquor pH	2.99	3.24	
Liquor Concentration (%w/v)	4.78	5.46	2.76
Scutch (g)			160
Gelatine (g)	668.2	397.5	197.6
Gelatine % Proportion	52.9	31.5	15.6
Total Gelatine Recovered (g)			1263.3
Total Gelatine Yield (%)			26.9
Anhydrous Gelatine Recovered (g) (f 0.875)			1105.4
Anhydrous Gelatine Corrected (g) (f 0.95)			1050.1
Anhydrous Gelatine Yield on Raw Material (%)			59.1

Gelatine Quality.

Run No	1	2
Bloom	275	272
Colour (DGI)	6.4	7.2
Clarity (DGI)	10.5	8.0
pH	5.4	4.5
Moisture (%)	11.31	10.9
Ash (%)	0.62	0.83
SO_2 (ppm)	64	72
Viscosity (ms @ 60°C)	53.1	48.7
Corrected* Bloom	272	267
Corrected* Viscosity	53	48

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C2. The effect of Conditioning Time.

EXPERIMENT CT

Raw Material and Conditioning.

Raw Material Brahman Bull - Feed Lot animal approx 18 months old.

Washed Salted Hide:

Moisture Content	69.2 ± 0.5%
Ash Content	0.18 ± 0.08%
Sample 1 to 6 Mass (kg)	5.75 kg
Sample 7 Mass (kg)	1.85 kg
Anhyd: Hide Subs:	1760 ± 32 g

Conditioning.

Conditioning Liquor: CaO 640 g
Na_2S 60% 70 g
Water to 20 kg.

Sample No	CT1	CT2	CT3
Cond: Time (Weeks)	1	2	3
Cond: Temperature (°C)	21.0	21.5	21.9
Init. Sulphide (g Na_2S/l)	1.7	1.76	1.83
Final Sulphide (g Na_2S/l)		1.12	1.13
Final Liquor Total Solids (%)		1.373	1.54
Final Liquor Volatile Solids (%)		0.794	1.09
Final Liquor Organic Solids (g)		159	218
Ex-Lime Wash for 18hrs			
Limed Mass (kg)	9.1	9.7	11.1
Swelling (%)	160	168	193
Acidulation 5 coats of H_2SO_3 soln. (days)	4	4	4
Wash 1hr.			
Soak in fresh water ± 22hr.			
Wt: for Extraction (kg)	10.25	15.55	12.15
Soak Water pH	2.54	2.24	2.33

Sample No	CT4	CT5	CT6
Cond: Time Weeks	4	5	6
Cond: Temperature (°C)	21.9	22.0	22.0
Init: Sulphide (g Na_2S/l)	1.77	1.7	1.76
Final Sulphide (g Na_2S/l)	0.95	0.80.	0.81
Final Liquor Total Solids (%)	1.62	1.91	1.93
Final Liquor Volatile Solids (%)	1.15	1.44	1.43
Final Liquor Organic Solids (g)	230	288	286
Ex-Lime Wash for 18hrs			
Limed Mass (kg)	11.05	11.1	10.35
Swelling (%)	192	193	180
Acidulation 5 coats of H_2SO_3 soln. (days).	4	4	4
Wash 1hr.			
Soak in fresh water ± 22hr.			
Wt: for Extraction (kg)	12.0	12.55	11.55
Soak Water pH	2.11	2.14	2.02

ADDENDUM C2. Continued...

Sample No	CT7
Cond: Time (Weeks)	3
Cond: Temperature (°C)	21.9
Init: Sulphide (g Na_2S/l)	1.72

Conditioning liquor (20 l) changed at the end of week 1 and week 2.

Final Sulphide (g Na_2S/l)	1.61	Errors ±0.015 to 0.003%
Final Liquor Total Solids (%)	0.478	Errors ±0.02 to 0.002%
Final Liquor Volatile Solids (%)	0.019	Errors ±0.02 to 0.003%
Final Liquor Organic Solids (g)	4 g	

Ex-Lime Wash for 18hrs

Limed Mass (kg)	3.45
Swelling (%)	186

Acidulation 5 coats of H_2SO_3 soln. (days).	4
Wash 1hr.	
Soak in fresh water ± 22hr.	
Wt: for Extraction (kg)	3.85
Soak Water pH	2.73

ADDENDUM C2. Continued...

Extraction & Quality Data. Experiment CT1. (1 weeks liming)

Extraction.

Run No	1	2	3	4
Time hrs	5	5	5	7
Temperature (°C)	45	50	55	Boil
Liquor Volume (l)	9.44	9.69	7.04	11.32
Liquor pH	2.78	3.48	3.69	
Liquor Concentration (%w/v)	0.60	1.42	2.79	11.63
Scutch (g)				390
Gelatine (g)	56.5	137.6	196.4	1316.5
Gelatine (%) Proportion	3.3	8.1	11.5	77.1
Heavy Liquor Volume (ml)	700	1300	600	
Heavy Liquor Conc: (%)				
5% H_2O_2 (ml)	9	8	2	
5% NH_3 (ml)	12	10	8	

Total Gelatine Recovered (g)	1707
Total Gelatine Yield (%)	29.7
Anhydrous Gelatine Recovered (g) (f 0.875)	1493.6
Anhydrous Gelatine Corrected (g) (f 0.95)	1418.9
Anhydrous Gelatine Yield on Raw Material (%)	80.6

Gelatine Quality.

Run No	1	2	3	4
Bloom	285	332	331	
Colour (DGI)	5.6	5.6	5.2	6.8
Clarity (DGI)	5.0	9.5	11.5	7.5
pH	5.6	5.6	5.9	4.4
Moisture (%)	11.44	9.5	10.72	
Ash (%)	5.29	1.82	0.80	
SO_2 (ppm)	**	56	272	
Viscosity (ms @ 60°C)	30.5	33.6	33.2	
Corrected* Bloom	314	323	323	
Corrected* Viscosity	32	33	32	
Overall Colour.		6.5		

** Insufficient Sample

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C2. Continued...

Extraction & Quality Data. Experiment CT2. (2 weeks liming)

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	55	Boil
Liquor Volume (l)	11.22	6.76	7.65	12.03
Liquor pH	2.76	3.37	3.66	
Liquor Concentration (%w/v)	1.72	3.57	4.28	7.82
Scutch (g)				290
Gelatine (g)	193.0	241.3	327.4	940.7
Gelatine % Proportion	11.3	14.2	19.2	55.2
Heavy Liquor Volume (ml)	1200	1050	1050	
5% H_2O_2 (ml)	8	2	2	
5% NH_3 (ml)	10	12	22	

Total Gelatine Recovered (g)	1702.4
Total Gelatine Yield (%)	29.7
Anhydrous Gelatine Recovered (g) (f 0.875)	1489.6
Anhydrous Gelatine Corrected (g) (f 0.95)	1415.1
Anhydrous Gelatine Yield on Raw Material (%)	80.4

Gelatine Quality.

Run No	1	2	3	4
Bloom	332	330	302	
Colour (DGI)	5.2	5.2	6.0	6.4
Clarity (DGI)	10.0	12.0	11.5	13.5
pH	5.4	5.6	5.2	4.2
Moisture (%)	11.16	11.22	11.30	
Ash (%)	1.92	0.74	0.55	
SO_2 (ppm)	88	24	192	
Viscosity (ms @ 60°C)	36.7	37.3	41.2	
Corrected* Bloom	336	325	297	
Corrected* Viscosity	37	37	41	
Overall Colour.		6.0		

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C2. Continued...

Extraction & Quality Data. Experiment CT3. (3 weeks liming)

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	55	Boil
Liquor Volume (l)	12.36	8.84	8.99	7.08
Liquor pH	2.78	3.21	3.51	
Liquor Concentration (%w/v)	3.00	4.81	5.28	6.79
Scutch (g)				180
Gelatine (g)	370.8	425.2	474.7	480.7
Gelatine % Proportion	21.2	24.3	27.1	27.4
Heavy Liquor Volume (ml)	1600	1900	1400	
Heavy Liquor Conc: (%)			11	
5% H_2O_2 (ml)	5	6	3	
5% NH_3 (ml)	11	35	30	
Total Gelatine Recovered (g)			1751.4	
Total Gelatine Yield (%)			30.5	
Anhydrous Gelatine Recovered (g) (f 0.875)			1532.5	
Anhydrous Gelatine Corrected (g) (f 0.95)			1455.9	
Anhydrous Gelatine Yield on Raw Material (%)			82.7	
Gelatine Quality.				
Run No	1	2	3	4
Bloom	317	309	291	
Colour (DGI)	5.2	5.6	6.4	7.2
Clarity (DGI)	12.0	11.0	10.0	13.0
pH	5.5	5.3	5.3	4.3
Moisture (%)	9.89	10.84	11.26	
Ash (%)	0.94	0.52	0.46	
SO_2 (ppm)	312	104	264	
Viscosity (ms @ 60°C)	42.8	43.5	39.5	
Corrected* Bloom	305	301	286	
Corrected* Viscosity	42	43	39	
Overall Colour.		6.2		

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C2. Continued...

Extraction & Quality Data. Experiment CT4. (4 weeks liming)

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	55	Boil
Liquor Volume (l)	13.02	8.85	9.36	8.20
Liquor pH	2.89	3.22	3.45	
Liquor Concentration (%w/v)	3.47	5.36	4.95	4.35
Scutch (g)				40
Gelatine (g)	376.3	474.4	463.3	356.7
Gelatine % Proportion	22.5	28.4	27.7	21.4
Heavy Liquor Volume (ml)	1400	1300	1500	
Heavy Liquor Conc: (%)	9	11	9	
5% H_2O_2 (ml)	6	3	2	
5% NH_3 (ml)	27	25	30	
Total Gelatine Recovered (g)				1670.7
Total Gelatine Yield (%)				29.1
Anhydrous Gelatine Recovered (g) (f 0.875)				1461.8
Anhydrous Gelatine Corrected (g) (f 0.95)				1338.8
Anhydrous Gelatine Yield on Raw Material (%)				78.9

Gelatine Quality.				
Run No	1	2	3	4
Bloom	310	324	299	
Colour (DGI)	5.6	5.6	5.6	**
Clarity (DGI)	9.0	11.0	11.5	**
pH	5.4	5.4	5.3	4.4
Moisture (%)	8.68	8.93	11.15	
Ash (%)	1.21	0.58	0.56	
SO_2 (ppm)	24	168	128	
Viscosity (ms @ 60°C)	46.3	48.0	39.4	
Corrected* Bloom	292	302	294	
Corrected* Viscosity	45	46	39	

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C2. Continued...

Extraction Data. Experiment CT5. (5 weeks liming)

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	55	Boil
Liquor Volume (l)	14.37	9.10	9.14	1.24
Liquor pH	2.87	3.23	3.39	
Liquor Concentration (%w/v)	4.03	5.62	5.03	8.23
Scutch (g)				Nil
Gelatine (g)	579.1	511.4	459.7	102.1
Gelatine % Proportion	35.0	31.0	27.8	6.2
Heavy Liquor Volume (ml)	1500	1100	1500	
Heavy Liquor Conc: (%)	9	13	11	
5% H_2O_2 (ml)	4	2	2	
5% NH_3 (ml)	20	30	40	
Total Gelatine Recovered (g)				1652.3
Total Gelatine Yield (%)				28.7
Anhydrous Gelatine Recovered (g) (f 0.875)				1445.8
Anhydrous Gelatine Corrected (g) (f 0.95)				1373.5
Anhydrous Gelatine Yield on Raw Material (%)				78.0

Gelatine Quality.				
Run No	1	2	3	4
Bloom	324	298	275	
Colour (DGI)	6.0	6.4	6.4	8.0
Clarity (DGI)	11.5	11.5	12.5	13.5
pH	5.7	5.4	5.6	4.5
Moisture (%)	9.59	11.78	9.96	
Ash (%)	0.94	0.49	0.53	
SO_2 (ppm)	40	144	464	
Viscosity (ms @ 60°C)	51.5	49.5	50.1	
Corrected* Bloom	310	296	263	
Corrected* Viscosity	51	49	49	
Overall Colour.		6.4		

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.

ADDENDUM C2. Continued...

Extraction Data. Experiment CT6. (6 weeks liming)

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	55	Boil
Liquor Volume (l)	13.70	9.58	8.14	2.90
Liquor pH	2.84	3.30	3.57	
Liquor Concentration (%w/v)	4.45	5.20	4.42	2.60
Scutch (g)				Nil
Gelatine (g)	609.7	498.2	359.8	75.4
Gelatine % Proportion	39.5	32.3	23.3	4.9
Heavy Liquor Volume (ml)	1400	1700	1300	
Heavy Liquor Conc: (%)	9	9	11	
5% H_2O_2 (ml)	4	3	2	
5% NH_3 (ml)	20	35	35	
Total Gelatine Recovered (g)				1543.1
Total Gelatine Yield (%)				26.8
Anhydrous Gelatine Recovered (g) (f 0.875)				1350.2
Anhydrous Gelatine Corrected (g) (f 0.95)				1282.7
Anhydrous Gelatine Yield on Raw Material (%)				72.9

Gelatine Quality.				
Run No	1	2	3	4
Bloom	327	308	277	
Colour (DGI)	5.2	5.6	6.0	**
Clarity (DGI)	11.5	11.5	12.5	**
pH	5.3	5.6	5.6	4.4
Moisture (%)	10.14	9.34	9.62	
Ash (%)	0.85	0.64	0.59	
SO_2 (ppm)	360	352	296	
Viscosity (ms @ 60°C)	48.0	55.6	49.7	
Corrected* Bloom	316	291	263	
Corrected* Viscosity	47	54	48	

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.

ADDENDUM C2. Continued...

Extraction Data. Experiment CT7. (3 weeks liming)

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	55	Boil
Liquor Volume (l)	2.92	3.22	3.74	4.20
Liquor pH	2.89	3.17	3.45	
Liquor Concentration (%w/v)	3.70	3.90	3.73	3.35
Scutch (g)				50
Gelatine (g)	108.0	125.6	139.5	157.5
Gelatine % Proportion	20.4	23.7	26.3	29.6
Heavy Liquor Volume (ml)	1000	1200	900	
Heavy Liquor Conc: (%)		8	7	
5% H_2O_2 (ml)	2	2	2	
5% NH_3 (ml)	10	17	17	
Total Gelatine Recovered (g)				530.6
Total Gelatine Yield (%)				28.7
Anhydrous Gelatine Recovered (g) (f 0.875)				464.3
Anhydrous Gelatine Corrected (g) (f 0.95)				441.1
Anhydrous Gelatine Yield on Raw Material (%)				77.8

Gelatine Quality.				
Run No	1	2	3	4
Bloom	333	318	279	
Colour (DGI)	5.6	5.6	6.4	**
Clarity (DGI)	9.0	11.0	11.5	**
pH	5.6	5.5	5.4	
Moisture (%)	10.19	10.72	10.87	
Ash (%)	0.86	0.60	0.61	
SO_2 (ppm)	56	144	192	
Viscosity (ms @ 60°C)	43.8	44.6	39.7	
Corrected* Bloom	322	310	273	
Corrected* Viscosity	43	44	39	

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C3. Old animal hide & conditioning time.

EXPERIMENT CTO.

Raw Material and Conditioning Data.

Salted Hide ex ADSRI from a beef animal 13 years of age. The hide was cut into small pieces, washed in a tumbler overnight, drained and then separated into 4 lots.

Moisture Content	69.3 ± 0.3%
Ash Content	0.28 ± 0.07%

Sample No	CTO1	CTO2	CTO3	CTO4
Sample Mass (kg)	4.6	4.8	4.8	4.8
Anhyd: Hide Subs: (g)	1399	1460	1460	1460

Conditioning.

Conditioning Liquor: CaO	640 g
Na_2S 60%	70 g
Water	to 20 kg.

Sample No	CTO1	CTO2	CTO3	CTO4
Cond: Time Weeks	2	4	7	10
Cond: Temperature (°C)	21.8	22.0	21.8	21.8
Init: Sulphide (Na_2S g/l)	1.81	1.84	1.85	1.81
Final Sulphide (Na_2S g/l)	1.14	0.68	0.46	0.27
Sulphide consumed (g/l)	0.67	1.16	1.39	1.54
Spent Liquor Solids (%w/v)	1.69	2.25	2.41	2.56
" " Ash (%w/v)	0.53	0.66	0.85	0.76
" " Volatiles (%w/v)	1.16	1.59	1.56	1.79
" " Organic Matter (g)	232	318	312	358
" " Absorb: (470nm)		0.12	0.08	0.08
Ex-Lime Wash for 16hrs.				
Limed Mass (kg)	6.1	7.5	6.9	.15
Swelling (%)	132	156	144	149
Acidulation. 5 coats of H_2SO_3 soln. over 4 days.				
Wash 1hr.				
Soak in fresh water ± 22hr.				
Wt: for Extraction (kg)	7.05	7.65	7.35	7.3
Swelling (%)	153	159	153	152
Soak Water pH	2.33	2.41	2.43	2.45

ADDENDUM C3. Continued...

CTO1 Extraction and Quality Data.

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	55	Boil
Liquor Volume (l)	7.76	7.44	6.58	9.32
Liquor pH	2.76	3.31	3.39	
Liquor Concentration (%w/v)	0.55	0.96	1.58	8.03
Fat (ml)	10	4	6	
Scutch (g)				280
Gelatine (g)	42.7	71.4	103.9	748.4
Gelatine % Proportion	4.4	7.4	10.8	77.4
Heavy Liquor Volume (ml)	1100	700	800	
Heavy Liquor Conc:	1.5	6	7.5	
5% H_2O_2 (ml)	6.5	3	--	
5% NH_3 (ml)	5	5	--	

Total Gelatine Recovered (g)	966.4
Total Gelatine Yield (%)	21.0
Anhydrous Gelatine Recovered (g) (f 0.875)	845.6
Anhydrous Gelatine Corrected (g) (f 0.95)	803.3
Anhydrous Gelatine Yield on Raw Material (%)	57.4
Total Anhydrous Solids Recovered (g)	1035.3
Total Anhydrous solids Recovered (%)	74.0

Gelatine Quality.

Run No	1	2	3	4
Bloom	300	306	286	
Colour	6.4	7.2	10.0	16.0
Clarity	10.5	11.5	10.5	10.5
pH	5.5	6.1	5.9	4.3
Moisture (%)	10.26	10.90	9.66	
Ash (%)	4.63	1.76	1.43	
SO_2 (ppm) ϕ	---	48+	216	
Viscosity (ms @ 60°C)	30.8	30.6	28.4	
Corrected* Bloom	317	294	277	
Corrected* Viscosity	31.6	30.0	27.9	
Overall Colour.		12.4		

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ϕ + Indicates peroxide positive on Starch/KI test.

ADDENDUM C3. Continued...

CTO2 Extraction and Quality Data.

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	55	Boil
Liquor Volume (l)	8.40	7.69	7.44	7.36
Liquor pH	2.88	3.27	3.53	
Liquor Concentration w/v	0.98	1.36	1.72	9.11
Fat (ml)	3.5	2.0	1.0	
Scutch (g)				40
Gelatine (g)	82.3	104.6	128.0	670.5
Gelatine % Proportion	8.4	10.6	13.0	68.0
Heavy Liquor Volume (ml)	1200	800	1000	
Heavy Liquor Conc:	3	6	-	
5% H_2O_2 (ml)	4	1.4	1	
5% NH_3 (ml)	6	8	10	
Total Gelatine Recovered (g)			985.4	
Total Gelatine Yield (%)			20.5	
Anhydrous Gelatine Recovered (g) (f 0.875)			862.2	
Anhydrous Gelatine Corrected (g) (f 0.95)			819.1	
Anhydrous Gelatine Yield on Raw Material (%)			56.1	
Total Anhydrous Solids Recovered (g)			1137.1	
Total Anhydrous solids Recovered (%)			77.9	

Gelatine Quality.				
Run No	1	2	3	4
Bloom	330	306	262	
Colour	8.0	8.9	10.0	20.0
Clarity	4.0	10.5	10.5	12.5
pH	5.8	5.9	5.8	4.3
Moisture (%)	8.66	8.90	8.85	
Ash (%)	2.51	1.32	1.10	
SO_2 (ppm) ϕ	80+	64	64	
Viscosity (ms @ 60°C)	38.3	32.9	31.1	
Corrected* Bloom	320	291	247	
Corrected* Viscosity	37.7	32.1	30.2	
Overall Colour.		16.5		

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ϕ + Indicates peroxide positive on Starch/KI test.

ADDENDUM C3. Continued...

CT03 Extraction and Quality Data.

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	55	Boil
Liquor Volume (l)	8.78	6.85	6.3	5.67
Liquor pH	2.88	3.26	3.45	
Liquor Concentration (%w/v)	1.12	1.59	2.15	9.94
Fat (ml)	2.0	1.5	1.5	
Scutch (g)				Nil
Gelatine (g)	98.3	108.6	135.1	563.6
Gelatine % Proportion	10.9	12.0	14.9	62.2
Heavy Liquor Volume (ml)	1000	800	1200	
Heavy Liquor Conc: (%)	4	5	6	
5% H_2O_2 (ml)	3	1	1	
5% NH_3 (ml)	7	8	20	
Total Gelatine Recovered (g)			905.6	
Total Gelatine Yield (%)			18.9	
Anhydrous Gelatine Recovered (g) (f 0.875)			792.4	
Anhydrous Gelatine Corrected (g) (f 0.95)			752.8	
Anhydrous Gelatine Yield on Raw Material (%)			51.6	
Total Anhydrous Solids Recovered (g)			1064.8	
Total Anhydrous solids Recovered (%)			72.9	
Gelatine Quality.				
Run No	1	2	3	4
Bloom	311	273	236	
Colour	9.4	10.7	12.3	17.8
Clarity	8.5	10.5	9.0	12.0
pH	5.6	5.6	5.6	4.2
Moisture (%).	8.31	7.98	9.29	
Ash (%)	1.6	1.17	0.96	
SO_2 (ppm) ϕ	160	64+	80	
Viscosity (ms @ 60°C)	37.8	27.5	30.7	
Corrected* Bloom	293	253	224	
Corrected* Viscosity	36.7	26.5	29.9	
Overall Colour.		15.2		

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ϕ + Indicates peroxide positive with Starch/KI test.

ADDENDUM C3. Continued...

CTO4 Extraction and Quality Data.

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	55	Boil
Liquor Volume (l)	8.5	7.11	6.16	4.89
Liquor pH	2.84	3.41	3.54	
Liquor Concentration (%w/v)	1.03	1.33	2.08	11.99
Fat (ml)	6	2	1	
Scutch (g)				Nil
Gelatine (g)	87.6	94.6	128.1	586.3
Gelatine (%) Proportion	9.8	10.5	14.3	65.4
Heavy Liquor Volume (ml)	900	800	1050	
Heavy Liquor Conc:	4	4.4	5	
5% H_2O_2 (ml)	1.5	1.0	0.5	
5% NH_3 (ml)	3.5	8.0	11.0	
Total Gelatine Recovered (g)			896.6	
Total Gelatine Yield (%)			18.7	
Anhydrous Gelatine Recovered (g) (f 0.875)			784.5	
Anhydrous Gelatine Corrected (g) (f 0.95)			745.3	
Anhydrous Gelatine Yield on Raw Material (%)			51.0	
Total Anhydrous Solids Recovered (g)			1103.3	
Total Anhydrous solids Recovered (%)			75.5	

Gelatine Quality.				
Run No	1	2	3	4
Bloom	302 Φ	246	213	
Colour	10.0	9.4	10.0	20.0
Clarity	8.5	10.0	9.0	6.0
pH	5.4	5.5	5.2	4.1
Moisture (%)	8.32	11.444	9.72	
Ash (%)	1.98	1.33	0.89	
SO_2 (ppm)	32	64	80	
Viscosity (ms @ 60°C)	39.1	32.0	28.9	
Corrected* Bloom	287	247	204	
Corrected* Viscosity	38.1	32.1	28.3	
Overall Colour.		16.4		

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

Φ Evaporation temperature rose to 50°C for a short time due to a leaf in the water vacuum pump.

ADDENDUM C4. Time & sodium sulphide concentration.

EXPERIMENT ST.

Raw Material.

Two salted hides were supplied by ADSRI from 12 year-old Afrikaners. These were cut into 100 x 100 mm pieces and randomised by tumbling. Equal amounts were taken from each hide to make up the 9 x 5.7 kg lots required for the experiment.

Mass of hide for each experiment.	5700 g.
Moisture Content	38.6 ± 1.4%
Ash Content	17.1 ± 0.7%
Organic Content	44.3 %
Organic Content of samples.	2525 g

Prewashing 18hrs minimum.

Hide Conditioning with 50 g Sodium Sulphide

Conditioning Liquor: CaO 640 g
Na_2S 50 g
Water to 20.0 kg.

Experiment No	ST1-2	ST1-4	ST1-6
Washed Hide (kg)	7.1	7.2	7.3
Conditioning Time -Weeks.	2	4	6
Cond: Temperature (°C)	22.2	21.9	22.0
Init: Sulphide (g Na_2S/l)	1.13±0.00	1.12±0.01	1.14±0.01
Ex-Lime Wash for 18hrs			
Limed Mass (kg)	10.15	11.25	11.2
Swelling (%)	143	156	153
Acidulation 5 coats of H_2SO_3 soln. over 4 days.			
Wash 1hr.			
Soak in fresh water ± 22hr.			
Wt: for Extraction (kg)	10.9	11.65	11.6
Swelling (%)	153	162	159
Soak Water pH	2.28	2.24	2.19
Spent Conditioning Liquor:			
Mass (kg)	20	19.7	19.55
Na_2S Conc: (g/l)	0.72±0.01	0.54±0.01	0.38±0.01
Sulphide consumed (g/l)	0.41	0.58	0.76
Spent Liquor Solids (g/l)	13.73±0.01	16.37±0.03	19.55±0.1
" " Ash (g/l)	3.53±0.03	5.32±0.04	4.8 ±0.6
" " Volatiles (g/l)	10.2	11.05	14.8
" " Organic Matter (g)	204	221	296
Organic Matter (%)	8.1	8.8	11.7

ADDENDUM C4. Continued...

Hide Conditioning with 74 g Sodium Sulphide.

Conditioning Liquor:	CaO	640 g
	Na_2S	74 g
	Water to	20.0 kg.

Experiment No	ST2-2	ST2-4	ST2-6
Washed Hide (kg)	7.05	6.8	7.4
Conditioning Time (Weeks)	2	4	6
Cond: Temperature (°C)	21.6	21.8	21.9
Init: Sulphide (g Na_2S/l)	1.91±0.01	1.90±0.01	1.84±0.01
Ex-Lime Wash for 18 hr.			
Limed Mass (kg)	10.45	10.8	10.75
Swelling (%)	148	159	145
Acidulation 5 coats of H_2SO_3 soln. over 4 days. Wash 1hr. Soak in fresh water ± 22hr.			
Wt: for Extraction (kg)	11.1	11.2	11.3
Swelling (%)	157	164	152
Soak Water pH	2.22	2.36	2.24
Spent Conditioning Liquor:			
Mass (kg)	20.0	19.75	19.8
Na_2S Conc: (g/l)	1.17±0.01	0.90±0.00	0.50±0.01
Sulphide consumed (g/l)	0.74	1.00	1.34
Spent Liquor Solids (g/l)	18.26±0.08	20.1±0.1	26.14±0.01
" " Ash (g/l)	5.80±0.1	6.3±0.1	9.00±0.01
" " Volatiles (g/l)	12.46	13.8	17.14
" " Organic Matter (g)	249	276	343
Organic Matter (%)	9.9	10.9	13.6

ADDENDUM C4. Continued...

Hide Conditioning with 98 g Sodium Sulphide.

Conditioning Liquor:	CaO	640 g		
	Na_2S	98 g		
	Water to	20.0 kg.		
Experiment No		ST3-2	ST3-4	ST3-6
Washed Hide (kg)		7.0	7.8	7.35
Conditioning Time (Weeks)		2	4	6
Cond: Temperature (°C)		21.6	21.9	21.9
Init: Sulphide (g Na_2S/l)		2.59±0.01	2.64±0.1	2.38±0.01
Ex-Lime Wash for 18hrs				
Limed Mass (kg)		9.9	10.6	10.55
Swelling (%)		141	135	144
Acidulation 5 coats of H_2SO_3 soln. over 4 days. Wash 1hr. Soak in fresh water ± 22hr.				
Wt: for Extraction (kg)		10.9	11.3	10.9
Swelling (%)		155	145	148
Soak Water pH		2.24	2.05	2.2
Spent Conditioning Liquor:				
Mass (kg)		19.6	19.4	19.9
Na_2S Conc: (g/l)		1.72	0.95	0.59
Sulphide consumed (g/l)		0.87	1.69	1.79
Spent Liquor Solids (g/l)		19.33±0.1	29.28±0.09	30.21±0.03
" "	Ash (g/l)	6.11±0.05	11.59±0.2	11.53
" "	Volatiles (g/l)	13.22	17.69	18.68
" "	Organic Matter (g)	264	354	374
	Organic Matter (%)	10.4	14.0	14.8

ADDENDUM C4. Continued...

Extraction & Quality Data. Experiment ST1-2.

Extraction.

Run No	1	2	3	4	5
Time (hrs)	5	5	5	1	6
Temperature (°C)	45	50	60	93	93
Liquor Volume (l)	9.23	8.58	7.90	5.81	3.26
Liquor pH	2.57	3.00	3.48	3.83	4.2
Liquor Concentration (%w/v)	0.79	1.46	3.68	5.95	26.73
Fat (ml)	60	20	25		
Scutch (g)					325
Gelatine (g)	72.9	125.2	290.3	345.7	871.4
Gelatine % Proportion	4.3	7.3	17.0	20.3	51.1
Heavy Liquor Volume (ml)	600	900	1050	900	
Heavy Liquor Conc:	7	9	11	10	
5% H_2O_2 (ml)	40	20	8	4	
5% NH_3 (ml)	15	8	7	10	

Total Gelatine Recovered (g)	1705.5
Total Gelatine Yield (%)	29.9
Anhydrous Gelatine Recovered (g) (f 0.875)	1492.3
Anhydrous Gelatine Corrected (g) (f 0.95)	1417.7
Anhydrous Gelatine Yield on Raw Material (%)	56.1
Total Anhydrous Solids Recovered (g)	1726.7
Total Anhydrous solids Recovered (%)	68.3

Gelatine Quality.

Run No	1	2	3	4	5
Bloom	179	92	237	146	
Colour	6.4f	10.0f	10.7	17.8	22.8
Clarity	12.5	12.5	12.5	6.5	9.0
pH	5.1	5.0	5.3	5.6	4.2
Moisture (%)	14.7	12.1	11.8	11.0	
Ash (%)	7.84	4.45	2.23	1.96	
SO_2 (ppm)	+	224	96	152	
Viscosity (ms @ 60°C)	15.8	13.9	19.8	19.2	
Corrected* Bloom	228	101	246	147	
Corrected* Viscosity	17.8	14.6	20.1	19.3	
Overall Colour.	18.1				

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.
f Bloom sample filtered; Whatman GF/A
\+ H_2O_2 Positive.

ADDENDUM C4. Continued...

Extraction & Quality Data. Experiment ST1-4.

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	10.95	8.80	7.49	8.64
Liquor pH	2.63	3.13	3.56	
Liquor Concentration (%w/v)	1.47	2.73	5.73	10.96
Fat (ml)	25	20	15	
Scutch (g)				75
Gelatine (g)	160.9	239.8	428.8	946.9
Gelatine % Proportion	9.1	13.5	24.1	53.3
Heavy Liquor Volume (ml)	600	1000	1200	
Heavy Liquor Conc:	8	10	13	
5% H_2O_2 (ml)	5	6	3	
5% NH_3 (ml)	3	7	18	

Total Gelatine Recovered (g)	1776.4
Total Gelatine Yield (%)	31.2
Anhydrous Gelatine Recovered (g) (f 0.875)	1554.4
Anhydrous Gelatine Corrected (g) (f 0.95)	1476.6
Anhydrous Gelatine Yield on Raw Material (%)	58.5
Total Anhydrous Solids Recovered (g)	1757.6
Total Anhydrous solids Recovered (%)	69.6

Gelatine Quality.

Run No	1	2	3	
Bloom	332	303	247	
Colour	10.0	11.4	13.3	16.0
Clarity	12.5	12.5	11.8	12.5
pH	5.4	5.4	5.5	4.4
Moisture (%)	9.6	9.4	11.3	
Ash (%)	2.81	2.39	1.39	
SO_2 (ppm)	nd	64	360	
Viscosity (ms @ 60°C)	33.2	29.8	27.5	
Corrected* Bloom	331	298	248	
Corrected* Viscosity	33.1	29.5	27.5	
Overall Colour.	14.2			

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.

ADDENDUM C4. Continued...

Extraction & Quality Data. Experiment ST1-6.

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	11.27	9.03	7.54	6.35
Liquor pH	2.69	3.20	3.61	
Liquor Concentration (%w/v)	1.81	3.23	6.00	12.50
Fat (ml)	15	15	10	
Scutch (g)				Nil
Gelatine (g)	204.4	291.2	452.4	793.8
Gelatine % Proportion	11.7	16.7	26.0	45.6
Heavy Liquor Volume (ml)	900	1000	1000	
Heavy Liquor Conc:	7	12	11	
5% H_2O_2 (ml)	4	8	2	
5% NH_3 (ml)	6	8	15	
Total Gelatine Recovered (g)				1741.8
Total Gelatine Yield (%)				30.6
Anhydrous Gelatine Recovered (g) (f 0.875)				1524.1
Anhydrous Gelatine Corrected (g) (f 0.95)				1447.9
Anhydrous Gelatine Yield on Raw Material (%)				57.3
Total Anhydrous Solids Recovered (g)				1783.9
Total Anhydrous solids Recovered (%)				70.6

Gelatine Quality.				
Run No	1	2	3	
Bloom	331	288	240	
Colour	10.7	12.3	14.5	13.3
Clarity	11.1	11.8	10.5	11.1
pH	5.5	5.4	5.5	4.1
Moisture (%) .	9.3	9.7	11.5	
Ash (%)	1.74	2.72	1.29	
SO_2 (ppm)	nd	200	456	
Viscosity (ms @ 60°C)	36.3	31.7	32.1	
Corrected* Bloom	320	288	241	
Corrected* Viscosity	35.7	31.7	32.2	
Overall Colour.	13.1			

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.
nd Not determined.

ADDENDUM C4. Continued...

Extraction & Quality Data. Experiment ST2-2.

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	10.66	10.30	8.07	8.83
Liquor pH	2.56	3.03	3.38	4.27
Liquor Concentration (%w/v)	0.61	1.37	3.91	14.70
Fat (ml)	35	15	20	
Scutch (g)				335
Gelatine (g)	65	141.1	315.5	1298.0
Gelatine (%) Proportion	3.6	7.8	17.3	71.3
Heavy Liquor Volume (ml)	600	1150	1150	
Heavy Liquor Conc:	6	8	10	
5% H_2O_2 (ml)	18.5	23	5.5	
5% NH_3 (ml)	12.5	15	8	
Total Gelatine Recovered (g)			1819.6	
Total Gelatine Yield (%)			31.9	
Anhydrous Gelatine Recovered (g) (f 0.875)			1592.2	
Anhydrous Gelatine Corrected (g) (f 0.95)			1512.5	
Anhydrous Gelatine Yield on Raw Material (%)			59.9	
Total Anhydrous Solids Recovered (g)			1831.7	
Total Anhydrous solids Recovered (%)			72.5	

Gelatine Quality.				
Run No	1	2	3	
Bloom	146	265	204	
Colour	11.4f	10.7f	11.4	16.0
Clarity	12.5	13.3	12.5	9.5
pH	5.1	5.3	5.2	4.1
Moisture (%)	10.2	10.1	10.9	
Ash (%)	8.39	4.29	1.92	
SO_2 (ppm)	+	40+	320	
Viscosity (ms @ 60°C)	15.0	20.7	17.8	
Corrected* Bloom	168	277	206	
Corrected* Viscosity	16	21.1	17.9	
Overall Colour.	14.6			

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.
f Bloom sample filtered; Whatman GF/A
+ H_2O_2 Positive.
ns Not set.

ADDENDUM C4. Continued...

Extraction & Quality Data. Experiment ST2-4.

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	11.36	9.21	8.59	7.08
Liquor pH	2.73	3.26	3.47	4.1
Liquor Concentration (%w/v)	1.49	2.50	5.42	12.67
Fat (ml)	15	10	20	
Scutch (g)				30
Gelatine (g)	169.3	230.3	465.6	897.0
Gelatine % Proportion	9.6	13.1	26.4	50.9
Heavy Liquor Volume (ml)	1200	1150	1050	
Heavy Liquor Conc:	9	9	15	
5% H_2O_2 (ml)		15	5	
5% NH_3 (ml)	10	5	12	
Total Gelatine Recovered (g)			1762.2	
Total Gelatine Yield (%)			30.9	
Anhydrous Gelatine Recovered (g) (f 0.875)			1541.9	
Anhydrous Gelatine Corrected (g) (f 0.95)			1464.8	
Anhydrous Gelatine Yield on Raw Material (%)			58.0	
Total Anhydrous Solids Recovered (g)			1785.8	
Total Anhydrous solids Recovered (%)			70.7	
Gelatine Quality.				
Run No	1	2	3	
Bloom	322	315	230	
Colour	8.9	12.3	13.3	17.8
Clarity	11.1	12.5	11.1	12.5
pH	5.9	5.3	5.2	4.1
Moisture (%)	8.4	8.1	10.9	
Ash (%)	3.64	3.16	1.56	
SO_2 (ppm)	+	8+	64	
Viscosity (ms @ 60°C)	32.5	31.7	23.2	
Corrected* Bloom	318	306	230	
Corrected* Viscosity	32.3	31.3	23.2	
Overall Colour.	15.0			

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.
\+ H_2O_2 Positive.

ADDENDUM C4. Continued...

Extraction & Quality Data. Experiment ST2-6.

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	11.63	9.87	8.42	5.04
Liquor pH	2.80	3.23	3.60	4.21
Liquor Concentration (%w/v)	1.70	2.88	5.49	15.35
Fat (ml)	20	10	15	
Scutch (g)				Nil
Gelatine (g)	197.7	284.3	462.6	772.9
Gelatine % Proportion	11.5	16.6	26.9	45.0
Heavy Liquor Volume (ml)	1450	900	1000	
Heavy Liquor Conc:	6	13	12	
5% H_2O_2 (ml)	15	5	3	
5% NH_3 (ml)	7	7	8	
Total Gelatine Recovered (g)				1717.5
Total Gelatine Yield (%)				30.1
Anhydrous Gelatine Recovered (g) (f 0.875)				1502.8
Anhydrous Gelatine Corrected (g) (f 0.95)				1427.7
Anhydrous Gelatine Yield on Raw Material (%)				56.5
Total Anhydrous Solids Recovered (g)				1815.7
Total Anhydrous solids Recovered (%)				71.9

Gelatine Quality.				
Run No	1	2	3	
Bloom	307	280	246	
Colour	11.4	11.4	16.0	20.0
Clarity	11.8	11.8	10.0	14.3
pH	5.2	5.1	5.2	4.15
Moisture (%)	12.5	12.4	9.6	
Ash (%)	3.44	1.82	2.14	
SO_2 (ppm)	40	104	144	
Viscosity (ms @ 60°C)	30.5	28.9	31.5	
Corrected* Bloom	332	291	242	
Corrected* Viscosity	31.8	29.5	31.2	
Overall Colour.	16.5			

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C4. Continued...

Extraction & Quality Data. Experiment ST3-2.

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	10.60	9.26	8.18	8.49
Liquor pH	2.60	3.02	3.49	4.14
Liquor Concentration (%w/v)	0.62	1.56	3.74	14.41
Fat (ml)	40	30	10	
Scutch (g)				430
Gelatine (g)	65.7	144.5	305.9	1223.4
Gelatine % Proportion	3.8	8.3	17.6	70.3
Heavy Liquor Volume (ml)	550	1000	1350	
Heavy Liquor Conc:	7	8	9	
5% H_2O_2 (ml)	20	10	11.5	
5% NH_3 (ml)	15	7.5	10	
Total Gelatine Recovered (g)			1739.5	
Total Gelatine Yield (%)			30.5	
Anhydrous Gelatine Recovered (g) (f 0.875)			1522.1	
Anhydrous Gelatine Corrected (g) (f 0.95)			1446.0	
Anhydrous Gelatine Yield on Raw Material (%)			57.3	
Total Anhydrous Solids Recovered (g)			1790.0	
Total Anhydrous solids Recovered (%)			70.9	
Gelatine Quality.				
Run No	1	2	3	
Bloom	267	287	265	
Colour	10.0	11.4	12.3	16.0
Clarity	8.0	12.5	12.5	5.5
pH	5.7	5.5	5.3	4.2
Moisture (%)	11.2	10.0	10.5	
Ash (%)	8.28f	2.93	2.66	
SO_2 (ppm)	nd	48+	88	
Viscosity (ms @ 60°C)	25.4	22.4	25.1	
Corrected* Bloom	315	290	269	
Corrected* Viscosity	27.6	22.5	35.3	
Overall Colour.	14.7			

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.
f Bloom sample filtered; Whatman GFA
+ H_2O_2 Positive.
nd Not determined.

ADDENDUM C4. Continued...

Extraction & Quality Data. Experiment ST3-4.

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	10.97	9.80	7.51	5.76
Liquor pH	2.72	3.07	3.48	4.16
Liquor Concentration (%w/v)	1.32	2.63	5.22	17.11
Fat (ml)	15	10	10	
Scutch (g)				55
Gelatine (g)	144.8	257.7	392.0	985.5
Gelatine % Proportion	8.1	14.5	22.0	55.4
Heavy Liquor Volume (ml)	1000	1100		
Heavy Liquor Conc:	8	8		
5% H_2O_2 (ml)	19	10		
5% NH_3 (ml)	10	8		

Total Gelatine Recovered (g)	1780.0
Total Gelatine Yield (%)	31.2
Anhydrous Gelatine Recovered (g) (f 0.875)	1557.5
Anhydrous Gelatine Corrected (g) (f 0.95)	1479.6
Anhydrous Gelatine Yield on Raw Material (%)	58.6
Total Anhydrous Solids Recovered (g)	1868.6
Total Anhydrous solids Recovered (%)	74.0

Gelatine Quality.

Run No	1	2	3	
Bloom	305	295	252	
Colour	9.4	12.3	13.3	16.0
Clarity	12.5	12.5	111.1	12.5
pH	5.1	5.3	5.2	4.1
Moisture (%)	10.13	9.7	10.5	
Ash (%)	4.81	2.94	1.40	
SO_2 (ppm)	144	224	32	
Viscosity (ms @ 60°C)	29.0	26.2	27.4	
Corrected* Bloom	322	296	248	
Corrected* Viscosity	29.8	26.2	27.2	
Overall Colour.	14.3			

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.

ADDENDUM C4. Continued...

Extraction & Quality Data. Experiment ST3-6.

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	11.85	8.92	7.84	7.63
Liquor pH	2.60	3.35	3.64	4.20
Liquor Concentration (%w/v)	1.79	3.01	5.80	9.78
Fat (ml)	30	15	30	
Scutch (g)				Nil
Gelatine (g)	212.1	268.5	454.7	746.2
Gelatine % Proportion	12.6	16.0	27.0	44.4
Heavy Liquor Volume (ml)	1250	1000	1600	
Heavy Liquor Conc:(%)	8	10	12	
5% H_2O_2 (ml)	14	6	5	
5% NH_3 (ml)	10	8	25	

Total Gelatine Recovered (g)	1681.5
Total Gelatine Yield (%)	29.5
Anhydrous Gelatine Recovered (g) (f 0.875)	1471.3
Anhydrous Gelatine Corrected (g) (f 0.95)	1397.7
Anhydrous Gelatine Yield on Raw Material (%)	55.4
Total Anhydrous Solids Recovered (g)	1846.7
Total Anhydrous solids Recovered (%)	73.1

Gelatine Quality.

Run No	1	2	3	
Bloom	305	290	222	
Colour	11.4	13.3	16.0	17.8
Clarity	11.1	11.8	10.0	14.3
pH	5.3	5.4	4.8	4.14
Moisture (%)	9.0	9.0	11.6	
Ash (%)	3.42f	2.39	1.53	
SO_2 (ppm)	152	120	48	
Viscosity (ms @ 60°C)	30.4	31.3	29.0	
Corrected* Bloom	305	283	225	
Corrected* Viscosity	30.4	30.9	29.2	
Overall Colour.	15.8			

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.

ADDENDUM C5. Conditioning temperature & sulphide concentration.

EXPERIMENT WT.

Raw Material.

The raw material for this experiment was a salted hide from a 12 year-old Afrikaner animal supplied by ADSRI. The hide had been stored for 72 days before being used.

Mass of hide for each experiment.	4000 g.
Moisture Content	41.1± 0.6%
Ash Content	17.2± 0.2%
Organic Content	41.7%
Organic Content of samples.	1668 g ± 32 g
Prewashing	18hrs minimum.

Sundry Data.

pH of Lime Slurry.

18 g CaO (commercial) + 90 ml Water to slake. Cooled overnight.
pH @ ± 20°C = 12.69
$Ca(OH)_2$ Dissociation Constant $Ka_2 = 3.1 \times 10^2$
Solubility @ 20°C = 1.64 g/l = 2.2×10^2M
$[Ca] \times [OH^-]^2 = 3.1 \times 10^{-2} \times [Ca(OH)_2]$
Hence Theoretical pH = 12.94

pH of Na_2S Solutions:

18 g comm/l. (0.14M) pH = 12.79
0.33 g comm / 100 ml = 2 g Na_2S/l. pH = 12.8
0.6 g AR $Na_2S.9H_2O$(99%) = 2 g Na_2S/l. pH = 12.15

pH of NaOH Solutions:

2.0 g/l = 0.05M Theoretical pH = 12.70
Measured pH = 12.84
20.0 g/l = 0.50M Theoretical pH = 13.70
Measured pH = 13.57
(Error of pH measurements was < ±0.2 pH units.)

Dissolved Oxygen Measurements.

33 g CaO/l as freshly made. DO = 7.8 mg/l
33 g CaO/l boiled & cooled. Do = 1.0 mg/l
WT1 Spent conditioning liquor. DO = 0.1 mg/l
WT2 Spent conditioning liquor. DO = 0.1 mg/l

ADDENDUM C5. Continued...

Hide Conditioning at 22 deg C.

Conditioning Liquor:	CaO 510 g Na_2S qs Water to 16.0 kg.		
Experiment No	WT1	WT2	WT3
Washed Hide (kg)	5.3	5.3	5.25
Conditioning Time (Weeks)	4	4	4
Cond: Temperature (°C)	24.1	22.0	22.0
Init: Sulphide (g Na_2S/l)			1.77±0.03
Init: pH		12.23	12.79
Ex-Lime Wash for 18hrs			
Limed Mass (kg)	6.95	6.8	7.85
Swelling (%)	131	128	150
Acidulation 5 coats of H_2SO_3 soln. over 4 days. Wash 1hr. Soak in fresh water ± 22hr.			
Wt: for Extraction (kg)	7.8	7.2	8.15
Swelling (%)	147	136	155
Soak Water pH	2.33	2.31	2.16
Spent Conditioning Liquor:			
Mass (kg)	16	15.5	15.7
pH	12.39	12.46	12.37
Na_2S Conc: (g/l)	0.03	0.02	0.73
Sulphide consumed (g/l)			1.04
Spent Liquor Solids (g/l)	11.45±0.02	8.74±0.07	18.87±0.05
" " Ash (g/l)	3.87±0.07	2.85±0.5	6.07±0.3
" " Volatiles (g/l)	7.6	5.9	12.8
" " Organic Matter (g)	152	118	256
Organic Matter (%)	9.1	7.0	15.3

ADDENDUM C5. Continued...

Hide Conditioning at 12 deg: C.

Conditioning Liquor:	CaO 510 g Na_2S qs Water to 16.0 kg.	
Experiment No	WT4	WT5
Washed Hide (kg)	4.95	5.1
Conditioning Time -Weeks.	4	4
Cond: Temperature (°C)	12.4±2.7	11.9±3.4
Init: Sulphide (g Na_2S/l)	1.83±0.01	11.1 ±0.0
Init: pH	12.78	12.89
Ex-Lime Wash for 18hrs		
Limed Mass (kg)	6.95	7.6
Swelling (%)	140	149
Acidulation 5 coats of H_2SO_3 soln. over 4 days. Wash 1hr. Soak in fresh water ± 22hr.		
Wt: for Extraction (kg)	7.5	7.75
Swelling (%)	151	152
Soak Water pH	2.18	2.28
Spent Conditioning Liquor:		
Mass (kg)	15.5	15.9
pH	12.64	12.99
Na_2S Conc: (g/l)	0.91±0.01	7.66±0.00
Sulphide consumed (g/l)	0.92	3.44
Spent Liquor Solids (g/l)	15.05±0.00	24.42±0.01
" " Ash (g/l)	4.67±0.03	13.84±0.03
" " Volatiles (g/l)	10.38	10.58
" " Organic Matter (g)	208	212
Organic Matter (%)	12.5	12.7

ADDENDUM C5. Continued...

Extraction & Quality Data Experiment WT1.

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	93
Liquor Volume (l)	7.92	7.77	6.12	8.95
Liquor pH	2.83	3.16	3.55	4.23
Liquor Concentration (%w/v)	0.92	1.63	4.46	9.24
Fat (ml)	15	5	11	
Scutch (g)				155 (Mainly hair)
Gelatine (g)	72.9	126.7	273.0	827.0
Gelatine % Proportion	5.6	9.7	21.0	63.6
Heavy Liquor Volume (ml)	600	900	900	
Heavy Liquor Conc:	8	8	15	
5% H_2O_2 (ml)	15	12	5	
5% NH_3 (ml)	10	7.5	10	

Total Gelatine Recovered (g)	1299.6
Total Gelatine Yield (%)	32.5
Anhydrous Gelatine Recovered (g) (f 0.875)	1137.2
Anhydrous Gelatine Corrected (g) (f 0.95)	1080.3
Anhydrous Gelatine Yield on Raw Material (%)	64.7
Total Anhydrous Solids Recovered (g)	1262.9
Total Anhydrous solids Recovered (%)	75.6

Gelatine Quality.

Run No	1	2	3	4
Bloom	263	253	230	
Colour	12.3f	11.4	12.3	17.8 (Darker than)
Clarity	12.5	12.5	12.5	4.0 (WT2/4)
pH	5.8	5.4	5.3	4.2
Moisture (%)	12.5	12.8	13.8	
Ash (%)	5.60	3.19	1.44	
SO_2 (ppm)		40	32	
Viscosity (ms @ 60°C)	23.8	26.1	25.2	
Corrected* Bloom	300	274	245	
Corrected* Viscosity	25.4	27.1	26.0	

Overall Colour. 15.7

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.
f Bloom sample filtered; Whatman GFA

ADDENDUM C5. Continued...

Extraction & Quality Data Experiment WT2.

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	8.43	8.41	6.87	7.33
Liquor pH	2.74	3.11	3.48	4.15
Liquor Concentration (%w/v)	0.65	1.54	3.87	12.13
Fat (ml)	6	2	2	
Scutch (g)			120 (epidermis)	
Gelatine (g)	54.8	129.5	265.9	889.1
Gelatine % Proportion	4.1	9.7	19.8	66.4
Heavy Liquor Volume (ml)	600	800	1200	
Heavy Liquor Conc:	6	11	12	
5% H_2O_2 (ml)	16	12	5	
5% NH_3 (ml)	10	7.5	10	

Total Gelatine Recovered (g)	1339.3
Total Gelatine Yield (%)	33.5
Anhydrous Gelatine Recovered (g) (f 0.875)	1171.9
Anhydrous Gelatine Corrected (g) (f 0.95)	1113.3
Anhydrous Gelatine Yield on Raw Material (%)	66.6
Total Anhydrous Solids Recovered (g)	1241.1
Total Anhydrous solids Recovered (%)	74.3

Gelatine Quality.

Run No	1	2	3	
Bloom	240	257	227	
Colour	11.4f	10.0	12.3	13.0
Clarity	7.0	12.5	12.5	nm
pH	5.2	5.4	5.4	4.0
Moisture (%)	12.39	13.50	12.98	
Ash (%)	6.16	3.20	1.76	
SO_2 (ppm)	40	64	64	
Viscosity (ms @ 60°C)	21.9	24.8	25.3	
Corrected* Bloom	277	283	239	
Corrected* Viscosity	23.5	26.1	25.9	
Overall Colour.	12.7			

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.
f Bloom sample filtered; Whatman GFA
nm Not measurable.

ADDENDUM C5. Continued...

Extraction & Quality Data Experiment WT3.

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	8.63	6.90	7.14	7.83
Liquor pH	2.69	3.17	3.57	4.25
Liquor Concentration (%w/v)	1.53	2.58	4.80	7.88
Fat (ml)	3	0	3	
Scutch (g)				30
Gelatine (g)	132.0	178.0	342.7	617.0
Gelatine (%) Proportion	10.4	14.0	27.0	48.6
Heavy Liquor Volume (ml)	750	1150	1500	
Heavy Liquor Conc:	10	9	12	
5% H_2O_2 (ml)	10	12	10	
5% NH_3 (ml)	8	10	20	

Total Gelatine Recovered (g)	1269.7
Total Gelatine Yield (%)	31.7
Anhydrous Gelatine Recovered (g) (f 0.875)	1111.0
Anhydrous Gelatine Corrected (g) (f 0.95)	1055.4
Anhydrous Gelatine Yield on Raw Material (%)	63.2
Total Anhydrous Solids Recovered (g)	1317.4
Total Anhydrous solids Recovered (%)	78.9

Gelatine Quality.

Run No	1	2	3	
Bloom	295	273	241	
Colour	10.7	13.3	16.0	14.5
Clarity	13.3	12.5	4.8	12.5
pH	5.5	5.4	5.5	4.1
Moisture (%)	12.67	10.13	10.60	
Ash (%)	2.99	3.18	1.74	
SO_2 (ppm)	nd	184	88	
Viscosity (ms @ 60°C)	27.9	30.1	32.1	
Corrected* Bloom	316	278	240	
Corrected* Viscosity	28.8	30.4	32.0	
Overall Colour.	14.3			

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.
nd Not determined.

ADDENDUM C5. Continued...

Extraction & Quality Data Experiment WT4.

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	7.92	7.86	7.49	6.67
Liquor pH	2.61	3.04	3.55	4.35
Liquor Concentration (%w/v)	0.61	1.35	3.06	14.05
Fat (ml)	10	3	3	
Scutch (g)				230
Gelatine (g)	48.3	106.1	229.2	937.1
Gelatine % Proportion	3.6	8.0	17.4	71.0
Heavy Liquor Volume (ml)	600	900	1250	
Heavy Liquor Conc:	4	6	10	
5% H_2O_2 (ml)	14	16	8	
5% NH_3 (ml)	10	10	8	

Total Gelatine Recovered (g)	1320.7
Total Gelatine Yield (%)	33.0
Anhydrous Gelatine Recovered (g) (f 0.875)	1155.6
Anhydrous Gelatine Corrected (g) (f 0.95)	1097.8
Anhydrous Gelatine Yield on Raw Material (%)	65.7
Total Anhydrous Solids Recovered (g)	1321.4
Total Anhydrous solids Recovered (%)	79.1

Gelatine Quality.

Run No	1	2	3	
Bloom	262	278	267	
Colour	11.4f	12.3f	13.3	17.8
Clarity	12.5	13.3	12.5	nm
pH	5.6	5.5	5.4	4.2
Moisture (%)	10.00	9.84	9.97	
Ash (%)	7.99	4.88	2.08	
SO_2 (ppm)	---	24+	128	
Viscosity (ms @ 60°C)	26.9	26.2	28.2	
Corrected* Bloom	298	293	264	
Corrected* Viscosity	28.7	26.9	18.1	
Overall Colour.	16.3			

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.
f Bloom sample filtered; Whatman GFA
\+ H_2O_2 Positive.
nm Not measurable.

ADDENDUM C5. Continued...

Extraction & Quality Data Experiment WT5.

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	8.42	8.88	7.04	7.13
Liquor pH	2.78	3.31	3.44	---
Liquor Concentration (%w/v)	0.80	1.47	3.47	11.06
Fat (ml)	10	5	3	
Scutch (g)				210
Gelatine (g)	67.4	130.5	244.2	788.6
Gelatine % Proportion	5.5	10.6	19.8	64.1
Heavy Liquor Volume (ml)	600	1100	1300	
Heavy Liquor Conc:	7	7	10	
5% H_2O_2 (ml)	15	14	6	
5% NH_3 (ml)	8	8	8	

Total Gelatine Recovered (g)	1230.7
Total Gelatine Yield (%)	30.8
Anhydrous Gelatine Recovered (g) (f 0.875)	1076.8
Anhydrous Gelatine Corrected (g) (f 0.95)	1023.0
Anhydrous Gelatine Yield on Raw Material (%)	61.2
Total Anhydrous Solids Recovered (g)	1252.6
Total Anhydrous solids Recovered (%)	75.0

Gelatine Quality.

Run No	1	2	3	
Bloom	271	294	261	
Colour	11.4f	13.3f	16.0	17.8
Clarity	12.5	12.5	11.1	nm
pH	5.5	5.4	5.2	4.2
Moisture (%)	11.63	9.05	9.44	
Ash (%)	5.48	3.94	1.86	
SO_2 (ppm)	0	24	176	
Viscosity (ms @ 60°C)	23.4	29.1	29.4	
Corrected* Bloom	301	297	254	
Corrected* Viscosity	24.7	29.3	29.0	
Overall Colour.	16.6			

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.
nm Not measurable.
f Bloom sample filtered; Whatman GF/A

ADDENDUM C6. Young (10.5 month old) animal hide and sodium sulphide level.

EXPERIMENT YS.

Raw Material and Conditioning.

For 2 g/l Na_2S 67 g sodium sulphide flake was used.
For 6 g/l Na_2S 200 g sodium sulphide flake was used.

Conditioning Liquor data.

Bin	Na2S g/l Analyzed	Tare Wt	Gross Wt + liquor	Gross Wt + hide
A	1.63	3.00	23.0	29.05
B	5.70	3.25	23.25	29.15
C	1.60	3.20	23.20	29.10
D	5.73	3.70	23.70	29.50
E	Nil	3.70	23.70	29.65

Raw Material.

Young - 10.5 month old - animal salted hide ex ADSRI.

Prewashing	18hrs minimum.
Mass of hide for each experiment.	6000 g.
Moisture Content	61.01± 0.4%
Ash Content	0.23± 0.03%
Organic Content	38.76%
Organic Content of samples.	2326 g

ADDENDUM C6. Continued...

Hide Conditioning at 22 degree Centigrade.

Conditioning Liquor:	CaO	640 g	
	Na_2S	qs	
	Water to	20.0 kg.	
Experiment No	YSA	YSB	YSC
Washed Hide (kg)	6.0	6.0	6.0
Conditioning Time -Weeks.	2	2	3
Cond: Temperature (°C)	21.4	21.4	21.6
Init: Sulphide (g Na_2S/l)	1.63	5.70	1.60
Ex-Lime Wash for 17 hrs			
Limed Mass (kg)	9.0	9.9	9.3
Swelling (%)	150	165	155
Acidulation 5 coats of H_2SO_3 soln. over 4 days. Wash 1hr. Soak in fresh water ± 22hr.			
Wt: for Extraction (kg)	10.35	11.0	11.85
Swelling (%)	173	183	198
Soak Water pH	2.44	2.35	2.23
Spent Conditioning Liquor:			
Mass (kg)	19.65	19.80	19.95
pH	12.28	12.48	12.30
Na_2S Conc: (g/l)	1.17	4.30	1.00
Sulphide consumed (g/l)	0.46	1.40	0.60
Spent Liquor Solids (g/l)	13.94±0.03	21.47±0.03	16.14±0.00
" " Ash (g/l)	5.01±0.02	9.38±0.03	5.10±0.05
" " Volatiles (g/l)	8.93	12.09	11.04
" " Organic Matter (g)	179	241	220
Organic Matter (%)	7.7	10.4	9.5

ADDENDUM C6. Continued...

Hide Conditioning at 22 degree Centigrade.

Conditioning Liquor:	CaO	640 g	
	Na_2S	qs.	
	Water to	20.0 kg.	
Experiment No		YSD	YSE
Washed Hide (kg)		6.0	6.0
Conditioning Time -Weeks.		3	4
Cond: Temperature (°C)		21.6	22.0
Init: Sulphide (g Na_2S/l)		5.73	Nil
Ex-Lime Wash for 18hrs			
Limed Mass (kg)		9.3	9.1
Swelling (%)		155	152
Acidulation 5 coats of H_2SO_3 soln. over 4 days. Wash 1hr. Soak in fresh water ± 22hr.			
Wt: for Extraction (kg)		12.25	10.2
Swelling (%)		204	170
Soak Water pH		2.31	2.38
Spent Conditioning Liquor:			
Mass (kg)		19.95	19.85
pH		12.53	12.46
Na_2S Conc: (g/l)		3.90	0.04
Sulphide consumed (g/l)		1.83	-0.04
Spent Liquor Solids (g/l)		22.82±0.07	9.4±0.6
" "	Ash (g/l)	9.81	3.07±0.04
" "	Volatiles (g/l)	13.01	6.36
" "	Organic Matter (g)	260	127
	Organic Matter (%)	11.2	5.5

ADDENDUM C6. Continued...

Extraction & Quality Data Experiment YSA.(2 weeks liming, 1.63 g/l Na_2S)

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	55	Boil
Liquor Volume l	12.45	6.04	5.36	4.45
Liquor pH	3.06	3.08	3.28	
Liquor Concentration (%w/v)	4.41	6.85	6.06	6.02
Fat (ml)	40	45	35	
Scutch (g)				360
Gelatine (g)	549.0	413.7	324.8	267.9
Gelatine % Proportion	35.3	26.6	20.9	17.2
Heavy Liquor Volume (ml)	1000	1400	1650	
Heavy Liquor Conc:(%)	10	13	9	
5% H_2O_2 (ml)	4	6	4	
5% NH_3 (ml)	10	20	20	

Total Gelatine Recovered (g)	1555.4
Total Gelatine Yield (%)	25.9
Anhydrous Gelatine Recovered (g) (f 0.875)	1360.9
Anhydrous Gelatine Corrected (g) (f 0.95)	1292.9
Anhydrous Gelatine Yield on Raw Material (%)	55.6
Total Anhydrous Solids Recovered (g)	1591.5
Total Anhydrous solids Recovered (%)	68.4

Gelatine Quality.

Run No	1	2	3	4
Bloom	326	313	286	
Colour	3.6	4.4	4.8	4.8
Clarity	8.0	8.0	7.5	12.5
pH	5.8	5.6	5.6	4.5
Moisture (%)	9.1	9.9	10.8	
Ash (%)	0.46	0.28	0.58	
SO_2 (ppm)	120	632	600	
Viscosity (ms @ 60°C)	55.1	50.8	47.5	
Corrected* Bloom	305	297	279	
Corrected* Viscosity	53	49	47	
Overall Colour.		4.3		

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C6. Continued...

Extraction & Quality Data Experiment YSB. (2 weeks liming, 5.7 g/l Na_2S)

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	55	Boil
Liquor Volume (l)	13.04	6.12	5.90	4.04
Liquor pH	3.03	3.02	3.23	
Liquor Concentration (%w/v)	4.70	6.71	5.24	5.16
Fat (ml)	20	45	50	
Scutch (g)				255
Gelatine (g)	612.9	410.7	309.2	208.5
Gelatine % Proportion	39.8	26.6	20.1	13.5
Heavy Liquor Volume (ml)	1250	1600	1400	
Heavy Liquor Conc:(%)	11	13	10	
5% H_2O_2 (ml)	4	6	3	
5% NH_3 (ml)	12.5	25	17.5	

Total Gelatine Recovered (g)	1541.3
Total Gelatine Yield (%)	25.7
Anhydrous Gelatine Recovered (g) (f 0.875)	1348.6
Anhydrous Gelatine Corrected (g) (f 0.95)	1281.2
Anhydrous Gelatine Yield on Raw Material (%)	55.1
Total Anhydrous Solids Recovered (g)	1638.0
Total Anhydrous solids Recovered (%)	70.4

Gelatine Quality.

Run No	1	2	3	
Bloom	314	312	287	
Colour	<3.2	5.2	4.8	4.4
Clarity	10.0	7.5	9.0	11.8
pH	5.5	5.5	5.6	4.5
Moisture (%)	9.08	9.76	10.79	
Ash (%)	0.47	0.43	0.42	
SO_2 (ppm)	504	616	184	
Viscosity (ms @ 60°C)	53.7	52.6	38.7	
Corrected* Bloom	294	296	279	
Corrected* Viscosity	52	51	38	
Overall Colour.		4.2		

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C6. Continued...

Extraction & Quality Data Experiment YSC. (3 weeks liming, 1.6 g/l Na_2S)

Extraction.				
Run No	1	2	3	
Time (hrs)	5	5	7	
Temperature (°C)	45	50	Boil	
Liquor Volume (l)	14.32	7.15	3.56	
Liquor pH	3.11	3.30		
Liquor Concentration (%w/v)	4.81	6.65	9.45	
Fat (ml)	6	27		
Scutch (g)			310	
Gelatine (g)	688.8	475.5	336.4	
Gelatine % Proportion	45.9	31.6	22.4	
Heavy Liquor Volume (ml)	1350	1400		
Heavy Liquor Conc:(%)	-	10.5		
5% H_2O_2 (ml)	4	4		
5% NH_3 (ml)	15	17.5		
Total Gelatine Recovered (g)				1500.7
Total Gelatine Yield (%)				25.0
Anhydrous Gelatine Recovered (g) (f 0.875)				1313.1
Anhydrous Gelatine Corrected (g) (f 0.95)				1247.5
Anhydrous Gelatine Yield on Raw Material (%)				53.6
Total Anhydrous Solids Recovered (g)				1495.5
Total Anhydrous solids Recovered (%)				64.3

Gelatine Quality.

Run No	1	2	3
Bloom	326	323	
Colour	3.2	4.0	4.4
Clarity	11.1	10.5	11.8
pH	5.5	5.6	4.4
Moisture (%)	9.14	8.60	
Ash (%)	0.34	0.31	
SO_2 (ppm)	160	680	
Viscosity (ms @ 60°C)	53.1	51.9	
Corrected* Bloom	305	298	
Corrected* Viscosity	51	50	
Overall Colour.		4.0	

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.

ADDENDUM C6. Continued...

Extraction & Quality Data Experiment YSD. (3 weeks liming, 5.7 g/l Na_2S)

Extraction.			
Run No	1	2	3
Time (hrs)	5	5	7
Temperature (°C)	45	50	Boil
Liquor Volume (l)	15.34	8.00	4.20
Liquor pH	2.97	3.20	
Liquor Concentration (%w/v)	4.81	5.73	4.22
Fat (ml)	3	35	
Scutch (g)			110
Gelatine (g)	737.9	458.4	177.2
Gelatine % Proportion	53.7	33.4	12.9
Heavy Liquor Volume (ml)	1350	1300	
Heavy Liquor Conc:(%)	10	12	
5% H_2O_2 (ml)	4	2	
5% NH_3 (ml)	15	20	
Total Gelatine Recovered (g)			1373.5
Total Gelatine Yield (%)			22.9
Anhydrous Gelatine Recovered (g) (f 0.875)			1201.8
Anhydrous Gelatine Corrected (g) (f 0.95)			1141.7
Anhydrous Gelatine Yield on Raw Material (%)			49.1
Total Anhydrous Solids Recovered (g)			1441.9
Total Anhydrous solids Recovered (%)			62.0

Gelatine Quality.

Run No	1	2	3
Bloom	322	323	
Colour	3.2	3.2	4.8
Clarity	11.1	11.8	13.3
pH	5.5	5.4	4.4
Moisture (%)	9.21	8.55	
Ash (%)	0.37	0.26	
SO_2 (ppm)	96	1184	
Viscosity (ms @ 60°C)	55.5	48.7	
Corrected* Bloom	332	297	
Corrected* Viscosity	54	47	
Overall Colour.		3.4	

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C6. Continued...

Extraction & Quality Data Experiment YSE. (4 weeks liming, No Na_2S)

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	55	Boil
Liquor Volume (l)	13.23	8.24	6.50	2.48
Liquor pH	3.14	3.27	3.41	
Liquor Concentration (%w/v)	3.51	5.02	6.12	6.04
Fat (ml)	30	20	Not Measurable.	
Scutch (g)		161.7 Dry. **		
Gelatine (g)	464.4	413.6	397.8	(274.2)
Gelatine % Proportion	30.0	26.7	25.7	17.6
Heavy Liquor Volume (ml)	1000	1400	1300	
Heavy Liquor Conc:(%)	10	9	14	
5% H_2O_2 (ml)	2	2	2	
5% NH_3 (ml)	10	17.5	25	

Total Gelatine Recovered (g)	1550 estimated.
Total Gelatine Yield (%)	25.8
Anhydrous Gelatine Recovered (g) (f 0.875)	1356.3
Anhydrous Gelatine Corrected (g) (f 0.95)	1288.4
Anhydrous Gelatine Yield on Raw Material (%)	55.4
Total Anhydrous Solids Recovered (g)	1465.6
Total Anhydrous solids Recovered (%)	63.0

Gelatine Quality.

Run No	1	2	3
Bloom	340	319	292
Colour	<3.2	4.0	5.2
Clarity	10.5	7.5	7.5
pH	5.6	5.5	5.3
Moisture (%)	9.56	10.73	10.2
Ash (%)	0.62	0.40	0.36
SO_2 (ppm)	48	40	80
Viscosity (ms @ 60°C)	44.0	46.8	43.4
Corrected* Bloom	323	309	279
Corrected* Viscosity	43	46	42
Overall Colour.		±4.0	

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.

**The residue boil nearly boiled dry, thus some of the gelatine was rendered insoluble. For this reason the insoluble residue was air dried and weighed. The total recovery was 1587.3 g which included all the insolubilized gelatine and scutch. For this reason a gelatine yield of 1550 g was taken for calculation purposes.

ADDENDUM C7. Gelatine from various layers of a hide.

EXPERIMENT KTO.
(Krugersdorp Tannery Old animal hide).

Raw Material and Conditioning Data.

Sample	KT01 Whole Hide	KT02 Flesh Split	KT03 Middle Split	KT04 Grain Split
Moisture Content (%)	69.84	71.6	68.26	71.77
Ash Content (%)	3.82	17.70	2.69	2.24
Sample Mass (kg)	8.55	2.40	2.75	3.15
Anhyd: Hide Subs: (g)	2315	257	799	819

Conditioning.

Conditioning Liquor: 640 g CaO in 20 kg water.

Sample No	KT01	KT02	KT03	KT04
Cond: Time (Weeks) *	6	6	6	6
Cond: Temperature (°C)	21.5	21.5	21.0	21.0
Init: Sulphide Na_2S (g/l)	2.19	2.07	1.94	1.99
Final Sulphide Na_2S (g/l)	1.18	1.33	1.29	1.19
Sulphide consumed (g/l)	1.01	0.74	0.65	0.80
Spent Liquor Solids (%w/v)	1.96	1.00	0.83	1.00
" " Ash (%w/v)	0.98	0.68	0.64	0.74
" " Volatiles (%w/v)	0.98	0.32	0.19	0.26
" " Organic Matter (g)	196	64	37	51
" " Absorb: (470nm)	0.050	0.000	0.001	0.006
Ex-Lime Wash for 16hrs.				
Limed Mass (kg)	14.2	5.85	4.10	4.6
Swelling (%)	167	244	149	146
Acidulation. 5 coats of H_2SO_3 soln. over 4 days. Wash 1hr. Soak in fresh water ± 22hr.				
Wt: for Extraction (kg)	13.5	5.5	5.1	5.1
Swelling (%)	158	229	185	162
Soak Water pH	2.82	2.12	2.91	2.83

* See experimental procedures.

ADDENDUM C7. Continued...

KT01 Whole Hide Extraction & Quality Data.

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	13.66	10.80	10.05	5.66
Liquor pH	2.73	3.36	3.66	
Liquor Concentration (%w/v)	1.49	2.24	4.63	7.94
Fat (ml)	Nil	Nil	Nil	
Scutch (g)				Nil
Gelatine (g)	203.5	241.9	465.3	1015.1
Gelatine % Proportion	10.5	12.6	24.2	52.7
Heavy Liquor Volume (ml)	1200	1200	1300	
Heavy Liquor Conc:	7	5.5	8	
5% H_2O_2 (ml)	2.5	1	0.5	
5% NH_3 (ml)	9	12	24	

Total Gelatine Recovered (g)	1925.8
Total Gelatine Yield (%)	22.5
Anhydrous Gelatine Recovered (g) (f 0.875)	1685.1
Anhydrous Gelatine Corrected (g) (f 0.95)	1600.8
Anhydrous Gelatine Yield on Raw Material (%)	69.1
Total Anhydrous Solids Recovered (g)	1796.8
Total Anhydrous solids Recovered (%)	77.6

Gelatine Quality.

Run No	1	2	3	4
Bloom	324	271	222	
Colour	8	7.2	9.4	13.3
Clarity	11.8	11.8	9.0	14.3
pH	5.5	5.5	5.5	4.08
Moisture (%)	8.06	11.81	12.12	
Ash (%)	1.23	0.71	0.40	
SO_2 (ppm) ϕ	16	376	272	
Viscosity (ms @ 60°C)	35.0	30.8	30.9	
Corrected* Bloom	301	271	222	
Corrected* Viscosity	33.7	30.8	30.9	

Overall Colour.	11.6

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.

ϕ + Indicates peroxide positive on Starch/KI test.

ADDENDUM C7. Continued...

KT02 Flesh Split Extraction & Quality Data.

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	6.95	4.66	4.88	1.44
Liquor pH	3.35	3.69	3.9	
Liquor Concentration (%w/v)	0.72	1.035	1.33	6.795
Fat (ml)	Nil	Nil	Nil	
Scutch (g)				Nil
Gelatine (g)	50	48.2	64.9	97.8
Gelatine % Proportion	19.2	18.5	24.9	37.4
Heavy Liquor Volume (ml)	600	600	900	
Heavy Liquor Conc:	5	5	3	
5% H_2O_2 (ml)	0.5	0.5	0.5	
5% NH_3 (ml)	5	6	8	

Total Gelatine Recovered (g)	260.9
Total Gelatine Yield (%)	10.9
Anhydrous Gelatine Recovered (g) (f 0.875)	228.3
Anhydrous Gelatine Corrected (g) (f 0.95)	216.9
Anhydrous Gelatine Yield on Raw Material (%)	84.3
Total Anhydrous Solids Recovered (g)	280.9
Total Anhydrous solids Recovered (%)	109.3

Gelatine Quality.

Run No	1	2	3	4
Bloom	310	267	234	
Colour	8.4	10.0	16.0	NM
Clarity	11.1	7.0	3.5	NM
pH	5.3	5.2	5.3	4.3
Moisture (%)	8.58	12.12	11.05	
Ash (%)	2.14	1.62	1.30	
SO_2 (ppm) ϕ	368	272	464	
Viscosity (ms @ 60°C)	41.0	39.4	41.6	
Corrected* Bloom	298	274	233	
Corrected* Viscosity	40.2	39.9	41.5	
Overall Colour.		---		

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.
ϕ + Indicates peroxide positive on Starch/KI test.

ADDENDUM C7. Continued...

KT03 Middle Split Extraction & Quality Data.

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	6.8	5.25	4.3	2.4
Liquor pH	3.27	3.5	3.74	
Liquor Concentration (%w/v)	0.86	1.44	3.73	20.15
Fat (ml)	Nil	Nil	Nil	
Scutch (g)				Nil
Gelatine (g)	58.5	75.6	160.4	483.6
Gelatine % Proportion	7.5	9.7	20.6	62.2
Heavy Liquor Volume (ml)	650	450	950	
Heavy Liquor Conc:	6	12	9	
5% H_2O_2 (ml)	0.5	0.25	0.1	
5% NH_3 (ml)	8	14	23	

Total Gelatine Recovered (g)	778.1
Total Gelatine Yield (%)	28.3
Anhydrous Gelatine Recovered (g) (f 0.875)	680.8
Anhydrous Gelatine Corrected (g) (f 0.95)	646.8
Anhydrous Gelatine Yield on Raw Material (%)	80.9
Total Anhydrous Solids Recovered (g)	683.8
Total Anhydrous solids Recovered (%)	85.6

Gelatine Quality.

Run No	1	2	3	4
Bloom	313	252	218	
Colour	6.4	6.0	6.4	16.0
Clarity	12.5	12.5	11.1	15.45
pH	5.3	5.3	5.3	4.15
Moisture (%)	9.98	10.21	9.46	
Ash (%)	2.02	1.08	0.36	
SO_2 (ppm) ϕ	160	192	240	
Viscosity (ms @ 60°C)	30.3	28.8	28.8	
Corrected* Bloom	309	245	205	
Corrected* Viscosity	30.1	28.4	27.9	

Overall Colour.	12.3

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.
ϕ + Indicates peroxide positive with Starch/KI test.

ADDENDUM C7. Continued...

KT04 Grain Split Extraction & Quality Data.

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	6.65	4.92	4.01	3.36
Liquor pH	3.13	3.51	3.68	
Liquor Concentration (%w/v)	1.01	1.71	3.93	13.95
Fat (ml)	Nil	Nil	Nil	
Scutch (g)				Nil
Gelatine (g)	67.2	84.1	157.6	468.7
Gelatine % Proportion	8.6	10.8	20.3	60.3
Heavy Liquor Volume (ml)	675	600	800	
Heavy Liquor Conc:	7	11	10	
5% H_2O_2 (ml)	0.5	0.25	0.1	
5% NH_3 (ml)	10	16	20	

Total Gelatine Recovered (g)	777.6
Total Gelatine Yield (%)	24.77
Anhydrous Gelatine Recovered (g) (f 0.875)	680.4
Anhydrous Gelatine Corrected (g) (f 0.95)	646.4
Anhydrous Gelatine Yield on Raw Material (%)	78.9
Total Anhydrous Solids Recovered (g)	697.6
Total Anhydrous solids Recovered (%)	85.1

Gelatine Quality.

Run No	1	2	3	4
Bloom	316	259	216	
Colour	6.0	6.0	6.8	16.7
Clarity	12.5	12.5	11.1	9.0
pH	5.5	5.5	5.5	4.15
Moisture (%)	9.96	10.01	9.73	
Ash (%)	1.49	0.91	0.34	
SO_2 (ppm)	32	144	160	
Viscosity (ms @ 60°C)	30.0	29.8	29.7	
Corrected* Bloom	308	249	204	
Corrected* Viscosity	29.6	29.3	28.9	

Overall Colour.	12.6

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C8. Type A calf skin gelatine.

EXPERIMENT CALF-A

Conditioning.

Raw Material Weight. 7.6 kg.
Moisture content. 44.75%
Ash content. 17.03%
Organic Matter. 38.22% = 2905 g.

Tumbler washed for 17 (hrs).
Dehairing. Solution (3%) = 20 kg containing 1 kg 60% Na_2S.
Time. 30 minutes with occasional stirring.
Tumbler wash. 6 (hrs).

Dehaired & Washed Weight. 11.25 kg.
Moisture content. 85.93%
Ash Content. 0.13%
Organic Matter. 13.94% (= 1568 g).
Hair + epidermis 1337 g (= 17.6% of Raw Material).

Acidulation.

57 (ml) Conc: H_2SO_4 diluted to 20 kg. By titration = 0.103N.
= 2.06 Eq.
Acidulation time 17 hr.
Spent acid = 0.019N. (pH = 1.7).
" " Concentration = 0.33% acid soluble collagen.
or ±66 g.
Total water volume = 20 (l) acid solution
+ 9.67 (l) in washed skin.
Residual acid = 29.66 (l) @ 0.019N = 0.564 Eq.
Acid consumed = 2.06 - 0.56 = 1.50 Eq
or 0.96 Eq/kg collagen.

ADDENDUM C8. Continued...

CALF-A

Extraction.

Weight for extraction = 17.0 kg.
Soak water pH = 3.2.

Run No	1	2	3	4
Extraction Time. (Hours)	5	5	5	7
Extraction Temperature. (°C)	50	60	70	Boil
Extract Volume. (l).	16.24	6.84	5.42	4.41
Extract pH.	3.63	3.73	3.78	
Extract Concentration. (%)	3.40	4.58	3.97	4.46
Extract proportion (%).	43.2	24.5	16.8	15.4
Fat. (ml).	40	15		
Residue (kg).				2.1
Heavy Liquor Volume. (l).	2.4	1.8	1.05	
Heavy Liquor Conc: (%).	10.5	9	8.5	
5% NH_3 added to >pH 5	5 (ml)	0 (ml)	*45 (ml)	
* 0.1N H_2SO_4				
Total Gelatine Produced.		1277 g		
Yield		16.8 %		
Corrected Yield (f= 0.92)		15.5 %		
Yield on Anhyd: Organic matter.		43.9 % (Normally 60% min:)		

Gelatine Quality

Run No.	1	2	3
Bloom	351	333	284
Colour DGI	4.0	3.6	5.2
Clarity DGI	7.5	10.5	9.0
pH	6.2	6.3	6.5
Moisture (%).	10.08	11.07	13.79
Ash (%)	0.27	0.21	0.11
SO_2 ppm Titre.	S 22	S 31	S 17
SO_2 ppm Distillation.	72	128	56
Viscosity ms.	55.9	63.5	32.6
Isoelectric Point	9.4	9.2	8.9

ADDENDUM C9. Three & six year-old Friesland's hides ex ADSRI.

EXPERIMENTS 3Y and 6Y.

3 Year-old - Raw Material and Conditioning.

Raw Material: Hide No 8901 - Washed and Drained.
3 year-old Friesland hide.

Moisture Content	65.6 ± 3.8%
Ash Content	0.28 ± 0.1%
Sample Mass (kg)	5.0
Anhyd: Hide Subs:	1705 g

Conditioning.

Conditioning Liquor: CaO 640 g
Na_2S 60% 75 g (150 g)
Water to 20 kg.

Sample No	3Y2	3Y4A	3Y4B
Cond: Time Weeks	2	4	4
Cond: Temperature (°C)	21.7	21.8	21.8
Init: Sulphide (g Na_2S/l)	1.99	1.95	4.25
Final Sulphide (g Na_2S/l)	1.43	1.12	2.78
Sulphide consumed (g/l)	0.56	0.83	1.47
Spent Liquor Solids (%w/v)	1.62	1.90	2.10
" " Ash (%w/v)	0.60	0.44	0.66
" " (%w/v) Volatiles	1.02	1.46	1.44
" " Organic Matter (g)	204	292	288
Evaporative Loss (kg)	0.35	0.3	0.45
Ex-Lime Wash for 16hrs			
Limed Mass (kg)	7.1	7.75	7.55
Swelling (%)	142	155	151
Acidulation 5 coats of			
H_2SO_3 soln. over 4 days.			
Wash 1hr.			
Soak in fresh water ± 22hr.			
Wt: for Extraction (kg)	7.3	9.8	8.15
Soak Water pH	2.34	2.1	2.04

Sample No	3Y6A	3Y6B	3Y8
Cond: Time Weeks	6	6	8
Cond: Temperature (°C)	21.8	21.8	21.8
Init: Sulphide (g Na_2S/l)	1.99	0.01	2.03
Final Sulphide (g Na_2S/l)	0.96	0.05	0.69
Sulphide consumed (g/l)	1.03	-0.04	1.34
Spent Liquor Solids (%w/v)	2.24	1.12	2.44
" " Ash (%w/v)	0.56	0.30	0.75
" " pH	12.38	12.27	
" " (%w/v) Volatiles	1.68	0.83	1.69
" " Organic Matter (g)	336	166	338
Evaporative Loss (kg)			0.6
Ex-Lime Wash for 16hrs			
Limed Mass (kg)			7.05
Swelling (%)			141
Acidulation 5 coats of			
H_2SO_3 soln. over 4 days.			
Wash 1hr.			
Soak in fresh water ± 22hr			

ADDENDUM C9. Continued...

Six Year-old - Raw Material and Conditioning.

Raw Material: Hide No 8551 - Washed and Drained.
6 year-old Friesland hide.

Moisture Content	59.3 ± 3.8%
Ash Content	0.59 ± 0.01%

Sample No	6Y2	6Y4A	6Y4B
Sample Mass (kg)	6.0	6.0	6.0
Anhyd: Hide Subs: (g)	2406	2406	2406

Conditioning.
Conditioning Liquor: CaO 640 g
Na_2S 60% 75 g (150 g)
Water to 20 kg.

Sample No	6Y2	6Y4A	6Y4B
Cond: Time (Weeks)	2	4	4
Cond: Temperature (°C)	21.6	21.7	21.7
Init: Sulphide (g Na_2S/l)	2.05	2.02	4.41
Final Sulphide (g Na_2S/l)	1.37	1.03	2.44
Sulphide consumed (g/l)	0.69	0.99	1.96
Spent Liquor Solids (%w/v)	1.78	2.14	2.83
" " Ash (%w/v)	0.65	0.43	0.78
" " (%w/v) Volatiles	1.13	1.71	2.05
" " Organic Matter (g)	226	332	394
Evaporation Loss (kg)	0.15	0.6	0.8
Ex-Lime Wash for 16hrs			
Limed Mass (kg)	8.55	8.75	9.10
Swelling (%)	143	146	152
Acidulation 5 coats of			
H_2SO_3 soln. over 4 days.			
Wash 1hr.			
Soak in fresh water ± 22hr.			
Wt: for Extraction (kg)	8.85	9.05	8.9
Soak Water pH	2.07	2.25	2.14

ADDENDUM C9. Continued...

Sample No	6Y6A	6Y6B	6Y8
Sample Mass (kg)	6.0	2.4	6.0
Anhyd: Hide Substance (g)	2406	962	2406
Cond: Time Weeks	6	6	8
Cond: Temperature (°C)	21.8	21.8	21.7
Init: Sulphide (g Na_2S/l)	2.04	0.0	2.03
Final Sulphide (g Na_2S/l)	0.81	0.02	0.70
Sulphide consumed (g/l)	1.23	-0.02	1.33
Spent Liquor Solids (%w/v)	2.48	0.59	2.69
" " Ash (%w/v)	0.61	0.19	0.62
" " (%w/v) Volatiles	1.87	0.40	2.07
" " pH	13.09	13.31	
" " Organic Matter (g)	374	80	414
Evaporation Loss (kg)			0.1
Ex-Lime Wash for 16hrs			
Limed Mass (kg)	8.45	3.00	
Swelling (%)	141	125	
Acidulation 5 coats of H_2SO_3 soln. over 4 days. Wash 1hr. Soak in fresh water ± 22hr.			
Weight for Extraction (kg)	9.0	3.25	8.6
Soak Water pH	2.07	2.22	2.26

ADDENDUM C9. Continued...

Extraction & Quality Data. Experiment 3Y2. (2 weeks liming)

Extraction.				
Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	55	Boil
Liquor Volume (l)	8.98	7.35	7.05	5.24
Liquor pH	2.65	3.21	3.57	
Liquor Concentration (%w/v)	1.27	2.42	3.82	11.99
Fat (ml)	35	15	10	
Scutch (g)				210
Gelatine (g)	114.0	177.9	269.3	628.3
Gelatine % Proportion	9.6	15.0	22.6	52.8
Heavy Liquor Volume (ml)	600	1200	1000	
Heavy Liquor Conc: (%)	8	9	12	
5% H_2O_2 (ml)	14	14	10	
5% NH_3 (ml)	7	7	5	

Total Gelatine Recovered (g)	1189.5
Total Gelatine Yield (%)	23.8
Anhydrous Gelatine Recovered (g) (f 0.875)	1040.8
Anhydrous Gelatine Corrected (g) (f 0.95)	988.8
Anhydrous Gelatine Yield on Raw Material (%)	58.0
Total Anhydrous Solids Recovered (g) (Fat + Cond:)	1252.8
Total Anhydrous solids Recovered (%)	73.5

Gelatine Quality.

Run No	1	2	3	4
Bloom	295	296	292	
Colour	6.4	6.0	6.4/7.2	4.8
Clarity	12.5	11.1	11.1	10.5
pH	5.5	5.4	5.4	4.5
Moisture (%)	13.25	12.58	12.3	
Ash (%)	4.58	2.69	2.51	
SO_2 (ppm)				
Viscosity (ms @ 60°C)	23.0	32.5	38.5	
Corrected* Bloom	335	316	308	
Corrected* Viscosity	25	34	40	
Overall Colour.		5.5		

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.

ADDENDUM C9. Continued...

Extraction & Quality Data. Experiment 3Y4A. (4 weeks liming, 2 g/l Na_2S)

Extraction.			
Run No	1	2	3
Time (hrs)	5	5	7
Temperature (°C)	45	50	Boil
Liquor Volume (l)	10.01	7.47	3.97
Liquor pH	2.89	3.31	
Liquor Concentration (%w/v)	3.33	4.72	12.39
Fat (ml)	15	15	
Scutch (g)			50
Gelatine (g)	333.3	352.6	491.9
Gelatine % Proportion	28.3	29.9	41.8
Heavy Liquor Volume (ml)	1250	1000	
Heavy Liquor Conc: (%)	9	11	
5% H_2O_2 (ml)	15	7	
5% NH_3 (ml)	10	8	
Total Gelatine Recovered (g)			1177.8
Total Gelatine Yield (%)			23.6
Anhydrous Gelatine Recovered (g) (f 0.875)			1030.6
Anhydrous Gelatine Corrected (g) (f 0.95)			979.0
Anhydrous Gelatine Yield on Raw Material (%)			57.4
Total Anhydrous Solids Recovered (g) (Fat + Cond:)			1301
Total Anhydrous solids Recovered (%)			76.3

Gelatine Quality.

Run No	1	2	3
Bloom	266	272	
Colour	6.4/6.8	6.0	3.6
Clarity	10.0	11.1	14.3
pH	5.4	5.7	4.3
Moisture (%)	15.5	14.8	
Ash (%)	3.01	2.74	
SO_2 (ppm)	448	624	
Viscosity (ms @ 60°C)	37.5	40.6	
Corrected* Bloom	307	306	
Corrected* Viscosity	40	43	
Overall Colour.		5.2	

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.

ADDENDUM C9. Continued...

Extraction & Quality Data. Experiment 3Y4B. (4 weeks liming, 4g/l Na_2S)

Extraction.			
Run No	1	2	3
Time (hrs)	5	5	7
Temperature (°C)	45	50	Boil
Liquor Volume (l)	9.82	7.96	3.47
Liquor pH	3.00	3.29	
Liquor Concentration (%w/v)	3.58	5.02	13.44
Fat (ml)	5	15	
Scutch (g)			50
Gelatine (g)	351.6	399.6	466.4
Gelatine % Proportion	28.9	32.8	38.3
Heavy Liquor Volume (ml)	1200	1200	
Heavy Liquor Conc: (%)	9	10	
5% H_2O_2 (ml)	12	12	
5% NH_3 (ml)	8	5	

Total Gelatine Recovered (g)	1217.6
Total Gelatine Yield (%)	24.3
Anhydrous Gelatine Recovered (g) (f 0.875)	1065.4
Anhydrous Gelatine Corrected (g) (f 0.95)	1023.1
Anhydrous Gelatine Yield on Raw Material (%)	59.4
Total Anhydrous Solids Recovered (g) (Fat + Cond:)	1331.1
Total Anhydrous solids Recovered (%)	78.1

Gelatine Quality.

Run No	1	2	3
Bloom	277	277	
Colour	6.4	6.4	5.6
Clarity	9.5	10.5	14.3
pH	5.4	5.3	4.4
Moisture (%)	15.89	12.74	
Ash (%)	2.97	3.15	
SO_2 (ppm)	440	384	
Viscosity (ms @ 60°C)	34.3	41.2	
Corrected* Bloom	322	300	
Corrected* Viscosity	37	43	
Overall Colour.		6.1	

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C9. Continued...

Extraction & Quality Data. Experiment 3Y6A. (6 weeks liming)

Extraction.

Run No	1	2	3
Time (hrs)	5	5	7
Temperature (°C)	45	50	Boil
Liquor Volume (l)	10.51	8.18	4.51
Liquor pH	2.90	3.24	
Liquor Concentration (%w/v)	3.98	Est*4.92	7.48
Fat (ml)	1	Nil	
Scutch (g)			Nil
Gelatine (g)	418.3	402.4	337.3
Gelatine % Proportion	36.1	34.8	29.1
Heavy Liquor Volume (ml)	1300	1250	
Heavy Liquor Conc: (%)	9	10	
5% H_2O_2 (ml)	13	10	
5% NH_3 (ml)	7.5	5	

Total Gelatine Recovered (g)	Est*1158.0
Total Gelatine Yield (%)	23.2
Anhydrous Gelatine Recovered (g) (f 0.875)	
Anhydrous Gelatine Corrected (g) (f 0.95)	
Anhydrous Gelatine Yield on Raw Material (%)	
Total Anhydrous Solids Recovered (g)	(1322.1)
Total Anhydrous solids Recovered (%)	(77.5)

* Due to loss of the sample, estimates were based on the average yields for the other 5 lots of hide from the same animal.

Gelatine Quality.

Run No	1	2	3
Bloom	305	270	
Colour	6.0	6.8	
Clarity	12.5	11.8	
pH	5.4	5.4	
Moisture (%)	11.3	12.55	
Ash (%)	3.58	2.67	
SO_2 (ppm)	936	96	
Viscosity (ms @ 60°C)	29.5	39.2	
Corrected* Bloom	322	288	
Corrected* Viscosity	34	41	
Overall Colour.		Not Available.	

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C9. Continued...

Extraction & Quality Data. Experiment 3Y6B. (6 weeks liming, no Na_2S)

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	8.76	8.31	6.81	1.92
Liquor pH	2.89	3.22	3.5	
Liquor Concentration (%w/v)	2.34	3.00	6.61	15.3
Fat (ml)	20	7	20	
Scutch (g)				Nil
Gelatine (g)	205.0	249.3	450.1	293.8
Gelatine % Proportion	17.1	20.8	37.6	24.5
Heavy Liquor Volume (ml)	1050	1050	1115	
Heavy Liquor Conc: (%)	6	11		
5% H_2O_2 (ml)	9	14	5	
5% NH_3 (ml)	5	7	7	

Total Gelatine Recovered (g)	1198.2
Total Gelatine Yield (%)	24.0
Anhydrous Gelatine Recovered (g) (f 0.875)	1048.4
Anhydrous Gelatine Corrected (g) (f 0.95)	996.0
Anhydrous Gelatine Yield on Raw Material (%)	58.4
Total Anhydrous Solids Recovered (g) (Fat + Cond:)	1208.6
Total Anhydrous solids Recovered (%)	70.9

Gelatine Quality.

Run No	1	2	3
Bloom	296	270	223
Colour	6.0	6.0	6.8
Clarity	11.1	12.5	10.5
pH	5.3	5.3	5.3
Moisture (%)	12.16	12.74	13.66
Ash (%)	6.44	2.85	2.01
SO_2 (ppm)	80	56	240
Viscosity (ms @ 60°C)	25.0	28.4	39.4
Corrected* Bloom	342	290	240
Corrected* Viscosity	27	29	41

Overall Colour. Not Available.

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.

ADDENDUM C9. Continued...

Extraction & Quality Data. Experiment 3Y8. (8 weeks liming)

Extraction.

Run No	1	2	3
Time (hrs)	5	5	7
Temperature (°C)	45	50	Boil
Liquor Volume (l)	10.0	6.48	3.24
Liquor pH	2.86	3.29	
Liquor Concentration (%w/v)	3.69	5.16	9.12
Fat (ml)	Nil	Nil	
Scutch (g)			Nil
Gelatine (g)	369.0	334.4	295.5
Gelatine % Proportion	36.9	33.5	29.6
Heavy Liquor Volume (ml)	1050		
Heavy Liquor Conc: (%)	10.5		
5% H_2O_2 (ml)	10	8.5	
5% NH_3 (ml)	7	7.5	

Total Gelatine Recovered (g)	998.9
Total Gelatine Yield (%)	20.0
Anhydrous Gelatine Recovered (g) (f 0.875)	874.0
Anhydrous Gelatine Corrected (g) (f 0.95)	830.3
Anhydrous Gelatine Yield on Raw Material (%)	48.7
Total Anhydrous Solids Recovered (g) (Fat + Cond:)	1167.3
Total Anhydrous solids Recovered (%)	68.4

Gelatine Quality.

Run No	1	2	3
Bloom	288	236	
Colour	6.4	6.8	
Clarity	11.8	12.5	
pH	5.4	5.3	
Moisture (%)	12.74	13.14	
Ash (%)	3.19	2.72	
SO_2 (ppm)	80	24	
Viscosity (ms @ 60°C)	35.9	31.6	
Corrected* Bloom	312	255	
Corrected* Viscosity	37	33	
Overall Colour.		Not Available.	

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.

ADDENDUM C9. Continued...

Extraction & Quality Data. Experiment 6Y2. (2 weeks liming)

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	7.62	7.92	7.08	3.51
Liquor pH	2.72	2.99	3.42	
Liquor Concentration (%w/v)	1.11	2.26	4.85	19.31
Fat (ml)	100	35	25	
Scutch (g)				315
Gelatine (g)	84.6	179	343.4	677.8
Gelatine % Proportion	6.6	13.9	26.7	52.8
Heavy Liquor Volume (ml)	625	1100	1400	
Heavy Liquor Conc: (%)	7	8	13	
5% H_2O_2 (ml)	11	14	10	
5% NH_3 (ml)	10	7	7	

Total Gelatine Recovered (g)	1284.8
Total Gelatine Yield (%)	21.4
Anhydrous Gelatine Recovered (g) (f 0.875)	1121.5
Anhydrous Gelatine Corrected (g) (f 0.95)	1065.5
Anhydrous Gelatine Yield on Raw Material (%)	44.28
Total Anhydrous Solids Recovered (g) (Fat + Cond:)	1451.5
Total Anhydrous solids Recovered (%)	60.3

Gelatine Quality.

Run No	1	2	3	4
Bloom	98	303	289	
Colour	5.2(F)	6.8	7.6/8.0	7.6
Clarity	10.0	11.1	10.5	10.0
pH	6.1	5.3	5.3	4.5
Moisture (%)	11.3	10.94	9.79	
Ash (%)	5.37	3.08	2.22	
SO_2 (ppm)				
Viscosity (ms @ 60°C)		27.0	31.9	
Corrected* Bloom	108	314	286	
Corrected* Viscosity		28	32	
Overall Colour.		7.4		

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.
(F) Filtered using GFA paper before colour/clarity determination.

ADDENDUM C9. Continued...

Extraction & Quality Data. Experiment 6Y4A. (4 weeks liming, 2 g/l Na_2S)

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	9.00	7.16	7.41	5.47
Liquor pH	2.73	3.4	3.62	
Liquor Concentration (%w/v)	2.09	3.57	5.70	6.63
Fat (ml)	50	15	35	
Scutch (g)				40
Gelatine (g)	188.1	255.6	422.4	362.7
Gelatine % Proportion	15.3	20.8	34.4	29.5
Heavy Liquor Volume (ml)	900	1100	1200	
Heavy Liquor Conc: (%)	6	9	10	
5% H_2O_2 (ml)	5	8	9	
5% NH_3 (ml)	2.5	5	7	

Total Gelatine Recovered (g)	1228.8
Total Gelatine Yield (%)	20.5
Anhydrous Gelatine Recovered (g) (f 0.875)	1075.2
Anhydrous Gelatine Corrected (g) (f 0.95)	1021.4
Anhydrous Gelatine Yield on Raw Material (%)	42.4
Total Anhydrous Solids Recovered (g)	1453.4
Total Anhydrous solids Recovered (%)	60.4

Gelatine Quality.

Run No	1	2	3	4
Bloom	312	284	249	
Colour	8.4	7.6	8.9	6.8
Clarity	10.5	13.3	12.5	14.3
pH	5.4	5.5	5.5	4.4
Moisture (%)	9.8	11.42	11.26	
Ash (%)	2.82	2.45	2.90	
SO_2 (ppm)	120	8	240	
Viscosity (ms @ 60°C)	38.4	29.4	33.7	
Corrected* Bloom	313	293	259	
Corrected* Viscosity	39	30	34	
Overall Colour.		7.9		

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C9. Continued...

Extraction & Quality Data. Experiment 6Y4B. (4 weeks liming, 4 g/l Na_2S)

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	8.91	7.50	7.02	4.83
Liquor pH	2.75	3.17	3.53	
Liquor Concentration (%w/v)	2.48	3.63	6.13	7.80
Fat (ml)	25	10	25	
Scutch (g)				30
Gelatine (g)	221	272.3	430.3	376.7
Gelatine % Proportion	17.0	20.9	33.1	29.0
Heavy Liquor Volume (ml)	1100	1600	1100	
Heavy Liquor Conc: (%)	8	7	13	
5% H_2O_2 (ml)	13	17	7	
5% NH_3 (ml)	5	7.5	4	

Total Gelatine Recovered (g)	1300.3
Total Gelatine Yield (%)	21.7
Anhydrous Gelatine Recovered (g) (f 0.875)	1137.8
Anhydrous Gelatine Corrected (g) (f 0.95)	1080.9
Anhydrous Gelatine Yield on Raw Material (%)	44.9
Total Anhydrous Solids Recovered (g) (Fat + Cond:)	1550.9
Total Anhydrous solids Recovered (%)	64.5

Gelatine Quality.

Run No	1	2	3	4
Bloom	289	288	247	
Colour	6.4	8.0	9.0/10.0	4.8
Clarity	11.1	11.8	12.5	15.4
pH	5.3	5.2	5.2	4.4
Moisture (%)	13.18	12.36	11.44	
Ash (%)	3.21	2.96	2.52	
SO_2 (ppm)	216	56	184	
Viscosity (ms @ 60°C)	35.3	33.1	34.0	
Corrected* Bloom	317	308	255	
Corrected* Viscosity	37	34	35	

Overall Colour. 7.3

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.

ADDENDUM C9. Continued...

Extraction & Quality Data. Experiment 6Y6A. (6 weeks liming)

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	9.19	9.03	6.45	4.05
Liquor pH	2.77	3.25	3.6	
Liquor Concentration (%w/v)	2.88	3.37	6.74	5.73
Fat (ml)	40	4	25	
Scutch (g)				Nil
Gelatine (g)	264.7	304.3	434.7	232.1
Gelatine % Proportion	21.4	24.6	35.2	18.8
Heavy Liquor Volume (ml)	1300	1150	1400	
Heavy Liquor Conc: (%)	8	9	11	
5% H_2O_2 (ml)	15	5	11	
5% NH_3 (ml)	7	5	7	

Total Gelatine Recovered (g)	1235.8
Total Gelatine Yield (%)	20.6
Anhydrous Gelatine Recovered (g) (f 0.875)	1081.3
Anhydrous Gelatine Corrected (g) (f 0.95)	1027.3
Anhydrous Gelatine Yield on Raw Material (%)	42.7
Total Anhydrous Solids Recovered (g) (Fat + Cond:)	1470.5
Total Anhydrous solids Recovered (%)	61.1

Gelatine Quality.

Run No	1	2	3	4
Bloom	300	282	235	
Colour	8.0	7.2	8.4/9.0	6.4
Clarity	10.5	10.0	11.1	12.5
pH	5.2	5.3	5.1	4.4
Moisture (%)	11.33	11.77	8.43	
Ash (%)	3.26	1.83	2.59	
SO_2 (ppm)	72	256	296	
Viscosity (ms @ 60°C)	34.8	40.7	29.4	
Corrected* Bloom	315	289	227	
Corrected* Viscosity	36	41	29	

Overall Colour. 7.7

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C9. Continued...

Extraction & Quality Data. Experiment 6Y6B. (6 weeks liming, no Na_2S)

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	2.66	3.00	3.39	3.13
Liquor pH	2.70	3.13	3.50	
Liquor Concentration (%w/v)	1.80	2.49	4.66	7.39
Fat (ml)	45	6	6	
Scutch (g)				Nil
Gelatine (g)	47.9	74.7	158.0	231.3
Gelatine % Proportion	9.4	14.6	30.8	45.2
Heavy Liquor Volume (ml)	600	600	1000	
Heavy Liquor Conc: (%)	4	9	9	
5% H_2O_2 (ml)	9	6	3	
5% NH_3 (ml)	4	3	5	

Total Gelatine Recovered (g)	511.9
Total Gelatine Yield (%)	21.3
Anhydrous Gelatine Recovered (g) (f 0.875)	447.9
Anhydrous Gelatine Corrected (g) (f 0.95)	425.5
Anhydrous Gelatine Yield on Raw Material (%)	44.2
Total Anhydrous Solids Recovered (g) (Fat + Cond:)	562.5
Total Anhydrous solids Recovered (%)	58.4

Gelatine Quality.

Run No	1	2	3	4
Bloom	No Material	279	248	
Colour		6.4	10.0	NM
Clarity		10.5	7.0	NM
pH		5.1	5.3	4.2
Moisture (%)	10.54	10.96	8.12	
Ash (%)	5.27	2.94	2.64	
SO_2 (ppm)	128	96	504	
Viscosity (ms @ 60°C)		28.5	37.1	
Corrected* Bloom		288	238	
Corrected* Viscosity		29	36	

Overall Colour. Not Available.

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.

ADDENDUM C9. Continued...

Extraction & Quality Data. Experiment 6Y8. (8 weeks liming)

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	9.26Est*	10.75	6.96	2.29
Liquor pH	2.75	3.24	3.55	
Liquor Concentration (%w/v)	2.68	3.16	6.40	10.10
Fat (ml)	3	Nil	9	
Scutch (g)				Nil
Gelatine (g)	248.2	(339.7)	445.4	231.3
Gelatine % Proportion	19.6	(26.9)	35.2	18.3
Heavy Liquor Volume (ml)	1000	1000	950	
Heavy Liquor Conc: (%)	10	8	11	
5% H_2O_2 (ml)	11	14	5	
5% NH_3 (ml)	5	7.5	5	

Total Gelatine Recovered (g)	Est *1264.8
Total Gelatine Yield (%)	21.1
Anhydrous Gelatine Recovered (g) (f 0.875)	
Anhydrous Gelatine Corrected (g) (f 0.95)	
Anhydrous Gelatine Yield on Raw Material (%)	(1051.4)
Total Anhydrous Solids Recovered (g) (Fat + Cond:)	(1477.4)
Total Anhydrous solids Recovered (%)	(61.4)

* Light Liquor spilled. Estimate based on the average Yield of the other five samples.

Gelatine Quality.

Run No	1	2	3	4
Bloom	293	238	221	
Colour	6.4	8.9	9.4	
Clarity	12.5	11.8	11.1	
pH	5.2	5.2	5.2	
Moisture (%)	8.8	10.82	10.0	
Ash (%)	3.44	4.70	2.36	
SO_2 (ppm)	408	-	120	
Viscosity (ms @ 60°C)	29.6	24.8	31.9	
Corrected* Bloom	291	255	220	
Corrected* Viscosity	30	26	32	

Overall Colour. Not Available.

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C10. Five year-old Chianina hide ex ADSRI.

EXPERIMENT 5YC.

Raw Material and Conditioning.

Raw Material:	Chianina Hide No 11.	
Moisture Content		46.25 ± 3.1%
Ash Content		14.84 ± 1.03%
Sample Mass (kg)		5.6
Anhyd: Hide Subs:		2179 ± 116 g
Washed Weight (kg).		7.15
Conditioning.		
Conditioning Liquor:	CaO 640 g	
	Na_2S 60% 75 g	
	Water to 20 kg.	

Sample No	5Y2	5Y4	5Y6	
Conditioning Time (Weeks)	2	4	6	
Cond: Temperature (°C)	21.5	22.2	22.3	
Init: Sulphide (g Na_2S/l)	2.01	1.97	2.00	
Evaporative Loss (kg)	0.2			
Ex-Lime Wash for 16hrs				
Limed Mass (kg)	10.1	10.3	9.6	
Swelling (%)	141	144	134	
Sample			5Y6A	5Y6B
Weight (kg)			4.8	4.7
Acidulation. 5 coats of H_2SO_3 soln. over 4 days. Wash 1hr. Soak in fresh water ± 22hr.				
Wt: for Extraction (kg)	10.35	10.9	5.4	5.3
Soak Water pH		2.55	2.76	2.52

ADDENDUM C10. Continued...

Extraction & Quality Data. Experiment 5Y2. (2 weeks liming)

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	10.14	10.27	9.30	7.46
Liquor pH	2.74	3.32	3.58	
Liquor Concentration (%w/v)	1.27	2.42	3.82	11.99
Fat (ml)	21	9	3	
Scutch (g)				460
Gelatine (g)	143.0	230.0	423.2	1118.3
Gelatine % Proportion	7.5	12.0	22.1	58.4
Heavy Liquor Volume (ml)	750	750	1000	
Heavy Liquor Conc: (%)	7	10.5	12.2	
5% H_2O_2 (ml)	18	15	2	
5% NH_3 (ml)	10	7.5	6	
Isoionic point	5.2			

Total Gelatine Recovered (g)	1914.5
Total Gelatine Yield (%)	34.2
Anhydrous Gelatine Recovered (g) (f 0.875)	1675.2
Anhydrous Gelatine Corrected (g) (f 0.95)	1591.4
Anhydrous Gelatine Yield on Raw Material (%)	73.0

Gelatine Quality.

Run No	1DI$^{\Phi}$	1	2	3	4
Bloom	377	280	280	291	
Colour	4.8	6.4	5.6	8.0	6.0
Clarity (NTU)	36	15	22	23	11
Clarity (DGI)	9.0	12.0	11.0	11.0	12.5
pH	5.7	5.4	5.2	5.0	4.1
Moisture (%)	8.3	8.93	10.16	8.57	
Ash (%)	0.19	6.24	4.17	1.58	
SO_2 (ppm)	17	528	344	232	
Viscosity (ms @ 60°C)	38.4	32.6	25.9	33.7	
Corrected* Bloom	345	297	292	276	
Corrected* Viscosity	37	34	26	33	

Overall Colour. 6.4

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.
Φ Mixed bed ion exchanged.

ADDENDUM C10. Continued...

Extraction & Quality Data. Experiment 5Y4. (4 weeks liming)

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	13.02	9.14	6.05	5.57
Liquor pH	2.99	3.37	3.61	
Liquor Concentration (%w/v)	2.90	4.31	8.91	10.42
Fat (ml)	6	5	28	
Scutch (g)				20
Gelatine (g)	377.6	393.9	539.1	580.4
Gelatine % Proportion	20.0	20.8	28.5	30.7
Heavy Liquor Volume (ml)	800	1000		
Heavy Liquor Conc: (%)	11.5	10		
5% H_2O_2 (ml)	5	7.5		
5% NH_3 (ml)	5	12		

Total Gelatine Recovered (g)	1891.0
Total Gelatine Yield (%)	33.8
Anhydrous Gelatine Recovered (g) (f 0.875)	1654.6
Anhydrous Gelatine Corrected (g) (f 0.95)	1571.9
Anhydrous Gelatine Yield on Raw Material (%)	72.1

Gelatine Quality.

Run No	1	2	3	4
Bloom	311	269	226	
Colour	5.6	6.8	8.4	7.2
Clarity (NTU)	19	21	20	9
Clarity (DGI)	11.0	11.0	11.0	13.0
pH	5.9	5.8	5.6	4.12
Moisture (%)	10.0	9.9	11.5	
Ash (%)	2.32	3.94	2.41	
SO_2 (ppm)	8	48	80	
Viscosity (ms @ 60°C)	35.7	34.5	33.2	
Corrected* Bloom	310	277	233	
Corrected* Viscosity	35	35	34	

Overall Colour. 7.2

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C10. Continued...

Extraction & Quality Data. Experiment 5Y6A. (6 weeks liming)

NB Experiment 5Y6A - Acidulation over 4 days.

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	6.53	2.76	2.94	1.78
Liquor pH	2.91	3.30	3.62	
Liquor Concentration (%w/v)	2.94	6.75	9.27	13.96
Fat (ml)				
Scutch (g)				Nil
Gelatine (g)	192.0	186.3	272.5	248.5
Gelatine % Proportion	21.4	20.7	30.3	27.6
Heavy Liquor Volume (ml)	900	800	1200	
Heavy Liquor Conc: (%)	10.5	13	12.5	
5% H_2O_2 (ml)	6	6	4	
5% NH_3 (ml)	5	10	15	

Total Gelatine Recovered (g) 899.3

Gelatine Quality.

Run No	1	2	3	4
Bloom	317	288	225	
Colour	6.4	8.4	9.4	11.4
Clarity (NTU)	12	23	32	43
Clarity (DGI)	12.5	10.5	9.5	8.0
pH	5.8	5.3	5.3	4.03
Moisture (%)	10.1	10.8	11.0	
Ash (%)	2.57	1.51	1.36	
SO_2 (ppm)	32	0	48	
Viscosity (ms @ 60°C)	41.5	37.5	24.6	
Corrected* Bloom	318	287	224	
Corrected* Viscosity	42	37	25	

Overall Colour. 9.1 (Boil clarity was poor)

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C10. Continued...

Extraction & Quality Data. Experiment 5Y6B. (6 weeks liming)

NB Experiment 5Y6B - Acidulation over 3 days.

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Liquor Volume (l)	6.04	2.71	2.94	1.60
Liquor pH	2.96	3.26	?	
Liquor Concentration (%w/v)	3.0	6.89	9.06	14.26
Fat (ml)				
Scutch (g)				Nil
Gelatine (g)	181.2	186.7	267.2	228.2
Gelatine % Proportion	21.0	21.6	31.0	26.4
Heavy Liquor Volume (ml)	1200	700	1100	
Heavy Liquor Conc: (%)	10.4	14	13	
5% H_2O_2 (ml)	9	4	5	
5% NH_3 (ml)	5	10	15	
Total Gelatine Recovered (g)			863.9	

Gelatine Quality.

Run No	1	2	3	4
Bloom	321	279	226	
Colour	6.8	7.2	8.9	9.4
Clarity (NTU)	20	29	29	7
Clarity (DGI)	11.0	10.0	10.0	13.0
pH	5.3	5.3	5.3	4.20
Moisture (%) .	9.43	11.5	11.8	
Ash (%)	2.61	1.35	1.47	
SO_2 (ppm)	48	64	64	
Viscosity (ms @ 60°C)	32.1	37.4	30.4	
Corrected* Bloom	310	277	233	
Corrected* Viscosity	35	35	34	
Overall Colour.		8.2		

* Corrected to 12.5% non gelatine (moisture + ash) using √ for gel strengths.

ADDENDUM C10. Continued...

Extraction Data. Experiment 5Y6. (6 weeks liming)

NB Combined data for Experiment 5Y6.

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	45	50	60	Boil
Scutch (g)				Nil
Gelatine (g)	373.2	373.0	539.7	476.7
Gelatine % Proportion	21.2	21.2	30.6	27.0

Total Gelatine Recovered (g)	1762.6
Total Gelatine Yield (%)	31.5
Anhydrous Gelatine Recovered (g) (f 0.875)	1542.3
Anhydrous Gelatine Corrected (g) (f 0.95)	1465.1
Anhydrous Gelatine Yield on Raw Material (%)	67.2

ADDENDUM C11. 12 year-old Inguni's hide ex ADSRI.

EXPERIMENT INO.

Raw Material.

Hide from Inguni Cow, 143 months of age.
Cut into 100 x 100 mm pieces.

Moisture Content	41.4 ± 0.2%
Ash Content	16.5 ± 0.13%
Hide Substance	42.1 ± 0.1%
Each Lot Weight	3700 g; Hide Substance 1558 ± 3 g.

Conditioning.

Lot	E
Na_2S (g)	75
CaO (g)	640
Water to (kg)	20
Time (days).	28
Ave. Temp. (°C)	22.2
Tumbler Wash (hours).	16
Cond. Weight (kg)	6.65
Swelling (%)	180

Acidulation.

Lot	**INOE**
5 Coats of H_2SO_3 soln. over 4 days.	
Wash 1 hr.	
Soak in 40 (l) for ± 20 hr.	
Wt. for Extn.	6.75 kg
Soak Water pH	2.48

ADDENDUM C11. Continued...

Lot INOE. Extraction and Quality.

Extraction.

Run No	1	2	3	4
Time (hrs)	5	5	5	7
Temperature (°C)	50	60	70	Boil
Liquor Volume (l)	8.90	6.00	4.98	3.18
Liquor pH	3.00	3.45	3.57	
Liquor Concentration (%w/v)	1.40	4.18	8.14	9.80
Fat (ml)	8	2	-	
Scutch (g)				95
Gelatine (g)	124.2	250.5	405.1	311.5
Gelatine % Proportion	11.4	23.0	37.1	28.5
Heavy Liquor Volume (ml)	1200	1200	1600	
Heavy Liquor Conc: (%)	6.8	13.5	15	
5% H_2O_2 (ml)	2	1	1	
Initial pH	<4	4.1	3.9	
5% NH_3 (ml)	6.5	20	34	
Final pH	5.4	5.2	5.2	

Total Gelatine Recovered (g)	1091.3
Total Gelatine Yield (%)	29.5
Anhydrous Gelatine Recovered (g) (f 0.875)	954.9
Anhydrous Gelatine Corrected (g) (f 0.95)	907.1
Anhydrous Gelatine Yield on Raw Material (%)	58.2

Gelatine Quality.

Run No	1	2	3	4
Bloom	302	233	154	
Colour	9.4	12.3	16.0	17.8
Clarity (NTU)	13	21	25	20
Clarity (DGI)	12	11	10.5	11
pH	5.5	5.3	5.1	4.1
Moisture (%)	10.38	10.89	10.72	
Ash (%)	1.69	0.62	0.38	
SO_2 (ppm)	96	48	64	
Viscosity (ms @ 60°C)	29.9	26.9	24.3	
Corrected* Bloom	299	228	149	
Corrected* Viscosity	29.8	26.6	23.9	
Overall Colour.		14.9		

* Corrected to 12.5% non gelatine (moisture + ash) using ✓ for gel strengths.

ADDENDUM C12. The effect of animal age and processing on gelatine colour.

EXP	AGE MONTHS	CONDIT. TIME WKS.	COLOURS 1ST	2ND	3RD	BOIL	OVERALL
YSA	10	2	3.6	4.4	4.8	4.8	4.3
YSB	10	2	<3.2	5.2	4.8	4.4	4.2
YSC	10	3	3.2	4.0		4.4	4.0
YSD	10	3	3.2	3.2		4.8	3.3
YSE	10	4	<3.2	4.0	5.2	?	?
		AVE	3.3			AVE	4.0
CT	18	1	5.6	5.6	5.2	6.8	6.5
		2	5.2	5.2	6.0	6.4	6.0
		3	5.2	5.6	6.4	7.2	6.2
		4	5.6	5.6	5.6	?	?
		5	6.0	6.4	6.4	8.0	6.4
		6	5.2	5.6	6.0	?	?
		AVE	5.5			AVE	6.3
3Y	40	2	6.4	6.0	6.8	4.8	5.5
		4	6.6	6.0		3.6	5.2
		4	6.4	6.4		5.6	6.1
		6A	6.0	6.8		LOST	?
		6B	6.0	6.0	6.8	?	?
		8	6.4	6.8		LOST	?
		AVE	6.3			AVE	5.8
5Y	58	2	6.4	5.6	8.0	6.0	6.4
		4	5.6	6.8	8.4	7.2	7.2
		6	6.4	8.4	9.4	11.4	9.1
		AVE	6.1			AVE	7.6
6Y	78	2	5.2	6.8	7.8	7.6	7.4
		4A	8.4	7.6	8.9	6.8	7.9
		4B	6.4	8.0	9.5	4.8	7.3
		6A	8.0	7.2	8.7	6.4	7.7
		6B	?	6.4	10.0	NM	?
		8	6.4	8.9	9.4	?	?
		AVE	6.9			AVE	7.6
INOE	143	4	9.4	12.3	16.0	17.8	14.9
CTO	152	2	6.4	7.2	10.0	16.0	12.4
		4	8.0	8.9	10.0	20.0	16.5
		7	9.4	10.7	12.3	17.8	15.2
		10	10.0	9.4	10.0	20.0	16.4
		AVE	8.5			AVE	15.1
KTO	144	6	8.0	7.2	9.4	13.3	11.6
ST1	144	2	6.4	10.0	10.7	22.8	18.1
		2	11.4	10.7	11.4	16.0	14.6
		2	10.0	11.4	12.3	16.0	14.7
		4	10.0	11.4	13.3	16.0	14.2
		4	8.9	12.3	13.3	17.8	15.0
		4	9.4	12.3	13.3	16.0	14.3
		6	10.7	12.3	14.5	13.3	13.1
		6	11.4	11.4	16.0	20.0	16.5
		6	11.4	13.3	16.0	17.8	15.8
		AVE	10.0			AVE	15.1

ADDENDUM C12. Continued...

EXP.	AGE MONTHS	CONDIT. TIME WKS.	COLOURS 1ST	2ND	3RD	BOIL	OVERALL
ST2/WT	144	4	12.3	11.4	12.3	17.8	15.7
		4	11.4	10.0	12.3	13.0	12.7
		4	10.7	13.3	16.0	14.5	14.3
		4	11.4	12.3	13.3	17.8	16.3
		4	11.4	13.3	16.0	17.8	16.6
		AVE	11.4			AVE	15.1
CALF-A	3 (ACID PROC)		4.0	3.6	5.2	?	?

ADDENDUM C13. Amino acid analysis of 6 samples of gelatine by Stevens and Stevens - Consultants.

AMINO ACID AND PROTEIN CONTENT BY WEIGHT. mg/g sample.

		SAMPLES														
Mw	AMINO ACID	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	E1	E2	E3	F1	F2	F3
133.1	ASP	42.7	42.1	51.2	46.3	47.6	48.6	37.5	51.1	48.5	45.7	42.9	41.9	41.4	46.6	42.6	46.7
165.2	GLU	84.5	85.6	96.5	87.3	87.3	94.0	87.3	90.2	90.7	84.6	89.2	82.5	80.5	87.2	81.9	86.0
131.1	HOPRO	104.6	108.8	107.2	102.8	105.3	111.8	117.9	113.0	107.1	105.5	109.7	104.9	105.5	105.1	103.4	109.0
105.1	SER	27.3	27.6	30.4	27.8	27.7	29.2	30.6	29.4	30.1	28.3	28.6	27.2	26.8	28.8	27.6	28.2
75.1	GLY	193.6	201.1	212.5	192.8	197.2	206.9	201.5	203.0	205.0	191.3	201.0	187.3	189.2	199.7	187.2	197.3
155.2	HIS	5.6	0.0	0.0	6.5	0.0	0.0	6.1	0.0	0.0	7.1	0.0	7.2	0.0	0.0	6.7	0.0
174.2	ARG	89.4	89.6	78.9	84.3	86.2	75.4	83.4	87.1	73.4	83.5	70.5	85.4	77.8	70.3	77.6	81.5
165.2	METSO1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.7	0.0	0.0	4.9	0.0	0.0
165.2	METSO2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0
119.1	THR	12.6	13.0	17.2	14.6	15.0	16.3	14.9	14.8	15.9	14.1	14.8	13.9	14.1	15.0	14.5	14.5
89.1	ALA	80.9	83.2	80.8	77.9	78.5	76.0	77.5	78.4	74.7	76.3	70.9	72.3	72.2	72.8	76.3	74.8
115.1	PRO	120.9	129.1	124.1	124.5	125.2	115.8	123.7	124.0	116.0	125.4	108.1	115.9	116.7	114.8	127.4	122.9
181.2	TYR	0.0	0.0	4.5	3.5	0.0	4.9	4.5	0.0	5.4	4.8	2.8	2.8	0.0	3.3	2.9	0.0
117.2	VAL	20.0	20.2	21.3	19.8	19.6	18.2	18.8	18.6	18.9	19.0	17.4	18.4	17.9	18.6	19.1	18.2
149.2	MET	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0
121.2	CYS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
131.2	ILE	12.9	13.0	13.6	12.4	12.3	12.8	11.9	12.6	13.1	13.9	12.1	12.7	12.1	13.0	13.5	12.4
131.2	LEU	24.9	25.6	25.4	26.5	26.3	22.2	23.7	25.2	23.1	25.6	21.6	24.5	23.7	22.6	26.0	24.4
162.2	HOLYS1	9.8	10.0	9.5	11.0	10.7	8.8	9.0	11.6	8.8	10.3	9.5	10.8	9.2	8.1	9.8	9.9
162.2	HOLYS2	3.0	2.7	2.1	2.8	2.6	1.8	2.0	2.8	1.8	2.7	1.7	2.6	1.9	1.6	2.5	2.4
165.2	PHE	15.8	16.0	18.1	16.4	16.5	16.1	15.0	16.3	17.2	17.2	14.5	14.7	14.3	16.1	16.6	14.8
146.2	LYS	34.9	35.0	34.6	31.9	31.7	32.1	29.9	34.7	33.2	34.6	29.9	31.5	30.7	32.0	33.9	33.4
132.2	ORN	1.0	1.2	0.0	1.6	1.8		1.4	1.8		1.6		3.9	3.9		3.8	3.8
	% PROTEIN	88.4	90.4	92.9	89.1	89.2	89.1	89.7	91.5	88.3	89.2	85.2	86.0	83.8	86.1	87.6	88.0

ADDENDUM C13. Continued.

Mean values and Relative Standard Deviation %-RSD of amino acids in gelatine.

AMINO ACID	MEAN ALL	%-RSD ALL	MEAN -A	%-RSD -A
ASP	45.2	8.1	45.6	8.3
GLU	87.2	4.7	87.5	4.9
HOPRO	107.6	3.6	107.7	3.7
SER	28.5	4.0	28.6	4.0
GLY	197.9	3.6	198.0	3.7
HIS	2.5	129.8	2.4	134.5
ARG	80.9	7.5	79.7	6.9
METSO1	0.5	267.5	0.6	247.7
METSO2	0.2	387.3	0.2	360.6
THR	14.7	7.5	15.0	5.9
ALA	76.5	4.4	75.7	3.7
PRO	120.9	4.6	120.3	4.5
TYR	2.5	83.0	2.8	69.2
VAL	19.0	5.0	18.8	4.9
MET	0.3	273.9	0.3	253.7
CYS	0.0	ERR	0.0	ERR
ILE	12.8	4.3	12.7	4.6
LEU	24.5	6.0	24.3	6.2
HOLYS1	9.8	9.1	9.8	9.8
HOLYS2	2.3	19.3	2.2	18.8
PHE	16.0	6.5	16.0	7.0
LYS	32.8	5.2	32.4	4.9
ORN	2.2	59.8	2.6	42.1
% PROTEIN	88.4		86.1	

ADDENDUM C14. Transformation of amino acid data from ADDENDUM 13.

MOLAR % OF AMINO ACIDS IN GELATINES.

AMINO ACID	SAMPLES A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	E1	E2	E3	F1	F2	F3
ASP	4.03	3.87	4.57	4.35	4.45	4.52	4.46	4.66	4.55	4.30	4.17	4.08	4.10	4.48	4.07	4.41
GLU	6.42	6.34	6.94	6.61	6.57	7.04	6.46	6.63	6.85	6.41	6.98	6.48	6.42	6.76	6.30	6.55
HOPRO	10.01	10.15	9.70	9.81	9.98	10.55	10.98	10.45	10.19	10.07	10.82	10.37	10.60	10.26	10.02	10.45
SER	3.26	3.21	3.43	3.31	3.28	3.44	3.56	3.39	3.57	3.37	3.52	3.36	3.36	3.51	3.34	3.37
GLY	32.38	32.78	33.60	32.13	32.66	34.11	32.79	32.81	34.08	31.89	34.63	32.35	33.22	34.05	31.69	33.04
HIS	0.45	0.00	0.00	0.52	0.00	0.00	0.48	0.00	0.00	0.57	0.00	0.60	0.00	0.00	0.55	0.00
ARG	6.44	6.29	5.38	6.05	6.15	5.36	5.85	6.07	5.26	6.00	5.23	6.36	5.89	5.17	5.66	5.88
METSO1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.00	0.00	0.38	0.00	0.00
METSO2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00
THR	1.33	1.34	1.71	1.53	1.57	1.69	1.53	1.51	1.67	1.48	1.61	1.51	1.56	1.61	1.55	1.53
ALA	11.40	11.43	10.77	10.94	10.96	10.56	10.63	10.68	10.47	10.72	10.29	10.52	10.68	10.46	10.88	10.55
PRO	13.18	13.72	12.80	13.53	13.52	12.45	13.13	13.07	12.58	13.63	12.14	13.05	13.36	12.76	14.06	13.42
TYR	0.00	0.00	0.29	0.24	0.00	0.33	0.30	0.00	0.37	0.33	0.20	0.20	0.00	0.23	0.20	0.00
VAL	2.14	2.11	2.16	2.11	2.08	1.92	1.96	1.93	2.01	2.03	1.92	2.04	2.01	2.03	2.07	1.95
MET	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.00
CYS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ILE	1.23	1.21	1.23	1.18	1.17	1.21	1.11	1.17	1.25	1.33	1.19	1.26	1.22	1.27	1.31	1.19
LEU	2.38	2.39	2.30	2.53	2.49	2.09	2.21	2.33	2.20	2.44	2.13	2.42	2.38	2.21	2.52	2.34
HOLYS1	0.76	0.75	0.70	0.85	0.82	0.67	0.68	0.87	0.68	0.79	0.76	0.86	0.75	0.64	0.77	0.77
HOLYS2	0.23	0.20	0.15	0.22	0.20	0.14	0.15	0.21	0.14	0.21	0.14	0.21	0.15	0.13	0.20	0.19
PHE	1.20	1.19	1.30	1.24	1.24	1.21	1.11	1.20	1.30	1.30	1.14	1.15	1.14	1.25	1.28	1.13
LYS	3.00	2.93	2.81	2.73	2.70	2.72	2.50	2.88	2.83	2.96	2.64	2.79	2.77	2.80	2.95	2.87
ORN	0.09	0.11		0.15	0.17		0.13	0.17		0.15		0.38	0.39		0.37	0.36
	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	90.1	90.4	90.6	89.8	90.2	90.7	91.3	90.1	90.7	89.6	90.8	89.6	90.6	90.8	89.8	90.4

ADDENDUM C14. Continued...

AMINO ACID	EXPERIMENTAL ERROR %-RSD C-D	MEAN ALL	%-RSD ALL		MEAN -A	%-RSD -A	
ASP	2.6	4.3	5.2	*	4.4	4.3	*
GLU	3.6	6.6	3.5		6.6	3.4	
HOPRO	3.0	10.3	3.3		10.3	3.5	
SER	2.4	3.4	3.0		3.4	2.6	
GLY	2.6	33.0	2.5		33.1	2.7	
HIS	123.3	0.2	130.1		0.2	134.8	
ARG	5.9	5.8	7.1	*	5.7	6.6	
METSO1	ERR	0.0	267.3		0.0	247.5	
METSO2	ERR	0.0	387.3		0.0	360.6	
THR	5.5	1.5	6.7	*	1.6	4.4	*
ALA	0.8	10.7	2.9	*	10.6	1.7	*
PRO	3.3	13.1	3.8		13.1	3.9	
TYR	50.7	0.2	82.4		0.2	68.5	
VAL	2.2	2.0	3.7	*	2.0	3.5	
MET	ERR	0.0	276.3		0.0	256.0	
CYS	ERR	0.0	ERR		0.0	ERR	
ILE	6.1	1.2	4.4	*	1.2	4.7	
LEU	5.3	2.3	5.6		2.3	6.0	
HOLYS1	10.8	0.8	9.1		0.8	9.7	
HOLYS2	19.6	0.2	18.9		0.2	18.5	
PHE	5.9	1.2	5.2		1.2	5.6	
LYS	5.8	2.8	4.5		2.8	4.2	
ORN	9.9	0.2	51.4	*	0.3	44.0	*

ADDENDUM C15. Mean molar % amino acids in gelatine.

AMINO ACID	SAMPLES. A	B	C	D	E	F	EXPERIMENTAL ERROR %RSD C-D	MEAN ALL	% RSD ALL		MEAN -A	%RSD -A	
ASP	3.9	4.5	4.5	4.4	4.1	4.3	2.6	4.3	5.2	*	4.4	4.3	*
GLU	6.4	6.7	6.7	6.6	6.6	6.5	3.6	6.6	3.5		6.6	3.4	
HOPRO	10.1	9.8	10.7	10.1	10.6	10.2	3.0	10.3	3.3		10.3	3.5	
SER	3.2	3.3	3.5	3.5	3.4	3.4	2.4	3.4	3.0		3.4	2.6	
GLY	32.6	32.8	33.2	33.0	33.4	32.9	2.6	33.0	2.5		33.1	2.7	
HIS	0.2	0.2	0.2	0.3	0.2	0.2	123.3	0.2	130.1		0.2	134.8	
ARG	6.4	5.9	5.8	5.6	5.8	5.6	5.9	5.8	7.1	*	5.7	6.6	
METSO1	0.0	0.0	0.0	0.0	0.1	0.1	ERR	0.0	267.3		0.0	247.5	
METSO2	0.0	0.0	0.0	0.0	0.1	0.0	ERR	0.0	387.3		0.0	360.6	
THR	1.3	1.6	1.6	1.6	1.6	1.6	5.5	1.5	6.7	*	1.6	4.4	*
ALA	11.4	10.9	10.6	10.6	10.5	10.6	0.8	10.7	2.9	*	10.6	1.7	*
PRO	13.5	13.3	12.9	13.1	12.9	13.4	3.3	13.1	3.8		13.1	3.9	
TYR	0.0	0.2	0.2	0.4	0.1	0.1	50.7	0.2	82.4		0.2	68.5	
VAL	2.1	2.1	1.9	2.0	2.0	2.0	2.2	2.0	3.7	*	2.0	3.5	
MET	0.0	0.0	0.0	0.0	0.0	0.1	ERR	0.0	276.3		0.0	256.0	
CYS	0.0	0.0	0.0	0.0	0.0	0.0	ERR	0.0	ERR		0.0	ERR	
ILE	1.2	1.2	1.2	1.3	1.2	1.3	6.1	1.2	4.4	*	1.2	4.7	
LEU	2.4	2.4	2.2	2.3	2.3	2.4	5.3	2.3	5.6		2.3	6.0	
HOLYS1	0.8	0.8	0.7	0.7	0.8	0.7	10.8	0.8	9.1		0.8	9.7	
HOLYS2	0.2	0.2	0.2	0.2	0.2	0.2	19.6	0.2	18.9		0.2	18.5	
PHE	1.2	1.3	1.2	1.3	1.1	1.2	5.9	1.2	5.2		1.2	5.6	
LYS	3.0	2.7	2.7	2.9	2.7	2.9	5.8	2.8	4.5		2.8	4.2	
ORN	0.1	0.2	0.1	0.2	0.4	0.4	9.9	0.2	51.4	*	0.3	44.0	*