



Scientific Research

Enzymatic assisted extraction of gelatin from *Thunnus tonggol* skinParisa Feizi¹, Alireza Salarzadeh^{2*}

1-, Ph.D in Food Science & Technology , Dept. of Food Science & Technology, college of Food Technology,
Gorgan University of Agricultural Sciences & Natural Resources.

2*-, Ph.D in Fisheriere , Associate Professor, Dept. of Fisheriere , Bandar Abbas Branch, Islamic Azad University,
Bandar Abbas , Iran

ARTICLE INFO	ABSTRACT
Article History: Received:2024/10/23 Accepted:2025/6/9 Keywords: Enzymatic extraction, Gelatin, Gel strength, Color, Yield DOI: 10.22034/FSCT.22.166.56. *Corresponding Author E- Reza1375bandar@yahoo.com	Gelatin is a water-soluble component and is achievable as a result of partial hydrolysis of collagen and it has many applications in food industry and nutrition science. Therefore, the current research was conducted in relation to gelatin extraction from inexpensive and available sources. The aim of this study was to evaluate the effects of temperature at three levels of 30, 40 and 50 °C, enzyme at 0.055%, 0.01% and 0.1% (w/w) levels and pH at 2, 3 and 4 levels on the quality of extracted gelatin from <i>Thunnus tonggol</i> skin. Accordingly, the firmness, yield and color of resultant gelatin were determined. Based on the results, despite form temperature, by increasing of pH and enzyme percentage, the firmness of gelatin was increased ($p<0.05$). The highest firmness was recorded at 30 °C, 0.1% enzyme at pH=4. The color data revealed that lightness (L^*), redness (a^*) and whiteness of enzymatic extracted gelatin powder was higher that the chemical extracted one ($p<0.05$). The highest lightness was observed at 40 C, 0.055% enzyme at pH=3. According to the results, the highest gelatin yield as 21.65% was related to the enzymatic assisted method at 50 °C, 0.01% enzyme at pH=2, whereas it was obtained as 10% at the chemical extraction method. In conclusion, by application of 30 C and 0.1% enzyme at pH=4 the highest gelatin yield is achievable, while using 50 C and 0.01% enzyme at pH=2 could result the gelatin with the highest firmness.

1.Introduction

Gelatin is a water-soluble protein obtained through the partial hydrolysis of collagen, which is the primary fibrous protein found in bones, cartilage, and skin. The properties of gelatin are influenced by its source, the animal's age, and the type of collagen [1]. Gelatin is widely used across various industries, including food, pharmaceuticals, and photography [2]. Its unique chemical and physical properties make it particularly valuable in the food and pharmaceutical sectors [3], offering capabilities such as gel formation, adhesion, clarification, and thickening [4].

The production of gelatin in America dates back to 1850. After porcine skin was introduced as a raw material in 1930, the industry experienced significant growth. In Europe, this industry flourished from 1996 onwards. Globally, gelatin from fish skin was historically produced in Saxon Harbor, Scotland [5]. The first company to produce gelatin in England was established in 1745, marketing gelatin free from additives and preservatives. Gelatin derived from collagen fibers is digestible, cholesterol-free, fat-free, and carbohydrate-free, offering distinct positive effects on human health.

Gelatin is a unique protein known for its ability to form a thermoreversible gel with a melting temperature close to body temperature, while also being soluble in water. The broad range of its potential applications stems from its specific structure, which is composed of 20 amino acids [6]. Gelatin is one of the most widely used hydrocolloids across various industries. Its main edible applications include bakery products (for emulsification, gelling, and stability), dairy products (for stabilization and texture improvement), confectionery (for foam stability and texture enhancement), dietetic products (for creaminess, fat reduction, and

improved mouthfeel), and meat products (to prevent syneresis) [6]. Additionally, it is utilized in the cosmetic, pharmaceutical, and chemical industries.

Fish gelatin differs from mammalian gelatin in properties such as gelling temperature, melting point, and gel strength. These differences are attributed to variations in amino acid composition, particularly **proline** and **hydroxyproline**. Hydroxyproline is an amino acid derived from proline; both are responsible for the stability and integrity of the collagen structure. They form hydrogen bonds that stabilize the triple-helix structure through tight coiling of the collagen. The presence of proline and hydroxyproline is also responsible for the melting and setting temperatures of various types of gelatin. The content of these amino acids varies across different gelatin sources. Lower levels of proline and hydroxyproline lead to a reduction in the melting point and gel setting temperature of gelatin [6].

Although animal-derived gelatin is consumed globally in various forms and on a large scale, there are significant concerns and warnings regarding its use [7]. This is often due to religious beliefs (e.g., prohibition of bovine products for Hindus and porcine products for Muslims and Jews) and the widespread adherence to vegetarianism worldwide. Furthermore, researchers are increasingly concerned about the potential transmission of pathogenic vectors [8]. Further research indicates that animal gelatin is directly linked to the emergence of Bovine Spongiform Encephalopathy (BSE), or "mad cow disease," in Europe. Consequently, efforts to find suitable alternatives to gelatin for food products are increasing.

Fish gelatin, particularly with characteristics like a lower melting temperature, rapid dissolution in the mouth, and absence of a rubbery texture, is

considered one of the most suitable alternatives to mammalian gelatin and is accepted as a halal food. Although the production of fish gelatin is in its early stages of development, accounting for only about 1% of annual global production, the annual waste from fish processing is approximately 3–7 million tons. This waste can serve as a rich and valuable source for fish gelatin production. Utilizing this waste reduces disposal costs for fish processing factories and creates economic value for them [9].

Fish gelatin has a low gel formation and melting temperature, which limits its industrial application. Nevertheless, the functional properties of gelatin can be improved chemically and enzymatically [6]. The quality of gelatin and collagen depends on their physical, chemical, and rheological properties, suitable extraction conditions, and the type of source. Commercially, fish gelatin has been shown to be less competitive than mammalian gelatin due to its lower gel strength, lower melting point, and slower gel formation. To address or mitigate some of these issues, solutions such as enzymatic extraction have been proposed, which aim to improve the strength and characteristics of the extracted gelatin [10].

The animal enzyme **pepsin**, also known as EC 3.4.23.2, is a crucial acidic protease widely used in protein hydrolysis. It is an important aspartic protease with many unique features for industrial applications and medical research. Its optimal pH is 2.0–4.0, with pH stability at ≤ 6.0 . The optimal temperature range is 30–55 °C, with specific thermal stability at ≤ 40 –50 °C. It has a molecular weight of 36 kDa. Pepsin is synthesized and secreted in the gastric mucosa. The inactive form of pepsin is called **pepsinogen (PG)**, which has a molecular weight of 40 kDa [11].

Pepsin is utilized in **collagen extraction** [12, 13, 14], **gelatin extraction** [15], and as

a **rennet substitute** [16]. Primarily found in the gastric lumen's digestive fluid, pepsin has been isolated and studied from various mammals including humans [17], Japanese monkeys [18], pigs [19], cattle [20, 21], rats [22], and rabbits [23].

In a 2011 study by Metta Maro et al., the characteristics of **acid-soluble collagen (ASC)** and **pepsin-soluble collagen (PSC)** extracted from spotted golden goatfish (*Parupeneus heptacanthus*) were investigated. They concluded that both ASC and PSC were identified as Type I collagen, containing distinct $\alpha 1$ and $\alpha 2$ chains. Additionally, β and γ components were found in both collagens. Based on FTIR spectra, limited digestion by pepsin did not disrupt the collagen's triple-helix structure.

Feng et al. (2013) explored the optimization of enzymatic extraction and characterization of collagen from Chinese sturgeon (*Acipenser sturio Linnaeus*) skin. Their SDS-PAGE gel electrophoresis patterns indicated that sturgeon fish skin contained Type I collagen, composed of α and β chains. The collagen's infrared spectrum also demonstrated that pepsin hydrolysis did not affect the collagen's secondary structure, particularly its three-dimensional conformation.

Based on the existing body of research, no studies have yet investigated the enzymatic extraction of gelatin from yellowfin tuna skin using pepsin, nor have they examined its functional properties or the enzyme's effect on improving gelatin's textural characteristics. Therefore, this research explored the functional properties of pepsin-extracted gelatin and the enzyme's impact on it using enzymatic extraction techniques.

2. Materials and Methods

2.1. Materials and Equipment

Sodium chloride, pepsin enzyme (**TITRACHEM**, Iran), sodium carbonate, 37% hydrochloric acid, and sodium hydroxide (**Merck**, Germany) were obtained. All other laboratory reagents used were of analytical grade and high purity, procured from reputable chemical and laboratory supply stores.

2.2. Sample Preparation

Yellowfin tuna (*Thunnus tonggol*) skin was acquired from Bandar Abbas Fishery Products Company in the Bandar Abbas Industrial City. It was transported in a Styrofoam cooler containing ice to the laboratory at Islamic Azad University, Bandar Abbas Branch. Undesirable materials were separated from the skins, which were then rinsed with water and stored at -18 °C for two weeks prior to extraction.

2.3. Pre-treatment Process

The pre-treatment of the skin was performed following the method of [24] and extraction by [25], with minor modifications. A 3.5% sodium chloride (NaCl) solution was used to remove non-collagenous proteins, and a 0.5% sodium carbonate (Na₂CO₃) solution was used to remove fat from the skin. Initial pre-treatment involved stirring the skin with a 3.5% sodium chloride solution at a 1:10 (w/v) ratio at 180 rpm for 6 continuous hours using an orbital shaker (**IKA**, Germany), with solution changes every 3 hours. After rinsing the skins with distilled water, they were stirred with a 0.5% sodium carbonate solution at a 1:10 (w/v) ratio at 180 rpm for 6 continuous hours, with solution changes every 3 hours. The skins were then rinsed with distilled water, and the extraction process commenced.

2.4. Extraction Procedure

Gelatin extraction from the pre-treated skin was carried out in distilled water at a 1:2.8 (w/v) ratio, at temperatures of 30, 40, and 50 °C, with enzyme concentrations of 0.01%, 0.055%, and 0.1% (w/w), and at pH levels of 2, 3, and 4. The extraction process lasted for 6 hours and 45 minutes in a water bath (**Fan Azma Gostar**, Iran). To maintain the optimal pH for enzyme activity during extraction, the pH was measured every 30 minutes using a pH meter (**CB 16 NW**, U.K.), and adjusted to the desired value with 1 N hydrochloric acid or 1 N sodium hydroxide.

After extraction, the mixture was placed in a boiling water bath for 5 minutes to inactivate the enzymes. Subsequently, the solution was passed through cheesecloth and then centrifuged (**Universal Centrifuge**, Iran) at 3500 rpm for 20 minutes for purification. Finally, to dry the filtered material and obtain a powder, a freeze dryer (**Operon, FDU-8624**, South Korea) was used. For this purpose, the gelatin solution was poured into disposable containers and initially placed in a conventional freezer at -18 °C. Once the samples were frozen, they were transferred to the freeze dryer chamber at -85 °C for lyophilization under vacuum. The resulting lyophilized powder was identified as yellowfin tuna skin gelatin. The produced powders were stored in zip-lock bags at -18 °C in a freezer until their characteristics were evaluated.

2.5. Analyses

2.5.1. Gel Properties Evaluation

Gel Strength Determination: Gel strength was determined by the method of [26] with slight modifications, using a **texture analyzer**. To measure gel strength, a 3% (w/v) gelatin solution was first prepared. Three grams of gelatin powder were weighed using a laboratory balance with

0.0001 precision (A&D GR200, Japan) and diluted to 100 mL. The solution was then left at room temperature for 20-30 minutes for swelling, followed by dissolution in a water bath at 45 °C for 30 minutes. Subsequently, 3% gelatin solution samples were poured into plastic containers with a diameter of 5.5 cm and a height of 3 cm. The samples were stored at 4 °C for 16-18 hours to allow gel setting. The bloom value of the gel samples was measured and recorded under specific instrument conditions: a cylindrical probe with a diameter of 1.27 cm, a 10 kN load cell, a penetration speed of 1 mm/s, and a penetration depth of 10 mm into the gel. The bloom number was calculated using the following equation:

$$\text{Gel Strength (g)} = \frac{\text{Load (g)} \times \text{Distance (mm)}}{10}$$

Gelatin Yield

The yield of extracted gelatin was calculated according to the method described in [27] using the following equation:

$$\text{Yield (\%)} = \frac{\text{وزن ژلاتین خشک شده}}{\text{وزن پوست اولیه}} \times 100$$

Color Determination

Color was measured using a colorimeter (IMG-Pardazesh Cam-Systeme XI) with a 45-degree light source and a 0-degree observer, following the method described in [28]. For color measurement, a quantity of dried gelatin sample was placed in a plate and inserted into the device. This measurement is known as CIE L*a*b* colorimetry, where the measured parameters are:

- **L***: Lightness
- **a***: Red (+) to Green (-) axis
- **b***: Yellow (+) to Blue (-) axis

2.6. Statistical Analysis

In this research, **Response Surface Methodology (RSM)** was used to optimize the experimental treatments. A **Central Composite Rotatable Design** was employed to optimize the enzymatic gelatin extraction process. Optimization was based on **gel strength** as the primary index. The effects of independent variables—temperature (30, 40, and 50 °C), enzyme concentration (0.01%, 0.055%, and 0.1% w/w), and pH (2, 3, and 4)—were evaluated at three levels.

The statistical validation of the obtained model for each response was performed using **Analysis of Variance (ANOVA)**. **Design Expert software (version 7.00)** was utilized for experimental design, data analysis, graph plotting, and optimization.

3. Results and Discussion

3.1. Gel Strength Measurement

Table 1 indicates that gel strength varied significantly across different treatments, ranging from 483.08 g to 2466.36 g. The highest gel strength was observed in treatment 5 (2466.36 g). This suggests that the α and β chain structures of the extracted gelatin were better preserved under the optimized extraction conditions of this treatment, leading to increased gel strength. Conversely, the lowest gel strength was recorded for treatment 19 (483.08 g), likely due to extensive degradation of the α and β chains.

Table 1- The firmness and yield data related to the extracted gelatin from *Thunnus tonggol* skin by enzymatic method in RSM desing

Treatment	Temperature(°C)	(%) Enzyme ratio	pH	Firmness (g)	(%)Yield
1	4.	0/1	3	10.79/7	17/1±6.5
2	3.	0/1	2	9.49/2	19/1±4.25
3	5.	0/1	4	13.16/4.8	14/1±8
4	5.	0/0.55	3	9.63/2.7	18/1±0.25
5	3.	0/1	4	24.66/3.6	±4.5/7
6	4.	0/0.55	3	5.3/9	18/1±1.2
7	5.	0/0.1	4	10.11/9	16/1±6.25
8	3.	0/0.1	4	10.51/0.5	10/1±0.5
9	4.	0/0.55	3	9.93/5.5	18/1±0.125
10	4.	0/0.55	2	8.99/6	16/1±2.5
11	3.	0/1	2	9.41/4.2	17/1±5.5
12	4.	0/0.55	3	8.89/3	18/1±9
13	4.	0/0.55	3	8.28/6	17/1±8.75
14	5.	0/1	2	7.99/9	20/1±4.25
15	4.	0/0.55	3	9.16/8	16/1±5.75
16	3.	0/0.55	3	9.13/2.5	12/1±5.5
17	4.	0/0.55	3	9.9/9.2	18/1±1.5
18	4.	0/0.55	4	20.87/7	12/1±8
19	5.	0/0.1	2	48.3/0.8	21/1±6.5
20	4.	0/0.1	3	67.9/4.5	18/1±7.25
Chemical method	75	.	.	1124/7.6	1±1.

As seen in Table 2, the **Response Surface Methodology (RSM)** model for **gel strength** is significant at the 99% confidence level ($p < 0.01$). Furthermore, the lack-of-fit test is not significant at the 95% confidence level ($p > 0.05$). This indicates that the chosen model effectively represents the data, and there are no significant differences between the experimental units in terms of experimental error.

The p-values demonstrate the effects of the independent variables. **Enzyme concentration** shows a linear effect on gel

strength at the 95% confidence level, while **pH** exhibits a linear effect at the 99% confidence level. Additionally, pH has a quadratic effect on the extracted gel strength at the 95% confidence level ($p < 0.05$).

The coded polynomial equation for this experiment revealed that both the linear and quadratic effects of **pH** are highly effective and impactful on the gel strength response. Specifically, a decrease in pH led to a significant reduction in the strength of the extracted gelatin.

Table 2- the ANOVA results of gelatin gel strength in respond surface method extracted from *Thunnus tonggol* skin by enzymatic method

Source of change	Sum of squares	Degree of freedom	Mean square	F Value	P Value Probe>F	
Regression model	3/43.E+0.06	9	3/811E+0.05	5/22	0/0.82	Significance**
Temperature:A	3/0.51E+0.05	1	3/0.51E+0.05	4/18	0/0.681	
Enzyme ratio:B	5/915E+0.05	1	5/915E+0.05	8/11	0/0.173	Significance*
pH:C	1/49.E+0.06	1	1/49.E+0.06	20/43	0/0.011	
AB	77250/0.5	1	77250/0.5	1/0.6	0/7732	Significance**
AC	42251/79	1	42251/79	0/58	0/4642	
BC	2/488E+0.05	1	2/488E+0.05	3/41	0/0.945	
A ²	19676/27	1	19676/27	0/27	0/6148	

B ²	57637/28	1	57637/28	0/79	0/3949	Significance*
C ²	6/096E+0.05	1	6/096E+0.05	8/36	0/0161	
lack of fit	7/295E+0.05	10	72949/63	3/87	0/0820	
Pure error	1/499E+0.05	5	29979/09			Lack of significance

Significance at 99% confidence level,* * Significance at 95% confidence level*

Based on the ANOVA of the gel strength data, the following mathematical relationship was obtained using coded values:

$$Y_{\text{Gel Strength}} = 10246/71B - 2363/64C + 470/8C^2$$

The **ANOVA** confirmed that the **quadratic polynomial model** adequately represents the response (Table 3). The **coefficient of determination (R²) of 0.8246** indicates that the regression model explains the reaction well. Specifically, the linear and quadratic effects of **pH**, and the linear effect of **enzyme concentration**, account for 82.46% of the variability in gel strength.

The suitability of the model was further assessed using the **lack-of-fit test** (Table 3). This test was significant for the linear model but not for the quadratic model ($p >$

0.05). Therefore, the **quadratic model was deemed appropriate for prediction within the range of variables used**. Although the cubic model also exhibited a very high R², it was not recommended by the software due to potentially misleading results. A cubic model is typically suitable only when there is at least a minimal significance among all factors under investigation. While the linear model offers higher precision, its significant lack-of-fit makes it unsuitable for use. A **precision value greater than 4 (9.324)** suggests the chosen and proposed model is adequate and suitable. For a model to have good predictive power, its **adjusted coefficient of determination** must be high, which was indeed the case for the proposed quadratic model compared to the linear model.

Table 3- The statistical model for gelatin gel strength

Sources of change Model	standard deviation	R ²	R ² adjustment	R ² predicted	The accuracy of the decision	PRESS	
linear	332/81	0/5739	0/4940	0/2607	10/802	3/075E±0.09	suggested
interference	328/62	0/6624	0/5066	-0/4946	-	6/216E±0.09	
second degree	270/09	0/8246	0/6667	-1/0003	9/324	8/319E±0.09	suggested
cubic	208/08	0/9375	0/8022	-31/5123	-	1/352E±0.08	Aliased

Model Optimization

Optimal conditions were determined by the software using the **Numerical**

Optimization Test (NOT), as presented in Table 4.

Table 4- Optimization parameters for dependent variables and surface respond of gelatin gel strength

parameter	objective	The lowest level	The highest level	Lowest weight	Highest weight	Validity
Temperature	in range	۳۰	۵۰	۱	۱	۳
Enzyme ratio	in range	۰/۰.۱	۰/۱	۱	۱	۳
pH	in range	۲	۴	۱	۱	۳
Gel strength	the most	۴۸۳/۰.۸	۲۴۶۶/۳۶	۱	۱	۵

After defining the ranges, the software identified the optimal points. The **optimal conditions** for gel strength, with a **desirability** of 0.919 (close to one), were: **30 °C temperature, 0.1% w/w enzyme concentration, and pH 4**, yielding a predicted gel strength of 2306.005 g. Notably, in the present study, the actual gel strength obtained under these same conditions was even higher (2466.36 g). This demonstrates a good fit between the predicted and actual experimental data, indicating a logical correlation between the experimental results and the selected model.

Gel strength is a crucial functional property of gelatin, serving as a key determinant of its quality [29]. It is a complex interplay of factors, dependent on amino acid distribution and the ratio of alpha and beta chains, as well as the content of beta compounds [30]. It appears that cross-links between alpha chains are more stable than those between beta chains [31], and gelatin with a higher percentage of alpha chains exhibits superior gel strength [32]. Gelatin quality is generally classified by gel strength, or bloom index, into low bloom (<150), medium bloom (150-220), and high bloom (220-300) [33]. High-bloom gelatin is considered the best type and has a wide range of applications in the food industry, particularly in the production of jellies, canned meats, marshmallows, and yogurts [34].

Gel strength was measured after the gel set for 16-18 hours at 4 °C. In the enzymatic extraction method, the highest gelatin gel strength (2466.36 g) was observed in

gelatin extracted at 30 °C with 0.1% enzyme concentration and pH 4. It was observed that as the hydrolysis pH increased, the gel strength improved due to lower enzyme activity at this pH, resulting in less hydrolysis of polypeptide chains by the enzyme. This finding aligns with the results of [35], who reported that increasing pH decreased the hydrolytic effect of papain enzyme on the extracted chain, thereby improving gel strength. The strength of this gelatin was significantly higher than that extracted chemically from yellowfin tuna skin (1124.76 g). The lower gel strength observed in the chemical method is likely due to the destructive effect of chemicals on the protein chains, leading to chain shortening. This result is consistent with [12], who studied gelatin prepared from bigeye snapper skin using pepsin and concluded that among all samples, chemically prepared gelatin exhibited the lowest gel strength, possibly because the gelatin chain length was reduced during chemical extraction, forming a weaker gel network.

Referring to the data from this study, the lowest gel strength was observed in treatment 19 (temperature 50 °C, enzyme ratio 0.01%, pH 2). The gel strength of this treatment was proportionally lower (0.05%) compared to the best treatment. This is attributed to excessive hydrolysis and enzyme activity at this pH, leading to shorter polypeptide chains and consequently lower gel strength. Conversely, the central treatment (treatment 17) showed a gel strength of 909.92 g. Compared to the best treatment, it had proportionally lower gel strength (0.028%), and proportionally higher gel

strength (0.02%) compared to the worst treatment.

Significant differences in gel strength can be attributed to intrinsic characteristics, such as molecular weight distribution. Gelatin with a high molecular weight distribution typically exhibits high bloom, while gelatin with shorter chains has lower gel strength [36]. Achieving high strength can be attributed to the use of enzymes and the preservation of alpha chains, which play a role in preventing excessive peptide degradation [24]. The improvement in gel strength and properties of gelatin extracted enzymatically from bighead carp scales was investigated by [25], who found that enzymatically prepared gelatin had higher gel strength (318 ± 7 g) than chemically prepared gelatin (216 ± 3 g).

3.2. Gel Yield Measurement

Table 1 shows that the yield of extracted gelatin powder varied between 7.45% and 21.65% across different treatments. The highest yield (21.65%) was observed in **treatment 19**, which used a temperature of 50 °C, an enzyme concentration of 0.01%, and a pH of 2. The higher enzymatic

hydrolysis at this pH led to an increased amount of extracted gelatin powder. Conversely, the lowest yield of extracted gelatin powder was found in **treatment 5** (temperature 30 °C, enzyme concentration 0.1%, pH 4), attributed to the lower enzymatic hydrolysis at this pH.

As shown in Table 5, the model for the gelatin powder yield response was significant at the 95% confidence level ($p < 0.05$). Additionally, the lack-of-fit test was not significant at the 95% confidence level ($p > 0.05$), indicating that the model accurately represents the data, and there's no significant difference in experimental error among the units. The p-values for the independent variables show that **pH** has a linear effect on gelatin powder yield at the 95% confidence level, while **temperature** and **pH** exhibit an interactive effect at the 99% confidence level. The coded polynomial equation confirmed that the linear effect of **pH** and the interaction effect between **pH** and **temperature** are highly effective on the yield of extracted gelatin powder. Specifically, a decrease in pH led to a significant increase in the yield of extracted gelatin powder.

Table 5- the ANOVA results of gelatin yield in respond surface method extracted from *Thunnus tonggol* skin by enzymatic method

Source of change	sum of squares	degree of freedom	mean square	F Value	P Value Probe>F	
Regression model	214/56	9	23/84	14/40	0/0001	significance**
Temperature:A	60/02	1	60/02	36/26	0/0001	significance**
Enzyme ratio:B	7/40	1	7/40	4/47	0/0606	
pH:C	112/73	1	112/73	68/10	<0/0001	significance**
AB	0/25	1	0/25	0/15	0/7036	
AC	9/74	1	9/74	5/88	0/0357	significance*
BC	0/22	1	0/22	0/13	0/7234	
A ²	4/24	1	4/24	2/56	0/1404	
B ²	7/56	1	7/56	4/57	0/0584	
C ²	11/05	1	11/05	6/68	0/0272	significance*
remaining lack of fit	16/55	10	1/66			
	13/68	5	2/74	4/77	0/0558	Lack of significance
Pure error	2/87	5	0/57			

Significance at 99% confidence level,* * Significance at 95% confidence level*

Based on the ANOVA of the extracted gelatin powder yield data, the following mathematical relationship was obtained using coded values:

$$Y_{\text{Yield}} = 3.24611 + 0.88620A + 4.46175C + 0.11031A \\ C - 2.00489C^2$$

The ANOVA confirmed that the **quadratic polynomial model** adequately represents the response (Table 6). The **coefficient of determination (R²) for the predicted yield was 0.9284**, and the **p-value for the lack-of-fit test was 0.0558**. This indicates that the proposed model provides a good fit for the response and can predict it

accurately. In other words, 92.84% of the variability in the dependent variable can be explained by the independent variables. Notably, **pH and temperature** show a strong correlation with this explanation ($P < 0.01$).

An **adequate precision value greater than 4 (14.658)** further confirms the suitability and adequacy of the selected model. The high adjusted coefficient of determination reinforces this, as no non-significant conditions were observed in the model ($p < 0.05$). Overall, this model was suitable for prediction within the range of the variables used.

Table 6- The statistical model for extracted gelatin yield

Sources of change Model	standard deviation	R ²	R ² adjustment	R ² predicted	Precision decision	PRESS	
linear	1/78	0.7795	0.7381	0.6504	-	80/80	
interference	1/77	0.8237	0.7423	0.5599	-	101/71	
<u>second degree</u>	1/29	0.9284	0.8639	0.9290	14/658	86/42	suggested
<u>cubic</u>	1/25	0.9592	0.8709	0.33/8399	-	80.51/92	Aliased

Model Optimization

The optimal conditions were determined by the software using the **Numerical Optimization Test (NOT)**. Table 7

presents the optimal parameters for both the independent variables and the dependent variable's response level.

Table 7- Optimization parameters for dependent variables and surface respond of gelatin yield

parameter	objective	The lowest level	The highest level	Lowest weight	Highest weight	Validity
Temperature	in range	30	50	1	1	3
Enzyme ratio	in range	0.01	0.1	1	1	3
pH	in range	2	4	1	1	3
Yield	the most	7/45	21/65	1	1	5

After setting the parameters, the software identified the optimal points. The **best optimal conditions for yield**, with a **desirability of 0.997** (very close to one), are as follows: **temperature of 46.396 °C**, **enzyme concentration of 0.01% w/w**, and

pH of 2.381, resulting in a predicted gelatin powder yield of 21.608%.

Interestingly, in the current study, a slightly higher yield (21.65%) was achieved at a temperature of 50 °C, an enzyme concentration of 0.01% w/w, and pH 2.

This indicates a good fit between the predicted data and the actual experimental results, confirming a logical correlation between the experimental data and the chosen model.

The **highest yield of enzymatically produced gelatin from yellowfin tuna skin** was 21.65%, obtained from the treatment with a temperature of 50 °C, an enzyme concentration of 0.01% w/w, and pH 2. In contrast, the yield using the chemical method was 10%. This study revealed that employing the lowest enzyme concentration (0.01%) and the highest temperature (50 °C) for gelatin extraction more than doubled the yield compared to the chemical method.

One might expect that at low pH and the lowest enzyme level, coupled with the highest temperature—which yielded the highest efficiency—increasing the enzyme concentration from 0.01% to 0.1% at the same pH would lead to even higher yields. However, this study observed the opposite. This suggests that at **pH 2**, enzyme activity is significantly reduced, and the primary hydrolytic activity is due solely to the **pH** and **temperature**, rather than the enzyme itself. This is supported by **treatment 11**, which, with a 0.1% enzyme concentration and pH 2, yielded only 17.55%, confirming this observation.

Reviewing the study's data, the **lowest yield of extracted gelatin** came from **treatment 5** (30 °C, 0.1% enzyme, pH 4). Its yield was proportionally lower (0.03%) than that of the highest-yielding treatment (treatment 19). Conversely, the central treatment (treatment 17) yielded 18.15% gelatin. Compared to the highest-yielding treatment (treatment 19), this was proportionally lower (0.012%), but proportionally higher (0.02%) than the lowest-yielding treatment (treatment 5).

The **low yield of chemically prepared gelatin** might stem from the low solubility

of cross-links formed via aldehyde reactions with lysine and hydroxylysine in the helical parts of the terminal peptides [37]. Collagen saturation in the skin and its degradation during extraction and conversion to gelatin could also contribute to the low yield in chemical extraction [38]. When pepsin's limited digestion is enhanced, there's a higher likelihood of breaking molecules involved in telopeptide cross-linking, thereby increasing collagen extraction efficiency [12].

A study on acid and pepsin-soluble collagen extracted from goatfish scales found that the yield of pepsin-extracted collagen was 1.2% (dry weight basis), which was about 2.61 times higher than the 0.46% obtained by the chemical method [39]. Similarly, acid-extracted and pepsin-extracted collagen from carp scales yielded 0.86% and 2.32% (dry weight basis), respectively [13]. Variations in yield can be attributed to differences in fish species, biological conditions, and preparation methods [40].

Regarding collagen yield extracted by pepsin from various fish parts (skin, swim bladder, fins, scales, and bones), studies found the highest yields were, in descending order, from skin, then swim bladder, followed by fins, scales, and bones. Overall, the results indicate that **pepsin was effective in increasing the yield of gelatin extracted from yellowfin tuna skin**.

3.3. Gel Color Measurement

Table 8 presents the color measurements of the extracted gelatin powder. To evaluate the color of the produced powders, **L***, **a***, **b***, and **W** values were measured. **L*** represents lightness, **a*** indicates the red-green spectrum, **b*** indicates the blue-yellow spectrum, and **W** represents whiteness.

The highest and lowest **lightness (L*)** values were observed in treatments 13 and 11, respectively. The highest and lowest **redness (a*)** values were found in treatment 7 and the chemically extracted gelatin, respectively. The highest **yellowness (b*)** was observed in chemically extracted gelatin, while the

lowest was found in treatments 8, 13, and 20. Lastly, the highest and lowest **whiteness (W)** values corresponded to treatments 6 and 11, respectively.

Table 8- The color data of gelatin powder extracted by enzymatic method from *Thunnus tonggol* skin

Treatment	(°C) Temperature	Enzyme (%) ratio	pH	L	a*	b*	W
1	40	0.1	3	67/3±97/39	-0.2±12/65	2/2±4/89	68/3±68/43
2	30	0.1	2	77/2±29/86	-0.1±76/0.6	1/1±89/67	77/2±13/86
3	50	0.1	4	71/4±24/27	-0.2±0.9/0.4	1/2±36/59	71/4±0.3/35
4	50	0.55	3	73/1±42/0.8	-1/1±98/19	4/2±87/16	72/1±81/36
5	30	0.1	4	75/2±0.5/33	-2/1±52/57	5/2±97/29	74/2±13/71
6	40	0.55	3	79/1±39/0.4	-0.0±61/57	1/1±0.3/0.2	79/1±36/0.3
7	50	0.1	4	76/4±65/0.9	0.2±42/19	1/1±52/55	73/4±5/14
8	30	0.1	4	69/2±16/4	.	.	69/2±16/4
9	40	0.55	3	77/1±87/13	-2/1±12/58	5/2±52/14	77/1±46/32
10	40	0.55	2	68/2±5/64	-1/1±38/35	9/4±60/77	66/2±75/69
11	30	0.1	2	64/3±84/7	-1/2±89/44	8/1±83/71	63/3±58/35
12	40	0.55	3	77/1±45/17	-1/1±36/0.7	3/1±27/58	77/1±0.2/35
13	40	0.55	3	79/1±81/0.1	.	.	79/1±81/0.1
14	50	0.1	2	71/2±78/0.4	-5/0±99/44	12/1±72/39	68/1±42/65
15	40	0.55	3	78/1±98/0.8	-0.0±61/57	1/1±0.3/0.2	78/1±92/0.6
16	30	0.55	3	74/1±24	-1/0±0.9/77	2/1±14/78	74/1±0.7/1
17	40	0.55	3	75/1±93/26	-2/1±12/58	5/2±52/14	75/1±12/64
18	40	0.55	4	73/4±71/49	-1/1±0.2/39	1/2±94/66	73/4±51/56
19	50	0.1	2	75/1±8/24	-1/1±24/27	4/1±35/79	75/1±31/35
20	40	0.1	3	78/1±86/24	.	.	78/1±86/24
chemic	75	.	.	69/4±47/41	-5/2±46/99	14/3±81/38	65/3±30/41

As shown in Table 9, the model for the **whiteness (W)** response is significant at the 95% confidence level ($p < 0.05$). Additionally, the lack-of-fit test is not significant at the 95% confidence level ($p > 0.05$), indicating that the model accurately represents the data, and there's no significant difference in experimental error among the units.

The p-values for the independent variables reveal that enzyme concentration has a

significant effect at the 99% confidence level, and there's a significant interaction effect between enzyme concentration and pH on the whiteness of the extracted gelatin powder, also at the 99% confidence level. The coded polynomial equation from the analysis shows that the effect of enzyme concentration on the whiteness response is highly efficient and impactful; specifically, decreasing the enzyme concentration significantly increased the whiteness of the extracted gelatin powder.

Table 9- the ANOVA results of gelatin powder whiteness in respond surface method extracted from *Thunnus tonggol* skin by enzymatic method

Source of change	sum of squares	degree of freedom	mean square	F Value	P Value Probe>F	
Regression model	۱۵۶/۵۱	۶	۲۶/۰.۸	۴/۰.۵	۰/۰.۱۶۶	significance*
Temperature:A	۰/۹۰	۱	۰/۹۰	۰/۱۴	۰/۷۱۴۷	
Enzyme ratio:B	۷۹/۰.۷	۱	۷۹/۰.۷	۱۲/۲۶	۰/۰.۰۳۹	Significance**
pH:C	۱۰/۲۸	۱	۱۰/۲۸	۱/۵۹	۰/۲۲۸۸	
AB	۰/۰.۷۶	۱	۰/۰.۷۶	۰/۰.۱۲	۰/۹۱۵۲	
AC	۰/۴۰	۱	۰/۴۰	۰/۰.۶۱	۰/۸۰۸۱	
CB	۶۵/۷۸	۱	۶۵/۷۸	۱۰/۲۰	۰/۰.۰۷۰	Significance**
remaining lack of fit	۸۳/۸۲	۱۳	۶/۴۵			
	۷۳/۰.۶	۸	۹/۱۳	۴/۲۴	۰/۰.۶۴۱	Lack of significance
Pure error	۱۰/۷۶	۵	۲/۱۵			
Core Total	۲۴۰/۳۳	۱۹				

Significance at 99% confidence level,* * Significance at 95% confidence level*

Based on the ANOVA of the extracted gelatin powder whiteness data, the following mathematical relationship was obtained using coded values:

$$Y_{\text{Whiteness}} = -244.98889B + 63.72222BC$$

The ANOVA confirmed that the **interactive polynomial model** adequately represents the response (Table 10). The **coefficient of determination (R²) of 0.6512** indicates that the regression model effectively explains the reaction. Specifically, the interaction effect between **temperature and pH**, along with the linear effect of **enzyme concentration**, accounts

for 65.12% of the variability in the gel strength response.

The model's suitability was further assessed using the **lack-of-fit test** (Table 9), which was not significant for this model ($p > 0.05$). Therefore, the **interactive model was appropriate for prediction within the range of variables used**. An **adequate precision value greater than 4 (8.257)** further indicates the suitability and adequacy of the chosen and proposed model.

Table 10- The statistical model for whiteness of extracted gelatin powder

Sources of model change	standard deviation	R ²	R ² adjustment	R ² predicted	Accuracy of decision	PRESS	
linear	۳/۰.۶	۰/۳۷۵۵	۰/۲۵۸۵	-۰/۲۵۳۵	-	۳۰.۱/۲۶	
<u>interference</u>	۲/۵۴	۰/۶۵۱۲	۰/۴۹۰۲	-۱/۰.۴۴۳	۸/۲۵۷	۴۹۱/۳۱	<u>suggested</u>
second degree	۲/۵۸	۰/۷۲۴۰	۰/۴۷۵۵	-۲/۴۴۳۶	-	۸۲۷/۶۱	
cubic	۱/۴۴	۰/۹۴۸۶	۰/۸۳۷۲	-۷/۲۰.۳۴	-	۱۹۷۱/۵۲	Aliased

Model Optimization

Optimal conditions were determined by the software using the **Numerical**

Optimization Test (NOT). Table 11 presents the optimal parameters for the independent variables and the corresponding response level of the dependent variable.

Table 11- Optimization parameters for dependent variables and surface respond related to the whiteness of gelatin powder

parameter	objective	The lowest level	The highest level	Lowest weight	Highest weight	Validity
Temperature	in range	۳۰	۵۰	۱	۱	۳
Enzyme ratio	in range	۰/۰/۱	۰/۱	۱	۱	۳
pH	in range	۲	۴	۱	۱	۳
W	the most	۶۳/۵۸	۷۸/۸۶	۱	۱	۵

After setting the parameters, the software identified the optimal points. The **best optimal conditions for the whiteness of extracted gelatin powder**, with a **desirability of 0.892** (close to one), are as follows: **temperature of 50 °C, enzyme concentration of 0.01% w/w, and pH 2**, resulting in a predicted whiteness of 77.217. In our study, under these same conditions, the actual whiteness was close to this predicted value (75.31). This shows a good fit between the predicted data and the actual experimental results, indicating a logical correlation between the experimental data and the chosen model.

Gelatin's color generally depends on the extraction conditions and the raw materials. While color is an aesthetic property and depends on its application, it doesn't affect the functional properties of gelatin [41].

The maximum values for **L* (lightness)**, **a* (redness)**, **b* (yellowness)**, and **W (whiteness)** for enzymatically extracted gelatin were 79.81 (Treatment 13), 0.42 (Treatment 7), 12.72 (Treatment 14), and 79.81 (Treatment 13), respectively. For chemically extracted gelatin, these values were 69.47, -5.46, 14.82, and 65.3, respectively. These results indicate that the **lightness (L*)**, **redness (a*)**, and **whiteness (W)** of enzymatically extracted gelatin were higher than those of chemically extracted gelatin, while the **yellowness (b*)** of enzymatically prepared gelatin was lower than that of chemically prepared gelatin. This difference is statistically significant ($p < 0.05$).

Similarly, a study by [42] comparing enzymatically extracted gelatin from fish shield skin with chemically extracted bovine gelatin found that the enzymatically extracted gelatin had higher lightness (L^*) than the chemically prepared gelatin.

4. Conclusion

The findings of this research indicate that **yellowfin tuna skin** can serve as an inexpensive and accessible source for **gelatin extraction** using an appropriate **enzymatic method**. Our results show that the extracted gelatin exhibited a high **gel strength** of 2466.36 g when measured after 16-18 hours of setting at 4 °C. This high strength was achieved under optimal conditions of 30 °C, 0.1% w/w enzyme concentration, and pH 4, suggesting that controlled enzymatic hydrolysis preserved the alpha chains.

Furthermore, the enzymatically produced gelatin yielded an acceptable **yield** of 21.65%, which is more than double that obtained by the chemical method. The highest yield was achieved at 50 °C, 0.01% w/w enzyme concentration, and pH 2. In addition, the enzymatically extracted gelatin demonstrated superior color characteristics (L^* , a^* , W) compared to gelatin prepared using the chemical method.

5-References

- [1]. BAILEY, A. J., PAUL, R. G., & KNOTT, L. 1998. Mechanisms of maturation and ageing of collagen. *Mechanisms of ageing and development*, 108, 439-445.

- [2]. RAHMAN, M. S., AL-SAIDI, G. S., & GUIZANI, N. 2008. Thermal characterisation of gelatin extracted from yellowfin tuna skin and commercial mammalian gelatin. *Food Chemistry*, 108, 472-481.
- [3]. YANG, H., WANG, Y., JIANG, M., OH, J. H., HERRING, J., & ZHOU, P. 2007. 2-Step Optimization of the extraction and subsequent physical properties of channel catfish (*ictalurus punctatus*) skin gelatin. *Food Science*, 72.
- [4]. GILSENAN, P. M., AND ROSS-MURPHY, S.B 2000b. Rheological characterization of gelatins from mammalian and marine sources. *J.Food Hydrocolloids*, 12, 191- 195.
- [5]. KOŁODZIEJSKA, I., KACZOROWSKI, K., PIOTROWSKA, B., & SADOWSKA, M. 2004. Modification of the properties of gelatin from skins of Baltic cod (*Gadus morhua*) with transglutaminase. *Food Chemistry*, 86, 203-209.
- [6]. A., A. Q. M. 2011. Application of fish gelatin as one of the most important sources of halal gelatin in the world. . first national food security seminar. [in Persian]
- [7]. ASHER, D. M. 1999. The transmissible spongiform encephalopathy agents: concerns and responses of United States regulatory agencies in maintaining the safety of biologics. *Developments in biological standardization*, 100, 103-118.
- [8]. WILESMITH, J. W., RYAN, J. B., & ATKINSON, M. J. 1991. Bovine spongiform encephalopathy: epidemiological studies on the origin. *The veterinary record*, 128, 199-203.
- [9]. KARIM, A. A., & BHAT, R 2009. Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. *Food hydrocolloids*, 23, 563-576.
- [10]. TONG, Y., & YING, T. 2013. Gelling strength improvement and characterization of a gelatin from scales of bighead carp (*Aristichthys nobilis*). *Journal of Food, Agriculture & Environment*, 11, 146-150.
- [11]. ZHAO, L., BUDGE, S. M., GHALY, A. E., BROOKS, M. S., & DAVE, D. 2011. Extraction, purification and characterization of fish pepsin: a critical review. *J Food Process Technol*, 2, 6-2.
- [12]. NALINANON, S., BENJAKUL, S., VISESSANGUAN, W., & KISHIMURA, H. 2007. Use of pepsin for collagen extraction from the skin of bigeye snapper (*Priacanthus tayenus*). *Food Chemistry*, 104, 593-601.
- [13]. ZHANG, Y., LIU, W., LI, G., SHI, B., MIAO, Y., & WU, X. 2007. Isolation and partial characterization of pepsin-soluble collagen from the skin of grass carp (*Ctenopharyngodon idella*). . *Food chemistry*, 103, 912-906.
- [14]. Jongjareonrak, A., Benjakul, S., Visessanguan, W., Nagai, T., & Tanaka, M. (2005). Isolation and characterisation of acid and pepsin-solubilised collagens from the skin of Brownstripe red snapper (*Lutjanus vitta*). *Food chemistry*, 93(3), 475-484.
- [15]. Nalinanon, S., Benjakul, S., Visessanguan, W., & Kishimura, H. (2008). Improvement of gelatin extraction from bigeye snapper skin using pepsin-aided process in combination with protease inhibitor. *Food hydrocolloids*, 22(4), 615-622.
- [16]. JONGJAREONRAK, A., BENJAKUL, S., VISESSANGUAN, W., NAGAI, T., & TANAKA, M. 2005. Isolation and characterisation of acid and pepsin-solubilised collagens from the skin of Brownstripe red snapper (*Lutjanus vitta*). *Food Chemistry*, 93, 475-484.
- [17]. SOGAWA, K., FUJII-KURIYAMA, Y., MIZUKAMI, Y., ICHIHARA, Y., & TAKAHASHI, K. 1983. Primary structure of human pepsinogen gene. *Biological Chemistry*, 258, 5306-5311.
- [18]. KAGEYAMA, T., & TAKAHASHI, K 1976. Pepsinogens and pepsins from gastric mucosa of japanese monkey Purification and characterization. *The Journal of Biochemistry*, 79, 455-468.
- [19]. NIELSEN, P. K., & FOLTMANN, B. 1995. Purification and characterization of porcine pepsinogen B and pepsin B. *Archives of biochemistry and biophysics*, 322, 417-422.
- [20]. MARTIN P. TRIEU-CUOT P., C. J., RIBADEAU DUMAS B 1982. Purification and characterization of bovine gastricsin. *European Journal of Biochemistry*, 122, 31-39.
- [21]. SUZUKI, M., NARITA, Y., ODA, S. I., MORIYAMA, A., TAKENAKA, O., & KAGEYAMA, T. 1999. Purification and characterization of goat pepsinogens and pepsins. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 122, 453-460.
- [22]. MUTO, N., & TANI, S. 1979. Purification and characterization of rat pepsinogens whose contents increase with developmental progress. *The Journal of Biochemistry*, 85, 1143-1149.
- [23]. KAGEYAMA, T., TANABE, K., & KOIWAI, O. 1990. Structure and development of rabbit pepsinogens. Stage-specific zymogens, nucleotide sequences of cDNAs, molecular evolution, and gene expression during development. *Journal of Biological Chemistry*, 265, 17031-17038.
- [24]. FENG, W., ZHAO, T., ZHOU, Y., LI, F., ZOU, Y., BAI, S., ... & WU, X. 2013. Optimization of enzyme-assisted extraction and characterization of collagen from Chinese sturgeon (*Acipenser sturio* Linnaeus) skin. *Pharmacognosy magazine*, 9, S32.
- [25]. MOHTAR, N. F., PERERA, C. O., QUEK, S. Y., & HEMAR, Y. 2013. Optimization of gelatine gel preparation from New Zealand hoki (*Macruronus novaezelandiae*) skins and the effect of transglutaminase enzyme on the gel properties. *Food hydrocolloids*, 31, 204-209.

- [26]. El Mohtar, C. S., Bobet, A., Santagata, M. C., Drnevich, V. P., & Johnston, C. T. (2013). Liquefaction mitigation using bentonite suspensions. *Journal of geotechnical and geoenvironmental engineering*, 139(8), 1369-1380.
- [27]. Alfaro, E. C., Umaña-Taylor, A. J., Gonzales-Backen, M. A., Bámaca, M. Y., & Zeiders, K. H. (2009). Latino adolescents' academic success: The role of discrimination, academic motivation, and gender. *Journal of adolescence*, 32(4), 941-962.
- [28]. CHANARAT, S., & BENJAKUL, S. 2012. Comparative study on protein cross-linking and gel enhancing effect of microbial transglutaminase on surimi from different fish. *Journal of the Science of Food and Agriculture*, 92, 844-852.
- [29]. GILSENAN, P. M., & ROSS-MURPHY, S. B. 2000a. Rheological characterisation of gelatins from mammalian and marine sources. *Food Hydrocolloids*, 14, 191-195.
- [30]. CHO, S. M., KWAK, K.S., PARK, D.C., GU, Y.S., JI, C. I., JANG, D. H., LEE, Y. B., AND KIM, S. B. 2004. Processing optimization and functional properties of gelatin from shark (*Isurus oxyrinchus*) cartilage. *J.Food Hydrocolloids*, 18, 573-579.
- [31]. HAIYING LIU., D. L., AND SHIDONG GUO, 2008. Rheological properties of channel catfish (*Ictalurus punctatus*) gelatin from fish skins preserved by different methods. *J. Science Direct*, 41, 1425-1430.
- [32]. CHEOW, C. S., NORIZAH, M.S., KYAW, Z.Y., AND HOWELL, N.K. 2007. Preparation and characterisation of gelatins from the skins of sin croaker (*johnius dussumieri*) and shortfin scad (*Decapterus macrosoma*). *J.Food chemistry*, 101, 386-391.
- [33]. JOHNSTON-BANKS, F. A. 1990. *Food gels*, London, Elsevier Applied Science Publ.
- [34]. D., H. 1996. Gelatin production. *US patent*, 5, 484,888.
- [35]. SIRIPORN DAMRONGSAKKUL , K. R. & KITTINAN KOMOLPIS , W. T. 2008. Enzymatic hydrolysis of rawhide using papain and neutrase. *Journal of Industrial and Engineering Chemistry*, 14, 202-206.
- [36]. VEIS, A. 1964. *The macromolecular chemistry of gelatin*, New York, Academic Press.
- [37]. KNOTT, L., & BAILLEY, A. J. 1998. Collagen cross-links in mineralizing tissues: A review of their chemistry, function, and clinical relevance. *Review Article*, 22.
- [38]. JAMILAH, B. H., KG 2002. Properties of gelatins from skins of fish: black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*). *Food Chemistry*, 77, 81-84.
- [39]. KANOKWAN MATMAROH , S. B., THUMMANOON PRODPRAN , ANGEL B. ENCARNACION , & KISHIMURA, H. 2011. Characteristics of acid soluble collagen and pepsin soluble collagen from scale of spotted golden goatfish (*Parupeneus heptacanthus*). *Food Chemistry*, 129, 1179-1186.
- [40]. REGENSTEIN, J. M., & ZHOU, P. 2007. *Collagen and gelatin from marine by-product*, Maximising the value of marine by-products.
- [41]. OCKERMAN, H. W., & HANSEN, C. L. 1988. *Glue and gelatin animal by-product processing*, New York, Ellis Horwood.
- [42]. Lassoued, I., Jridi, M., Nasri, R., Dammak, A., Hajji, M., Nasri, M., & Barkia, A. (2014). Characteristics and functional properties of gelatin from thornback ray skin obtained by pepsin-aided process in comparison with commercial halal bovine gelatin. *Food hydrocolloids*, 41, 309-318.
- [30] Chrzęszcz, M, Krzemińska, B, Celiński, R, and Szewczyk, K. 2021. Phenolic Composition and Antioxidant Activity of Plants Belonging to the *Cephalaria* (Caprifoliaceae) Genus. *Plants*, 10(5), 952. <https://doi.org/10.3390/plants10050952>
- [31] Wang, S. Y, and Lin, H.S. 2000. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *Journal of agricultural and food chemistry*, 48(2), 140-146. <https://doi:10.1021/jf9908345>.
- [32] Kavak, C, and Baştürk, A. 2020. Antioxidant activity, volatile compounds and fatty acid compositions of *Cephalaria syriaca* seeds obtained from different regions in Turkey. *Grasas y Aceites*, 71(4), 379. <https://doi.org/10.3989/gya.0913192>
- [33] Caliskan, O, Radusiene, J, Temizel, K. E, Staunis, Z, Cirak, C, Kurt, D., & Odabas, M. S. 2017. The effects of salt and drought stress on phenolic accumulation in greenhouse-grown *Hypericum pruinatum*. *Italian Journal of Agronomy*, 12(3). <https://doi.org/10.4081/ija.2017.918>
- [34] Bettaieb, I, Hamrouni-Sellami, I, Bourgou, S, Limam, F, and Marzouk, B. 2011. Drought effects on polyphenol composition and antioxidant activities in aerial parts of *Salvia officinalis* L. *Acta Physiologiae Plantarum*, 33(4), 1103-1111. <https://doi.org/10.1007/s11738-010-0638-z>
- [35] Rebey, I.B, Zakhama, N, Karoui, I.J, and Marzouk, B. 2012. Polyphenol composition and antioxidant activity of cumin (*Cuminum cyminum* L.) seed extract under drought. *Journal of food science*, 77(6), C734-C739. <https://doi.org/10.1111/j.1750-3841.2012.02731.x>.
- [36] Bartsch, H. and Montesano, R., 1984. Relevance of nitrosamines to human cancer. *Carcinogenesis*, 5, 1381-1393. <https://doi.org/10.1136/oem.57.3.180>
- [37] Zhonggao, C, Felgines, O, Texier, C, Besson, D.J, Liu, J. and Wang, S. 2005. Antioxidant activities of total pigment extract from blackberries.

Food Technology and Biotechnology, 43(1), 97-102. ISSN 1330-9862

[38] Nickavar, B, Alinaghi, A. and Kamalinejad, M. 2008. Evaluation of the antioxidant properties of five *Mentha* species. Iranian Journal of Pharmaceutical Research, 7(3), 203-209. <https://doi.org/10.22037/ijpr.2010.766>

[39] Tsuda, T, Kato, Y. and Osawa, T. 2000. Mechanism for the peroxynitrite scavenging activity by anthocyanins. Federation of European Biochemical Societies Letters, 484, 207-210. [https://doi.org/10.1016/s0014-5793\(00\)02150-5](https://doi.org/10.1016/s0014-5793(00)02150-5).

[40] Pergola, C, Rossi, A, Dugo, P, Cuzzocrea, S. and Sautebin, L. 2006. Inhibition of nitric oxide biosynthesis by anthocyanin fraction of blackberry extract. Nitric Oxide, 15(1), c30-39. <https://doi.org/10.1016/j.niox.2005.10.003>.

[41] Yu, L, Zhao, M, Wang, J.S, Cui, C.h, Yang, B, Jiang, Y. and Zhao, Q. 2007. Antioxidant, immunomodulatory and anti-breast cancer activities of phenolic extract from pine (*Pinus massoniana* Lamb.) bark. Innovative Food Science and Emerging Technologies, 9, 122-125. <https://doi.org/10.1016/j.ifset.2007.06.006>

[42] Marcocci, L, Packer, L, Droy-Lefai, MT, Sekaki, A. and Gardes-Albert, M. 1994. Antioxidant action of Ginkgo biloba extracts EGb 761. Methods of Enzymology, 234, 462-475. [https://doi.org/10.1016/0076-6879\(94\)34117-6](https://doi.org/10.1016/0076-6879(94)34117-6).



استخراج ژلاتین به کمک آبکافت آنزیمی از پوست ماهی هوور (*Thunnus tonggol*)

پریسا فیضی^۱، علیرضا سالارزاده^{۱*}

۱-دکترای فناوری مواد غذایی دانشکده صنایع غذایی دانشگاه علوم کشاورزی و منابع طبیعی گرگان.

*۲-دانشیار گروه شیلات، واحد بندرعباس، دانشگاه آزاد اسلامی، بندرعباس، ایران

اطلاعات مقاله	چکیده
تاریخ های مقاله :	ژلاتین یک ترکیب پروتئینی قابل حل در آب است که توسط هیدرولیز جزئی از کلاژن به دست می آید و کاربردهای بسیاری در صنایع غذایی و علوم تغذیه دارد. بنابراین پژوهش حاضر در رابطه با استخراج ژلاتین از منابع ارزان و در دسترس انجام شد. در این مطالعه تأثیر سه فاکتور دما در سه سطح ۳۰، ۴۰ و ۵۰ درجه سانتی گراد، آنزیم در سه سطح ۰/۰۱، ۰/۰۵۵ و ۰/۱٪ وزنی-وزنی و pH در سه سطح ۲، ۳ و ۴ بر روی استخراج ژلاتین از پوست ماهی هوور بررسی گردید. در این راستا استحکام ژل، بازده و رنگ پودر ژلاتین های استخراج شده اندازه گیری شد. نتایج نشان داد با افزایش pH و درصد آنزیم، استحکام ژل افزایش یافت ($P<0/05$) اما دما اثر معنی داری بر آن نداشت ($P>0/05$). بیشترین استحکام ژل مربوط به تیمار با دمای ۳۰ درجه سانتی گراد و درصد آنزیم ۰/۱ وزنی-وزنی و $pH=4$ مشاهده شد. بررسی رنگ ژلاتین نشان داد که میزان روشنایی (L^*)، قرمزی (a^*) و سفیدی (W) در پودر ژلاتین استخراج شده به روش آنزیمی بیشتر از روش شیمیایی بوده ($P<0/05$) اما میزان شاخص رنگ زرد (b^*) در استخراج به روش شیمیایی نسبت به روش آنزیمی افزایش یافت ($P<0/05$). بیشترین روشنایی در ژلاتین استخراج شده با شرایط دمایی ۴۰ درجه سانتی گراد، درصد آنزیم ۰/۰۵۵ وزنی-وزنی و $pH=3$ مشاهده شد. بر اساس نتایج بیشترین بازده ژلاتین تولید شده در فرآیند آنزیمی با میزان ۲۱٪/۶۵ مربوط به تیمار با دمای ۵۰ درجه سانتی گراد و نسبت آنزیم ۰/۰۱٪ و $pH=2$ بود در حالی که این عدد در فرآیند شیمیایی ۱۰٪ به دست آمد. نتیجه اینکه استفاده از دمای ۳۰ درجه سانتی گراد و درصد آنزیم ۰/۱ وزنی-وزنی و $pH=4$ منجر به بازدهی بیشتر ولی استفاده از دمای ۵۰ درجه سانتی گراد و نسبت آنزیم ۰/۰۱٪ و $pH=2$ منجر به تولید ژلاتین با استحکام ژل بیشتر می شود.
تاریخ دریافت: ۱۴۰۳/۸/۲	
تاریخ پذیرش: ۱۴۰۴/۳/۱۹	
کلمات کلیدی:	
استخراج آنزیمی،	
ژلاتین،	
استحکام ژل،	
رنگ،	
بازده.	
DOI: 10.22034/FSCT.22.166.56.	
* مسئول مکاتبات:	
Reza1375bandar@yahoo.com	