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Comparison of the Physicochemical Properties of Gelatin Extracted from the *Caspian Kutum* Scales by Conventional Water-Bath and Ultrasound-Assisted Extraction

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ABSTRACT

Due to its unique role , phycocyanin pigment extracted from the cycanobacterium *Spirulina platensis* can play an important role in enriching traditional cheese. In this study , the amount of protein , fat , total sugar, aeration, melting speed of fortified ice cream with different concentrations of phycocyanin pigment were determined. In addition , the counting of bacteria was done along with the assessment of antioxidant properties. Also , GC/MS test was performed to identify volatile compounds . The results of tests showed that the amount of fat , total sugar , melting speed , DPPH of fortified ice cream has decreased significantly compared to the control . Also , the amount of protein , aeration , FRAP and ABTS significantly compared to the control . sensory evaluation, the acceptability of the smell decreased with increasing concentration, but there was no significant difference in other parameters compared to the control . In addition no sighns of *Escherichia coli*, *Staphylococcus aureus* and *salmonella* were found during different days . However , the presence of *coliform bacteria* 42 to 56 days in enriched and control ice cream . The presence of mold and yeast on days 28 to 56 was evident only in the control sample . The presence of *phychrophilic bacteria* on days 14 to 56 of fortified ice cream has decreased significantly compared to the control. Also, the results of the vaolatile compounds obtained from GC/MS test in control ice cream and enriched ice cream with 2% phycocyanin pigment show the presence of antioxidant and antimicrobial properties that played an important role in the shelf life and quality of ice cream. It is hoped that the results of this study will be the basis for empowering the food industry in using of pigments obtained from cyanobacteria

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1. Introduction

Gelatin is a high molecular weight polymer that is produced by thermal denaturation and partial hydrolysis of collagen present in the skin, bone and cartilage. This combination has high commercial application in different industries.nowadays , due to high commercial consumption of gelatin and hygienic or religious limitations in relation to consumption of gelatin and bacon , researchers are looking for new sources of gelatin.One of the potential sources of this compound is skin , bone , and scale .[1] Gelatin is commercially obtained from the bones and skins of animals such as cows and pigs, and these two are important commercial sources of gelatin extraction [1]. It has been reported that fish processing leads to large amounts of waste , between 50 and 75 percent of the total weight of the fish , and the scale of the fish scale alone accounts for 3 % of the total weight of the fish[2-4]. The fish scale contains approximately 0.8 - 6 % collagen ; however,the extraction of gelatin from this part of collagen has been studied since this part of fish wastes , in addition to collagen (mainly collagen type i) ,contains different amounts of minerals , especially hydroxyapatite , which cause scaling to be easily extracted from chemical treatment and collagen and consequently gelatin [5-6] . kutum (rutilus frisii kutum) is a member of the cyprinidae family and is the habitat of the caspian sea . Rutilus frisii kutum is one of the fish which is grown easily in iran due to its abundance and price throughout the year. Approximately 4 - 6 % of the weight of the fish is scales that have a high potential for use as a source of gelatin[7]. This fish is traditionally consumed and has no industrial production . For this reason , almost all of its waste contains scale that is discarded before consumption . For this reason , in order to decrease the lesions , the scale of this fish scale was used to produce gelatin . The usual method for extraction of gelatin from animal tissues is extraction with hot water this method(Bain-marie)is low cost but in order to achieve high extraction yield requires a long time and relatively high temperature.Today , new techniques have been introduced for extraction of gelatin , which can reduce the time needed for extraction and increase extraction efficiency while preserving the quality of the product[8].One of these new methods is extraction with the help of ultrasound. The basis of the ultrasonic method is the production of high-frequency sound waves that cause mechanical vibrations in the material and produce acoustic cavitation , which is called cavitation. In the following , these holes are destroyed , and strong stress waves can be created , which can break , large particles and even cell walls and therefore better extraction of intracellular compounds such as collagen and gelatin[9-10]. In general , ultrasound assisted extraction could be done in shorter time and lower temperatures which can reduce the damage caused by the high time and temperature in traditional extraction[8].In comparison with the conventional method which is used only from temperature for extraction and hydrolysis of collagen , the mechanism of cavitation in extraction with ultrasound may affect the collagen molecules and consequently the gelatin and change their structure. in a better expression , the molecules of collagen are made up of three polypeptide chains , the alpha chain , which together form the triple helix. These helices are connected to each other by covalent bonds at the ends of the chains with cross-linking. During the traditional method and during thermal denaturing, the covalent crosslinks are destroyed and this triple helix is opened and free alpha chains are produced, which is gelatin[13-11]. The mentioned amino acids have different functional groups, the most important of which are amide functional groups. Amides are nitrogen compounds that are attached to a carbonyl group[14].Among the most important amide groups in gelatin, we can mention amide I, amide II, amide III, amide A and amide B[15]. These amides have the role of influencing the physical properties of gelatin, including the melting point and gel

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strength. Therefore, it is expected that the type of extraction used in this study will affect the amount of these amides and consequently the final properties of gelatin. For this reason, the main goal of this study was to investigate the physicochemical properties and structure of gelatin extracted by two traditional methods and extraction with the help of ultrasound during 1, 2 and 3 hours. The resulting gelatin treatments were compared with each other to determine the effect of this new method on the structure of gelatin compared to its traditional counterpart.

2-materials and methods

kutum scales, which are almost the only waste before consumption, were obtained from the local markets of Gilan.. The fish used for weighing had an average weight of 850 grams to 1 kg. In order to prevent spoilage, the scales separated from the fish were transported to the laboratory in ice storage tanks at -18 degrees Celsius. Sodium hydroxide and hydrochloric acid used for pre-treatment of gelatin were also obtained from Dr. Majalli Company Iran

2-2- preparation of white fish scale

At first, the scales were thawed overnight in the refrigerator and then soaked with 5% salt water at a ratio of 1:5 for 24 hours at refrigerator temperature to remove interfering proteins. After this time, the scales were completely rinsed and dried in the oven at a temperature of 30 degrees[16].

2-3-Pre-treatment of scales

Pretreatment of the scales was done based on the method of Fahim et al. (2017) with a slight change.In order to remove non-collagenous proteins, 50 grams of washed and dried scales were soaked in 500 ml of cold sodium hydroxide solution (4 C°) with a concentration of 1 normal for 2 hours and gently stirred on a shaker at 100 rpm. (changed with fresh solution every 1 hour). After 2 hours, the scale/NaoH net mixture was completely washed with water until the pH of the solution reached neutral.

After treatment with NaoH, the washed scales were soaked in 500 ml of cold hydrochloric acid solution (4 C°) with a concentration of 1 normal for 2 hours and were gently stirred on a shaker with a speed of 100 rpm (every 1 hour with fresh solution replaced). After 2 hours, the scale/acid mixture is completely washed with water until the pH of the solution reaches neutral[17].

4-2- gelatin extraction from white fish scale

To extract gelatin, two traditional methods and ultrasonic extraction were used[20].In the first method, 50 grams of treated scales were added to 750 ml of distilled water and placed in a Bain-marie at 60 degrees Celsius for 1, 2, and 3 hours.

In the ultrasonic assisted extraction method , 50 g of scale specimens were added to 750 ml distilled water. water and scale containers were placed in the ultrasound (30 h - 1 s - germany) bath (30 hz) and the temperature was adjusted at 60 ° c. In this method, the samples were subjected to ultrasound for 1, 2 and 3 hours (Table 1 shows the different gelatin treatments). After the specified times, the extracted gelatin solution was passed through a filter to remove the non-gelatinous part of the scales. The strained gelatin was placed in a fan oven (Memmert, UF 110, Germany) at 30°C for 24 hours to dry completely. After drying, the extracted gelatin was powdered with the help of a grinder and kept in polyethylene bags until the test[15].

2-5-Approximate analysis

Proximate analysis of gelatin , including moisture content, protein percentage ,and gelatin ash, was measured based on Iranian National Standard No. 3474 [18]

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The moisture percentage of gelatin powder was calculated using an oven and based on equation (1)

$$\text{moisture metrical percent} = \frac{(w_1 - w_2)}{((w_1 - w_2))}$$

where w-1 is the weight of the container and sample before drying (grams) and w_2 is the weight of the container and sample after drying (grams). W is the weight of the empty container (grams.(

Gelatin protein percentagewas measured by Kjeldahl based on gelatin nitrogen percentage ,and its amount was calculated using equation(2):

$$\text{Nitrogen metrical percent} = \frac{\text{Acid used volume} \times \text{Normality Acid} \times 100 \times 14}{\text{sample weight gram} \times 1000}$$

The amount of gelatin ash was also measured using an electric furnace, and the relation (3) was used to calculate it:

$$\text{Ash metrical percent} = \frac{(\boldsymbol{w_1 - w_2})}{((\boldsymbol{w_1 - w_2}))}$$

where w-1: is the weight of the plant and ash of the sample and the weight of the empty plant ,and w-2 is the weight of the tested sample

2-6-Extraction efficiency

To determine the efficiency of gelatin extraction, the weight of extracted gelatin powder and the dry weight of the scales were measured, and the efficiency of gelatin extraction was obtained from the following equation (4)

2-7-infrared spectroscopy of Fourier transform infrared spectroscopy

The Fourier transform infrared spectroscopy was carried out by apparatus (Cary 630 , inc. , inc. , Danbury , ct USA) in the range of 600 to 4000 cm - 1 and resolution 4 cm^{-1}

2-8- gel strength

The gelatin solution with a concentration of 6 / 67 (weight/volume) was prepared and poured into cylindrical containers with a volume of 25 ml .The solution was then kept at 10 degrees Celsius in the refrigerator for 16 to 18 hours ,and its strength was measured using a hictometer (Koopa, TA, Iran) and a cylindrical probe with a diameter of 12.7 mm with a smooth end, with a load. 100 N cell, 1 mm/s head speed was measured. For a probe with this diameter, the gelatin samples in the container must have a minimum diameter of 33 mm and a depth of 22 mm. Gel strength in terms of Bloom was defined as the force required to sink the probe to a depth of 4 mm in the gel (in grams)[15].

2-9-Electrophoresis pattern (SDS-PAGE)

Gelatin samples with a concentration of 5 mg/ml were dissolved in distilled water at a temperature of 60 degrees Celsius for 20 minutes . A buffer containing 20% beta mercaptoethanol was added to it .After denaturation at 95°C for 5 minutes, gelatin samples (20 µg) and marker (30 µl) were injected into the wells with a micro syringe and resolved by SDS-PAGE using 10% gel and 5% gel. Accumulated was analyzed. The electrophoresis was carried out at 50 and 70 mV voltage ,and the power flow was interrupted when the dye reached the end of

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the glass. After the isolation of the supernatant from the bottom for 30 min, the gel was stained with Mord blue dye, which was clearly seen in this phase [19].

2-10-melting point

Gelatin solution with a concentration of 6.67% (weight/volume) was poured into capped test tubes and kept in the refrigerator (7 degrees Celsius) for 16 to 18 hours.Then the tubes were transferred to a cold water bath (4°C) and a drop containing 0.5% methyl red-chloroform was poured on their surface. The water bath was heated at a rate of 0.2°C per minute. As the temperature increases, the gelatin slowly turns into a liquid. The temperature at which the gel melts and allows the color to fall was reported as the melting point of gelatin [15].

2-11-Differential scanning calorimetry (DSC)

Thermal properties of gelatins were performed by differential scanning calorimetry (DSC, 400-Ci, Sanaf, Iran) according to the method of Rezaei and Motamedzadegan (2015) with a slight change. in summary , 15 mm of the sample was placed inside the aluminum pan and the sample was heated from 35 to 400 ° c with heating rate of 10 ° per minute and under nitrogen atmosphere . An empty aluminum pan was used as a reference [21].

3- statistical analysis

All statistical analyses were performed using infoSTAT software. The resulting data were evaluated in a 2*3 factorial experiment in the form of a completely randomized design to determine the difference between the treatments. The comparison of duncan 's mean between treatments was considered at 95 % level . All treatments were done in three replications

4- Results and discussion

4-1-Proximate analysis and gelatin extraction efficiency

The results of approximate analysis as well as the yield of gelatin are shown in table 1 . The results showed that there was a significant difference between different treatments in terms of moisture content , protein and ash content ($p < 0.05$).According to the results of the statistical analysis of the samples, both the simple effect of the type of treatment, the simple effect of time, and the interaction of these two factors had a significant impact on the yield factor ($P<0.05$). The yield of gelatin obtained by ultrasound was higher than the gelatin obtained by the Bain-marie method at all extraction times. Also, by increasing the reaction time from 1 to 3 hours, the yield of gelatin increased significantly, so that the maximum amount of gelatin after 1 and 3 hours of reaction in the ultrasonic method was 22.94 and 25.51%, respectively, and in the traditional method after 1 and 3 The reaction time was 18.5 and 22.52%, respectively (Table 1)Increased efficiency as a result of increased reaction time has been reported by many researchers [15, 22. the long reaction time (along with the applied temperature) can provide the required energy to remove triple helix bonds and thus,the amorphous and easily amorphous gelatin molecules can be removed from the connective tissue matrix . Also , the increase in the yield of gelatin in the ultrasonic method can be attributed to the effect mechanism of this method , i.e., mechanical effect and cavitation. In a better expression , these effects of ultrasound led to the disintegration of the fortified tissue and helped to infiltrate more water into these areas , thereby further mass transfer and energy transfer, increasing the extraction efficiency of gelatin. These results were similar to those reported by Tu et al. (2015) and Mirzapour-Kouhdasht et al. (2019) [15, 23].

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The results of the statistical analysis showed that the simple effect of the type of treatment on the humidity factor had a significant difference (P<0.05) .In contrast ,the effect of time was not significant. Also, the results showed that, in general, the moisture content of ultrasonic samples was higher than that of Bain-marie samples, and the highest moisture content was 93.1% and the lowest was 4.7%.The results of the statistical analysis of ash also showed that the type of treatment had a significant effect on the amount of ash . At the same time,the simple effect of extraction time and also the interaction impact of treatment*extraction time had no significant effect on the samples (Table 1).in the case of better expression , the ash content of all ultrasound samples was higher than the other samples.while in Ben-Marie samples, the value of this parameter increased significantly with increasing reaction time from 1 to 3 hours, so that the lowest amount of ash(0.97) was observed in the sample that was extracted for one hour in a Bain-marie bath (P<0.05)in relation to the amount of protein , the results of statistical analysis were similar to yield factor and the simple effect of time , treatment and interaction of time * treatment on the protein content of the samples were significant (p < 0.05) on the other hand , the amount of protein in both ultrasound and Bain-marie treatments increased with increasing reaction time in a way that the amount of protein after 1 h reaction was increased . In contrast, with increasing the time to 2 and 3 hours , the amount of protein in the sample increased. However, in general, the protein of the samples treated with ultrasound was higher. These results were similar to those reported by Tu et al. (2015) . These researchers stated that the amount of protein as well as ash of samples extracted with ultrasonic bath is higher than gelatin extracted by traditional method [15].On the other hand , the maximum allowable moisture and ash content in gelatin according to the national standard of iran are 15 % and 2 % ,respectively. And the results of all the treatments presented in the paper are below the allowed value [18].

Table: proximate composition of gelatin extracted by conventional and ultrasound

Extraction conditions		Parameters				
Type	Time (h)	Moisture (%)	Protein (%)	Ash (%)	Yield (%)	Melting point (°C)
Conventional	1	4.77 ± 1.27^{b}	68.03 ± 0.22^{d}	0.97 ± 0.02^{c}	18.5 ± 1.34^{c}	28.0 ± 0.0^{a}
Conventional	2	5.05 ± 1.2^{b}	70.87 ± 0.87^{bc}	0.99 ± 0.006^{b}	19.88 ± 0.14^{c}	27.33 ± 0.58^{abc}
Conventional	3	4.7 ± 1.25^{b}	71.09 ± 0.21^{bc}	1 ± 0.006^{b}	22.52 ± 0.11^{b}	27.67 ± 0.58^{ab}
Ultrasound	1	8.93 ± 1.67^{a}	69.56 ± 2.18^{cd}	1.49 ± 0.0^{a}	22.94 ± 1.72^{b}	26 ± 0.0^{d}
Ultrasound	2	10.93 ± 1.57^{a}	71.87 ± 0.12^{a}	1.48 ± 0.006^{a}	25.79 ± 0.05^{a}	26.33 ± 0.58^{cd}
Ultrasound	3	10.6 ± 1.1^{a}	85.0 ± 1^{a}	1.5 ± 0.0^{a}	25.51 ± 0.27^{a}	26.67 ± 1.15^{bcd}

Different lowercase letter represents statistical differences among treatments (P<0.05)

infrared spectroscopy fourier transform infrared spectroscopy 4-2-

Figure 1 shows the FTIR spectra of gelatin samples extracted by ultrasonic bath and bain-marie. The indicator peaks in all spectra

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are similar to each other and the most important ones that represent the functional groups of gelatin are as follows:

The sharp peak appearing around 1625 corresponds to amide I and indicates the hydrogen bond in the stretching vibration of the C=O hydrogen bond coupled with COO, which at the same time contains CN stretching, CNN deformation and NH in-plane bending mode. Is also [15]. In Figure 1, it is clear that the intensity of this peak increased with the increase of the reaction time in both the Bain-marie treatment and the ultrasound treatment.On the other hand, the gelatin obtained from the Bain-marie treatment had the least intense reaction after one hour. It has been reported that the peak related to amide I can give information about the secondary structure of gelatin.On the other hand , reducing the intensity of this peak can be attributed to an irregular increase in the structure of the gelatin and consequently the loss of the triple helix structure. In this regard , Muyonga et al. (2004) reported that with increasing the extraction temperature of gelatin , the intensity of amide increased which was similar to the results of this study[24].

In this study, the extraction temperature did not change, but on the other hand , the higher extraction time meant that the gelatin was kept at this temperature for a longer time.As a result, it is possible that its triple structure was more destroyed. The amide II peak was observed in the range of 1521, which indicates a combination of asymmetric CH stretching and asymmetric deformation within the NH plane of the peptide group. Also, the amide III peak appeared at 1234. Amide III has a complex vibrational state, which is caused by combined peaks and CN stretching vibrations and NH in-plane bending vibrations related to amide bonds, as well as significant absorption due to CH2 group mobility vibrations related to the glycine skeleton and proline side chains[15].Like amide I, the intensity of the peaks associated to amide II and amide III also increased in both Ben-Marie and ultrasound treatments with increasing reaction time, so that this peak almost disappeared after one hour of reaction in Bain-marie treatment. As mentioned, the increase in the intensity of these peaks as a result of the longer duration of contact with high temperature leads to the destruction of the triple helix .On the other hand, the amount of molecular order in gelatins extracted at a higher time is higher[24].Two other gelatin indicator peaks are related to amide A and amide b, which were observed at 3275 and 3079,. Generally, the NH group shows an absorption peak around 3400 ; however, when the NH group establishes a hydrogen bond, it shifts to lower wavelengths, around 3200-3300 [15].

In this study, the peak of amide A in Bain-marie treatment after 1 hour of response was observed around 3258, while increasing the reaction to 3 hours in Bain-marie treatment, it shifted to 3282 and in ultrasound treatment to 3275 (higher wavelengths). It shows that with increasing reaction time, the number of free NH groups of gelatin has increased. The amide B peak, which is one of the gelatin indicator peaks, indicates the interaction of the NH3 group between the peptide chains. And the process of its increase was similar to the groove of gelatin index peaks, which means that with the increase in reaction time, the intensity of this peak also increased [15]. The results of FTIR spectra confirm the effect of ultrasound and reaction time on the change of gelatin structure.

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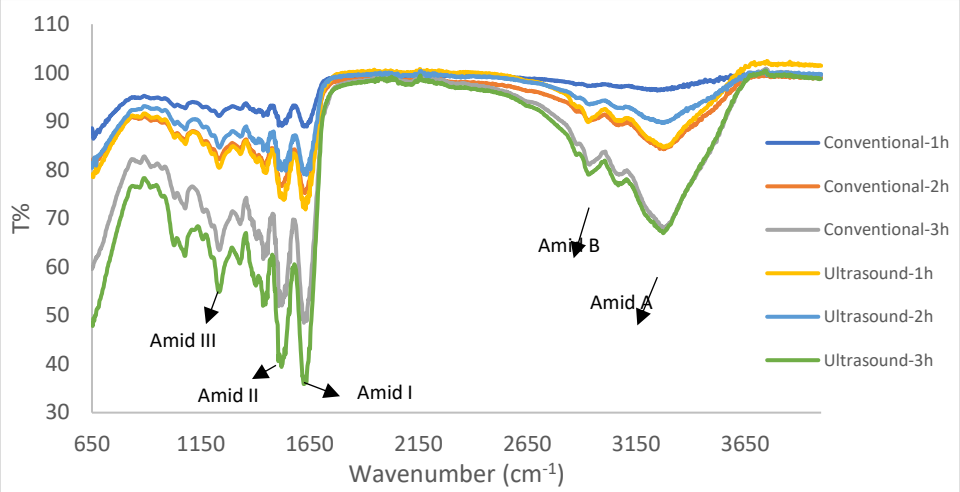

Figure1. FTIR spectra of gelatin extracted by conventional and ultrasound

4-2- The strength of the gel

The strength of the gel is the most important physical property that is important in the case of gelatin, and its value is in the grading criteria of commercial gelatins, which is usually between 100 and 300 degrees Bloom. The reason for the different bloom values of gelatins can be considered the difference in the initial origin of gelatin, the size of protein chains, and the interactions caused by the combination of different amino acids in the structure of gelatin. Also, this parameter can reflect the protein weight, amino acid composition, and ratio of alpha chains to beta chains [23]. The data of gel strength or bloom are shown in fig. According to the results , there was no significant difference between the type of treatment or extraction time. However, in terms of the numerical value, at the reaction time of 1 hour, the blooming gel of the ultrasound treatment was higher than the Bain-marie treatment. However, this trend was different in the samples after 2 and 3 hours of extraction, and its value in the ultrasound treatments was lower than the Ben-Marie treatment. Marie Bode (Figure 1).The low gel bloom in ultrasonic samples that had an extraction time of 2 and 3 hours can be due to the destruction of gelatin during extraction, which causes long chains to be destroyed and shorter chains to be produced. Which ultimately reduces the ability of the alpha chain to reconstitute the gel during cooling. Generally it can be said that lowmolecular weight peptides producedduring extraction with ultrasound cannot make multiple intracellularbinding sites and cause formation ofcoherent gelatin chains. On the other hand, the samples extracted with Bain-marie probably contain longer alpha and beta chains and probably for the same reason they can form a stronger gel [15-25]. Also, our results showed that using an ultrasonic bath for 1 hour did not affect the weakening of the gel. In this regard, Senarathna et al. (2021) compared the effects of two ultrasonic and Bain-marie methods at a temperature of 60 degrees Celsius for 3 hours . They reported that the strength of the gel extracted from these two methods was not significantly different from each other. However, numerically, gelatin extracted with ultrasound had a lower gel strength, which was similar to the result of our work [26]. In another study conducted by Asih et al. (2019) on gelatin obtained from fish bones using ultrasound, it was also reported that a longer extraction time with ultrasound could lead to the breaking of the amino acid chain because it creates more energy in the environment. and produce shorter chains, which in turn reduces the strength of the gel. These researchers

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obtained the amount of bloom gel between 1.74 and 173.94 g, which was lower than the amount received in our study [27]. This difference can be attributed to the difference in fish species and the origin of gelatin (which was bone in the study of these researchers and scales in the present study.

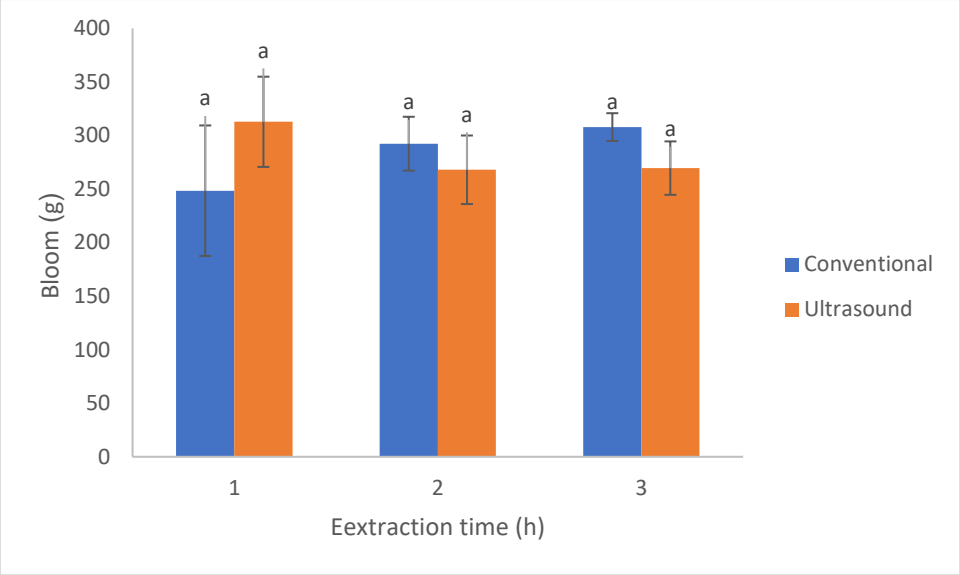

Figure2. Bloom of gelatin extracted by conventional and ultrasound (Different lowercase letters represent statistical differences among treatments)

4-3- Electrophoresis pattern

Using SDS-PAGE, it is possible to determine the distribution of molecular weight as well as the amounts of α chains and β chains. It has also been reported that the amount of these chains can affect gelatin properties , such as gel strength and melting point [15]. Figure 3 shows the electrophoresis images of gelatin samples. β, α1, and α2 chains are seen at 250 and 120 to 125, respectively. In general, according to the figure, it can be seen that the intensity of the band related to theβ chain is lower than the sum of α1 and α2 chains in all samples. On the other hand, several researcheshave reported that high amounts ofalpha chains in gelatin make the gelstrength and also its melting pointhigher[15-23]. The results of the analysis of the bandsappearing in the image using imageJsoftware showed that the total amountof alpha chains in the treatment withBain-marie was similar to each other,and the ultrasound treatment after 1hour of extraction also had the sameamount of alpha chains as the treatments with Ben-Marie. On the contrary, with the increase of extraction time to 2 and 3 hours, the amount of these chains decreased. These electrophoresis results confirm the results obtained from bloom gel .And as mentioned earlier, the bloom gelin ultrasound samples after 2 and 3hours of extraction was lower than in Bain-marie samples, which can be due to the decrease in the amount of chains.Alpha is in gelatin. These results are in agreement with what Asih et al. (2019) reported. These researchers stated that at longer extraction times with ultrasound (which was 7 hours in their study), the intensity of the band corresponding to alpha chains decreased, which was consistent with what we observed [27]. Also, in the survey conducted by Tu et al. (2015), it was reported that gelatin extracted by ultrasonic bath after 5 hours had a lower alpha band intensity than gelatin extracted by ultrasonic bath after 3 hours, which was consistent with our findings.,..Also, the same researchers

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reported that the amount of alpha chains in the treatments extracted with Bain-marie was higher than the samples extracted with ultrasound, which confirms the results of our work [15].

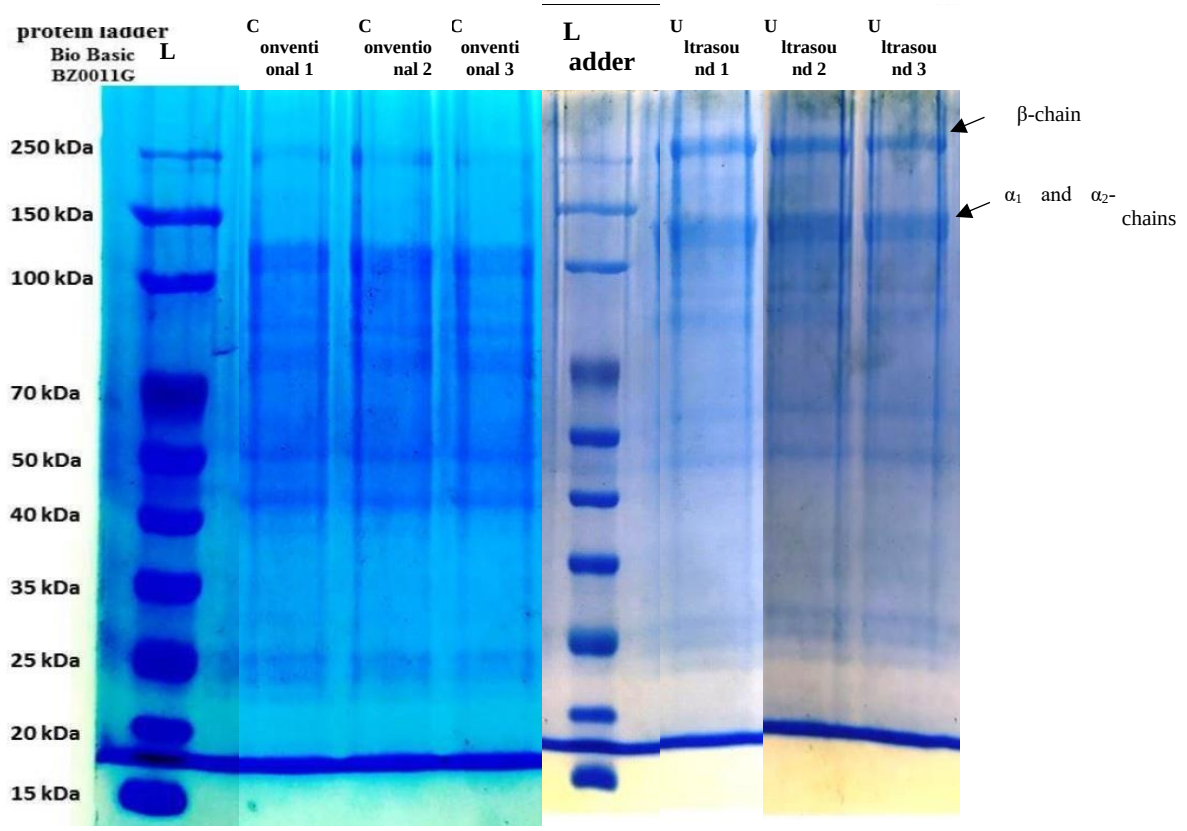

Figure3. SDS-PAGE image of gelatin extracted by conventional and ultrasound

4-4- Melting point

The melting point of gelatin samples are given in Table 1, and the statistical analysis of this factor showed that the simple effect of treatment type had a significant effect on the melting point of the samples . In contrast,the simple effect of extraction time and the interaction effect of treatment type*extraction time had a considerable impact. Did not pay attention. Melting point is one of the main functional properties of gelatin, and it has been reported that gelatins obtained from aquatic organisms have a lower melting point than gelatin extracted from mammals [26.[Usually, the factors affecting the melting point of gelatin are the complex interactions caused by the amino acid composition and the ratio of α/β chains, as well as the molecular weight of gelatin [15].However, it is said that various external factors may also affect the melting point of gelatin, such as heating speed, gel concentration, and heat transfer speed between the heating environment and the gel [26]. The results showed that there is a significant difference between all treatments ($P<0.05$). According to the data, the melting point of all Ben-Marie treatments was significantly higher that of ultrasound.The lower melting point of the ultrasonic samples compared to the Bain-marie samples can be seen as a result of the greater power of the ultrasound to destroy parts and long chains with high molecular weight and reduce their size, which ultimately weakens the gelled network and its melting point. Reduce [8-15].These results were consistent with what

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we obtained in the bloom gel section for gelatin. In other words, the melting point was directly related to the gel strength, and the samples with higher gel strength also had a higher melting point [28]. On the other hand, the results ofelectrophoresis also confirmed thedata obtained from the melting point. These results were similar to those reported by Tu et al. (2015).These researchers noted that although the melting point value of sonicated gelatin was slightly lower than that of Bain Marie gelatin, there was no significant difference between them, which was due to the short time used in their work [15].

4-5- Differential Scanning Calorimetry (DSC)

Figure 4 shows DSC thermograms of gelatin samples. All graphs show a broad endothermic peak between 35 and 200 °C, which is related to the evaporation of bound and free water in the gelatin structure. Considering the hydrophilic nature of this polymer, the presence of such a large endothermic peak can be justified. In this regard , Apostolov et al. (1999)reported that the extendedendothermic peak was observed from 25 to 175 oC . They attributed to theevaporation of crystalline and non - crystalline water of gelatin and noted that the more the watercontent of the gelatin , the higher thepeak.. Also, these researchers observed in some gelatin samples that had a higher water content that their primary endothermic peak has two peaks. In this study, the double-peak endothermic peak was not observed, but some thermograms had a break at the beginning of the peak, which may be related to the melting of small and incomplete pieces of gelatin crystals. Also, the glass transition temperature was not observed in the thermograms of this research because the glasstransition temperature of gelatin was covered by the endothermic peak of water evaporation. These results were similar to what Apostolov et al. observed regarding gelatin thermograms. Also, by examining the TGA graphs of gelatins, these researchers stated that, in general, crystallized water between 25 and 100 degrees and non-crystallized gelatin from 25 to about 300 degrees is gradually removed from the structure, which was similar to the results of our work.,.. In Figure 4, there is also an endotherm peak in all the samples that started at a temperature of approximately 300 degrees and continued until 400 degrees, which can be attributed to the thermal decomposition of gelatin. In this regard, Emamverdian et al.(2020) by examining the TGA graphs ofgelatin reported that the major weightloss of gelatin occurs at a temperatureof about 400 degrees, which was consistent with the results obtainedfrom our DSC graphs. . These researchers reported that the weight loss of gelatin at this temperature occurred as a result of water evaporation and also the thermal decomposition of protein.[30] Also, investigating the thermal stability of gelatin powder by Dang et al. (2017), it was found that gelatin powder loses about 80% of its weight during heating from 230 to 400 degrees Celsius. Considering that the process of weight loss is the result of the destruction and conversion of organic molecules to carbon, these researchers concluded that the majority of gelatin undergoes thermal decomposition at 400 degrees Celsius. These results were similar to those shown in the gelatin thermograms in Figure 4.[31]

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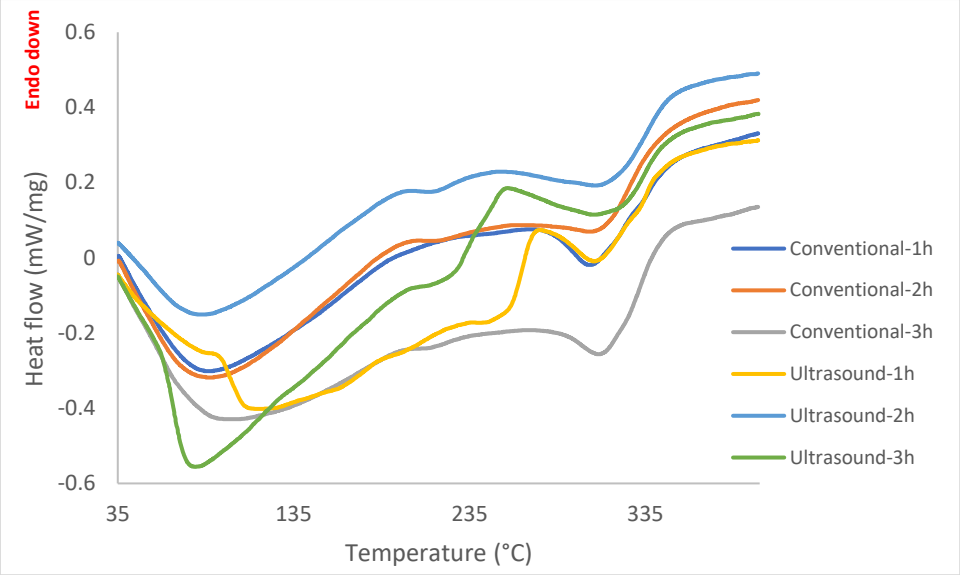

Figure4. DSC thermograms of gelatin extracted by conventional and ultrasound methods

Conclusion

The results of the analysis of extracted gelatins by Bain-marie and ultrasound methods differed from each other in terms of physicochemical properties. Electrophoresis of gelatin samples showed that the amount of alpha chains was higher in gelatins extracted by the Bain-marie method. Also, FTIR spectra showed that the intensity of the peaks related to the indicator amides in gelatin increased as a result of applying ultrasound and improving the extraction time. These changes made the bloom gel and also the melting point of gelatins extracted by Bain-marie method to be higher. In fact, ultrasound weakened the gelatin especially during higher extraction time. On the other hand, the use of ultrasound to extract gelatin increased the extraction efficiency, protein content, and ash of the samples. All samples showed two index peaks, endothermic peak between 35 and 200 related to water evaporation and exothermic peak between 300 and 400 degrees Celsius related to thermal decomposition. The results of this study generally showed that ultrasound can change the protein structure of gelatin and thus affect its physical properties. In general, according to the results of melting point, gel strength, and efficiency, it seems that extraction by ultrasonic method and reaction time of 1 hour in terms of extraction efficiency as well as melting point and gel strength are almost equivalent to gelatin extracted by traditional method after 2 and 3 hours is the reaction time . It can be considered the best example among the treatments.

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مقاله علمی_پژوهشی

مقایسه خصوصیات فیزیکوشیمیایی و حرارتی ژلاتین استخراج شده از فلس ماهی سفید(*Caspian kutum*)به دو روش سنتی و فراصوت

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چکیده

به جهت کاهش ضایعات و افزایش ارزش افزوده فراورده های دریایی، در این مطالعه فلس ماهی سفید به عنوان دور ریز جهت استخراج ژلاتین مورد استفاده قرار گرفت و تاثیر دو روش سنتی و فراصوت بر ویژگی های فیزیکوشیمیایی ژلاتین حاصله مقایسه شد. در این مطالعه ژلاتین از فلس ماهی سفید به دو روش سنتی و فراصوت در دمای ۶۰ درجه سانتیگراد و طی ۱، ۲ و ۳ ساعت استخراج شدند. نتایج نشان داد که به طور کلی فراصوت سبب افزایش معنی دار بازده استخراج، درصد پروتئین و درصد خاکستر نمونه ها شده است ($P<0.05$). نتایج آنالیز الکتروفورز و همینطور FTIR تایید کردند که فراصوت توانست ساختار پروتئین های ژلاتین را تحت تاثیر قرار دهد و در این راستا زمان استخراج طولانی تر (۲ و ۳ ساعت) باعث شد که تعداد زنجیره های آلفا در مقایسه با زنجیره های بتا ژلاتین کاهش یابد. طیف FTIR نیز نشان داد تمامی پیک های مربوط به آمیدهای A, B, I, II, III, درنتیجه تیمار فراصوت و افزایش زمان واکنش شدت بیشتری یافتند. همچنین نقطه ذوب و قدرت ژل ژلاتین استخراج شده با فراصوت به ترتیب برابر با ۲۶.۶۷ °C و ۲۶۹ g بود به صورت معنی داری پایین تر از ژلاتین استخراج شده به روش سنتی (به ترتیب ۲۷.۶۷ °C و ۳۰۷ g) بود ($P<0.05$). نتایج حاصل از آنالیز حرارتی ژلاتین ها نشان داد که تمام نمونه ها یک پیک گرماگیر وسیع بین ۳۵ تا ۲۰۰ درجه سانتیگراد مربوط به تبخیر آب و یک پیک گرماده بین ۳۰۰ تا ۴۰۰ درجه سانتیگراد مربوط به تجزیه حرارتی ژلاتین داشتند.

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