

REVIEW: GELATIN, SOURCE, EXTRACTION AND INDUSTRIAL APPLICATIONS

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ABSTRACT

Gelatin is an important functional biopolymer widely used in foods to improve elasticity, consistency, and stability. It can be obtained not only from the skin and bones of land animals, but also from fish and insects. In recent years gelatins from fish and edible insects provide an alternative source that is acceptable for halal (Muslim) and kosher (Jewish) products, gelatin extraction has been reported for different fishes and insects. In gelatin manufacture, two methods are usually used: the acid and the alkaline processes, to produce type A and type B gelatins, respectively.

Key words: gelatin, insect, extraction, type A & B gelatin, collagen, fish skin, halal, kosher

INTRODUCTION

Gelatin is a substantially pure protein food ingredient, obtained by the thermal denaturation of collagen, which is the structural mainstay and most common protein in the animal kingdom [Bailey and Paul 1998]. Gelatin is a water soluble proteinaceous substance prepared by processes, which involve the destruction of the tertiary, secondary and to some extent the primary structure of native collagens [Fernandez-Diaz et al. 2001], specifically by the partial hydrolysis of collagen derived from the skin, white connective tissue and bones of animals [Morrison et al. 1999]. Gelatin is a high molecular weight polypeptide and an important hydrocolloid, which has proved popular with the general public and finds its uses in a wide range of food products largely because of its gelling and thickening properties. It differs from other hydrocolloids because most of them are polysaccharide, whereas gelatin is a digestible protein containing all the essential amino

acids except tryptophan. The amino acid composition particularly with respect to proline and hydroxyproline can vary from species to species, as a result of exposure to a wide range of environmental conditions, particularly temperature [Ladislaus et al. 2007]. Cattle bones, hides, pig skins, fish and recently insects are the main commercial sources of gelatine. Many foods use gelatine as source for texture and binding agent, gelatine from insect can be used to produce ice cream by using 0.5% insect's gelatine and compared with that made using 0.5% commercial gelatine as stabilizing agent. The properties of the obtained ice cream produced using insects gelatine were found to be acceptable for the panelists, and no significant differences between ice cream made using insect gelatine when compared with that made using commercial gelatine in their general preferences [Abdelfadeel 2012]. Thus the current study was carried out to review gelatin

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methods of extraction of its main sources and uses, as well as its industrial applications.

GELATIN SOURCES

Gelatin can be made from many different sources of collagen. Cattle bones, hides, pig skins, and fish are the principle commercial sources. As such, it may come from either agricultural or non-agricultural sources. There are no plant sources of gelatin, and there is no chemical relationship between gelatin and other materials referred to as vegetable gelatin, such as seaweed extracts [GMIA 2012]. Mariod et al. [2011 b] prepared and characterised edible Halal gelatins from two Sudanese edible insects that are melon and sorghum bugs (Fig. 1).



Fig. 1. Sorghum bug adult (left) and melon bug adult (right) [Abdelfadeel 2012]

Mammalian gelatin

Mammalian gelatin is derived from collagen which is the principal constituent of connective tissues and bones of vertebrate animals. Study of two different mammalian gelatins, i.e. from bovine (type B) and porcine (type A) sources revealed that both sources contained components of different molecular weights with wide distribution ranging from 10 to 400 kDa. The result also showed strong correlation between average molecular weight and gel strength of gelatin, with high isoelectric and melting points [Lim and Mohammad 2011]. 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 [Karim and Bhat 2009]. The gelatin can be prepared either by the acid process (type A gelatine), or by the alkaline process (type B gelatine) [Mariod et al. 2011 b].

Fish gelatin

Fish gelatin can be obtained from the skin and bones of fish. The waste from fish processing after filleting can account for as much as 75% of the total catch weight [Shahidi 1995]. About 30% of such waste consists of skin and bones with high collagen content that can be used to produce fish gelatin [Gómez-Guillén et al. 2002]. Extraction of gelatins from fish skins may provide an alternative source that is acceptable for kosher (Jewish) and halal (Muslim) products and serve as an alternative for markets concerned about bovine spongiform encephalopathy (BSE).

Gelatin extraction has been reported for cod [Gudmundsson and Hafsteinsson 1997], hake [Montero et al. 1999], lumpfish [Osborne et al. 1990], megrim [Montero and Gómez-Guillén 2000], and tilapia [Grossman and Bergman 1992, Jamilah and Harvinder 2002]. However, Alaskan pollock, which is an important fishery resource and accounts for more than 1/3 of the U.S. domestic fish catch, has not been examined as a source of raw material for edible gelatin. The yield and quality of gelatin are influenced not only by the species or tissue from which it is extracted but also by the extraction process, which may depend on pH, temperature, and time during both pretreatment and extraction [Montero and Gómez-Guillén 2000].

Insect gelatin

Insect gelatin may provide an alternative source that is acceptable for Muslims products, in Sudan many edible insects consumed and desert locust considered the most famous one in many parts of the country beside sorghum and melon bugs [Mariod et al. 2004, 2006]. *Aspongopus viduatus* (melon bug) and *Agonoscelis pubescens* (sorghum bug), commonly known in Sudan as Um-bugga and Dura andat, respectively. In some areas of Sudan the collected bugs were extracted and the obtained oil was used for cooking and some medicinal uses. Mustafa et al. [2008] reported that the crude oil and the phenolic compounds-free

oil of melon bug showed high antibacterial activities against some test species. The two bugs' protein contained 16 known amino acids, including all of the essential amino acids. Compared with the amino acid profile recommended by FAO/WHO, the bug protein was of medium quality due to its medium content of essential amino acids [Mariod et al. 2011 a]. Three different methods of extraction were used to extract gelatin from *A. viduatus* and *A. pubescens*, mild acid and distilled water extraction method, distilled water extraction method and extraction with hot water. Extraction of insect gelatin using hot water gave higher yield reached up to 3.0% followed by mild acid extraction 1.5% and distilled water extraction 1.0%, respectively.

The *A. viduatus* gave high yield of 293 mg/g representing 1.45% followed by *A. pubescens*, of 263 mg/g representing 1.3%. The yield of gelatine extracted from *A. viduatus* and *A. pubescens* using distilled water with pretreatment of NaOH with varying concentrations 0.1, 0.2 and 0.5 mol/L was low compared with that obtained from *A. viduatus* and *A. pubescens* using mild acid and distilled water extraction method [Abdelfadeel 2012]. Mariod et al. [2011 a] reported that, during insect gelatine extraction, alkaline and acid pre-treatment showed effects on removing non-collagenous proteins with minimum collagen loss. Generally, extraction of gelatine from sorghum bug using hot water extraction pretreated by varying concentrations (0.1, 0.2, and 0.5 mol/L) of NaOH gave high yield and that is highly significant differ ($P \leq 0.05$) in amount of three gelatines and normal significant differ in yield percentage [Abdelfadeel 2012]. Gelatin obtained from insects was characterised by FTIR, and

its spectra seem to be similar with commercial gelatin. Amide II bands of gelatins from melon and sorghum bugs appeared around at 1542-1537 cm^{-1} [Mariod et al. 2011 b].

EXTRACTION AND PREPARATION OF GELATIN

The term "gelatin" is applied to a series of food protein products derived by hydrolysis of animal collagen, contained in bones and skins, and from cold-blooded animals such as fish [Norland 1990, Osborne et al. 1990, Grossman and Bergman 1992, Gudmundsson and Hafsteinsson 1997], insects [Mariod et al. 2011 b]. The fish and insect gelatin can be extracted using 2 categories: an acid process and an alkaline process. During fish gelatin extraction, the acid process refers to the extraction that is carried out in an acid medium [Gómez-Guillén and Montero 2001], and in some cases an acid pre-treatment before extraction is applied. The alkaline process refers to a pre-treatment of fish skin with an alkaline solution, in most cases followed by neutralization with an acid solution, so the extraction may be carried out in an alkaline, neutral, or acid medium [Osborne et al. 1990, Grossman and Bergman 1992, Gudmundsson and Hafsteinsson 1997, Montero and Gómez-Guillén 2000, Jamilah and Harvinder 2002]. Recently three different methods were used to extract gelatine from two edible insects' (Sorghum and melon bugs) these methods were mild acid, distilled water and hot water extraction (Table 1). Gelatin extracted from sorghum bug gave higher yield than that extracted from melon bug. During insect gelatin extraction, alkaline pretreatment followed by hot

Table 1. Yield of gelatin (expressed as mg dry gelatin per g of dried powdered defatted insect) extracted from two edible insects using different methods of extraction

Method of extraction	Weight of sample g	Melon bug mg	Yield %	Sorghum bug mg	Weight of sample g	Yield %
Mild acid extraction	5	125 ±0.34	2.5	134 ±0.42	5	2.68
Distilled water extraction	5	33 ±0.21	0.66	66 ±0.2	5	1.32
Hot water extraction	5	150 ±0.46	3.0	152 ±0.45	5	3.04

Values are means ±standard deviations of three (n = 3) measurements.

Source: Mariod et al. [2011 b].

water extraction showed better effect than acid pre-treatment and even better than alkaline pre-treatment followed by distilled water extraction [Mariod et al. 2011 b].

Because of the acid liability of cross-linking in fish skin collagen, reasonably mild treatment with acid should be enough to affect solubilization [Norland 1990]. Such treatment leads to a type-A gelatin with an isoelectric point between approximately pH 6 and 9, which carries a net positive charge in most food uses. Numerous studies on collagen from different species have focused on acetic-acid extractions. However, for the manufacture of food-grade gelatin from fish, citric acid is widely used because it does not impart objectionable colour or odour to the gelatin [Grossman and Bergman 1992, Gudmundsson and Hafsteinsson 1997]. Swelling properties and solubilisation of collagen affected by the type of acid used, the pH of this acid and the ionic strength. The type of acid used influenced the gelatin viscoelastic and gelling properties [Gómez-Guillén and Montero 2001]. Gelatin is results in a wide variety of peptide chain species since it is obtained by the degradation of a larger structure. This degradative process is not completely random, thus most gelatin preparations are not homogenous with respect to molecular weight or weight distribution [Gómez-Guillén et al. 2002].

GELATIN STRUCTURE

Primary structure of gelatin

The primary structure and composition of gelatin resembles the parent collagen. This similarity has been substantiated for several tissues and species [Fernandez-Diaz et al. 2001]. Slight differences are due to the source of raw material in combination with the pre-treatment and extraction procedures used.

Secondary structure of gelatin

Various aspects of gelatin behavior in solution and gels have been explained in relation to its molecular weight. Gelatin is not polydispersed completely, but has a definite molecular weight distribution pattern, which corresponds to the α -chain and its oligomers [Buice et al. 1995]. Gelatin is a mixture of different polypeptide chains including α -chains, β (dimers of α -chain) and γ (trimers of α -chain) components with

a molar mass of around 90, 180 and 300 $\times 10^3$ g/mol respectively [Rbii et al. 2011]. The presence of oligomers with increasing numbers of α -chains becomes more complex and difficult to read [Buice et al. 1995]. Thus it becomes necessary to separate these molecular weight fractions. Polyacrylamide gel electrophoresis (PAGE) is used to obtain highly accurate molecular weight spectra of both commercial and laboratory gelatins, giving quantitative separation. Figure 2 below depicts a modern picture of gelatin structure.

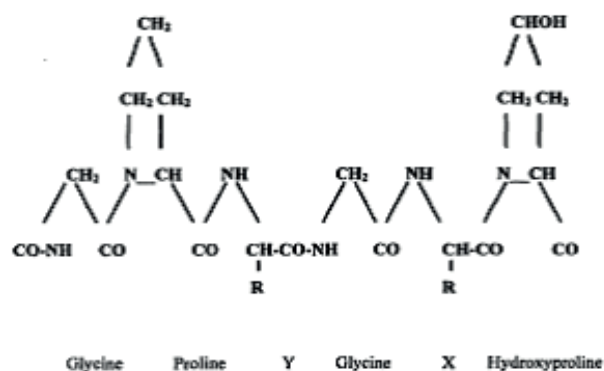


Fig. 2. Chemical configuration of gelatin [Ofori 1999]

DENATURATION OF SOLUBLE COLLAGEN TO GELATIN

The simplest way to transform collagen to gelatin is to denature soluble collagen. It involves hydrolysis catalysed by enzymes, acid or alkali. Thermal denaturation takes place in mild conditions by heating the collagen in neutral or slightly acidic conditions to about 40°C [Gimenez et al. 2005]. The transition is sharp and complete within a few minutes over a small temperature interval. The activation energy for denaturation is approximately 81 Kcal [Jusila 2004]. At that point only the hydrogen bonds and hydrophobic bonds that help to stabilize the collagen helix are broken causing the fibers and fibrils of collagen to dissociate into tropocollagen units. The next step, in the hydrolysis of collagen consists in breaking the intramolecular bonds between the three chains of the helix [Nishimoto et al. 2005].

HYDROLYTIC PRODUCT OBTAINED

If there are additional restraining bonds between the chains, then the hydrolysis can achieve the following three results:

- formation of three randomly coiled independent α -chains
- formation of a β -chain (two α -chains linked by one or more covalent bonds) and an independent α -chain
- formation of a γ -chain (three chains linked by covalent bonds).

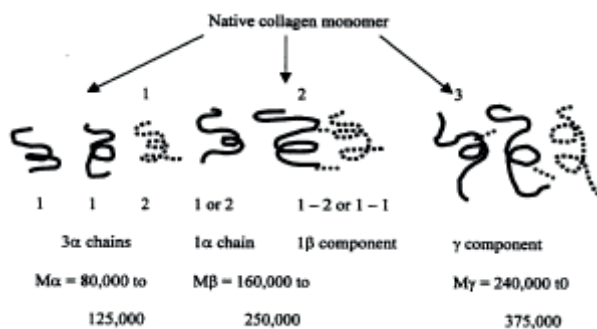


Fig. 3. Possible paths of collagen conversion to gelatin [Ofori 1999]

The α , β and γ forms of gelatin differ mainly in their molecular weight. For the α form molecular weight varies from 80,000 to 125,000 and for the β form 160,000 to 250,000. The molecular weight variation for the γ form is from 240,000 to 375,000 [Nishimoto et al. 2005].

FUNCTIONAL AND PHYSICAL PROPERTIES OF GELATIN

The functional properties of gelatin are related to their chemical characteristics. The gel strength, viscosity, setting behaviour and melting point of gelatin depend on their molecular weight distribution and the amino acid composition, the imino acids proline and hydroxyproline are important in the renaturation of gelatin subunits during gelling. As a result, gelatin

with high levels of amino acids tends to have higher gel strength and melting point [Johnston-Banks 1990]. Significant difference ($P \leq 0.05$) was found in the amount of total amino acids of insects' gelatin. The total of amino acids of insect gelatine was lower than that of the commercial gelatin (Table 2). Total amount of amino acids of sorghum bug gelatine was higher than that of water melon bug. Commercial gelatine had higher amount of glycine (123.87 mg/g N) than sorghum bug gelatine extracted using mild acid (51.76 mg/g N) and gelatine from melon bug using distilled water (51.47 mg/g N), and gelatine from melon bug using mild acid (50.70 mg/g N), the least amount of glycine was reported in gelatine extracted from sorghum bug using distilled water and hot water methods as 48.49 and 48.24 mg/g, respectively [Abdelfadeel 2012].

During conversion of collagen to gelatin, the inter- and intra-molecular bonds linking collagen chains, as well as some peptide bonds, are broken [Cole and McGill 1988]. There are differences in the extent and type of cross linking found in bones and skins [Sims and Bailey 1992]. The functional properties of gelatin are influenced by the distribution of the molecular weights, structures, and compositions of its subunits. SDS-PAGE pattern of insect gelatin showed low molecular weight chains, and the two gelatins contained protein with molecular weight of 40 kDa as main component of gelatin produced from denatured collagen [Mariod et al. 2011 b]. 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 et al. 2006]. 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 [Karim and Bhat 2009].

QUALITY CONTROL OF GELATIN

Gelatin production would be incomplete without a brief mention of the main aspect of routine quality control. The criteria for good food-grade gelatins are not as demanding as those for photographic gelatin.

Table 2. Amino acid composition (mg/g) of gelatin extracted from sorghum and melon bugs

Amino acid mg/100 g	Samples					
	control (CG)	SBG (DW)	SBG (AA)	SBG (HW)	MBG (DW)	MBG (AA)
Aspartic acid	23.5 ^b ± 1.9	18.4 ^c ± 1.5	14.6 ^d ± 1.5	27.6 ^a ± 2.9	13.3 ^f ± 1.2	13.9 ^e ± 1.3
Threonine	13.0 ^a ± 1.2	8.4 ^c ± 1.1	9.5 ^d ± 1.1	12.6 ^b ± 1.2	7.6 ^f ± 1.1	9.9 ^e ± 1.1
Serine	8.2 ^a ± 1.1	6.1 ^c ± 1.0	5.6 ^d ± 1.0	7.9 ^b ± 1.1	4.3 ^f ± 0.9	4.5 ^e ± 0.9
Glumatic acid	54.0 ^b ± 4.7	43.5 ^c ± 4.6	35.7 ^d ± 4.6	63.9 ^a ± 4.7	27.1 ^e ± 4.4	24.9 ^f ± 4.4
Glycine	123.9 ^a ± 8.9	48.5 ^c ± 4.7	51.8 ^b ± 4.6	48.2 ^f ± 4.5	51.5 ^c ± 4.6	50.7 ^d ± 4.7
Alanine	117.0 ^a ± 7.5	18.1 ^f ± 1.6	21.4 ^d ± 4.2	24.1 ^b ± 4.3	21.1 ^e ± 4.2	23.5 ^c ± 4.3
Valine	38.5 ^b ± 3.9	25.9 ^f ± 3.9	31.7 ^d ± 3.8	43.3 ^a ± 4.0	28.9 ^e ± 3.9	37.3 ^c ± 3.9
Methionine	5.2 ^a ± 2.5	3.8 ^d ± 2.7	2.9 ^f ± 2.6	5.1 ^b ± 3.5	3.2 ^e ± 2.6	4.6 ^c ± 2.6
Isoleucine	13.5 ^b ± 1.3	9.9 ^f ± 0.9	11.4 ^d ± 1.1	14.8 ^a ± 1.3	10.3 ^e ± 1.0	12.9 ^c ± 1.2
Leucine	34.9 ^a ± 3.9	20.2 ^f ± 2.7	23.7 ^d ± 3.7	31.8 ^b ± 3.9	20.6 ^e ± 2.7	25.6 ^c ± 2.9
Tyrosine	1.3 ^f ± 0.9	3.6 ^d ± 1.2	5.6 ^b ± 2.5	6.6 ^a ± 3.1	3.2 ^e ± 1.1	4.2 ^c ± 1.3
Phenylalanine	15.9 ^c ± 2.1	10.9 ^f ± 1.9	11.8 ^d ± 1.7	16.3 ^a ± 1.7	11.5 ^e ± 1.3	16.2 ^b ± 1.7
Histidine	23.8 ^a ± 3.3	7.4 ^f ± 0.5	9.9 ^c ± 0.9	14.3 ^b ± 2.9	8.5 ^e ± 0.9	9.8 ^d ± 0.9
Lysine	35.4 ^a ± 2.6	21.0 ^e ± 2.5	24.9 ^c ± 2.4	32.2 ^b ± 2.6	18.4 ^f ± 1.7	23.3 ^d ± 2.3
Ammonia	18.1 ^f ± 1.6	30.6 ^c ± 2.5	35.6 ^a ± 2.7	31.2 ^b ± 2.6	28.5 ^d ± 2.5	28.0 ^e ± 2.5
Arginine	100.2 ^a ± 6.7	35.1 ^e ± 2.8	51.0 ^c ± 4.9	58.1 ^b ± 2.9	33.9 ^f ± 2.7	40.9 ^d ± 3.9
Proline	200.7 ^a ± 13.7	52.7 ^f ± 4.7	62.6 ^d ± 5.9	90.4 ^b ± 7.2	58.9 ^e ± 6.0	70.0 ^c ± 6.5
Total	626.5	311.6	347.1	438.2	291.9	330.4

Mean ±SD value(s) bearing same superscript letter(s) within rows (for each amino acid) are not differ significantly ($P \leq 0.05$). SBG (DW) – gelatin from sorghum bug used distilled water extraction. SBG (MA) – gelatin extracted from sorghum bug using mild acid method. SBG (HW) – gelatin extracted from sorghum bug using hot water method. MBG (DW) – gelatin extracted from melon bug using distilled water method and MBG (MA) – gelatin extracted from melon bug using mild acid method. Source: Abdelfadeel [2012].

Viscosity and gel strength are the main physical properties used for grading any gelatin under carefully standardized conditions. Viscosity is determined at 60°C at a concentration of 6.67% (w/w) air-dried gelatin [Simon et al. 2003].

THE MECHANISM OF GELATIN GELATION

Gelatin forms gels similar to those of carbohydrates by forming a micro-structural network. It is unique in that, at concentration as low as 1.0% it will

form a thermoreversible gel. The gel converts to a solution as the temperature rises to 30°C to 40°C, thus gelatin gels tend to melt in the mouth [Morimura et al. 2002]. This is the desirable properties in ready to eat food such as clear dessert jellies and marshmallows. The well accepted mechanism of gelatin gelation is the random coiled helix reversion. The amino acid rich regions of the different polypeptide chains act as potential junction zones in that, upon cooling they take up a helical conformation resulting in the three-dimensional gel [Nishimoto et al. 2005].

GEL STRENGTH/BLOOM

The most important property of gelatine is the gel strength or “bloom”, which is a function of the molecular weight of the gelatine. The gel strength properties are related to the α - and β -chain components in the gelatine. The bloom strength, which is also related to viscosity, is an important property in the food industry as it is a good guide to the behavior of the gel. The bloom strength usually determines the grade of the gelatine. The gelatine must be tested under strict guidelines to be referred to as gel strength/bloom, which is defined as, “the weight required to push a cylindrical plunger, of 13 mm diameter, 4 mm into a previously prepared gel of 6 $\frac{2}{3}$ % w/w concentration matured at 10°C for 16-18 h [Johnson-Banks 1990]. The gel strengths typically range from 50-300. Different gel strengths are used for different applications. For example, Type B gelatine with gel strengths from 125-250 is commonly used for confectionary product. Type A gelatine with low gel strength (70-90) can be used for the fining of wine and juice [Wittich 2005]. The ability to form heat-reversible gel is one of the most important properties of gelatin. The jelly used should be conditioned for 16 to 18 hours at 10°C and should have a concentration of 6.67% (w/w) air-dried gelatin [Gómez-Guillén et al. 2002]. Commercially gelatins vary from 50 to 300 bloom, hydrolysis of gelatin gels can be initiated by numerous factors including pH, temperatures, enzymes, acids, bases and as well as bacteria. These cause a reduction in the gelling properties of the gelatin [Park et al. 2001].

SOLUBILITY

Gelatin swells upon contact with cold water forming large visible swollen particles, when heated above the melting point, the hydrated gelatin will rupture and go into solution, and form a gel upon cooling. Gelatin is practically insoluble in alcohol and non-polar solvents such as sorbitol, mannitol and glycerine [Jamilah and Harvinder 2002].

VISCOSITY

The viscosity produced by gelatin solutions is one of its most important functional properties generally

viscosity measurements are read from the flow time of a gelatin solution through calibrated viscosity pipettes. The calibrated Ostwald viscometers are highly recommended for gelatin viscosity determinations, the results are expressed in milipoise. This unit of expression is calculated from the kinetic viscosity by the following equation:

$$V = \eta / 10 \cdot \rho$$

Where ρ has a value of 1.00 and is the density of the 6.67% gelatin solution. Thus, a reading in centipoises is equivalent to the value in centistokes [Nishimoto et al. 2005].

MELTING POINT

The melting point is the temperature at which a gelatin gel softens sufficiently and allowing carbon tetrachloride drops to sink through it. Factors such as the maturing temperature and the concentration of the gelatin gel tend to affect its melting point [Gómez-Guillén et al. 2002].

SETTING POINT

The setting point of a gelatin solution is dependent on its thermal and mechanical history. Higher setting temperatures are encountered when the solution is cooled slowly in comparison to rapid chilling, Mechanical action hinders or delay setting [Simon et al. 2003].

PRODUCTION OF GELATIN

There are a large number of unit processes used in the production of gelatin from ossein, pig skin, cow hide, and fish skin. The production process for gelatin includes following steps: pretreatment, washing, extraction, purification, drying, grinding, blending and quality control [<http://www.pbgelatins.com>].

There are basically two processes by which collagen is processed to gelatin:

- The acid process is mainly used with pig skin and fish skin and sometimes bones raw materials. In this process collagen is acidified to about pH 4 and then heated, denatured, defatted, filtered, concentrated, then drying by passing dry air over the gel.

The obtained product is grinded and blended to customer requirements and packed. The resulting gelatin has an isoionic point of 7 to 9 based on the severity and duration of the acid processing of the collagen which causes limited hydrolysis of the asparagine and glutamine amino acid side chains [Cole 2000].

- The alkali process is used on bovine hide and collagen sources; in this process collagen is submitted to a caustic soda or lengthy liming process prior to extraction. After the alkali processing, the collagen is washed and treated with acid to the desired extraction pH. The collagen is then denatured and converted to gelatin by heating, then vacuum evaporated, filtered, gelled, dried, grind and blended [Cole 2000].

REMOVING OF PROTEINS DURING PRE-TREATMENTS

NaOH or Ca(OH)₂ with OH concentrations varying from 0.01 to 0.5 mol/L were used for alkaline

pre-treatment's. The total protein in the pre-treatment solutions was determined by the biuret method (Table 3), and it suggests that small amounts of proteins could be extracted from the source by alkaline pre-treatment. Then the noncollagenous proteins can be confirmed by SDS-PAGE.

GELATIN USES AND APPLICATIONS

General uses

Gelatin is used as a stabiliser (yoghurt), thickener (jam), and texturizer and emulsifier (oil-in-water emulsions). Gelatin is used as a foaming, emulsifying, and wetting agent in food, pharmaceutical, medical, and technical applications due to its surface-active properties [Lobo 2002]. Gelatin was among the first commercial raw materials suitable as a contact preservative for meat and meat products. The literature contains few references to methods to clarify tea, including the use of gelatin. Gelatin capsules have been used to encapsulate nutritional supplements as well as medications,

Table 3. Total protein extracted by pre-treatments and the yields and properties of gelatin extracts (mean ±SD, based on 2 determinations)

Pre-treatment	Total protein in pre-treatment solution mg	Extraction yield %	Gel strength g	pH of gelatin extracts
NaOH 0.01	54.2 ±2.0	7.1 ±0.3	62 ±2	8.48 ±0.05
NaOH 0.1	82.9 ±2.3	6.9 ±0.1	61 ±6	9.69 ±0.08
NaOH 0.2	76.6 ±3.2	6.9 ±0.2	77 ±3	9.90 ±0.03
NaOH 0.5	83.9 ±1.3	6.9 ±0.3	37 ±2	9.94 ±0.05
Ca-0.01	54.4 ±2.0	8.8 ±0.1	19 ±4	8.08 ±0.12
Ca-0.1	89.1 ±2.6	8.0 ±0.3	50 ±2	9.66 ±0.05
Ca-0.2	–	8.2 ±0.2	54 ±2	9.66 ±0.06
Ca-0.5	–	7.1 ±0.4	47 ±1	9.56 ±0.10
AH-0.05	22.0 ±1.9	16.0 ±0.6	251 ±1	4.88 ±0.03
Ca-0.1, AH 0.05	–	13.8 ±0.4	401 ±4	6.07 ±0.06
Original	–	13.4 ±0.6	33 ±3	7.01 ±0.05
Inhibitor	–	14.9 ±0.2	112 ±7	6.44 ±0.07

Presence of high concentration of Ca₂₊ may affect the total protein determination by the biuret method. Source: Zhou and Regenstein [2005].

the emulsion stabilizing properties of a set of commercial casein and whey protein ingredients, under neutral pH conditions, were compared with the properties of commercial fish gelatin as an emulsifying agent in oil soluble vitamin encapsulation, it was 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 [Dickinson and Lopez 2001].

Industrial applications of gelatin

Gelatin is an important hydrocolloid in the food, pharmaceutical and photographic industries.

Food

Gelatin has been widely used in food additives and healthy food due to its high content of protein and amino acid. Edible gelatin is made from animal hide and bone through the processing techniques such as defatting, pulverization, freezing and drying [Fu et al. 2000]. The unique hydrocolloidal nature of gelatin has enabled it to find numerous applications in the food industry. These can be divided into four main groups namely; confectionery (mainly for providing chewiness, texture, and foam stabilization) and jelly deserts (to provide creaminess, fat reduction, and mouth feel), dairy products (to provide stabilization and texturization), meat products (to provide water-binding), and hydrolyzed gelatin applications [Nishimoto et al. 2005, Karim and Bhat 2009]. General saying gelatine is used in foods as a beverage and juice clarifier, deserts and yogurt thickener. Further uses include fruit toppings for pastry, instant gravy, instant sauces and soups, edible films for confectionery products [Karim and Bhat 2008], as a stabilizer in ice cream, cream cheese, and cottage cheese, as well as in food foams and fruit salads [McWilliams 2001]. Gelatin is also used in coating meat products to reduce color deterioration and gelatin coat is equally effective during light and dark storage [Antoniewski et al. 2007, Tyburcy 2010]. Gelatin is used in canned meat products such as hams, loaves, frankfurters, Vienna sausages, and cured, canned pork), to hold juices lost during cooking and to provide a good heat transfer medium during cooking. Gelatin is also used in emulsified meats and jellied products at levels ranging from 3 to 15%, but more typically from 0.5 to 3% [Sams 2001].

Gelatin is truly remarkable in terms of its many functional properties in food applications. A variety of examples are given in Table 4.

Table 4. Functional properties of gelatin in foods

Function	Function
Gel former	gelled desserts, lunch meats, confectionery, pate, consommé, aspics
Whipping agent	marshmallows, nougats, mousses, soufflés, chiffons, whipped cream
Protective colloid	confectionery, icings, ice creams, frozen desserts and confections
Binding agent	meat rolls, canned meats, confectionery, cheeses, dairy products
Clarifying agent	beer, wine, fruit juices, vinegar
Film former	coating for fruits, meats, deli items
Thickener	powdered drink mixes, bouillon, gravies, sauces, soups, puddings, jellies, syrups, dairy products
Process aid	microencapsulation of colors, flavors, oils, vitamins
Emulsifier	cream soups, sauces, flavorings, meat pastes, whipped cream, confectionery, dairy products
Stabilizer	cream cheese, chocolate milk, yogurt, icings, cream fillings, frozen desserts
Adhesive agent	to affix nonpareils, coconut and other items to confections, to bond layered confections together, to bind frostings to baked goods, to bind seasonings to meat products.

Source: Turner [1988].

Gelatin in aqueous food systems readily forms a hydrogen bond with water because of many exposed polar regions. As gelatin binds with water, it swells and absorbs water. It can then be dispersed in hot water and with other ingredients. The formation of a gelatin gel is endothermic and occurs gradually as the energy of the system dissipates. As the concentration of gelatin increases, the rate of gelation also increases, thereby increasing the firmness and decreasing tenderness [McWilliams 2001]. Insect gelatine was used

to produce ice cream, the ice cream produced using commercial gelatine gave better taste and melt mouse than that with insect gelatine. The difference between the two developed ice cream products was significant ($P \leq 0.05$) as regard to their texture which means the commercial gelatine ice cream has a better texture than that of insects gelatine ice cream, and the ice cream made used gelatine with 0.5% a better than that used 1% insects gelatine. By comparing the calculated scores of panelist, it was found that there was significant difference ($P \leq 0.05$) between the developed ice cream products at the 5% level. But also the ice cream made with commercial 0.5% was best followed by ice cream made by insects gelatine 0.5% and ice cream made by insects gelatine 1%, that means no high difference between three products ice cream as general preference [Abdelfadeel 2012].

Halal and kosher gelatin

Halal food, as opposed to *haram* food, is food that is “ritually fit for use” because it has been “sanctioned by Islamic law”. The Qur’an forbids Muslims from eating anything except food defined as being *halal* [Milne 2007]. The kosher dietary laws derived from the Torah, determine which foods are “fit or proper” for Jews and deal predominantly with 3 issues: allowed animals, the prohibition of blood, and the prohibition of mixing milk and meat [Regenstein et al. 2003]. Important in many food products, gelatin is probably the most controversial of all modern kosher and halal ingredients. Gelatin can be derived from beef bones, or beef skin and fish products. These gelatins would be fully kosher and halal, and acceptable to almost all of the mainstream religious supervision organisations [Sams 2001]. More than 1.3 billion people in the world are Muslims; they trade on about 150 billion dollars as halal products. More than 10 million Americans are deliberate consumers of kosher food and they are purchasing almost 7 billion dollars worth of kosher products [Regenstein et al. 2003]. Gelatins and both soft and hard capsules of various sizes that produced from plants are considered halal gelatins. These products are available at competitive prices. This is an important new development that should be of interest to the nutraceutical and drug markets. Similarly, currently many companies producing certified halal

gelatin from cattle bones of animals that have been slaughtered by Muslims [Regenstein et al. 2003].

Gelatin alternatives for the food industry

The issue of gelatin alternatives has recently gained increased interest especially within Europe with the emergence of the bovine spongiform encephalopathy virus that has infected cattle. Many gelatin alternatives proposed for the food industry are polysaccharides, such as many gellan, alginate or carrageenan based gels. These alternatives generally have less flexible molecular backbones, leading to higher hot viscosities than gelatin [Morrison et al. 1999]. Some hydrocolloids considered for gelatin replacement include, mixed high-methoxyl/low-methoxyl pectin gels, which is not considered a good candidate as a gelatin alternative, since it forms thermally irreversible gel and requires a low pH and high-soluble solids. However, low-methoxyl (LM) pectin appears to be more flexible in terms of manipulation of gelling conditions, although at high sucrose concentrations. Modified starch/wheat fiber gel, is another gelatin alternative used a combination of a dual modified starch and wheat fiber gel to replace gelatin in yogurt. The starch-to-wheat-fiber-gel ratio was critical, with the optimum ratio at 60% starch to 40% wheat fiber gel. Yogurts with gelatin replacer showed higher stability against storage temperatures over 20°C. High acyl gellan gum which produces soft, elastic, thermoreversible gels for applications such as cultured dairy, dressings, jams and jellies, dessert gels, dairy and fruit beverages, milk puddings and confectionery. Carrageenan is a new iota carrageenan extract by using a new, proprietary extraction process was developed to be used for gummi-type, or molded candies. The new iota carrageenan-based products allow for shorter conditioning times, easier demolding and alternate molding processes [Karim and Bhat 2008].

Pharmaceutical

The largest proportion of gelatin procured by the pharmaceutical industry is used mainly for hard and soft gelatin capsules (Softgels) and for tableting, tablet coating, granulation, encapsulation and microencapsulation [GIMA 2012]. Where it helps prevent oxidation and makes the preparation more palatable [Nishimoto et al. 2005]. The capsules are formed on

mould pins, the surface of which carries a lubricant to facilitate the subsequent removal of the capsule. Gelatins with bloom in the range of 0 to 140 are offered for the microencapsulation of vitamins A, D and E. Fish gelatins have exceptionally good film forming properties and are offered for microencapsulation where religious reasons require the use of non-mammalian products [Morimura et al. 2002]. The earliest reference to gelatin capsules makes no specific mention of any ingredients other than the medicines encapsulated. However, current gelatin capsule formulations contain a wide variety of other ingredients. Each ingredient needs to be addressed on its own merits [Gold et al. 2001]. Gelatin capsules (gel-caps) are commonly used to encapsulate various foods, nutritional supplements, and medicines, applications for this technique have increased in the food industry since the encapsulated materials can be protected from moisture, heat or other extreme conditions, thus enhancing their stability and maintaining viability [Gibbs et al. 1999]. Gelatin is used as excipients in pharmaceutical formulations, including vaccines, and is used as a binder for tablets, excipients may be originating from quite distinct sources, including gelatin [Sam 2000]. 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 compared to values reported for pigskin gelatin [Gómez-Guillén et al. 2007].

Photographic

The collodion wet process was replaced with a gelatin emulsion which could be dried and was not required to be used immediately. Gelatin emulsions have, through the years, been continually improved in quality and speed. Gelatin is still the best medium known for making photographic emulsions [GMIA 2012]. Gelatin is used as a component in a photographic developer during the processing of the exposed film material. A photographic developer is usually an alkaline solution that contains a reducing agent which reduces silver halide rapidly when the reaction is catalyzed by the latent image formed during exposure, and slowly

when the silver halide has not been exposed. The gelatin enhances the ability of the developer to distinguish between the exposed and the unexposed crystals [Park et al. 2001].

CONCLUSIONS

Increase in demand for halal and kosher foods have created a demand for new sources of gelatin for food applications. A number of studies have addressed properties of fish skin gelatins showing that their properties differ from those of mammalian gelatins and vary between species. The functional properties of gelatin are related to their chemical characteristics. The gel strength, viscosity, setting behaviour and melting point of gelatin depend on their molecular weight distribution and the amino acid composition. Extraction of gelatin from melon and sorghum bugs gave high yield, melon and sorghum bugs gelatin had similar molecular weight profiles compared to bovine gelatin. The two gelatins contained 40 kDa as the main components of gelatin. Microstructures of the insect gelatin examined with the scanning electron microscope showed that melon bug exhibited the finest gelatin network with very small voids.

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Received – Przyjęto: 12.10.2012

Accepted for print – Zaakceptowano do druku: 3.01.2013

For citation – Do cytowania

Mariod A.A., Adam H.F., 2013. Review: gelatin, source, extraction and industrial applications. Acta Sci. Pol., Technol. Aliment. 12(2), 135-147.