OPTIMIZATION OF EXTRACTION CONDITIONS AND CHARACTERIZATION OF GELATIN FROM BUFFALO (*Bubalus bubalis*) SKIN

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FACULTY OF FOOD SCIENCE AND NUTRITION

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ABSTRACT

Gelatin is a partially hydrolyzed form of collagen and a water soluble, thermos-reversible and multifunctional hydrocolloid. Buffalo (*Bubalus bubalis*) is a type of bovidae that has not been associated with any health diseases and have abundant high supply or raw skins for production of gelatin. This study was aimed to optimize the extraction conditions of Type B for gelatin production from buffalo skin using Response Surface Methodology and characterize the functional and physicochemical properties of optimized gelatin. A central composite design (CCD) was performed to evaluate the effects of NaOH concentration (x_1), pre-treatment time (x_2), extraction temperature (x_3), and extraction time (x_4) on the yield, gel strength and hydroxyproline content of the extracted gelatin. The lack of fit value was found to be not significant (p>0.05) with satisfactory R^2 value for all of the dependent variables. The optimized extraction parameters were x_1, 0.77 M, x_2, 5.08 h, x_3, 62.93 °C and x_4, 11.62 h with predicted values of 16.91% yield, 236.5 g gel strength and 41.4 g/100g of hydroxyproline. The verifications of experiments that have been conducted showed that both actual and predicted values were found to be nearly coincide, confirming that the estimation models were capable of reasonably and accurately predicting the dependent variables. Analysis such as proximate composition, viscosity, colour, pH, rheology, foaming and emulsifying properties were carried out for optimized buffalo skin gelatin (BFS) and compared with standard bovine gelatin (STB). The protein content of the optimized BFS showed no significant different (p>0.05) with STB. The ash content of BFS was higher (p<0.05) than STB, while the moisture content of BFS was slightly lower (p<0.05) than STB. Nevertheless, the results are still in line with the Gelatin Manufacturers Institute of America (GMIA) requirements. BFS gelatin was colourless with L^* value of 88.02 ± 0.79 and b^* value of 6.44 ± 2.97. The pH value of BFS was in line with the GMIA standard which is 3.6 – 6.0. In addition, BFS were found to have significantly higher (p<0.05) viscosity; 63.05 ± 1.80 cP, melting; 33.4 ± 1.23 °C and cooling; 19.08 ± 0.02 °C point, and also higher emulsion stability and activity index than STB. However, BFS possessed significantly lower (p<0.05) foaming expansion and foaming stability at 30 min than STB. The secondary results from fourier-transform infrared spectrum (FTIR) and SDS - page found that both buffalo skin and standard bovine gelatin showed cleared bands of α- and β-chain components, and several bands for low molecular weight peptides were present in BFS. Taken all together, the results showed that buffalo skin gelatin complied with the industry standard set by the GMIA, hence has a potential as alternative source for gelatin manufacture.

ABSTRAK

PENGOPTIMUMAN KEADAAN PENGEKSTRAKAN DAN PENCIRIAN GELATIN DARIPADA KULIT KERBAU (Bubalus bubalis)

Gelatin merupakan hidrokoloid larut air yang terhasil daripada kolagen yang dihidrolisis, serta merupakan termos-terbalik dan hidrokoloid yang mempunyai pelbagai fungsi. Kerbau (Bubalus bubalis) adalah bovidae yang tidak mempunyai sejarah penyakit dan mempunyai bekalan kulit mentah yang tinggi untuk penghasilan gelatin. Kajian ini bertujuan untuk mengoptimumkan keadaan pengekstrakan gelatin Jenis B untuk penghasilan gelatin daripada kulit kerbau menggunakan Kaedah Respon Permukaan dan menilai sifat berfungsi dan fizikokimia bagi gelatin yang terhasil. Reka bentuk pusat komposit (CCD) digunakan untuk menilai kesan kepekatan NaOH (x_1), masa pra-rawatan (x_2), suhu pengekstrakan (x_3), dan masa pengekstrakan (x_4) ke atas hasil gelatin, kekuatan gel dan kandungan hidroksiprolin gelatin yang diekstrak. Nilai padanan kurang tepat didapati tidak signifikan ($p>0.05$) dengan nilai R^2 yang memuaskan bagi semua pembolehubah bersandar. Jangkaan respon bagi parameter pengekstrakan gelatin yang didapati adalah x_1, 0.77 M, x_2, 5.08 h, x_3, 62.93 °C dan x_4, 11.62 h dengan nilai ramalan sebanyak 16.91% hasil, 236.5 g kekuatan gel dan 41.4 g/100g kandungan hidroksiprolin. Ujian pengesahan mendapati nilai sebenar dan nilai ramalan adalah hampir sama ($p>0.05$), dan mengesahkan bahawa model anggaran ini mampu meramalkan pembolehubah bersandar secara tepat. Analisis seperti komposisi proksimat, kelikatan, warna, pH, reologi, sifat berbuih dan pengemulsi telah dijalankan untuk gelatin kulit kerbau (BFS) yang optimum dan dibandingkan dengan gelatin lembu piawai (STB) Kandungan protein BFS tidak menunjukkan perbezaan yang signifikan ($p>0.05$) dengan STB. Kandungan abu BFS adalah lebih tinggi ($p<0.05$) daripada STB, manakala kandungan lembapan BFS lebih rendah ($p<0.05$) daripada STB, tetapi nilainya masih mematuhi piawaian Institut Pengilang Gelatin Amerika (GMIA). Gelatin BFS mempunyai nilai L 88.02 ± 0.79 dan nilai b* 6.44 ± 2.97. Nilai pH BFS juga selari dengan piawaian GMIA iaitu 3.6 – 6.0. BFS juga mempunyai nilai kelikatan*; 63.05 ± 1.80 cP, takat lebur; 33.4 ± 1.23 °C dan penyejukan; 19.08 ± 0.02 °C*, yang tinggi ($p<0.05$), dan juga kestabilan emulsi dan indeks aktiviti yang lebih tinggi daripada STB. Namun, BFS mempunyai pengembangan berbuih dan kestabilan berbuih yang lebih rendah ($p<0.05$) pada minit ke-30 berbanding STB. Keputusan sekunder daripada spektrum inframerah transformasi fourier (FTIR) dan SDS mendapati bahawa kedua-dua BFS dan STB menunjukkan jalur komponen α- dan β yang jelas, dan beberapa jalur peptida berat molekul yang rendah hadir dalam BFS. Secara keseluruhan, keputusan yang diperolehi menunjukkan gelatin kulit kerbau mematuhi piawaian industri yang ditetapkan oleh GMIA, justeru mempunyai potensi sebagai sumber alternatif untuk pembuatan gelatin.*

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LIST OF ABBREAVIATIONS

ANOVA	-	Analysis of Variance
AOAC	-	Association of Official Analytical Collaboration
BFS	-	Buffalo Skin Gelatin
BSE	-	Bovine Spongiform Encephalopathy
EAI	-	Emulsion Activity Index
ESI	-	Emulsion Stability Index
FE	-	Foaming Expansion
FMD	-	Foot and Mouth Disease
FS	-	Foaming Stability
FTIR	-	Fourier transform infrared
GME	-	Gelatin Manufacturers of Europe
GMIA	-	The Gelatin Manufacturers Institute of America
HPLC	-	High Performance Liquid Chromatography

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CHAPTER 1

INTRODUCTION

1.1 Background Study

Gelatin is a water-soluble protein with a high molecular weight polypeptide produced by thermo-hydrolysis of bovine collagen (Mohtar *et al.,* 2010). Gelatin is sometimes described to as edible, photographic, technical, or pharmaceutical grade, however these words refer to their applications rather than their manufacturing method. The prevailing production process for gelatin involves various stages, including washing, partial hydrolysis using acids or alkalis, neutralisation, extraction, filtration, demineralization, concentration, and drying of the raw materials (Mariod *et al.,* 2013). Moreover, the physical properties of gelatin are significantly influenced by two key factors: the amino acid composition and the distribution of molecular weight, which primarily arise from various processing conditions, such as pH, temperature, and duration during both pretreatment and extraction procedures. Extensive research is conducted on gelatin due to its ability to retain the structural characteristics of collagen while eliminating the immunogenic response that collagen can induce (Wei & Kumbar, 2020). In 2021, the global gelatin market reached a value of 1.83 billion USD and was anticipated to grow at a rate of approximately 3.30%, reaching 2.15 billion USD by 2026 (Mordor Intelligence, 2022). The size of the global market has grown due to how well gelatin makes gels in jellies, foams and stabilises ice cream, stabilises emulsions in meat products, and makes capsules and films (Eryılmaz e*t al.,* 2017).

Buffalo, or its scientific name (*Bubalus bubalis*), has served as Borneo's primary supply of red meat, particularly for frozen meat exporting. In Malaysia, the buffalo population is distributed unevenly, with Sabah contributing 46.7% of the total population, while Sarawak accounts for a much smaller portion at 6.4%. The significant presence of buffaloes in these eastern Malaysian states can be attributed to the longstanding tradition of breeding them as a supplementary source of income (Khalex *et al.,* 2021). However, the skins are frequently thrown away in great quantities, despite the fact that they contain a high concentration of collagen, which is the precursor to gelatin. The perception of waste by the public has evolved from viewing it as an inevitable byproduct of industrialised economies to recognising its potential as a valuable and reusable resource, particularly in the face of increasing urbanisation and consumption rates and the depletion of natural resources. In this context, waste management services play a crucial role in achieving the objectives and targets set by Sustainable Development Goals 11 and 12. These goals emphasize the importance of implementing measures to prevent, reduce, recycle, and reuse waste effectively. The overarching aim is to ensure proper collection and disposal of municipal solid waste and to significantly reduce global food waste by the year 2030. The utilisation of buffalo skin is further justified by its thickness, which is approximately twice that of cowhide, ranging between 6 to 8 mm. This thickness indicates a high density of collagen bundles present in buffalo skin, making it a valuable resource for various applications (Mulyani *et al.,* 2021). Furthermore, buffalo demonstrated remarkable resilience to local ecological conditions, showcasing exceptional immunity to diseases (Nanda *et al.,* 2003). Thus, the goal of this study was to take the opportunity to investigate the reliability and functionality of transforming waste of buffalo skin to a beneficial product; gelatin.

Generally, the functional properties of gelatin such as gel strength, emulsifying, melting points, and viscosity are the typically evaluated characteristics of a good quality of gelatin. These inherent characteristics are significantly impacted not only by the source of raw materials, encompassing species, breed, age, and feed variety (Tabarestani *et al.* 2010; Amertaning *et al.* 2019), but as well as the processing method and parameters (Mahmoodani *et al.,* 2014) such as extraction time, extraction

temperature, soaking time and concentration of acid and alkaline used to treat the animal skin (Jakhar *et al.,* 2014). In order to produce gelatin from buffalo skin, Arsyanti *et al.* (2018) utilised a variety of various concentrations of sodium hydroxide as well as a mixture of sodium hydroxide and citric acid. The study revealed that increasing the concentration of NaOH led to an increase in gelatin yield; however, it resulted in poor physicochemical properties of the gelatin. On the other hand, a study conducted by Rabiatul *et al.* (2020) compared different concentrations of sodium hydroxide (NaOH) and calcium hydroxide (CaOH) during the extraction process of gelatin from buffalo skin. The study showed that NaOH yielded better results in terms of gelatin yield compared to CaOH. Furthermore, Aprizal *et al.* (2019) attempted to use enzyme treatment in buffalo skin gelatin, and their findings indicated that the gelatin yield ranged from 5.99 to 7.33 %. Additionally, the viscosity property of the gelatin was reported to be below the GMIA (Gelatin Manufacturers Institute of America) standard. These findings demonstrate that altering treatment conditions can result in various characteristics of gelatin, including yield, physicochemical, and functional properties. However, due to the suboptimal quality of gelatin produced in previous studies, there is a significant interest in delving deeper into the fundamental of understanding the processing conditions for gelatin derived from buffalo skin, where such exploration holds promise for future industrial applications of buffalo gelatin.

A statistical and mathematical technique called Response Surface Methodology (RSM) is widely used for development, improvement, and optimization of varying processes. In order to attain the highest possible output and desired functionalities of gelatin, it is essential to emphasize factors such as alkaline concentration, pretreatment time, extraction time, and extraction temperature. Therefore, optimization proves to be a suitable technique for developing extraction methods, since the extracted material's quality is entirely dependent on the manipulation of processing parameters. From previous studies, Silva *et al.* (2017) reported that yield of gelatin from kumakuma (*Brachyplatystoma filamentosum*) skin increase with the increment of extraction temperature yet caused a reduction in gel strength values. While longer curing and extraction time results in a high yield in cattle skin gelatin and also an increase in ash

content (Bahar *et al.,* 2018). Optimization of gelatin extraction from fish skin such as pangasius rainbow trout (*Onchorhynchus mykiss*) and catfish (*Pangasius sutchi*) has been carried out (Tabarestani *et al.* 2010; Fatemeh *et al.* 2014). It was reported that the optimum extraction conditions of sodium hydroxide and acetic acid concentration were corresponding which is 0.2 N and 0.1 N which showed impact on yield of extraction, viscosity, molecular weight distribution, melting point, and gel strength. However, few studies have investigated procedures to optimize extraction conditions from bovine skins, whereas it is one of the important tools to understand the effect of processing conditions on the final product with desired characteristics.

Through the utilization of RSM, significant interactions between variables can be distinguished and measured, allowing for the production of high-quality gelatin with specific characteristics tailored for various industrial applications. This research aims to optimize the pre-treatment conditions of buffalo skin gelatin to achieve the highest physicochemical characteristics, including yield, gel strength, and hydroxyproline content, as the primary responses. Additionally, the study will explore the physicochemical and functional properties of the extracted gelatin to gain a comprehensive understanding of its properties.

1.2 Problem Statement

Based on Commersonnianus 2020, the global gelatin production amounts to approximately 326,000 tonnes per year, with pigskin gelatin contributing the highest production at 44%, followed by bovine hides at 28%, bones at 27%, and the remaining one percent sourced from other species (Cho *et al.,* 2005). Nonetheless, these prevalent sources of gelatin present a contentious matter of concern. For example, adherents of Judaism and Islam abstain from including pork in their dietary practices and bovine gelatin is only permissible for Muslims if its slaughter according to Syariah compliance which will vary among scholars. Moreover, contemporary consumers are showing growing apprehension regarding the recurrent occurrences of health ailments, including bovine sponge encephalopathy (BSE), transmissible spongiform encephalopathy (TSE),

and foot-and-mouth disease (FMD) (Kaewdang *et al.,* 2014). While poultry gelatin has been associated with avian influenza. Hence, the production of gelatin from diverse animal sources becomes imperative to meet the demands of the market.

Moreover, the existing extraction conditions of the gelatin present several pressing issues that demand further research and optimization. Over the past few decades, gelatin obtained from non-mammalian origins, including marine and poultry sources, has experienced significant growth in popularity as a viable substitute for conventional gelatin. Nonetheless, marine and poultry-derived gelatin often requires additional refinement due to its lower quality in comparison to mammalian counterpart. This discrepancy arises from the fact that marine and poultry gelatin typically possesses inferior gelation and melting properties owing to its low hydroxyproline content. For this reason, extensive studies into the possibility of obtaining gelatin from a variety of other sources need to be carried out.

In support of SDG, it is important to take the opportunity to investigate the reliability and functionality of buffalo waste, such as hides and bones that contains a substantial amount of collagen that can be hydrolysed into gelatin. However, there are not much studies on buffalo skin gelatin that has been developed, while the current extraction process might not be the most efficient in terms of time, energy consumption, and chemical usage. By delving into the complexity of the buffalo skin's collagen characteristics and tissue composition, it is important to develop optimized extraction condition that minimises resources consumption and environmental impact, hence making the process more sustainable.

1.3 Justification of Studies

The optimization characterization of gelatin from buffalo skin holds significant importance and relevance for various reasons, such as the current global demand for gelatin necessitates exploring alternative and sustainable sources to meet industrial needs. Buffalo skin, a byproduct of the meat industry, presents an opportunity to utilise