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(54) Title: COLLAGEN AND METHOD FOR PRODUCING SAME

(57) Abstract: The present invention relates to a method for producing collagen from an animal skin. In particular, the present invention a method for producing collagen from an animal skin comprising the step of applying to said skin an organic acid buffer for sufficient time to enable the extraction of collagen.

COLLAGEN AND METHOD FOR PRODUCING SAME

FIELD OF THE INVENTION

[0001] The present invention relates to a method for producing collagen from an animal skin. In particular, the present invention relates to a method for producing collagen by applying an organic acid buffer to the skin of an animal.

BACKGROUND OF THE INVENTION

[0002] Collagen is a substance which accounts for about 30% of the total protein of mammals (See, L. C. Junqueira and J. Carneiro, Basic Histology, 4th ed., Lange Medical Publications, Los Altos, Calif. [1983], pp. 89-119). Collagen is the major structural protein of connective tissues such as skin, tendon cartilage and bone and has a unique amino acid composition and structure. Importantly, the major portion of the helical portion of collagen varies little between mammalian species.

[0003] Collagen from mammals is actually a family of proteins produced by several cell types. Within this protein family, the collagen types are distinguishable by their chemical compositions, different morphological and pathological features, distribution within tissues, and their functions.

[0004] There exist at least twelve genetically distinct types of collagen. The most familiar, type I, consists of three polypeptide chains. Two chains are identical and are called $\alpha 1$ and the third is called $\alpha 2$. Type I collagen forms the major portion of the collagen of both soft (skin, tendon) and hard (bone and dentine) connective tissue. Type II collagen is the major collagen of

cartilage and is composed of three α1 chains. Type III collagen is composed of three α1 chains and is found in blood vessels, wounds, and certain tumours.

[0005] There are many properties of collagen that make it an attractive substance for various medical applications, such as implants, transplants, organ replacement, tissue equivalents, vitreous replacements, plastic and cosmetic surgery, surgical suture, surgical dressings for wounds, burns, etc. (See e.g., U.S. Pat. Nos. 5,106,949, 5,104,660, 5,081,106, 5,383,930, 4,485,095, 4,485,097, 4,539,716, 4,546,500, 4,409,332, 4,604,346, 4,835,102, 4,837,379, 3,800,792, 3,491,760, 3,113,568, 3,471,598, 2,202,566, and 3,157,524, all of which are incorporated herein by reference; J. F. Prudden, Arch. Surg. 89:1046-1059 [1964]; and E. E. Peacock et al. Ann. Surg., 161:238-247 [1965]). For example, by itself, collagen is a relatively weak immunogen, at least partially due to masking of potential antigenic determinants within the collagen structure. Also, it is resistant to proteolysis due to its helical structure. In addition, it is a natural substance for cell adhesion and the major tensile load-bearing component of the musculoskeletal system. Thus, extensive efforts have been devoted to the production of collagen suitable for use in medical, as well as veterinary applications.

[0006] Collagen can be produced in a number of forms, from a variety of tissues by a variety of methods. One of the most sought after collagens is "native" collagen, which has a number of unique properties, including low antigenicity and resistance to enzymic digestion. Finely divided dispersed native collagen has a wide range of actual and potential application in the cosmetic, food and pharmaceutical industries.

[0007] However, despite its significant advantages, the

commercial success of collagen-based materials has been limited to a few specialised products that make use of collagen's unique properties. The major reasons for the lack of commercially available collagen-based medical and biotechnology products includes the high cost of preparing pure collagen, the variability of isolated collagen and the difficulties of handling and storing collagen.

[0008] The applicant has now surprisingly found a very simple technique that may be used on animal skins, which is capable of producing high yields of native soluble collagen and dispersed insoluble collagen cheaply, while overcoming or at least alleviating some of the problems encountered previously. In particular, the applicant has found that by treating animal skins with suitable organic acid buffers, the yields of enzymatically solubilised native collagen are increased.

SUMMARY OF THE INVENTION

[0009] In its broadest aspect the present invention provides a method for producing collagen from an animal skin comprising the step of applying to said skin an organic acid buffer for sufficient time to enable the extraction of collagen.

[0010] A further aspect of the invention is to provide collagen for medical and biotechnology products useful for general surgery, dermatology, dentistry, plastic and reconstructive surgery, neurosurgery, orthopaedics, ophthalmology urology, vascular surgery, veterinary medicine, and other related fields.

[0011] Preferably, the collagen is native soluble collagen and finely dispersed native collagen.

[0012] It will be appreciated by those skilled in the

art that the methods disclosed herein may be useful for extracting collagen from any animal skin. However, the present invention is particularly useful for extracting collagen from animal skins taken from animals from the mammalian orders Artiodactyla, Lagomorpha, Rodentia, Perissodactyla, Carnivora and Marsupialia. More preferably, the animal skin is removed from an animal selected from the group consisting of ovine, bovine, caprine, equine, porcine and marsupial. Most preferably, the skin is a sheep skin.

[0013] The organic acid buffer may comprises any suitable organic acid known in the art. Preferably, the organic acid buffer is an aqueous solution or suspension of one or more acids selected from the group consisting of acetic acid, citric acid, pyruvic acid, lactic acid and formic acid. More preferably, the organic acid buffer is composed of glacial acetic acid in water and has a pH between 3 and 5. Even more preferably, the organic acid buffer has a pH between 3.2 and 4.5. Most preferably, the organic acid buffer has a pH of between 4.0 to 4.2.

[0014] Once the organic acid buffer has been applied to the animal skin it is allowed to incubate for sufficient time to enable the wool or hair to be loosened or removed. Preferably, the incubation is undertaken at a temperature between 20 and 40°C for between 8 and 27 hours. The animal skin may then be chopped or minced into small pieces.

[0015] Preferably, the chopped or minced skin is then mixed with organic acid buffer in a suspension under non-denaturing conditions and incubated at temperature between 0 and 30°C with stirring. Preferably, the skin is also incubated in the presence of one or more proteases. More preferably, the protease is selected from the group consisting of ESPERASE®, ALCALASE®, DURAZYM® and SAVINASE®, MAXATASE®, MAXACAL®, PROPERASE®, MAXAPEM®, and pepsin. Most

preferably, the protease is pepsin.

[0016] After incubation of the skin pieces in the organic acid buffer and protease suspension, the suspension is centrifuged and/or filtered and the soluble and/or dispersed collagen is removed.

[0017] The collagen may be dried and ground to form a powder. An alternate to drying and grinding to form a powder, the collagen can be redispersed with organic acid, and this dispersion can then be used to form a collagen matrix or sponge by freeze-drying in the manner disclosed in the Silyer et al. U.S. Pat. No. 4,703,108.

[0018] It is further contemplated that the purified collagen may be comprised of additional compounds, including but not limited to antimicrobials, antivirals, growth factors, anti-dehydration compounds, antiseptics, or other compounds suitable for biomedical and/or veterinary uses.

DETAILED DESCRIPTION OF THE INVENTION

[0019] Before the present methods are described, it is understood that this invention is not limited to the particular materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "an organic acid" is a reference to one or more organic acids and equivalents thereof known to those skilled in the art, and so forth. Unless defined otherwise, all technical and

scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any materials and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred materials and methods are now described.

[0020] All publications mentioned herein are cited for the purpose of describing and disclosing the methods, protocols and reagents which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0021] The present invention relates to methods for preparing collagen from animal skins. In particular, the present invention provides methods for the preparation of collagen suitable for biomedical, veterinary, and other applications.

[0022] The term "animal" as used herein means any animal from which collagen may be extracted by the methods disclosed. For example, the animals may by selected from the mammalian orders Artiodactyla, Lagomorpha, Rodentia, Perissodactyla, Carnivora and Marsupialia.

[0023] Artiodactyls comprise approximately 150 living species distributed through nine families: pigs (Suidae), peccaries (Tayassuidae), hippopotamuses (Hippopotamidae), camels (Camelidae), chevrotains (Tragulidae), giraffes and okapi (Giraffidae), deer (Cervidae), pronghorn (Antilocapridae), and cattle, sheep, goats and antelope (Bovidae). Many of these animals such as goats, sheep, cattle and pigs have very similar biology and share high degrees of genomic homology. More importantly, it is well known that certain animals such as goats and sheep and

horses and donkeys can interbreed.

[0024] Perissodactyla are "odd-toed" or "odd-hoofed" mammals and include rhinos, tapirs and horses. Lagomorpha includes rats, mice, rabbits, hares and other rodents, while the order Marsupialia includes kangaroos and wallabies.

[0025] All members of the above animal groups have skin composed of two layers, the dermis and the epidermis. The epidermis consists of several layers, representing successive stages of development. The oldest part of the skin is an expendable outer layer of tough, protective, dead cells, continually worn off at the surface and continually replaced from below. As the cells age and mature, they eventually lose their nuclei and convert most of their cell contents to keratin, a protein that makes not only the protective layer of skin, but such structures as nails, hooves, hair, and horns.

[0026] The dermis lies below the epidermis and is a thick layer of connective tissue with associated muscles, nerves, and blood vessels. The connective tissue consists to a great degree of collagen.

[0027] As used herein, the term "collagen" is used in reference to the extracellular family of fibrous proteins that are characterised by their stiff, triple-stranded helical structure. Three collagen polypeptide chains ("α-chains") are wound around each other to form this helical molecule. The term is also intended to encompass the various types of collagen, although the preferred forms are type I and type III collagen. The term "native collagen" means that the triple helix region of the collagen remains intact.

[0028] As stated above, the major portion of the

helical portion of collagen varies little between mammalian species. Indeed, a number of collagen types have high degrees of nucleotide and amino acid sequence homologies. For example, the nucleotide sequence homology for collagen alpha I type II is at least 88% when comparing humans, equines and murine. Humans and equines have 93% sequence homology at the nucleotide level, while mouse and equine have 89% sequence homology. The nucleotide sequence homology for human and mouse is 88% (see, NCBI accession numbers U62528 (Equine), NM033150 (Human) and NM031163 (mouse) http://www.ncbi.nlm.nih.gov). Other types of collagen have similar levels of amino acid homology. For example, the nucleotide sequence homology between porcine collagen alpha I type I and ovine collagen alpha I type I is 90% (see, NCBI accession numbers AF29287 (Ovine) and AF201723 (Porcine) http://www.ncbi.nlm.nih.gov).

[0029] Given the level of common ancestry and biology for many of the above animals, the similar morphology ie skin structure, the high degree of amino acid and nucleotide sequence homology for collagen across a number of species such as cattle, sheep, mice and pigs, a person skilled in the art would appreciate that the methods disclosed herein are applicable for all animals.

[0030] Once an animal has been selected from which collagen is to be extracted, the skin is removed. Any process known in the art for removing the animal skin may be used. Moreover, the skin may be removed from an animal of any age; however, in one preferred embodiment the animal is a lamb less than 14 weeks old.

[0031] The removed skin may be used immediately in the methods of the invention, or alternatively stored at less than 20°C, more preferably between 4°C and 20°C. However, it will be appreciated that prolonged storage at a

temperature outside of this range may adversely affect the quality and quantity of the collagen extracted.

[0032] In one preferred embodiment, one or more organic acid based buffers are applied to the flesh side of a fresh animal skin. The term "fresh" as used herein means an animal skin that was removed no more than 1 day before use, preferably, no more than 12 hours before use, even more preferably, the animal skin is used within 8 hours of removal.

[0033] The organic acid buffer is preferably either a lactic acid buffer, acetic acid buffer, citric acid buffer, pyruvic acid buffer, formic acid buffer or combination thereof; however, other organic acid buffers known in the art may also be used.

[0034] The organic acid buffers are preferably adjusted to a pH of between 3-5, more preferably between 3.2 and 4.6 with sodium acetate, sodium citrate, sodium pyruvate, sodium hydroxide, sodium bicarbonate and sodium carbonate, potassium salts and the like may also be used.

[0035] In one especially preferred embodiment the buffer consists of an aqueous solution or suspension of one or more of acetic acid, lactic acid or formic acid mixed together with an alkaline metal (including ammonium) or an alkaline metal salt. A mixture of mineral and organic acid may be used.

[0036] The most preferred buffer is composed of 40% (w/w) glacial acetic acid in water and the pH adjusted to the preferred pH (4.0 - 4.2) by addition of around 20% anhydrous sodium acetate or the required amount of sodium hydroxide.

[0037] Application of the organic acid buffer may be by

any method known in the art. For example, the organic acid buffer may be applied to the skin by spraying, painting, dipping or the like. Preferably, the organic acid buffer is applied by painting at a rate of around 50 to 100mL per skin. If the organic acid buffer is be used a dip or the skin is immersed in the organic acid buffer, the organic acid buffer may be diluted 10 fold with water.

[0038] After application, the animal skin may be incubated at a temperature between 20 and 40°C for between 8 and 27 hours. It is also preferably that the relative humidity during incubation is sufficient high so that the animal skin does not dry out. For example, a relative humidity of greater than 60%, preferably greater than 80%, even more preferably greater than 90% should be used.

[0039] Without wishing to be bound by any particular theory or hypothesis, the applicant believes that the use of organic acid buffer at this stage of the extraction process results not only in the loosening of the wool and hair, but also in the substantial digestion of the glycosaminoglycans (GAGs) present in the skin. The removal of the GAGs results in the opening up of the collagen structure, which in turn facilitates the enzymic solubilisation of the collagen present at later stages.

[0040] The terms "substantial" and "substantially" as used herein when referring to the removal of the GAG means that, relative to the amount of GAG normally present in an animal skin, by the end of the initial incubation period with the organic acid buffer preferably greater than 70%, even more preferably greater than 80%, even more preferably greater than 90% of all GAG has been digested.

[0041] Following incubation the wool or hair is removed mechanically or by hand to produce a de-haired skin, from herein termed "pelt". If the hair or wool has an economic

value it may be dried and baled or merely discarded.

[0042] The pelt is then fleshed either mechanically or by hand. Any pelt containing residual wool or hair is removed and discarded. The pelt is then chopped or minced into small pieces. Any device capable of breaking up the pelts may be used including industrial mincers, grinders or food processors.

[0043] The pelt pieces are then added to a non-denaturing solution to produce a suspension. The non-denaturing solution comprises an organic acid buffer and the suspension is incubated under non-denaturing conditions. The term "non-denaturing conditions" as used herein means that the triple helix structure of the collagen is preserved. Accordingly, in one preferred embodiment the organic acid buffer used is preferably, about pH 2 at a temperature of less than 25°C.

[0044] In one preferred embodiment, the organic acid buffer comprises acetic, lactic, formic acids or combination thereof. The concentration of the organic acid buffer may be between 0.01M to 0.5M, more preferably between 0.1M to 0.05M. The preferred acid is acetic acid at concentration between 0.1M and 0.05M.

[0045] The ratio of pelt pieces to non-denaturing solution can be between 10:1 to 1000:1, respectively. Once the suspension is produced it is preferably slowly agitated. The amount of agitation depends upon the size of the pelt pieces in suspension; however, preferably the agitation is sufficient to gently move the pelt pieces. The suspension is also preferably incubated at a temperature of between 0 and 30°C for at least 1 hour. In one preferred embodiment the incubation is allowed to proceed for up to 7 days; however, the incubation length will be determined by the pH, the temperature and type of

pelt.

[0046] In one especially preferred embodiment, a protease is introduced the suspension. There are a very large number of suitable protease available commercially or known in the art. For example, suitable proteases are the subtilisins which are obtained from particular strains of *B. subtilis* and *B. licheniformis* (subtilisin BPN and BPN'). One suitable protease is obtained from a strain of *Bacillus*, having maximum activity throughout the pH range of 8-12, developed and sold as ESPERASE® by Novo Industries A/S of Denmark, hereinafter "Novo". The preparation of this enzyme and analogous enzymes is described in GB 1,243,784 to Novo. Other suitable proteases include ALCALASE®, DURAZYM® and SAVINASE® from Novo and MAXATASE MAXACAL®, PROPERASE® and MAXAPEM® (protein engineered Maxacal) from Gist-Brocades. Proteases also encompass modified bacterial serine proteases, such as those described in European Patent Application Serial Number 87 303761.8, filed April 28,1987 (particularly pages 17, 24 and 98) and in European Patent Application 199,404, Venegas, published October 29,1986, which refers to a modified bacterial serine proteolytic enzyme.

[0047] Other suitable proteases include the alkaline serine protease described in EP 90915958: 4, corresponding to WO 91/06637, Published May 16, 1991. Also suitable for the present invention are proteases described in patent applications EP 251 446 and WO 91/06637, protease BLAPS described in W091/02792 and their variants described in WO 95/23221.

[0048] Depending on the type and amount of enzyme digestion required the conditions used and the time required to obtain a maximum yield of soluble collagen will vary greatly, from 1 to 10 days. The ratio of the weight of the enzyme to the weight of pelt can vary

greatly from 1:10 to 1:1000 (w/w). Pepsin is the preferred enzyme and the ratio of the weight of the enzyme to the weight of pelt is around 1:100.

[0049] After incubation the soluble collagen and the dispersed collagen can be separated by centrifugation or filtration. With centrifugation, the supernatant contains the solubilised collagen and the precipitate contains the dispersed collagen. With filtration the soluble collagen is in the filtrate.

[0050] The solubilised collagen may be recovered from the supernatant by any known means including precipitation, filtration and the like. Precipitation of the soluble collagen with salt is the preferred method. Salt, such as NaCl may be added to the supernatant (filtrate) to a final concentration of between 5 and 20% w/v, preferably 10%. The suspension can then centrifuged. The precipitate can be retained and the supernatant discarded. The precipitate may then be re-dissolved in dilute (0.1M) acetic or lactic acid (ratio of precipitate to solution of around 1:100). The soluble collagen can then be re-precipitated by the addition of salt. The precipitate may then be re-dissolved in dilute acid and centrifuged. The collagen concentration in the supernatant can then be measured and adjusted to the required concentration (from 1 to 10 mg/mL). The collagen can be used in this form or the dissolved collagen can be dialysed against water and then converted to dehydrated collagen.

[0051] As used herein, the term "dehydrated collagen" refers to collagen that has been dehydrated using any method commonly known in the art. In preferred embodiments, dehydrated collagen is produced by lyophilization, freeze-drying or desiccation.

[0052] The suspension containing the dispersed collagen can be filtered to remove any large particles. The concentration of collagen in the filtrate can be adjusted to that required and used in this form. Alternatively, the dispersed collagen can be washed with water and then dried.

[0053] As used herein, the term "dried" refers to any method for the removal of water from the collagen. It is intended that the term encompasses methods including, but not limited to, air-drying or freeze-drying.

[0054] From the above, it is clear that the various embodiments of collagen prepared according to the methods of the present invention are suitable for various biomedical applications that require collagen. It is contemplated that the collagen of the present invention can be used in multiple settings and for numerous applications, including but not limited to, collagen sutures, collagen soft tissue replacements including wound and burn coverings, arterial vessel replacements, hemostatic agents, drug delivery matrices, vitreous replacement for ophthalmologic therapy, endodontic therapy, cell culture supports, etc. It is further contemplated that various embodiments of the present invention will find use in any form, including, but not limited to fibrous or membrane films, bags, sponges, suture threads, and aqueous suspensions, as well as composite materials. In addition, collagen prepared according to the present invention may be further modified as necessary for the desired application and to provide an improved bioactive response. It is also contemplated that the methods of the present invention will be applicable to the preparation of other biomolecules as well as collagen.

[0055] Throughout the specification, the word "comprise" and variations of the word, such as

"comprising" and "comprises", means "including but not limited to" and is not intended to exclude other additives, components, integers or steps.

[0056] The invention will now be further described by way of reference only to the following non-limiting examples. It should be understood, however, that the examples following are illustrative only, and should not be taken in any way as a restriction on the generality of the invention described above. For example, while the majority of the examples relate to sheep skins, it is to be understood that the invention can also be applied to other animal skins as disclosed herein, including for example, bovine, porcine and marsupial skins.

EXAMPLE 1 EXTRACTION OF TYPE I AND TYPE III COLLAGEN FROM A SHEEP SKIN

[0057] A fresh merino sheepskin with approximately 5cm length wool was obtained from a local abattoir immediately after slaughter. The skin was transported to the laboratory in a chilled container. The skin was fleshed mechanically and then sprayed with approximately 50mL of organic acid buffer. The buffer used was 40% glacial acetic acid in water adjusted to pH 4.0 with sodium hydroxide.

[0058] The skin was incubated at 35°C, at 85% relative humidity for 16 hours. The wool was removed by hand and trimmed to remove any skin containing residual hair or wool. The resulting pelt weighed approximately 1.2kg.

[0059] The pelt was then washed with water and chopped into small pieces using an industrial grade food processor.

[0060] The chopped pelt was then introduced to a non-

denaturing solution comprising 0.1M acetic acid. The ratio of pelt to non-denaturing solution used was 50:1 (w/w). Pepsin (1:2000) was then added to the suspension at a ratio of pepsin to tissue of 1:100. The suspension was then incubated for 7 days at 5°C, with occasional stirring.

[0061] After incubation the suspension was centrifuged at 10,000g, for 60 minutes. The supernatant, containing the solubilised collagen and the precipitate containing the insoluble dispersed collagen were recovered.

[0062] The soluble collagen was then precipitated by the addition of salt (NaCl) to a concentration of 10% (w/w). The resulting suspension was then centrifuged and the precipitate was retained and the supernatant discarded. The precipitate was re-dissolved in dilute (0.1M) acetic acid at a ratio of precipitate to acetic acid of 1:100. The resulting solution was then centrifuged. The dissolved collagen was dialysed against several changes of water until a desired level of salt concentration was obtained and then freeze-dried.

[0063] Using this process, 30g pepsinised soluble collagen of comparable purity to the commercially available bovine collagen was obtained. The soluble collagen produced consisted of 95% type I and 5% type III collagens. Based on the hydroxyproline content, it was estimated that approximately 270g of dispersed collagen was also produced.

EXAMPLE 2 EXTARCTION OF TYPE I AND TYPE III COLLAGEN FROM A LAMB SKIN

[0064] The procedure shown in Example 1 was performed using the skin from a "sucker" lamb ie a lamb less than 12 to 14 weeks old. This procedure resulted in over 50% of

the collagen present being isolated as acid soluble collagen. The ratio of type I to type III was found to be 90% to 10%, respectively.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for producing collagen from an animal skin comprising the step of applying to said skin an organic acid buffer for sufficient time to enable the extraction of collagen.

2. A method according to claim 1, wherein the animal skin is removed from an animal selected from the group consisting of ovine, bovine, caprine, equine, porcine and marsupial.

3. A method according to claim 1 or claim 2, wherein the step of applying said organic acid buffer to said skin comprises applying organic acid buffer to the flesh side of the animal skin.

4. A method according to any one of claims 1 to 3, wherein the organic acid buffer has a pH between 3 and 5.

5. A method according to any one of claims 1 to 3, wherein the organic acid buffer has a pH between 3.2 and 4.5.

6. A method according to any one of claims 1 to 5, wherein the organic acid buffer comprises an aqueous solution or suspension of one or more acids selected from the group consisting of acetic acid, citric acid, pyruvic acid, lactic acid and formic acid.

7. A method according to claim 6, wherein the organic acid buffer is composed of 40% (w/w) glacial acetic acid in water.

8. A method according to claim 7, wherein the organic acid buffer has a pH of between 4.0 to 4.2.

9. A method according to any one of claims 1 to 8, wherein the organic acid buffer is applied at a rate of around 50 to 100mL per skin.

10. A method according to any one of claims 1 to 9, further comprising the step of incubating the animal skins at a temperature between 20 and 40°C for between 6 and 48 hours.

11. A method according to claim 10, wherein the animal skin is de-haired.

12. A method according to claim 10 or claim 11, wherein residual flesh on the animal skin is removed either mechanically or by hand.

13. A method according to claim 12, wherein the animal skin is chopped or minced into small pieces.

14. A method according to claim 13, further comprising the step of mixing said animal skin pieces with organic acid buffer as a suspension under non-denaturing conditions.

15. A method according to claim 14, wherein organic acid is selected from the group consisting of acetic acid, lactic acid and formic acid.

16. A method according to claim 15, wherein the organic acid is acetic acid at concentration between 0.1 and 0.05M.

17. A method according to any one of claims 14 to 16, wherein the skin pieces and organic acid are incubated at temperature between 0 and 30°C with stirring.

18. A method according to claim 17, wherein the

suspension further comprises one or more proteases.

19. A method according to claim 18, wherein the protease is selected from the group consisting of ESPERASE®, ALCALASE®, DURAZYM® and SAVINASE®, MAXATASE®, MAXACAL®, PROPERASE®, MAXAPEM®, and pepsin.

20. A method according to claim 18, wherein the protease is pepsin.

21. A method according to any one of claims 14 to 20, further comprising a separation step.

22. A method according to claim 21, wherein the separation step involves centrifugation and/or filtration, wherein soluble collagen and dispersed collagen are separated.

23. A method according to claim 22, wherein the separation step involves centrifugation and the supernatant contains the solubilised collagen.

24. A method according to claim 22, wherein the separation step involves filtration and the filtrate contains the soluble collagen.

25. A method according to claim 23 or claim 24, wherein the soluble collagen is precipitated with the use of salt.

26. A method according to claim 25, wherein the salt is added to a final concentration of between 5 and 20% w/v.

27. A method according to claim 25, wherein the salt is added to a final concentration of about 10%.

28. A method according to any one of claims 1 to 27, wherein the collagen is freeze dried.

29. A method for producing purified native collagen comprising the steps of:

a). providing an animal skin;

b). contacting said skin with organic acid buffer for sufficient time to substantially digest the glycosaminoglycans present in the skin to produce a pelt;

c). mincing said pelt and exposing to one or more protease(s) to produce a preparation comprising soluble and dispersed collagen;

d). centrifuging said preparation to isolate soluble native collagen from dispersed collagen; and

e). precipitating said soluble native collagen with salt to produce purified collagen.

30. Purified collagen prepared according to a method according to any one of claims 1 to 29.

31. A purified collagen according to claim 30, wherein said purified collagen contains less than 0.1 percent dry weight of insoluble non-collagenous protein.

32. A purified collagen according to claim 30, wherein said purified collagen contains less than 0.0001 percent dry weight of phospholipids.

33. A purified collagen according to claim 30, wherein said purified collagen contains less than 0.0001 percent dry weight of neutral lipids and glycolipids.

34. A purified collagen according to claim 30, wherein said collagen comprises no more than 95% type I collagen and no less than 5% type III collagen.

35. A purified collagen according to claim 30,

wherein said collagen comprises no more than 90% type I collagen and no less than 10% type III collagen.

36. A purified collagen according to claim 30, wherein said collagen comprises no more than 80% type I collagen and no less than 20% type III collagen.

37. A method for producing purified biocompatible collagen comprising the steps of:

a). providing an animal skin;

b). contacting said skin with organic acid buffer for sufficient time to substantially digest the glycosaminoglycans present in the skin to produce a pelt;

c). mincing said pelt and exposing to one or more protease(s) to produce a preparation comprising soluble and dispersed collagen;

d). centrifuging said preparation to isolate soluble native collagen from dispersed collagen; and

e). precipitating said soluble native collagen with salt to produce biocompatible collagen.

38. A biocompatible collagen prepared by a method according to claim 37.

39. A purified biocompatible collagen according to claim 38, further comprising an additional compound suitable for biomedical uses.

40. A purified biocompatible collagen according to claim 39, wherein said additional compound is selected from the group consisting of an antimicrobial, an antiviral, an antiseptic, a growth factor and an anti-dehydration compound.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU03/00606

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C07K 14/78; A61K 38/39

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, keywords: acetate, citrate, lactate, pyruvate, formate, collagen, buffer, prepar+, isolat+, extract+
Medline, keywords: collagen, buffer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 201 15 753 U1(INSTITUT FÜR AGRAR- UND STADTÖKOLOGISHE PROJECKTE AN DER HUMBOLT-UNIVERSITÄT ZU BERLIN) 10 January 2002 See whole document	1 - 40
X	US 2,631,942 A (HIGHBERGER) 17 March 1953 See whole document, especially Example 1	1 - 40
X	WO 83/03673 (A/S N. FOSS ELECTRIC) 27 October 1983 See whole document	1, 6

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 June 2003	26 JUN 2003
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **DAVID GRIFFITHS** Telephone No : (02) 6283 2628

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU03/00606

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Derwent Abstract Accession No. 93-161128/20, Classes B04, D21; & JP 05-093000 A (NIPPON KASEI KK) 16 April 22 June 1993 See abstract	1
X	Derwent Abstract Accession No.93-232354/29, Class P34; & JP 05-155900 A (NIPPON KASEI KK) 22 June 1993 See abstract	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU03/00606

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
DE	20115753	NONE			
US	2631942	FR	1055317	GB	721312S
WO	8303673	EP	105898		

END OF ANNEX