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Extraction of collagen and gelatine from animal wastes

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Abstract: Collagen is the structural protein found in connective tissues in mammals and comprises about 60% of whole body protein. Gelatine is the protein product obtained from collagen via various procedures such as thermal denaturation, partial hydrolysis and chemical treatments. Animals such as bovine, sheep, chicken and fish are most commonly used for the production of foods for human nutrition. The wastes such as skins and bones raised during manufacturing of meat products are considered as rich sources for collagen. Various procedures are developed to extract collagen and gelatine as well from those wastes. The resultant protein materials with different purities can be served as value added products promising various application fields based on their characteristics. Many types of collagen are present based on structural differences however, most of the collagen in the body consists the types of I, II and III. Type A and type B are also the two common types of gelatine derived from collagen via acid or alkali treatments at different isoelectric points. Differences in amino acid composition can be encountered in different gelatine types obtained from different sources as well. This study briefly summarizes recovery of collagen and gelatine from animal wastes. Well-defined collagen and gelatine obtained from those wastes can be used to develop biomaterials available for cell scaffolding in tissue engineering besides serving opportunity in manufacturing edible products.

Keywords: collagen, gelatine, purification, animal wastes

1 Introduction

Gelatine produced by partial hydrolysis of collagen is not considered as a food additive in our country as in many countries (Anon, 2008). Gelatine is used for the development of textural and rheological properties in various foods (Baziwane and He, 2003). Due to its many technological features, gelatine is widely used in the production of food cosmetics, medicine, photography, pharmacy and agricultural products (Yetim, 2011). Collagen, the raw material of gelatine, is a structural protein that is quite common in animals. It is found in high amounts in tendons, skin and bones (Balian and Bowes, 1977; DeMan, 1999). These wastes that occur during the processing of animals such as cattle, sheep, goats and poultry after slaughter can be shown as an inexpensive and potential source for gelatine production. The gelatine obtained from those has a wide range of uses due to its technological and functional properties. This review aims to give brief information about recovery of collogen and gelatine from various animal wastes.

2 Raw material of gelatine: collagen

Collagen is one of the most common proteins in animals. Its ratio to all proteins in the body is %60 (Aberle et al. 2001; Schrieber and Gareis 2007). Collagen fibers consist of protein called tropocollagen. With the crystallization of tropocollagen, microfibrils, collagen fibrils from microfibrils, and collagen fibers from collagen fibrils. There are 27 different types of collagen. Type 1 collagen is found is bones, skin and tendons, type 2 colagen cartilage tissue and type 3 collagen in young tissues (Gomez-Guillen et al., 2011). The structure of collagen contains %12 proline, %11 alanine, %35 glycine and %9 hydroxyproline. The most important feature that distinguishes collagen from other proteins is the proline and hydroxyproline ratio (Gözükara 2001). The amount of hydroxyproline directly gives information about the amount of collagen and/or the amount of gelatine. There are cross-molecular cross-links in collagen. The number of these bonds increases as the animal ages. With the collagen molecule aging, trivalent bonds are established instead of divalent. Soluble gelatine can be obtained by subjecting these crosslinks to a more severe process (Ledward 2000). Collagen, which is the raw material of gelatine, is a water-insoluble protein with a molecular weight of 330 kDa, unlike gelatine.

3 Gelatine

Gelatine is a protein produced by controlled hydrolysis of collagen from the skin, bone and connective tissues of animals such as cattle, sheep, goats, pigs, poultry and fish. Gelatine, with 60-65 kDa in weight, is produced by breakig the cross bones in the collagen molecule weighing 300-500 kDa (Ockerman and Hansen 1988). It consists %51 carbon, %7 hdyrogen, %25 oxygen, %17 nitrogen molecules. About %85-92 of the gelatine molecule is consisted of the collagen molecule (Nur Hanani et al. 2014). Gelatine considered as GRAS (generally regarded as safe) status has superior properties compared to other gelling carbohydrates. It has properties such as thickener, gelling, emulsifier, foaming and film forming. It is easy to digest and can melt at the body temperature of human. Besides, gelatine can also provide numerous health benefits such as maintaining body tissues, giving healthy and youthful appearance to skin, strengten joints and bones, improving hair and nail growth, and helping to regulate blood sugar (Fernandez and Perez 1998; Rubio et al., 2008). Since gelatine has many technological, functional and health promoting features, it has a wide range of applications in food industry and medicine as well. The fact that enabling to produce from cheap and available raw materials affects the availability of gelatine positively.

4 Gelatine production

Gelatin production for targeted purposes includes sequential steps of raw material preparation, demineralization, acid and alkali treatments, and solvent extraction with varying process conditions. Major stages are given below and figure1 presents a general scheme for obtaining gelatine from animal wastes.

4.1 Raw (waste) materials

Cattle, sheep, goats, poultry and pig skin and bones are mostly used as raw materials for the production of collagen and gelatine. Hairs, blood, meat residues, fat and connective tissues were removed from bones and skins before the treatment. They are cut into small pieces in order to increase surface area and enhance recovery. In case of bone samples, calcium carbonate in the structure is removed with the help of concentrated HCl solution. As a result of demineralization, bone material called as ossein is obtained. After the raw materials are prepared properly, they are ready for acid and alkali treatments in the following step.

4.2 Alkali and acid application (pre-treatments)

Cross links in the structure of collagen are broken down by acid and alkali treatment. In the hydrolysis of collagen, the cross links are cleaved with dilute alkali and/or dilute acid, while the protein chains in the collagen remain intact (Hattrem et al. 2015). Type A and B gelatine produced from collagen are two common types of gelatine with different isoelectric points. The isoelectric point of A type gelatine is between 8,9 – 9,4 while B type gelatine is between 4,8 – 5,5. While chemical applications are made in the production of gelatine from collagen, a moderate temperature application can also be made. The cruciate ligaments which are broken down by chemical application and moderate temperature application have been prepared for extraction as well.

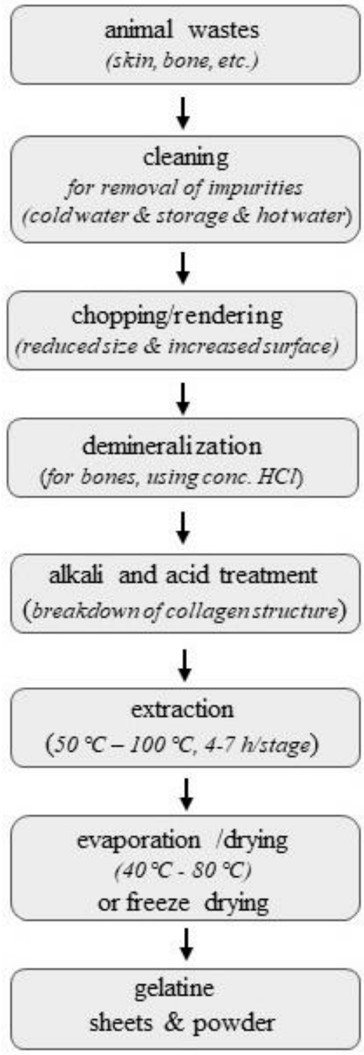

Figure1. A scheme of gelatine production from animal wastes

4.3 Extraction

Extraction is carried out with the help of acidic or alkaline solvents. Initial temperature can be accepted as 50 °C and it can be increased up to 100 °C in the following steps. Each stape takes an average of 4-7 hours. First, temperature applied is above the denaturation temperature of collagen (Erge and Zorba 2016). Oil emulsion is prevented by applying mixing during the extraction process. Due to the increase in temperature, maillard reaction may occur and dark colored gelatine could be obtained. (Duconseille et al. 2015). The extraction process is significantly affected by the age of the animal used, the particle size and the temperature applied. The age of animal and particle size prevent the occurrence of a homogeneous extraction process. By performing the extraction process at high temperature for a long time, high efficiency and gel strength can be obtained in the resultant gelatine product. In case of the lower temperature, extraction cannot occur properly and the yield decreases (Yetim 2011).

After extraction, gelatine containing solutions are filtered to remove oil and other suspended contaminants. Gelatine exract is concentrated by evaporation until it reaches a certain viscosity. High quality gelatine is aimed at about 20-25%, but if lower quality gelatine is targeted, it is reduced to around 40%. (Ledward 2000). Then gelatine is turned into gel by sterilization. Gelatine is pulverized by drying process. The moisture content of commercially produced gelatines varies between 8 and 12% (Yetim 2011). Physicochemical properties such as isoelectric points, moleculer weight distribution, gel strength, viscosity, melting and gelling temperature and color can be determined to assess the quality characteristics of the resultant gelatine product.

4.4 Physicochemical Properties of Gelatine

Physical and chemical properties of gelatine obtained after extraction should be evaluated in order to determine the quality of gelatine.

The amino acid composition of the gelatine is different from the collagen amino acid composition if treated with alkali. In alkaline treatment, non-ionized asparagine and glutamine amino acids turn into carboxyl groups. As a result, gelatine has an acidic property. For this reason, the isoelectric point of alkali-treated gelatine is between 4.8-5.5, and the isoelectric point of acid-treated gelatine is between 7.0-9.4 (Ledward 2000).

Gelatine produced after various processes consists of polypeptides with different molecular weights. The molecular weight distribution varies according to the non-hydrolyzed crosslinks in polypeptides in gelatine, intra-molecular and intermolecular covalent bonds in the material used as the source, and polypeptide chain length (Eysturskaro et al. 2010; Schrieber and Gareis 2007). High temperature and concentrated chemicals applied in the pretreatment and extraction stages cause the primary structure to break down and the molecular weight to decrease.

The molecular weight distribution of gelatine significantly affects viscosity, bloom value and melting/gelling temperature value. High molecular weight is the determinant of high viscosity, high bloom value and high gel strength (Ledward 2000).

Gel strength is an expression of gelatine gel resistance or hardness, and it is an important quality feature that allows gelatine to be classified as industrial. As bloom value of gelatine increases, market value and quality increase as well (Sarbon et al. 2013). Bloom value varies depending on the molecular weight distribution of gelatine. Molecular weight varies depending on the chemicals used and the temperature used in the pretreatment and extraction stages as well. In chemical processes under moderate conditions gelatine with high bloom value can be obtained. On the contrary, as a result of long-lasting and high-temperature processes gelatine with low bloom value can be obtained due to the hydrolysis of the polypeptide chains (Eysturskaro et al. 2010). Other factors affecting the bloom value of gelatine are the amount of proline and hydroxyproline amino acids, amino acid distribution and the source of collagen to be obtained.

Viscosity is one of the most important quality features of gelatine. Molecular weight distribution is one of the most important factors affecting the viscosity of gelatine. The viscosity increases as the molecular weight increases. The concentration and temperature of the chemicals applied in the pre-treatment and extraction stages affect the molecular weight of the gelatine, as well as the physicochemical properties such as viscosity (Uriarte-Montoya et al. 2011). The viscosities of the commercial gelatines can vary between 2-7 cP. Specially produced gelatines can have 13 cP viscosity. High viscosity gelatines are stronger but more flexible whereas low viscosity gelatines are weak and soft (Karayannakidis and Zotos 2014).

The temperature at which the gelatine gel becoming a solution is defined as the melting point, and the temperature at which the gelatine solution turning a gel is considered as the gelling point (Schrieber and Gareis 2007). Factors affecting the melting and gelling temperature of gelatine are bloom value, concentration, amino acid distribution, source of collagen and amount of proline and hydroxyproline.

The transparency and color of the gelatine obtained as a result of the processes is one of the quality criteria that significantly affects the technical properties, commercial capacity and consumer preferences (Du et al. 2013). As a result of the maillard reaction between the proteins and carbohydrates can provide medium the color of gelatine becomes brown. The intensity of this color can vary depending on the extraction time and temperature (Duconseille et al. 2015). Thus, the color of gelatine differs based on the formation of the maillard reaction, the increase in pH, the source of the gelatine and the concentration of chemicals used in the pre-treatment stage.

All of the physicochemical properties are important factors that determine the market value of gelatine. By calculating and explaining these factors quantitatively, gelatine is used in accordance with the intended purpose.

5 Conclusion

The need for gelatine is gradually increasing in the food industry. Wastes and by-products such as skin, bone, hair and tendon obtained from animal sources such as cattle, sheep, chicken and fish can be evaluated as the raw materials for collagen and gelatine production. However, as these raw material resources are not sufficient, searches for alternative resources are still ongoing. In addition to the food industry, gelatine can find wide use in the fields of medicine, cosmetics and pharmacy. Advantages that increase the use of gelatine are easy production, ability to melt at body temperature its own desirable brightness and transparency, GRAS status and being rich source of protein supporting human health. The disadvantages can be stated as the limitation of its use as an animal waste product in terms of religious rules. When the advantages and disadvantages are evaluated together, the researches on gelatine production and application are gradually increasing. Considering the physicochemical properties advanced researches are needed to optimize the yield and quality of gelatine.

Authors' contributions

This study is based on a part of Master Thesis of Ayşegül Gündem and Dr. Özgür Tarhan is the supervisor of her thesis. Equal contribution is provided by the authors.

Conflict of interest disclosure

The authors did not declear any conflict of interest.

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