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# Effect of extraction methods on the yield and gel strength of gelatin extracted from jellyfish *Acromitus flagellatus*

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**ABSTRACT**

*This study assessed the effects of different extraction methods and conditions on the yield and the gel strength of gelatin extracted from jellyfish Acromitus flagellatus, which were collected from the Southern and Western coasts of Sri Lanka. Gelatin was extracted using three methods; hot water extraction (HWE) (at 55, 60, and 65 ℃for 4 and 8 hours), microwave-assisted extraction (MAE) (900W microwave power at 10, 30 and 50 % power levels for 3 and 5 minutes) and ultrasound-assisted extraction (UAE) (at 55, 60 and 65 ℃for 1 and 2 hours), and their gel strength was tested. In the preliminary studies, no gel formation was observed in gelatin extracted from fresh dried jellyfish. Gelatin extracted from salted jellyfish exhibited gel-forming ability. Pretreatment of salted jellyfish at pH 2 resulted in gelatin with better gel properties. The yield and gel strength of jellyfish gelatin varied in different extraction methods and depending on the extraction conditions of the same method. Of all extraction methods and conditions, the highest and the lowest yields were reported in MAE at 30% power level for 3 minutes (34.75 ± 0.02%) and UAE at 55 ℃for 1 hour (6.19 ± 0.02%), respectively. No gel formation was observed in the UAE. When considering all extraction methods and conditions, the highest gel strength was reported for HWE at 60 ℃for 8 hours (565.0 ± 7.1 g). Of the MAE conditions, a 50 % power level for 3 minutes rendered gelatin with the highest gel strength (362 ± 24.75 g), which was significantly lower than that of the highest value of HWE ($p < 0.05$). The results indicate the yield and the gel strength of jellyfish Acromitus flagellatus-based gelatin may vary depending on the extraction methods and the conditions.*

**KEYWORDS:** *Gel strength, Gelatin, Jellyfish, Microwave extraction, Ultrasound extraction, Yield*

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## 1 INTRODUCTION

Gelatin is a protein produced by partial hydrolysis of collagen in animal skins and bones either in acidic or alkaline media. It is an extensively used ingredient in food products, as well as in cosmetics, pharmaceuticals, and photographic industries (Tabarestani et al. 2010; Haug & Draget 2011). Generally, pig and bovine hide and bones are widely utilized for gelatin production on a commercial scale (Tabarestani et al. 2010). Due to religious ideologies, pig and bovine-based gelatins are not acceptable in Muslim and Hindu cultures, respectively (Nagarajan et al. 2012). Moreover, animal-spread diseases; for example, Bovine Spongiform Encephalopathy (BSE) and Foot and Mouth Disease (FMD) have triggered concern over the use of bovine gelatin (Chancharern et al. 2016). Hence, at present, there is a major concern about the production of gelatin from alternative sources.

Jellyfish are marine invertebrates belonging to the Phylum Cnidaria, that possess nematocysts located on their tentacles or sometimes on their umbrella, which can inject venom into their prey and cause stings (Fernando, 2001). Jellyfish are infamous for their negative impacts on fisheries and aquaculture, tourism, coastal power plants, and desalination plant operations (Ranasinghe et al. 2022). However, few edible jellyfish species have been consumed in Asian countries, mainly in China for centuries and they have become a global delicacy in recent times (Zhuang et al. 2010). Meanwhile, in recent years, Sri Lanka experienced many jellyfish washed-up events, that negatively affected the socio-economic well-being of coastal populations. Still, the jellyfish fishery in Sri Lanka is not familiar, and it is considered a useless bycatch by fishermen. Collagen is the key protein found in jellyfish (Hsieh, Leong, and Rudloe, 2001), which can be a potential source of gelatin (Zhuang et al., 2010). There are previous studies based on the extraction of gelatin from jellyfish species including sand jellyfish *Rhopilema hispidum* (Cho et al., 2014), white jellyfish *Lobonema smithii* (Chancharern et al., 2016; Rodsuwan et al., 2016; Charoenchokpanich et al., 2021; Lueyot et al., 2021; Lueyot et al., 2022) and *Stomolophus meleagris* (Chiarelli et al., 2021; Esparza-Espinoza et al., 2023). Sri Lanka spends millions of Dollars on gelatin imports. Therefore, the exploitation of jellyfish for gelatin production can be considered a feasible approach.

The method of collagen pretreatment may impact the extraction efficacy and the characteristics of gelatin (Tkaczewska et al. 2018). Most of the studies found in the literature have followed hot water extraction for gelatin extraction (Ranasinghe et al. 2020). However, different methods such as ultrasound-assisted extraction (Tu et al. 2013; Widyasari and Rawdkuen 2014; Ahmad et al. 2018; Mirzapour-Kouhdasht et al. 2019) and microwave-assisted extraction (Park et al. 2013; Liu et al. 2019; Mirzapour-Kouhdasht et al. 2019) have also been studied recently. Previous studies have reported that the gelatin yield and gel strength varied depending on the extraction methods and conditions (Tabarestani et al. 2010; Ahmad and Benjakul 2011;

Sinthusamran, Benjakul & Kishimura 2014; Chancharern et al. 2016; Rodsuwan et al. 2016; Pan et al. 2018). Hence, this study focused on the effects of different extraction methods and conditions on the yield and the gel strength of gelatin extracted from jellyfish *Acromitus flagellatus*. To the best of our knowledge, this is the first attempt to extract gelatin from jellyfish in Sri Lanka and from the jellyfish *Acromitus flaellatus* in the world.

## 2 MATERIALS AND METHODS

### 2.1 Sample collection

The fresh samples of jellyfish *Acromitus flagellatus* were collected from Negombo (7°12′ N, 79°50′ E), Gampaha District, and Koggala (5°59′ N, 80°19′ E), Galle District, Sri Lanka. The collected samples were rapidly transferred into an ice box and transported to the Department of Food Science and Technology, University of Sri Jayewardenepura, Sri Lanka. Thereafter, the samples were thoroughly cleaned with water and the gonads were removed.

### 2.2 Preparation of salted jellyfish

Salted jellyfish preparation was based on the method described by Sloan & Gunn (1985), with a few modifications, by stepwise processing with mixtures of salt and alum. First, jellyfish were separated into umbrellas and oral arms, and they were salted separately. For the first step of salting, jellyfish were stacked with 25% (w/w) of salt/alum mixture (Salt: alum = 100:5) in alternative layers for 24 hours. Then, the jellyfish were drained, and the second salting step was performed by stacking the jellyfish in alternative layers with 20% (w/w) of salt/alum mixture (salt: alum: $NaHCO_3$ = 100: 10: 1) and left for 3 days. After 3 days, the jellyfish parts were drained and again stacked with 18% (w/w) of salt/alum mixture (salt: alum: NaHCO3=100:10:0.5) for 7 days.

### 2.3 Preparation of dried jellyfish powder

The method described by Chancharern et al. (2016) was followed for the preparation of dried jellyfish powder. Salted jellyfish was minced and washed with tap water for a considerable time until the salt content reached below 1%. Then, the samples were vacuum-dried at 50 ℃ (-0.1 MPa), until the moisture content reached below 9%. The dried jellyfish were ground and stored in an air-tight container and refrigerated.

### 2.4 Selection of pretreatment pH

Gelatin pretreatment was conducted according to the method described by Chancharern et al. (2016), with small modifications. Based on the previous literature, four pH values (pH 1, 2, 12, and 14) were initially tested for their suitability for pretreatment. The dried jellyfish powder was soaked in sulfuric acid solution (pH 1 and 2) for acid-treated (type A) and sodium hydroxide solution (pH 12 and 14) for alkaline-treated (type B), with a ratio of dried sample: solution = 1: 15 (w/v) for 24 hours at room temperature. After 24 hours, pH was adjusted to 7 using 1 N sodium hydroxide and 1 N sulphuric acid for type A and type B pretreatments, respectively. Then, the mixtures were held at 60 ℃ for 8 hours in a water bath. The extracts were centrifuged at 6000 rpm for 30 minutes, and the filtrates were collected and

vacuum-dried at 50 ℃ (-0.1 MPa). The dried gelatins obtained were stored in air-tight containers.

For the gel formation, gelatin samples were dissolved in distilled water at 6.67% (w/v) concentration and heated at 60 °C for about 1 hour until the gelatin was completely dissolved. The gelatin solutions were placed in a refrigerator at 10 °C for 16-18 hours for the gel formation.

**2.5 Pretreatment**

Acid pretreatment at pH 2 was conducted following the method described in section 2.4, followed by gelatin extraction by different extraction methods.

**2.6 Hot water extraction (HWE)**

The pretreated solutions were heated in a water bath at 55, 60, and 65 ℃ for 4 and 8 hours, with a total of six treatment combinations.

**2.7 Microwave-assisted extraction (MAE)**

The pretreated solutions were subjected to microwave-assisted extraction (Singer microwave oven, SMW928AS3, frequency 2450 MHz, 900 W microwave power) at three power levels (50, 30, and 10 %) for 3 and 5 minutes, with a total of six treatment combinations.

**2.8 Ultrasound-assisted extraction (UAE)**

The pretreated solutions were subjected to ultrasonication using an ultrasonic instrument (EMAG Ultrasonic cleaner Emmi-D130, Germany) at 400 W and 45 kHz, at 55, 60, and 65 ℃ for 1 hour and 2 hours, with a total of six treatment combinations.

**2.9 Sample drying**

The samples of each extraction method and condition were centrifuged at 6000 rpm for 30 minutes, and the filtrates were collected and vacuum-dried at 50 ℃ (-0.1 MPa). The dried gelatins obtained were stored in air-tight containers.

**2.10 Yield of gelatin**

The dried gelatin yield was calculated using the following equation.

$$\text{Gelatin yield (\%)} = \frac{\text{Weight of dry gelatin}}{\text{Weight of dried jellyfish}} \times 100$$

**2.11 Determination of gel strength**

The gel strength of the extracted gelatins was determined following Senarathna and Marapana (2021), with modifications. A gelatin solution of 6.67% (w/v) concentration was heated at 60 °C for about 1 hour until the gelatin was completely dissolved. Then, it was placed in a refrigerator at 10 °C for 16-18 hours to allow the gel formation. The gel strength was determined using a texture analyzer (CT3, Brookfield, USA) equipped with a 1.27 cm diameter flat-faced cylindrical probe. Gel strength was expressed as maximum force (in g) when the plunger had penetrated 4 mm into the gelatin gel at a load cell of 100 g. The pre-test and the post-test speeds of the plunger were 1 mm/s.

**2.12 Statistical analysis**

The statistical analysis of data was carried out using one-way ANOVA to test the significance of each variable ($\alpha$ = 0.05), followed by comparisons performed using the Tukey test by the statistical software MINITAB®17. All data

were presented as mean value ± standard deviation.

## 3 RESULTS & DISCUSSION

### 3.1 Preparation of salted jellyfish

In trial experiments, first, gelatin was extracted from fresh dried jellyfish at different extraction conditions. However, none of them formed gels at 6.67 – 25 % (w/v) gelatin concentrations. Almost all previous studies, which have extracted gelatin from jellyfish have used salted jellyfish (Cho et al. 2014; Chancharern et al. 2016; Rodsuwan et al. 2016; Charoenchokpanich et al. 2021; Lueyot et al. 2021; Lueyot et al. 2022). Therefore, a jellyfish salting process using salt and alum before gelatin extraction was performed and gel formation could be observed under the naked eye. Previous studies have reported that salting can precipitate the proteins in jellyfish. However, the impact of salting on the collagen in jellyfish and the resulting gelatin is not adequately elaborated in the literature.

Before the drying step, salted jellyfish were subjected to a desalting process to remove excess salt and alum, since NaCl can break both hydrogen and hydrophobic bonds, which may negatively impact the gelatin network, thereby reducing the gel strength (Monsur et al. 2014; Lueyot et al. 2022).

### 3.2 Selection of pretreatment pH

Tkaczewska et al. (2018) have found that the pre-treatment method has a major impact on the characteristics of the resulting gelatin. Figure 1 demonstrates the gel formation of gelatin pretreated at different pH values.


**Figure 1:** Gel formation of gelatins extracted with different pretreatment conditions (pH 1, 2, 12, and 14)

Among the four tested pH conditions, gelatin pretreated at pH 1 and 2 exhibited gel formation. However, a stable gel was present only in pH 2 pretreatment, while pH 1 pretreated gel was not stable at room temperature. A decrease in the gel strength of white jellyfish (*Lobonema smithii*) gelatin with an increasing acid concentration in the pretreatment has been reported by Charoenchokpanich et al. (2021). No gel formation was observed in gelatin subjected to alkali pretreatments at pH 12 and 14. Therefore, pH 2 was selected for the pretreatment of jellyfish before gelatin extraction. Similar to the present study, alkali pretreatment of jellyfish *Lobonema smithii* at pH 14 resulted in gelatin with no gel-forming ability (Chancharern et al. 2016). Monsur et al. (2014) have stated that fish collagen contains low amounts of non-reducible intra and inter-chain cross-links, enabling the application of mild acid pretreatments for the gelatin extraction from fish species, which is reinforced by the findings of the present study.

### 3.3 Gelatin yield

The yield of gelatin extracted from jellyfish at different extraction methods is depicted in Figure 2.

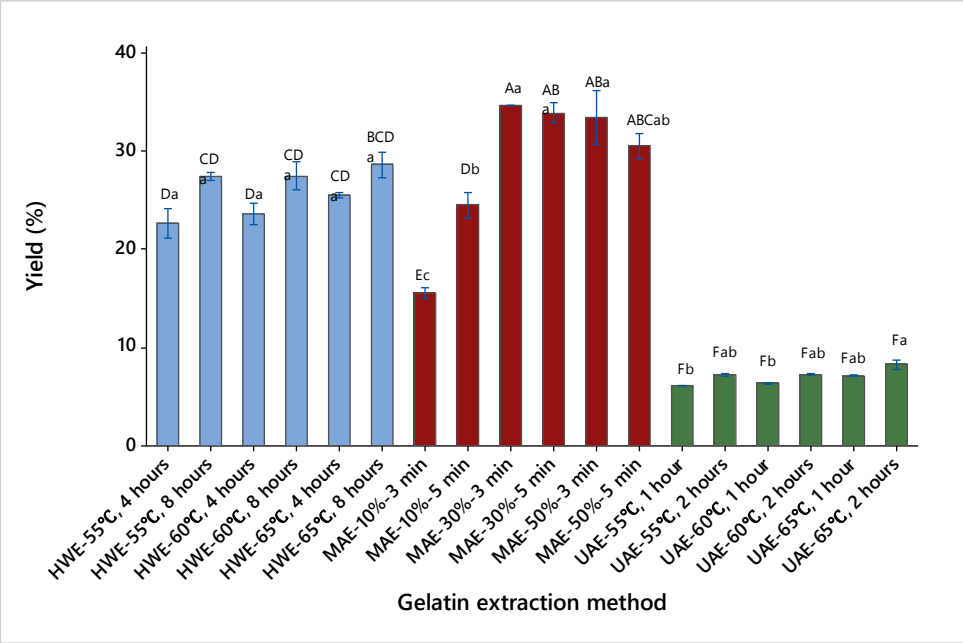


**Figure 2:** Effect of extraction method on the yield (%) of jellyfish gelatin (Data presented as mean ± standard deviation (N = 3))

HWE- Hot Water Extraction, MAE- Microwave-Assisted Extraction, UAE- Ultrasound-Assisted Extraction

Bars bearing different superscripts (A-F) indicate significant differences ($p < 0.05$) in yield (%) among different extraction methods. Bars bearing different superscripts (a-c) indicate significantly different ($p < 0.05$) yields among different extraction conditions within the same extraction method.

The yield of gelatin was significantly different in different extraction methods ($p < 0.05$). When considering all extraction methods and conditions, the highest yield was reported in gelatin extracted from MAE at 30% power level for 3 minutes (34.75 ± 0.02%), while the lowest yield was obtained by UAE at 55 ℃ for 1 hour (6.19 ± 0.02%).

In the HWE method, the highest yield (28.66 ± 1.85%) was recorded at 65 ℃/8 hours condition while the lowest yield (22.67 ± 2.10%) was reported by extraction of gelatin at 55 ℃ for 4 hours. The yields of type A gelatin extracted from jellyfish *Lobonema smithii* umbrella at 45 – 75 ℃ for 6-12 hours have ranged between 19.43 – 40.54 % (Rodsuwan et al. 2016), while Chancharern et al. (2016) have observed 24.39 ± 0.19 – 39.47 ± 0.04 % yield for type A gelatin extracted from the same species at 60 – 75 ℃ for 6 – 12 hours. Also, a comparatively higher yield (38.5 %) has been reported for gelatin extracted from sand jellyfish *Rhopilema hispidum* at 60 ℃ for 5 hours (Cho et al., 2014). In contrast, Esparza-Espinoza et al., (2023) reported a 10.49 ± 0.18% yield of gelatin extracted from jellyfish *Stomolophus meleagris* at 60 ℃ for 12 hours, followed by an alkali pretreatment, which is lower compared to that of the present study. The results in the present study comply with previous literature, as gelatin yields increased with increasing extraction time and temperatures for jellyfish *Lobonema smithii* (Chancharern et al. 2016; Rodsuwan et al. 2016; Charoenchokpanich et

al. 2022; Lueyot et al. 2022). Karim and Bhat (2009) state that the extent of collagen-to-gelatin conversion is governed by the properties and the preservation methods of raw materials, pretreatment, and processing conditions. At higher extraction temperatures, more energy is supplied for the destabilization of hydrogen and covalent bonds which are responsible for collagen matrix stabilization, resulting in an amorphous triple helix structure, which can be easily extracted to the medium. Prolonged extraction times also provide more energy to destroy bonds, releasing more free α or β-chains (Sinthusamran, Benjakul & Kishimura 2014). Furthermore, hydrolysis of amide bonds at higher extraction temperatures and prolonged extraction time can result in higher gelatin yields (Kaewruang, Benjakul & Prodpran 2013; Pan et al. 2018).

In the MAE method, the yield significantly increased ($p < 0.05$) from 10 – 30 % microwave levels, but slightly decreased at 50% microwave level, but that was not significant ($p > 0.05$). Although it was expected an increasing trend of yield with microwave extraction time, based on the findings of previous studies (Liu et al. 2019; Mirzapour-Kouhdasht et al. 2019), the yield slightly decreased with prolonged extraction at 30 and 50 % microwave levels, but that was not significant ($p > 0.05$). This can be a result of a higher degree of surface drying caused by prolonged exposure to microwaves, which may hinder the efficient extraction of gelatin (Park et al. 2013). Previous studies have reported lower yields of gelatin extracted from duck feet (Park et al. 2013), duck skin (Kim et al. 2020), and rabbit skin (Liu et al. 2019) using MAE compared to that of HWE. However, in the present study, the yield of MAE was significantly higher ($p < 0.05$) than that of HWE. This can be due to the higher microwave power (900 W) applied in the present studies compared to the former studies (350 W and 200 W).

Within the UAE method, the highest and the lowest yields were recorded for extraction conditions of 65 ℃ for 2 hours and 55 ℃ for 1 hour, respectively. Although the gelatin yield increased significantly ($p < 0.05$) with increasing ultrasonication temperature, and within the same temperature the yield was not significantly different ($p > 0.05$) in different extraction times. However, previous studies have reported increased gelatin yield with increasing ultrasonication time at the same temperature (Ahmad et al. 2018; Mirzapour-Kouhdasht et al. 2019). A significantly lower ($p < 0.05$) yield in UAE compared to HWE was observed. This can be due to the shorter extraction times employed in our study to preserve the gel strength. Widyasari and Rawdkuen (2014) have also reported a lower yield of chicken feet gelatin extracted using UAE (3.96% wet basis) than the HWE method (4.05% wet basis). However, Tu et al. (2013) have reported a higher yield of bighead carp (*Hypophthalmichthys nobilis*) scales gelatin extracted using the UAE method (30.94 – 46.67 %) than that of HWE (19.15 – 36.39 %). This is mainly a result of the mechanical effects and acoustic cavitation of ultrasound, which loosens the collagen matrix and allows more penetration of the liquid medium, thus,

increasing the release of gelatin (Ahmad et al, 2018; Tu et al. 2013).

### 3.4 Gel strength

Figure 3 shows the effect of different extraction conditions on the gel strength of gelatin extracted from jellyfish.

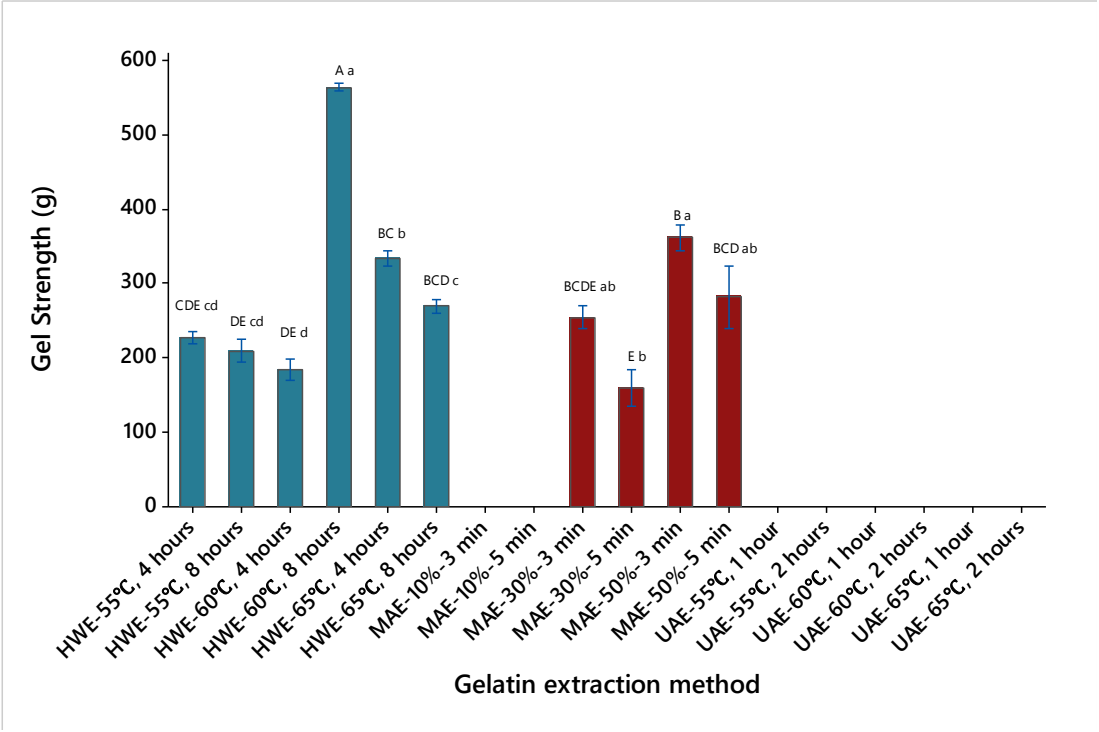


**Figure 3:** Effect of extraction method on the gel strength of jellyfish gelatin (Data presented as mean ± standard deviation (N = 3))

HWE- Hot Water Extraction, MAE- Microwave-Assisted Extraction, UAE- Ultrasound-Assisted Extraction

Bars bearing different superscripts (A-E) indicate significant differences ($p < 0.05$) in gel strengths among different extraction methods. Bars bearing different superscripts (a-d) indicate significantly different ($p < 0.05$) gel strengths among different extraction conditions within the same extraction method.

The gel strength is the most important physical property of gelatin. In the present study, the gel strength was significantly different in different extraction methods ($p < 0.05$). When considering all extraction methods and conditions, the highest gel strength was reported for gelatin extracted using HWE at 60 ℃ for 8 hours (565 ± 7 g).

In the HWE method, the gel strength decreased with increasing extraction time except at 60 ℃. The lowest gel strength was obtained in 60 ℃/ 4 hours extraction condition (185.0 ± 21.2 g). Except for this condition, the gel strength increased with increasing extraction temperature. Charoenchokpanich et al. (2022) have also observed that the gel strength of gelatin extracted from the jellyfish *Lobonema smithii* byproducts increased with increasing extraction time from 12 hours to 48 hours at 60 ℃. Moreover, Chancharern et al. (2016) reported no gel formation in *Lobonema smithii* gelatin extracted at 60 ℃, but the gel strength of gelatin extracted at 75 ℃ increased with the prolonged extraction time from 6 to 12 hours.

However, lower gel strengths in gelatin extracted at higher temperatures and prolonged time has been reported in *Lobonema smithii* (Rodsuwan et al. 2016; Lueyot et al. 2021; Lueyot et al. 2022) and gelatin extracted from the skins of fish species including seabass (*Lates calcarifer*) (Sinthusamran, Benjakul & Kishimura 2014), chum salmon (*Oncorhynchus keta*) (Liu et al. 2017) and tiger puffer (*Takifugu rubripes*) (Pan et al. 2018). Higher temperatures and prolonged extraction times lead to greater gelatin hydrolysis, resulting in low molecular weight fragments. These contain lower amounts of inter-junction zones for interactions and form a weaker gel (Gómez-Guillén et al. 2002; Liu et al. 2017; Pan et al. 2018).

Of the MAE conditions, 50 % power level for 3 minutes rendered the gelatin with the highest gel strength (362 ± 25 g). No gel formation was observed in microwave-extracted gelatin at a 10 % power level. This can be due to the insufficient microwave power for the conversion of collagen to gelatin. The gel strength increased with increasing microwave power level but decreased with prolonged extraction time in the same power level (Figure 3). Similarly, former studies have reported a decrease in the gel strength of Hyla rabbit skin gelatin (Liu et al. 2019) and common carp gelatin (Mirzapour-Kouhdasht et al. 2019) which were subjected to MAE for longer times. This can be due to the degradation of high molecular-weight gelatin subunits to low molecular-weight fragments during extended microwave treatments (Liu et al. 2019). In the present study, the highest gel strength of MAE gelatin was noticeably lower ($p < 0.05$) than that of the highest gel strength of HWE. However, previous studies have reported higher gel strength of MAE duck feet gelatin (Park et al. 2013), Hyla rabbit skin gelatin (Liu et al. 2019), and duck skin gelatin (Kim et al. 2020) compared to that of HWE.

No gel formation was observed in any of the UAE gelatin. Lower gel strengths of gelatin extracted using ultrasonication compared to the conventional method have been reported in bighead carp (*Hypophthalmichthys nobilis*) scales gelatin (Tu et al. 2013) and chicken feet gelatin (Widyasari & Rawdkuen 2014). This can be due to the greater degradation of gelatin due to the higher energy supplied during ultrasonication.

The most common method of gelatin extraction is Hot Water Extraction. However, it is time-consuming. Hence, there are former researchers who have focused on gelatin extraction using Microwave-Assisted Extraction and Ultrasound-Assisted Extraction attributed to their shorter extraction times. Although there are numerous studies devoted to the extraction and characterization of gelatin from fish offal, only a few studies have focused on the extraction of gelatin from jellyfish in the world, and all of them have adhered to Hot-Water Extraction. Therefore, to the best of our knowledge, this is the first attempt to extract gelatin from jellyfish using Microwave-Assisted Extraction and Ultrasound-Assisted Extraction in the world. Furthermore, previous studies have tested the gelatin extraction potential of commercially important edible

jellyfish species. This study revealed the potential to extract gelatin from jellyfish *Acromitus flagellatus,* which is considered a bycatch.

Sri Lanka spends millions of dollars on gelatin imports annually, which is mostly used in the food industry. The commercially available gelatins are usually produced from porcine skins or bovine hides, and their consumption is prohibited in Islam and Hindu religions, which account for more than 20% of the country's population. To the best of our knowledge, there is no previous study that has focused on the extraction of gelatin from jellyfish of Sri Lanka, and this study filled the gap of scientific knowledge in the area, encouraging the utilization and value-addition of jellyfish within the country, which may generate a novel avenue for people living in the fishing areas to earn from bycatch species. Moreover, jellyfish-based gelatin would be a better alternative to increase the gelatin consumption of Hindu and Islamic religious groups, as well as for the reduction of gelatin imports to Sri Lanka.

## 4 CONCLUSION & RECOMMENDATIONS

No gel formation occurred in gelatin extracted from jellyfish without salting, but gelatin extracted from salted jellyfish exhibited gel-forming ability. This can be a result of the modification of the collagen of jellyfish after salting. Previous studies have reported that salting can precipitate the proteins in jellyfish. Still, sufficient explanations for the impact of salting on the collagen structure could not be found. Therefore, an in-depth investigation of the impact of salting on the collagen of jellyfish is required.

Pretreatment of salted jellyfish at alkaline pH resulted in no gel formation while pretreatment at pH 1 and 2 resulted in gelatin with gel-forming ability, with pH 2 pretreatment exhibiting better gel properties. The highest and the lowest yields were reported in MAE at 30% power level for 3 minutes and UAE at 55 ℃ for 1 hour, respectively. No gel formation was observed in any of the gelatins extracted using an ultrasound-assisted process. Hot water extraction at 60 ℃ for 8 hours and microwave extraction at 50 % power level for 3 minutes resulted in the highest gel strengths of the respective methods. It can be concluded that the yield and the gel strength of jellyfish gelatin may vary depending on the extraction methods and the conditions.

In this study, gelatin was extracted from only one species, *Acromitus flagellatus*. The potential to extract gelatin from other jellyfish species found in Sri Lanka can be investigated.

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