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Comparative analysis of red skin Tilapia and bovine gelatins as halal alternatives in food industry

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Abstract

Gelatin plays a vital role in the food industry, serving as a thickening agent, emulsifier, wetting agent, and stabiliser. However, conventional sources like mammalian gelatin pose health and societal issues, while poultry gelatin can present risks related to avian flu. Our work was motivated by recent studies focusing on alternative gelatin sources, which prompted further investigation. Our study aimed to extract gelatin from red-skin Tilapia and bovine sources. Both types of gelatin underwent pre-treatment using 0.2 M sodium hydroxide (NaOH) and 0.05 M acetic acid (CH_3COOH) at 27°C, followed by water extraction at 60°C for 3 hours. Fourier-transform infrared (FTIR) analysis confirmed that the extracted gelatins exhibited peaks similar to commercial gelatin. The extracted fish gelatin (EFG) demonstrated superior gel strength compared to commercial fish gelatin (CFG), whereas commercial bovine gelatin (CBG) exhibited superior gel strength than extracted bovine gelatin (EBG). The protein content of EFG and EBG was comparable, but the fat content was significantly higher in EFG. The foaming capacity was also evaluated, with EFG showing greater capacity than EBG. Our work demonstrates excellent potential of alternative gelatin for usage in various applications and creates new opportunities for the food sector, particularly for halal food production.

1. Introduction

Global demand for gelatin is expected to increase by 230 million metric tonnes over the next five years, with a consistent compound annual growth rate (CAGR) of 5.6%. The global gelatin market is projected to grow from $3.20 billion in 2024 to $5.51 billion by 2032, reflecting a CAGR of 7.03% (Gelatin Market Report, 2023). Recent research has explored various gelatin sources, including camel. (Al-Hassan, 2020), rabbit (Liu *et al.*, 2019), goat (Zilhadia *et al.*, 2022), and porcine (Sha *et al.*, 2019). Despite its rich collagen content, mammalian gelatin faces significant limitations for Muslim consumers due to its halal status and the risk of diseases such as foot and mouth disease (FMD).

Different sources of gelatin, such as fish, bovine, and pig, exhibit unique compositions and structures that significantly impact their physicochemical and functional properties, such as protein content, gel strength, foaming, and emulsifying abilities. Cold-water fish commonly have low gelling properties due to low amino acid compositions, low molecular weight distributions, and low melting points, resulting in less stable gels at room temperature (Wu *et al.*, 2023). However, recent advancements in processing methods have begun to address these limitations. Techniques such as suitable acid/alkaline ratios, enzymatic catalysis, and cross-linking fish gelatin with transglutaminase (TGase) have enhanced fish gelatins' gel strength and stability (Huang *et al.*, 2020). Furthermore, acidic pre-treatments have proven effective in improving gelatin yield and consistency, as demonstrated in studies involving smooth hound tissue (*Mustelus mustelus*) (Silva *et al.*, 2014). These improvements underscore the potential for broadening the applicability of fish gelatin in various industrial applications.

Warm-water fish such as Black Tilapia possess qualities similar to mammalian gelatin, including comparable gel strength and thermal stability, making it a viable alternative for halal food applications (Zheng *et al.*, 2024). This suitability is essential given the dietary guidelines observed in Muslim communities, which restrict the use of traditional mammalian sources. The study on gelatin extraction from Red Tilapia is still limited compared to Black Tilapia. Our previous work indicated that acid-alkaline pre-treatments yield gelatin with high gel strength and excellent functional properties (Fazial *et al.*, 2024). This indicates the practicality of Red Tilapia as an alternative gelatin source and highlights the effectiveness of specific processing techniques in optimising its properties for broader commercial use. Further studies could explore the difference between Tilapia species and refine extraction methods to maximise yield and functional characteristics, thereby broadening the scope for its application in the halal food industry.

This study focuses on the potential of using crude collagen derived from Red Tilapia skin as an alternative to traditional mammalian and poultry-based gelatins, comparing its properties with those of local Malaysian bovine skin. Given that Malaysia is rich in aquaculture and livestock activities, which are significant contributors to the national income, the

potential utilisation of by-products like gelatin presents significant economic benefits. Additionally, this approach could decrease the need to export bovine skin to neighbouring countries, such as Thailand, by maximising local value creation. Our research explores the chemical compositions and structure-function interactions in gelatins derived from warm-water fish and mammals, aiming to assess their potential to deliver improved gelling and functional properties.

2. Materials and methods

2.1 Chemicals

Sodium hydroxide (NaOH), acetic acid (CH_3COOH) 96%, sulfuric acid (H_2SO_4) 98%, and hydrochloric acid (HCl) 36% were analytical grade and obtained from MERCK. Red Tilapia and bovine skins sourced from a local aquaculture farm and a regional abattoir were used to extract gelatin.

2.2 Chemical pre-treatment

Initially, the Red Tilapia and bovine skin are thoroughly cleaned with water to remove any dirt, debris, or other contaminants. The pre-treatment process involved a two-step protocol. First, the skins were soaked in a 0.20 M NaOH solution in a 1:3 ratio for approximately 2 hours, performed twice under continuous stirring at 27°C. Subsequently, the skins were thoroughly rinsed until they reached a pH of 7. A second pre-treatment was conducted using a 0.05 M CH_3COOH solution for an additional hour. The pre-treated Red Tilapia and bovine skin were washed with tap water until they reached a pH of 7, ensuring that any residual chemicals were removed and the skins were prepared for gelatin extraction.

2.3 Water extraction

Pre-treated Red Tilapia and bovine skin were then subjected to hot water extraction (60°C) in a beaker filled with distilled water for 3 hours in a 1:10 ratio. The gelatin solution was then filtered and dried before further analysis.

2.4 ATR-FTIR analysis

Fourier Transform Infrared (FTIR) Spectroscopy analysis was conducted using an FTIR spectrometer (Perkin Elmer) according to the method of Abedinia *et al.*, (2020)The sample was prepared by placing 5 mg of gelatin powder on the ATR plate. Scanning was conducted in the range of 400-4000 cm^{-1} with 16 scans at a resolution of 4.

2.5 Gel strength

The method by Fazial *et al.*, (2024) It was used to determine the gel strength of gelatin. Gel strength was determined using a TA. XT-Plus texture analyser (Stable *et al.*, UK) where the maximum force (g) at a probe penetration at a depth of 4 mm of the gelatin gel.

2.6 Protein content

Determination of protein was conducted using AOAC International (2016) (AOAC International, 2016) using the Kjedahl method. For the digestion step, the digestion unit was heated up to 420°C. Sample (2 g), two Kjedahl tablets and 12 ml concentrated H_2SO_4 were inserted in the digestion tube and subjected to the digestion process for 1 hour. After digestion, the solution was left to cool for 10 minutes. For the distillation process, 80 ml deionised water and 50 ml 40% NaOH was dispensed into the tube. This distillation process separates ammonia (nitrogen) from the digestion mixture. The last step was the titration process, where a volume of HCl was titrated into the distillate solution. The amount of HCl used was then recorded. The percentage of protein was calculated according to the following formula:

$$\%N = \frac{(T - B) \times 14.007 \times 100}{\text{sample weight}}$$

$$\%P = N \times F$$

T = Titrant volume for sample (ml).
B = Titrant volume for blank (ml).
N = Normality for HCl acid (0.1 N)
F = Conversion factor for Nitrogen to Protein-6.25 for General food and feed application.

2.7 Fat content

Fat was determined using AOAC International (2016) (AOAC International, 2016) using the Soxhlet method. Gelatin powder (2 g) was weighed and recorded as W1. Then, pre-dried extraction cups were weighed and recorded as W2. After the extraction (90 min), the cups were dried at 130°C for 30 minutes or until constant weight. The dried cups were weighed and recorded as W3. The fat content in the sample was calculated using the following formula:

$$\text{Fat (\%)} = \frac{(W3 - W2)}{W1} \times 100$$

W1 = Sample weight
W2 = Weight of empty extraction cup
W3 = Weight of extraction cup containing fat.

2.8 Gelatin colour and clarity analysis

The gelatin colour and clarity gel were measured using a Hunter Lab colour meter. (Tinrat & Sila-asna, 2017). The analysis of the colour and clarity was based on CIE L * for lightness, a* indicates redness or greenness and b* for yellowness or blueness colour system.

2.9 Functional properties

2.9.1 Foaming capacity

Foaming capacity (FC) was assessed according to Tinrat & Sila-asna, (2017) Each sample of about 5 ml was homogenised and centrifuged for 1 minute. The percentage of increased protein scattered throughout blending was measured as the capacity for foaming following equation 1:

$$\text{Foam capacity} = \frac{\text{Volume of foam}}{\text{Volume of total solution}}$$

2.9.2 Emulsifying capacity

The emulsifying capacity was determined using a modified method following the Tinrat & Sila-and (2017) technique, where 1%, 2%, or 3% of each sample would be emulsified in sterile water to produce a gelatin solution. For 30 minutes, the solution was then homogenised with soybean oil in a 3:1 ratio and then centrifuged for about 15 minutes. The thickened coating height can be expressed as a proportion of the total tube

height of the material determined by equation 2:

$$\text{Foam capacity} = \frac{\text{Height of emulsion layer}}{\text{Height of whole layer}} \times 100$$

2.10 Statistical analysis

SPSS 26 was used to analyse the data in this investigation. Duncan's test was used for one-way variance analysis, with a confidence level of $p \leq 0.05$. All analyses and measurements were at least triplicate.

3. Results and discussion

3.1 FTIR analysis

FTIR spectroscopy is advantageous for identifying the intermediate structure, confirmation of rearrangements, structural dynamics, and the stability of gelatine (Mao *et al.*, 2022). Furthermore, comparing spectra with those of commercial standard gelatine samples can further confirm the identity and quality of the extracted gelatine. Matching peaks with known gelatin spectra can validate the extraction method and the integrity of the gelatin structure post-extraction. Figure 1 shows the FTIR analysis of extracted bovine skin gelatin (EBG), extracted fish gelatin (EFG), commercial fish gelatin (CFG) and commercial bovine gelatin (CBG).

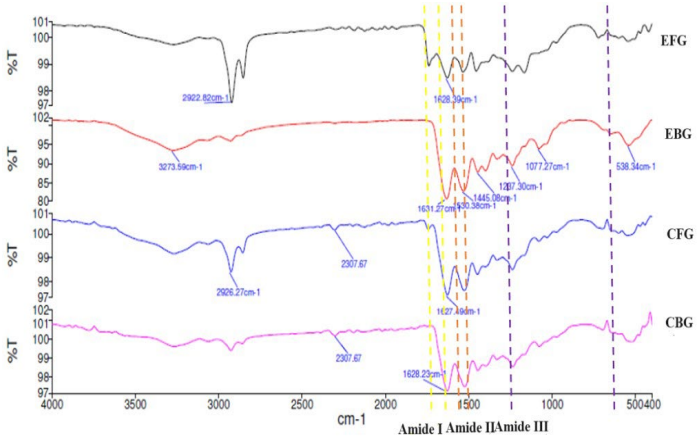

Figure 1: FTIR spectra of extracted fish gelatin (EFG), extracted bovine gelatin (EBG), commercial fish gelatin (CFG) and commercial bovine gelatin (CBG) along with represented amide I, amide II and amide III from wave numbers of 400-4000 cm^{-1}.

According to the FTIR spectrum, the extracted gelatin from bovine and Red Tilapia skin displayed a pattern comparable to that of commercial fish and bovine gelatin. Both extracted gelatins exhibited prominent protein functional groups at Amide A, Amide B, and Amide I, II, and III. In more detail, the extracted bovine gelatin (EBG) displayed peaks at 3273.59 cm^{-1}, 2918.35 cm^{-1}, 1631.27 cm^{-1}, 1530 cm^{-1}, and 1077.27 cm^{-1} for Amide A, B, I, II, and III, respectively. On the other hand, the extracted fish gelatin (EFG) exhibited peaks at 3198.34 cm^{-1}, 2922.82 cm^{-1}, 1631.27 cm^{-1}, 1530 cm^{-1}, and 1237.30 cm^{-1} for Amide A, B, I, II, and III, respectively. Amide I shows that the gelatin derivative with a characteristic coiled conformation contributes to the stability of the triple helical structure. This implies that a loose hydrogen bond created by N-H bonding during the acid solution soaking period may be the reason for reducing the C=O stretching vibration (Wang *et al.*, 2024). The amide group's C=O stretching vibration and the C-N stretching vibration were disclosed by the absorption in the Amide I region, whereas the N-H bending and the C-N stretching vibration were revealed in the Amide-II region. The combination of C-N stretching vibrations, N-H deformations resulting from amide linkages, and absorptions brought on by CH_2 wagging vibrations out of the glycine backbone with proline side chains corresponded to the Amide III peaks. Solid amide I and II peaks are typical of gelatin and indicate that the extraction process successfully yielded a product containing proteinaceous material, likely gelatin from collagen sources (Hajlaoui *et al.*, 2024).

3.2 Gel strength

The most essential functional attribute of fish gelatin is its gel strength, which directly affects the quality of packaged foods. Figure 2 shows the gel strength of gelatin obtained from Red Tilapia skin, bovine skin, fish commercial, and bovine commercial.

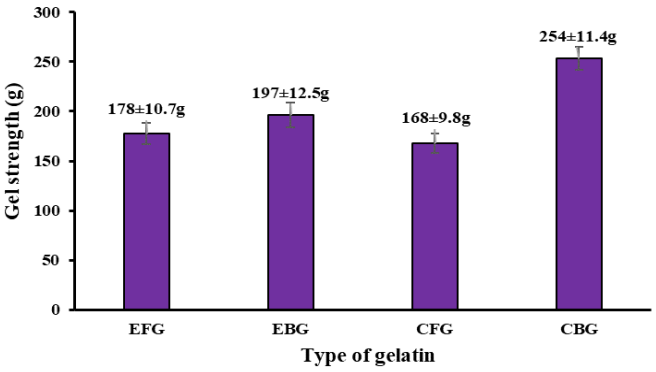

Figure 2: Gel strength (g) of extracted fish gelatin (EFG), extracted bovine gelatin (EBG), commercial fish gelatin (CFG) and commercial bovine gelatin (CBG).

The gelatin derived from CBG displayed the maximum gel strength (254 g), followed by EBG (197 g), EFG (178 g), and CFG (168 g). The gel strength of EFG was considerably higher than that of CFG. Due to its high gel strength, gelatin is valuable for promoting chewiness, texture, and foam stability in various culinary products, including confections (Tinrat & Sila-asna, 2017).

Because of pressure treatment, gelatin derived from acid extraction techniques often has more incredible gel strengths and viscosities (Zhang *et al.*, 2020). Due to gelatin's strong ability to make hydrogen bonds with water molecules, a robust three-dimensional gel is formed (Derkach *et al.*, 2020). They asserted that fish gelatin had a smaller molecular weight and a lower concentration of proline and hydroxyproline amino acids needed to stabilise collagen-like triple helices than mammalian gelatin. As a result, compared to mammalian gelatin, fish gelatin gels are often less robust and have lower gelation and melting temperatures.

Sulfuric acid and acetic acid help break down collagen's complex structures into simpler forms that can be more easily converted into gelatin. These acids also assist in the hydrolysis of peptide bonds within the collagen structure. Acetic acid, on the other hand, being a weaker acid compared to sulfuric acid, is often used to gently modify the collagen without overly degrading it, which can be crucial for maintaining the integrity of gel-forming sites (Kendler *et al.*, 2024). This controlled modification can lead to gelatin with better gelling properties.

Regarding consumers' interest in safe and excellent gelatin properties, Red Tilapia fish gelatin, which is being explored in this study, has promising potential future uses due to its improved gelling behaviour compared to CFG. This result can ensure that the extraction process can be scaled up efficiently without compromising the quality and properties of the gelatin.

3.3 Protein and fat compositions

In principle, the protein content of the gelatin produced may be classified as high when it exceeds 95% and low when it is less than 75% (Casanova *et al.*, 2020). The extracted gelatin's protein and fat content are presented in Table 1.

The findings indicated that both EFG and EBG have a high protein level of 98.78%, which attests to the high purity of gelatin, which is significantly higher than the protein content value of 21.3% for African catfish (*Clarias gariepinus*) skin gelatin as previously reported (Alfaro *et al.*, 2013). The high protein content in both extracted samples is due to the suitability of the pre-treatment process and efficient extraction procedure. In principle, the protein concentration of collagenous material indicates the maximum amount of gelatin that may be extracted from the material (Lan *et al.*, 2024). Thus, a high protein concentration in the extracted gelatin indicates a high gelatin yield. The results indicated that the high gelatin content of Red Tilapia skin (18.5 %) results in a high protein content (98.78%). Other chemically pre-treated connective tissues were found to have a high imino acid (total proline and hydroxyproline amino acid) concentration similar to that of Labeo Rohita and Cod Japanese (Wu *et al.*, 2023) and Black Drum (*Pogonia cromis*) as well as Sheepshead Seabream (*Archosargus probatocephalus*) bone collagens (Ogawa *et al.*, 2003).

Table 1: Protein content (%) and fat content (%) of extracted fish gelatin (EFG) and extracted bovine gelatin (EBG). Using an independent T-test, values are given as mean ± standard deviation with different superscripts within the column indicating significant differences (p≤0.05)

Gelatin sample	Protein	Fat
EFG	98.78 ±0.98[a]	4.01 ±0.294[a]
EBG	97.91 ±1.52[a]	1.36 ±0.618[b]

As for fat content, EFG has a higher fat content (4.01%) than EBG (1.36%). Gelatin standards certified by certain countries, like the Indonesian National Standard (SNI), allow the fat content of gelatin not to exceed 5% (Taufik *et al.*, 2010). This might be due to specific conditions or types of fish used, which may inherently have higher fat content in their skins or other by-products. Moreover, modifying the concentrations of NaOH and acetic acid in pre-treatment could optimise gelatin recovery, colour, and solubility, suggesting that the manipulation of the acid-alkaline ratio can also impact the extraction efficiency and possibly the encapsulation and protection of lipids within the gelatin structure (Shahiri Tabarestani *et al.*, 2014). Collagen obtained from younger animals is more soluble in hot water; these qualities diminish with age. The extraction temperature used was also very low, preventing the degradation of fat contained in the skin (Delikanlı Kıyak *et al.*, 2024). Chemical compositions of skin change according to the animal's age and sex and how the skin is treated once it is removed from the carcass. Besides, gelatin's functional qualities depend on processing factors such as temperature, time, and pH, as well as the pre-treatment methodology and the characteristics and preservation method of the original raw material. Fat content for other extracted gelatin, such as tuna head bones, was 3.2%± 0.5, and duck feet was 3.35% ±0.26 (Aksun Tümerkan *et al.*, 2019; Kuan *et al.*, 2017). It was also confirmed that if the gelatin is over-solubilized, the fat content will be washed away during the washing process.

3.4 Color and clarity of gelatin

According to the result acquired in Table 2, EFG has about 28.16 ±0.09 for lightness colour (L*) and 2.01 ±0.31, indicating yellowness colour (b*). As for EBG, the lightness colour (L*) and yellow colour (b*) are slightly lower at 27.89±0.39 and 1.68±0.05, respectively. Positive b* values indicate a degree of yellowness, while positive L* values indicate the lightness of the sample. The results show that EFG is lighter in colour and more yellowish than EBG.

Table 2: L* and b* values of extracted fish gelatin (EFG) and extracted bovine gelatin (EBG). Using an independent T-test, values are given as mean ± standard deviation with different superscripts within the column indicating significant differences (p≤0.05)

Colour	Sample	
	EGG	EBG
L*	28.16 ±0.09[a]	27.89 ±0.39[b]
b*	2.01 ±0.31[a]	1.68 ±0.05[b]

When extracting gelatin, factors including the source material and extraction stage impact the final product's colour, as does how the gelatin was made. Colour is a significant factor in the consumer's acceptance of food goods. It is connected to the physical qualities of extracted gelatin that must be reported (Utomo & Suryanti, 2018). However, those functional aspects are unaffected by colour (Haddar *et al.*, 2011). Because the process of soaking in an acetic acid solution was prolonged, it created several loose triple helix chains within collagen molecules, which degraded the pigment in the Red Tilapia skin and resulted in a brighter tone.

A previous study discovered that bovine gelatin has better b* values (19.05) than tuna head bones gelatin (5.02) *(Ahmad et al.*, 2017). The L* and b* values of huge grouper skin gelatin gel were much higher than those of commercial Tilapia skin gelatin by Lin *et al.* (2015). Additionally, the same analysis suggested that the gelatin gel extracted from giant grouper skin was lighter in colour and had more yellowness than commercial gelatin.

3.5 Foaming capacity

Foaming capacity is the ability to incorporate air into a solution and stabilise the resulting foam. It is essential in various food products where air bubbles such as whipped creams, marshmallows, desserts and beverages enhance texture and volume. Figure 3 presents a detailed analysis of the foaming capacity of gelatin derived from EFG (extracted fish gelatin) and EBG (extracted bovine gelatin) across three gelatin concentrations: 1%, 2%, and 3%. The foaming capacity for EFG increased progressively with concentration, recording values of 1.25%, 1.35%, and 1.4% at 1%, 2%, and 3% concentrations, respectively. In contrast, EBG demonstrated a foaming capacity of 1.01% at 1% concentration, which gradually increased to 1.26% at 2% and peaked at 1.47% at 3%. This comparative analysis underscores the influence of source material and concentration on the foaming properties of

gelatin.

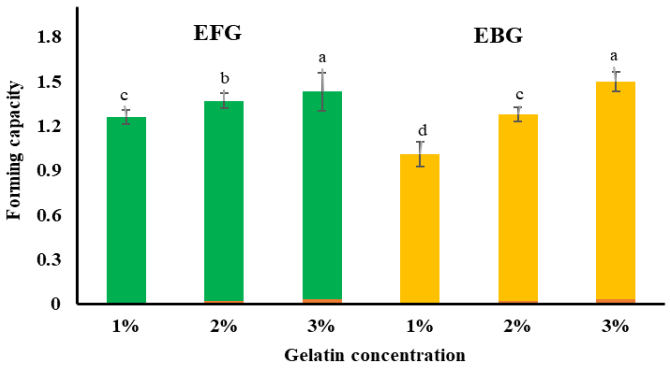

Figure 3: Foaming capacity (%) of EFG and EBG at 1%, 2% and 3% gelatin concentration.

The findings indicate that the foaming capacity of both EFG and EBG improves as the gelatin concentration increases. This enhancement in foaming capacity is facilitated by the dynamic behaviour of protein molecules in the gelatin when interacting with air and water. Transport, penetration, and structural modification of protein molecules at the air-water interface are necessary to produce foam. The exceptional foaming capacity of proteins allows them to quickly spread into the air-water interface, unfold, and reorganise themselves there. Because EFG contains more hydrophobic amino acids than EBG, it may have a somewhat larger foaming capacity. Adding hydrophobic residues that form a massive hydrophobic sphere on the polypeptide's surface may improve the foaming ability (Li *et al.*, 2024).

Sulfuric and acetic acids used during pretreatments help unfold the protein structure and expose hydrophobic and hydrophilic groups. Some of the lower molecular weight peptides tend to be more surface active, which enhances their ability to reduce surface tension, thus facilitating foam stability (Dong *et al.*, 2024).

3.6 Emulsifying capacity

Emulsifying capacity is essential in creating and maintaining the stability of mixtures containing oil and water. It is used in mayonnaise, ice cream, and salad dressings to ensure a uniform and palatable texture. An emulsifier's ability to keep these immiscible phases mixed enhances the product's shelf life, appearance, and texture. The emulsifying capacity of gelatin recovered from EFG and EBG was investigated at three different gelation concentrations: 1%, 2%, and 3%, as shown in Figure 4.

The emulsifying capacity of EFG is 4.33%, 7.65%, and 8.24% for 1%, 2%, and 3% gelatin concentrations. On the other hand, EBG has an emulsifying capacity of 5%, 8.24 %, and 11.9% at concentrations of 1%, 2%, and 3%, respectively. As observed, EFG has a slightly poorer emulsifying capacity than EBG. Even though EFG and EBG both exhibit a rising trend, there is a slight variation in the emulsifying capacity. As observed, the emulsifying capacity of EBG is slightly higher than EFG's at all gelatin concentrations. However, both gelatin emulsifying capacities increased from 1% to 3%. The hydrophobic regions on the peptide chains of gelatin give it its emulsifying and foaming capacity (Heidary & Soltanizadeh, 2024). Because of its exceptional active surface qualities, gelatin can be used as an emulsifier, foaming agent, and moisturising agent in culinary, pharmaceutical, medical, and technical applications. Enormous droplets occur when gelatin emulsifiers are employed alone in homogenisation because their surface-active qualities are lower than typical surface-active agents such as globular proteins and gum Arabic.

The capacity of a protein to form adsorption films on oil globules and reduce interfacial tension at the oil-water interface is referred to as its emulsifying ability. Solubility in the dispersion phase is a critical factor in increasing emulsifying effectiveness (Aksun Tümerkan *et al.*, 2019). This is because the protein molecules should be able to quickly migrate to the lipid droplets' surfaces. Because of their differing amino acid compositions, polar and nonpolar amino acids, EBG was shown to have more extraordinary emulsifying ability and emulsion stability ($P<0.05$) than duck feet gelatin (Kuan *et al.*, 2017). Studies for emulsion and foaming properties, the emulsion stability (ES) of chicken bone gelatin, which ranged from 9.82 to 61.19 %, has shown that increased concentration and extraction times resulted in higher emulsion stability (ES) (Abedinia *et al.*, 2020)The results of the bovine skin gelatin emulsifying capacity satisfied this statement as the emulsifying capacity and gelatin concentration increased. As for the treatment used for extracting the gelatin, gelatines recovered from mackerel and blue whiting bones after using alcalde and flavoursome pre-treatment had a greater EAI than gelatines produced with the lowest EAI after the chemical pre-treatment (Khiari *et al.*, 2013).

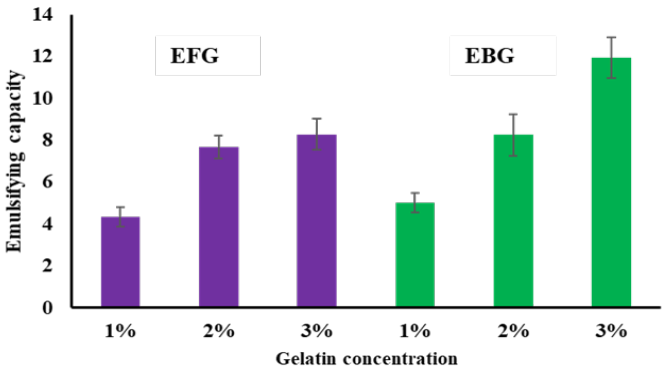

Figure 4: Emulsifying capacity of EFG and EBG at 1%, 2% and 3% gelatin concentration.

4. Conclusion

In conclusion, this study explored the potential of gelatin extracted from red-skin Tilapia and bovine sources, demonstrating that extracted Red Tilapia fish gelatin could serve as a viable alternative to traditional gelatins in the food industry, especially for halal products. Both fish and bovine showed high protein content (>90%). EFG has superior gel strength compared to CFG, which contradicts EBG, which has lesser gel strength than CBG. The high gel strength of EFG shows the high potential of EFG to be commercialised and could be applied for various applications. Foaming and emulsifying capacity increased as gelatin concentrations increased, signifying the stability of gelatin in reducing the surface tension of water and oil. However, challenges must be addressed, including fish gelatin's inherently lower gel strength and stability than mammalian sources, which could limit its use in specific applications. The colour and clarity of the gelatins also varied, potentially affecting consumer acceptance. Future research should focus on improving the functional properties of these gelatins through advanced processing techniques and

conducting consumer acceptance studies to understand market potential better. Overall, the study highlights the significant potential of alternative gelatin sources to meet the needs of the halal food industry and contribute to more sustainable food production technologies.

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6. Copyright

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