CHAPTER ONE. Introduction and literature review.

GELATINE AND ITS USES.

Gelatine is a protein derived from the natural mammalian protein, collagen, which is most abundant in connective tissues. According to Siebert (1992) the world consumption was estimated at 200 thousand tons per annum with a growth rate of about 2% per annum. Although gelatine was used in a very wide range of products, some two thirds of the production was used in the food sector of the economy where it was considered to be an ingredient rather than an additive, and of the food sector, some 70% of the usage was for confectionary (sweets).

Gelatine had a wide range of properties which could be exploited in foods. The most important property was that of reversible gelation at about blood temperature (37°C) which gave products "melt in the mouth" characteristics accompanied by excellent flavour release. Associated with gelation was the property of water binding. Gelatine gels were formed at about 2% concentration in water hence gelatine could bind up to 50 times its own weight of water. This property was used particularly in canning and in cake and pie fillings. Being a high molecular weight polymer gelatine could be used to impart body to soups. Gelatine, having positive and negative charges on the molecule, was amphoteric and had hydrophilic and hydrophobic areas on the polymer chain so it had emulsifying properties which complemented the gelation properties when it was used as a stabiliser in low fat spreads and the manufacture of toffees. The emulsifying properties of gelatine extend beyond oil and water and one of the main uses was as a foaming agent - air in water emulsion - where small quantities (2 - 3%) were used to generate and stabilise the foam of marshmallows and similar products. In yoghurt gelatine complexed with casein to overcome the problems of syneresis (Modler and Kalab, 1983). The oldest use of gelatine was as an animal glue. Its adhesive properties could be used for sticking together the layers of "liquorice all-sorts" as well as in pharmaceutical tableting. One property of gelatine that was partly surpassed by superior products was that of film former. However, that property could be coming back into use in the microencapsulation of flavours and vitamins. The edible film forming property of gelatine had long been utilized

in the manufacture of both hard and soft pharmaceutical capsules and the property was essential in forming the light sensitive film on an acetate or paper backing used in photography. The one property of gelatine that was not used in the food industry was that of flame retarder which was most important in the use of gelatine for match heads (Ward and Courts, 1977).

RAW MATERIAL FOR GELATINE MANUFACTURE.

As diverse as were the uses of gelatine, so were the sources from which it was obtained. Gelatine was traditionally manufactured from materials rich in Type I collagen which were "ossein" from degreased, demineralized bone and from the skin of pigs, bovines, and in China, donkeys. To a lesser extent sheep, goat and game skins were used in the manufacture of gelatine and recently, due to the Kosher implications, fish skin gelatine was gaining some prominence in the market place. In all cases the raw material for gelatine was waste material unwanted or unsuitable for more profitable uses. For example, the gelatine manufacturer received the skin from the head, legs and tails of bovines (2.5 to 5 kg per animal), while the rest of the hide which could vary between 20 to 36 kg per animal (Forrest, Aberle, Hendrik, Judge, and Merkel, 1975a) was sold at a much higher price to the leather manufacturer. Pigskin in excess of requirements for food manufacture (sausages) and leather was disposed of to the gelatine manufacturer. The implication was that in some instances the gelatine manufacturer, as a waste disposal industry, did not have much control over what was received as raw material.

The range of sources of raw material was further complicated by the fact that all skins could be fresh off the animal, or preserved by drying, salting, or other treatment. In the case of material of bovine origins, the gelatine manufacturer had to handle fresh hairy hide from the abattoir, salted hide from more distant sources, hide subjected to short term preservation using sodium metabisulphite, frozen sinews, dry hide and hide that had been pretreated in a tannery with lime and sodium sulphide, fleshed and then split to the required thickness. Unwanted corium (splits) and trimmings were used for gelatine manufacture and could be received either direct from the tannery or preserved by drying. Furthermore; a low grade gelatine could be recovered from chrome tanned leather waste using the SPA process (Drew, 1930). Under the circumstances it was not surprising that detailed analytical data on the precise nature of the raw material used in gelatine manufacture was not

considered of importance, hence its unavailability. However some proximate analyses would be forthcoming from this study.

This study, was primarily concerned with the colour of gelatine derived from wet salted bovine hide (cowhide) using a lime-sulphide conditioning process.

COLLAGEN.

According to Bailey and Light (1989a) and Forrest, Aberle, Hendrik, Judge, and Merkel (1975b) collagen, which was the protein from which gelatine was derived, was the most abundant protein in the animal body and constituted 20-25% of the total protein. It was the principal structural protein of connective tissue and was a major component of tendons, ligaments, bones and cartilage. It was classified as a glycoprotein by Forrest et al. (1975b) as it contained a small amounts of galactose and glucose. Collagen was characterized by the fact that one third of it was composed of glycine and another third of proline or hydroxyproline. It was also the only protein, besides elastin, to contain hydroxyproline (13-14%) and the amount of collagen or gelatine in a product or tissue could be reliably estimated from the hydroxyproline content. The structure and development of collagen was well covered in these as well as a number of other texts like Swatland (1985). Collagen was the subject of much current research mainly in the field of medicine, as collagen defects lead to many diseases (Bailey, 1992 or Igarashi, Uzuka and Nakajima, 1989). Knowledge of collagen chemistry had led to the mitigation of some of these effects, such as the treatment of the accelerated ageing in diabetes (Ulrich and Cerami, 1992). Furthermore, Bailey (1994) in a lecture to the Northampton group of the Society of Leather Technologists and Chemists was reported as saying that "if collagen was not hydroxylated, using vitamin C, scurvy resulted, (Peterkofsky, 1991), and that other problems could occur if collagen was not formed correctly, such as the hypermobile joints of the so called India rubber men in circus acts".

Collagen was a family of large extracellular proteins of closely related molecular structure. It was composed of three polypeptide chains (called α-chains) each of which contained long sequences of repeating tripeptides based on the general structure Gly-X-Y, where X was commonly proline and Y could represent any amino acid but was often the modified amino acid hydroxyproline. All collagens also contained varying quantities of the amino acid hydroxylysine which was formed by the enzyme-catalysed hydroxylation of lysine

residues at C5 in newly synthesised α-chains. Many of the hydroxylysine residues were subsequently glycosylated during intracellular processing and contained O-linked β-D-galactopyranoside or β-D-glucopyranosyl-β-D-galactopyranoside. The individual polypeptide α-chains were unstable but when three chains were wrapped round each other they formed a very stable right-handed triple helix characteristic of collagen (Bailey and Light, 1989b).

In order for the collagen triple helices to form fibres it was necessary that the helix be further stabilised by inter- and intramolecular cross-links. Most of the cross-links had been shown to follow the oxidative deamination of lysine or hydroxylysine by the enzyme lysyl oxidase. This enzyme replaced the ε-amino group of lysine with an aldehyde group to produce lysine aldehyde commonly called allysine. Adjacent aldehyde groups in the N-terminus telopeptide region of collagen α-chains had been shown to form aldol links between chains thus increasing the stability of the triple helix (Bailey and Light, 1989c).

In order to give stability to the collagen fibre, intermolecular cross-links were necessary. An example of this was the reaction of allysine in the telopeptide of one collagen helix with an ε-amino group of hydroxylysine (residue 103 or 946 of the α-chain) in the overlaps with an adjacent helix to form dehydro-hydroxylysinonorleucine (dehydro-HLNL). In the case of extensive hydroxylation of lysine the double bond formed between lysine and aldehyde could undergo the Amadori rearrangement. The C=N bond became a C-N bond while the adjacent C-OH became C=O giving the oxo-imine hydroxylysino-5-oxo-norleucine (HLONL). Both HLONL and dehydro-HLNL could be reduced by sodium borohydride to give acid stable products which had been isolated by chromatography. (Bailey and Light, 1989c)

With age, mature collagen matrices became progressively stronger and more rigid and the quantity of sodium borohydride reducible cross-links was reduced. It had been suggested that histidine combined with and stabilised dehydro-HLNL cross-links. Similarly in hydroxylysine rich collagens, hydroxylysine aldehyde reacted with the C-N bond of an oxo-imine cross-link to form the fluorescent pyridinoline trivalent cross-link between α-chains. As skin Type I collagen was not rich in hydroxylysine it was said that the fluorescent mature pyridinoline cross-link was not found in skin but was only found in Type I collagen from bone. (Bailey and Light, 1989c). Sell and Monnier, (1989) had shown the formation with age of a non-enzymic cross-link

in collagen producing the fluorescent product pentosidine. This linked the amino acids lysine and arginine by means of a 5-carbon chain derived from a sugar.

From the gelatine manufacturer's point of view the variation in the response of hide collagen to chemical pretreatment could be loosely attributed to variations in the cross-linking of the collagen. However, other than from the study of Reich, Walther, and Stather (1962b) there was no precise information on the effect of animal age and collagen cross-linking on the manufacturing process or the quality of gelatine produced. It was known that the divalent cross-links dehydro-HLNL and HLONL were acid labile (Bailey and Light, 1989c) but more important to the bovine hide gelatine manufacturer would be to know what links were alkali labile.

RAW MATERIAL COMPOSITION.

The constituents of cowhide and gelatine raw materials were variable as indicated below:

1. According to Bailey and Light (1989d) the collagen content of skin was approximately 50% (with 5% elastin) whereas tendon contained 95% collagen (based on dry masses).

2. According to Cooper, Russel, Shuttleworth and Boast (1984) cowhide consisted of 65% moisture and 35% dry substance. Salted hide consisted of 44% moisture, 14% salt and 42% hide substance. During salting there was a byproduct, brine, derived from the moisture in the hide which contained some salt soluble organic matter.

3. In the twentieth Proctor Memorial Lecture, Haines (1984) reviewed the subject of "The skin before tannage-Proctor's view and now." This review dealt with the micro-structure of skin as revealed in SEM micrographs and how this was affected by animal breed and age. Scott (according to Haines, 1984) had shown that the proteoglycan, dermatan sulphate was distributed over the surface of collagen fibrils in a regular and highly organized manner. Dermatan sulphate had a marked influence on collagen stability and its removal by liming was accompanied by a reduction in shrinkage temperature. A schematic diagram of the cross-section of the epidermal / dermal boundary of skin

showing 9 layers was given. This included layers of keratinous cells of the epidermis, layers of hyparin sulphate, laminin, and Types III and IV collagen in the "basement membrane". The latter, were all outside the dermis proper and it was apparent that the Type III collagen was responsible for the smooth grain layer of leather (Thompson, 1988 and Bailey, 1992).

4. Bowes, Elliot and Moss (1957) presented the constituents of fresh skin as:

Water 60-65%
Protein 30-35% - excluding hair.
Grease 1-10%
Carbohydrate (Sugars) 1%
Mucopolysaccharide 0.5-1%

Bowes, Elliot and Moss (1958) studied both acid and alkaline extracts of calf skin. Acid extraction yielded, besides acid soluble collagen which could be precipitated by salt, at least two other proteins. Furthermore, there were "plasma proteins" extractable using 0.1M phosphate buffer. Based on their study, calf skin also contained non-collagenous proteins soluble in alkali at 4° to 20°C. In summary, they showed that calf skin contained plasma proteins (8-10%), other non-collagenous proteins (10-15%) and collagen (75%). The amounts of hexosamine and hexose in the alkali extracts were relatively small and the greater part of the material extracted by alkali was non-collagenous protein rather than polysaccharide. "Of the 30-35% protein about 4-6% was made up of the interfibrillar proteins such as albumins and globulins of the tissue fluids, about 0.5-1% represented the epidermis and there were small amounts of muscle protein, elastin and reticulin.....but by far the greater part of the protein was collagen (90-95%)".

5. Shirai and Wada (1981) found that on liming pigskin, about 7.5 % of the protein as nitrogen was dissolved after 60 days. After 7 and 14 days, amino acid analysis of the protein in solution showed it to be different from collagen in that extraordinarily large amounts of tyrosine and low amounts of hydroxyproline were found. Thereafter the amino acid analysis was closer to that of collagen. The non-collagenous protein was estimated at 1% of total nitrogen and was shown to contain

glucosaminoglycan or acid mucopolysaccharide and to be of a molecular size far smaller than the α-chain released from collagen.

6. Komanowsky (1989) stated that "hide" contained at least 0.8% glucosaminoglycan (GAG) chondroitin sulphate in addition to about 0.5% hexose which was responsible for the cross-linking of hide during drying (particularly at high temperatures). Furthermore, there were indications that hyaluronic acid (a polymer of a repeating disaccharide unit of glucuronic acid and N-acetyl glucosamine) in salted hides was converted enzymically into smaller polysaccharides which exposed additional carbonyl groups for cross-linking reactions.

Ultrafiltration trials using a 2000 Dalton (unit of atomic mass) nominal cut-off membrane, were conducted at Davis Gelatine Industries (Cole, 1988) on spent conditioning liquor containing 1.5% ash and 1.5% organic matter. The liquor was concentrated to 9.5% solids and this concentrate contained 65% of the organic matter in the original solution. After drying, this organic matter was insoluble in water and it was concluded that spent conditioning liquor contained mainly non-collagenous protein which was denatured and rendered insoluble by drying at up to 60°C. It was felt that the findings in this study confirmed the findings of Bowes *et al*. (1958) that most of the low temperature alkali soluble proteins from skin were non-collagenous as long as the pH did not exceed 12.7 and the temperature did not go above 20-25°C. In this instance, the alkaline liquors contained sodium sulphide, hence the products of the hydrolysis of keratin (hair) must have also contaminated the alkali soluble proteins from the skin.

THE MANUFACTURE OF GELATINE.

This subject was covered in the literature (Bailey and Light, 1989d; Hinterwaldner, 1977; Glicksman, 1969; Veis, 1964a). There were essentially two main types of gelatine: Type A and Type B. Type A gelatine was produced by the acid process in which the conditioning function of the acid was simply one of pH adjustment to that required for extraction (Reich, Walther and Stather, 1962a). Type B gelatine was the product of an alkali conditioning process which largely hydrolysed the glutamine and asparagine residues to glutamic and aspartic acids. This resulted in a drop in iso-ionic pH from about 9 to about 5 (Veis, 1964b). In the traditional alkali process the conditioned material was extracted at a "neutral" pH of about 6. Variation of the extraction pH had

a dramatic effect on the relationship between gel strength and viscosity and this could be used to produce a gelatine most suited to its application. (Fysh and Goodwin, 1955).

The details of the laboratory manufacturing procedure follow later.

THE COLOUR OF GELATINE.

Commercial gelatin in solution had a colour varying from a very pale yellow to dark amber. The same variation was observed with the dry material but the particle size influenced the apparent dry colour.

It was accepted at Davis Gelatine Industries (DGI) that gelatines derived from pigskin or ossein (demineralised bone) had paler colours than those derived from bovine hide. Furthermore, tannery waste from bovine hide was recognised as yielding a paler gelatine than the whole hide. In gelatine derived from whole hide, there was found to be also a large variance in gelatine colour which was correlated with the other quality parameters of gel strength and viscosity. There was, in addition, a smaller random variance in the colour of gelatines with the same gel strength and viscosity. The availability of indigenous raw material dictated that in South Africa gelatine was made largely from the whole hide.

Although not a "functional property" of gelatine, the presence or absence of colour affected its use in many applications. Generally gelatine colour was an unwanted attribute in confectionary both because lack of colour was associated with "purity" and because it was impossible to make colourless articles with a yellow to amber coloured gelatine. Also, it stood to reason that variable colour militated against the production of a uniform product or the variation in results obtained with added colourants would be minimised if the gelatine was colourless. Colour was also important in some other uses of gelatine as in the production of art papers and string where gelatine was used as a size and in the production of decorative picture frame mouldings. For these reasons bovine hide gelatin could find itself disadvantaged in the market place. Hence, it was decided to try and approach the matter systematically.

Saunders and Ward (1953) studied the measurement of gelatine colour and made some suggestions for the instrumental measurement of colour but they had not

correlated their instrumental values with visual colour measurements. Blake and Plaster (1950) in a series of experiments involving heating gelatine solution with sugars at 134°C, found that colour was produced and therefore they suggested that the colour of gelatine could be as a result of protein/sugar reaction *in vivo*, however, they thought that lactose was the most likely sugar to be involved and they said that "if sugars were removed from raw material then the resulting gelatines would be better in colour ...."

From a comprehensive literature search it was found that most references covered the colouring of products containing gelatine (Hegasy and Winter, 1987) and the effects of gelatine on the colour of beverages due to the fining effects of gelatine (Weissenbach, 1985). One or two references mentioned colour as an attribute of gelatine. These were by the employees of a European manufacturer who made their readers aware of the perceived advantages of pigskin gelatine (Hoffmann, 1984). Otherwise there was no indication that gelatine colour had been a subject of study either from the point of view of its origins or its measurement.

At the start of this study the thinking with regard to gelatine colour was:

1. DGI production statistics showed that paler gelatines were produced at the start of a production cycle at the lowest extraction temperatures. These gelatines also had the best Bloom gel strength and viscosity. The darkest gelatines were produced at the end of a production cycle at the higher extraction temperatures. These gelatines also had the lowest Bloom strength and viscosity.

2. Gelatine produced from chrome tanned leather by the SPA process (Drew, 1930), at relatively high temperatures, had very low Bloom strength and viscosity and it was almost colourless. The results of Heidemann (1982) showed that chromium was attached exclusively to the aspartic and glutamic acid side chains of collagen. In the SPA process the chromium-collagen complex did not dissolve hence the aspartic and glutamic acid groups must have been in some way associated with gelatine colour.

3. Neutralisation with sulphurous acid after alkali conditioning produced gelatine of better colour than processes using sulphuric or hydrochloric acid, prior to extraction.

4. An enzymic conditioning process seemed to produce darker gelatine even with the sulphurous acid acidulation process.

5. Raw material soaked in hydrogen peroxide solution after alkaline conditioning and before acidulation gave a gelatine of better colour. The cost was high and the effect was variable.

6. Gelatine solution (liquor) passed through an anion exchange column exhibited variable colour reductions.

7. From the little data available in the author's M.Sc. thesis, (Cole, 1986), there was an indication that animal age could play a significant role in gelatine colour.

8. Investigations done at DGI, Cole (1983, 1983-1984, 1986, 1988) had shown that, in twinned experiments (duplicate experiments on raw material that had been carefully halved to eliminate raw material as a variable), wet salted hide stored for 1 month exhibited a small decrease in extractability and an increase in gelatine colour which was not considered significant. However, wet salted hide stored for 12 months in a sealed plastic bag exhibited decreased extractability from 24% to 16% at 50°C and increased gelatine colour from 6.4 to 11.2 for the first extraction. In a parallel experiment, wet limed splits showed a small improvement in gelatine colour accompanied by a drop in extractability but the material had been allowed to dry out because wet splits did not have an adequate shelf life.

9. Iron contamination of gelatine had been shown to have an adverse effect on colour (Williams, 1957), but as current iron contents of gelatine were less than 50 ppm this was not considered to be a factor in the colour of gelatine today. An experiment was conducted to re-establish the effect of iron contamination on colour. Chromium was also known to be a possible cause of colour but the colour was greenish whereas the colour of gelatine was yellowish to brown. Also from regular analyses, because of the use of chrome tanned leather as a raw material, it was known that chromium levels in gelatine were below 5 ppm at which level any colour produced by chromium was not visible. From experience it was known that the colour produced by 200 ppm Cr as $Cr_2(SO_4)_3$ was only just visible to the eye.

There were many subjective observations on which theories with regard to gelatine colour were based, but there was a lack of scientific investigation to verify these theories:

(a) One of the more plausible theories was a relationship between the observation of dark coloured fat during extraction and dark gelatine production. Dark fat could be due to breed or to the oxidation or decomposition of glycerides with the production of peroxides. If the latter was the cause of dark fat then the Maillard type of reactions with the protein could be envisaged. Dark fat due to breed, age or nutrition was believed to be due to the accumulation of a derivative of carotene (lutein - Xanthophyll). Because carotene was fat soluble, it was not considered a likely source of colour in gelatine.

(b) A second possible source of gelatine colour could be the melanocytes associated with the hair follicles which were part of the dermis, (Lyne and Short, 1965). These would not be lost during conditioning depilation. The melanin pigments causing skin pigmentation were found above the "basement membrane". If not completely removed by liming, they could also be a source of gelatin colour, particularly, in lower quality gelatines which were derived at higher temperatures. At these temperatures the complete disintegration of the skin occurs. On the other hand melanin pigments were water insoluble polymers (Brady, Duncan and Russell, 1989), hence their effect on the colour of a water soluble polymer like gelatine should be negligible.

OBJECTIVES.

The reason for undertaking this study was the fact that the colour of gelatine produced from bovine hide was dark in comparison to competitive gelatines produced from different raw materials and it was necessary to know the parameters that were responsible for the dark product. The primary objectives were to determine the parameters influencing the occurrence of gelatine colour and to overcome the problem of the instrumental measurement of colour. Thereafter, the gelatines produced from precisely known origins and by known methods were studied by fluorescence spectrophotometry and by electrophoresis in order to try to determine on the molecular origins of gelatine colour.

1. The origins of gelatine colour.

Gelatine colour had always been a concern to DGI and continuous efforts to try to improve colour, had resulted in few significant achievements. In most cases improvements had been small and not always reproducible. It was felt that in view of the little that was known about gelatine colour, the first requirement was to establish why gelatines from different sources exhibited different colours. With this knowledge it was possible that efforts at colour improvement could be made systematically or logically rather than in the random or *ad hoc* approaches of the past.

Firstly, it was necessary to determine how to produce dark coloured gelatine on a consistent basis. (It might appear trite to say it, but it was often forgotten that if one wanted to examine the effect of any process modification on colour one needed to know that the unmodified process would produce a coloured product, or in other words, taken to absurdity, one could not improve the colour of a colourless product). The essence of the problem was not only the production of dark gelatine *per se* but also the variation in the colour of the palest top quality gelatine produced by a standardised process.

Secondly, it was required to establish how far the alkali conditioning process affected colour. Production gelatine colours seemed to be cyclic as was the annual change in alkali conditioning with ambient temperature, hence it seemed probable that conditioning was a factor in gelatine colour.

1.1. Fluorescence and Electrophoresis.

As the study progressed the indications were that animal age (which was accompanied by reduced gelatine extractability) was at the root of the colour problem. In addition the increased degree of collagen cross-linking with senescence, (Sell and Monnier, 1989; Uchiyama, Ohishi, Takahashi, Kushida, Inoue, Jugie and Horiuchi, 1991), was appearing repeatedly in the literature as was collagen cross-linking and meat toughness (Bailey and Light, 1989e). The presentation by Monnier, Sell, Miyata, and Nagaraj (1990), showed that collagen cross-linking, senescence and the Maillard reaction were related. This was the basis on which a reasoned theory on the cause of colour in gelatine could be developed. There was no known literature on gelatine fluorescence although much had been published on collagen by Tanaka, Avigad, Eikenberry and Brodsky (1988), Sell and Monnier (1989) and others. Hence, it

was decided to study the fluorescence spectrum of gelatine with a view to establishing whether the pentosidine cross-link described by Sell and Monnier (1989) could be present in Types A and B gelatines. If so, was the fluorescence related to colour and/or animal age?

Electrophoresis had been used successfully by Tanaka *et al.* (1988), Chalepakis, Tanay and Heidemann (1985), Koepff (1984) to study collagen and gelatine at the molecular level, hence it was decided that this technique could yield additional insights into the effects of cross-linking and animal age on gelatine colour if applied to the range of gelatines produced above.

2. Gelatine colour measurement.

The measurement of colour was usually based on the absorption of light as measured by a spectrophotometer. With gelatine the variable scatter of light, due to molecular size and imperfections in clarity, interfered with the light absorption due to colour. This resulted in there being no generally accepted instrumental method for measuring the colour of gelatine. The measurement of gelatine colour by visual comparison to gelatine standards was the current subjective method used by internal quality control staff. As this comparison method was subjective it could not be used for specification purposes. Hence one of the objectives of this study was to investigate alternative instrumental methods for the measurement of gelatine colour, bearing in mind that a high degree of correlation with the visual methods was desirable.

REFERENCES.

Bailey, A.J., and Light, N.D. 1989a. Connective tissue in meat and meat products. p. 4. Elsevier Applied Science. London and New York.

Bailey, A.J. and Light, N.D. 1989. Molecular and fibre structure of collagen. Ch. 2, In *Connective tissue in meat and meat products*. Elsevier Applied Science. London & New York.

Bailey, A.J. and Light, N.D. 1989. Fibre formation and stabilisation of collagen. Ch. 4, In *Connective tissue in meat and meat products*. Elsevier Applied Science. London & New York.

Bailey, A.J., and Light, N.D. 1989d. Connective tissue in meat and meat products. p. 239-242. Elsevier Applied Science. London and New York.

Bailey, A.J., and Light, N.D. 1989e. Connective tissue in meat and meat products. p. 180-182. Elsevier Applied Science. London and New York.

Bailey, A.J. 1992. Proctor Memorial Lecture. Collagen - Nature's framework in the medical, food and leather industries. Journal of the Society of Leather Technologists and Chemists 76: 111-127.

Bailey, A.J. 1994. Reported in a news item. Journal of the Society of Leather Technologists and Chemists 78: 126-127.

Blake, J.N., and Plaster, F.H. 1950. Colour formation in gelatin manufacture. Journal of the Society of Leather Trades' Chemists 34: 177-186.

Bowes, J.H., Elliot, R.G., and Moss, J.A. 1957. Collagen and the more soluble constituents of skin. Journal of the Society of Leather Trades' and Chemists XLI(7): 249-266.

Bowes, J.H., Elliot, R.G., and Moss, J.A. 1958. The extraction of alkali-soluble protein from calf skin. British Leather Manufacturers' Research Association Laboratory Reports 37(1): 148-162.

Brady, D., Duncan, J.R., and Russell, A.E. 1989. Wool yellowing studies: The formation of melanin-like structures in solutions of tryptophan or tyrosine during exposure to sunlight. Leather Industries Research Institute Research Bulletin No 980.

Chalepakis.Von, G., Tanay, I., and Heidemann, E. 1985. Wie spezifisch ist der kollagenabbau bei der gelatineherstellung. Das Leder 36: 2-10.

Cole, B. 1983. Enzyme conditioning of wet salted hide - SA DAVAC 9 - The effect of ageing on drying and conditioning. Davis Gelatine Industries Internal Report dated 20 July 1993. Krugersdorp. RSA.

Cole, B. 1984. The effect of ageing raw material for 1 year. Davis Gelatine Industries Internal Reports SA DAVAC 13 dated 21 September 1983. and follow-up report dated 30 October 1984. Krugersdorp. RSA.

Cole, C.G.B. 1986. A study of the properties of gelatines derived by various chemical and enzymic methods. M.Sc Thesis. University of Pretoria. Pretoria. RSA.

Cole, B. 1988. The Application of Ultrafiltration to Pit Liquor. Davis Gelatine Industries Internal Report dated 7 January 1988. Krugersdorp. RSA.

Cooper, D.R., Russel, A.E., Shuttleworth, S.H., and Boast, D.A. 1984. Closed systems for salt and saline waste water in curing and tanning. Leather Industries Research Institute Research Bulletin No 877.

Drew, R.B. 1930. An improved process for extracting glue and gelatine from chrome leather. British Patent Specification 338,584. Nov.17, 1930. His Majesty's Stationery Office. Redhill. UK.

Forrest, J.C., Aberle, D.E., Hendrik, H.B., Judge, M.D., and Merkel, R.A. 1975a. In *Principles of meat science*. Schweigert, B.S. (Ed.). p. 356. W.H. Freeman and Company. New York.

Forrest, J.C., Aberle, D.E., Hendrik, H.B., Judge, M.D., and Merkel, R.A. 1975b. In *Principles of meat science*. Schweigert, B.S. (Ed.). p. 53. W.H. Freeman and Company. New York.

Fysh, D., and Goodwin, J.W. 1955. The influence of pH on the extraction of hides and the gelatine obtainable from them. Gelatine and Glue Research Association Research Report C6. August 1955.

Glicksman, M. 1969. Gum technology in the food industry. p. 364-366. Food Science and Technology, 8. Academic Press. New York.

Haines, B.M. 1984. The skin before tannage-Proctor's view and now. Journal of the Society of Leather Technologists and Chemists 68: 57-70.

Hegasy, A., and Winter, M. 1987. Beta-Carotin enthaltende gelatine. European Patent Application EP0221277A2. [In Food Science and Technology SilverPlatter 88-01-V0291]

Heidemann, E. 1982. Newer developments in the chemistry and structure of collagenous connective tissues and their impact on leather manufacture. The Journal of the Society of Leather Technologists and Chemists 66: 21-29.

Hinterwaldner, R. 1977. Technology of gelatine manufacture. In *The science and technology of gelatine*. Ward, A.G., and Courts, A. (Ed.). p. 315-361. Academic Press. London, New York, San Francisco.

Hoffmann, P. 1984. Der einsatz von gelatinprodukten in lebensmitteln. Deutsche Milchwirtschaft. 35: 269-273. [In Food science and Technology Abstracts SilverPlatter 85-12-T0026]

Igarashi, A., Uzuka, M., and Nakajima, K. 1989. The effects of vitamin E deficiency on rat skin. British Journal of Dermatology 121: 43-49.

Koepff, P. 1984. The use of electrophoresis in gelatine manufacture. In *International Working Group for Photographic Gelatin Reports 1970-1982*. Amman-Brass, H., and Pouradier, J. (Ed.). p. 198-208

Komanowsky, M 1989. The Maillard Reaction - Its possible influence on the physical properties of leather. Journal of the American Leather Chemists Association 84: 369-380.

Lyne, A.G., and Short, B.F. 1965. Biology of skin and hair growth. pp. 252-256. Angus and Robertson. Sydney.

Modler, H.W., and Kalab, M. 1983. Microstructure of yogurt stabilized with milk proteins. Dairy Science 66: 430-437.

Monnier, V.M., Sell, D.R., Miyata, S., and Nagaraj, R.H. 1990: The Maillard reaction as a basis for a theory of ageing. In *The Maillard reaction in food processing, human nutrition and physiology*. Finot, P.A., Aeschbacher, H.K., Hurrell, R.F., Liardon, R. (Ed.). p. 393-414. Advances in Life Sciences. Birkhauser Verlag Basel.

Peterkofsky, B. 1991. Ascorbate requirements for hydroxylation of procollagen: relationship to inhibition of collagen synthesis in scurvey. American Journal of Clinical Nutrition 54: 1135S-1140S.

Reich, G., Walther, S., and Stather, F. 1962a. (Translated) On some of the laws governing the production of gelatine from pigskin according to the acid conditioning process. Deutsche Lederinstitut, Freiberg/SA. 18: 15-23.

Reich, von G., Walther, S., und Stather, F. 1962b. Über den einÆuß des alters von rinds- und Schweinshäuten auf ausbeute und eigenschaften daraus nach dem sauren aufschlußverfahren gewonnener gelatine. Untersuchungen über kollagen und gelatine IV. [The influence of the age of cattle and pigskin on the yield and the quality of the gelatines obtained after the acid conditioning process. Investigation of collagen and gelatine IV.] Deutsche Lederinstitut, Freiberg/SA. 18: 24-30.

Saunders, P.R., and Ward, A.G. 1953. The colour and clarity of gelatin and glue solutions. Journal of the Science of Food and Agriculture 4: 523-527.

Sell, D.R., and Monnier, V.M. 1989. Structure elucidation of a senescence cross-link from human extracellular matrix. Journal of Biological Chemistry 264: 21597-21602.

Shirai, K., and Wada, K. 1981. Changes in the properties of pigskin collagen by liming. Japanese Journal of Zootech Science 52(10): 819-831.

Siebert, J. 1992. Ein stoff mit vielen eigenschaften. Chemische Industrie 115: 32-34.

Swatland, H.J. 1985. Structure and development of meat animals. p. 57-63. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Tanaka, S., Avigad, G., Eikenberry, E.F., and Brodsky, B. 1988. Isolation and partial characterization of collagen chains dimerized by sugar-derived cross-links. Journal of Biological Chemistry 236: 17650-17657.

Thompson, G.A. 1988. The Bacteriology of hide and skin preservation: Past, Present and Future. Leather Industries Research Institute Research Bulletin No 961.

Uchiyama, A., Ohishi, T., Takahashi, M., Kushida, K., Inoue, T., Jugie, M., and Horiuchi, K. 1991. Fluorophores from ageing human articular cartilage. Journal of Biochemistry (Tokyo). 110: 714-718.

Ulrich, P.C., and Cerami, A. 1992. Amino acids useful as inhibitors of advanced glycosylation of protein. US Patent 825 598.

Veis, A. 1964a. The Macromolecular Chemistry of Gelatin. p. 121-227. Academic Press-New York and London.

Veis, A. 1964b. The Macromolecular Chemistry of Gelatin. p. 194-198. Academic Press-New York and London.

Ward, A.G. and Courts, A. (Ed.). 1977. The Science and Technology of Gelatin. Ch. 10 to 12. 315-413. Academic Press. London, New York, San Francisco.

Weissenbach. M.J. 1985. Gelatine-Bierschoenung in der praxis. Brauwelt. 125: 1030-1031, 1034, 1036, 1038, 1040, 1042-1044. [In Food Science and Technology Abstracts SilverPlatter 85-10-H0200].

Williams, H.V. 1957. The effect of iron on the colour of gelatine. DGI Research Report No 32. Dated 14 March 1957.