Dosimetric impact of interplay effect in lung IMRT and VMAT treatment using in-house dynamic thorax phantom

S Julia, Nurlely and D S Soejoko

1 Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok 16424, Indonesia

E-mail: s.julia2805@gmail.com

Abstract. The objective of this research was to determining of protein from connective tissue (protein) in the inner layer of organic and broiler chicken gizzard. The presence of collagens was observed by submersion with 0.1 M NaOH base pH 8 and without submersion. The physiochemical properties and characteristic of collagen from inner layer chicken gizzard such as FTIR spectra, element content, morphology and SDS-PAGE pattern were evaluated. FTIR spectrum in the sample without submersion shown collagen components had wave number that are in the range of wave number (3289-1078 cm\(^{-1}\)) bovine collagen. While, FTIR spectrum in the sample with submersion exhibited removal of molecular functional groups and decreasing intensity of infrared absorption. The element content and morphology of two samples indicated that the elements found in the samples were recognized as the elements generally found in proteins and had little structure at SEM micrograph. SDS-PAGE pattern demonstrated that collagen from inner layer chicken gizzard had >120 kDa on molecular unit weight indicating that type 1 collagen is a major component of inner layer chicken gizzard collagen. This study demonstrated the existence of collagen from exploited bio-waste to be an alternative possible source for collagen production.

1. Introduction

Collagen is a material which has a ranged strength and fibrous shape structure. Collagen is the most abundant animal protein with the amount about 30% of total protein in the body [1,2,3]. Nearly, a third of the protein in the body of vertebrates are collagen [2]. Collagen presents in all organ showing strength and stiffness. Collagen is a major fibrous component in bones, teeth, cartilage, dermis and tendons. This structure consists of collagen fibril that looked like transverse lines, which are organized by biological functions of a cell. Collagen fibrils consist of repetitive polypeptide subunit with a triple helix shaped and are called tropocollagen. In one piece of collagen, chains consist of more than 1000 amino acid residue [4].

Collagen has many gene types which involved in the biological function of a cell such as survival and differentiation [4]. Each type of collagen has different gene types which shown in the composition of the polypeptide chain. Due to its unique characteristic, collagen has been used in various fields such as medical and biotechnology [1, 2]. Collagen has been used as a raw material of cosmetics and pharmaceutical industries. Non-denatured collagens can be used as the component of food, cosmetics, biomedical, and pharmaceutical substances. Denatured-collagen known as gelatin is commonly used in food and many biomedical substances. Biomedical and pharmaceutical substances consisting of
collagen can be used in various treatments such as hypertension, urinary incontinence and pain associated with osteoarthritis and also in tissue engineering for implants in humans [5].

Recently, most commercial collagens come from skin and bone from bovine and porcine [1, 2, 3, 5]. Due to the outbreak of bovine spongiform encephalopathy (BSE) and foot and mouth disease (FMD) [1, 2, 3, 6, 7, 8], so that the use of cow collagen has a risk of contracting this disease. Scientists have found that skin, bone, fin and scales of freshwater and marine fishes, chicken skin, marine sponge, and bullfrog skin can be used as alternative collagen source, which have no ethnic or safety-related consumer concern [1].

A greater amount of chicken consumption and a large number of chicken cuts produce wastes. Although, there is an attempt to decrease the waste, the quantity of the waste produced is increasing annually [5]. Facing of the above problems, recently, there has been much interest in investigating the most effective use of under-utilized resources wastes. Based on the results of research conducted by Kiruthiga et al. proved that the waste from the broiler chicken can be beneficial as a source of collagen production [6]. Additionally, Bonifer et al. also said that there are about 3% of collagen in the chicken skin that may provide the potential for increasing the value of products [9]. In this study, an alternative collagen production has been explored from the inner layer of chicken gizzard. The result indicated that chicken gizzard collagen was almost similar with bovine collagen.

2. Methodology
The chemicals that were used in this study were NaOH base 0.1 M pH 8, tris buffer HCl 0.5 M pH 6.8 and 200 mg KBr pellets. All chemicals used in this study were analytical grade and are from Merck-Germany. The samples were the inner layer of organic and broiler chicken gizzard and obtained from the traditional market.

2.1. Sample treatment
Sample from organic chicken (sample I) and those from broiler chicken (sample II) are divided into four groups, sample I without submersion (sample I.1) and with submersion (sample I.2), sample II without submersion (sample II.1) and with submersion (sample II.2).

All samples were initially cleaned and heated at the low temperature at about 60°C. Sample I.2 and II.2 were soaked in NaOH base 0.1 M pH 8 for 16 hours and changed the solvent twice a day. The resultant solvent was washed with aqua dest for 8 hours until sample in the neutral condition. All of the samples (sample I.1, I.2, II.1 and II.2) were ground to be a powder with 50 mesh and 120 mesh particle size. Samples with 120 mesh particle size were further called sample I.1a and sample II.1a and the others with 50 mesh were called sample I.2b and II.2b.

2.2. Fourier transform infrared (FTIR) spectroscopy
Molecular functional group and its modification in the sample were observed with ABB FTIR MB3000 in Material Analysis Laboratory, Physics Department – FMIPA IPB. For infrared spectroscopy, 2 mg of samples and 100 mg KBr were pressed into a pellet. Measurements were performed in the range of wavenumber 4000 – 400 cm⁻¹.

2.3. Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX)
Surface morphology was studied by SEM equipped with EDX (SEM-EDX EVO 50 ZEISS) in Puslitbang, Forest Departement, Gunung Batu - Bogor. SEM was used to examine the morphology and the microstructure of the collagen in four types of samples, and EDX identified the elements. The magnifications were 100x, 250x, 500x and 1000x.

2.4. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)
The examination of the sample unit weight protein was performed by SDS-PAGE ATTO Electrophoresis Page Run R WSE-110 in LIPI Biotechnology-Biological Research Centre. The band was observed using polyacrylamide gel. Gel consist of 3.9% stacking gel and 8% separating gel. For
this examination 0.25 gram of sample was dissolved in 4 ml of 0.1 M NaOH base and kept in -10°C temperature. The sample was inserted into the plate wells. The plate containing the samples was connected to the cathode and the anode which was connected to the Tris buffer HCl. This was observed for 120 minutes and 300 mA electrical current. Electrophoresis results can be visualized by staining gel. This staining using a staining solution with 0.1% Coomassie Brilliant Blue R-250.

3. Results and discussion
Sample with submersion (sample I.2 and II.2) were clearer and thinner than sample without submersion (sample I.1 and II.1). The submerged sample had a larger size may be because of increasing fibre size. FTIR spectra of samples in Figure 1 exhibited the characteristic peaks of amides A, B, I, II and III (amide A at wavenumber 3367 cm\(^{-1}\), amide B at wavenumber 2924 and 2854 cm\(^{-1}\), amide I at wavenumber in the range of 1600-1700 cm\(^{-1}\), amide II at wavenumber 1529 cm\(^{-1}\) and amide III at wavenumber 1232 and 1234 cm\(^{-1}\)). It was found that IR spectra of the sample I.1 and II.1 had similar wavenumber peaks that are in the range of wave number bovine collagen [8]. The absorption characteristics of amide A commonly associated OH -bond in hydroxyproline. Amide B peaks, representing the asymmetrical stretch of CH\(_2\), for sample I.1 and II.1, were in the wave number of 2924 and 2854 cm\(^{-1}\), whereas bovine collagen was at 2920 and 2853 cm\(^{-1}\) [8], and fish collagen was at the wavenumber of 2926 and 2928 cm\(^{-1}\) [2]. Amide I is mainly associated with CO-stretch vibration in the chain peptide group that are owned by amino acids in collagen. Amide I that attributed groups with C- or O- stretching vibration along the polypeptide backbone, is a sensitive marker of the peptide secondary structure [2]. Amide II is associated N-H bending vibration and C-N stretching vibration. The amide III are in the range of wave number from cow collagen (1260 cm\(^{-1}\)) [2] and fish collagen (1242 and 1244 cm\(^{-1}\)) [8]. This is associated with NH bending corresponded to glycine residue.

![Figure 1. FTIR for sample without submersion (sample I.1 and II.1).](image1)

![Figure 2. FTIR for sample with submersion (sample I.2 and II.2).](image2)
FTIR for the sample with submersion (sample I.2 and II.2) in figure 2 exhibited removal of molecular functional groups and decreasing intensity of infrared absorption. Both of samples has lost amide A. Moreover, amide B for sample II.2 did not appear but still occur on sample II.1 at the wavenumber of 2931 cm\(^{-1}\). Differ with the sample without submersion, this sample shown amide II peaks at different wave number (1404 cm\(^{-1}\)). This wave number is similar to the amide II from fish collagen at the wavenumber of 1403 cm\(^{-1}\), that originated from COO- stretching symmetry. While, amide III only existed in sample II.2 with skeletal stretching. Amide lost from these samples was predicted due to the submersion in 0.1 M NaOH base and temperature treatment. Many studies that avoid amide lost were conducted at 4\(^{\circ}\)C in treatment process [1, 2, 5, 7, 8, 10, 11].

![Figure 3. SDS-PAGE of the sample without and with submersion. M is high molecular weight marker, a is sample I.2, b is sample I.1, c is sample II.2 and d is sample I.2.](image)

The SDS-PAGE pattern in figure 3 shown that samples had molecular weight more than 120 kDa. Based on P Sivakumar’s study that type I collagen had large molecular weight with α1 chain [10, 11]. Therefore, collagen from samples had type I collagen.

The surface morphology of the samples in figure 4 was observed with low (100x) and high (1000x) magnification. Morphology of samples were fibres and there were pores in 250x and 500x magnification. Moreover, in high magnification shown was performed in Figure 5 had small fiber structure in the presence of granules which indicate as the impurities. These impurities were related with the drying process in the outdoors.

![Figure 4. SEM of sample I.1a and II.1 a with 100x magnification.](image)
4. Conclusions
Collagen samples isolated from the inner layer of organic and broiler chicken gizzards were successfully observed and there existed no significant difference between the contents of the two sample groups. The characteristic of collagen samples was also similar with bovine collagen from FTIR analysis. However, samples submerged in NaOH base had lost functional amide group shown in the range of 3300-3400 cm$^{-1}$ of wave number. The existence of both samples is evidence that major collagen from inner layer chicken gizzard is type I collagen. The result of the present study reveals the existence of collagen and it may be concluded that the inner layer of chicken gizzard as waste may become an additional and alternative possible source for alternative sources of collagen production.

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Corrigendum: Dosimetric impact of interplay effect in lung IMRT and VMAT treatment using in-house dynamic thorax phantom

S Julia¹, Nurlely¹, D S Soejoko¹
¹Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok 16424, Indonesia

E-mail: s.julia2805@gmail.com

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**Title** should be replaced by Characterization of organic material from inner layer of chicken gizzard