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Review

Application of Green Technology in Gelatin Extraction: A Review

Nor Qhairul Izzreen Mohd Noor [1], Rina Syafinaz Razali [1], Nur Khairina Ismail [1], Rabiatul Amirah Ramli [1], Umi Hartina Mohamad Razali [1,2,*], Ahmad Riduan Bahauddin [1], Nazikussabah Zaharudin [3], Ashari Rozzamri [4], Jamilah Bakar [4] and Sharifudin Md. Shaarani [5]

1 Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Kota Kinabalu 88450, Malaysia; qhairul@ums.edu.my (N.Q.I.M.N.); syafinash@gmail.com (R.S.R.); mn1922025t@student.ums.edu.my (N.K.I.); rabiatulamirah96@gmail.com (R.A.R.); a_riduan@ums.edu.my (A.R.B.)
2 School of Chemical & Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia (UTM), Johor Bahru 81310, Malaysia
3 Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang, Gambang 26300, Malaysia; nazikussabah@ump.edu.my
4 Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang 43400, Malaysia; rozzamri@upm.edu.my (A.R.); jamilah@upm.edu.my (J.B.)
5 Food Biotechnology Programme, Faculty of Food Science and Technology, Universiti Sains Islam Malaysia, Nilai 71800, Malaysia; sharifudinms@usim.edu.my
* Correspondence: umi.hartina@ums.edu.my

Abstract: Growing demands for green and sustainable processing that eliminates the utilization of toxic chemicals and increases efficiency has encouraged the application of novel extraction technologies for the food industry. This review discusses the principles and potential application of several green technology for gelatin extraction. Several novel technologies and their processing efficiency are discussed in this review. Furthermore, factors that affect the quality of the gelatin produced from different sources are also highlighted. The potential application of ultrasound-assisted extraction (UAE), subcritical water extraction, high-pressure processing, and microwave-assisted extraction (MAE) to improve gelatin extraction are addressed. These technologies have the potential to become an efficient extraction method compared to the conventional extraction technologies. Several combinations of green and conventional technologies have been reported to yield promising results. These combinations, especially using conventional pre-treatment and green technologies for extraction, have been found to be more effective in producing gelatin. Since gelatin could be produced from various sources, it exhibits different characteristics; thus, different approaches and extraction method should be identified for specific types of gelatin. Although these technologies have limitations, such as overhydration and sophisticated systems explicitly designed for large-scale production, they are nonetheless more efficient in the long run to safeguard the environment as they reduce solvent usage and carbon footprint along the way.

Keywords: ultrasound extraction; high-pressure processing; subcritical water extraction; microwave extraction; novel technology

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1. Introduction

Gelatin is a flavourless, transparent, colourless, or pale-yellow thickener derived from animal collagen. It is a high-molecular-weight biopolymer obtained from the process of hydrolytic degradation of proteins. Gelatins are commonly extracted from the collagen of pigs, cattle, fish, and poultry. It can be found in the skin, bones, tendons, and ligaments of animals. Gelatin has a distinct amino-acid composition dominated by Gly-Pro-Hyp, which provides functional properties such as gelling ability, binding capacity, increased viscosity, and film-foaming properties, making it useful as a food ingredient and in other industrial applications [1]. In the food industry, gelatin is utilised as an ingredient to enhance food

properties such as elasticity, consistency, and stability of the product. Gelatin also acts as a gelling agent and is incorporated in foods like jellies, cakes, aspics, and marshmallows [2]. In addition, gelation provides chewiness, foam stability and good textural properties for confectionery products. It also provides creaminess, reduces fat, and increases mouth-feel taste for low-fat spreads [3]. For applications in baked goods, gelatin aids the emulsification, gelling and stabilisation process during baking [4].

In order to obtain high-quality gelatin, the extraction method used plays an important role. Solid-liquid extraction (SLE), acidification, maceration extraction (ME), and percolation are extensively used in the gelatin industry as extraction methods. However, these enzyme-, acid-, and base-isolation procedures are time-consuming and labour-intensive, with various limitations that impede process scaling. The requirement for multiple operational steps, high energy consumption, and the use of significant volumes of water and other solvents are factors that concern manufacturers and those concerned about the environment. Furthermore, the selectivity and recovery of these methods are extremely low. These conventional extraction methods are not eco-friendly, as they require long extraction time, produce low yields, cause quality degradation, and have higher solvent requirements. They also require significant consumption of energy during solvent extraction and mechanical extraction and cause thermal effects and in the aqueous phase of conventional methods, which have negative impacts on the environment [5].

Innovative and novel technologies for extraction have been developed to help increase yield and reduce time and cost while sustaining the environment by lowering energy consumption. Green technologies have recently been introduced into the ingredient-processing business to improve the extraction of non-eco-friendly elements. This concept and its success and application have sparked an interest in the gelatin industry. As a result, the development effective extraction processes that entail fewer operational phases and more environmentally friendly practices and adhere to the green idea is encouraged. Additionally, there are numerous advancements in technology that could propel the development of green and sustainable processing. These alternative technologies may reform rapid extraction methods with lower solvent consumption rates and encourage the use of environmentally friendly solvents. One of the common green methods used to produce gelatin is enzyme hydrolysis. This technique has been widely used and has undergone several modifications throughout the years. In recent years, various conditions have been explored to minimise the steps for this biocatalyst. Therefore, enzyme hydrolysis is not included in this review; however, the combination of this biocatalyst and current technologies, such as ultrasound-assisted extraction (UAE), high-pressure processing (HPP), microwave-assisted extraction (MAE), and subcritical water extraction (SWE) will be discussed. This review aims to summarise the principles and application of novel technologies to improve conventional gelatin extraction methods from various sources and increase extraction efficiency. The parameters and conditions of these technologies, as well as their effect on the characteristics of gelatin produced will also be discussed.

2. Gelatin

Gelatin is made from native, insoluble collagen that has been denaturised and partially hydrolysed. It is a soluble, amorphous mixture made up of different free chains, which are α-chains, β-chains, and γ-chains stabilised by hydrogen bonds. During the gelatin manufacturing process, the hydrogen bonds in collagen molecules are disrupted. Next, the intramolecular (aldol condensation and Schiff base), intermolecular, and main-chain peptide bonds are hydrolysed, causing their triple helices to unravel. Subsequently, the collagen fibrils are disassembled [6] and produce a viscous colloidal solution known as gelatin. Figure 1 shows the general structure of gelatin produced from various sources.

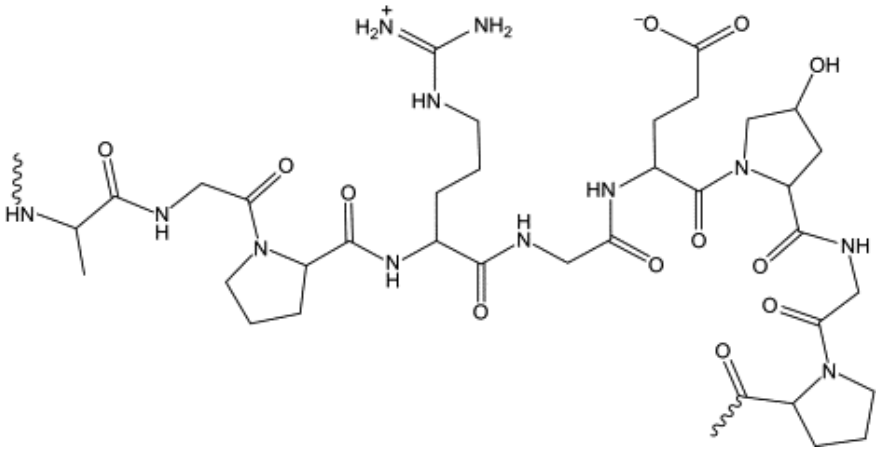

Figure 1. Gelatin structure.

Gelatin is mainly produced from skins and bones of animals melted upon heating and reformed upon cooling. Gelatin is predominantly made from skins and bones of pigs, cows, and calves under a regulated stepwise process that begins with chemical hydrolysis of collagen and continues with thermal denaturation. There are three steps to manufacture gelatin. The first step is to remove all impurities, minerals, and non-collagenous material. The next step is to perform hydrolysis to convert the collagen into gelatin, and the final step is to recover the final product, which is gelatin. There are two types of gelatin that can be produced by manipulating the processing conditions: Type A and Type B (Table 1) [7].

Table 1. Types of gelatin.

Gelatin	Type A	Type B
Sources	Porcine skin, fish skin	Bovine hide and bone
Extraction	Pre-treatment with acid	Alkali pretreatment with alkali
Raw material matrices	Weakly crosslinked collagen	Strongly crosslinked collagen
Isoelectric points	8–9	4.8–5.5
Molecular weight	Wider distributions of molecular weight fractions (10,000–200,000 Da)	The central part of the molecular weight fractions is in the region of 100,000 Da
Amino-acid composition	Mostly similar to the parent collagen molecule	Differs from parent collagen, especially in the content of glutamic acid and aspartic acid.

Type A gelatin is commonly found in pig and fish skins, which require only mild acid conditioning due to their less complex crosslinked structures. Due to the differences in amino-acid composition, Type A gelatin has an isoelectric point of approximate pH 8–9, whereas Type B gelatin has an isoelectric point of about pH 4.8–5.5 [8]. On the other hand, Type B gelatin is frequently associated with highly crosslinked raw material that has been treated with an alkali, such as calcium hydroxide. Gelatin extracts contain α-, β-, and γ-chains, as well as larger and intermediate-sized molecules. Different hydrolysis and extraction procedures and different types of raw materials influence the molecular spectrum, thus having an impact on the functional properties of the extracted gelatin [9,10]. For instance, Type A gelatin has a broader distribution of molecular weight fractions, with a smaller peptide size compared to Type B. Most of the molecular weight fractions in the 100 kDa region contribute to the high gelling strength of Type B gelatin [8].

3. Gelatin Extraction

Gelatin is commonly extracted with similar procedures; however, methods keep evolving, depending on sources. The gelatin extraction procedure for pig skin and bones, bovine hides, and fish skin is already established and commercially applied; however, emerging potential new sources require alternative methods that are derived and modified, based on the existing protocol. In general, gelatin processing consists of three stages: pre-treatment, extraction, and recovery. Samples are usually treated with acid or an alkali

to remove non-collagenous proteins and fat. The demineralisation step is also necessary for raw materials with high mineral content, such as fish scales and bones and bovine and porcine bones.

Moreover, the pre-treatment step is essential to prepare the collagenous matrices for hot-water hydrolysis, known as 'swelling'. The extraction step, in which the swollen materials are subjected to hot-water denaturation, needs to be critically monitored to liberate gelatin with the targeted qualities. The resulting extracts are then collected and purified by sequential filtration, clarification, deionisation and drying. Figure 2 shows the process flow of a typical gelatin extraction process. New technologies such as ultrasound, subcritical water extraction, high-pressure processing, and microwave are used to assist during the pre-treatment or extraction stages, or both.

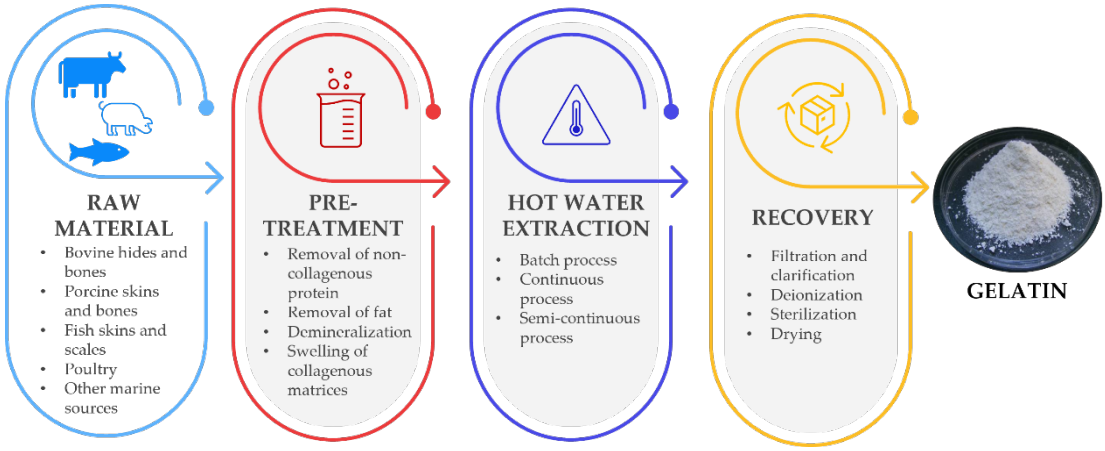

Figure 2. The general gelatin extraction procedure.

There is a broad possibility of applying these technologies in the gelatin industry, as some of the equipment already exists in the market and is being applied in several industries. The challenge is to establish the effectiveness of these technologies in assisting gelatin extraction or, better yet, whether they could be employed individually in the extraction process.

4. New Technology for Gelatin Extraction

4.1. Ultrasound-Assisted Extraction

Ultrasound-assisted extraction (UAE) is a physical, non-thermal approach that is considered environmentally safe. It utilises ultrasonic waves (above 20 kHz) to break the complex molecules into smaller compounds and enhance the interface between solvents and the targeted compounds. Ultrasound can be used in the extraction of compounds that do not require heating, as it physically breaks down molecules. The most popular applications of ultrasound are in the production peptides and the extraction of bioactive compounds from plants. The ultrasound frequency range is between 20 Hz and 20 kHz, and lower frequencies are known as infrasound [11].

4.1.1. Principle

UAE uses ultrasound pressure waves that produce cavitation, which can damage the structure of the matrix and enhance gelatin extraction. The frequencies depend on the purpose of the analytical techniques; for instance, higher frequencies are needed to control the chemical effects (200 to 500 kHz).

These acoustic waves travel through the solvent, creating a change in pressure and temperature that increases the analytes' net movement transfer rate. As a result, it requires a specialised medium to spread. Great energy production is the result of massive changes

that lead to implosion. This mechanism consists of two steps: first, the transmission into the food matrix; and second, after the matrix structure is disrupted, the content is let out [12].

The mechanism of this process occurs by the dispersion imputed by collision between particles and ultrasonic waves, which causes particle-size reduction and assists in mass transfer. Due to the permeability alteration, it allows easy penetration for the compound to escape and interact. Secondly, the shear mechanism of the oscillation movement causes the cavitation bubble to collapse, thus improving accessibility. The solvent diffuses in the sample, while the compound is released [12]. Ultrasonic equipment has two applications: the ultrasonic bath system (indirect) and the ultrasound-with-probe system (direct). However, the ultrasound-with-probe system is more efficient, wherein direct contact with the samples allows 100 times more power than an ultrasonic bath system.

4.1.2. Extraction of Gelatin Using Ultrasound-Assisted Extraction

- Combination of Ultrasound and Acidification

A combination of ultrasound with other assisted methods, such as enzyme and acidification, appears to be more effective than ultrasound alone. The extraction includes lengthy pre-treatment procedures with acid and alkali, regardless of the raw materials used, whether fish or poultry. Collagen is commonly extracted by acid, but it produces a lower yield of gelatin. Ultrasound alone may not be enough to hydrolyse the protein for extraction. A pre-treatment method is required to weaken the collagen structure, solubilise non-collagenous proteins, and hydrolyse several the peptide bonds while maintaining the consistency of collagen fibres [13]. Thus, combining ultrasound and acid catalysts might produce better results. Acid catalysis is a technique used to speed up chemical reactions by applying specific acid to break down a substance, which entails the transfer of protons from the acid to the reactant. The molarity of the acid, the temperature of the solution, and the size of reactants could amplify the reaction for acid catalysts.

Tu et al. (2015) [14] have reported that the ultrasound-assisted water bath (UWB) method could improve the yield and gel strength of fish bighead carp (*Hypophthalmichthys nobilis*) scale gelatin. The gelatin yield increased to 47% when it was extracted for 5 h compared to the application of a normal water bath (37%), with an extraction time ranging from 1 to 5 h at 60 °C with ultrasound at 200 W. Extraction time up to 1 h displayed the highest value of gel strength (634.7 g). However, as extraction time increased, the gel strength was found to decrease. This was expected, as more prolonged exposure to ultrasound provided more energy to disrupt long chains and convert them into short chains, leading to gelatin with lower gel strength, α-chain, and β -chain. Gel strength is hugely affected by the molecular weight distribution, which is primarily determined by processing conditions. A short extraction time of up to one hour does not show any significant difference in yield and gel strength. It was determined that ultrasound causes a high level of protein aggregation [15]; however, extraction time of one-hour indicates that ultrasound did not affect the gel strength.

Huang et al. (2016) [16] extracted gelatin from bighead carp scales using an ultrasound frequency of 200 W/40 kHz at different temperatures (60, 70 and 80 °C) for 1 h. They found that extraction at 60 °C had a high gelation point, a high melting point, and a high viscosity compared to other samples treated with a water bath and UAE at temperatures of 70 and 80 °C. However, this extraction condition produces a lower yield. At high extraction temperature (80 °C), combined with the cavitation effect of ultrasound, the triple helices of the sample were easily broken down to smaller α-chain and β-chain proteins. Therefore, long extraction periods at high temperatures were found to reduce the gelling properties of gelatin.

Application of UAE could reduce extraction time whilst maintaining the quality of gelatin. No significant difference in gelatin yield was reported when water baths were used for 5 h and ultrasonic baths were applied for 3 h. This indicates that ultrasound could decrease extraction time. However, UAE at higher temperatures (70–80 °C) causes more damage to the triple-helix content of gelatin than extraction using water bath. High

temperature also decreases irregular aggregates and heights influenced by hydrogen bonds and hydrophobic interactions due to the crosslinking of gelatin under the effect of ultrasound and high temperatures [16].

Acidification increases the water absorption rate, which affects the electrostatic force between charged polar groups by sending hydrogen ions to collagen molecules. Incorporation of ultrasonication causes fish skin to swell, enhancing the yield of gelatin. Asih et al. (2019) [17] extracted gelatin from patin fishbone using UAE with different extraction times (3, 5, and 7 h) and reported that ultrasound frequency at 700 W for 5 h showed the best treatment, as it obtained the highest extraction yield (5 ± 1.03%), gel strength (147.74 ± 0.83 g), and viscosity (14.63 ± 0.31 cP). Longer extraction time recorded the lowest yield and gel strength. This finding contradicts that of Gomez (2011) [18], who reported that gelatin yield would increase with increased extraction time.

Generally, the gel-strength values reported by Asih et al. (2019) decreased with longer extraction time, similar to other previous findings. However, this value is still considered low compared to the gel strength reported by Tu et al. (2015) [14]. Widyasari and Rawdkuen (2014) [19] compared the application of acid extraction only and acid extraction pre-treated with UAE to produce gelatin from chicken feet. A similar percentage of extraction yield was obtained from acid-extracted gelatin (4.05%) and UAE (3.96%) at 70 °C with ultrasonic power at 300 W for 100 min. The reported gel strength of 147.74 ± 0.83 g was considered low, similar to Asih et al. (2019) [17]. They suggested that this slight difference in yield was due to the loss of collagen during washing in pre-treatment processes or incomplete collagen hydrolysis. However, UAE produces gelatin with a low moisture content, which is an advantage, as it increases the shelf life of gelatin and provides benefits in food applications. The protein, fat, and ash content produced by both methods was similar to commercial bovine gelatin. Ultimately, ultrasound has the ability to reduce extraction time at lower temperature whilst maintaining gelatin quality.

- Combination of ultrasound and enzyme

Previously, ultrasound was used to inactivate enzymes due to the mechanical impacts of sound waves that break the hydrogen bonds and van der Waals bonds of enzymes. Disrupting these linkages affects the secondary and tertiary structures of enzymes, eliminating the enzyme's biological activity [20]. However, it has been found that ultrasound does not inactivate all enzymes. The use of ultrasonic treatment at specific frequencies and intensity levels can lead to enhanced enzyme activity due to favourable conformational changes in protein molecules without altering their structural conformation [21]. Therefore, the application of ultrasound for the extraction of gelatin fully utilises the latter condition as an efficient tool to activate enzymes by modifying the structural conformation of the molecule under controlled conditions. Enzymes are biological catalysts that lower the required activation energy for reactions. When the required activation energy is reduced, it increases the rate of reaction. Thus, a specific enzyme is needed to bind to active sites and initiate the chemical reaction. However, enzyme extraction of gelatin is a traditional process that requires more time and has a degradation limit. Ahmad et al. (2018) [22] studied the effects of UAE conjugated with actinidin enzyme on the physicochemical properties of bovine-hide gelatin. They found that bovine hide treated with UAE and actinidin extraction exhibited a 20% higher gelatin yield, and the increase was significant ($p < 0.05$) compared to other treatments. The mechanical impact of ultrasound increases the contact-surface area between the food matrix and the solvent.

Furthermore, due to the higher amount of energy reflected by this mechanical wave, the content of amino acids in the gelatin increased, as the length of the ultrasonic treatment increased. Ahmad et al. (2018) [22] reported a substantial loss of molecular order in ultrasound-treated samples, which could be caused by thermal uncoupling of intermolecular crosslinks resulting from a longer duration of ultrasound exposure and actinidin pre-treatment. A similar study by Ahmad et al. (2018) [23] on gelatin extraction from bovine skin found that the gelatin extracted using ultrasound (53 kHz and 500 W) at 60 °C for 6 h with bromelain pre-treatment produces a higher yield (almost 20% difference) and exhibits

better quality compared to bovine skin treated without enzyme and ultrasound. The results showed that the gelatin possessed high gel strength, high viscosity, and high amino-acid content, especially glycine (27.06%) and hydroxyproline (17.21%), with increased duration of ultrasound treatment.

Ultrasound, a green and novel physical-processing technology, contributes to the extraction, release, and diffusion of substances of solutes [24,25]. It also increases enzyme activity by affecting the activation energy of the immobilised enzyme. Furthermore, ultrasound could change the structure of the carrier and affect the environment surrounding the immobilised enzyme, which would affect the specificity of the immobilised enzyme to the substrate.

Besides producing gelatin, ultrasound could be used to solubilise gelatin, as reported by Farahnaky et al. (2016) [26]. They used different parameters compared to the general thermal treatment for this process to set the ultrasound to solubilise gelatin. Internal aspects, such as ultrasound frequency and power, and external factors, such as temperature, time, and collagen concentration, are among the parameters that facilitate the breakdown of gelatin. Additionally, the combination of ultrasound with other technologies, such as HPP [27] and MAE [28], has been proven to increase gelatin extraction efficiency. Kim et al. (2019) [29] extracted gelatin from duck skin using an ultrasound frequency of 40 kHz at 60 °C for 10 min and high pressure, resulting in a high yield of 26.15%, 33.25 °C melting point, 220 g gel strength, and 65.33 mPa.s viscosity. The yield obtained was relatively high compared to the gelatin extracted from bovines [23]. On the other hand, the gel strength was reported to be low, which is consistent with the notion that ultrasound cavitation and high pressure generate a large amount of energy, which damages protein structure during extraction. The characteristics of gelatin using ultrasound - assisted extraction from various sources are summarized in Table 2.

4.2. Subcritical Water Extraction

Subcritical water extraction (SWE) has been utilised to separate bioactive compounds from parent protein molecules, such as collagen. Previous research has shown a high likelihood of obtaining high-quality collagen or gelatin prior to SWE application in the process. This demonstrates the feasibility of utilising SWE to further hydrolyse collagen to generate gelatin.

4.2.1. Principle

Water has a distinct feature due to two strong hydrogen bonds in the water molecule. The properties of water change in response to variations in temperature and pressure [30]. Subcritical water is a fluid that occurs in a phase with both liquid and gas characteristics above its critical temperature and pressure. Subcritical water is described as hot water under sufficient pressure to keep the liquid state at a critical temperature between 100 °C and 374 °C, which are the boiling point and critical point of water, respectively, under the required pressure (1–22.1 MPa) [31].

Water can be held in liquid form by varying the pressure and temperature, and the dielectric constant can be adjusted. The energy from subcritical water can disrupt the interaction between adhesive molecules (solute matrix) and cohesive molecules (solute-solute) by lowering the activation energy required for the desorption process [32]. At the same time, elevated pressure can aid extraction by forcing water to penetrate the matrix (pores), which is impossible to do under normal pressure [32]. The polarity of subcritical water decreases as the temperature increases. As a result, polar, medium-polar, low-polar, and nonpolar substances can be distinguished [31]. This is beneficial for protein extraction since protein is made from building blocks of amino acids, containing a side-chain R group with different polarity.

In general, the SWE extraction mechanism consists of four sequential steps. In the first stage, the solute is first desorbed at various active sites in the matrix of the sample under high increased temperature and pressure conditions. The second phase primarily concerns

the diffusion of the extracts into the matrix. The sample matrix determines the third step, and the solutes can be partitioned from the sample matrix into the extraction fluid. The sample solution is then eluted and collected from the extraction cell using chromatography (Figure 3) [33]. Holgate & Tester, 1994 [34] developed a thermodynamic model for the extraction mechanism. The extraction of chemicals from their matrix is accomplished in two processes in this paradigm. The substances must first be desorbed from the original binding site of the sample matrix and then eluted from the sample in a manner similar to front-elution chromatography. The efficiency of subcritical water extraction could be improved as follows: (1) improved solubility and mass transfer effects and (2) increased damage to surface equilibrium [33].

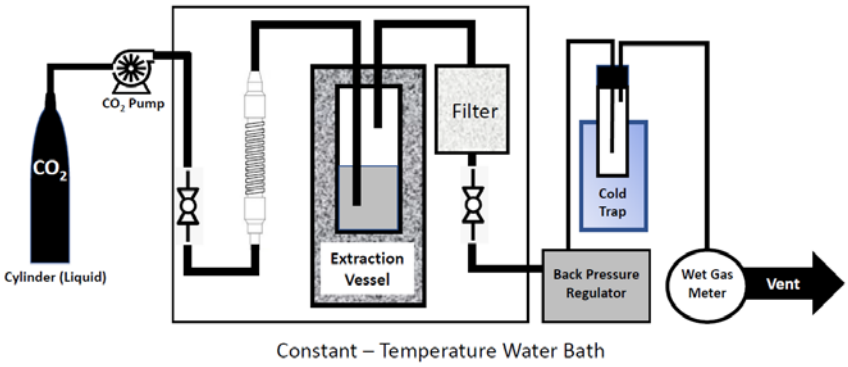

Figure 3. Schematic diagram of subcritical water extraction (SWE).

The characteristics of subcritical water vary continuously as temperature rises and its capacity to dissolve analytes changes. This process is accompanied by a decrease in viscosity and increase in infusibility, allowing the penetration of matrix particles to improve. Fresh water is continually injected into the dynamic extraction process of subcritical water, which improves mass-transfer efficiency and increases extraction yield. Furthermore, under high temperatures and pressures, the material's surface could be affected. The temperature increase can overcome the solute-matrix interaction mediated by hydrogen bonds, van der Waals forces, active spots in the matrix, and solute dipole attraction [35]. When water temperature exceeds the boiling point, adequate pressure must be applied to keep it in a liquid state. Applying pressure can aid in the dissolution of analytes from matrix pores [36].

4.2.2. Extraction of Gelatin/Hydrolysed Collagen Using SWE

Records of previous work on the production of gelatin using SWE are quite limited. Most of the studies focused mainly on collagen and collagen hydrolysates, without explicitly mentioning gelatin. Therefore, the products of collagen and fish hydrolysates are also be discussed here. Kyung et al. (2014) [37] found that the application of trypsin with SWE (375 bar, 200 °C, 90 min holding time) effectively produced low-molecular-weight collagen peptides from porcine placenta compared to standard heating pre-treatment at 90 °C for 1 h. The gelatinised hydrolysates possessed abundant proline, followed by glycine, which could also increase the enzyme reaction during hydrolysis.

Meanwhile, the application of pepsin and chymotrypsin exhibited less impact on collagen hydrolysis compared to that of trypsin. This indicates that besides SWE, the effect of enzymes is also critical in the process, and it shows similar behaviour to enzymes in any biochemical reaction. However, Park et al. (2015) [38] used ethanol and SWE at a temperature of 170 °C and a pressure of 10 bar for 30 min to extract the porcine placenta. They reported that a longer extraction time improved the efficiency of porcine placenta hydrolysis. Solubilised gelatin had a high molecular weight over 20 KDa, and with increasing extraction temperature, the high-molecular-weight (MW) peptides shifted to low-molecular-weight peptides. On the contrary, Park et al. (2015) [38] found that the SWE pressure did not influence the extraction but the hydrolysis temperature, as they discovered

that extraction at 170 °C for 30 min with a pressure of 10 bar was the optimal condition for the hydrolysis of porcine placenta compared to the 375 bar used in the previous study [37].

Furthermore, SWE was more efficient when water was used as an extraction medium compared to ethanol [38]. Prior to these previous studies, Chun et al. (2014) [39] tested various pressure levels (0.1–300 MPa) to determine their effect on the characteristics of porcine placenta hydrolysates. They found that trypsin had better placental-hydrolysing activities under high pressure (particularly at 200 MPa), with a lower MW distribution of the hydrolysates. The obtained peptides were mainly composed of glycine (Gly), alanine (Ala), hydroxyproline (Hyp), and proline (Pro). Furthermore, they concluded that high pressure could enhance the placental-hydrolysing activity of selected proteases, and the optimal pressure at which the maximum protease activity occurs was around 200 MPa.

Recent work by Melgosa et al. (2021) [40] using SWE in Atlantic codfish frames reported that they could obtain collagen and collagen fragments with more than 50% extraction yield and almost 100% protein recovery rate at a temperature of 90 °C and 100 bar. They also found that this condition showed the highest anti-inflammatory potential in a human intestinal epithelium cell model. When extracted at 250 °C, the free amino-acid content was the highest, and 22% of the identified amino acids were in free form. Previous work reported that the temperature range of 220–260 °C was optimal for amino-acid production using the SWE process; however, it depends on the raw material and the mode of operation. Furthermore, Melgosa et al. (2020) [41] tested different temperatures from 90 °C to 190 °C in sardine waste from fish canning and found the highest yield: 7.7% free amino acids at 140 °C. However, amino-acid content decreased at higher temperatures (190 °C) due to the thermal decomposition of organic acids and volatile carbon. Sukkwai et al. (2011) reported similar results [42], as the amino-acid hydroxyproline content on the skin of large eye snapper using the conventional method increased from 50.79% to 76.36%, with an increase in temperature from 80 °C (1 h) to 120 °C (30 min). The same pattern was observed by Lee et al. (2013) [43] in porcine placenta, where the levels of glycine, alanine, hydroxyproline, and proline increased when treated with HPP (high pressure/high temperature) [43]. As a result, regardless of the extraction process chosen, a similar trend of increased temperature results in a high level of amino acids. However, optimal conditions need to be identified to break down peptide bonds into amino acids. If the process had adequate energy provided by temperature and pressure, it could break down peptides in a shorter period.

Under elaborate pressure and temperature (subcritical region), collagen converts into gelatin and undergoes partial or complete hydrolysis, depending on the subcritical conditions, such as temperature. Previous studies indicate that subcritical water processing could hydrolyse collagen within a few minutes [37,38]. From these results, it is evident that the amino-acid profile and the nature of the proteins present in the raw material, together with the operating temperature and extraction or holding time, exert a significant influence on the production of free amino acids and the distribution of peptides. These previous results also recommend the subcritical extraction system as an effective hydrolysis method.

Apart from peptides and amino acids, a high yield of collagen/gelatin mixtures with 32–36% per weight (g) was observed in different types of marine sponges when extracted using SWE at 50 bar and a temperature of 37 °C for 16 h. Similarly, collagen extracted from cod skin with SWE showed a high yield of 13.8% compared to other conventional acid- and enzyme-aided extraction methods [44]. It was observed that a longer extraction time (30 min) improved the efficiency of hydrolysis. Subcritical conditions break down the hydrogen bonds of water; thus, water becomes less polar and imitates the properties of an organic solvent. Therefore, the solubility of organic materials could be increased by this condition of subcritical water. The characteristics of gelatin using subcritical water extraction from various sources are summarized in Table 2.

4.3. High-Pressure Processing

High-pressure processing (HPP), pioneered by Hite (1899) [45] and Bridgman (1912) [46], has been used for centuries. The application of this high-pressure technology has been established as one of the most important commercial food-preservation technologies. Elevated pressure is the primary lethal agent to reduce pathogens without conceding to food's nutritional and organoleptic properties. This technology utilises pressure instead of heat to induce a preservation effect similar to pasteurisation that inactivates harmful pathogens, as well as vegetative food-spoilage agents, thus preserving food-quality parameters and enhancing the microbial safety, nutritional, and functional properties of food products [47]. Therefore, it can maintain most foods with minimal changes in flavour, texture, appearance, or nutritional content [48]. In addition, it has been used in the application of food proteins and the isolation of bioactive compounds. Only recently, the application of HPP has been extended to the production of bioactive peptides to hydrolyse food proteins, including the extraction of collagen and gelatin from various sources [49].

4.3.1. Principle

HPP processing technology is a green and non-thermal technology that uses isostatic pressures between 100 and 1500 MPa. [47]. High-pressure processing is sometimes called pascalization. Pascalization is based on an activation volume that uses a transferring medium applied only in batch processing. HPP is based on Le Chatelier's principle, indicating that an application of pressure shifts the equilibrium of the system to the state that occupies the lowest volume. Therefore, any chemical or physical changes (phase transitions, chemical reactions, and changes in molecular configuration) accompanied by a decrease in volume are enhanced by applying pressure. Consequently, the inter- and intramolecular interactions (non-covalent bonds) change. High pressure shortens hydrogen bonds; however, hydrogen bonds can be completely ruptured at extreme pressure. In addition, at high pressure, electrostatic and hydrophobic interactions are disrupted, which affects protein tertiary and quaternary structure. Conversely, high pressure strengthens the interactions of Van der Waals forces, thus maximising packing density and reducing the volume of proteins. As a result, conformation and solvation of protein molecules are affected [50], while crucially retaining food quality. However, enzyme reactions can occur (e.g., during the pressure build-up phase before inactivation) other than the occurrence of adiabatic heating (approx. 1–2 °C per 100 MPa). Temperature and pressure distribution are not uniform and not entirely homogenous in processing units.

Pressure vessels are the most crucial part in the entire HPP system. Other compartments within the instrumentation include the generation pressure system, temperature, device control, a high-pressure vessel and its closure(s), and the material-handling system. HPP is a batch system in which water, as a medium, transmits pressure to the sample in the vessel to accelerate the operation and compatibility of food items [47,51]. During the process, the loaded container is filled with fluid to transmit pressure during isostatic processing. The air present in the system is expelled using a low-pressure fast-fill-and-drain pump and an automatic deaeration valve. The pressure medium is then heated or directly compressed to achieve high hydrostatic pressure [52] (Figure 4).

Multiple alterations can be observed when HPP is applied in the process, such as the reconstruction of proteins, gelation, hydrophobic reactions, lipid phase shifts in cell membranes, and an escalation in the ionisation of dissociable molecules due to electrostriction [53].

During pressure treatment, physical compression causes a volume reduction and an increase in energy and temperature [53]. The combination of heat and pressure causes physical, chemical, and biological changes in food compounds, including protein-conformational changes. Yordanov and Angelova (2010) [54] studied the behaviour of food under pressure based on high-pressure processing principles. They concluded that high pressure leads to a low level of reaction, conformational change, and phase transition, compromising weak interactions and bonds in the molecules. At constant temperature, high pressure increases the ordering structure of molecules. The isostatic principle entails compressing

food products from all directions with consistent pressure. Unlike heat treatment, the effects of high pressure are reversible, especially for the denaturation process [49].

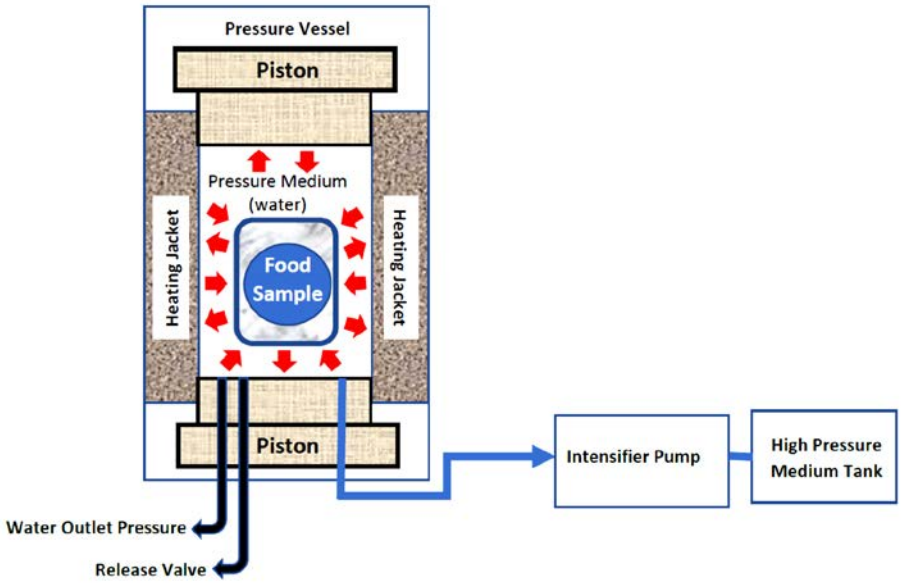

Figure 4. Diagram of high-pressure processing (HPP).

However, the high-pressure procedure has limiting factors, which are temperature (T), pressure (*p*), and duration of exposure (t). The pressure employed in food is typically between 300 and 800 MPa, and pressurisation above 1500 MPa causes protein to denaturate by weakening the non-covalent bond's crosslink interaction [55]. Furthermore, high pressure facilitates the infiltration of more acid in the skin, allowing collagen to expand [56].

4.3.2. High-Pressure Processing during Pre-Treatment and Extraction

Jaswir et al. (2017) [57] used HPP to aid in the gelatin extraction process during pre-treatment, following the Gómez-Guillén et al. (2005) [58] approach with a few alterations. Jaswir et al. (2017) [57] and Yusof et al. (2017) [59] pre-treated the fish skins before using HPP by first soaking the skin in NaOH and acetic acid, followed by a swelling technique by which the citric acid and skins were packed in a polythene bag. The was then placed into the pressure chamber before it was thermally extracted in distilled water at a constant temperature. The application of acid during pre-treatment allows mild acid to penetrate the skin, interrupt the non-covalent bond of the gelatin structure, and obtain sufficient swelling to facilitate the extraction process [60]. High pressure applied during treatment helps force the acid to permeate into the skin quickly and increase swelling activity [59]. According to Chen et al. (2014) [56], the combination of acid and HPP treatments may prevent the degradation of collagen/gelatin since the HHP treatment disrupts the balance of non-covalent interactions in collagen. However, brief acid treatment was insufficient to break down the peptide bonds of the collagen molecule. Oher than that, thermal hydrolysis quickly destroys the crosslinkage in the gelatin structure due to heat. In addition, pressurisation facilitates the breakage of the non-covalent bond; hence, warm extraction could extract more gelatin and thus reduce extraction time [61].

According to Gómez-Guillén et al. (2005) [58], the extraction process starts with mild acid treatment (50 mM acetic acid), the swelling step for 3 h, followed by distillation in water-gelatin extraction overnight (16–18 h) at a moderate temperature (45 °C). The gelatin extracted was then dried up to approximately 15% of the moisture content. Following the approach described in the conventional procedures, NaOH, acetic acid, and citric acid were applied to the fish skins for the soaking process [57]. Following that, fish skin samples were placed in distilled water in a sealed polyethene bag, then placed in the pressure chamber, and the HPP procedure was initiated at 250 MPa for 10 min.

Gómez-Guillén and Montero (2001) [62] described a method to extract the high gelling capacity of gelatin from fish skins that involved a mild acid pre-treatment for collagen swelling, followed by extraction in water at 45 °C [58]. The purpose of using high pressure during the extraction process of fish gelatin is to help reduce the treatment time and increase the quality of gelatin. Gómez-Guillén et al. (2005) [58] also clarified that extraction in water at 45 °C helped accelerate collagen hydrolysis.

4.3.3. Extraction of Gelatin Using HPP

HPP was employed to aid in gelatin extraction through two different stages: either in the pre-treatment procedure or during the extraction, or a combination of both stages. During pre-treatment, acidification at 10 °C accelerates the destabilisation of labile acid crosslinks, and extraction at 45 °C in water speeds up the hydrolysis of collagen. Gómez-Guillén et al. (2005) [58] found that extraction with HPP at 250 MPa/10 min increased gelatin yield (22.8%) when compared to the conventional method (21.3%). The advantage of this step is that it helps reduce the initial swelling step during the pre-treatment process from 3 h to 10 min at 250 MPa. However, the increase in pressure (250 MPa to 400 MPa) and the pressurisation time (10 min to 20 min) during the extraction process decreased the yield of the extracted fish skin by up to 50%. Jaswir et al. (2017) [57] showed that HPP-assisted pre-treatment increased the extractability of gelatin by up to 25% in the skins of red tilapia, black tilapia, grouper, and threadfin bream, in addition to forming a solid gel with a high melting point. Unfortunately, the use of HPP during the extraction time yielded the lowest results for gelatin yield, melting point, and gel strength. They found that HPP-assisted pre-treatment produced a higher yield than the conventional acid-based pre-treatment process for red tilapia and black tilapia fish skin.

In contrast, grouper and threadfin bream produced similar yields for both processes. The increase in gelatin yield from the skin of red tilapia extracted using the HPP-assisted method (from 258 mg/g to 321 mg/g) and from the skin of black tilapia (217.5 mg/g from 201.5 mg/g) was caused by the pressurisation of this technology. The applied pressure induces protein denaturation by destabilising the inter- and intramolecular bond, which increases the yield of gelatin during thermal extraction [61]. In addition, the presence of higher pressure during the pre-treatment or extraction process allows more acid to penetrate the skin structure. The conditions in both treatments are in line with the mass transfer theory, where the rate of mass transfer is in accordance with the applied pressure/resistance. Hence, higher pressure increases the amount of solvent penetrating the cell membrane. Under the process of HPP extraction, the differential pressure between the interior and exterior of the cell membranes leads to rapid permeation. Based on this, it was also reported that the type of acid used during extraction also influences the gelatin yield. However, Chang et al. (2013) [63] found that the gel strength of collagen increased with HPP, although the yield did not show any difference between using the acid/base extraction method. Using HPP at 150 MPa was also found to shorten the extraction time and the amount of acid used, and this was in line with Gómez-Guillén et al. (2005) [58] and his work using fish skin. However, Yusof et al. (2017) [59] found that increasing the pressure from 150 MPa to 250 MPa increased the gelatin yield from 30% to 32%. However, the yield decreased (26%) when they further increased the pressure to 350 MPa, which indicates that high pressure does not necessarily produce high yield.

Interestingly, the 32% yield obtained at 250 MPa was relatively high, given that it was extracted from fish skin. Regardless of pressure and extraction time, Yusof et al. (2017) [59] postulated that to obtain a higher yield of fish-gelatin extraction, a higher amount of acid during pre-treatment is required compared to mammalian gelatin. As fish gelatin has a lower proline and hydroxyproline concentration than Type A and Type B gelatin, it is easily denatured at low temperatures. However, high pressure (250 MPa) did not degrade the protein but enhanced the protein structure [57]. The concentration of pre-treated HPP-assisted gelatin was higher compared to the conventional method. Furthermore, high pressure was proven to increase gelatin concentration, which indicates an improvement in gelatin

properties [57]. However, due to the different sources and the variety of extraction methods, gelatin extraction assisted by HPP results in lower protein content than the conventional approach. This statement is supported by Chen et al. (2014) [56] and Zhang et al. (2016) [64] and their work extracting gelatin from pig skin. The gelatin yield extracted from the pig skin was remarkably high, as Chen et al. (2014) [56] obtained a yield of 63% when HPP was applied at 300 MPa for 15 min, assisted with 1% HCl. Zhang et al. (2016) [64] improved the method by adding pepsin during extraction and obtained an 80% yield from pig skin. The pressure of 300 MPa was optimal for HPP-assisted pig-skin extraction. This might be due to the different extents of damage caused by the intermolecular covalent crosslinks that form between collagen molecules in collagen fibres.

Jaswir et al. (2017) [57] described that gels extracted from HPP-assisted pre-treatment produced fragile gel/no gel, similar to gelatin extracted using conventional methods. Furthermore, gelatin extracted from HPP-assisted pre-treatment was in solid form. In contrast, there are no gel formations from the extraction process administered with HPP after refrigeration. This observation shows that during the extraction process, HPP failed to produce gel formation when the temperature was low (4 °C), suggesting a low melting point. This result is also paralleled in the study by Gómez-Guillén and Montero (2001) [62], who described that the stability of gelatin made from fish skins is low, as they melt at a lower temperature. Both Chen et al. (2014) [56] and Zhang et al. (2016) [64] obtained similar results of gel strength between 390 and 400 g, respectively, in pig-skin-extracted gelatin. The same procedures adopted by Chen et al. (2014) [56] and Zhang et al. (2016) [64] might lead to similar results. However, a different extraction method in the porcine placenta was carried out by Lee et al. (2013) [43]. Their work found that the solubility of collagen extracted from porcine placenta increased when it was treated at 150–170 °C compared to the ground raw placenta. The insoluble collagen in the sample gradually changes into soluble gelatin in water following thermal treatment and might increase the sample solubility in the supernatant. The characteristics of gelatin using High pressure processing from various sources are summarized in Table 2.

4.4. Microwave-Assisted Extraction

Microwave is an extraction technology and a thermal-based approach that uses electromagnetic waves to increase temperature and evaporate intracellular fluids. This causes the breakdown of cells and the release of intracellular compounds into the medium. This extraction method uses electromagnetic radiation with a frequency range between 300 MHz and 300 GHz. Microwave-assisted extraction (MAE) is commonly applied to produce bioactive peptides from raw plants and animals. This process is efficient and considered a promising method to increase the yield of the product of interest. Compared to ultrasounds, MAE is an energy-assisted extraction method that uses less solvents and improves extraction yields [65]. MAE has several advantages over conventional extraction, including shorter extraction time, reduced solvent consumption, higher yield, greater precision, and suitability for thermolabile chemical components [66,67]. Moreover, MAE increases the bioactivity of protein hydrolysates due to enhanced proteolysis and the formation of lower-molecular-weight peptides. However, the heat generated during the extraction process may damage heat-sensitive compounds.

4.4.1. Principle

Once microwave radiation interacts with chemically bonded water molecules, high temperature and pressure are generated. High temperature could cause dehydration of the sample and consequently reduce the internal mechanical strength. The MAE process consists of several steps to perform efficient extraction. MAE begins with the generation of electromagnetic waves by a cavity magnetron inside the cell. Cell tissues and other subunits in the cell interact with the radiation waves produced. The absorption of photonic energy from electromagnetic waves generates electromagnetic energy, which heats up the water trapped inside the cell and evaporates it from the cell matrix. Cell swelling could

occur if significant pressure is imposed at the cellular and subcellular levels during the MAE process. This condition eventually alters cell structure in the matrix and enabls the more significant mass transfer of solutes due to cell rupture. This, in turn, accelerates cell hydrolysis during MAE extraction [5]. Figure 5 shows the schematic process of the MAE system.

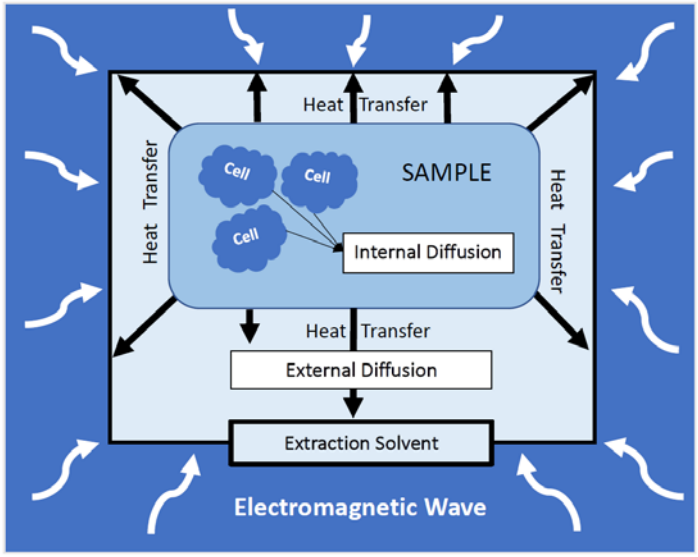

Figure 5. Schematic mechanism of the microwave-assisted extraction system.

4.4.2. Extraction of Gelatin Using MAE

Besides reducing extraction time and use of chemical solvents, MAE also helps to improve the physicochemical characteristics of gelatin, such as gel strength, viscosity, melting point, pH, and colour. To compare the extraction efficiency of different extraction methods, Park et al. (2013) [68] studied the effects of extraction by a microwave oven, water bath, and pressure cooker on the quality of duck-feet gelatin. They reported that the highest yield obtained from the experiment was the by conventional water-bath extraction (51.83 ± 1.27%), while the microwave oven reported the lowest yield (17.58 ± 1.42%). Since the heat was produced within the solvent medium in a microwave oven, it could have caused a drying effect on the surface of the duck feet that retarded gelatin extraction, resulting in a low gelatin yield [68]. The higher gelatin yield was obtained from duck skin, with a recovery of 28.51% [29], and the lowest was from rabbit skin (6.44%) [69]. Gelatin extracted from duck skin, duck feet, and rabbit skin recorded a lower yield than the conventional method, which was 11.71% in duck skin, 51.83% in duck feet, and 7.50% in rabbit skin. However, Liu et al. (2019) [69] reported that the gelatin yield increased with increased extraction time from 60 to 90 min at 65 °C using 500 W. However, the yield was still considered low when compared with conventional water-bath extraction.

The microwave parameters are factors that influence the size of yield obtained. Higher frequency might not affect the yield, as gelatin yield from duck skin using the lowest microwave frequency (200 W) was 28.52% compared to duck feet (310 W) and rabbit skin (500 W). A short extraction time of 5 min may not be sufficient for collagen to completely hydrolyse to extract the gelatin from duck feet [68], and a longer extraction time of 60 min [69] may over-hydrolyse the protein structure. Since collagen crosslinks are unstable at high thermal levels, a low yield of gelatin is generally obtained because there is a loss of the three-dimensional structure of the protein. Thus, an optimal duration of extraction needs to be identified.

Interestingly, Park et al. (2013) [68] reported that the values of gel strength (700 g), viscosity (0.015 Pa.s), and melting point (39.38 ± 0.25 °C) of gelatin extracted were higher with a microwave oven compared to the conventional method (620 g gel strength, 0.0074 Pa.s viscosity and 38.69 ± 0.31 °C melting point). The microwave oven required less extraction

time than the traditional method, which preserves the collagen molecules from undergoing complete denaturation, resulting in high gel strength, high viscosity, and high melting point. Similar results were reported by Liu et al. (2019) [69], as gelatin obtained by microwave at 60 min showed equal gel strength to water-bath extraction, indicating that a short microwave time could produce better gel strength. This might be ascribed to the fact that a short microwave extraction time could properly release subunit components to achieve higher gel strength. Prolongation of microwave time (60–90 min) caused the gel strength decreased significantly. However, gelatin extracted from duck skin with a shorter extraction time of 10 min showed low gel strength of 260 g and 77.86 ± 3.64 mPa.s viscosity compared to duck feet and rabbit skin, though the values were still higher than the conventional method (250 g gel strength, 56.92 ± 6.01 mPa.s viscosity). The molecular weight of the protein was the lowest using this method because of degradation, which reduces the quantity of proline and hydroxyproline; therefore, the physicochemical properties of the gelatin were also affected. The decrease in gelatin gel strength could be due to the reduction in amino-acid content or the degradation of high-molecular-weight subunits in gelatin [10]. These two aspects were verified by Liu et al. (2019) when they reported on the reduction in gel strength as the duration of the microwave was prolonged and different types of raw material were used [69].

Kim et al. (2019) [29] also studied the effect of various heating methods, such as a water bath, ultrasound, HPP, and microwave oven on duck-skin gelatin. In accordance with the finding on duck feet, the pH, gel strength, and viscosity of duck-skin gelatin using a microwave oven were higher than other extraction methods, such as ultrasound (220 gel strength, 65.33 ± 1.52 mPa.s viscosity, 33.25 ± 0.65 °C melting point) and HPP (210 gel strength, 74.89 ± 3.91 mPa.s viscosity, 31.25 ± 0.29 °C melting point). However, the melting point using water-bath extraction was higher than that of the microwave oven: 33.88 °C and 32.75 °C, respectively. Other than that, the quality and quantity of gelatin depended greatly on the type and species of the animals [7]. Mirzapour et al. (2019) [28] demonstrated that microwave extraction on common carp (*Cyprinus carpio*) produces lower gelatin yield, gel strength, viscosity, melting point, and gelling point compared to the ultrasonic extraction method.

According to Liu et al. (2019) [69], the content of amino acids (glycine (Gly), proline (Pro), and hydroxyproline (Hyp)) from rabbit-skin gelatin was not influenced by MAE extraction time, which indicates that the amino-acid content remained relatively stable, regardless of the microwave extraction time or method. Gelatin obtained by MAE from 5 to 30 min extraction recorded numerous high-molecular-weight subunits, suggesting that these conditions are optimum for polymer subunits to be released; thus, higher gel strength could be obtained. However, a longer extraction time of 60 to 90 min degrades the high-molecular-weight subunits and causes the gel strength to become weaker [69]. Breaking of covalent bonds during the MAE process disintegrates collagen due to the vibrational power of the water molecules upon generation of thermal energy during the extraction process. Hence, the collagen polymer subunit might be released rapidly; however, extended exposure to MAE could also cause excessive breakdown of the polymer subunits.

Consequently, high-molecular-weight subunits created via MAE may be rapidly dissolved and be destroyed with the extension of MAE exposure duration. Research conducted by Binsi et al. (2017) [70] evaluated the gelation and thermal characteristics of microwave-extracted fish-scale gelatin blended with natural gums; however, the specific parameters for MAE were not indicated in their manuscript. Thus, no conclusion could be made on the condition of MAE for gelatin extraction. In addition, Feng et al. (2021) [71] reported that the solubility of commercial pig-skin gelatin improved tremendously at 25 °C due to the destruction of polymer subunits. Likewise, more hydrophobic groups were exposed when treated with microwaves, and the produced gelatin was more hydrophobic, improving the amphiphilic property and the interfacial properties of gelatin. The summary of the characteristics of gelatin using microwave - assisted extraction from various sources are shown in Table 2.

Table 2. Characteristics of gelatin using green-technology extraction from various sources.

Sample Origin	Technology	Optimum Extraction Conditions	Characteristics	Major Findings	Limitation	References
Bighead carp (*Hypophthalmichthys nobilis*) scales	Ultrasound-assisted extraction (UAE)	200 W, 60 °C, 5 h.	46.7% yield, 490.6 g gel strength, 27.25 °C melting point, 89.61 total protein, high free amino acid.	High yield. Short extraction time with HCl and NaOH.	Need a pre-treatment method.	[14]
Bighead carp (*Hypophthalmichthys nobilis*) scales		200 W/40 kHz, 60 °C, 1 h,	22.94 gelation points, 29.54 °C melting point, 5000 Pa max modulus.	Produced gelatin with the highest viscosity.	Need a pre-treatment method, low gelatin yield.	[16]
Patin fish bone (*Pangasius hypophthalmus*)		700 W, 70 °C, 5 h	5.33 ± 1.03 yield, 147.74 ± 0.83 g gel strength, 30.27 cP viscosity.	Simple setup for the process.	Long period for the pre-treatment method and extraction process, low yield, low gel strength.	[17]
Chicken feet		300 W, 70 °C, 100 min	3.97% yield, 88.35 total protein, 79.23 g gel strength.	Simple setup, short extraction time.	Low yield, low gel strength	[19]
Bovine hide		500 W/53 kHz, 60 °C, 6 h + actinidin	19.65% yield, 502.2 g gel strength, 15.6 mPa.s viscosity, 13.65 hydroxyproline, 20.6 glycine.	Combination of UAE and enzyme increased the extraction efficiency of gelatin yield and quality.	Loss of molecular order (polypeptide chains) in extracted gelatin, enzyme-aided extraction.	[22]
Bovine skin		500 W/53 kHz, 60 °C, 6 h + bromelin	19.71% yield, 595.51 g gel strength, 16.37 mPa.s viscosity, 10.53 turbidity, 20.06 glycine, 17.21 hydroxyproline.			[23]
Duck skin		40 kHz, 60 °C, 10 min	26.15% extraction yield, 33.25 °C melting point, 220 g gel strength, 65.33 mPa.s viscosity	High pressure and high temperature extracted gelatin efficiently, very short extraction time	Produced different MW peptides according to different extraction method.	[29]
Porcine placenta	Subcritical water extraction (SWE)	375 bar, 200 °C, 90 min + trypsin	Mostly low MW peptides.	Save time and energy without pre-treatment methods. SWE substitutes pre-treatment and trypsin to effectively hydrolyse the placenta.	SWE alone was unable to produce low MW peptides, with the major peak being 10 kDa peptides.	[37]
Porcine placenta		10 bar, 170 °C, 30 min	Mostly low MW peptides (434 Da), 45% crude protein, low amount of free amino acid.	No pre-treatment method. Quick procedures. Do not need other medium, as water was the most efficient medium for the system.	Complex procedures and setup. Information was limited only to protein and amino acids.	[38]
Porcine placenta		200 MPa, 25 °C, 5 min + trypsin	Produced hydrolysates with MW below 20 kDa.	Short extraction time to hydrolyse collagen.	Complex procedures and setup.SWE used during the extraction step. Produced high MW (>5 kDa) gelatin. Enzyme-aided to get maximum results.	[39]
Atlantic codfish (*Gadus morhua*) frames		100 bar, 250 °C, 30 min	47 g/100 g protein, 100.27 mg/g hydroxyproline, 88.11 mg/g proline, 145.04 mg/g glycine, 53.9 g/100 g extraction yield.	Short extraction time. No chemical was used during extraction.	Hydrolysates was collagen/gelatin. Applied several modes of operation condition.	[41]
Duck skin		15 °C, 10 min	44.02% extraction yield, 31.25 °C melting point, 210 g gel strength, 74.89 mPa.s viscosity	Optimal physicochemical properties	Pressure was not mentioned. Produced different MW peptides according to different extraction method.	[29]

Table 2. *Cont.*

Sample Origin	Technology	Optimum Extraction Conditions	Characteristics	Major Findings	Limitation	References
Porcine placenta	High-pressure processing (HPP)	375 bar, 170 °C, 30 min	77% solubility, 0.31 mmol/g free amino acids, low MW (<55 kDa), 30% glycine, >10% hydroxyproline and proline.	Efficient energy savings.	The protein hydrolysate was mostly collagen.	[43]
Red tilapia, black tilapia, grouper, threadfin bream skins		250 MPa, 10 min,	HPP—pre-treatment: high yield, solid gel, high melting point HPP-extraction: low yield, no gel, low melting point.	Reduced pre-treatment time and saved energy.	HPP assisted during pre-treatment and extraction.	[57]
Dover sole (*Solea vulgaris*) skin		250 MPa, 10 min (pretreatment), 45 min (extraction)—overnight extraction in water	HPP pre-treatment: 10.2 g/100 g yield, gelling temperature of 8 °C, melting temperature of 17 °C melting temperature, $M_{W.} \leq 100$ kDa.	Short extraction time, high yield. Most of the peptides were low MW peptides	Low yield and gel strength compared to conventional method.	[58]
Red tilapia (*Oreochromis niloticus*) skin		250 MPa, 10 min (pretreatment), 45 min (extraction)—12 h extraction in water + 7.5 mL HCl	Optimum condition produced 32% yield.	Short extraction time and reduced amount of acid in the process.	Amount of acid influenced hydrolysis process. Response for the optimisation was yield and concentration of gelatin. Not much information can be obtained.	[59]
Pig skin		300 MPa, 15 min + 1% HCl (extraction)	80% yield, 400 g gel strength.	Saves time (several hours if in conventional method) and energy.	Acid- and pepsin-aided. HPP cannot degrade collagen subunit.	[64]
Pig skin		300 MPa, 15 min	63% yield, 390 g gel strength, high MW peptides.	Had better physical properties of gelatin compared to the conventional method.	Utilisation of acid for pre-treatment, required system setup.	[56]
Duck feet	Microwave-assisted extraction (MAE)	310 W/2450 kHz, 5 min	17.58% extraction yield, 700 g gel strength, 0.015 Pa.s viscosity, 39.38 ± 0.25 melting point, contain α-1 and α-2 chains that were derived from type I collagen and the β chains. The molecular weight of the α-1 and α-2 chains is approximately 120–130 kDa.	Short extraction time and high-quality gelatin.	Laborious and long pre-treatment method.	[68]
Rabbit skin		500 W, 65 °C, 60 min	6.44% yield, 400 g gel strength, 235.13 ± 2.39 g/1000 g glycine, 94.81 ± 8.52 g/1000 g hydroxyproline.	Simple extraction procedures, short extraction time (5 min). Obtained high gel strength and hydroxyproline.	Need a pre-treatment method.	[69]
Duck skin		200 W/2450 kHz, 10 min	28.51% yield, 260 g gel strength, 77.86 ± 3.64 mPa.s viscosity, 32.75 °C melting point	Short extraction time, high gel strength, and viscosity compared to other extraction methods	Need a pre-treatment method.	[29]
Common carp (scales and fin)		350 W/2450 kHz, 60 °C, 1 min	0.82% yield, 88.19% protein, 367.46 g gel strength, 6.66 mPa.s viscosity, 25.38 °C melting point	Short extraction time when using high frequency.	Long pre-treatment time. Obtained a very low yield.	[28]

5. Challenges and Future Perspectives

The deployment of modern technology to ensure long-term viability is critical for the gelatin industry. The traditional/conventional method has been longstanding, and based on the increased application gelatin in various industries, the demand for this product will continue to rise. Therefore, innovations are needed to explore new technologies and improve existing methods. In line with the global agenda of sustainability, these improvements should consider efficiency, cost, and environmental safety.

Gelatin is conventionally produced in two stages: pre-treatment and extraction. Both stages require acid or alkali treatment to hydrolyse collagen and subsequently produce gelatin. Acid and/or alkali hydrolysis are the approved economic processes; however, hydrolysis requires longer processing times, usually about 24 h for fish and up to 48 h for bovine and pig. The limitation in gelatin production is mainly to obtain more yield and maintain good physicochemical properties for the industry. Pig-skin and bone gelatin have undergone tremendous modifications to achieve the highest commercial production output and are still evolving. However, pig gelatin is less preferable due to religious concerns, and fish gelatin is currently receiving more attention. Unfortunately, modifications and improvements of fish gelatin are still ongoing due to some drawbacks. For example, the quality of pig gelatin is still superior. To tackle this matter, not only do new sources of potential gelatin need to be explored, but extraction methods must also be improvised, and new technology must be incorporated in production process.

Technology like high-pressure processing (HPP) has been used for centuries in food applications and sometimes in gelatin extraction. Similarly, ultrasound and microwave are widely used in food analysis; however, only limited work has been done on gelatin production for the past ten years. Interestingly, subcritical water extraction (SWE) has opened up new possibilities in this industry. SWE has been adopted in other industries, such as palm-oil extraction, extraction of bioactive compounds and bioactive peptides, extraction of antioxidant and phenolic compounds from various sources, as well in the production essential oils. Interestingly, the application of SWE was recently introduced in the extraction of porcine hydrolysates, and this opens up new possibilities for the application of SWE for gelatin extraction.

The purpose of integrating ultrasound, microwave, HPP technology, and SWE is to minimise the extraction time, reduce chemical consumption, and improve extraction efficiency while producing good-quality gelatin. However, based on previous studies, all of these methods cannot hydrolyse peptides on their own, and a pre-treatment process is needed. These pre-treatment extractions usually consume some amount of acid and alkalis in these procedures, which should be reduced if possible. Although acid and alkali waste poses a low risk to the environment after being treated, it is of both environmental and economic interest to eliminate this procedure. The sources of gelatin influence the method and time consumed by extraction. Fortunately, processing time could be markedly cut down from several hours to a few minutes by using several methods. Implementation of these technologies also varies. Some are applied as pre-treatment processes, and others as extraction processes. SWE recorded the shortest time (3–5 min) to achieve desirable results, while other technologies can take from 5 to 60 min. Lengthier operational procedures are costly, and they degrade the yield and quality of gelatin due to extensive hydrolysis.

The limitations of ultrasound, microwave, and HPP to produce low-molecular-weight peptides are not as substantial as those of SWE. However, SWE alone cannot produce low MW peptides (mostly MW more than 10 kDa), reflecting its limited collagen-hydrolysing effect. The advantage of this limitation is that SWE could be used as an efficient, inexpensive, and quick alternative to enzymatic digestion in protein processing due to the ability of SWE to provide high protein-sequence coverage (>90%) throughout the hydrolysis process. Subcritical water extraction was also used to determine the molecular changes of hydrolysates. The basic fundamentals of protein hydrolysates, such as their structure and function, are still not well understood.

SWE has the potential to be developed as a stable and efficient extraction technique to support scientific research towards environmental sustainability. Its simplicity throughout the extraction process, with the help of advanced IT technologies, can reduce waste and help the environment. Conventional methods are traditionally conducted by thermal treatment; however, the technologies discussed, except for microwave extraction, use a non-thermal approach for extraction and allow for effective hydrolysis of protease. These technologies could be categorized eco-friendly methods. In terms of solvents, these extraction approaches only utilise water as the extraction medium, which is easier and cheaper to obtain, as it is reproducible and readily available. Not only does SWE use water, but HPP also practically uses steam and pressure for extraction. In addition, the extraction medium used does not affect the environment, as it is safe, and no chemical waste is produced at the end of the process. As a result, continuous extraction could be achieved when all these technologies are applied in the industry. Food manufacturers could meet a higher demand with these technologies involved in the industry, as they are safe and environmentally friendly methods for the extraction of compounds.

However, the use of these technologies in industry faces several challenges. HPP and SWE are made of delicate systems, and even a minor mishap could cause the machine to malfunction, thus impairing the process. Both HPP and SWE require a complex system setup, especially for the production line. Factors such as water and critical pressure medium for the extraction are crucial. Due to their system complexity, these processes must be supervised by trained and qualified personnel, in addition to accurately setting up the system to deliver the desired result. Unlike SWE, HPP has been well established in the market for various processes. Therefore, HPP would be easier to install, and implementation of a scaling-up process is feasible. As for ultrasound and microwave technology, although both systems are not as complicated as HPP and SWE, factors that can affect ultrasound and microwave energy, such as frequency, power, temperature, and time, need to be critically analysed before extraction. In addition, the whole production line still requires a conventional method, especially for the pre-treatment and extraction process, as ultrasound and microwave work best to assist and improve conventional methods. Therefore, scaling up of production with ultrasound and microwave in a safe, sustainable, and economical manner is particularly challenging in order to ensure the objective of practicing green technology is achievable.

The potential of using ultrasound, microwave, HPP, and SWE is very promising for the gelatin industry. The capacity of these technologies to shorten the operating time and reduce the usage of solvents during extraction can overcome long operational time, pollution, and waste management issues. The advantages and limitation of all procedures mentioned above are summarized in Table 3.

Table 3. Summary of advantages and disadvantages of green extraction technology.

Extraction Technology	Advantages	Limitations
Ultrasound-assisted extraction	Reduce energy consumption, minimize extraction time, assist during pretreatment and/or extraction, produce low-molecular-weight peptides, reduce solvent requirements and waste materials, produce heat during extraction but easily control by applying interval resting time.	Industrial-scale bulk treatment plant is needed, where the uniformity of energy flux for a continuous treatment must be controled, high-power process design, ease of installation and maintenance costs must be strengthened.
Subcritical water extraction	Reduce energy consumption, very short extraction time, applied during pretreatment and/or extraction stages, no solvent usage, reduce waste, does not produce heat during extraction, produce high-molecular-weight peptides.	Delicate system, complex system setup.
High-pressure processing	Time-saving, higher extraction yields, fewer impurities in the extraction solution, minimal heat and can avoid thermal degradation, no solvent usage, reduce waste, produce low-molecular-weight peptides.	Delicate system, complex system setup, produce heat.
Microwave-assisted extraction	Minimize pretreatment or/and extraction time, assist during pretreatment and/or extraction, reduce solvent usage, produce low-molecular-weight peptides.	Thermal (uses energy), produce heat.

6. Conclusions

The application of novel extraction methods, such as ultrasound-assisted extraction (UAE), subcritical water extraction, high-pressure processing (HPP) and microwave-assisted extraction (MAE), has excellent potential, as these methods are eco-friendly and provide an efficient alternative compared to conventional extraction technologies. Although these technologies have their drawbacks, such as overhydration and complex system setups, especially for large-scale production, they are still more efficient in the long run to protect the environment, as they minimise solvent consumption and reduce carbon footprint along the way. Combining these technologies with conventional extraction methods has proven effective and achieved promising results. However, more research should be carried out to determine the optimal conditions to develop efficient processing procedures and produce high-quality gelatin. Since gelatin sources are of various origins and exhibit different characteristics, different approaches and novel technologies could be used to find the method best-suited for specific resources.

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