REYES & REYES
A B O G A D O S

SEÑOR
SUPERINTENDENTE DE INDUSTRIA Y
E. S. D.

REF: **Expediente 04-129-358**

TÍTULO SOLICITADO: **"UN AGENTE PARA HACER CÁPSULAS DE GELATINA BLANDA Y LAS CÁPSULAS ASÍ OBTENIDAS".**

PUBLICACIÓN: **Gaceta 557 de 31-16-2005, número de publicación 657**

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SOLICITANTE: **R. P. SCHERER TECHNOLOGIES INC.**

APODERADO: **LUIS FELIPE CASTILLO GIBSONE**

TRÁMITE: **REMISIÓN DE INFORMACIÓN.**

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **TECNOQUIMICAS S.A.**, dentro del trámite de la referencia, por medio del presente escrito, procedo a presentar argumentos que demuestran que la solicitud de la referencia incumple los requisitos de patentabilidad establecidos por los artículos 14, 16 y 18 de la Decisión 486 de 2000, y que por ello no merece recibir el beneficio patentario:

I. REMISION DE INFORMACIÓN.

En primer lugar, es importante señalar que la modificación en el capítulo reivindicatorio, presentada 18 de Noviembre de 2010, corresponde a un cambio de

forma, que por esa condición, no logra superar los argumentos planteados en contra por el suscrito, ni aquellos formulados por el examinador técnico. Es así como al revisar dicho nuevo capitulo reivindicatorio, persiste la carencia de nivel inventivo, al mantenerse en esencia las mismas características técnicas (capsulas de gelatina blanda que comprenden la gelatina, plastificante y un agente antiahdesión).

Entrando en materia, y teniendo en cuenta que en la respectiva respuesta al requerimiento de información el solicitante argumenta, en relación a las anterioridades que afectan el nivel inventivo y que fueron mencionadas por el examinador técnico, que *"no hacen referencia al uso de gelatina de pescado, formulaciones de concha terminadas con alto contenido de agua o a la producción de capsulas para ser masticadas"*, podemos demostrar que de ninguna manera las composiciones para obtener dichas capsulas blancas poseen nivel inventivo, veamos:

En primer lugar cualquier persona medianamente versada en la materia debe reconocer que las técnicas de formulación y el conjunto de compuestos que se pueden utilizar para desarrollar productos farmacéuticos viables, son elementos bien conocidos para una persona capacitada en la producción de dichos productos farmacéuticos. Por lo tanto no puede considerarse inventivo que dicha persona capacitada en la formulación de productos farmacéuticos escoja dentro de los diferentes compuestos que se pueden encontrar en el comercio para obtener formulaciones del tipo capsulas blandas masticables, formulaciones descritas y utilizadas ampliamente en el área farmacéutica.

A.A. Karim, Rajeev Bhat y colaboradores[1] nos indican que la producción de gelatina de pescado en realidad no es nueva ya que ha sido producida desde 1960 por la extracción de ácido, aunque la mayor parte ha utilizado para aplicaciones industriales (Norland, 1990) y que los procedimientos de extracción y caracterización de las propiedades de la gelatina de pescado fueron descritos por Grossman y Bergman (1992) en una patente de Estados Unidos. Así mismo nos indican que muchas compañías han desarrollado experiencia para manejar la

[1] A.A. Karim*, Rajeev Bhat. Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocolloids 23 (2009) 563–576.

gelatina de pescado, y que volúmenes menores de gelatina de pescado se utilizan para hacer cápsulas de gelatina blanda.

En cuanto a la novedad de capsulas blandas diseñadas para ser masticadas, también existen referencias que hacen evidente que con anterioridad se habían conseguido formulaciones farmacéuticas con estas mismas características, veamos:

La patente Japonesa 05238954 A publicada el 17 de Septiembre de 1993 revela:

(54) **CARRIER SUITABLE FOR CHEWING ADMINISTRATION**

(57) Abstract:

PURPOSE: To obtain a carrier, capable of being chewed and administered without causing an oily taste or touch and useful for a medicine, etc., sealed in a soft capsule.

CONSTITUTION: The objective carrier, useful for a medicine or a food material and suitable for chewing administration is characterized in that it is composed of 50-99 pts.wt. uniformly mixed edible oil and 1-20 pts.wt. surfactant having 1-20HLB.

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Por su parte la patente Japonesa 10273436 publicada el 13 de Octubre de 1998 revela:

(54) **SOFT CAPSULE FOR CHEWING**

(57) Abstract:

PROBLEM TO BE SOLVED: To provide soft capsule for chewing that can be readily crunched into pieces in mouth with little sticking to teeth or cavity and shows excellent solubilization behavior.

SOLUTION: This soft capsule for chewing has the skin that is composed of the following components (A), (B) and (C): (A) gelatin, (B) totally 100-600 pts.wt., per 100 pts.wt. of gelatin, of one or two or more kinds of plasticizers selected from the components (b1)-(b3): (b1) glycerol, (b2) saccharides selected from D-sorbitol, sucrose, mannitol, fructose, sucrose alcohol and isomerized sugar, (b3) glycol selected from propylene glycol and polyethylene glycol, and (C) a water-soluble cellulose.

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Como se puede apreciar, estas dos anterioridades exponen cápsulas de gelatina blandas con las características necesarias para ser masticables, y que al igual que las cápsulas reivindicadas por la solicitud colombiana en trámite, contienen el mismo tipo de plastificantes y una proporción elevada de agua.

Para finalizar, podemos hacer aun más evidente la versatilidad del uso de la gelatina de pescado en el mismo tipo de capsulas de gelatina blanda a través de la publicación internacional WO/1999/033924 publicada el 08 de Julio de 1999 y titulada "GELATINE COMPOSITIONS" que revela lo siguiente:

The invention concerns gelatine compositions containing an additional setting system for the use in pharmaceutical, veterinary, food, cosmetic or other products like films for wrapping food, aspics or jellies, preferably for predosed formulations like soft or hard gelatine capsules wherein the gelatine used is of non-bovine and non-pig origin and preferably derived from fish, poultry or plant sources. Especially preferred are film compositions for hard gelatine capsules prepared from fish gelatine.

(...)

Surprisingly it has been found that fish gelatine can be used to produce gelatine compositions with conventional properties by adding a setting system to the aqueous fish gelatine solution.

(...)

The setting system consist of a hydrocolloid or mixtures of hydrocolloids and may contain in addition cations and / or sequestering agents.

(...)

The setting system consist of a hydrocolloid or mixtures of hydrocolloids and may contain in addition cations and / or sequestering agents.

Suitable hydrocolloids or mixtures producing synergistic properties may be selected from natural seaweeds, natural seed gums, natural plant exudates, natural fruit extracts, bio-synthetic gums, bio-synthetic processed starch or cellulosic materials, preferred are the polysaccharides.

The preferred polysaccharides are alginates, agar gum, guar gum, locust bean gum (carob), carrageenan, tara gum, gum arabic, ghatti gum, Khaya grandifolia gum, tragacanth gum, karaya gum, pectin, arabian (araban), xanthan, gellan, starch, Konjac mannan, galactomannan, funoran, and other

(...)

The inventive gelatine compositions may contain additionally acceptable plasticizers in an range from about 0 to 40 % based upon the weight of the gelatine. Suitable plasticizers are polyethylene glycol, glycerol, sorbitol, sucrose, corn syrup, fructose, dioctyl-sodium sulfosuccinate, triethyl citrate, tributyl citrate, 1,2-propyleneglycol, mono-, di- or triacetates of glycerol, natural gums or the like as well as mixtures thereof.

(...)

2. Gelatine compositions according to claim 1, wherein the gelatine is derived from fish, poultry or plants.

3. Gelatine compositions according to claim 1, wherein the gelatine is fish gelatine.

Como se puede apreciar, en este documento se revela con anterioridad la utilidad de la gelatina de pescado en la elaboración de capsulas de gelatina blanda. Pero lo mas relevante del documento es que se utiliza la misma estrategia tecnológica que reivindica la solicitud colombiana para hacer las películas de gelatina de pescado mas adecuadas al proceso productivo de producción de capsulas, que resulta ser la adición de hidrocoloides de compuestos tales como almidones procesados y almidón. Así mismo se hace mención del mismo tipo de plastificantes utilizados por la formulación de la solicitud colombiana en cuestión.

En conclusión, podemos afirmar que la solicitud colombiana en tramite carece irrefutablemente de nivel inventivo, puesto que absolutamente todas las características técnicas a través de las cuales se puede llegar a una formulación de gelatina de pescado blanda masticable, se encuentran descritas con anterioridad en el estado de la técnica, y su obtención no resulta ser mas que la aplicación de toda la información referida con anterioridad acerca de dicha tecnología.

Por todo lo anterior, ruego se analicen los argumentos expuestos con el rigor y detenimiento habituales, y que en consecuencia el Despacho decrete, además de la negación de la solicitud en comento, el archivo del expediente que lo contiene.

III. ANEXOS.

- Copia de todas las publicaciones mencionadas en el acápite de Remisión de información.

Señor Superintendente,

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(54) Title: GELATINE COMPOSITIONS

(57) Abstract

The invention concerns Gelatine compositions containing an additional setting system for the use in pharmaceutical, veterinary, food, cosmetic or other products like films for wrapping food, aspics or jellies, preferably for predosed formulations like soft or hard gelatine capsules wherein the gelatine used is of non-bovine and non-pig origin and preferably derived from fish, poultry or plant sources. Especially preferred are film compositions for hard gelatine capsules prepared from fish gelatine.

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Gelatine compositions

The invention concerns gelatine compositions containing an additional setting system for the use in pharmaceutical, veterinary, food, cosmetic or other products like films for wrapping food, aspics or jellies, preferably for predosed formulations like soft or hard gelatine capsules wherein the gelatine used is of non-bovine and non-pig origin and preferably derived from fish, poultry or plant sources. Especially preferred are film compositions for hard gelatine capsules prepared from fish gelatine.

A second embodiment of the invention is the use of the film composition for the manufacturing of hard gelatine capsules by conventional dip moulding processes.

The gelatine used for hard gelatine capsules is traditionally produced by extraction from collagen containing mammalian tissues, particularly such as pig skin and bovine bone. Gelatine from pig and bovine origin are preferably used for their gelling, film forming and surface-active properties. The manufacture of hard gelatine capsules by dip moulding process exploits fully its gelling and film forming abilities. Such capsules are manufactured by dipping mould pins into a hot solution of gelatine, removing the pins from the gelatine solution, allowing the gelatine solution attached on pins to set by cooling, drying and stripping the so-formed shells from the pins. The setting of the solution on the mould pins after dipping is the critical step to obtain a uniform thickness of the capsule shell.

Fish collagen is a further source of gelatine. However, it has long been known that gelatine derived from fish collagen lacks much of the gelling and setting ability of mammalian gelatines which limits the fish gelatine application. It is only applicable for products where a high viscosity of the solution without gel formation is desired, for example, in glue or food manufacturing. In the field of predosed pharmaceuticals, the fish gelatine can be used for micro-encapsulation (WO 9620612).

A. N. Fraga et al. describe in J. Polym. Mater. 5 (1988) 49-55 the mechanical properties from fish Gelatines as a brittle behaviour characteristic of a glassy material at normal temperatures. Such a brittleness is very undesired property for a gelatine capsule.

Norland Products Inc. describe in Research Disclosure 1987, 788 that water solutions of fish gelatine remain liquid down to 10°C, whereas water solutions of mammalian gelatine must be heated to temperatures over 30°C to remain liquid. This behaviour of fish gelatine will not allow the use in the conventional dip moulding process at conventional temperatures because of its too low gelling temperature.

B. Leuenberger describes in Food Hydrocolloids 1991, 353-361 viscosity and gelation properties of different mammalian and fish gelatines with the conclusion that fish gelatine may be useful in applications where high solution viscosity without gel formation is desired.

Surprisingly it has been found that fish gelatine can be used to produce gelatine compositions with conventional properties by adding a setting system to the aqueous fish gelatine solution.

The aim of the invention is therefore the provision of gelatine compositions for the use in pharmaceutical, veterinary, food, cosmetic or other products like films for wrapping food, aspics or jellies, preferably for predosed formulations like soft or hard gelatine capsules wherein the gelatine used is of non-bovine and non-pig origin and preferably derived from fish, poultry or plant sources, and wherein a setting system is added to the aqueous gelatine solution. Especially preferred are gelatine compositions for hard gelatine capsules prepared from fish gelatine.

Surprisingly this is achieved by the addition of a setting system and this allows the use of a wide range of gelatines from other sources than pigs or cattle for gelatine products for human consumption avoiding ethical and cultural problems.

The addition of a setting system to gelatine solutions with normally insufficient gelling behaviour, enables the adaptation of specific and desired gelling properties, especially for the production of hard gelatine capsules. For the production of such capsules it is extremely important that the film forming gelatine solution remaining on the mould pins after dipping is prohibited from flowing down the pins. Otherwise the obtained film will not have the desired uniform thickness.

exocellular polysaccharides. Preferred are exocellular polysaccharides.

The preferred exocellular polysaccharides are xanthan, acetan, gellan, welan, rhamsan, furcelleran, succinoglycan, scleroglycan, schizophyllan, tamarind gum, curdlan, pullulan, dextran.

The preferred hydrocolloids are kappa-carrageenan or gellan gum or combinations like xanthan with locust bean gum or xanthan with konjac mannan.

Among the setting systems mentioned above, the systems of kappa-carrageenan with cation and gellan gum with cation are specifically preferred. They produce high gel strength at low concentrations and have excellent compatibility with gelatine.

The amount of the hydrocolloid is preferably in the range of 0.01 to 5 % by weight and especially preferred 0.03 to 1.0 % in the aqueous gelatine solution.

The cations are preferably selected from , K^+, Na^+, Li^+, NH_4^+, Ca^{++} or Mg^{++}, for kappa-carrageenan are preferred K^+, NH_4^+ or Ca^{++}. The amount of cations is preferably lower than 3 %, especially 0.01 to 1 % by weight in the aqueous gelatine solution.

The preferred sequestering agents are ethylenediaminetetraacetic acid, acetic acid, boric acid, citric acid, edetic acid, gluconic acid, lactic acid, phosphoric acid, tartaric acid or salts thereof, methaphosphates, dihydroxyethylglycine, lecithin or beta

cyclodextrin and combinations thereof. Especially preferred is ethylenediaminetetraacetic acid or salts thereof or citric acid or salts thereof. The amount is preferably lower than 3 %, especially 0.01 to 1 % by weight of the dipping solution.

The gelatine capsules or films produced from the solutions as described will consequently contain by weight of 7 to 17 % of water, 83 to 93 % of gelatine, 0.01 to 10 %, preferably 0.05 to 5 % of hydrocolloids, less than 5 %, preferably 0.01 to 3 % of cations depending on the hydrocolloids used, and optionally less than 5 %, preferably 0.01 to 3 % of sequestering agents.

Capsules or films with the inventive gelatine compositions may be manufactured with conventional machines by the conventional processes like extrusion moulding, injection moulding, casting or dip moulding.

The inventive gelatine compositions may contain additionally acceptable plasticizers in an range from about 0 to 40 % based upon the weight of the gelatine. Suitable plasticizers are polyethylene glycol, glycerol, sorbitol, sucrose, corn syrup, fructose, dioctyl-sodium sulfosuccinate, triethyl citrate, tributyl citrate, 1,2-propyleneglycol, mono-, di- or triacetates of glycerol, natural gums or the like as well as mixtures thereof.

The inventive gelatine compositions may contain in a further aspect additionally pharmaceutically or food acceptable coloring agents in the range of from 0 to 10 % based upon the weight of the gelatine. The coloring agents may be selected from azo-, quinophthalone-, triphenylmethane-, xanthene- or

indigoid dyes, iron oxides or hydroxides, titanium dioxide or natural dyes or mixtures thereof. Examples are patent blue V, acid brilliant green BS, red 2G, azorubine, ponceau 4R, amaranth, D+C red 33, D+C red 22, D+C red 26, D+C red 28, D+C yellow 10, yellow 2 G, FD+C yellow 5, FD+C yellow 6, FD+C red 3, FD+C red 40, FD+C blue 1, FD+C blue 2, FD+C green 3, brilliant black BN, carbon black, iron oxide black, iron oxide red, iron oxide yellow, titanium dioxide, riboflavin, carotenes, anthocyanines, turmeric, cochineal extract, clorophyllin, canthaxanthin, caramel, or betanin.

The shaped final product from gelatine compositions of the invention may be coated with a suitable coating agent like cellulose acetate phthalate, polyvinyl acetate phthalate, methacrylic acid gelatines, hypromellose phthalate, hydroxypropylmethyl cellulose phthalate, hydroxyalkyl methyl cellulose phthalates or mixtures thereof to provide e.g. enteric properties.

The gelatine compositions of the invention may be used for the production of containers for providing unit dosage forms for example for agrochemicals, seeds, herbs, foodstuffs, dyestuffs, pharmaceuticals, flavouring agents and the like.

The inventive gelatine compositions may be useful for the encapsulation of caplets in a capsule, especially in a tamper-proof form. The encapsulation of a caplet in a capsule is preferred processed by cold shrinking together capsule parts, which are filled with a caplet, which comprises the steps providing empty capsule parts, filling at least one of said capsule parts with one or more caplets, putting said

capsule parts together, and treating the combined capsule parts by cold shrinking.

The inventive gelatine compositions are also useful for encapsulating and sealing the two capsule halves in a process in which one or more layers of the composition are applied over the seam of the cap and body, or by a liquid fusion process wherein the filled capsules are wetted with a hydroalcoholic solution that penetrates into the space where the cap overlaps the body, and then dried.

A specific embodiment of the instant invention is a hard gelatine capsule from fish gelatine filled with fish oil.

The improved properties of the gelatine compositions are demonstrated by the following examples:

Example 1

To 3.39 kg of deionised water is added 5 g of potassium acetate (0.10% by weight in the solution), followed by addition of 10 g kappa-carrageenan (0.20% by weight) under stirring at about 70°C. When a clear solution is obtained 1.60 kg of fish gelatine (32% by weight) are added at 60°C under slow stirring until the gelatine is completely dissolved and the solution is defoamed.

The fish gelatine solution thus prepared is then poured into a dipping dish of a pilot machine of conventional hard gelatine capsule production equipment. While keeping the temperature of dipping fish gelatine solution at about 50°C, natural transparent hard fish gelatine capsules of size 1 were produced according to the conventional process with the

same dimensional specifications to the conventional hard gelatine capsules.

Example 2

To 5 kg of fish gelatine solution at 60°C, prepared according to example 1, are added 32.6 g of titanium dioxide previously dispersed into a small quantity of water. After homogenising the solution, it is poured into the dipping dish, and white opaque hard fish gelatine capsules of size 1 were produced as in the example 1.

The capsules from both examples have excellent dissolution properties as demonstrated in Fig. 1, showing the percentage of acetaminophen dissolved from capsules immersed in deionised water at 37°C (USP XXIII) as a function of dissolution time.

Claims

1. Gelatine compositions consisting of a gelatine of non-bovine and non-pig origin and an additional setting system.

2. Gelatine compositions according to claim 1, wherein the gelatine is derived from fish, poultry or plants.

3. Gelatine compositions according to claim 1, wherein the gelatine is fish gelatine.

4. Gelatine compositions according to claim 1, wherein the setting system consists of hydrocolloids.

5. Gelatine compositions according to claim 1, wherein the setting system contains optionally cations and / or sequestering agents.

6. Gelatine compositions according to claim 1, wherein the gelatine is contained in an amount of 83 to 93 % by weight with a water content of 7 to 17 % by weight and the hydrocolloids are contained in an amount of 0.01 to 10 %, preferably 0.05 to 5 % by weight and cations in an amount of less than 5 %, preferably 0.01 to 3 % by weight.

7. Gelatine compositions according to claim 1, wherein the setting system contains optionally sequestering agents in an amount of less than 5 %, preferably 0.01 to 3 % by weight.

8. Gelatine compositions according to claim 1, wherein the hydrocolloids of the setting system are selected from polysaccharides.

9. Gelatine compositions according to claim 1, wherein the hydrocolloids of the setting system are selected from alginates, agar gum, guar gum, locust bean gum (carob), carrageenan, tara gum, gum arabic, ghatti gum, Khaya grandifolia gum, tragacanth gum, karaya gum, pectin, arabian (araban), xanthan, gellan, starch, Konjac mannan, galactomannan, or funoran.

10. Gelatine compositions according to claim 1, wherein the hydrocolloids of the setting system are selected from exocellular polysaccharides.

11. Gelatine compositions according to claim 1, wherein the hydrocolloids of the setting system are selected from xanthan, acetan, gellan, welan, rhamsan, furcelleran, succinoglycan, scleroglycan, schizophyllan, tamarind gum, curdlan, pullulan, or dextran.

12. Gelatine compositions according to claim 1, wherein the hydrocolloids of the setting system are selected from gellan gum or kappa-carrageenan.

13. Gelatine compositions according to claim 1, wherein the optional sequestering agent or mixture of sequestering agents of the setting system is selected from ethylenediaminetetraacetic acid, acetic acid, boric acid, citric acid, edetic acid, gluconic acid, lactic acid, phosphoric acid, tartaric acid or salts thereof, methaphosphates, dihydroxyethylglycine, lecithin or beta cyclodextrin.

14. Gelatine compositions according to claim 13, wherein the sequestering agent or mixture of sequestering agents is selected from ethylenediaminetetraacetic acid or salts thereof or citric acid or salts thereof.

15. Gelatine compositions according to claims 1 to 14 containing additionally plasticizers in an range from about 0 to 40 % based upon the weight of the gelatine.

16. Gelatine composition according to claim 15 wherein the plasticizer or mixture of plasticizers is selected from polyethylene glycol, glycerol, sorbitol, sucrose, corn syryp, fructose, dioctyl-sodium sulfosuccinate, triethyl citrate, tributyl citrate, 1,2-propyleneglycol, mono-, di- or triacetates of glycerol, or natural gums.

17. Gelatine compositions according to claims 1 to 16 containing additionally coloring agents in an range from about 0 to 10 % based upon the weight of the cellulose ether.

18. Gelatine compositions according to claim 17 wherein the coloring agent or mixture of coloring agents is selected from azo-, quinophthalone-, triphenylmethane-, xanthene- or indigoid dyes, iron oxides or hydroxides, titanium dioxide or natural dyes.

19. Gelatine compositions according to claim 17 wherein the coloring agent or mixture of coloring agents is selected from patent blue V, acid brilliant green BS, red 2G, azorubine, ponceau 4R, amaranth, D+C red 33, D+C red 22, D+C red 26, D+C red 28, D+C yellow 10, yellow 2 G, FD+C yellow 5, FD+C yellow 6, FD+C red 3, FD+C red 40, FD+C blue 1, FD+C blue 2, FD+C green 3, or brilliant black BN.

20. Gelatine compositions according to claim 17 wherein the coloring agent or mixture of coloring agents is selected from carbon black, iron oxide black, iron oxide red, iron oxide yellow, titanium dioxide, riboflavin, carotenes, anthocyanines, turmeric, cochineal extract, clorophyllin, canthaxanthin, caramel, or betanin.

21. Containers for unit dosage forms for agrochemicals, seeds, herbs, foodstuffs, dyestuffs, pharmaceuticals, or flavouring agents produced from the gelatine compositions according to claims 1 to 20.

22. Container according to claim 21 which is a pharmaceutical capsule.

23. Containers according to claims 21 or 22, characterized in that it has a coating.

24. Coated container according to claim 23 wherein the coating is selected from cellulose acetate phthalate, polyvinyl acetate phthalate, methacrylic acid gelatines, hypromellose phthalate, hydroxypropylmethyl cellulose phthalate hydroxyalkyl methyl cellulose phthalates or mixtures thereof.

25. Caplets encapsulated in Gelatine compositions according to claims 1 to 20.

26. Capsules according to claim 21 or 22 characterized in that the capsule halves are sealed with one or more layers of the gelatine composition according to claims 1 to 20.

27. Capsules according to claim 21 or 22 characterized in that the capsule halves are sealed by a liquid fusion process.

28. Capsules according to claim 21 or 22 containing products derived from fish, preferred fish oil.

29. Aqueous solutions of gelatine compositions according to claims 1 to 20 for the manufacturing of gelatine capsules.

30. Aqueous solutions according to claim 29, containing gelatine in an amount of 10 to 60 %, preferably 20 to 40 % by weight, hydrocolloids in an amount of 0.01 to 5 %, preferably 0.03 to 1.0 % by weight and cations in an amount less than 3 %, preferably 0.01 to 1 % by weight of the aqueous solution.

31. Aqueous solutions according to claim 29 or 30, containing optionally sequestering agents in an amount of less than 3 %, preferably 0.01 to 1 % by weight of the aqueous solution.

32. Use of aqueous gelatine solutions according to claims 29 to 31 for the manufacturing of hard gelatine capsules in a dip moulding process.

33. Manufacturing of hard gelatine capsules from aqueous gelatine solutions according to claims 29 to 31 in a dip moulding process with conventional hard gelatine capsules process parameters and equipment.

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(71) Applicant: **TOKAI CAPSULE KK**
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ITO MAKOTO
NAKAJIMA MITSUTERU
ENOMOTO ITSUMI

(54) SOFT CAPSULE FOR CHEWING

(57) Abstract:

PROBLEM TO BE SOLVED: To provide soft capsule for chewing that can be readily crunched into pieces in mouth with little sticking to teeth or cavity and shows excellent solubilization behavior.

SOLUTION: This soft capsule for chewing has the skin that is composed of the following components (A), (B) and (C): (A) gelatin, (B) totally 100-600 pts.wt., per 100 pts.wt. of gelatin, of one or two or more kinds of plasticizers selected from the components (b1)-(b3): (b1) glycerol, (b2) saccharides selected from D-sorbitol, sucrose, mannitol, fructose, sucrose alcohol and isomerized sugar, (b3) glycol selected from propylene glycol and polyethylene glycol, and (C) a water-soluble cellulose.

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(71) Applicant: **TOYO CAPSULE KK**
(72) Inventor: **ITOU YUKA**
TAKAHASHI MASAHITO
MOCHIZUKI HIROYUKI

(54) CARRIER SUITABLE FOR CHEWING ADMINISTRATION

(57) Abstract:

PURPOSE: To obtain a carrier, capable of being chewed and administered without causing an oily taste or touch and useful for a medicine, etc., sealed in a soft capsule.

CONSTITUTION: The objective carrier, useful for a medicine or a food material and suitable for chewing administration is characterized in that it is composed of 50-99 pts.wt. uniformly mixed edible oil and 1-20 pts.wt. surfactant having 1-20HLB.

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Fish Gelatin

Abstract

Gelatin is obtained by the hydrolysis of collagen which is the principal protein found in skin and bones. Fish gelatin is being extracted commercially in Nova Scotia by Kenney & Ross at Port Saxon. The raw material is the skin from deep water fish such as cod, haddock and pollock, and is obtained from local salt fish and frozen fish processors. The uniqueness of fish gelatin lies in the amino acid content of the gelatin. Although all gelatins are composed of the same 20 amino acids, there can be a variation in the amount of imino acids, proline and hydroxyproline. With lower amounts of these amino acids, there is less hydrogen bonding of gelatin in water solutions, and hence a reduction in the gelling temperature. Gelatin from cod skin gels at 10°C, whereas gelatin from carp skin would be more similar to animal gelatin, which gels above room temperature. Most people think of gelatin as a food additive or part of photographic film. With a lower gelling temperature, other commercial applications of fish gelatin have been developed. These applications are discussed.

Overview

The amount of information reported in the literature on fish gelatin is somewhat limited (Mees, 1966; Gustavson, 1956; Piez, 1965). Kenney & Ross has been producing fish gelatin since 1960, and this conference gives us the opportunity to share with you some of the information that we have developed. The product initially manufactured by us was marketed as a clarified fish glue, and essentially was a fish gelatin used in industrial application. We have been marketing an edible type of fish gelatin since 1981.

All gelatin is derived from collagen, the principal protein found in skin and bone. A simplified characterization of the applications of gelatin would be into the following four uses:

1. **Edible gelatin** - Free of heavy metals and aesthetically suitable for eating.
1. **Industrial gelatin** - Where the chemical and physical properties are uniquely suitable for an industrial application. A good example would be gelatin used for the microencapsulation of dye precursors for carbonless paper.
2. **Photographic gelatin** - The requirements being extremely critical. Photographic film requires a long shelf-life, and the gelatin has a major impact on the silver halide chemistry that requires the ability to take a picture and be able to develop it later with standard developing conditions.
3. **Glue** - Essentially for adhesive or gluing applications.

Fish gelatin, with the exception of photographic film, is used in all these applications. However, fish gelatin is used as the base for a light sensitive coating (or photoresist) for the electronics trade.

The precursor for gelatin is collagen. Collagen is the major structural protein found in the skin and bones of all animals. The collagen molecule consists of 3 individual polypeptide chains (alpha chains) which are wound around one another in a triple helix confirmation. This triple helix is stabilized by hydrogen bonds between collagen molecules, which happens as the animal ages. A collagen molecule of three alpha chains would measure 3000A° in length (0.3 microns) and 15A° in diameter. Each alpha chain has approximately 1050 amino acids connected together. There are twenty different amino acids in each alpha chain, and for each animal type of gelatin, these amino acids are in a specific repeated pattern. Glycine, which represents a third of the amino acids content, is in repeated sequence with two other amino acids. This might be represented as glycine-x-y. It is not unusual for x to be proline and y to be a hydroxyproline residue.

Table 1 compares the content of amino acids in fish gelatin with that of calf skin gelatin. We sell our gelatin as HiPure Liquid and Dry Gelatin. The amino acids are listed alphabetically together with their functionality. A common way of stating the content of amino acids is by indicating the number of residues per thousand amino acids. Note the comparison between fish gelatin and calf skin gelatin for the amino acids proline and hydroxyproline.

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Table 1

Comparative contents of amino acids in Hi-Pure liquid gelatin and animal gelatin.

Amino Acid	Function	Residues/1000 Amino Acids	
		HiPure Liquid Gelatin	Calf Skin Gelatin
Alanine	Non polar aliphatic	124	114
Arginine	Basic amino acid	55	51
Aspartic Acid	Dicarboxylic acid	37	45
Cysteine	Sulphur containing	--	--
Glutamic Acid	Dicarboxylic acid	77	71
Glycine	Non polar aliphatic	334	325
Histidine	Basic amino acid	9	5
Hydroxyproline	Imino acid	54	86
Isoleucine	Non polar aliphatic	10	11
Leucine	Non polar aliphatic	20	25
Lysine	Basic amino acid	35	34
Methionine	Sulphur containing	13	6
Phenylalanine	Aromatic	11	13
Proline	Imino Acid	106	135
Serine	Hydroxyl containing	65	37
Threonine	Hydroxyl containing	26	18
Tryptophan	Aromatic	--	--
Tyrosine	Hydroxyl containing	2	3
Valine	Non polar aliphatic	22	22

The role of proline and hydroxyproline in collagen is very critical. There appears to be a relationship between the temperature at which the animal (or fish) metabolizes and the properties of the skin and resultant extracted gelatin. Gelatin derived from the skin of deep cold water fish has lower amounts of proline and hydroxyproline, and as a result, water solutions will not gel at room temperature, but will remain liquid to 8 to 10°C.

Collagen in skin can be dissolved in dilute cold acid or salt solution, but with great difficulty and generally poor yields. This is because collagen molecules are covalently cross-linked into fibrils that may swell, but do not dissolve. If the skin and cold acid are heated, at a certain temperature the collagen molecule undergoes a helix coil transition. The helix coil literally unfolds, and the collagen becomes more readily soluble. The temperature at which this occurs depends upon the amount of proline and hydroxyproline in the alpha chain, and this temperature is the point of denaturing. For deep cold water fish collagen, this temperature is approximately 15°C. Bovine collagen is approximately 40°C. Another term, which covers the same effect in the skin itself, is the shrinkage t temperature. This is exactly what the term implies. At a certain temperature, the collagen in the raw skin will relax and the skin will shrink. The shrinkage temperature of the raw skin is higher than the denaturing temperature of the skin in acidified water, but the same relationship of proline and hydroxyproline to the shrinkage temperature still holds.

Table 2 shows a comparison of the amino acid composition of some fish collagens that have been published by Piez and Gross (1960). Glycine represents a third of the total amino acids residues. There are similarities in the composition, but what is important, is the total amount of proline and hydroxyproline, as well as the highest shrinkage temperature.

Table 2

Amino acid compositions and shrinkage temperatures of fish and calf gelatins.*

Amino Acid/ Shrinkage	Residues/1000 Amino Acids			
	Carp Skin Gelatin	Cod Skin Gelatin	Pike Skin Gelatin	Calf Skin Gelatin
Glycine	317	345	328	320
Alanine	120	107	114	112
Valine	19	19	18	20
Isoleucine	12	11	9.2	11
Leucine	25	23	20	25
Proline	124	102	129	138
Hydroxyproline	73	53	70	94
Phenylalanine	14	13	14	13
Tyrosine	3.2	3.5	1.8	2.6
Serine	43	69	41	36
Threonine	27	25	25	18
Methionine	12	13	12	4.3
Cystine	<1	<1	<1	<1
Hydroxylysine	4.5	6.0	7.9	7.4
Lysine	27	25	22	27
Histidine	4.5	7.5	7.4	5.0
Arginine	53	51	45	50
Aspartic Acid	47	52	54	45
Glutamic Acid	74	75	81	72
Shrinkage Temp (°C)	57	40	55	65

**Piez and Gross (1960)*

Commercial extraction of gelatin depends upon both dissolving and hydrolyzing the denatured skin. The gelatin may retain some covalent bonds between alpha chains, which would entail multiples of the single alpha chain length of 95,000 daltons. There are also major proportions of shorter chain polypeptide in the gelatin, as the chain is cleaved in the extraction process. This is not necessarily a problem, as the end product may not need very high molecular fractions in order to accomplish the specific application.

The molecular weight of four gelatins were compared using gel permeation chromatography and low angle light scattering (Berg and Frederick, private communication) in Fig. 1. The top curve is a standard gelatin, and underneath it is calf skin gelatin. The second curve is fish gelatin extracted with minimal hydrolysis. The lowest curve is commercial fish gelatin, which was hydrolyzed more to achieve certain viscosity characteristics. The animal gelatins and the low hydrolysis fish gelatin all have peaks at 190,000 and 95,000, characteristic of beta and alpha collagens respectively. About 10% of the wet weight of fish skin is Type I collagen. Fig. 2 is a comparison of molecular composition of calf skin collagen with fish skin collagen, done by using polyacrylamide gel electrophoresis (PAGE) according to the method of Laemmli (1970), whereby molecules of different molecular weights are separated using an electrical current. There are similarities between the two collagens. The fish collagen consisted primarily of alpha 1 and alpha 2 chains in a 2:1 ratio. Beta collagen would be two alpha collagens covalently linked together.

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Fig. 3 plots the viscosity of a 10% solution of animal gelatin vs fish gelatin with minimal hydrolysis and commercial fish gelatin. There are similarities between the gelatins, but note that the animal gelatin gels at 32°C, whereas the fish gelatin remains liquid down to 8°C.

Norland HiPure Liquid Gelatin is supplied at 45% solids in water and is pourable solution at 7000 to 8000cps, about the viscosity of honey. The gelatin is protected by a mixture of methyl and propyl hydroxybenzoates.

Fish gelatin has similar chemical reactivity to animal gelatin. Aldehydes such as formaldehyde, gluteraldehyde and glyoxal will cross-link and harden the gelatin under appropriate conditions. It can be reacted with anydrides under alkaline conditions, reducing or eliminating the effect of aldehydes as a hardening agent on the gelatin. Fish gelatin also provides a good medium for precipitating silver halide emulsions, as this can be done at lower temperatures than with animal gelatin.

Our factory is located on the southwestern tip of Nova Scotia, Canada, in an area rich in fishing grounds. Skins are obtained from frozen fish producers, as well as salt fish processors. Large quantities of skin are used in the plant, and 16,000 pounds of skins are handled daily.

I mentioned fish gelatin being used in a light sensitive coating. Our clarified fish glue was originally used as a base for a water soluble photoresist (Holahan, 1965). If you have a color television set there is a good possibility that the critical part of the television tube, the aperture mask, was made

using a photolithographic process with fish gelatin as the photoresist base. There are 400,000 holes or slots in the mask and the purpose of these holes is to delineate the color picture that is projected onto the phosphors on the inside of the television tube. The optics are so critical, that imperfections of a fraction of a micron can be visually seen on the screen, and would be a cause for rejection. These masks are made in a continuous process whereby thin metal is unwound from a role from one end of the equipment, and completed masks are stripped from the sheet at the other end 1,000 feet away.

Fish gelatin is also used in the manufacture of lead frames that hold the silicon chip in computers and microprocessors (American Machinist, 1971). The chip is mounted on a pad at the center portion of the lead frame, and each circuit on the chip is connected to a lead that surrounds it. The l lead frame are made using a photochemical machining process and a fish gelatin photoresist.

A necessary part of the optics of a color video camera is a color stripped filter that separates the color signals for the electronics of the camera. Each filter has a series of 3 different color stripes, 12 microns wide, put down on glass in a repeat pattern. The total size of the filter is approximately 1/2" x 3/4". The stripes are put on the glass using a photolithographic process in the fish gelatin.

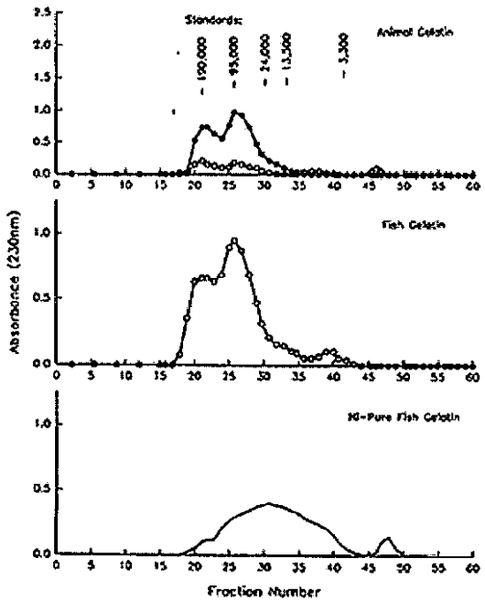

Fig. 1. Comparison of the column elution distributions based on the molecular weights of animal, fish and Hi-Pure fish gelatins.

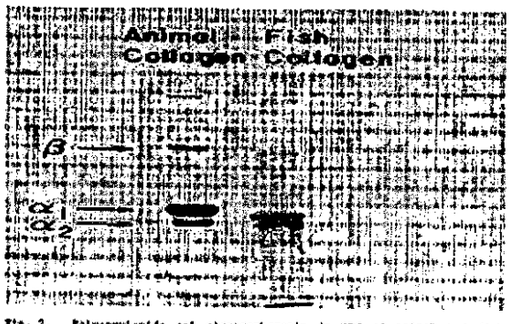

Fig. 2. Polyacrylamide gel electrophoresis in SDS of animal and fish collagens.

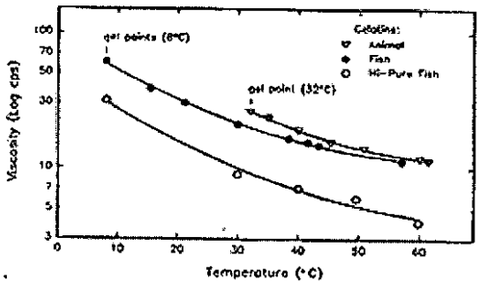

Fig. 3. Effect of temperature on the viscosities of 10% solutions of animal, fish and Hi-Pure fish gelatins.

Summary

1) Gelatin is made from collagen, which is part of the skin and bones of animals and fish.

2) All gelatins have same 20 different amino acids, in slightly different proportions for different species.

3) The amount of the imino amino acids, proline and hydroxyproline, determines the shrinkage temperature and the denaturing temperature, (The temperature at which the collagen helix

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unwinds), and as a result, the temperature at which solutions of the extracted gelatins gels.

4) Gelatin from cold deep water fish such as cod, haddock, pollock, hake and cusk, gels at 8 to 10°C compared to calf skin gelatin which gels at 30 to 35°C.

5) Fish gelatin is used in a variety of coating applications, the largest of which is a base for a water soluble photoresist.

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Review

Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins

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ABSTRACT

Food and pharmaceutical industries all over the world are witnessing an increasing demand for collagen and gelatin. Mammalian gelatins (porcine and bovine), being the most popular and widely used, are subject to major constraints and skepticism among consumers due to socio-cultural and health-related concerns. Fish gelatin (especially from warm-water fish) reportedly possesses similar characteristics to porcine gelatin and may thus be considered as an alternative to mammalian gelatin for use in food products. Production and utilization of fish gelatin not only satisfies the needs of consumers, but also serves as a means to utilize some of the byproducts of the fishing industry. This review focuses on the unique features, advantages, constraints, and challenges involved in the production and utilization of fish gelatin in order to provide a comprehensive look and deeper insight on this important food ingredient, as well as prospects for its future commercial exploitation and directions for future studies.

Contents

1. Introduction

Gelatin, one of the most popular biopolymers, is widely used in food, pharmaceutical, cosmetic, and photographic applications because of its unique functional and technological properties. In the food industry, gelatin is utilized in confections (mainly for providing chewiness, texture, and foam stabilization), low-fat spreads (to provide creaminess, fat reduction, and mouthfeel), dairy (to provide stabilization and texturization), baked goods (to provide emulsification, gelling, and stabilization), and meat

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products (to provide water-binding) (Johnston-Banks, 1990; Schrieber & Gareis, 2007). In the pharmaceutical and medical fields, gelatin is used as a matrix for implants, in injectable drug delivery microspheres, and in intravenous infusions (Pollack, 1990; Rao, 1995; Saddler & Horsey, 1987). There are also reports in which live attenuated viral vaccines used for immunization against measles, mumps, rubella, Japanese encephalitis, rabies, diphtheria, and tetanus toxin contain gelatin as a stabilizer (Burke, Hsu, & Volkin, 1999). In the pharmaceutical industry, gelatin is also widely used for the manufacture of hard and soft capsules, plasma expanders, and in wound care. Gelatin, being low in calories, is normally recommended for use in foodstuffs to enhance protein levels, and is especially useful in body-building foods. In addition, gelatin is also used to reduce carbohydrate levels in foods formulated for diabetic patients.

The global demand for gelatin has been increasing over the years. Recent reports indicate that the annual world output of gelatin is nearly 326,000 tons, with pig skin-derived gelatin accounting for the highest (46%) output, followed by bovine hides (29.4%), bones (23.1%), and other sources (1.5%) (GME, 2008). However, although gelatin has such a wide range of useful applications, pessimism and strong concerns still persist among consumers with regard to its usage (Asher, 1999). This is mainly due to religious sentiments (both Judaism and Islam forbid the consumption of any pork-related products, while Hindus do not consume cow-related products) as well as the enhanced and stricter adherence to vegetarianism throughout the world. In addition, there is increasing concern among researchers about whether animal tissue-derived collagens and gelatins are capable of transmitting pathogenic vectors such as prions (Wilesmith, Ryan, & Atkinson, 1991). However, studies conducted by various authorities have shown that the production process of gelatin is an effective barrier against possible BSE prions. For example, in March 2003, the Scientific Steering Committee of the European Union confirmed, "The risk associated with bovine bone gelatin is close to zero" (Schrieber & Gareis, 2007).

Although porcine gelatin accounts for the highest levels of production, a significant amount of gelatin used in the food and pharmaceutical industries is also derived from cows. The BSE episode, as well as religious concerns, has led to intensive research, especially in Europe, to identify and develop alternatives to mammal-derived gelatin. Furthermore, strong competition exists among manufacturers for the procurement of pigskin or other mammalian sources, which has created increased demand and raised costs. To date, however, few alternatives are available, and as a result it has not been possible to eliminate gelatin. Researchers from academia and industry are continually searching for an alternative to gelatin, and to find new sources of gelatin that might be more favorably viewed.

Within the past decade there has been intense interest in the market in gelatin derived from fish and poultry. Poultry skin and bones are expected to yield gelatin in the near future, but commercial production is currently limited by low yields. The skin obtained from poultry is also a coveted raw material for other food applications (Schrieber & Gareis, 2007). In this regard, fish gelatin has been highlighted as a better alternative to mammalian gelatins, particularly with qualities such as a lower melting point, resulting in faster dissolution in the mouth with no residual 'chewy' mouthfeel. However, the production of fish gelatin is still in its infancy, contributing only about 1% of the annual world gelatin production (Arnesen & Gildberg, 2006).

Fish catch is mainly used for human consumption and other minor uses, such as meal production and bait. Fish used as human food accounts for 78%, of the total fish catch in developed and developing countries, leaving about 21% for non-food uses (Vannuccini, 2004). Processing leads to the generation of a large biomass of fish waste (e.g., skin, bones, and fins), which is generally discarded (~7.3 million tons/year) (Kelleher, 2005). Consequently, research has been initiated to investigate an increased utilization of collagenous fish waste for the production of gelatin (Gilsenan & Ross Murphy, 1999; Holzer, 1996; Nagai & Suzuki, 2000; Wasswa, Tang, & Gu, 2007).

Although fish gelatin has been studied since the 1950s, most studies on gelatin refer to mammalian gelatin, and only in recent years have more intensive studies been carried out on fish gelatin and begun appearing in the literature. The increased research efforts might be directly correlated with the outbreak of BSE in Europe. This review is therefore aimed at providing more insight towards the possible exploration of fish gelatin as an alternative to mammalian gelatins, with more emphasis on extraction methods and physicochemical, functional, and sensory properties. In addition, challenges to and prospects for enhancing industrial utilization of fish gelatin, as well as future research directions, are also discussed.

2. Collagen and gelatin

Not a naturally occurring protein, gelatin is derived from the fibrous protein collagen, which is the principal constituent of animal skin, bone, and connective tissue. Gelatin is produced via the partial hydrolysis of native collagen. During the manufacturing of gelatin, raw animal material is treated with dilute acid or alkali, resulting in partial cleavage of the crosslinks: the structure is broken down to such an extent that "warm-water-soluble collagen", i.e. gelatin, is formed (Schrieber & Gareis, 2007).

To date, some 27 different types of collagen have been identified, and a simple classification is shown in Table 1 (Schrieber & Gareis, 2007). Collagen molecules, composed of three α-chains intertwined in the so-called collagen triple-helix, adopt a 3D structure that provides an ideal geometry for inter-chain hydrogen bonding (Te Nijenhuis, 1997). Each chain in the helix rotates counterclockwise. The triple-helix is approximately 300 nm in length, and the chain has a molecular weight of approximately 10^5 kDa (Papon, Leblon, & Meijer, 2007). The triple helices are stabilized by the aforementioned inter-chain hydrogen bonds. Collagen denaturation causes separation of the rods and total or partial separation of the chains due to destruction of the hydrogen bonds, causing loss of the triple-helix conformation, and following denaturation, the polymers exist in a coiled form. Industrial gelatins are mixtures of different compounds: α-chains (one polymer chain), β-chains (two α-chains covalently crosslinked), and γ-chains (three covalently crosslinked α-chains) (Papon et al., 2007).

The composition of collagen encompasses all 20 amino acids (Schrieber & Gareis, 2007). Although some differences in amino acid composition are apparent across collagens derived from different sources, there are certain features that are common to and uniquely characteristic of all collagens. It is the only mammalian protein to contain large amounts of hydroxyproline and hydroxylysine, and the total imino acid (proline and hydroxyproline) content is high (Balian & Bowes, 1977). The amino acid composition of gelatin is very close to that of its parent collagen, and is characterized by a repeating sequence of Gly-X-Y triplets, where X is

Table 1
Classification of collagen (Schrieber & Gareis, 2007)

Type	Description
Type I	This type occurs widely, primarily in connective tissue such as skin, bone, and tendons.
Type II	This type of collagen occurs practically exclusively in cartilage tissue.
Type III	This type strongly dependent on age: very young skin can contain up to 50%, but in the course of time this is reduced to 5–10%.
Other types	The other types of collagen are present in very low amounts only and mostly organ-specific.

mostly proline and Y is mostly hydroxyproline (Eastoe & Leach, 1977). Table 2 shows the typical amino acid composition of porcine gelatin.

3. Fish gelatin

Gelatin from marine sources (warm- and cold-water fish skins, bones, and fins) is a possible alternative to bovine gelatin (Kim & Mendis, 2006; Rustad, 2003; Wasswa et al., 2007). One major advantage of marine gelatin sources is that they are not associated with the risk of outbreaks of Bovine Spongiform Encephalopathy. Fish gelatin is acceptable for Islam, and can be used with minimal restrictions in Judaism and Hinduism. Furthermore, fish skin, which is a major byproduct of the fish-processing industry, causing waste and pollution, could provide a valuable source of gelatin (Badii & Howell, 2006). Fish skin contains a large amount of collagen: Nagai and Suzuki (2000) reported that the collagen contents in the fish skin waste of Japanese sea-bass, chub mackerel, and bullhead shark were 51.4%, 49.8%, and 50.1% (dry basis), respectively.

Production of fish gelatin is actually not new as it has been produced since 1960 by acid extraction, although most of it has been used for industrial applications (Norland, 1990). Detailed extraction procedures and characterization of the properties of fish gelatin were described by Grossman and Bergman (1992) in a United States patent. Since then, multiple research groups have further investigated the various aspects of fish gelatin. Gelatin has been extracted from skins and bones of various cold-water (e.g., cod, hake, Alaska pollock, and salmon) and warm-water (e.g., tuna, catfish, tilapia, Nile perch, shark, and megrim) fish. Table 3 lists the various reports currently available in the literature on the extraction and characterization of fish gelatin.

3.1. Extraction of fish gelatin

Conversion of collagen into soluble gelatin can be achieved by heating the collagen in either acid or alkali. Thermal solubilization of collagen (in the presence of acid or alkali) is due to the cleavage of a number of intra- and intermolecular covalent crosslinks that are present in collagen. In addition, some amide bonds in the elementary chains of collagen molecules undergo hydrolysis (Bailey & Light, 1989). The extraction process can influence the length of the polypeptide chains and the functional properties of the gelatin. This depends on the processing parameters (temperature, time, and pH), the pretreatment, and the properties and preservation method of the starting raw material.

All gelatin manufacturing processes consist of three main stages: pretreatment of the raw material, extraction of the gelatin, and purification and drying. Furthermore, manufactured gelatin is often blended to produce trade-quality gelatin, with specific properties for specific applications (de Wolf, 2003). Depending on the method in which the collagens are pretreated, two different types of gelatin (each with differing characteristics) can be produced. Type A gelatin (isoelectric point at pH 6–9) is produced from acid-treated collagen, and type B gelatin (isoelectric point at approximately pH 5) is produced from alkali-treated collagen (Stainsby, 1987). Acidic treatment is most suitable for the less covalently crosslinked collagens found in pig or fish skins, while alkaline treatment is suitable for the more complex collagens found in bovine hides.

Fish gelatin has been extracted using a number of different methods, and these are summarized in Table 4. The direct procedures used for preparing fish gelatin typically involve a mild chemical pretreatment of the raw material and mild temperature conditions during the extraction process. In general, a mild acid pretreatment of the fish skin is used prior to gelatin extraction.

Gómez-Guillén and Montero (2001) previously reported a procedure for extracting gelatin with high gelling capacity from fish skins: the procedure was essentially based on a mild acid pretreatment for collagen swelling, followed by extraction in water at moderate temperatures (45 °C). The entire process takes about 24 h. Because of the acid lability of the crosslinks found in fish skin collagen, mild acid treatment is sufficient to produce adequate swelling and to disrupt the non-covalent intra- and intermolecular bonds (Montero, Borderías, Turnay, & Lizarbe, 1990; Norland, 1990; Stainsby, 1987). Subsequent thermal treatment above 40 °C (well

Table 2
Amino acid content in some fish gelatins compared to pork gelatin (residues/1000 total amino acid residues)

Amino acid	Cod skin[a]	Alaska pollock skin[b]	Hake[a]	Megrim[a]	Tilapia skin[c]	Pork skin[d]
Ala	96	108	119	123	123	112
Arg	56	51	54	54	47	49
Asx	52	51	49	48	48	46
Cys	0	0	–	–	0	0
Glx	78	74	74	72	69	72
Gly	344	358	331	350	347	330
His	8	8	10	8	6	4
Hyl	6	6	5	5	8	6
Hyp	50	55	59	60	79	91
Ile	11	11	9	8	8	10
Leu	22	20	23	21	23	24
Lys	29	26	28	27	25	27
Met	17	16	15	13	9	4
Phe	16	12	15	14	13	14
Pro	106	95	114	115	119	132
Ser	64	63	49	41	35	35
Thr	25	25	22	20	24	18
Trp	0	0	–	–	0	0
Tyr	3	3	4	3	2	3
Val	18	18	19	18	15	26
Imino acid	156	150	173	175	198	223

Cod, Alaska pollock and Hake are examples of cold-water fish whereas Megrim and Tilapia are examples of warm-water fish.
[a] Gómez-Guillén et al. (2002).
[b] Zhou et al. (2006).
[c] Sarabia et al. (2000).
[d] Eastoe and Leach (1977).

Table 3
Examples of literature on extraction and characterization of fish gelatin

Variety of fish	Reference
Baltic cod (*Gadus morhua*), salmon (*Salmo salar*), herrings (*Clupea harengus*)	Kołodziejska, Skierka, Sadowska, Kołodziejska, and Niecikowska (2008)
Flounder (*Platichthys flesus*)	Fernández-Díaz et al. (2003)
Alaska pollock (*Theragra chalcogramma*)	Zhou and Regenstein (2005)
Megrim (*Lepidorhombus boscii*) (Risso), Hake (*Merluccius merluccius*), Dover sole (*Solea vulgaris*)	Fernández-Díaz et al. (2001), Gómez-Guillén et al. (2002), Sarabia et al. (2000)
Horse mackerel (*Trachurus trachurus*)	Badii and Howell (2006)
Cod (*Gadus morhua*)	Gudmundsson and Hafsteinsson (1997), Fernández-Díaz et al. (2001)
Catfish (*Ictalurus punctatus*)	Yang et al. (2007), Liu et al. (2008)
Sin croaker (*Johnius dussumieri*), shortfin scad (*Decapterus macrosoma*)	Cheow, Norizah, Kyaw, and Howell (2007)
Black tilapia (*Oreochromis mossambicus*), red tilapia (*Oreochromis nilotica*)	Jamilah and Harvinder (2002)
Bigeye snapper (*Priacanthus macracanthus*), brownstripe red snapper (*Lutjanus vitta*)	Jongjareonrak et al. (2006a)
Yellowfin tuna (*Thunnus albacares*)	Chiou et al. (2006)
Blue shark (*Prionace glauca*)	Yoshimura et al. (2000)
Nile perch (*Lates niloticus*)	Muyonga et al. (2004)
Grass carp (*Ctenopharyngodon idella*)	Kasankala et al. (2007)
Skate (*Raja kenojei*)	Cho, Jahncke, Chin and Eun (2006)
Atlantic salmon (*Salmo salar*)	Arnesen and Gildberg (2007)
Dover sole (*Solea vulgaris*)	Gómez-Guillén, Giménez, and Montero (2005), Giménez et al. (2005a)
Harp seal	Arnesen and Gildberg (2007)

Table 4
Procedures employed to extract fish gelatin

Fish type	Pretreatment	Extraction procedure	Reference
Megrim (*Lepidorhombus boscii*)	Pre-treatment of the skins with NaCl and dilute NaOH, followed by swelling with 0.05 M acetic acid	Extracted in water at 45 °C	Montero and Gómez-Guillén (2000).
Megrim (*Lepidorhombus boscii*)	Extractions with 0.05 M of each acid were also done after a pretreatment with 0.2 N NaOH (1:6 w/v) at 5 °C (30 min)	Cleaned skins were stirred for 16–18 h at 20 °C, with different solutions of 0.05 M, 0.1 M, and 0.5 M of acids viz., (1:20 w/v): formic, acetic, propionic, lactic, malic, tartaric, and citric acid.	Gómez-Guillén and Montero (2001)
Black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*)		Fish skins rinsed with excess water followed by soaking in 0.2% (w/v) NaOH solution (40 min). This was followed by soaking in 0.2% H_2SO_4 acid and 1.0% citric acid. Rinsing with distilled water was carried out between soakings followed by final extraction in distilled water at 45 °C (12 h)	Jamilah and Harvinder (2002)
Megrim (*Lepidorhombus boscii*)	Pre-stored at −20 °C until use	Extraction by acid treatment	Montero et al. (2002)
Flounder (*Platichthys flesus*)	Part of the samples frozen at −12 and −20 °C for 15 days before use	Gelatins obtained with mild acid treatment, and subsequent extraction in water at temperatures below 50 °C	Fernández-Díaz et al. (2003)
Nile perch (*Lates niloticus*)	Skins were pretreated by acidulation with 0.01 M H_2SO_4 solution (pH of 2.5–3.0). For bones, de-mineralised using 3% HCl	Extracted with warm-water at 60 °C	Muyonga et al. (2004)
Baltic cod (*Gadus morhua*)	Samples washed with NaCl solution (with the exception of salted and marinated herring skins) and water, followed by gentle stirring with water (1:6, w/v) for 15–120 min at 45, 70 or 100 °C. The samples were centrifuged at 10,000 *g* for 30 min at 15 °C	Extracted at 45 °C in water	Kołodziejska et al. (2004)
Pollock skins (Alaskan pollock)	Cleaned skins were treated with $Ca(OH)_2$ (1:6 w/v) at varying OH-concentrations for varying times + drained and rinsed with tap water (twice). Samples were then treated with acetic acid (1:6 w/v) with varying H^+ concentrations (45 min) + drained and rinsed with tap water.	Pretreated samples were mixed with distilled water and extracted at varying temperatures for varying times	Zhou and Regenstein (2004)
Dover sole (*Solea vulgaris*)	Frozen at 20 °C until use. High pressure applied at 250 and 400 MPa, for 10 or 20 min	Mild acid (50 mM acetic acid) swelling step for 3 h and subsequent overnight (16–18 h) gelatin extraction in distilled water at moderate temperature (45 °C).	Gómez-Guillén et al. (2005)
Alaska pollock skin	Pretreatments with NaOH or $Ca(OH)_2$ (1:6 w/v) with varying OH concentrations (0.01, 0.1, 0.2, and 0.5 mol/L) for 60 min, or treated with acetic acid (1:6 w/v) with H concentration at 0.05 mol/L for 60 min.	Direct extraction with distilled water. In some other cases, extracted directly with distilled water at 50 °C for 180 min in the absence or presence of a mixture of protease inhibitors, consisting of 5 mM EDTA disodium salt, 0.2 mM phenylmethanesulfonyl fluoride, and 2 μM pepstatin	Zhou and Regenstein (2005)
Yellowfin tuna (*Thunnus albacares*)	Washed, chopped and frozen at −15 °C until used. Further treated with 8 volumes (v/w) of alkali solution (1–3% NaOH) at 10 °C with shaking (200 rpm for 1–5 days to remove the non-collagen protein and subcutaneous tissue after they were swollen neutralized with 6 N HCl	Hot water extraction, six volumes (v/w) of distilled water was added and heated at temperature ranging 40–80 °C for 1–9 h. The extracted solution was centrifuged for 30 min (900 × g) at 30 °C	Cho et al. (2005)
Dover sole (*Solea vulgaris*)	Fish skins were either (a) frozen, (b) treated with absolute ethanol or a 80–20 absolute ethanol–glycerol mixture and dried, or (c) dried using marine salt	Mild acid swelling step in 0.05 M acetic acid and subsequent gelatin extraction in distilled water at 45 °C overnight	Giménez et al. (2005a)
Dover sole (*Solea vulgaris*)	Fish skins were washed with different salt solutions (5 °C, 2 min), followed by rinsing with abundant tap water. The salt solutions tested were NaCl, KCl, $MgCl_2$ and $MgSO_4$ (1:6 w/v), at a concentration of 0.8 M.	Same as above	Giménez et al. (2005b)
Dover sole (*S. vulgaris*)	Mild acid swelling step in 50 mM acetic acid. Lactic acid (25 mM) was also used.	Subsequent gelatin extraction in distilled water at 45 °C overnight	Giménez et al. (2005b)
Atlantic cod (*Gadus morhua*)		Extraction at room temperature at room temperature in dilute NaOH (pH 11) and HCl (pH 2–2.6).	Arnesen and Gildberg (2006)
Bigeye snapper (*Priacanthus macracanthus*) and brownstripe red snapper (*Lutjanus vitta*)		Skins soaked in 0.2 M NaOH (1:10, w/v) at 4 °C with gentle stirring. Skins were further washed with tap water until neutral basic pH and soaked in 0.05 M acetic acid with a skin/solution ratio of 1:10 (w/v) for 3 h at room temperature (25 °C) with gentle stirring to swell the collagenous material in fish skin matrix. Acid-treated skins were washed and swollen fish skins were soaked in distilled water with a skin/water ratio of 1:10 (w/v) at 45 °C (12 h) with a continuous stirring to extract the gelatin	Jongjareonrak et al. (2006a, 2006b, 2006c)

Table 4 (*continued*)

Fish type	Pretreatment	Extraction procedure	Reference
Grass carp (*Ctenopharyngodon Idella*)	Thawed skins were drained by cheesecloth and treated with 0.1–3.0% HCl and incubated at 7 °C. Further, skin samples were washed for removal of excessive chemicals before extraction.	Extraction was done at 40–80 °C in a shaking hot water bath (180 rpm)	Kasankala et al. (2007)
Atlantic salmon (*Salmo salar*) and Atlantic cod	Skin frozen at 20 °C	Gelatin prepared by acid extraction method. Skin washed in cold-water (about 8 °C) + incubated (twice) in cold NaOH solutions (0.04 N) (30 min) followed by acid incubations (twice) (30 min), in H_2SO_4 (0.12 M) and then in citric acid solution (0.005 M). After the final washing in cold-water, a two-step gelatin extraction (each step 2 h) was performed by gentle stirring, first in 1 L water at 56 °C and then in 1 L at 65 °C	Arnesen and Gildberg (2007)
Sin croaker (*Johnius dussumieri*) and shortfin scad (*Decapterus macrosoma*)	Samples stored at −20 °C until used	Rinsing and washing with water followed by alakali and acid treatments (method of Gudmundsson and Hafsteinsson (1997).	Cheow et al. (2007)
Channel catfish (*Ictalurus punctatus*)	Alkaline solution followed by an acid solution (NaOH and acetic acid)	Cleaned skins (30 g) treated with NaOH (1:6 w/v) for variable time. Then, samples drained using cheesecloth and rinsed with tap water (related two times). Afterwards the samples were treated with acetic acid (1:6 w/v) for variable times, followed by draining using cheesecloth and rinsed with tap water (1:6 w/v) (three times) (samples maintained at 4 °C). After the above pretreatment, ion-free water was added to the flasks and samples were extracted in a water bath for variable times.	Yang et al. (2007)
Nile tilapia (*Oreochromis niloticus*)		Skin-extracted gelatin in either acetic acid or formic acid aqueous solutions	Songchotikunpan, Tattiyakul, and Supaphol (2008)
Yellowfin tuna (*Thunnus albacares*)	Frozen skin thawed at room temperature for 1 h	Skin washed in running water + dipped in 0.5 M NaCl (5 min, 5 °C), followed by washing in tap water (three times) + treating with 0.1 N NaCl. The solution was further washed (three times) with distilled water and placed in 0.1 N acetic acid solution for heating and stirring at 50 °C on a hot plate for 18 h.	Rahman et al. (2008)
Baltic cod (*Gadus morhua*), skins of fresh and cold-smoked salmon (*Salmo salar*)	Partially frozen raw material was minced in a meat grinder with 3-mm diameter mesh, mixed thoroughly, and stored at −20 °C until use (washed with NaCl)	Extracted with water	Kołodziejska et al. (2008)
Bigeye snapper (*Priacanthus tayenus*)		Extraction by pepsin-aided process	Nalinanon et al. (2008)
Channel catfish (*Ictalurus punctatus*)	The channel catfish skin was soaked in eight volumes (v/w) of 50-mmol/L acetic acid at 15 °C (18 h.). The treated skin was further washed with distilled water until the pH of solution reached 3.5–4.0.	The gelatin extraction was carried out in distilled water in a water bath at 45 °C for 7 h.	Liu et al. (2008)

above helix-to-coil transition temperatures for fish gelatins) destroys hydrogen bonding and cleaves a number of covalent bonds, which destabilizes the triple-helix via a helix-to-coil transition and results in conversion to soluble gelatin (Djabourov, Lechaire, & Gaill, 1993). High-molecular weight polymers may occur in the resulting gelatin through the possible persistence of crosslinks, depending on the nature and degree of solubilization.

Kołodziejska, Kaczorowski, Piotrowska, and Sadowska (2004) showed that it is possible to omit the chemical treatment and to shorten the extraction time from skins of cold-water fish from 12 h to 30 min, but minced raw material must be used instead of whole skins. Because of the structural characteristics of collagen, fish skins are difficult to mince in a meat grinder. However, they can be comminuted easily after treatment with diluted acetic acid (1:6) at temperatures below 15 °C for 2 h (Sadowska, Kołodziejska, & Niecikowska, 2003).

Prior to extraction, the methods for preservation of the raw material have been found to affect some of the physical properties of fish gelatin. Fernández-Díaz, Montero, and Gómez-Guillén (2003) reported that gelatin from skins frozen at −12 °C had lower gel strength values compared to both fresh skins and skins frozen at −20 °C. In a study by Liu, Li, and Guo (2008), they looked at the properties of gelatins that were extracted (using 50 mmol/L acetic acid) from channel catfish skins preserved using different methods. They noted that compared to gelatins from fresh and frozen skins, gelatin from dried channel catfish skin exhibited higher gel strength, and they attributed this to the large α-chain content of gelatin from the dried skins. They also observed that the gelling and melting points of dried channel catfish skin gelatin solution were similar to those of fresh skin gelatin solution, but distinctly different from those of frozen skin gelatin.

On average, the extraction yield of fish gelatin is lower than mammalian gelatin, giving approximately between 6% and 19% (expressed as grams of dry gelatin per 100 g of clean skin). The lower extraction yield of fish gelatin could be due to the loss of extracted collagen through leaching during the series of washing steps or due to incomplete hydrolysis of the collagen (Jamilah & Harvinder, 2002). In addition, it has been reported that some heat-stable proteases endogenous to the skin are involved in the degradation of gelatin molecules (specifically the β- and α-chains) during the extraction process at elevated temperatures, which contribute to the low Bloom strength (Intarasirisawat et al., 2007). Thus, Nalinanon, Benjakul, Visessanguan, and Kishimura (2008) envisaged that the addition of an appropriate protease inhibitor together with the pepsin-aided process might be an effective means to obtain a higher yield with negligible hydrolysis of the peptides. Indeed, they showed that for extraction of gelatin from the bigeye snapper, the pepsin-aided process in combination with

a protease inhibitor (pepstatin A) markedly increased the yield from 22.2% to 40.3% (yield was calculated based on the hydroxyproline content of the gelatin in comparison with that of the skin prior to extraction).

Rahman, Al-Saidi, and Guizani (2008) have also reported a higher yield (18%) of gelatin from yellowfin tuna skin. The yield and quality of gelatin are not only influenced by the species or tissue from which it is extracted, but also by the extraction process itself (Montero & Gómez-Guillén, 2000). This was further examined in work by Zhou and Regenstein (2004), where they studied the optimization of extraction conditions for pollock skin gelatin. The observed yields for pollock skin gelatin in their studies varied from 3% to 19%, and were most sensitive to the pretreatment temperature and the concentration of H^+. The pretreatment at room temperature led to a high loss of gelatin, although it may have slightly increased the viscosity. They suggested that a low pretreatment temperature should be used during pollock skin gelatin extraction, and this result may possibly be applicable to other cold-water fish.

In comparison, Gudmundsson and Hafsteinsson (1997), using a prolonged extraction of whole cod skins, achieved a yield of gelatin between 11% and 14%, depending on the concentrations of the sodium hydroxide, sulfuric acid, and citric acid solutions used in the preliminary treatment of raw material. Gómez-Guillén, Fernaqndez-Diaz, Ulmo, Lizarbe, and Montero (2002) reported that the yield of extractions varied slightly among the fish species (sole: 8.3%; megrim: 7.4%; cod: 7.2%; hake: 6.5%). They also observed that squid skin requires higher extraction temperatures (80 °C), but even under these conditions, the yield was only 2.6%, lower than yields from fish skin extracted using a milder procedure.

In the cases of other species, the extraction yield of gelatin from skins ranged from about 5.5% to 21% of the starting weight of the raw material (Giménez, Gómez-Guillén, & Montero, 2005a; Giménez, Turnay, Lizarbe, Montero, & Gómez-Guillén, 2005b; Grossman & Bergman, 1992; Jamilah & Harvinder, 2002; Muyonga et al., 2004; Songchotikunpan et al. 2008). The variation in such values depends on the differences in both the proximate composition of the skins and the amount of soluble components in the skins (Muyonga et al., 2004), as these properties vary with the species and the age of the fish. In addition, variation in the extraction method can also have an effect on yields. The wide range in gelatin yields could also be attributed to differences in collagen content of the raw material; however, this information is often not available in published data (Songchotikunpan et al., 2008). Reporting gelatin yield as dry gelatin weight compared to the weight of wet skin is common, but not very reliable. Water content may vary because of different treatments to the skin (freezing, salting, scraping, draining, etc.). Therefore, gelatin yield should be reported as the amount of dry gelatin compared to the amount of dry matter in skin (Arnesen & Gildberg, 2007).

3.2. Chemical, physicochemical, and functional properties of fish gelatin

For food applications, the most important properties characterizing gelatin are gel strength, viscosity, gelling, and melting points. These properties are affected by many factors, such as the average molecular weight and molecular weight distribution, concentration of the gelatin solution, gel maturation time, gel maturation temperature, pH, and salt content. Some studies on the food properties of fish gelatin have been conducted (Choi & Regenstein, 2000; Norland, 1990; Osborne, Voight, & Hall, 1990). For example, Leuenberger (1991) directly compared fish and porcine gelatins. Furthermore, physicochemical and functional properties of fish gelatin have been studied extensively, especially with respect to its rheological (Badii & Howell, 2006; Gilsenan & Ross-Murphy, 2000a; Gudmundsson, 2002; Haug, Draget, & Smidsrod, 2004a; Jamilah & Harvinder, 2002; Muyonga et al., 2004; Zhou & Regenstein, 2007) properties as well as its emulsifying, foaming (Dickinson & Lopez, 2001; Surh et al., 2006), film-forming (Avena-Bustillos et al., 2006; Jongjareonrak, Benjakul, Visessanguan, & Tanaka, 2006b; Zhang, Wang, Herring, & Oh, 2007), and sensory properties (Choi & Regenstein, 2000).

3.2.1. Chemical and structural properties

Table 2 summarizes the amino acid composition of different types of fish gelatin. Generally, collagens present in fish skins show a wider variety in amino acid compositions than those of mammalian collagens. Their hydroxyproline and, to a lesser extent, proline contents are lower than those in mammalian collagens, and this is compensated for by higher serine and threonine contents (Balian & Bowes, 1977). In general, fish collagens have lower imino acid contents than mammalian collagens, and this may be the reason for the denaturation at low temperature (Grossman & Bergman, 1992). The source and type of collagen will influence the properties of the resulting gelatins.

Overall, fish gelatins have lower concentrations of imino acids (proline and hydroxyproline) compared to mammalian gelatins, and warm-water fish gelatins (such as bigeye-tuna and tilapia) have a higher imino acid content than cold-water fish (such as cod, whiting and halibut) gelatins (Eastoe & Leach, 1977). The proline and hydroxyproline contents are approximately 30% for mammalian gelatins, 22–25% for warm-water fish gelatins (tilapia and Nile perch), and 17% for cold-water fish gelatin (cod) (Muyonga et al., 2004).

Avena-Bustillos et al. (2006) reported similar trends in that they found that cold-water fish gelatins have significantly fewer hydroxyproline, proline, valine, and leucine residues than mammalian gelatins, but significantly more glycine, serine, threonine, aspartic acid, methionine, and histidine residues. However, both cold-water fish and mammalian gelatins have the same proportion of alanine, glutamic acid, cysteine, isoleucine, tyrosine, phenylalanine, homocysteine, hydroxylysine, lysine, and arginine residues.

Haug et al. (2004a), conducting a similar comparative study on the rheological properties of fish and mammalian gelatins, found that the main difference between fish and mammalian gelatins is the content of the imino acids, proline and hydroxyproline, which stabilize the ordered conformation when gelatin forms a gel network. The lower content of proline and hydroxyproline gives fish gelatin a low gel modulus, and low gelling and melting temperatures. It should be kept in mind that the super-helix structure of the gelatin gel, which is critical for the gel properties, is stabilized by steric restrictions. These restrictions are imposed by both the pyrrolidine rings of the imino acids in addition to the hydrogen bonds formed between amino acid residues (Te Nijenhuis, 1997).

Apart from amino acid composition, the functional properties of gelatin are also influenced by the distribution of the molecular weights, structures, and compositions of its subunits. During gelatin manufacturing, the conversion of collagen to gelatin yields molecules of varying mass, due to the cleavage of inter-chain covalent crosslinks and the unfavorable breakage of some intra-chain peptide linkages (Zhou, Mulvaney, & Regenstein, 2006). As a result, the gelatin obtained has a lower molecular weight than native collagen, and consists of a mixture of fragments with molecular weights in the range of 80–250 kDa (Poppe, 1997).

Fish and mammalian gelatins have a polydisperse molecular weight distribution related to the collagen structure and production process. In addition to different oligomers of the alpha subunits, intact and partially hydrolyzed alpha-chains are also present, giving rise to a mixture containing molecules of different molecular weights (Schrieber & Gareis, 2007). The polydispersity,

calculated as the ratio of the weighted average molecular weight (M_w) to the number average molecular weight (M_n), of gelatin always has a value over 2 (Schrieber & Gareis, 2007). However, in a rheological study on several types of fish gelatin, Gudmundsson (2002) reported polydispersity values in the range of 1.57–2.21. Gudmundsson (2002) also reported isoelectric point (pI) values for megrim (9.5), tilapia (9.1), and cod (8.9).

β-Chain and γ-chain aggregations are present in salmon and pollock skin gelatins as well as in commercial mammalian and fish skin gelatins. Large amounts of β- and γ-chains have been shown to negatively affect some of the functional properties of fish gelatins, such as lowering viscosity, lowering melting and setting points, and resulting in a longer setting time (Muyonga et al., 2004). Chiou et al. (2006) reported that pollock and salmon gelatins had slightly different molecular weight profiles compared to porcine gelatin, and that the fish gelatins had chains with slightly lower molecular weights. In addition, the fish gelatins contained lower molecular weight species that were not present in the porcine gelatin.

3.2.2. Rheological properties

Gelatin is categorized as a physical gel, i.e., the interactions or bonds between the chains that make up the material are physical in nature (van der Waal's interactions and hydrogen bonds, with an $E \approx 2$ kcal/mole). Some physical gels, such as alginate, are not thermally reversible. However, the bonding energy in gelatin is relatively weak, and as a result, gelatin is capable of forming thermoreversible gels. Apart from gelatin, casein (a milk protein that forms spherical aggregates approximately 20–300 nm in diameter), agarose, pectin, and carrageenans (polysaccharides extracted from algae) (Papon et al., 2007) also form thermoreversible gels.

Gel strength and gel melting point are the major physical properties of gelatin gels. These are governed by molecular weight, as well as by complex interactions determined by the amino acid composition and the ratio of α/β-chains present in the gelatin (Cho et al., 2004). According to Schrieber & Gareis (2007), the gel strength is mainly dependent on the proportion of fractions having a molecular weight of approximately 100,000 g mol^{-1}. In addition, there is a strong correlation between gel strength and the α-chain content in gelatin. Gelatin containing more α-chains would thus show higher gel strength. On the other hand, a high ratio of peptides with molecular weights higher or lower than the α-chains would decrease gel strength (Liu et al., 2008).

The gel strengths of commercial gelatins are expressed using Bloom values. The Bloom value is the weight in grams that is required for a specified plunger to depress the surface of a standard, thermostated gel to a defined depth under standard conditions (Schrieber & Gareis, 2007). The gelling strength of commercial gelatins ranges from 100 to 300, but gelatins with Bloom values of 250–260 are the most desirable (Holzer, 1996).

Table 5 shows the Bloom value of some fish gelatins as reported in the literature. Fish gelatin typically has a Bloom value ranging from as low as zero to 270 (tested under the conditions of the standard Bloom test), compared to the high Bloom values for bovine or porcine gelatin, which have Bloom values of 200–240. However, a Bloom value as high as 426 has been reported for yellowfin tuna skin (Cho, Gu, & Kim, 2005). Some species of warm-water fish gelatins have been reported to exhibit relatively high Bloom values, close to that of high Bloom pork gelatin (Gudmundsson & Hafsteinsson, 1997). Such high gel strength characterizes only those gelatins extracted from the skins of warm-water fish such as tilapia (Grossman & Bergman, 1992; Jamilah, & Harvinder, 2002; Zhou et al., 2006) and grass carp (Kasankala, Xue, Weilong, Hong, & He, 2007). For example, Bloom values ranging from 128 to 273 have been reported for tilapia gelatin (Jamilah, & Harvinder, 2002; Zhou et al., 2006). On the other hand, cold-water fish gelatin solutions may remain in a liquid state under the conditions of the standard Bloom test at 10 °C (Norland, 1990). Typical Bloom values ranging from 70 to 110 have been reported for cod, Alaska pollock, salmon, and hake (Table 5). The wide range of Bloom values found for the various gelatins arises from differences in proline and hydroxyproline content in collagens of different species, and is also associated with the temperature of the habitat of the animals. Badii and Howell (2006) have shown that hydrophobic amino acids (Ala, Val, Leu, Ile, Pro, Phe, and Met) could also contribute to the high Bloom value of tilapia fish gelatin. They found a lower number of hydrophobic amino acids in the commercial non-gelling cod gelatin compared to tilapia and horse mackerel gelatin. It has been suggested that extraction conditions

Table 5
Gel strength and melting point of various fish gelatins

Type of fish	Bloom strength (g)	Gelling temperature (°C)	Melting point (°C)	Reference
Alaska pollock	98	Not reported	21.2	Zhou et al. (2006)
Salmon	108	Not reported	Not reported	Arnesen and Gildberg (2007)
Cod	71 Porcine: 216			
Cod	~90	11–12	13.8	Gómez-Guillén et al. (2002)
Hake	~110	11–12	14	
Sole	350	18–19	19.4	Gómez-Guillén et al. (2002)
Megrim	340	18–19	18.8	
Sin croaker	124.9	7.1	18.5	Cheow et al. (2007)
Shortfin scad	176.9 Bovine: 239.9	9.9 Bovine: 19.6	24.5 Bovine: 28.9	
Red tilapia skin	128.1	Not reported	22.4	Jamilah and Harvinder (2002)
Black tilapia skin	180.7		28.90	
Tilapia (species unknown)	273 Porcine: 240	Not reported	25.4 Porcine: 31.2	Zhou et al. (2006)
Tilapia spp.	263	Not reported	Not reported	Grossman and Bergman (1992)
Nile tilapia	328	Not reported	Not reported	Songchotikunpan et al. (2008)
Tilapia spp	Not reported	18.2	25.8	Gudmundsson (2002)
Young Nile perch	222	13.8	21.4	Muyonga et al. (2004)
Adult Nile perch	229	19.5	26.3	
Grass carp	267	19.5 Porcine: 24.7 Bovine: 21.7	26.8 Porcine: 31.5 Bovine: 30.0	Kasankala et al. (2007)
Yellofin tuna skin	426 Porcine: 295 Bovine: 216	18.7 Porcine: 25.6 Bovine: 23.8	24.3 Porcine: 36.5 Bovine: 33.8	Cho et al. (2005)
Megrim	MG1[a]:220 MG2[a]:350	9 9–10	11 19	Montero and Gómez-Guillén (2000)
Hake	150	11	12–13	
Harp seal	260 Porcine: 200 Bovine: 210	Not reported	Not reported	Arnesen and Gildberg (2002)
Bigeye snapper	56.0[b] 135.3[c]	Not reported	Not reported	Nalinanon et al. (2008)
Bigeye snapper	105.7	Not reported	Not reported	Jongjareonrak et al. (2006b)
Brownstripe red snapper	218.6			
Catfish	252	Not reported	Not reported	Yang et al. (2007)
Catfish	243–256	15–18	23–27	Liu et al. (2008)

The values for bovine/porcine gelatins are given when available.
[a] MG1 – gelatin from megrim skin pretreated with 0.7% citric acid for 40 min; MG2 – gelatin from megrim skin pretreated with 0.05 N acetic acid for 3 h. The values of gelling and melting temperatures are estimated from the graph.
[b] Gelatin from bigeye snapper skin extracted by the typical process.
[c] Gelatin extracted from skin treated with bigeye snapper pepsin.

may affect the hydrophobic amino acid composition and distribution, which influences the physical properties of gelatin, even more than the imino acid content (Montero & Gómez-Guillén, 2000).

Apart from variables concerning the origin of the raw material, the extraction conditions also markedly affect the gelling point and strength. For example, the use of high concentrations of sulfuric acid, sodium hydroxide, and citric acid, has been reported to result in the lowest Bloom values for cod gelatin (Gudmundsson & Hafsteinsson, 1997), indicating that the gel-forming ability of the gelatin was sensitive to acid and alkali hydrolysis, as both affect the degree of crosslinking in the collagen.

Arnesen & Gildberg (2007) argued that the measurement of the standard Bloom value may give an incorrect impression of the potential gel strength of fish gelatins. It is well known that gelatin gels strengthen during storage. They noted that the relative strengthening of fish gelatins was found to be much higher than the strengthening of porcine gelatin. The gel strength of cod gelatin extracted at 65 °C increased by 250% with storage 6 days after the Bloom value was measured, whereas the corresponding strength increase of porcine gelatin gel was only 23%.

The most remarkable characteristics of gelatin are its solubility in water and ability to form thermally reversible gels. As a thermoreversible gel, gelatin gels will start melting when the temperature increases above a certain point, which is called the gel melting point, and is usually lower than human body temperature. This melt-in-the-mouth property has become one of the most important characteristics of gelatin gels, and is widely exploited in the food and pharmaceutical industries. The rheological properties of thermoreversible gelatin gels are primarily a function of temperature (below the melting point of the gel) and the concentration of gelatin for a given gelatin type (Zhou et al., 2006). The transformation of collagen to gelatin is interpreted as the disintegration of the helical structures into random coils (Kuijpers et al., 1999; Watanabe, Tezuka, & Tadahiro, 1997). Upon cooling, the random coils undergo a coil to helix transition (Kuijpers et al., 1999) during which they attempt to reform the original structure (Mackie, Gunning, Ridout, & Morris, 1998). The resulting three-dimensional network is responsible for the strength and integrity of the gelatin gel.

The main differences in the properties of mammalian and fish gelatins are that fish gelatins have lower gelling and melting temperatures, but relatively higher viscosities (Leuenberger, 1991). Typical gelling and melting points for porcine and bovine gelatins range from 20 to 25 °C and 28 to 31 °C, respectively. In comparison, typical gelling and melting points for fish gelatins range from 8 to 25 °C and 11 to 28 °C, respectively (Table 5). The wide range of gelling temperatures is greatly influenced by the origin of the raw material used in the process. Gilsenan and Ross-Murphy (2000b) compared the rheological properties and melting points of mammalian gelatin with gelatins from different types of fish. They observed that gelatins from cold-water fish have a much higher critical concentration and lower melting point than mammalian samples, due to the lower imino acid contents, which, in turn, reduces the propensity for intermolecular helix formation. Warm-water fish gelatins, however, have properties that are quite similar to mammalian samples. Similar studies have concluded that in general, the melting temperature of gelatins derived from the skins of cold-water fish are significantly lower than those of collagens and gelatins from the skins of mammals and fish living in warm-waters, due to the lower imino acid contents and less proline hydroxylation (Gómez-Guillén et al., 2002; Norland, 1990; Yamaguchi, Lavéty, & Love, 1976). Consequently, cold-water fish gelatins behave as a viscous liquid at room temperature, which limits their use in many applications.

Gómez-Guillén et al. (2002) studied the rheological characteristics (viscoelasticity and gel strength) and chemical/structural properties (amino acid composition, molecular weight distribution, and triple-helix formation) of gelatins extracted from the skins of several marine species. Gelatins from flat-fish species (sole and megrim) showed the best gelling ability, and the gels were more thermostable than those from cold-adapted fish (cod and hake). This difference in behavior was explained based on the amino acid composition, the α_1/α_2 collagen-chain ratio, and the molecular weight distribution. They pointed out that although the amino acid composition is important for determining the gelling properties of a given gelatin, the average molecular weight and, more specifically, the distribution of α-, β-, or γ-chains, is also important when considering the physical properties of the gelatin preparations. This is consistent with Liu et al. (2008), who noted that gelatin that contains more α-chains exhibits higher gel strength. Therefore, all processing steps during the extraction of gelatin should avoid extensive degradation of the peptide structure to obtain gelatin with high gel strength.

3.2.3. Emulsifying and foaming properties

Gelatin, and to some extent also collagen, is used as a foaming, emulsifying, and wetting agent in food, pharmaceutical, medical, and technical applications due to its surface-active properties. Previous studies have shown that gelatin is surface-active and that it is capable of acting as an emulsifier in oil-in-water emulsions (Lobo, 2002). The hydrophobic areas on the peptide chain are responsible for giving gelatin its emulsifying and foaming properties (Cole, 2000; Galazka, Dickinson, & Ledward, 1999). However, gelatin is generally a weaker emulsifier than other surface-active substances such as globular proteins and gum arabic. Therefore, when used on its own, gelatin often produces relatively large droplet sizes during homogenization (Chesworth, Dickinson, Searle, & Stainsby, 1985; Dickinson & Lopez, 2001; Lobo, 2002), and it has to be either hydrophobically modified by the attachment of nonpolar side-groups (Toledano & Magdassi, 1998), or used in conjunction with anionic surfactants to improve its effectiveness as an emulsifier (Muller & Hermel, 1994; Olijve, Mori, & Toda, 2001; Surh, Gu, Decker, & McClements, 2005).

The versatility of the emulsifying and foaming properties of gelatin is particularly valued in products like emulsified powders (Kläui, Hausheer, & Huschke, 1970). In such products, its surface-active and film-forming characteristics can be successfully exploited during the emulsification process. Its stabilization and gelation characteristics are useful during the subsequent drying and encapsulation stages. In marshmallows, the gel-forming properties of gelatins are used to stabilize the foam upon cooling. In most applications, gelatin is chosen not only for its surface-active properties, but rather because of its unique combination of surface-active, chemical, rheological, and gelling properties. For example, in gelatin-foamed foods and ice creams, the unique gel melting behavior in the range of 10–30 °C results in the melting of gelatin gels in the mouth (de Wolf, 2003).

In general, there have been very limited studies on the emulsifying and foaming properties of fish gelatin compared to the number of studies on its gelation properties. In general, fish gelatin emulsions are moderately stable to creaming. Surh et al. (2006) have studied whether physically stable oil-in-water emulsions could be produced using fish gelatin, and determined the influence of gelatin molecular weight (low molecular weight fish gelatin [LMW-FG] and high-molecular weight fish gelatin [HMW-FG]) and environmental stresses (pH, salt, and thermal processing) on the stability of such emulsions. They noted that emulsions with monomodal particle size distributions and small mean droplet diameters ($d_{43} \sim 0.35$ μm for LMW-FG and 0.71 μm for HMW-FG) could be produced at protein concentrations ≥4.0 wt% for both molecular weight fish gelatins. However, the presence of a small fraction of relatively large droplets (>10 μm) was observed in the

emulsions, even at relatively high protein concentrations. Surh et al. (2006) noted that the number of large droplets and the amount of destabilized oil was less in the HMW-FG emulsions than in the LMW-FG emulsions. This effect may be attributed to the fact that the thickness of an adsorbed gelatin membrane increases with increasing molecular weight. The researchers also report that emulsions of both low- and high-molecular weight fish gelatins were fairly stable when subjected to high salt concentrations (250 mM sodium chloride), thermal treatments (30 and 90 °C for 30 min), and different pH values (pH 3–8), demonstrating that fish gelatin may have limited use as a protein emulsifier for oil-in-water emulsions.

Dickinson and Lopez (2001) have compared the emulsion-stabilizing properties of a set of commercial casein and whey protein ingredients, under neutral pH conditions, with the properties of commercial fish gelatin as an emulsifying agent in oil-soluble vitamin encapsulation. They noted that when gelatin is used as an emulsifying agent, the protein/oil ratio should be optimized in order to avoid the presence of large droplets that could lead to coalescence, especially at high ionic strength. Conversely, where milk protein is intended as a replacement for gelatin in existing emulsion products, attention should be given to the effect of flocculation of whey protein-coated droplets on storage.

3.2.4. Film-forming properties

Studies on the production of films from fish gelatin and their characterization have been carried out, and it has been observed that all fish gelatins exhibited excellent film-forming properties (Avena-Bustillos et al., 2006; Gómez-Guillén, Ihl, Bifani, Silva, & Montero, 2007; Jongjareonrak et al., 2006b; Jongjareonrak, Benjakul, Visessanguan, Prodpran, & Tanaka, 2006a).

In general, gelatin films from the skins of a warm-water fish species, such as the Nile perch, have been reported to exhibit stress and elongation at break similar to that of bovine bone gelatin (Muyonga et al., 2004). Fish gelatin film, however, exhibits lower water vapor permeability than bovine gelatin. For example, films from tuna skin gelatin plasticized with glycerol showed lower water vapor permeability (WVP) compared to values reported for pigskin gelatin (Gómez-Guillén et al., 2007). The lower WVP values (compared to those from bovine or porcine) reported for films based on fish gelatins from several species, can be explained in terms of the amino acid composition: fish gelatins are known to have much higher hydrophobicity due to lower proline and hydroxyproline contents, as the hydroxyl group of hydroxyproline is normally available to form hydrogen bonds with water (Avena-Bustillos et al., 2006).

Gelatin films prepared from cold-water fish and warm-water fish also show different WVPs. According to Avena-Bustillos et al. (2006), the WVP of cold-water fish gelatin films was significantly lower than warm-water fish, and this was attributed to increased hydrophobicity, as explained above, due to reduced amounts of proline and hydroxyproline in cold-water fish gelatins. They suggested that the lower WVP of fish gelatin films can be particularly useful for applications related to reducing water loss from encapsulated drugs and refrigerated or frozen food systems.

3.2.5. Sensory properties

Choi and Regenstein (2000) studied the physicochemical differences between pork and fish gelatin and the effect of melting point on the sensory characteristics of a gelatin–water gel. Quantitative descriptive analysis (QDA) was performed to determine the effect of the melting point on the sensory characteristics of gelatin gels. They noted that flavored fish gelatin dessert gel product had less undesirable off-flavors and off-odors, with more desirable release of flavor and aroma than the same product produced with pork gelatin possessing equal Bloom values, but a higher melting point. The lower melting temperature of fish gelatin seems to assist in the release of fruit aroma, fruit flavor, and sweetness. In contrast, since pork gelatin melts more slowly than fish gelatin in the mouth, the perceived viscosity of pork gelatin might be expected to be higher than that of the fish gelatin under the same conditions. Choi and Regenstein (2000) further noted that since fish gelatin has a better release of aroma, it might offer new opportunities to product developers.

4. Applications of fish gelatin

The gelatin produced from the skins of fish living in cold-waters does not gel at room temperature – its gelling temperature is below 8–10 °C (Norland, 1990). Cold-water fish gelatin can also be used in applications that do not require a high Bloom value or gelling, relying instead in its other properties, such as prevention of syneresis and texturization. Cold-water fish gelatin can be used in frozen or refrigerated products that are consumed quickly following removal from the fridge or defrosting.

Low gelling temperatures also offer new potential applications for fish gelatin. Gelatins with low melting points could also be used in dry products (such as for micro-encapsulation), and in fact, one of the major applications of fish gelatin is in the microencapsulation of vitamins and other pharmaceutical additives such as azoxanthine. Fish gelatin may also be used in the microencapsulation of colorants. Soper (1999) described a method for microencapsulation of food flavors such as vegetable oil, lemon oil, garlic flavor, apple flavor, or black pepper with warm-water fish gelatin (150–300 Bloom). The microencapsulation process is conducted at temperatures of 33–35 °C by complex coacervation to form the microencapsulated capsules. Most encapsulators have developed the expertise to handle fish gelatin in the sophisticated process, and minor volumes of fish gelatin are used to make soft-gel capsules. The use of fish gelatin soft capsules is most common in nutrition supplements.

The low gelling temperature of gelatin from cold-water fish also makes it useful as the base for light-sensitive coatings that are important to the electronics trade. Cold-water fish gelatin is also a good medium for precipitating silver halide emulsions since this process can be carried out at a lower temperature with fish gelatin than with warm-blooded animal gelatin (Norland, 1990).

As a protein, gelatin is low in calories, and melts in the mouth to give excellent sensory properties resembling fat, making it ideal for use in low-fat products. With a favorable pricing structure, manufacturers may consider the use of fish gelatin in large volume, consumer-price driven products such as low-fat spreads and yogurts. In these products, cold-water-soluble grades of pigskin gelatins are often used, which are available at a price premium of approximately 25%. Alternatively, normal grades can be used in yogurts, but considerable agitation is required to prevent clumping. Fish gelatins with lower melting points would be easier to incorporate as an alternative.

Warm-water fish gelatin can have a Bloom value of 200–250 g. Tuna, for instance, is regarded as a good source, but the skin can be fatty, and gelatin must be fat-free. Tuna or tilapia gelatins have a melting point of 25–27 °C, and therefore, these gelatins are suitable for products stored at low room temperatures. These gelatins more closely resemble bovine or pig gelatin, which melts at 32–35 °C. A previous sensory study on gelatin gel desserts suggested that fish gelatin with lower gel melting temperatures had a better release of aroma and offered a stronger flavor (Choi & Regenstein, 2000). By increasing the concentration of gelatin or by using gelatin mixtures, desserts made from fish gelatins would be more similar to desserts made from high Bloom pork skin gelatin

(Zhou & Regenstein, 2007). Warm-water fish gelatin grades can therefore more readily compete in the traditional gelatin markets.

Kołodziejska et al. (2004) observed that enzymatically crosslinked fish gels did not melt during heating in the boiling water bath. They suggested that if the gel structure is not destroyed at higher temperatures, fish gelatin could be used as a gelling component of sterilized products. Combinations of fish gelatin with other common hydrocolloids can be used to extend the application of fish gelatin as a food ingredient. For example, fish gelatin and pectin have been used to make a low-fat spread (Cheng, Lim, Chow, Chong, & Chang, 2007). It was found that a decrease in the fish gelatin to pectin ratio resulted in an increase in bulk density, firmness, compressibility, adhesiveness, elasticity, and meltability.

Fish gelatin has also been used in the preparation of pharmaceutical products. Park, Joon, Bae, Kim, and Cha (2007) patented a process describing the preparation of a film-forming composition for hard capsules composed of fish gelatin. Using transglutaminase for crosslinking circumvented the problems caused by the low gelling temperature property of fish gelatin. Another patent (Hansen, Vilstrup, & Jensen, 2002) described the use of fish gelatin (Bloom value higher than 100) as an ingredient in drug tablets.

5. Challenges associated with fish gelatin

Compared to bovine and porcine gelatin, the market share of fish gelatin is still considered very small. Some limiting factors that hamper the large-scale development of the fish gelatin industry include:

- *Inferior rheological properties* – thus far, fish gelatin has limited applications because the gels formed tend to have inferior rheological properties as compared to gelatins from land mammals (Leuenberger, 1991). This limitation has been attributed mainly to the lack of proline-rich regions of the collagen or gelatin molecules in cold-water fish compared to warm-blooded animals (Ledward, 1986; Norland, 1990). In addition, the total glycine–proline–hydroxyproline sequence content is one of the main factors affecting collagen thermostability (Burjandze, 2000).
- *Insufficient availability of raw materials* – it has been estimated that fish-processing byproducts account for as much as 70–85% of the total weight of the catch, and these typically include processing wastes from fish, shellfish, and the by-catch of unutilized and underutilized species (Shahidi, 1994). However, gelatin manufacturers have found it difficult to provide adequate quantities of a particular fish type on a regular and consistent basis. This primarily applies to warm-water fish. In contrast, supplies of cold-water fish skins are more abundant. Another factor that could pose a problem is the difficulty to obtain certification on fish raw material. Certification is required for traceability, which has become an essential requirement for food additives, especially from animal sources.
- *Variable gelatin quality* – this is especially of concern when fish-processing byproducts are being used due to the variable composition of the source material. This issue could perhaps be resolved through careful quality control of the raw material or by custom blending.
- *Other intrinsic quality factors* – these include odor, color, Bloom strength, and viscosity of fish gelatin. The technical difficulties associated with producing fish gelatin for human consumption generally surround the elimination of the unpleasant fish odor from the product. Persisting residual odor in fish gelatin can cause problems especially when the fish gelatin is intended for use in mildly flavored products. In cases, the product is odor-free when produced, but when formulated into other products, the odor returns with generation of off-flavors. Gudmundsson and Hafsteinsson (1997) reported that for gelatin extraction from cod skin, the odor was absent or barely detectable if sulfuric acid and sodium hydroxide were used in concentrations $\geq 0.2\%$ (w/v) and citric acid was $0.7 \leq 1.2\%$ (w/v), and Grossman and Bergman (1992) have developed a method that can make the gelatin odorless. In terms of gel strength, fish gelatins with a wide range of Bloom strengths are generally less available than bovine or porcine gelatins.
- *Prices* – this is another sticky issue and is likely related to the economics of the production process (i.e., high production cost and low yield). Only a handful of manufacturers are involved in the production of fish gelatin worldwide, and for small manufacturers, the production volumes are small, probably not more than 100 tons. The price of fish gelatin varies considerably in the market and tends to be more expensive (4–5 times higher) than bovine or porcine gelatins. Currently, fish gelatin is regarded as a niche product, as the industry cannot support the higher costs. Under current conditions, producers of fish gelatin may find it difficult to lower the prices due to the low yield of fish skins and the lack of an economy of scale in the production process.

6. Prospects of fish gelatin as an alternative to mammalian gelatin

To address or minimize some of the problems associated with the inferior properties of fish gelatin compared to mammalian gelatins, three different approaches have been proposed:

- Enzyme crosslinking of gelatin using enzymes such as transglutaminase (Yi, Kim, Bae, Whiteside, & Park, 2006), or chemical crosslinking using chemicals such as genipin (Chiou et al., 2006);
- Creating mixed gelling systems consisting of fish gelatin combined with other high Bloom gelatins (Gilsenan & Ross-Murphy, 2000a, 2000b; Zhou et al., 2006) or with suitable plant hydrocolloids (Haug, Draget, & Smidsrod, 2004b; Pranoto, Lee, & Park, 2007) which may give higher gel strength, gelling and melting temperature;
- Manipulating the characteristics of gelatin by the addition of solutes, such as different salts (Elysée-Collen & Lencki, 1996).

Gelation of unmodified gelatin occurs by physical crosslinking, which generally leads to the formation of "junction zones," followed by the formation of a three-dimensional branched network (Gilsenan & Ross-Murphy, 2000a). The gel strength of such gelatin depends on the time and temperature of maturation, and is inversely related to changes in temperature (increases when temperature decreases) (Choi & Regenstein, 2000). In enzymatically modified gelatin gels, wherein covalent bonds also participate in the formation of the three-dimensional branched network, those parameters are very important since they also affect the activity of the enzyme.

Gómez-Guillén, Sarabia, Solas, and Montero (2001) reported that the addition of microbial transglutaminase to a fish skin gelatin can considerably raise the melting point, gel strength, and viscosity at 60 °C, depending on the concentration of the enzyme and incubation time. While increasing concentrations of transglutaminase increase the elasticity and cohesiveness of the gels, this can also result in lower strength and hardness due to excessively rapid gel network formation. In addition, it was found that whether or not a gelatin is thermoreversible depends primarily on the degree of enzyme inactivation. It was thus possible to thermally achieve partial inactivation of the enzyme without negatively affecting the properties of the gelatin. Kołodziejska et al. (2004)

also noted that the enzymatically crosslinked gels did not melt during heating in a boiling water bath. They suggested that this property might be useful when the activity of the enzyme must be inhibited. Babin and Dickinson (2001) also observed that the magnitude of the effect of transglutaminase treatment on gelation thermoreversibility depends on enzyme concentration and melting temperature. They noted a rather modest reduction in the thermoreversibility of the gelatin gel as a result of the limited degree of covalent crosslinking, which becomes rapidly arrested by high-temperature melting. In contrast, when more extensive covalent crosslinking occurs (both during cold-set gelation and following low temperature melting), the loss of thermoreversibility is dramatic. Kołodziejska et al. (2004) also found that the structures of enzymatically crosslinked fish gelatin gels were not destroyed during 30 min of heating in a boiling water bath.

The mechanical properties of gelatin can also be improved by introducing covalent 'chemical' crosslinks between single strand chain segments (McEvoy et al., 1989; Watanabe, Tezuka, & Tadahiro, 1997). Compared to enzymatic crosslinking, however, chemical crosslinking of gelatin has received less attention. Existing industrial processes using covalent crosslinking reactions include the production of hardened gelatin gels for photographic emulsions and the manufacture of hardened gelatin–acacia coacervates for ink encapsulation in pressure-sensitive paper (Green & Schleicher, 1957). Among the commercially available chemical crosslinkers, glutaraldehyde is one of the most widely used since it reacts rapidly with amine groups in the gelatin and is also relatively inexpensive. However, there have been concerns about the toxicity of the glutaraldehyde, and recently, genipin (isolated from the fruits of *Gardenia jasminoides* Ellis) has attracted interest as an alternative crosslinker to glutaraldehyde because of its lower toxicity. Chiou et al. (2006) studied the rheological and mechanical properties of fish gelatins (pollock and salmon) using genipin and glutaraldehyde as crosslinking agents. Both fish gelatins that contained genipin showed faster crosslinking rates for samples with higher pH values. However, salmon samples exhibited greater dependence on pH, and pollock gelatin crosslinked faster with glutaraldehyde than with genipin.

An interesting study by Strauss and Gibson (2004) described the use of plant phenolics as crosslinkers of gelatin gels and gelatin-based coacervates for future use as food ingredients. Polyphenols are known to react under oxidizing conditions with the amino side chains of peptides, leading to the formation of crosslinks in proteins. In this study, they used several phenolic acids (caffeic, chlorogenic, and ferulic), using instant coffee and commercial white grape juice as examples of plant-derived sources of polyphenols. Solutions of the phenolics were mixed with gelatin in various proportions and adjusted to the desired pH (mainly pH 8). They reported that gelatin gels crosslinked using these materials had higher mechanical strength with reduced swelling and fewer free amino groups. More interestingly, the availability of coffee, grape juice, and various other plant materials containing sufficient concentrations of phenolics made their direct use practical, thus eliminating the need to isolate the active components. Since crosslinked gelatin gels behave like non-crosslinked gels of higher concentrations, they offer the possibility of developing gelled foods with reduced gelatin content and lower calories. Although this study was carried out on porcine type A gelatin, it could likely be extended to fish gelatin and presumably, it could enhance the rheological properties of fish gelatin and increase its commercial value.

A mixed system combining a given fish gelatin with other high Bloom fish gelatins or other hydrocolloids could be exploited to compensate for the generally weak gel strength of that fish gelatin (Gilsenan & Ross-Murphy, 2000a, 2000b; Zhou et al., 2006). For example, Zhou et al. (2006) suggested that for cold-water fish gelatin, such as gelatin derived from the Alaska pollock, with a lower gelling ability (the Bloom value was only about 100), gel properties can be enhanced via mixing with high quality gelatins from warm-water fishes. Choi and Regenstein (2000) noted that, apart from enhancing the gel strength, the two-step melting process of gels comprised of pollock gelatin mixed with tilapia gelatin or pork gelatin may be useful in food product development to control the texture and flavor release during mastication.

In another study, Badii and Howell (2006) studied a mixture of fish (horse mackerel) gelatin with egg albumen proteins (3:10). They observed that fish gelatin produced synergistic interactions and compatible gel structures when combined with egg albumen protein, leading to a higher gel strength. The gelling properties and compatibility with egg proteins make the horse mackerel gelatin a potential alternative to the use of porcine and bovine gelatins in desserts and bakery products.

The addition of polysaccharides such as gellan and carrageenan can also be used to modify the properties of fish gelatin. Haug et al. (2004b) described a mixture containing fish gelatin–κ-carrageenan added to compensate for the low gelling/melting point and low gel strength of fish gelatin alone. Mixtures of fish gelatin and κ-carrageenan resulted in solutions and gels with various degrees of turbidity, depending on the concentration of polymers, pH, ionic strength, and the nature of the added salt. The turbidity is most likely a result of phase separation in the system. The interactions between the fish gelatin and κ-carrageenan at 60 °C are potentially stabilized by electrostatic interactions, and the system is believed to segregate when carrageenan adopts an ordered conformation and forms a gel network.

Another mixed system of fish gelatin with κ-carrageenan and gellan has been studied by Pranoto et al. (2007). The addition of gellan and κ-carrageenan increased the melting point of fish gelatin gels, with gellan being the more effective additive. According to Fonkwe, Narsimhan, and Cha (2003), gellan may form coupled networks with the gelatin molecule, wherein the anionic regions of the gellan form new heterolytic junction zones with cationic regions of the gelatin molecules, leading to increases in gelation temperature, gelation rate, and gel strength.

Improvement of the gel strength of gelatin using modified starch has also been described in several patents (Helmstetter, 1977; Szymanski & Helmstetter, 1975). Helmstetter (1977) described a process of enhancing gel strength of gelatin using chemically modified dialdehyde polysaccharides (starch or dextrin), claiming that the dialdehyde starch (degree of substitution of at least 0.005) provides a synergistic improvement in gel strength and hardness. These approaches have not been attempted with fish gelatin, but future consideration should be given to this, especially in cases where the formulation of fish gelatin and the modified starch is suitable for the product with respect to texture and other organoleptic properties.

The preparation of carboxymethylcellulose (CMC)–gelatin complexes has been described by Lii, Tomasik, Zaleska, Liaw, and Lai (2002). Using an electrochemical approach, the protein–polysaccharide complexes can be separated at the anode in a natural, non-forced, and autocontrolled manner (Lii et al., 2002). Analysis of the carboxymethylcellulose–gelatin complexes suggested that, apart from hydrogen bonds and dispersion forces, strong interactions between the carboxyl groups of CMC and the peptide moieties of gelatin were involved in the formation of the CMC–gelatin complexes. They suggested that such complexes could be potentially used as edible films and coatings, emulsion stabilizers, and biodegradable packagings.

Strauss and Gibson (2004) described coacervates of gelatin complexed with anionic polyelectrolytes, such as pectin, in the form of microparticles or microcapsules. These may be used as fat-mimetic additives or for flavor encapsulation. Coacervates are

formed when a mixed dilute solution of gelatin and an anionic polysaccharide (acacia, pectin, etc.) is brought to a pH at which the polyelectrolytes have opposite net charges. Presumably, similar coacervates of fish gelatin with other anionic polyelectrolytes could also be prepared and characterized.

Another potential means for manipulating the characteristics of a given gelatin is to modify existing interactions by the addition of solutes, such as salts (Elysée-Collen & Lencki, 1996). There are two points of view regarding the possible interactions between collagen (or gelatin) molecules and saline ions. Some workers believe that the ions directly interact with the peptide backbone of collagen, while others believe that the ions affect collagen folding indirectly by interacting with structurally bound water molecules (Asghar & Henrickson, 1982). Sarabia et al. (2000) found that it was possible to improve the functional properties of fish gelatins, such as megrim skin gelatin, to achieve properties similar to those of gelatins from warm-blooded animals, chiefly in terms of melting point, through the addition of neutral salts (such as $MgSO_4$, $(NH_4)_2 \cdot SO_4$, or NaH_2PO_4) under appropriate pH and ionic strength conditions. Similar observations were reported by Fernández-Díaz, Montero, and Gómez-Guillén (2001), who showed that the gel strength of gelatin from cod and hake was substantially increased by the addition of "coenhancers" such as magnesium sulphate. However, these compounds must be added in relatively high concentrations: 15% glycerol and 0.1–0.5 M $MgSO_4$. For this reason, the use of such a method for the modification of gelatin may be limited in the food industry. Haug et al. (2004a) observed that the gel modulus increases at low ionic strength and decreases with increasing ionic strength. In addition, the gelling and melting temperatures are also influenced by changes in ionic strength. This suggests that formation and stability of the junction zone in gelatin could be directly or indirectly influenced by electrostatic interactions.

7. Conclusions and future outlook

Increasing demand for fish gelatin may pave the way for further research and exploration of fish gelatin as an alternative for mammalian gelatin, as it fulfills the majority of consumer needs and complements the increasing global demand for gelatin. In the past decade, significant advances have been made with respect to the extraction of fish gelatin from various parts of the fish body, such as the skins, bones, and even offal. Processing conditions (solvent, time, and temperature) to produce the optimum yield and quality (gel strength and melting/gelling points) of fish gelatin have been identified for specific types of raw material, but scaling up the extraction and production process of fish gelatin and securing control of the extraction conditions during this process still pose a problem for processors. Therefore, more technological development research is warranted.

Significant progress has also been made in improving the functional properties of fish gelatin (especially gel strength). The results of various crosslinking studies indicate the potential of using food-grade transglutaminase to modulate the properties of fish gelatin gels. However, apart from the long reaction time and high cost of the enzyme, achieving a compromise between gel strength and thermoreversibility would depend on maintaining control over the degree of crosslinking and perhaps other factors, such as the molecular weight of gelatin. As noted by Kołodziejska et al. (2004), regarding enzymatic crosslinking, it is important to use the enzyme and substrate in the proper concentrations. Excessive enzymatic crosslinking of the material may confer no improvement and may even lead to worsening of the product properties. On the other hand, fish gelatin gels cannot be produced at all if the concentration of enzyme is too low. Therefore, in order to facilitate the practical use of transglutaminase preparations for the modification of fish gelatin, it would be important to determine the enzyme activity necessary to perform the reaction under the appropriate enzyme concentrations that ensure the desired properties of the product (Kołodziejska et al., 2004). Another issue regarding the use of transglutaminase is the uncertain halal/kosher status of the enzyme. Since the application of this enzyme to enhance the functional properties of fish gelatin would nullify the halal/kosher status of the gelatin, further research in this area is needed.

Gelatin from several species of cold- and warm-water fish has been well characterized. As Good Manufacturing Practices (GMP) and HACCP (Hazard Analysis and Critical Control Point) are becoming increasingly important in food manufacturing, future research has to be directed towards the development of low cost and high quality fish gelatins with minimal or no contaminants (chemical or microbial). Detailed investigations need to be carried out to standardize the purity of samples/raw material used (e.g., warm-water or cold-water fish) to ensure uniformity. Use of physical (ultrasound and ionizing radiation), enzymatic, and natural (plant phenolics and genipin) crosslinking agents will definitely enhance the gel strength of fish gelatin to compete with mammalian gelatin.

The current production of fish gelatin may not increase significantly, at least in the foreseeable future, as the availability of raw material, coupled with the relatively low yield will be limiting factors in fish gelatin production. However, though fish gelatin will be unable to completely replace mammalian gelatin, it is hopeful that one day, it might become a niche product offering unique and competitive properties to other biopolymers, as well as meeting the demand of global halal/kosher market.

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