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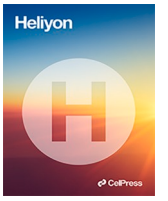

Research article

# Effect of seasonal variation and farming systems on the properties of Nile tilapia gelatin extracted from scales

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ABSTRACT

Although fish gelatin has become a research hotspot in recent years, researchers and manufacturers are still looking for high-quality sources of fish galatin to meet the commercial demand for safer gelatin.became This study aimed to evaluate the impact of seasonal variation and farming systems on the properties of gelatin extracted from Nile tilapia scales. Gelatin extracted from farmed tilapia had lowest impurities, higher clarity as well as desirable color characteristics (L* = 65.95 and a* = −0.33). The protein and fat composition of Wild (91.00 ± 0.00$^{c}$) and 1.94 ± 0.05$^{a}$ respectively were higher than farmed gelatin of protein (91.00 ± 0.00$^{c}$) and fat (0.84 ± 0.08$^{b}$) but gelatin from the farmed type were clearer (98.30 ± 0.28$^{a}$) than wild type (94.60 ± 0.28$^{b}$). In addition, the XRD analysis confirmed its amorphous structure (2θ = 11°, 21°. 29°, and 31°). The gelatin extracted from wild tilapia showed an average yield of 1.98 % and good physicochemical and functional properties. Furthermore, FTIR indicated a strong bond positioned in the amide I region (1650.88 cm-1) of the wild tilapia gelatin. Partial Least Square (PLS) confirmed that viscosity is positively correlated with melting temperature upon a unit change in gelatin yield. This work highlights the significance of farming systems and seasonal variation in extraction conditions and great parameter to comprehensively navigate the functional, biochemical, and physical properties of Nile tilapia gelatin for broadening both food and non-food industrial appliactions.

## 1. Introduction

Gelatin is denatured collagen obtained via thermal, acidosis, or enzymatic treatment. Its applications vary widely and include food, agriculture, pharmaceutical, and photographic industries [1]. In the food industry, it is regarded as an animal product with high emulsifying properties, stable textural characteristics, bland flavor, good mouthfeel, and strong thermo-reversible gel properties [2]. Additionally, it is recommended as a protein enhancement, adhesive, dietetic food, fat replacer, and salt reducer in the pharmaceutical industry [3,4].

Gelatin production globally keeps gaining ground closing to a 5 billion USD market in the coming years ([5]) and is projected to reach 6.67 billion USD in 2027 [6] The annual world output of gelatin has increased to 326,000 tons where pig skin (porcine) was the

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major source at 46 %, followed by bovine hides (29.4 %), bones (23.1 %), and others (1.5 %). Unfortunately, this mammalian gelatin is noted for spreading bovine spongiform encephalopathy (BSE), and foot and mouth disease (FMD) [4]. It has been also recently highlighted in reviews that the use of porcine or other mammalian gelatin can be restricted due to religious or cultural reasons [7] Among the other gelatin sources, fish shows promise as an excellent alternative source for mammalian gelatin [8]. The total fish production in the world in 2018 was approximately 179 million tons. It has been estimated that over 50 % of fish tissues, fins, heads, skin, and viscera are discarded as wastes [9]. Therefore, it is imperative that large quantities of fish waste generated are converted to value-added products such as fish gelatin. The increasing world population and looming scarcity of raw materials mean that in order to achieve both material and social-economic sustainability, what was earlier considered as wastewaste needs to be utilized much better [10].

The processing of fish by-products, such as skin and bones, has gained interest tremendously due to their value-adding potential [11]. Further utilization of these wastes is reported to be possible through various extraction methods. The conditions and types of extraction greatly influence the quality of fish gelatin [12]. According to Ref. [13] the most important indicators of the functional properties of gelatin are gelatin strength, viscosity, melting point, and gelation which are highly affected by extraction temperature, time and Ph [14,15] Other parameters affecting the functional and physicochemical properties of fish gelatin are habitation and seasonality. Gelatin obtained from warm-water fish species possesses superior physicochemical properties and functional characteristics compared to cold-water fish species [4]. A previous report by [12], indicated that fish gelatin quality is influenced by thermodynamic differences and seasonal variations. For example, fish gelatin extracted from silver carp fish caught in summer had higher viscosity, emulsion stability, melting point, and lower concentration for gelling compared to the winter silver carp fish variety. However, the study on temperature conditions of fish natural habitat on functional properties of fish gelatin is rare [12,16] and there should be intensive research on the impact of seasonal variation and farming systems on the physicochemical and functional properties of Nile tilapia fish gelatin.

Nile Tilapia (*Oreochromis niloticus*) is the fourth fast-growing aquaculture species in the world. It is classified as warm water fish species with fish gelatin of superior functional and physicochemical properties [6]. More recently, the production of tilapia has seen tremendous expansion in developing countries due to an increase in international trade and the development of new markets [17] Norman-López and Bjørndal, 2010). In Ghana alone, production grew rapidly, from 2000 to 30000 tonnes in 2013 where 90 % of households consume tilapia regularly or occasionally [18]. It is mostly grown on caged culturing and free range type or wild farming in rivers, lakes, or lagoons where 90 % of the total volume is generated from the former under two main weather patterns [19,20]. These two patterns are a hot, dry season and rainfall season which usually occurs from November to March and May/June and September respectively [21]. Meanwhile, there are some data available on tilapia wastes but the perspective of this utilization is biogas or bio-based chemical production [22,23], or a specific waste component [24]. Also, to the authors' knowledge, there is no existing data on the tilapia wastes from the African food systems. Additionally, knowledge of thermostability in seasonal differences and farming systems on the quality of fish gelatin extracted using scales of Nile tilapia is limited.

Previous studies have focused on extraction conditions, functional and physicochemical properties of fish gelatin. No experimental study about the effect of farming systems (wild or farmed) on the physico-chemical characteristics of Nile tilapia has been reported to our best knowledge. The present study sought to determine the impact of seasonal variation and farming systems on the structure, functional and physicochemical characteristics of fish gelatin extracted from scales of Nile Tilapia using sequential acid-alkali techniques. Also, statistical predictive modeling was carried out to examine the relationship between rheological and functional properties using Partial Least Square (PLS). The knowledge gained could provide a mechanism or channel to unravel the factors responsible for the functionality and physicochemical properties of fish scale gelatin, broaden the utilization possibilities of fish gelatin, and bring positive local socio-economic developments in African setting.

## 2. Materials and methods

### 2.1. Chemicals

Hydrochloric acid was bought from Fisher Scientific (Nepean, Ontario, Canada); sodium hydroxide was purchased from Merck (KGaA, Darmstadt, Germany), Sulphuric acid was purchased from Sigma Aldrich (Saint Louis, MO, USA); All chemicals and reagents were analytical grades.

### 2.2. Samples

Nile tilapia (*Oreochromis niloticus*) fish species were bought from Weija Lagoon in the Greater Accra Region and Akosombo in the Eastern Region of Ghana. The harvest took place during the rainy (R) and dry seasons (D) for both wild (W) and farmed (caged) (F) species of Nile tilapia. They were then transported on ice at a ratio (Scale/ice 1:2 (w/w)) to the CSIR-Food Research Institute in Accra. Residual scales were manually removed and isolated scales were washed with tap water. The scales were packed into polyethylene plastic bags and stored at a temperature of −20 °C in a freezer until they were used.

### 2.3. Gelatin extraction

The method of [25] was slightly modified and used for the gelatin extraction. Thawed scales stored at a temperature of −20 °C were cut into small pieces (1 cm × 1 cm) with a pair of scissors. Scales were thoroughly cleaned and rinsed with excess distilled water to

remove superfluous material. To extract the gelatin, they were soaked in an alkali solution (0.5 M NaOH) for 24 min followed by soaking in 0.4 % $H_2SO_4$ for 20 min. Digested scales were neutralized using distilled water until a neutral pH was attained. The soaking and neutralization processes were repeated twice. The final extraction was carried out in distilled water at 60 °C for 6 h and the extract was filtered with Whatman No. 4 filter paper. The purified extract was lyophilized in freeze-dryer (Bioevopeak, Shandong, China) at 50 °C for 24 h. The dried sample was ground and sieved with a 250 mesh screen to produce gelatin powder. The gelatin samples were named as indicated in Table 1.

Yield of gelatin extracts produced from each variety was determined according to Equation (1):

$$\%Yield\ (wb) = \frac{dry\ wt.\ of\ gelatin}{wet\ wt.\ of\ scales - mc} * 100\% \quad (1)$$

where *wb*: wet basis; *wt*: wight; *mc*: moisture content.

### 2.4. Chemical, physical, and structural properties of gelatin

The chemical properties of gelatin were analyzed by determining moisture (Air-oven circulation method), ash (high-temperature burning method), protein (Kjeldahl nitrogen determination method). and fat contents (Soxhlet method) according to Ref. [26]. Additionally, the pH value was measured using a 1 % (w/v) gelatin solution at 25 °C [27].

The physical properties of gelatin, including appearance (color and turbidity), melting and gelling temperature, viscosity, gel strength, and emulsifying capacity were assessed. The color of the gelatin gel was determined using a color meter (Minolta, CR-310, Osaka Japan) based on CIE L*(lightness), a* (redness/greenness), and b* (yellowness/blueness) color system [28] and SIIAASnA. Following Kittiphattanabawon [29], a 6.67 % (w/v) gelatin solution was prepared in distilled water at 60 °C. The turbidity of the gelatin solution was measured using a UV–vis spectrophotometer (UV mini-1240, Shimadzu Corporation, Japan) at an absorbance of 620 nm. To measure the melting temperature, 1 g of gelatin powder was heated and stirred with a spatula and subsequently allowed to gel to determine the gelation temperature. The viscosity of the gelatin was assessed using the modified method by the [30]. Gelatin was dissolved in distilled water (6.67 %, w/v) and heated in a water bath at 60 °C for 30 min. Then, the viscosity (cP) of 10 mL of gelatin solutions was measured using a rheometer (Brookfield, Engineering Laboratories Ltd., Middleboro, MA). The gel strength of the gelatin was determined according to Ref. [31] with slight modifications. Gelatin (7.5 g) was added to a bloom jar (150 ml capacity) containing 105 mL of distilled water to obtain a 6.67 % solution, stirred with a glass rod, and allowed to stand at room temperature for 3 h. The solution was heated at 40 °C for 20 min on a magnetic heater stirrer and allowed to cool for 15 min before being stored at 10 °C in a refrigerator for 17 h. The bloom strength was tested using a penetration test on a texture analyzer (TA.XTplus, Stable Microsystems, Surrey, UK) with a standard 0.5″ diameter cylinder probe. The probe was lowered to penetrate the gel to a depth of 4 mm at a test speed of 0.5 mm/s. The maximum force (resistance to penetration) during penetration was recorded as the bloom strength. The emulsifying capacity was analyzed according to the method of [29] with some modifications. A gelatin sample was solubilized in distilled water to form a 4.5 % gelatin solution. The solution was homogenized with sunflower oil at a ratio of 3:1 for 30 min and centrifuged at 2500×*g* for 15 min. The emulsifying capacity was calculated using Equation (2):

$$Emulsifying\ Capacity = \frac{Height\ of\ emulsion\ layer}{Total\ height\ of\ suspension} * 100 \quad (2)$$

The structural properties of gelatin were determined by FT-IR and X-ray diffractometer. The lyophilized sample was placed on the cell and clamped to the mount of the FT-IR spectrophotometer (Spectrum Two, PerkinElmer LTD. UK). Infrared spectra were recorded in the mid-region of 400–4000 $cm^{-1}$ in 64 scans. The resolution was 4 $cm^{-1}$ and the sample was analyzed in a solid state to avoid water absorption in the amide I region (Sinthusamran et al., 2014). X-ray diffraction pattern of gelatin was determined using a powder X-ray diffractometer (Malvern Panalytical Ltd, Malvern, UK) following the method of [32]. The pattern was recorded by Cu K α radiation in a θ-2θ configuration at a voltage of 45 kV and a current of 40 mA.

### 2.5. Statistical analysis and modeling of rheological and functional properties of gelatin using partial linear regression (PLS)

The PLS statistical modeling was employed to ascertain the changes in the relationship of rheological and functional properties of gelatin extracted.

The experimental data was presented as mean and standard deviation. Each group of samples was paralleled at least 3 times. One-way analysis of variance (ANOVA) and Fisher's least significant difference tests were performed by SPSS software version 21, Chicago Inco. USA) with $p < 0.05$ as the significance level. Origin 9.0 (OriginLab Corporation, Northampton, MA, USA) was used for the

**Table 1**
Naming of the fish gelatin samples based on seasonality and fish habitation.

| Season | Fish Habitation | Source of gelatin | Sample naming |
|---|---|---|---|
| Dry | Farmed | Tilapia scales | DSFFG |
| Rainy | Farmed | Tilapia scales | RSFFG |
| Dry | Wild | Tilapia scales | DSWFG |
| Rainy | Wild | Tilapia scales | DSWFG |

graphical presentation of infra-red spectra and X-ray diffractograms.

## 3. Results and discussion

### 3.1. Chemical composition and yield

The yield obtained from the wild and cultured Nile tilapia scale gelatin on a wet basis under rainy and dry seasons is shown in Table 2. In both seasons, the gelatin yield is consistently higher for farmed tilapia compared to wild tilapia. The highest gelatin yield of 7.95 % was recorded for Dry Season Farmed Fish Gelatin (DSFFG) whilst the lowest of 1.98 % was obtained by Dry season Wild Fish Gelatin (DSWFG) Additionally, there is a significant difference ($p < 0.05$) among farmed gelatin compared to that obtained from the wild.

The high yield recorded in gelatin extracted from farmed could be related to the easiness of hydrolysis of collagen in water [33] due to low gel strength (Fig. 2B). The extraction conditions regarding acid concentration, and time were severe in breaking the triple helix of collagen and hydrogen bond in α-chains in farmed gelatin. According to the report by Ref. [34], it is worth noting that the yield of pangasius skin gelatin increased when similar extraction conditions: time and citric acid concentration were increased from 6 to 8h and 0.1%-0.,3 % respectively as a result of the collapse of collagen structure Contrarily, wild-type tilapia scale gelatin had high gel strength as demonstrated in Fig. 2A. This is a result of the strong bond of triple helix in their collagen [31] and explains the reason for low gelatin yield noted among the wild species. This suggests that the origin of fish (farmed or wild) could be an explicitly determining factor in the yield of fish gelatin. Optimisation of extraction conditions and different farming systems and seasonal variations should be carried out in the future.

Gelatin extracted from farmed tilapia in the rainy season and from wild tilapia in the dry season showed the highest protein content, at 93.90 % and 94.90 %, respectively. These values are similar to those reported for Tunisian barbel (92.1 %), bigeye (92.6 %), and cuttlefish (91.5 %) [35]. Also [31], reported a protein content of 90.70 % when they investigated the effect of extraction variables on the physical and functional properties of tilapia gelatin. Protein increment was attributed to the removal of water bound to the gelatin structure by high temperature [36]. The moisture content recorded for fish gelatin extracted ranged from 2.51 to 6.08 %, which all meet the requirement of edible gelatin (<15 %) [37]. Generally, the fat and ash content of gelatin from farmed in both seasons was low compared to wild-variety extracted gelatin (Table 2). Gelatin consists of mainly water and protein, hence, the presence of ash, lipids, and other impurities at low contents is important for its quality [38]. The relatively low content of fat in farmed gelatin suggested that it is of good quality. The pH range of 5.3–9.2 recorded for gelatin extracted from the fish scale using the acid-alkali pretreatment method in this study is similar to pH 9.5 and 5.4 reported for type A and type B gelatin [39]. This amphoteric behavior of fish gelatin explains its versatility in industrial applications [4]. Gelatin obtained from the wild tilapia during the rainy season showed variation compared to other gelatins (Table 2) and can affect other characteristics such as melting, gelling temperatures, and viscosity, as seen in this study.

### 3.2. Physical characteristics of gelatin

#### 3.2.1. Gelatin turbidity and color

Table 3 shows the turbidity and colour of DSFFG, RSFFG, DSWFG, and RSWFG gel extracted at different farming systems at different seasons.

The turbidity is important in food applications as gelatin is used as a thickening agent [40]. The turbidity was high for all samples (94.55%–98.30 %). It is obvious that the clarity of gelatin obtained from wild tilapia in either season (rainy or dry) was lower ($p < 0.05$) than that obtained from farmed tilapia. The L*, a*, and b* color values indicate that the color of the gelatin was relatively whitish, with a slight yellow hue and practically no green or red pigmentation.

The gelatin from wild tilapia was more turbid compared to that from farmed tilapia due to the presence of contaminants in their scale exposed by an uncontrolled environment. The clarity and relatively whiter color of gelatin obtained from farmed tilapia could be due to the farming confinement type of practice, thereby limiting exposure of contaminants to their body. Low clarity of gelatin is most likely caused by residual fat, and non-collagen, organic, and inorganic substances [41]. It is no surprise that wild gelatin had a high-fat content of $1.94 \pm 0.05^{a}$ among other samples as confirmed by low clarity in Table 3. Similar work was reported by Ref. [42] who

**Table 2**
Proximate composition of gelatin from wild and farmed Nile tilapia.

| Sample | Component (% wet weight basis) | | | | | |
|---|---|---|---|---|---|---|
| | Yield (%) | Protein (%) | Moisture (%) | Ash (%) | Fat (%) | pH |
| DSFFG | $7.95 \pm 0.07^{a}$ | $91.00 \pm 0.00^{c}$ | $6.08 \pm 0.22^{a}$ | $2.05 \pm 0.13^{b}$ | $0.84 \pm 0.08^{b}$ | $5.25 \pm 0.01^{c}$ |
| RSFFG | $7.67 \pm 0.03^{b}$ | $93.90 \pm 0.06^{a}$ | $3.20 \pm 0.00^{b}$ | $1.19 \pm 0.26^{b}$ | $1.66 \pm 0.16^{a}$ | $5.56 \pm 0.04^{b}$ |
| DSWFG | $1.98 \pm 0.03^{c}$ | $94.05 \pm 0.38^{a}$ | $2.51 \pm 0.01^{c}$ | $1.46 \pm 0.35^{b}$ | $1.94 \pm 0.05^{a}$ | $5.58 \pm 0.04^{b}$ |
| RSWFG | $2.00 \pm 0.01^{c}$ | $92.03 \pm 0.35^{b}$ | $3.58 \pm 0.12^{b}$ | $3.18 \pm 0.22^{a}$ | $1.18 \pm 0.07^{b}$ | $9.18 \pm 0.04^{a}$ |

Values are presented as the means ± standard deviation of triplicate (n = 3). Superscript to means in the same column are significantly different $p < 0.05$. DSFFG: Dry season farmed fish gelatin extracted from the scale of Nile tilapia; RSFFG: Raining season farmed fish gelatin from Nile tilapia scale; DSWFG: Dry season wild fish gelatin of Nile tilapia scales; RSWFG: Raining season wild fish gelatin.

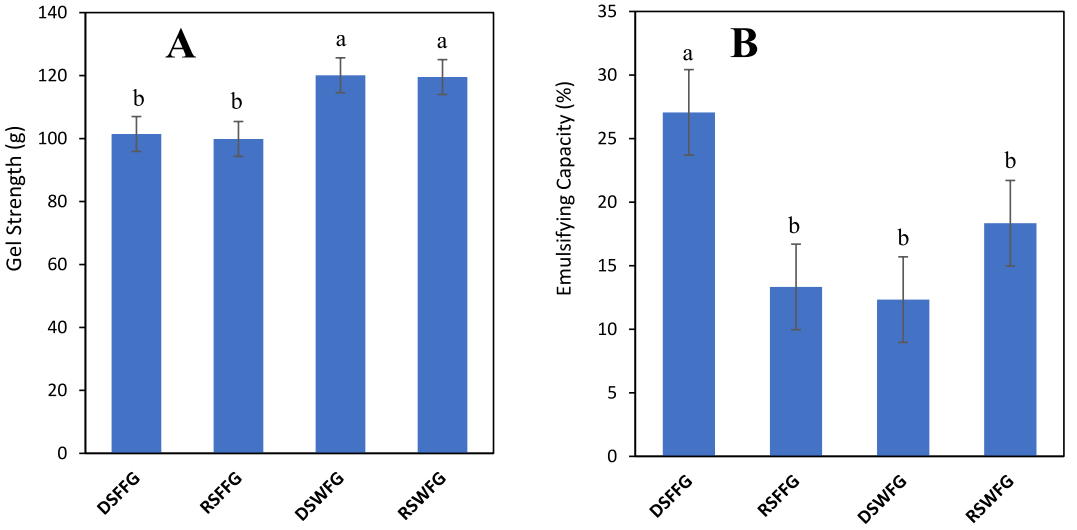


**Fig. 1.** Gel strength (A) and Emulsifying capacity (B) of gelatin extracted from Nile tilapia.

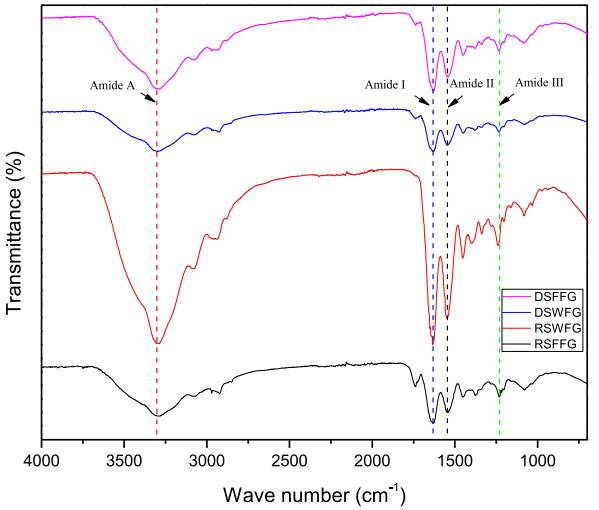


**Fig. 2.** FTIR spectra for gelatin isolated from Nile tilapia under different farming systems and seasons.

**Table 3**
Gelatin turbidity and color.

| Sample | Turbidity (%) | Colour | | |
|---|---|---|---|---|
| | | L* | a* | b* |
| DSFFG | 98.30 ± 0.28$^{a}$ | 65.95 ± 0.75$^{a}$ | −0.33 ± 0.02$^{b}$ | 4.09 ± 0.03$^{b}$ |
| RSFFG | 97.45 ± 0.21$^{a}$ | 65.69 ± 0.62$^{a}$ | −0.01 ± 0.01$^{b}$ | 4.46 ± 0.33$^{b}$ |
| DSWFG | 94.60 ± 0.28$^{b}$ | 64.79 ± 0.64$^{ab}$ | 0.04 ± 0.01$^{b}$ | 4.79 ± 0.23$^{b}$ |
| RSWFG | 94.55 ± 0.07$^{b}$ | 62.23 ± 0.62$^{b}$ | 0.64 ± 0.26$^{a}$ | 6.70 ± 0.32$^{a}$ |

Value are means ± standard deviation of triplicates; Means values with the different superscripts (a–b) within a same column are significantly different ($p < 0.05$).

indicated that pigment and other fat substances are mainly responsible for the turbidity of gelatin solution. Although other factors affecting the purification and clarity of gelatin such as the filtration process have been reported [33], farming system practice in this study seemed to play a greater role in the color and clarity of the fish gelatin.

### 3.2.2. Melting, gelling temperatures, and viscosity characterisation

The gelling and melting temperatures of DSFFG, RSFFG, DSWFG, and RSWFG are shown in Table 4. The results showed that the gelling and melting temperatures of DSWFG were higher than those of the other samples. This might be caused by different amounts of proline and hydroxyproline contents [43]. The gelatin obtained from wild species during the dry season may have had a good amount of proline and hydroxyproline, which can promote the formation of triple helix and physical folding kinetics [12]. This could increase the hydrogen bonds formed by the interaction of imino acid residues and water molecules, resulting in more stable intermolecular structures that enable easier gel formation [44]. Data from the present study were higher than the values (gelling temperature, 10 °C) reported by Ref. [45] for gelatin extracted from Nile tilapia living in clean water.

The gelatin DSWFG showed the highest viscosity of 27.95 Cp. Viscosity tends to increase with higher gelling and melting temperatures, as well as stronger gel strength. The viscosity of DSWFG and DSFFG were far higher than that reported by Ref. [45], which implies that these gels could withstand high temperatures and acid-alkali pretreatment applied during the collagen hydrolysis and resulted in low yield, high gel strength, high gelling, and melting temperatures. In general, the higher the molecular weight, the greater the viscosity of the gelatin solution [46]. Thus, the higher viscosity could be due to the high molecular weight of gelatin extracted from wild Nile tilapia scale under dry season. This difference could also be related to the physiological adaptation of fish to their environmental temperature at which stable collagen may be required for the survival of fish, which finally plays a key role in how collagen is structured under these conditions [47].

### 3.2.3. Gel strength and emulsifying properties of the extracted gelatins

The gel strength plays a critical role in determining the functional properties of gelatin [31]. It also forms the basis for classifying its overall quality into low bloom (<150), medium bloom (150–200), and high bloom (220–300).). The gel strength of RSFFG was less than 100 g, while that of other samples was in the range of 100–120 g (Fig. 1A)

These results reflect significant differences ($p < 0.05$) in the gel-forming ability of gelatin obtained from the scales of farmed Nile tilapia and Nile tilapia found in the wild. The seasonal variations and farming could not influence the gel strength significantly ($p > 0.05$) rather the temperature (60 °C) employed in this study affected the gel strength of DSFFG and RSFFG significantly ($p < 0.05$) for DSWFG and RSWFG. The decrease in gel strength was usually related to the degradation of α-, and β-protein due to high temperature [48]. Also, the gel strength was affected by pH. The pH at the isoelectric point (IEP) caused the gel to be denser and harder due to the reduction of the repulsive force between the protein's chains (α-, and β-) [36]. This scenario supported the DSWFG with the highest gel strength attributed to pH 5.58 as seen in Table 2. The low yield of RSWFG and DSWFG recorded in the present study could be another underlying factor responsible for denser gel strength.The triple helix in collagen of gelatins RSWFG and DSWFG was more organized and cohesive by intermolecular forces that existed between them as suggested by Ref. [42]. Also, the combination of moderate temperature and high pH contributed to the formation of gel with high strength which played a major role in gel formation of gel. The results were confirned by a similar report by Ref. [31] when the gel strength of tilapia skin gelatin was high at 45 °C/pH 5.

The emulsifying capacity of extracted gelatins was significantly affected by the farming system and seasonal differences ($p < 0.05$) as shown in Fig. 1B. However, only DSFFG and other samples showed significant differences. In addition, there were no significant differences in emulsification capacity between the other three samples despite differences in farming system and seasonality. the. The changes in emulsifying capacities in extracted gelatins are primarily attributed to protein content. According to Ref. [49], protein content plays a major role in the emulsification process. At the low level, the diffusion and adsorption of protein at the oil-water interface are favorable. It was observed that extracting gelatin during the dry season from farmed fish was ideal, especially when the emulsification property of fish gelatin is to be considered in food application. Furthermore, a correlation was found between the gel strength and emulsifying capacity of nilapia from different farming systems under different seasonal variations. A gel strength is dirently influenced by molecular weight [31] i.e. positive correlation had a negative correlation with emulsifying capacity of the samples in Fig. 2A and B. Obviously, gel strength directly impacted the emulsifying properties of samples. The lower the gel strength which is a mirror of molecular weight the higher the emulsifying capacity. This is because lower molecular weight diffuses and adsorbs to the interface resulting in lower interfacial tension leading to high emulsifying capacity result in lower interfacial tension [50]. Tan et al. (2020) [15] observed a similar phenomenon when lower molecular had a high surface activity with good emulsifying properties of black tilapia skin gelatin.

## 3.3. Structural properties of extracted gelatin

### 3.3.1. FTIR spectra of extracted gelatins

The spectra for the four samples appeared similar, suggesting no conformational differences among the gelatin samples studied

**Table 4**
Melting, gelling temperatures, and viscosity of gelatin extracted.

| Sample | Melting temperature (°C) | Gelling temperature (°C) | Viscosity (cP) |
|---|---|---|---|
| DSFFG | $27.95 \pm 0.07^{b}$ | $13.50 \pm 0.70^{b}$ | $26.56 \pm 0.73^{b}$ |
| RSFFG | $28.85 \pm 0.07^{a}$ | $11.50 \pm 0.70^{d}$ | $17.03 \pm 0.03^{c}$ |
| DSWFG | $28.95 \pm 0.07^{a}$ | $15.50 \pm 0.70^{a}$ | $27.95 \pm 0.07^{a}$ |
| RSWFG | $26.65 \pm 0.21^{c}$ | $12.50 \pm 0.70^{c}$ | $16.23 \pm 0.68^{c}$ |

Figures are presented as means ± standard deviation of triplicates; Superscripts to figures in the same row imply significant difference at $p < 0.05$.

(Fig. 2). Specifically, for the extracted fish gelatin samples, intense peaks representing Amide A, Amide I, Amide II, and Amide III were noticed around 3302, 1623, 1545, and 1230 $cm^{-1}$, respectively.

Despite the similarity in their FTIR pattern, nuanced differences in transmittance and slight peak shifts were observed. The Amide A characteristic peak, a stretching vibration of the N–H group of gelatins usually occurs in the range 3400–3440 $cm^{-1}$ [51,52] Wang et al., 2014 [29]. When an N–H group is involved in hydrogen bond formation, its position will shift to a lower frequency [52]. All the Amide A characteristic peaks of gelatins in this study centered around 3249.90 $cm^{-1}$, between DSWFG and RSWFG indicating that the N–H group was involved in the formation of hydrogen bonds and combined the collagen triple-helix structures through hydrogen bonds leading to 3D-networking structure. b structures in the gel-forming process, thus increasing the gel strength (Fig. 1A) [53]. The sample RSWFG had a broad spectrum which in the bands of 3000–3300 cm-1 reflects the –OH stretching vibration. Furthermore, in the Amide I region, RSWFG demonstrated a distinct peak at 1650.83 cm-1, which is designated to be an α-helix structure, a strong backbone for gelatin [54] and judged as triple-helix conformation attributed to the strong C–O, which accounted for low yield (Table 2) in wild type gelatin [31]. However, gelatin in DSFFG, DSWFG, and RSFFG recorded low wave numbers (Table 5) in the α-helix of Amide which indicates the transformation of α-helical to random coil structure resulting in collagen-gelatin formation [55].

#### 3.3.2. XRD pattern of extracted Nile tilapia scale fish gelatin

The diffractograms of DSFFG, RSFFG, DSWFG, and RSWFG are shown in Fig. 2. The results obtained for all the extracted gelatin demonstrated similarities except DSFFG, which showed conspicuous peaks around 11°, 21°, 29° and 31°. The diffractograms for all samples present an amorphous character, indicating no tendency toward recrystallization (see Fig. 3).

The amorphous structure of the gelatin in this study is similar to the typical fingerprints for gelatin powder [56]. Gelatin with peaks around 7–8° is regarded as gelatin with crystalline behavior, and a corresponding triple-helix content. This is the case of the RSWFG sample which maintains the integrity of collagen triple-helix structure during the extraction However, a different scenario was recorded in farmed gelatin which has amorphous nature corresponding to the disorded structure of collagen structure [57] underscoring the explanation of high yield which makes the collagen hydrolyze easily induced by weak bond of triple helix structure. The results of the present study is in agreement with earlier report by Ref. [7].

### 3.4. Statistical modeling for rheological and functional properties of fish gelatin

The rheological, functional, and physical properties of extracted fish gelatin were modeled to study how these properties affected the yield. Due to the nature of the independent variables, which are continuously measured variables with error, partial least squares (PLS) regression was adopted to build the models.

For the rheological properties, the gelling temperature, melting temperature, and viscosity were the measured predictors. The PLS regression output is summarized in Table 6. An optimum three-component model was derived and accounted for 92.5 % of the total variations in yield.

The first two components also accounted for 67 % of the total variance in the predictors. Gelling temperature and viscosity loaded strongly on the first component, which accounted for 70 % of variations in the yield whereas the melting temperature loaded strongly on component two (Fig. 4A)

The regression model was significant ($p < 0.05$) and showed that a unit increase in yield will result in a 1.09 decrease in gelling temperature of fish gelatin when yield is standardized with an intercept of zero and that all other predictors remain constant (Table 6). Though melting temperature and viscosity showed a positive correlation (an increase in yield will result in an increase in viscosity and melting temperature), their standardized coefficients were less than one, an indication that changes in yield will affect melting temperature and viscosity less than gelling temperature. The viscosity increases with melting and gelling temperature as reported by Ref. [46], however, the present study indicated that viscosity is more related to melting than gelling temperature upon unit change in gelatin yield.

The PLS model parameters for functional properties are presented in Table 7. A two-component model was developed that utilized turbidity and emulsifying capacity as predictors. These accounted for 98 % of total variations in the response (yield). The first component alone accounted for 83 % of total variations in yield and 74 % variance in the predictors. Both turbidity and emulsifying capacity loaded strongly on component 1 (Fig. 4B).

However, the higher effect of turbidity indicates that changes in fish gelatin yield affect the turbidity of fish gelatin than

**Table 5**
FTIR peak locations and assignment of sequential acid-alkali extraction of DSFFG, RSFFG, DSWFG, and RSWFG.

| Region | Peak wave numbers (cm-1) | | | | Assignment | Reference |
|---|---|---|---|---|---|---|
| | DSFFG | RSFFG | DSWFG | RSWFG | | |
| Amide A[1)] | 3289.98 | 3284.53 | 3299.05 | 3249.90 | N–H stretch coupled with H-bond | Muyonga et al., 2004 |
| Amide B[2)] | – | 2969.28 | 2928.68 | – | CH antisymmetric and symmetric stretching | Abe and Krimm, 1972 |
| Amide I[2)] | 1629.37 | – | 1629.77 | 1650.83 | C═O stretch/hydrogen bond coupled with COO- | Abe and Krimm, 1972 |
| Amide II | 1543.87 | 1543.92 | 1546.12 | 1548.66 | NH bend coupled with CN stretch | Payne and Veis, 1988 |
| Amide III | 1232.62 | 1231.34 | 1232.62 | 1238.41 | NH bend stretch coupled with CN stretch | Shurvell, 2002 |
| Fingerprint | 1079.26 | 1079.26 | 1081.51 | 1081.34 | C–O skeletal stretch | Jackson et al., 1995 |
| Esters | 1710 | 1737 | 1725 | – | C═O stretch with OCOR | Wu et a., 2019 |

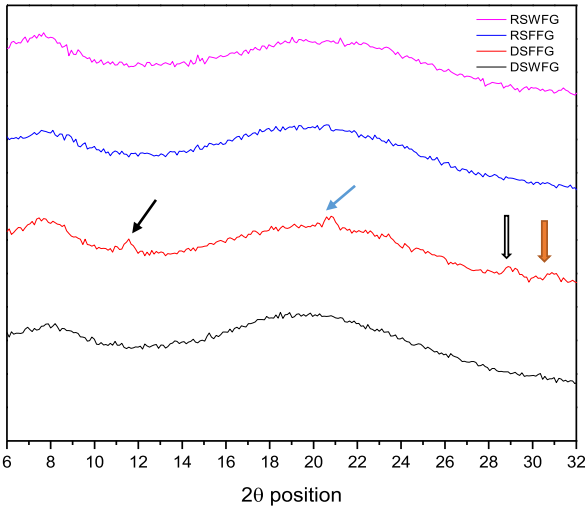


**Fig. 3.** The diffractograms of RSWFG, DSWFG, DSFFG, and RSFFG gelatins.

**Table 6**
PLS regression of rheological properties of fish gelatin and yield.

| Components | X Variance | $R^2$ |
|---|---|---|
| 1 | 0.40076 | 0.702457 |
| 2 | 0.67039 | 0.918525 |
| 3 | 1 | 0.925214 |
| | | |
| Coefficients | Standardized yield | p-value |
| Constant | 0 | 0.01 |
| Gelling temperature | −1.09359 | |
| Melting temperature | 0.60657 | |
| Viscosity | 0.05967 | |

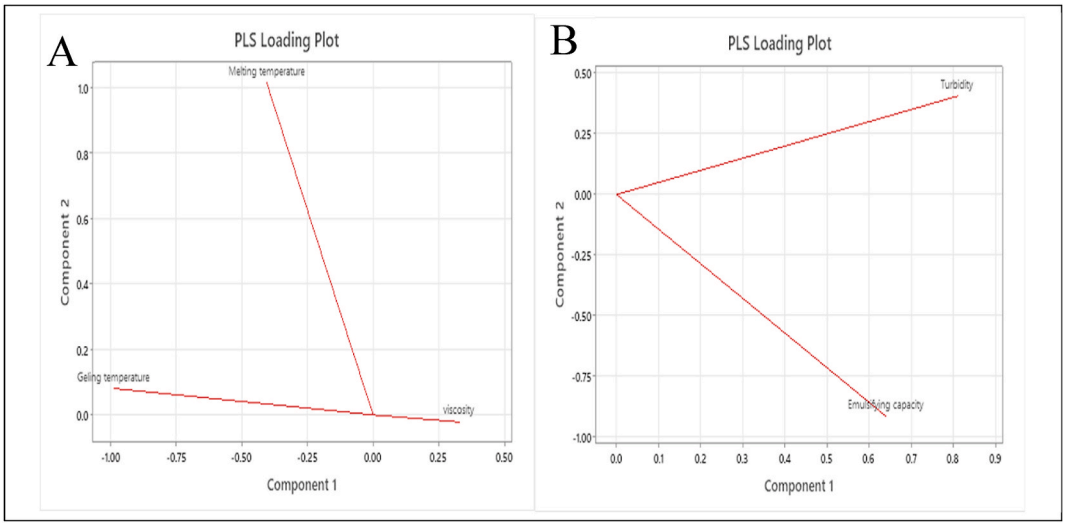


**Fig. 4.** Variable loadings plot for PLS regression. Rheological property (A) and functional property (B).

emulsifying capacity. The regression model was significant ($p < 0.05$) and standardized coefficients showed that an increase in the yield of fish gelatin leads to a subsequent increase in turbidity. For every unit change in yield will lead to a 1.05 increase in the turbidity of gelatin.

**Table 7**
PLS regression of functional properties of fish gelatin and yield.

| Components | X Variance | $R^2$ |
|---|---|---|
| 1 | 0.74435 | 0.831451 |
| 2 | 1 | 0.981913 |
| | | |
| Coefficients | Standardized yield | p-value |
| Constant | 0 | 0.000 |
| Turbidity | 1.05297 | |
| Emulsifying capacity | −0.12687 | |

## 4. Conclusion

Our study demonstrates that the combination of the fish farming system and seasonal differences can significantly affect the structural, physicochemical, and functional properties of Nile tilapia gelatin. Specifically, we have found that gelatin extracted from farmed tilapia using sequential acid-alkali extraction technique in both seasons exhibited higher yields than gelatin from wild tilapia. The physicochemical analysis revealed that gelatin extracted from farmed fish was significantly different ($p < 0.05$) from wild type regarding nutritional quality. The protein and fat composition of Wild ($91.00 \pm 0.00^{c}$) and $1.94 \pm 0.05^{a}$ respectively were higher than farmed gelatin of protein ($91.00 \pm 0.00^{c}$) and fat ($0.84 \pm 0.08^{b}$) except moisture content where latter recorded higher than former (farmed: $6.08 \pm 0.22^{a}$; wild ($2.51 \pm 0.01^{c}$) under dry season. For turbidity properties, farmed gelatin types were more clearer ($98.30 \pm 0.28^{a}$) than wild-type ($94.60 \pm 0.28^{b}$). In addition, the XRD analysis confirmed its amorphous structure ($2\theta = 11^{\circ}$, $21^{\circ}$. $29^{\circ}$, and $31^{\circ}$) as it contained less impurities (fat, ash). The results from FTIR and XRD indicated a gelatin gel with strong internal structures was obtained from wild tilapia. This gelatin from wild tilapia had high bloom strength, high melting, viscosity, and gelling temperature. The Partial Least Square (PLS) ascertained that viscosity is positively correlated to melting temperature more than gelling temperature upon a unit change in gelatin yield. These findings provide important insights into the factors that influence the quality and properties of gelatin and have implications for developing more efficient and sustainable processes for the extraction and utilization of gelatin from fish byproducts and advance the progress in research of temperature of natural fish habitat and farming systems on the industrial application of fish gelatin. Further studies on the interactive effect of farming systems and seasonal variations, and extraction optimisation on well-defined properties of different fish species for fish gelatin are encouraged.

## Ethics approval

This Manuscript was approved by Council for Scientific and Industry Research -Institutional Review Board with reference (RPN 005/CSIR-IRB/2021).

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## Data availability

Data will be made available on request.

## CRediT authorship contribution statement

**Ebenezer Asiamah:** Writing - original draft, Methodology, Formal analysis, Conceptualization. **Amy Atter:** Writing - review & editing, Resources, Data curation. **Hayford Ofori:** Writing - review & editing, Software, Data curation. **P.T. Akonor:** Writing - review & editing, Formal analysis, Data curation. **Stephen Nketia:** Writing - review & editing, Software. **Hanna Koivula:** Writing - review & editing, Funding acquisition. **Youngsun Lee:** Writing - review & editing, Resources, Funding acquisition. **Seth Agyakwah:** Writing - review & editing, Data curation.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Amy Atter reports financial support was provided by European Union, Horizon 2020. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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