IMPROVING GELATION PROPERTIES OF FISH GELATIN BY NON-THERMAL PROCESSES

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ABSTRACT

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Gelatin is mostly obtained from skin, bone or connective tissues of bovine or porcine land animals. Gelatin is commonly used in confectionery products as the main gelling agent and as a hydrocolloid and stabilizer in liquid food systems. Due to religious preferences, helal gelatin has started to be very common in Middle Eastern countries and bovine gelatin is mainly utilized for that purpose. Gelatin could also be obtained from fish which is very abundant in nature however it has weak gelation ability. There are different studies in the literature that explored the gelation ability of fish gelatin by adding additional substances as $CaCl_2$ or other hydrocolloids.

In this research, it is hypothesized that fish gelatin could be modified by using processing techniques as High Hydrostatic Pressure (HHP) and ultrasonication (US). HHP was applied at 400 MPa, at two different temperatures (10^o C and 30^o C) by keeping process time constant at 15 minutes. Ultrasonication experiments were conducted at 24 kHz at two different amplitudes (100% and 60%) for 5 and 10 minutes. In order to compare the gelation abilities of the fish gelatin, bovine gelatin is used as a commercial gelatin type. The results showed that HHP treatment on fish and bovine gelatin could stabilize gelatin network by organizing the structure and reduce free volume. Furthermore, US treatment could destroy gelatin network, change gelation mechanism and decrease the degree of aggregation. NMR spectroscopy

measurements were useful to monitor change in protein structures and intramolecular forces between protein and water molecules. Gelation properties and hydration of gelatins were identified by using T_1 and T_2 values. When the T_2 results of fish and bovine gelatin were compared, it concluded that the free water of fish gelatin was higher than bovine. Moreover, gelation capability of the bovine gelatin was higher than fish gelatin. In order to estimate the change in the amino acid structure of the gelatin after HHP and US treatments, FTIR measurements were used. FTIR results indicated that the secondary structure of the amino acids was rearranged after treatments.

Keywords: Fish Gelatin, HHP, US, FTIR, NMR Relaxometry, Gelation

ÖZ

ISIL OLMAYAN İŞLEMLERLE BALIK JELATİNİN JELLEŞME ÖZELLİKLERİNİN GELİŞTİRİLMESİ

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Yaygın olarak sığır ya da domuz gibi hayvanların kemik, deri veya bağ dokularından elde edilen jelatin gıda endüstrisinde oldukça sık kullanılan etken maddelerden biridir. Özellikle Orta Doğu ülkelerinde dini ve etik gerekliliklerden ötürü "helal" jelatin kullanımı önemli yere sahiptir. Gıda endüstrisinde jelatin özellikle şekerleme ürünlerinde stabilizatör, jelleşmeye yardımcı etken madde ve hidrokolloid olarak tercih edilmektedir. Balık jelatininin jelleşme özelliği bugüne kadar yapılan araştırmalarda $CaCl_2$ gibi ek maddeler veya diğer hidrokolloidler kullanılarak test edilmiştir.

Bu çalışmada yüksek hidrostatik basınç (YHB) ve ultrasonikasyon (US) metodları kullanılarak balık jelatininin modifikasyonu hedeflenmiştir. Çalışmada kullanılan jelatin konsantrasyonu 6.67% olup her basamakta su tutma kapasitesinin 100% olması esasına dayandırılmıştır. Yüksek basınç methodu için 400 MPa ve 10 ^{o}C ve 30 ^{o}C basınç ve sıcaklık kombinasyonları 15 dakika olan deney süresiyle çalışılmış, ultrasonikasyon deneyleri için 60% ve 100% güç, 24KHz frekansta 5 ve 10 dakika parametreleri uygulanmıştır. Araştırma dahilinde, balık jelatininin jelleşme özelliklerini analiz etmek adına sığır jelatinin karşılaştırma grubu olarak seçilmiştir. Balık jelatininin jelleşme yeteneği, hidrasyonu manyetik rezonans kullanılıp T_1 ve T_2 verileriyle ele alınmıştır. Elde edilen T_2 değerleri kıyaslandığı zaman balık jelatinde

sığır jelatinine oranla serbest suyun daha yüksek olduğu açıkca görülmüş, her iki jelatin türünün jelleşme yetenekleri arasındaki fark gözlemlenmiştir. Balık jelatinin jelleşme yeteneğinin daha düşük olduğu kontrol grupları kıyaslandığı zaman açıkca görülmüştür. Ayrıca ısıl olmayan işlemler olan YHB ve US methodlarının aminoasit zinciri üzerindeki etkisini gözlemleyebilmek adına Fourier-Dönüştürülmüş Kızılötesi (FTIR) Spekstroskopisi analizi yapılmıştır. FTIR spektroskopisine göre ısıl olmayan işlemler sonrası ikincil yapıdaki aminoasitlerin yeniden düzenlenme esnasında farklı yapılar oluşturduğu belirlenmiştir.

Anahtar Kelimeler: Balık Jelatini, YHB, US, FTIR, NMR Relaksometresi, Jelasyon

To my mom…

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HHP: High hydrostatic pressure

US: Ultrasonication

F-Ctrl: Untreated fish gelatin

F-P-400/10: HHP treated fish gelatin at 400 MPa – 10^{o}C for 15 min.

F-P-400/30: HHP treated fish gelatin at 400 MPa – 30^{o}C for 15 min.

F-US-60-5: US treated fish gelatin at 60% amplitude for 5 min.

F-US-60-10: US treated fish gelatin at 60% amplitude for 10 min.

F-US-100-5: US treated fish gelatin at 100% amplitude for 5 min.

F-US-100-10: US treated fish gelatin at 100% amplitude for 10 min.

B-Ctrl: Untreated bovine gelatin

B-P-400/10: HHP treated bovine gelatin at 400 MPa – 10^{o}C for 15 min.

B-P-400/30: HHP treated bovine gelatin at 400 MPa – 30^{o}C for 15 min.

B-US-60-5: US treated bovine gelatin at 60% amplitude for 5 min.

B-US-60-10: US treated bovine gelatin at 60% amplitude for 10 min.

B-US-100-5: US treated bovine gelatin at 100% amplitude for 5 min.

B-US-100-10: US treated bovine gelatin at 100% amplitude for 10 min.

WHC: Water holding capacity

W_I = initial weight of the sample

W_F = Final weight of the sample

τ = Turbidity

I_o = Initial radiation intensity

I = Final radiation intensity

L = The path of the light (1 mm)

T_G: Gelling Temperature

T_M: Melting Temperature

G': Elastic modulus

G": Viscous modulus

θ: Phase angle

CHAPTER 1

INTRODUCTION

1.1. Gelatin

Gelatin is the most commonly used biopolymer in pharmaceutical, cosmetic, and food applications. It is used as a thickener, stabilizer, emulsifier, and gelling in the food industry (Cai et al., 2017). Gelatin is derived from the fibrous collagen which is the main polymer found in the skin, bone, and connective tissues (Karim & Bhat, 2009). Collagen is a natural macromolecule and extracted from animal skin, bone and white connective tissues. Gelatin is obtained by heating and partial hydrolysis of collagen and obtaining soluble proteins after denaturing (Das, Suguna, Prasad, Vijaylakshmi, & Renuka, 2017). Additionally, the hydrolysis of the gelatin from collagen is made by acid or alkali treatments to breakdown crosslinks and convert collagen to water-soluble particle (Arfat et al., 2017). The general gelatin extraction method is also summarized in Fig.1.1. As shown in figure gelatin production process base on main steps as pretreatment of raw materials, extraction of gelatin and clarification and drying. Moreover, depending on how collagen is pretreated, two different types of gelatin is obtained. They are named as type A and type B gelatin. Each type has different characteristics and the main difference between these two are their isoelectric points. Type A gelatin has isoelectric point at pH 6-9 and type has nearly pH 5 (Pearson & Dutson, 1985). While type A is produced from acid-pretreated collagen, type B is produced from alkali-pretreated (Karim & Bhat, 2009). Different type of gelatin is used in the different food application area. In addition, type A gelatin with gel strength as 70-90 g which is relatively low, is used to fine wine and juice. Type B gelatin with gel strength as 125-250 g is used in confectionery products (Mariod & Adam, 2013).

Figure 1.1. Scheme for gelatin extraction

The gelation procedure can be explained by the transition of disordered to the ordered structure as a random coil to the triple helix. This transition is initiated mainly by hydrogen bonding and partially hydrophobic and ionic interactions (Benjakul et al., 2012). In that regard, gelatin network formation is stabilized by crosslinking due to hydrogen bonding and ionic interactions. Gelation of the gelatin solution depends on several factors like extraction method, temperature, pH, type of the raw material for extraction and addition of materials during extraction. The quality parameters of the gelatin are its textural and rheological properties as gel strength (bloom value), melting and gelling temperatures (Kaewruang et al., 2014). These properties are not only influenced by the initial collagen source but also the extraction method of the gelatin (Karim & Bhat, 2009). The other important property of the gelatin is its amino acid sequence. The amino acid composition and its length result in different characteristics among gelatins (Muyonga et al., 2004). The amino acid composition of the bovine and

porcine gelatin is different than fish gelatin. The bovine and porcine gelatin has higher amounts of proline (PRO), hydroxyproline (HYP) and glycine (GLY) imino acids which are less in fish gelatin (Gilsenan & Ross-Murphy, 2002).

Since glycine, proline, and hydroxyproline has a role in secondary structure formation, gelatin types with a higher amount of them are more stable (Jiang, 2015). The gelatin gelation mechanism depends on the connection of small gelatin molecules by hydrogen, covalent bonding, and van der Waals forces by immobilizing the liquid (Farahnaky et al., 2017). After that, fluid sol is converted to thermo-reversible elastic gelatin (Gilsenan & Ross-Murphy, 2002).

According to the latest report of IMARC as "Gelatin Market: Global Industry Trends, Share, Size, Growth, Opportunity and Forecast 2019-2023", gelatin has increasing share in the market. Gelatin market growth depends on its higher functionality and user-friendly attribute in industries. Thanks to these preferable properties of gelatin, Report also tells that compound annual growth rate (CAGR) of the gelatin has increased 8.1% from 2011 to 2018 and it is expected that it will grow 6% more till 2024.

Commercial gelatin is mostly extracted from porcine skin or bovine bone because collagen is found more in their bone, skin, and connective tissues and their preferable quality parameters. The major quality parameters are their higher gel strength, suitable melting and gelling temperatures for the food industry to use them as an additive. Due to the fact that porcine and bovine gelatins are less preferred due to religious preferences, safety concerns and economic considerations, using fish skin or bone materials to obtain gelatin has become popular in recent years (Sow & Yang, 2015; Yang & Wang, 2009). Consequently, fish gelatin has started to use as an alternative

to bovine and porcine gelatin in the food industry (Kaewruang et al., 2014). However, non-modified fish gelatin is not suitable for mammalian gelatin replacements. The common problems about fish gelatin can be summarized as its lower quality parameters, which have been mentioned previously on mammalian gelatins.

1.1.1. Fish Gelatin

Marketing studies have shown that more than 3 billion people consume halal and around 10 million preferred kosher products (Nurrachmi, 2017). The poultry gelatin production has a lower yield than fish gelatin and this difference boomed the fish gelatin production (Karim & Bhat, 2009). Fish gelatin is considered as an alternative to porcine and bovine gelatin but there are some differences between fish gelatin and land mammalian gelatin sources. The main consumption of the fish is human consumption and its catching for the non-consumption area is less. In that regard, general fish gelatin production sources are wastes of bone and skin (FAO, 2018).

Fish gelatin drawbacks make it less preferable in the food industry. Recent studies have shown that the main drawback of fish gelatin that is lower stability and lower rheological characteristics can be changed (Derkach et al., 2015; Gómez-Guillén et al., 2005; Karim & Bhat, 2009). Different characteristics of fish gelatin are lower gel strength, gelling temperature (T_G) and melting temperature (T_M) than bovine and porcine gelatin (Kaewruang et al., 2014). Gelatin extraction from bone, skin, and tissues starts with acid or alkali pre-treatment followed by partial modification of the crosslinking (Karim & Bhat, 2009). The rearrangement of the gelatin and its rheological and textural properties depend on the amino acid chain. Fish gelatin has lower proline (PRO) and hydroxyproline (HYP) imino acid content in its sequence which cause lower gelling and melting temperature and lower gel strength than bovine and porcine gelatin (Haug et al., 2004). The average amino acid compositions of fish and mammalian gelatins are given in Fig.1.2.

The drawbacks of the fish gelatin could be improved by modifying extraction methods. Modification of gel characteristics of the fish gelatin is possible by adding new steps to the extraction methods like the addition of polysaccharides (Sow et al., 2017) or the addition of salts (Giménez et al., 2005) during classical extraction method. Giménez et al. (2005) studied the salt-washing pre-treatment on skins of fish and afterward acid treatment before extraction. Another study showed that the addition of salt solutions (NaCl, KCl, $MgCl_2$, and $MgSO_4$) changed the yield of extraction, molecular weight distribution, gel strength and viscoelastic properties of the gelatin. In the study of Sow et al., 2017, they used a mixed fish gelatin system that includes polysaccharide as gellan and salt as $CaCl_2$. After finding optimum mixed system concentrations, fish gelatin, gellan, $CaCl_2$ mixture concludes feasible gelatin mixture that can be replaced with bovine gelatin.

There are many different methods that try to extract the higher quality gelatin from fish skins, bones, and connective tissues and obtain high-quality fish gelatin. Besides the addition of salts or acid-alkali pre-treatments, using novel processing methods have also been used to modify gelatin. These methods are not only used in the extraction but also used after gelatin is extracted (Gómez-Guillén et al., 2005b; Vega-Gálvez et al., 2011).

Table 1.1. *The Approximate Amino Acid Composition of Fish and Mammalian Gelatin (Haug et al., 2004)*

Amino acids	Residues/1000 amino acids	
	Fish gelatin	Mammalian gelatin
Ala	112	114
Arg	49	51
Asp	48	45
Cys	–	–
Glu	72	71
Gly	347	313
His	11	5
Hyl	5	11
Hyp	60	86
Ile	11	11
Leu	21	25
Lys	28	34
Met	13	6
Phe	13	13
Pro	96	135
Ser	63	37
Thr	24	18
Try	–	–
Tyr	9	3
Val	18	22

1.1.2. Characterization of Gelatin

In a general manner, it can be said that characterization of gelatin for using in the food industry is based on their gel strength, viscosity, gelling and melting temperatures. As has been commented before, these properties of gelatin are influenced by many factors like gel production method, raw materials, molecular weight distribution, amino acid composition, gel production time, temperature, pH and salt content. In that regard, characterizing gelatin is the main step before using it for food applications. Properties related with the gelling behavior of gelatin as gel-forming, texturizing, thickening and water holding are mainly depending on the structure, the molecular weight distribution of amino acids (Gomez-Guillen et al., 2011).

FTIR spectra analysis, rheological characterization and texture analysis are mainly used to characterize gelatin gels. When gelatin gels obtained from different raw materials are characterized by FITR, their spectra can be used to examine their protein conformation. FTIR analysis provides qualitative differentiation of different gelatin gels in a simple and rapid way (Gomez-Guillen et al., 2011). The main advantage of FTIR among all other protein characterization is its convenience. It gives wide protein spectra with a small amount of sample. Moreover, rheological characterization has been also used to characterize the physicochemical and functional properties of gelatin. Gelatin quality for food applications largely depends on its rheological properties as gel strength and thermal stability. One of the differences among different gelatin types obtained from different sources is their gelling and melting temperatures. Rheological characterization gives detailed information about its gelation mechanism and thermal stability as gelling and melting temperatures by proving loss modulus and storage modulus data. To standardize gel strength labeling, bloom value is used. Bloom is defined for a certain concentration of gelatin as 6.67% (w/w) and maturation time as 17h and weight in grams (Gomez-Guillen et al., 2011). The bloom value is generally determined by the gel strength test by texture analysis. Amino acid structure of the gelatins as α- and β- chain components are the main determinants of the gel strength. In other words, all characterizing methods as FTIR spectra, rheological determination, and texture analysis are used to identify food quality and where it can be used in food applications in different aspects.

1.2. Novel Processing Techniques

Novel processing is considered as an alternative for the traditional thermal food processing technologies. These methods use new technologies and improve the efficiency of processing among traditional methods. Conventional thermal methods have several drawbacks on product quality and freshness of which novel methods overcome. Thermal methods can destroy thermolabile nutrients, vitamins and other components which contribute to the flavor and taste of the products (Misra et al., 2017). All of this insufficiency of conventional methods makes novel processing techniques highlighted. Current methods known as emerging technologies are high hydrostatic pressure (HHP), ultrasonication (US), pulsed electric field (PEF), radio frequency (RF), cold plasma, etc. which have advantages on food preservation, extraction, homogenization and potential commercial applications (Misra et al., 2017).

1.2.1. High Hydrostatic Pressure

High hydrostatic pressure (HHP) is based on pressure transmission to foods in a liquid tank at a certain time and temperature. The effect of the HHP is a function of pressure, temperature and time. This method is not controlled by the size or shape of the materials. There is no unused or wasted energy or time in HHP processing which are the major advantages different than traditional heating methods. Since there is no overheating of environment and loss of heat in HHP, it reduces air pollution risk of heating methods. In terms of food production, high-pressure treatment has many advantages. It gives a higher chance to inactivate enzymes and microorganisms, denature proteins while all desired flavors, aromas, nutrients, and vitamins are remained (Kulisiewicz et al., 2007). In addition to these effects, pressurization can cause structural modification, aggregation, fragmentation or gelatinization of molecules (Meng et al., 2017). The major food types that arise structural changes are raw and high protein stuffed because of the high potential of proteins to denature by high pressure (Buzrul, 2015).

The pressurization device is isostatic and composed of the high-pressure vessel with its closure, pressure generator, pressure and temperature control monitors. Pressure is transferred with pressure transmission liquid which can be solutions of castor oil, silicone oil, sodium benzoate, ethanol, and glycol or water. Liquid transferring medium enables pressure to transfer sample without any edge effects. Subsequently, when pressure is released sample returns to its original shape due to pressurization from every direction.

HHP processing has two principles that underlie the pressurization effect. The first principle is Le Chatelier's principle which means that decrease in volume can develop any reaction, phase transition, and configuration changes in molecules after pressure is applied. The second principle is about pressure application (Walkenström & Hermansson, 1997). The uniform pressure is applied from every direction suddenly and it is not controlled by the size, shape, and food composition. This principle is known as Pascal or isostatic pressure principle. In conclusion, the determinants of the HHP process are pressure, temperature and time, not size, shape, and composition.

Achieving healthy and safe foods by HHP treatment was firstly come up by Hite (1899) which is the first report that gives milk without any microorganisms. Milk was pressured at 600 MPa and it resulted in microbiologically safe milk by reduction of viable microbes significantly. Later on, the pressurization method was started to use in many food products as jams, fruit juices, meat, oysters, salad dressings and poultry (Chawla et al., 2011). Market evolution of the HHP treated foods was started with jam which was revealed in Japan at the first by Meijiya Food Company (Buzrul, 2015). From the first product to nowadays, food processing by HHP has increased and pressurized products are more conveniently find in markets.

Pressure application above 150 MPa is thought protein denaturation enhancer and concludes stabilization of native conformations (Gómez-Guillén et al., 2005a). The main effect of the pressure can be explained by destroying the effect of pressure on non-covalent interactions in protein molecules which results firstly unfolding and then rearrangement of bonds between or within proteins (Messens et al., 1997).

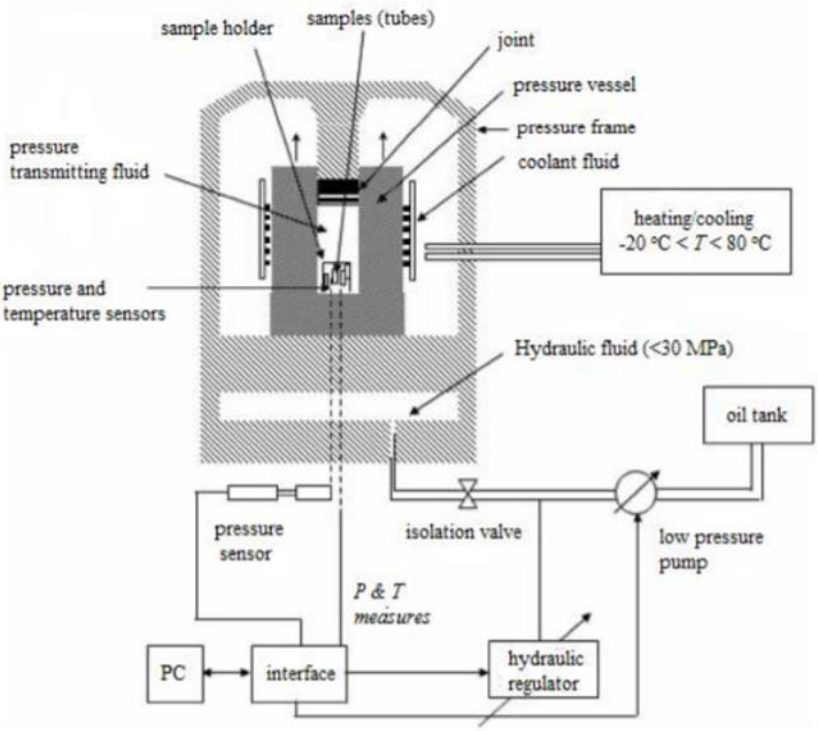

Figure 1.2. Schematic Diagram of High Hydrostatic Pressure Equipment

1.2.1.1. Effect of HHP treatment on gelatin

In recent years, studies about HHP and gelatin relation are based on gelatin extraction at different pressure, temperature, time combinations with some additive materials to sample. All these studies prove that HHP has effect on increasing gelatin extraction yield, quality and some textural and sensorial properties of the gelatin (Gómez-Guillén et al., 2005b; Ma et al., 2013; Moreno et al., 2015; Moreno et al., 2016). High hydrostatic pressure (HHP) can be the method for enhancing gelatin properties of the fish gelatin. Improvement of lower characteristics of the fish gelatin by pressurization is mainly based on enhancing protein-water interactions of the gelatin. Generally, high pressure induced gelatin solution results in greater aggregation, denaturation and gelation characteristics which mean improvement of the gelatin characteristics (Ma et al., 2013).

1.2.2. Ultrasonication (US)

Ultrasonic processing method without loss of any nutrient and sensorial properties as in the case of high hydrostatic pressure technique has increasing demand in recent years (O'Sullivan, Murray, Flynn, & Norton, 2016). This method basically depends on mechanical waves at a certain frequency and resulting in the formation of a cavity. Frequencies are higher than 16 kHz which means that the frequency range is above the human hearing level (Ravikumar, 2018). This technique is classified into two groups as low ($f>100$ kHz at intensities below 1 Wcm^{-2}) and high ($20\ kHz<f<500$ MHz at intensities above 1 Wcm^{-2}) energy levels (Majid, Nayik, & Nanda, 2015). The device with high energy is mainly used as a food processing device because it enables an alternative way to conventional methods. It provides a change in physicochemical properties of the food materials.

The development of US processing method had started in 1790 at which echo sounding was discovered. In recent years, US has become an alternative food processing method as emulsification, homogenization, extraction, and particle size reduction (Ravikumar, 2018). Especially, ultrasonication as the application of ultrasound in low-temperature method is used to prevent loss of nutrients like Vit-C, denaturation of protein and non- enzymatic browning (Zheng & Sun, 2006). Ultrasonication application range quite ranges in food materials. This wide range covers the enhancement of food preservation and alternative ways to traditional methods. The main topics that ultrasonication treatment takes place are the effect on proteins, microbial inactivation, meat tenderization of meat products, removing of fat from dairy wastewaters, extraction and hydrolysis methods, emulsification, etc. (Majid et al., 2015). All these applications are classified into three groups with respect to application mode as a direct application to the product, coupling with the device and submerge in an ultrasonic bath (Majid et al., 2015).

Ultrasonication has both advantages and disadvantages as a novel technology in the food industry. This method is nontoxic, safe and environmental friendly which make it emerging technology (Mcclements, 1995). Rather than its technological benefits, it has many applications based advantages on the food industry. The most known ones are higher extraction yield, enhancing of functional properties as emulsifying and solubility, minimum loss of flavor and aroma, and more homogenous end products (Ali, Kishimura, & Benjakul, 2018; Ravikumar, 2018). Although this method has many advantages in the food industry, the use of this device also has disadvantages. For example, when the ultrasonication process is made over than required, this can cause undesired degradation and results change in physical properties of the materials (Majid et al., 2015).

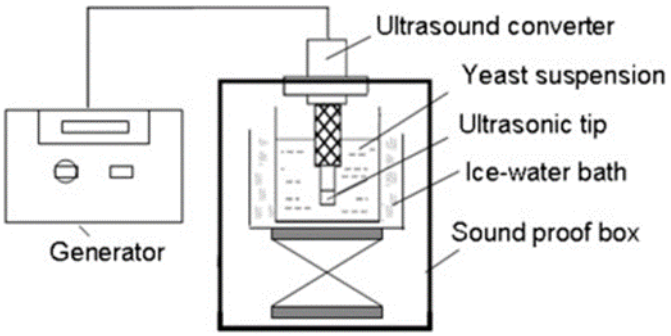

Figure 1.3. Schematic Diagram of Ultrasonication Equipment

1.2.2.1. Effect of US on gelatin

Ultrasonication (US) treatment is a method used to induce a crosslinking degree of proteins and enhance protein-protein interactions as in the case of heat treatment processing (Jiang et al., 2018). The main reason why ultrasonication has become novel technology is improving functional properties of proteins such as emulsifying, solubility and rheological by disintegrating the wall matrix and changing the conformation of the protein structure (Ali et al., 2018). Also, it is beneficial to get higher yield while gelatin extraction from skin, bone and connective tissues. The main reason for higher extraction yield is physical disruptions of wall and matrix of the tissues by resulting cavitation on the material (O'Sullivan et al., 2016). As a result, the ultrasonication process has accepted as an efficient method to increase the chemical and physical properties of the gelatin by affecting protein molecules (Majid et al., 2015).

1.3. Objective of the Study

There are several studies about increasing the gelling ability of fish gelatin by changing pH while extraction, using traditional heat induced processes or through the addition of mineral salt as $CaCl_2$ (Cai et al., 2017; Gómez-Guillén et al., 2005a; Nurul & Sarbon, 2015). These studies confirmed that the addition of salts not only increase the yield of extraction but also induce aggregation degree of proteins and increase gel quality by differentiating molecular weight distribution of the gelatin.

Hence, the major objective of this thesis is to enhance the poor functional properties of the fish gelatin by using HHP and US as an alternative to traditional heat induced treatments or use extra crosslinking agents. Moreover, comparing properties of the fish gelatin with another commonly used type as bovine gelatin is considered as a sub-objective during this study. Finally, the efficiency of HHP and US with different parameters is aimed to be found by statistical analyses. Since it is known that high hydrostatic pressure and ultrasonication mainly influence protein molecules, it is expected that gelation properties of the fish gelatin will improve after treatments.

Several methods were used to characterize fish and bovine gelatins and compare their quality parameters. At first, texture analysis was done to determine gel strength because it is the major component among gelatin quality parameters. FTIR analysis has explained the effect of HHP and US on molecular impact, qualitatively. Additionally, rheological characterization was done to determine how protein conformation and composition effects viscoelastic characteristics of both fish and bovine gelatins.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

Bovine gelatin was provided by Kervan Gıda Sanayi (Istanbul, Turkey). Fish gelatin was obtained from SG chemicals PTE LTD by Elvan Gıda Sanayi (Istanbul, Turkey).

2.2. Gel Preparation

All different types of gelatin solutions were prepared using the same procedure. In the beginning, dry gelatin and distilled water were mixed (6.67 % w/v) at 30^{o}C until they completely dissolved by a magnetic stirrer (Norziah et al., 2009). Since 100% water holding capacity was achieved at 6.67% w/v, gelatin samples were prepared in this concentration. Completely dissolved samples were indicated as a control (F- ctrl and B-ctrl). After preparation, all samples were kept in a refrigerator at 7^{o}C for 16-18h.

2.3. High Hydrostatic Pressure (HHP) Treatment

HHP treatments were performed with a 760.0118 type pressure equipment supplied by SITEC-Sieber Engineering, Zurich, Switzerland. The vessel had a volume of 100 mL with ID 24 mm and length 153 mm. Built-In heating–cooling system (Huber Circulation Thermostat, Offenburg, Germany) was used to maintain and control the required temperature, which is measured by a thermocouple type K in the vessel. The vessel was filled with a pressure-transmitting medium consisting of distilled water. Pressurization rate was 340 MPa/min for 400 MPa. Pressure release times were less than 20 s for each. Pressurization time reported in this study did not include the

pressure increase and release times. Completely dissolved solutions were poured into 25 ml sterile polyethylene cryotubes (LP Italiana SPA) and pressurized at 400 MPa for 15 min at 10^{o}C and 30^{o}C. Samples were coded as F-P-400/30, B-P-400/30, and F-P-400/10, B-P-400/10 respectively. F and B denote fish and bovine gelatins respectively and P denotes HHP processing. All pressurized samples were cooled in the refrigerator at 4^{o}C for 16-18h before analysis. Control group samples were not pressurized.

2.4. Ultrasonication (US) Treatment

Ultrasonication treatment on the gelatin solutions was followed by the device as Heilscher UP400S (Dr. Heilscher GmbH, Germany) with 24 kHz, 400W and 20-100% amplitude. In order to stabilize the temperature of the solutions, the ice bath was used and the temperature of the solutions was kept at approximately 30 ^{o}C until processing was performed. Completely dissolved gelatin solutions were processed under ultrasonication with constant frequency (24 KHz) at different amplitudes (60, 100 %) and different times (5, 10 min). Samples were named as F-US-60-5, B-US-60-5, F-US-60-10, B-US-60-10, F-US-100-5, B-US-100-5, F-US-100-10 and B-US-100-10.

2.5. Water Holding Capacity (WHC)

Water holding capacity of gel gels was determined by using the method of Nurul and Sarbon (2015) with some modifications. The nearly same amount (4 g) of samples were put into centrifuge tubes. Then samples were centrifuged at 2800 x g for 25 min (Nurul & Sarbon, 2015). The initial and final weight of gels were recorded. The difference between the weights of the sample was determined and named as gr of water absorbed per gram of gelatin sample. WHC of samples were calculated according to the following formula:

Water Holding Capacity (WHC) = $(W_I-W_F)/W_I$ (1)

where

W_I = initial weight of the sample

W_F = Final weight of the sample

2.6. Gel Strength

Gel strength of the gelatin gels was determined after 16-18 h maturation time. Brookfield Texture Analyzer CT310K, USA was used for determination of gel strength of gelatin samples. The cylindrical probe with 12.7 mm diameter and 34 mm length was used with 4 mm penetration depth and a speed of 0.5 mm/s into the samples (Sow & Yang, 2015). The gel strength of the samples was determined from the maximum force required, which was recorded as a unit of g, at the penetration depth of 4 mm.

2.7. Turbidity

Turbidity of the gelatin samples was determined by using spectrophotometer (Shimadzu UV-1700, Japan). Turbidity values of the samples were calculated from the equation by absorbance values recorded at the absorbance 600 nm (Sow et al., 2017). The samples were prepared for absorbance measurement and incubated for 1 h at 10^{o}C. The equation of turbidity was used as

$$\tau = -\frac{1}{L} * ln\frac{I}{I_o} \quad (2)$$

τ = Turbidity

I_o = initial radiation intensity

I = final radiation intensity

L = the path of the light (1 mm)

2.8. Fourier Transform Infrared Spectroscopy (FTIR) Analyses

Fourier transform infrared (FTIR) spectroscopy analyses were done by using dry samples. Dry gel samples were obtained after freeze-drying (Zhejiang Value Mechanical & Electrical Products Co. Ltd., Wenling City, China) for 2 days. After freeze-drying, IR Affinity-1 Spectrometer with Attenuated Total Reflectance (ATR) attachment (Shimadzu Corporation, Kyoto, Japan) was used to characterize the structure of the samples in the frequency range of 400-4000 cm^{-1} with 4 cm^{-1} resolution and 32 number of scans.

2.9. Nuclear Magnetic Resonance (NMR) Measurements

Nuclear magnetic resonance measurements as T_2 relaxation time were performed on a 0.32 T NMR system (Spin Track SB4, Mary El, Russia). For relaxation time measurements, samples were placed into the instrument and Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence was used. T_2 CPMG data were determined with 1500 ms echo time, 4000 echos, 600 ms period with 24 scan number. All NMR relaxation measurements were done after 16-18 h maturation time, like other measurements.

2.10. Rheological Characterization

In order to measure rheological properties, Kinexus dynamic rheometer (Malvern, Worcestershire, UK) was used. Testing was performed using the geometry of cone and plate with 40 mm diameter and 4^o conical surface and plate angle. Frequency sweep and temperature sweep experiments were conducted as the oscillatory tests for the characterization of viscoelastic behavior. The cooling and heating range of the

measurement was 10-40 ºC for heating and 40-10 ºC for cooling at a rate of 1 ºC/min (Norziah et al., 2009). The frequency of the temperature sweep test was determined as 1 Hz from the linear viscoelastic region which was found by frequency sweep testing of the sample. Results were recorded as elastic modulus (G'), viscous modulus (G") and phase angle (θ) as a function of temperature.

2.11. Statistical Analysis

Sigma Plot software package (SigmaPlot Ver.12, Chicago, IL, USA) was used to analyze the results. Two-way ANOVA was used to determine which parameters are statistically significant ($p \leq 0.05$) on the physicochemical properties of gelatin. If factors were found significant, Tukey's multiple range test was implemented to evaluate the significant differences among the different levels of the same factors ($\alpha \leq 0.05$).

2.12. Summary of Experimental Design

To summarize the parameters, experiments, and responses measured overall experimental design table is given.

Table 2.1. *Experimental Design Components*

Samples	Processing Techniques	Experiments
Fish Gelatin (6.67% w/v) Bovine Gelatin (6.67% w/v)	High Hydrostatic Pressure (HHP) 400 MPa-10^{o}C-15min 400 MPa-30^{o}C-15min Ultrasonication (US) 60% amplitude-5 min 60% amplitude-10 min 100% amplitude-5 min 100% amplitude-10 min	Texture Analysis Water Holding Capacity Turbidity measurement Fourier Transform Infrared (FTIR) Spectroscopy analysis Nuclear Magnetic Resonance (NMR) Relaxometry Rheometer Measurements

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Gel Strength

Effects of treatments on gel strength were shown in Fig.3.1. Results showed that the highest gel strength result was found for the gels of 400 MPa-30^{o}C for bovine gelatin (1083.3 g) and 400 MPa-10^{o}C for fish gelatin (634.025 g). For HHP treatment, gel strength values were significantly different with respect to pressure and temperature ($p \leq 0.05$). Gel strength values were also significantly different with respect to US time. Gel strength of the gelatin samples for a treatment time of 10 min. was both greater than 5 min for both 60% and 100% amplitudes.

Gel strength of the gelatin is one of the most important parameters used to evaluate the gelling properties of gelatin (Bhat & Karim, 2009). Traditional gelation procedure by using heating depends on the gelatin particle solubilization due to random coiling of gelatin chains after melting of the triple helix structures. Intermolecular forces between water and free hydroxyl groups of amino acid molecules, concentration, and molecular weight distribution are the major factors that affect the gel strength (Arnesen & Gildberg, 2002; Muyonga et al., 2004). Fish gelatin has lower proline and hydroxyproline compared to bovine and porcine gelatin (Haug et al., 2004). Both proline and hydroxyproline contributed to hydrogen bonding of water molecules with gelatin more and this causes lower stability and gel strength values when their occurrence decreases as in the case of fish gelatin (Ali et al., 2018). Figure 3.1 shows that HHP treatment has a promoting effect on the gel strength of the gelatin. Increase in gel strength could be related to enhanced crosslinking (Bhat & Karim, 2009). HHP treatment causes a decrease in the free volume of the samples. Reduction of the free

volume by pressurization results increase in intermolecular interactions and strengthen the hydrogen bonding between water and free hydroxyl groups of amino acid chains (Moreno et al., 2016). Free volume reduction not only increases the hydrogen bonding of the molecules, but it could also result in new physical interactions such as dipole-dipole interactions (Moreno et al., 2016).

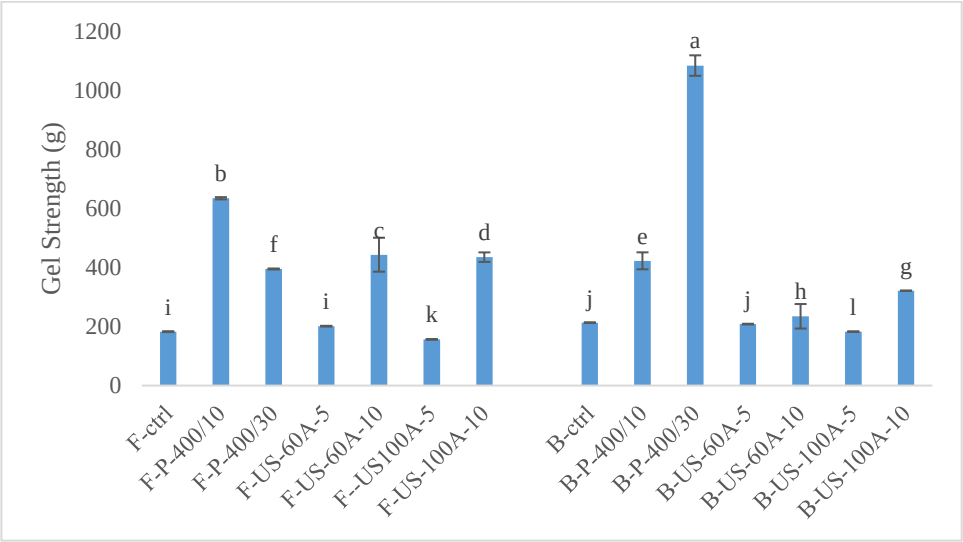

Figure 3.1. Gel Strength Results of Fish and Bovine Gelatin as affected by HHP and US

In the case of US, processing time as 5 min has no significant effect ($p \geq 0.05$) on gel strength rather than 10 minutes ($p \leq 0.05$). For US treatment, as processing time increased at a constant amplitude, higher gel strength was achieved. Effect of increased sonication time could cause higher gel strength with constant amplitude due to increase in water solubility of the exo-polysaccharides and hydrocolloids (Lii et al., 1999; Wang et al., 2010). Gel strength differences depend also on the chain length of the gelatins. When gelatins have longer chain length, alignment and self-aggregation become easier (Ali et al., 2018). Contrary to the HHP treatment, US has destructive effect on the gelatin network and results in shorter chain lengths (Ali et al., 2018).

Since the chain length of the gelatins were destroyed with US, lower gel strength values were obtained rather than the HHP treated samples were recorded.

3.2. Turbidity

Turbidity of the fish gelatin and its changes with respect to treatments in different processing combinations were shown in Table 3.1. As in the case of gel strength, there are relatively high values of turbidity when gelatin was treated. The highest turbidity value after HHP treatment was obtained at 400MPa-10^{o}C (2.12 cm^{-1}). The effect of HHP on turbidity with respect to pressure and temperature combinations were all statistically significant ($p \leq 0.05$). The turbidity of the HHP treated samples were greater. This meant that there was more interaction between amino acid molecules and it resulted in pressure-induced protein aggregation. Since intermolecular interactions were induced after the reduction in the free volume by pressurizing, it increased hydrogen bonding and protein aggregation accordingly (Moreno et al., 2016). For ultrasonication, it was found that US treatment was statistically significant for all amplitude and time combinations ($p \leq 0.05$). Also, statistical results showed that the effect of amplitude level depended on how long treatment was performed which also indicated that there was a statistically significant interaction between amplitude and time ($p \leq 0.05$). The interaction term was also found significant with respect to ANOVA results. Ultrasonication has a significant effect on cavitation of the molecules and collapse of gas bubbles (O'Sullivan et al., 2016). Moreover, it was found that the US also has an effect on unfolding on polymer chains and results in an increase in hydrophilic groups and water interaction (Farahnaky et al., 2017). As a result, US treatment caused higher interaction between water molecules and hydrophilic groups of gelatin molecules and it resulted in higher turbidity values as shown in Table 3.1. However, it was found that US treatment time had a lowering effect on the turbidity of the gelatin. It was decreased for both 60% and 100% amplitude treatments. Increase in sonication time with constant amplitude resulted in smaller protein size for the

gelatin (O'Sullivan et al., 2016). Decreasing of the protein size resulted in disruption of hydrophobic and electrostatic interactions which caused lowering on the degree of aggregation. In brief, ultrasonication treatment not only had the ability to breakdown intermolecular forces between gelatin and water but also had the ability to destroy gelatin network and decrease the degree of aggregation (Farahnaky et al., 2017).

Table 3.1. *Turbidity Results of the Fish Gelatin as Affected by Different Processing Combinations of HHP and US*

Sample	Turbidity
F-ctrl	1.181 ± 0.107^{g}
F-P-400-10	1.560 ± 0.211^{f}
F-P-400-30	2.122 ± 0.062^{e}
F-US-60A-5	7.264 ± 0.284^{a}
F-US-60A-10	3.481 ± 0.049^{d}
F-US-100A-5	5.425 ± 0.158^{b}
F-US-100A-10	3.842 ± 0.086^{c}

3.3. Fourier Transform Infrared Spectroscopy (FTIR)

In order to find the effects of HHP and US on the gelation properties of the fish and bovine gelatins, FTIR spectra were used and results are shown on Fig.3.2-3.3-3.4-3.5 and Table 3.2-3.3-3.4-3.5. Amide A, amide B, amide I, amide II and amide III were the major regions that were represented in figures. All the details extracted from the FTIR spectra (height and area) are shown in tables.

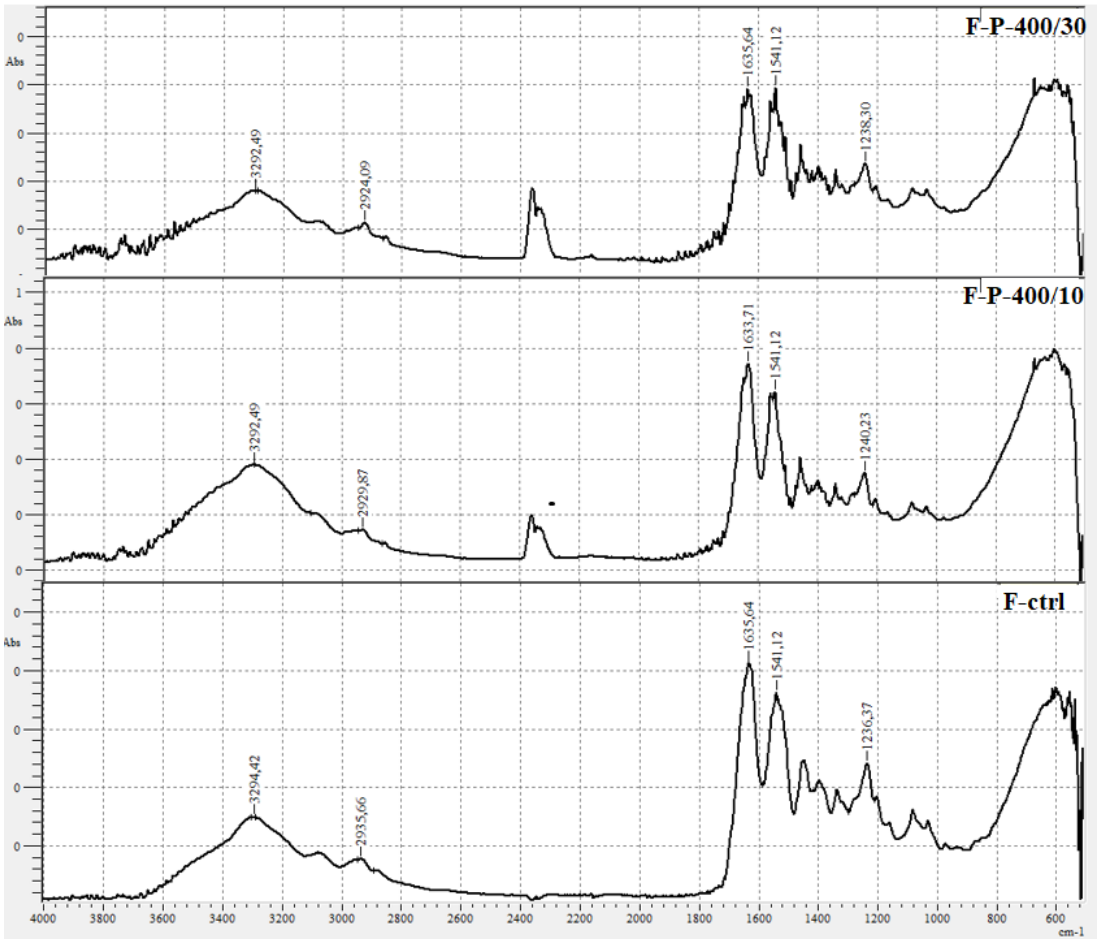

Figure 3.2. FTIR Results of Fish Gelatin as Affected by HHP

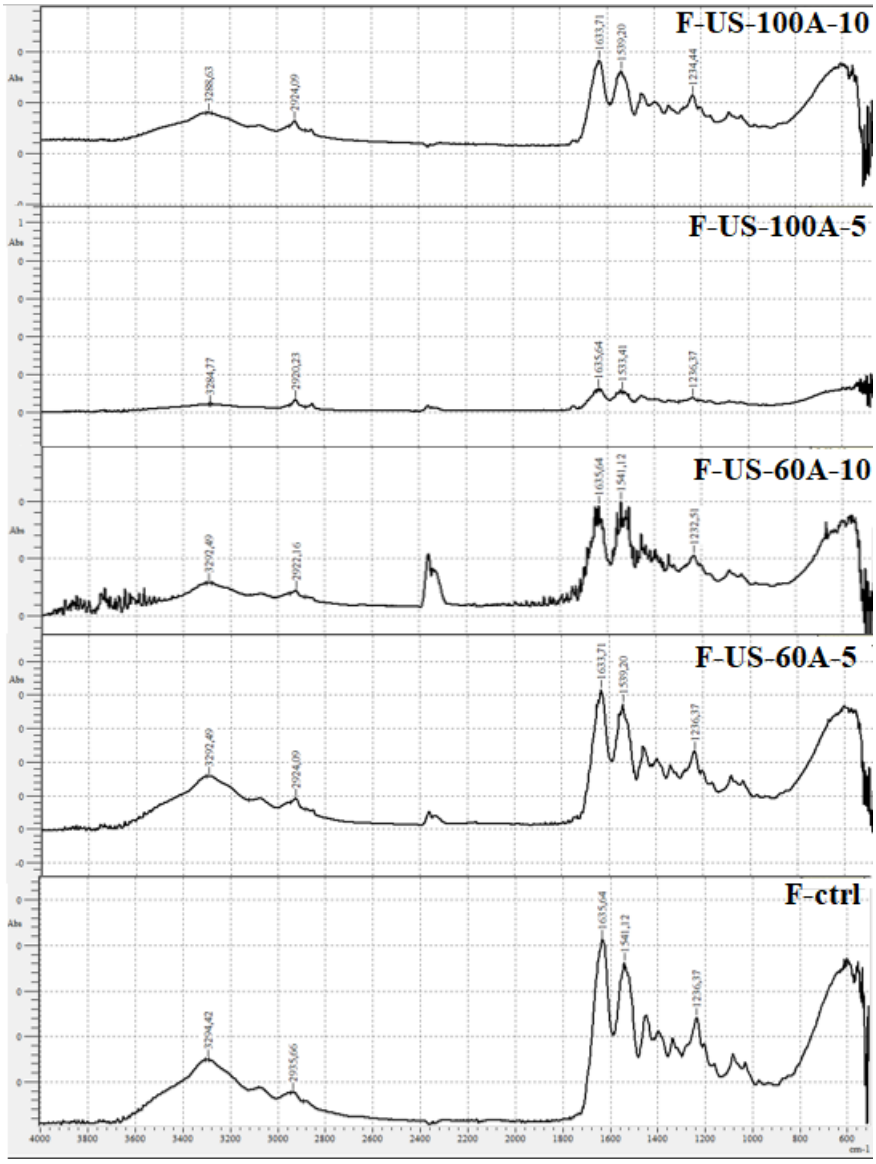

Figure 3.3. FTIR Results of Fish Gelatin as Affected by US

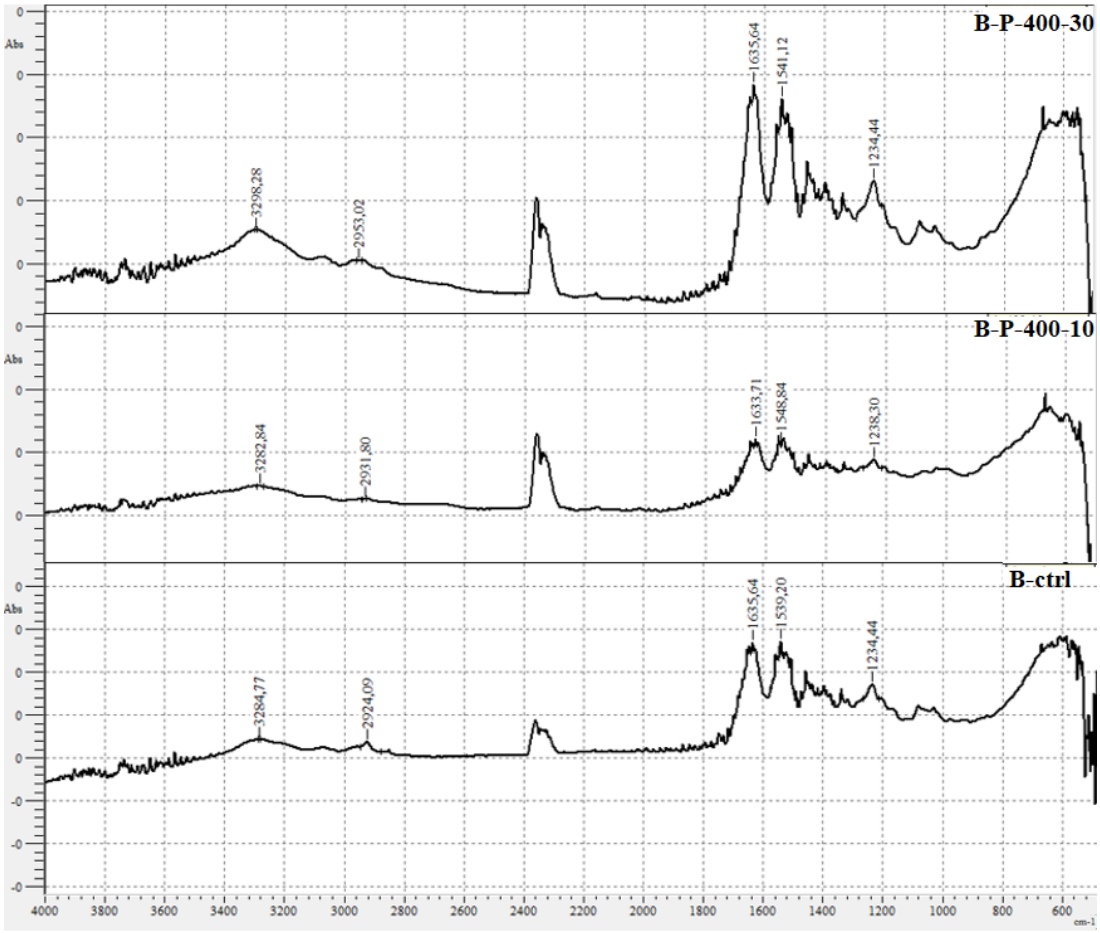

Figure 3.4. FTIR Results of Bovine Gelatin as Affected by HHP

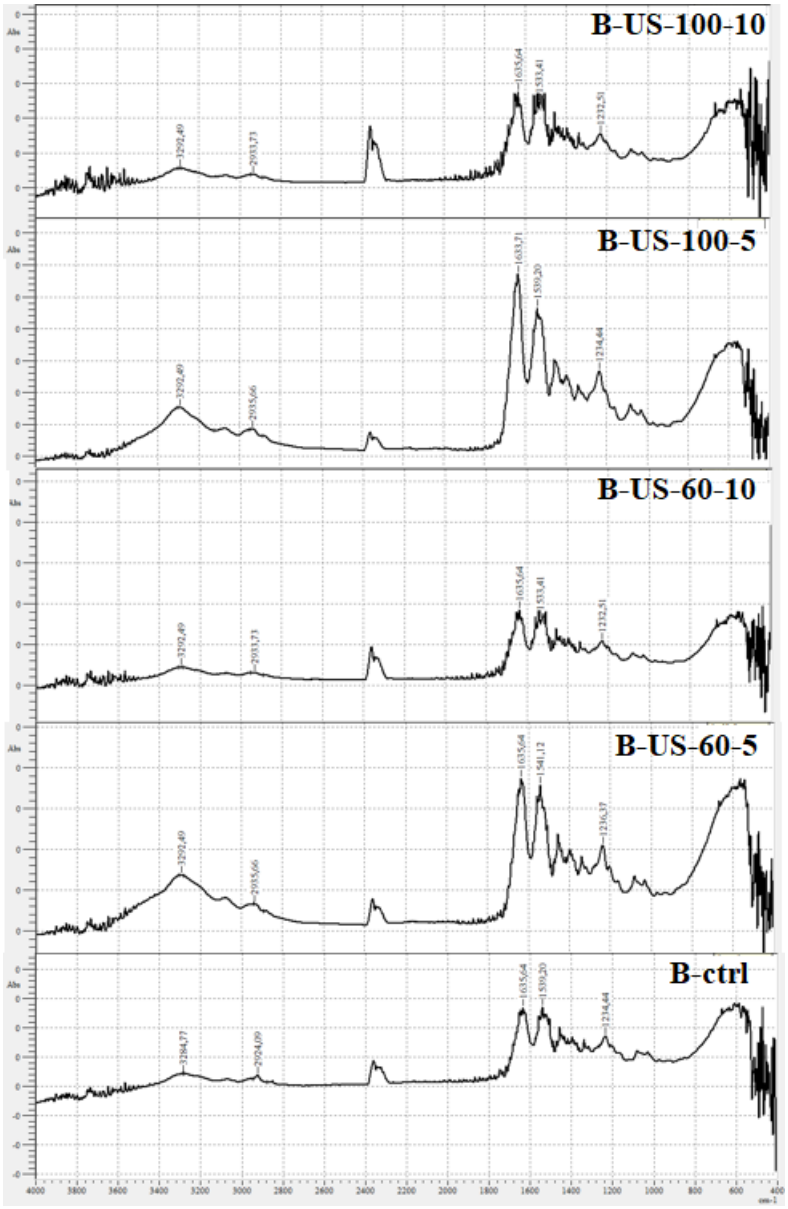

Figure 3.5. FTIR Results of Bovine Gelatin as Affected by US

Table 3.2. *FTIR height values of fish gelatin as affected by different processing combinations of HHP and US*

	Peak				
Sample	Amide A	Amide B	Amide 1	Amide 2	Amide 3
F-ctrl	3294.4±0.7	2935.6±1.9	1635.6±0.4	1541.1±1.1	1236.3±0.9
F-P-400-10	3292.4±1.2	2929.8±1.8	1633.7±1.0	1541.1±1.1	1240.2±1.5
F-P-400-30	3292.4±0.8	2924.0±1.1	1635.6±0.6	1541.1±0.6	1238.3±0.5
F-US-60A-5	3292.4±0.9	2924.0±1.3	1633.7±1.1	1539.2±1.0	1236.3±1.1
F-US-60A-10	3292.4±1.2	2922.1±0.9	1635.6±0.4	1541.1±1.0	1232.5±0.6
F-US-100A-5	3284.7±0.8	2920.2±1.0	1635.6±0.9	1533.4±0.7	1236.3±1.0
F-US-100A-10	3288.6±1.1	2924.0±0.7	1633.7±0.3	1539.2±1.1	1234.4±0.9

Table 3.3. *FTIR peak areas of fish gelatin as affected by different processing combinations of HHP and US*

	Area				
Sample	Amide A	Amide B	Amide 1	Amide 2	Amide 3
F-ctrl	0.573±0.01	0.936±0.10	4.628±1.04	3.74±0.95	4.282±0.04
F-P-400-10	28.599±6.23	4.945±0.57	4.17±1.00	8.961±1.5	8.895±1.12
F-P-400-30	0.697±0.02	4.104±0.55	1.812±0.06	2.131±0.8	6.317±0.88
F-US-60A-5	11.382±4.40	2.454±0.40	2.314±0.09	8.107±1.33	6.179±0.80
F-US-60A-10	0.61±0.01	1.241±0.15	0.989±0.01	1.002±0.09	2.706±0.40
F-US-100A-5	0.163±0.02	1.561±0.09	0.687±0.01	0.413±0.01	2.785±0.08
F-US-100A-10	0.839±0.09	1.751±0.07	4.03±1.10	0.92±0.01	3.088±0.01

Table 3.4. *FTIR height values of bovine gelatin as affected by different processing combinations of HHP and US*

	Peak				
Sample	Amide A	Amide B	Amide 1	Amide 2	Amide 3
B-ctrl	3284.77±2.12	2924.09±3.29	1635.64±0.36	1539.20±2.01	1234.44±0.78
B-P-400-10	3282.84±1.98	2931.80±1.04	1633.71±1.02	1548.84±1.99	1238.30±1.34
B-P-400-30	3298.28±1.12	2953.02±1.77	1635.64±0.76	1541.12±1.35	1234.44±0.66
B-US-60-5	3292.49±1.44	2935.66±2.06	1635.64±0.98	1541.12±1.04	1236.37±0.45
B-US-60-10	3292.49±1.09	2933.73±1.45	1635.64±1.01	1533.41±0.97	1232.51±0.91
B-US-100-5	3292.49±0.97	2935.66±2.76	1633.71±0.43	1539.20±0.78	1234.44±0.37
B-US-100-10	3292.49±0.66	2933.73±2.06	1635.64±0.80	1533.41±1.72	1232.51±1.09

Table 3.5. *FTIR peak areas of bovine gelatin as affected by different processing combinations of HHP and US*

	Area				
Sample	Amide A	Amide B	Amide 1	Amide 2	Amide 3
B-ctrl	0.082±0.01	0.431±0.05	0.74±0.001	0.742±0.01	2.082±0.28
B-P-400-10	0.538±0.03	0.204±0.00	0.663±0.00	0.218±0.00	2.422±0.71
B-P-400-30	0.590±0.01	0.916±0.02	8.211±0.6	2.89±0.07	8.573±1.07
B-US-60-5	1.442±0.04	1.394±0.01	2.081±0.2	1.972±0.05	5.557±1.01
B-US-60-10	0.379±0.00	0.821±0.02	0.986±0.05	0.457±0.00	2.869±0.00
B-US-100-5	10.48±1.04	2.363±0.05	10.717±0.7	4.138±0.94	9.439±1.21
B-US-100-10	0.215±0.01	0.514±0.00	0.725±0.03	0.346±0.00	1.989±0.09

In general, peak shapes and amide region patterns were similar for both fish and bovine gelatins with some minor changes. As shown in Table 3, wave numbers (cm-1) of control fish gelatin samples (non-treated) for amide A and B were at 3294.42 and 2935.66 while they were 3284.77 and 2924.09 for the control bovine gelatin. After HHP treatment, amide A region shifted for 400 MPa-30o C treated bovine gelatin sample from 3284. 77 cm-1 to 3298.28 cm-1 which also had the highest gel strength value. This 15 cm^{-1} shifting in amide A region indicated that intermolecular forces

between bovine gelatin and water increased (Sow et al., 2017). Likewise, in Amide A region, the effect of HHP on FTIR spectra was observed by shifting from 2924.09 cm-1 to 2931.8 cm-1 and 2953.02 cm^{-1} in Amide B region after treatment. Amide B band corresponds to the CH_2 stretch bands and shifting to higher values might be due to the dimeric molecular association of carboxylic groups after pressurization (Sow & Yang, 2015). In the spectrum of the fish gelatin, the peak height of the samples was not different but their intensity values were different. The intensity of the 400MPa-10ºC HHP treated sample had the highest value among the HHP treated samples. This amide A band intensity increase showed that HHP could enhance the hydrogen bonding between gelatin and water molecules. The effect of the temperature was observed from the intensity of the samples when 400 MPa-30◦C and 400 MPa- 10◦C treated fish gelatins were compared.

Furthermore, other common bands such as amide I, II and III were also observed. Peak height and area values from Table 3 and Fig. 3 showed the data from these regions for bovine and fish gelatins. Band between 1900 to 900 cm-1 were assigned to them (Al-Saidi at al., 2012). As shown in Table 3, peaks for amide I, II and III were at 1635.64 cm^{-1}, 1541.12 cm^{-1} and 1236.37 cm^{-1} for control fish gelatin and at 1635.64 cm-1, 1539.2 cm^{-1} and 1234.44 cm-1 for control bovine gelatin. After HHP treatment, the difference was not found in wave numbers. However, when peak and area results were compared, they showed that pressurizing did not affect bands chemically but it increased the intensity of the gelatins at specific wave numbers. Change in amide II peaks was generally considered more sensitive to hydration rather than secondary structure changes (Nurul & Sarbon, 2015). Since amide I band gives information about secondary structure (Sow et al., 2017) and amide III band about triple helical structure (Al-Saidi et al., 2012), the area under the band peaks could be used to estimate the structure of the gelatin and observe the loss of secondary structure and the random coil formation. When the area values were compared, the secondary structure of the gelatins was increased with pressurizing at 10◦C for fish gelatin and 30◦C for bovine

gelatin. This showed that HHP treatment helped to stabilize the gelatin and organize the structure by reduction of the free volume (Moreno et al., 2015). Different than HHP, US had a reducing effect on the intensity of amide I bands. US treatment is known to have a destructive effect on the protein backbone (Jiang et al., 2018) and it could disrupt the secondary structure of the gelatin. Also according to Sow et al., (2017), peaks at 1633 cm^{-1} shows the characteristic coil structure present in the gelatin. When the US treated fish gelatin samples are compared with control and HHP treated ones, it was clearly observed that the intensity of the coil structures decreased.

3.4. Nuclear Magnetic Resonance (NMR) Relaxometry

Nuclear magnetic resonance (NMR) relaxometry gives detail about proton mobility among materials and it is a non-destructive method. Therefore, physicochemical changes in food materials can be observed by using NMR (Kirtil & Oztop, 2016). Analysis of the NMR was based on taking signals to measure longitudinal and transverse relaxations times also known as T_1 and T_2 respectively. While T_1 value gives information about the time that spin realign along the longitudinal z-axis, T_2 is the time constant that characterizes the rate at which Mxy component decays. Since hydroxyl groups and amino group protons in protein gels affect T_2 relaxation time directly (Oztop et al., 2010), NMR measurements were based on T_2 values in this study. Fish and bovine gelatin T_2 results were shown in Table 3.6. Results clearly showed that bovine gelatin had lower T_2 values than fish gelatin. The highest T_2 values for pressurized fish gelatin and bovine gelatin samples were obtained at both 400 MPa-10 ^{o}C-15 minute. There was no significant difference between control and pressurized fish gelatin ($p>0.05$) while pressure had a significant effect on the T_2 value of the bovine gelatin ($p\leq0.05$).

Table 3.6. *T_2 results of fish and bovine gelatin according to different processing parameters*

Sample	T_2 (ms)
F-ctrl	811.7 ± 16.2
F-P-400-10	808 ± 0.6
F-P-400-30	800.85 ± 6.55
F-US-60-5	846.25 ± 1.45
F-US-60-10	801.95 ± 2.75
F-US-100-5	843.15 ± 4.35
F-US-100-10	843.75 ± 0.75
B-Ctrl	713.9 ± 2.5
B-P-400-10	764.83 ± 2.87
B-P-400-30	720.39 ± 0.01
B-US-60-5	738.05 ± 0.55
B-US-60-10	765.6 ± 1.8
B-US-100-5	817 ± 6.1
B-US-100-10	807.1 ± 4

Renou et al. (2003) considered that T_2 values of the samples were assigned to free or expelled water of the samples. In that regard, the longer T_2 values of the fish gelatin than bovine gelatin might be caused by different amino acid composition, length of amino acid chains and their association with water. According to Pranoto et al. (2016), the amount of glycine, glutamic acid, hydroxyproline, alanine, and arginine were

found in bovine gelatin higher than fish gelatin. Likewise, imino acids (hydroxyproline and proline) and glycine were found in bovine gelatin more than fish gelatin according to Cheow et al. (2006). The imino acid content and glycine amount were determinants of the stability and interaction between water and protein molecules of the gelatin (Binsi et al., 2009). Imino acids as hydroxyproline have the gel-forming ability by forming hydrogen bonding from their hydroxyl group. Moreover, FTIR results gave higher intensities on imino acid content for the bovine gelatin than fish gelatin that contributed to recent studies. These different protein structures of the fish and bovine gelatin results in different attributes on hydrogen bonding, free water and on T_2 relaxation times.

Pressure-induced bovine gelatin resulted in longer T_2 values for both 400 MPa – 30 ^{o}C and 400 MPa – 10 ^{o}C. Increase on the relaxation times after pressurization might be due to the ability of pressure to change the conformation of the protein structures, especially on the secondary structure (Moreno et al., 2015). The results of the FTIR spectrum also showed changing the secondary structure after pressurization. The increase of the T_2 values indicated a rearrangement of the water-protein interactions and it resulted in an increase in free water after pressurization.

Besides the effect of pressure on T_2 relaxation times, ultrasonication treatment on gelatin samples was significant ($p \leq 0.05$) when different levels of amplitudes applied at certain time values. Ultrasonication treatment on gelatin samples resulted in the longest T_2 values at 60% amplitude for 5 min. for fish gelatin and 100% amplitude 5 min. for bovine gelatin. The interaction parameter as time resulted significantly important ($p \leq 0.05$) for the ultrasonication treatment. While amplitude was constant and ultrasonication treatment time increased, T_2 time of both fish and bovine gelatin decreased. The shorter relaxation period showed might be due to the breakdown of the intermolecular forces between water and protein molecules and a decrease in the

degree of aggregation (Ma et al., 2013) as in the turbidity measurements. Both changes in intermolecular forces and the degree of aggregation on gelatin samples might result in changes in surface hydrophobicity (Poulsen et al., 2019).

Consequently, NMR relaxometry measurements of gelatin samples showed that T_2 values were good indicators to investigate the change in protein structures and intramolecular forces between protein and water molecules.

3.5. Rhelogical Characterization

The gelling and melting temperatures of the fish and bovine gelatin samples were determined by small-strain oscillatory measurements. While gelling temperatures of the samples were measured in the cooling period, melting temperatures were measured in the heating period at the crossover point. Different HHP and US parameters were studied and Tm and Tg of the samples were shown in Table 3.7. Subsequently, G' and G" values of the fish gelatin samples during heating and cooling as a function of T_M and T_G were shown in Fig.3.6 and Fig.3.7. Both Fig.3's and Table 3.7 showed that G' of the samples were greater than G" values during cooling and heating period. Additionally, control fish gelatin had the lowest G' and G" value during both heating and cooling periods among all processes. The higher G' values than G" during gelation showed that the gelation process was formed by the hydrogen and covalent bonds between water and side chains of amino acid groups (Huang et al., 2018).

Table 3.7. *Rheology results (T_G, T_M) of fish gelatin and bovine gelatin as affected by different processing combinations of HHP and US*

Sample	T_G (ºC)	T_m (ºC)
F-ctrl	22.385 ±0.31	30.685 ±0.1
F-P-400-10	22.89 ±0.405	32.37 ±0.195
F-P-400-30	22.14 ±0.105	31.215 ±0.3
F-US-60-5	20.36 ±0.31	30.75 ±0.1
F-US-60-10	19.09 ±0.305	29.795 ±0.78
F-US-100-5	19.49 ±0.415	30.435 ±0.3
F-US-100-10	18.725 ±0.305	29.855 ±0.585
B-Ctrl	22.995 ±0.04	32.16 ±0.205
B-P-400-10	22.23 ±0.2	30.44 ±0.3
B-P-400-30	22.53 ±0.41	31.84 ±0.59
B-US-60-5	20.41 ±0.51	31.01 ±0.1
B-US-60-10	20.34 ±0.41	30.13 ±0.59
B-US-100-5	20.05 ±0.405	30.895 ±0.2
B-US-100-10	18.745 ±0.4	31.33 ±0.095

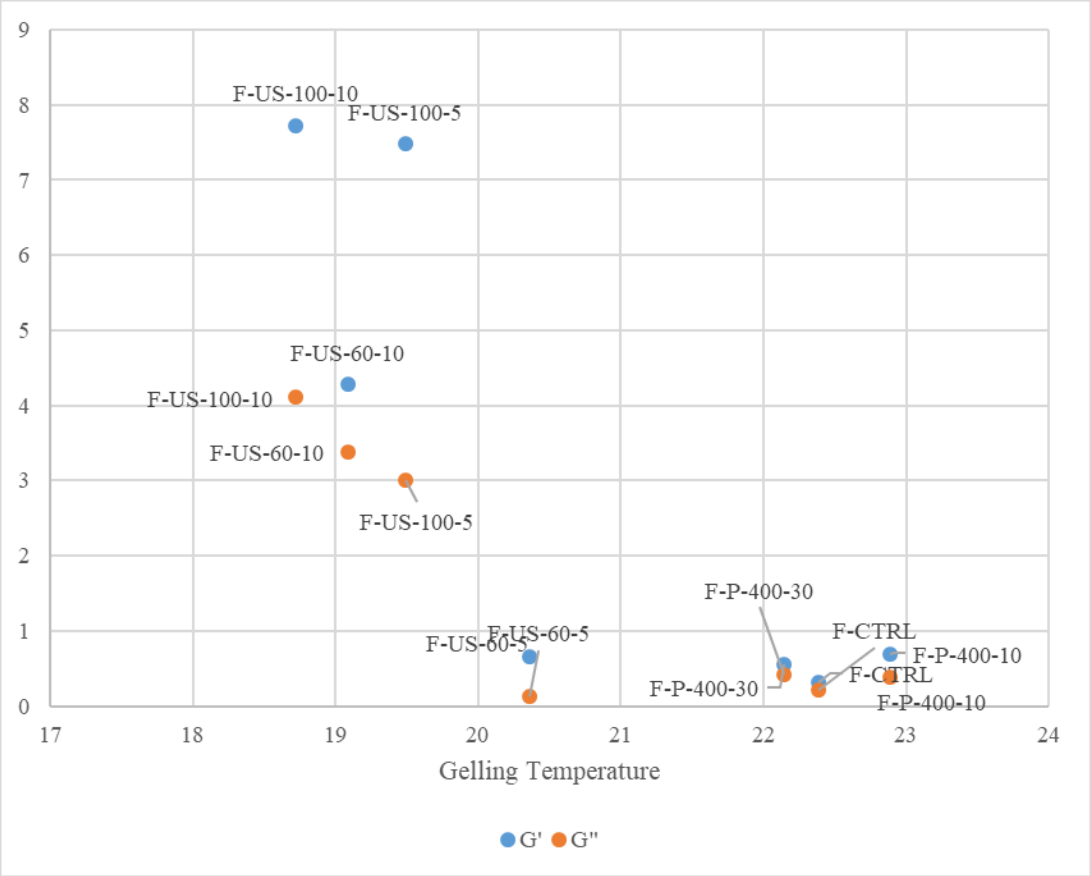

Figure 3.6. Rheology results (G', G'') of fish gelatin as affected by different processing combinations of HHP and US as a function of T_G

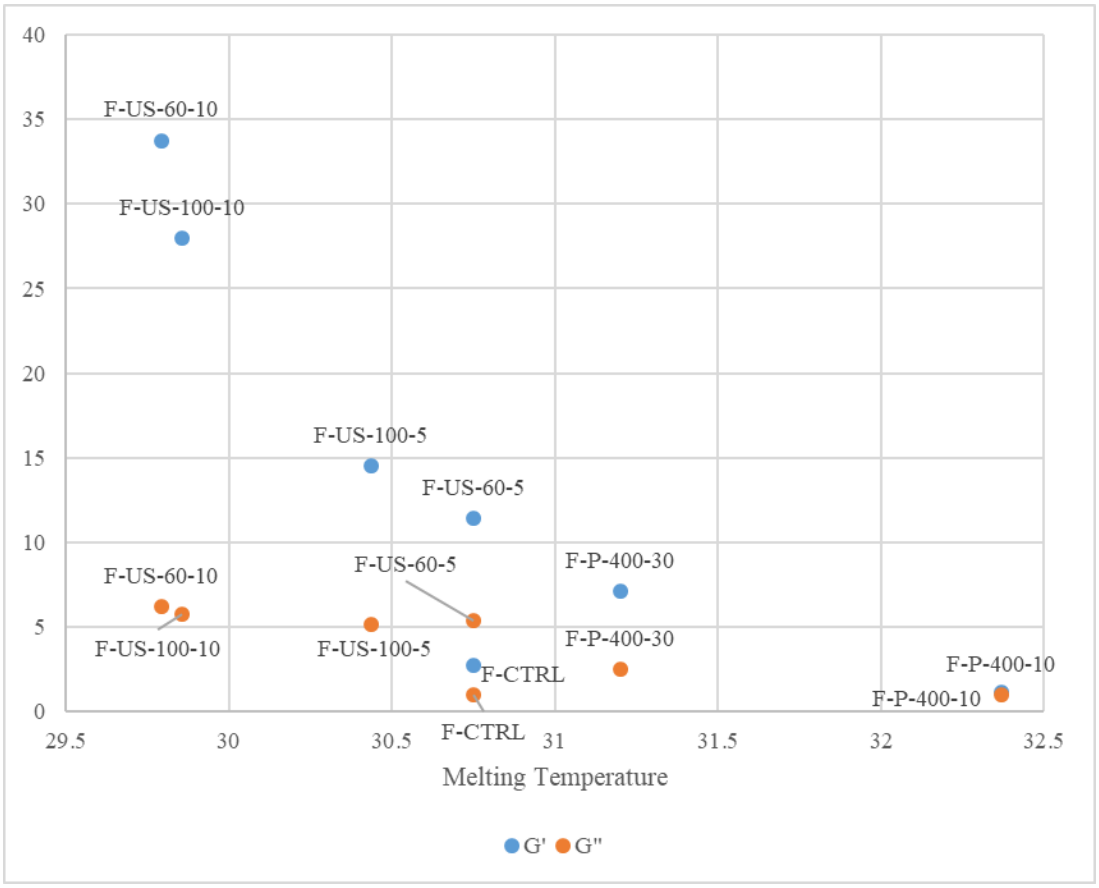

Figure 3.7. Rheology results (G', G'') of fish gelatin as affected by different processing combinations of HHP and US as a function of T_M

Both G' and G" values of the fish gelatin increased after HHP and US treatment. Although both HHP and US treatments on fish gelatin increased G' and G" values, US treatment had a greater effect on the G' and G". This increasing trend after US treatment might be due to a different conformation of the coil structures and breaking of H-bonds (Sow & Yang, 2015) after destroying the gelatin network (Farahnaky et al., 2017). In addition to the US treatment, HHP treatment increased the gelation parameters as G' and G" in both cooling and heating periods of fish gelatin. Increase in both G' and G" values of the fish gelatin might be due to the higher degree of unfolding after pressurization which enabled the gelation process (Meng et al., 2017).

In the case of gelling and melting temperatures of the fish and bovine gelatin, Table 3.7 clearly showed that pressurization increased both T_M and T_G. As in the gel strength results, fish gelatin and bovine gelatin showed a different behavior after pressurization at different temperatures as 10°C and 30°C. While the highest T_M and T_G values for fish gelatin was achieved at 400 MPa - 10°C, bovine gelatin had the highest value of T_M and T_G at 400 MPa – 30°C. In general, an increase in T_M and T_G of the gelatin gels showed the stabilization effect of the pressure treatment (Montero, Fernández-Díaz, & Gómez-Guillén, 2002). Slightly higher T_M and T_G values of both fish and bovine gelatin samples, which were an indicator of higher stability, could be explained by specific hydration structures in the crosslinking junctions (Walkenström & Hermansson, 1997). Additionally, while the gelation mechanism of control samples was based on stabilization by hydrophobic interactions, hydrogen bonding had less effect on the stabilization of the pressurized samples (Montero, et al., 2002). In conclusion, different gelation mechanisms, change in crosslinking junctions and amino acid content differences between fish and bovine gelatin results different effects on G', G", T_M and T_G after treatments.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

There have been many studies about increasing the gelling ability of fish gelatin by traditional heat induced methods and mineral salt ($CaCl_2$) addition. To the best of our knowledge, there has been no report on investigating the gelation of fish gelatin treated by HHP and US. In the scope of this study, HHP and US effects on fish and bovine gelatin samples were investigated. Results showed that both processing methods increased the gel strength of both fish and bovine gelatin so greater gelatin stability and quality were achieved after the treatments as compared to conventional methods. Greater stability and quality characteristics of the fish gelatin makes it more convenient to use in the food industry as in the case of other gelatins like bovine or porcine.

On the other hand, it was investigated that both HHP and US treatments had effects on amino acid structures and conformations of the gelatins which were examined by amide A, B, I, II and III bands of FTIR results. In addition to FTIR results, different imino acid content of fish and bovine gelatin were investigated. Change in amino acid sequence and structure was desired in both HHP and US treatments in order to increase lower characteristics of the fish gelatin.

Amino acid conformation difference resulted in different attributes of free water and also on T2 relaxation times. NMR Relaxometry was also shown as a useful method to detect protein-water interactions and change in protein structures after processing. NMR results firstly give information about different attributes of fish and bovine

gelatin on free water. Additionally, NMR data led enhancement on identifying HHP and US treatment effects on free water and gelatin relation.

Finally, rheology measurements were used to confirm the increase in stability after processing. G' and G" measurement was used to identify gelation properties of the gelatin and change in crosslinking junctions. Pressurized samples tended to stabilized by hydrogen bonding rather than hydrophobic interactions as in the case of control samples.

In brief, HHP and US had an effect on the stability of gelatin and improve gelation properties of the fish gelatin. This study proves the hypothesis at the beginning of the work. As future work, SDS-PAGE analysis can be suggested in order to identify molecular weight distribution the samples before and after treatment. These analyses can provide a better vision about the protein distribution of the gelatin samples. Besides, future work can be focused on the usage area of the fish gelatin and how it can be replaced by widely used types as porcine and bovine gelatin.

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APPENDICES

A. ANOVA Results of General Full Factorial Regressions

General Full Factorial Regressions: Gel Strength of Fish Gelatin for HHP Treatment;Pressure,Temperature

Two Way Analysis of Variance

Data source: Data 1 in fishgelstrength.JNB

General Linear Model (No Interactions)

Dependent Variable: Gel strength

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Pressure	1	22503.811	22503.811	(+inf)	<0.001
Temperature	1	28650.195	28650.195	(+inf)	<0.001
Residual	0	3.877E-026	0.000		
Total	2	102060.946	51030.473		

The difference in the mean values among the different levels of Pressure is greater than would be expected by chance after allowing for effects of differences in Temperature. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature is greater than would be expected by chance after allowing for effects of differences in Pressure.

There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.0500: for Pressure : 1.000

Power of performed test with alpha = 0.0500: for Temperature : 1.000

Least square means for Pressure :

Group Mean

0.1000 352.187

400.000 564.337

Std Err of LS Mean = 0.000

Least square means for Temperature :

Group Mean

30.000 338.575

10.000 577.950

Std Err of LS Mean = 0.000

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Pressure**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
400.000 vs. 0.100	212.150	2	(+inf)	1.000	No

Comparisons for factor: **Temperature**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
10.000 vs. 30.000	239.375	2	(+inf)	1.000	No

General Full Factorial Regressions: Gel Strength of Fish Gelatin for US Treatment;Amplitude,Time

Two Way Analysis of Variance

Data source: Data 1 in fishgelstrength.JNB

General Linear Model (No Interactions)

Dependent Variable: Gel strength

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Amplitude	1	702.250	702.250	2.097	0.385
Time	1	67704.040	67704.040	202.168	0.045
Residual	1	334.890	334.890		
Total	3	68741.180	22913.727		

The difference in the mean values among the different levels of Amplitude is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Time. There is not a statistically significant difference (P = 0.385).

The difference in the mean values among the different levels of Time is greater than would be expected by chance after allowing for effects of differences in Amplitude. There is a statistically significant difference (P = 0.045). To isolate which group(s) differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.0500: for Amplitude : 0.114

Power of performed test with alpha = 0.0500: for Time : 0.735

Least square means for Amplitude :

Group Mean

60.000 371.950

100.000 345.450

Std Err of LS Mean = 12.940

Least square means for Time :

Group Mean

5.000 228.600

10.000 488.800

Std Err of LS Mean = 12.940

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Time**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
10.000 vs. 5.000	260.200	2	20.108	0.045	Yes

General Full Factorial Regressions: Gel Strength of Bovine Gelatin for HHP Treatment;Pressure,Temperature

Two Way Analysis of Variance

Data source: Data 1 in bovinegelstrength.JNB

General Linear Model (No Interactions)

Dependent Variable: Gel strength

Normality Test (Shapiro-Wilk) Passed (P = 1.000)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Pressure	1	378711.045	378711.045	(+inf)	<0.001
Temperature	1	218493.551	218493.551	(+inf)	<0.001
Residual	0	9.370E-026	0.000		
Total	2	412731.585	206365.792		

The difference in the mean values among the different levels of Pressure is greater than would be expected by chance after allowing for effects of differences in Temperature. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature is greater than would be expected by chance after allowing for effects of differences in Pressure. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.0500: for Pressure : 1.000

Power of performed test with alpha = 0.0500: for Temperature : 1.000

Least square means for Pressure :

Group Mean

0.1000 -117.525

400.000 752.775

Std Err of LS Mean = 0.000

Least square means for Temperature :

Group Mean

30.000 648.150

10.000 -12.900

Std Err of LS Mean = 0.000

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Pressure**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
400.000 vs. 0.100	870.300	2	(+inf)	1.000	No

Comparisons for factor: **Temperature**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
30.000 vs. 10.000	**661.050**	**2**	**(+inf)**	**1.000**	**No**

General Full Factorial Regressions: Gel Strength of Bovine Gelatin for US Treatment;Amplitude,Time

Two Way Analysis of Variance

Data source: Data 1 in bovinegelstrength.JNB

General Linear Model (No Interactions)

Dependent Variable: Gel strength

Normality Test (Shapiro-Wilk) Failed ($P < 0.050$)

Equal Variance Test: Passed ($P = 1.000$)

Source of Variation	DF	SS	MS	F	P
Amplitude	1	930.250	930.250	0.297	0.683
Time	1	6806.250	6806.250	2.170	0.380
Residual	1	3136.000	3136.000		
Total	3	10872.500	3624.167		

The difference in the mean values among the different levels of Amplitude is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Time. There is not a statistically significant difference ($P = 0.683$).

The difference in the mean values among the different levels of Time is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Amplitude. There is not a statistically significant difference ($P = 0.380$).

Power of performed test with alpha = 0.0500: for Amplitude : 0.0926

Power of performed test with alpha = 0.0500: for Time : 0.115

Least square means for Amplitude :

Group Mean

60.000 221.250

100.000 251.750

Std Err of LS Mean = 39.598

Least square means for Time :

Group Mean

5.000 195.250

10.000 277.750

Std Err of LS Mean = 39.598

General Full Factorial Regressions: Turbidity of Fish Gelatin for HHP Treatment;Pressure,Temperature

Two Way Analysis of Variance

Data source: Data 1 in fish-turbidity.JNB

General Linear Model (No Interactions)

Dependent Variable: Turbidity

Normality Test (Shapiro-Wilk) Passed (P = 0.161)

Equal Variance Test: Passed (P = 0.521)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Pressure	1	1.801	1.801	32.121	0.001
Temperature	1	3.262	3.262	58.180	<0.001
Residual	6	0.336	0.0561		
Total	8	3.856	0.482		

The difference in the mean values among the different levels of Pressure is greater than would be expected by chance after allowing for effects of differences in Temperature. There is a statistically significant difference (P = 0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature is greater than would be expected by chance after allowing for effects of differences in Pressure. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.0500: for Pressure : 0.997

Power of performed test with alpha = 0.0500: for Temperature : 1.000

Least square means for Pressure :

Group	**Mean**	**SEM**
0.1000	1.919	0.167
400.000	0.823	0.0967

Least square means for Temperature :

Group	**Mean**	**SEM**
30.000	0.634	0.0967
10.000	2.108	0.167

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Pressure**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
0.100 vs. 400.000	1.096	2	8.015	0.002	Yes

Comparisons for factor: **Temperature**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
10.000 vs. 30.000	1.475	2	10.787	<0.001	Yes

General Full Factorial Regressions: Turbidity of Fish Gelatin for US Treatment;Amplitude,Time

Two Way Analysis of Variance

Data source: Data 1 in fish-turbidity.JNB

Balanced Design

Dependent Variable: Turbidity

Normality Test (Shapiro-Wilk) Passed (P = 0.859)

Equal Variance Test: Passed (P = 0.377)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Amplitude	1	1.640	1.640	18.939	0.002
Time	1	21.589	21.589	249.313	<0.001
Amplitude x Time	1	3.628	3.628	41.901	<0.001
Residual	8	0.693	0.0866		
Total	11	27.550	2.505		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of Amplitude depends on what level of Time is present. There is a statistically significant interaction between Amplitude and Time. (P = <0.001)

Power of performed test with alpha = 0.0500: for Amplitude : 0.966

Power of performed test with alpha = 0.0500: for Time : 1.000

Power of performed test with alpha = 0.0500: for Amplitude x Time : 1.000

Least square means for Amplitude :

Group Mean

60.000 5.373

100.000 4.633

Std Err of LS Mean = 0.120

Least square means for Time :

Group Mean

5.000 6.344

10.000 3.662

Std Err of LS Mean = 0.120

Least square means for Amplitude x Time :

Group Mean

60.000 x 5.000 7.264

60.000 x 10.000 3.482

100.000 x 5.000 5.425

100.000 x 10.000 3.842

Std Err of LS Mean = 0.170

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Time within 60**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
5.000 vs. 10.000	3.782	2	22.263	<0.001	Yes

Comparisons for factor: **Time within 100**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
5.000 vs. 10.000	1.583	2	9.317	<0.001	Yes

Comparisons for factor: **Amplitude within 5**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
60.000 vs. 100.000	1.839	2	10.825	<0.001	Yes

Comparisons for factor: **Amplitude within 10**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
100.000 vs. 60.000	**0.360**	**2**	**2.121**	**0.172**	**No**

General Full Factorial Regressions: T_G of Fish Gelatin for US Treatment;Amplitude,Time

Two Way Analysis of Variance

Data source: Data 1 in Notebook1

General Linear Model (No Interactions)

Dependent Variable: TG

Normality Test (Shapiro-Wilk) Failed ($P < 0.050$)

Equal Variance Test: Passed ($P = 1.000$)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Amplitude	1	0.0163	0.0163	0.462	0.620
Time	1	0.589	0.589	16.755	0.153
Residual	1	0.0352	0.0352		
Total	3	0.640	0.213		

The difference in the mean values among the different levels of Amplitude is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Time. There is not a statistically significant difference ($P = 0.620$).

The difference in the mean values among the different levels of Time is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Amplitude. There is not a statistically significant difference ($P = 0.153$).

Power of performed test with alpha = 0.0500: for Amplitude : 0.0926

Power of performed test with alpha = 0.0500: for Time : 0.251

Least square means for Amplitude :

Group Mean

60.000 30.273

100.000 30.145

Std Err of LS Mean = 0.133

Least square means for Time :

Group Mean

5.000 30.593

10.000 29.825

Std Err of LS Mean = 0.133

General Full Factorial Regressions: G' of Fish Gelatin for US Treatment;Amplitude,Time

Two Way Analysis of Variance

Data source: Data 1 in Notebook1

General Linear Model (No Interactions)

Dependent Variable: G'

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Amplitude	1	1.891	1.891	0.0961	0.809
Time	1	319.873	319.873	16.263	0.155
Residual	1	19.669	19.669		
Total	3	341.433	113.811		

The difference in the mean values among the different levels of Amplitude is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Time. There is not a statistically significant difference (P = 0.809).

The difference in the mean values among the different levels of Time is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Amplitude. There is not a statistically significant difference (P = 0.155).

Power of performed test with alpha = 0.0500: for Amplitude : 0.0926

Power of performed test with alpha = 0.0500: for Time : 0.248

Least square means for Amplitude :

Group Mean

60.000 22.600

100.000 21.225

Std Err of LS Mean = 3.136

Least square means for Time :

Group Mean

5.000 12.970

10.000 30.855

Std Err of LS Mean = 3.136

General Full Factorial Regressions: G'' of Fish Gelatin for US Treatment;Amplitude,Time

Two Way Analysis of Variance

Data source: Data 1 in Notebook1

General Linear Model (No Interactions)

Dependent Variable: G"

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Amplitude	1	0.0930	0.0930	5.954	0.248
Time	1	0.511	0.511	32.718	0.110
Residual	1	0.0156	0.0156		
Total	3	0.620	0.207		

The difference in the mean values among the different levels of Amplitude is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Time. There is not a statistically significant difference (P = 0.248).

The difference in the mean values among the different levels of Time is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Amplitude. There is not a statistically significant difference (P = 0.110).

Power of performed test with alpha = 0.0500: for Amplitude : 0.165

Power of performed test with alpha = 0.0500: for Time : 0.339

Least square means for Amplitude :

Group	**Mean**
60.000	5.790
100.000	5.485

Std Err of LS Mean = 0.0884

Least square means for Time :

Group	**Mean**
5.000	5.280
10.000	5.995

Std Err of LS Mean = 0.0884

General Full Factorial Regressions: T_G of Fish Gelatin for HHP Treatment;Pressure,Temperature

Two Way Analysis of Variance

Data source: Data 1 in Notebook1

General Linear Model (No Interactions)

Dependent Variable: Tg

Normality Test (Shapiro-Wilk) Failed ($P < 0.050$)

Equal Variance Test: Passed ($P = 1.000$)

Source of Variation	DF	SS	MS	F	P
Pressure	1	0.684	0.684	(+inf)	<0.001
Temperature	1	0.125	0.125	(+inf)	<0.001
Residual	0	2.777E-028	0.000		
Total	2	1.469	0.735		

The difference in the mean values among the different levels of Pressure is greater than would be expected by chance after allowing for effects of differences in Temperature. There is a statistically significant difference ($P = <0.001$). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature is greater than would be expected by chance after allowing for effects of differences in Pressure.

There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.0500: for Pressure : 1.000

Power of performed test with alpha = 0.0500: for Temperature : 1.000

Least square means for Pressure :

Group	**Mean**
0.1000	32.120
400.000	30.950

Std Err of LS Mean = 0.000

Least square means for Temperature :

Group	**Mean**
30.000	31.785
10.000	31.285

Std Err of LS Mean = 0.000

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Pressure**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
0.100 vs. 400.000	1.170	2	(+inf)	1.000	No

Comparisons for factor: **Temperature**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
30.000 vs. 10.000	0.500	2	(+inf)	1.000	No

General Full Factorial Regressions: G' of Fish Gelatin for HHP Treatment;Pressure,Temperature

Two Way Analysis of Variance

Data source: Data 1 in Notebook1

General Linear Model (No Interactions)

Dependent Variable: G'

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Pressure	1	9.724	9.724	(+inf)	<0.001
Temperature	1	18.060	18.060	(+inf)	<0.001
Residual	0	1.775E-030	0.000		
Total	2	19.376	9.688		

The difference in the mean values among the different levels of Pressure is greater than would be expected by chance after allowing for effects of differences in Temperature. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature is greater than would be expected by chance after allowing for effects of differences in Pressure. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.0500: for Pressure : 1.000

Power of performed test with alpha = 0.0500: for Temperature : 1.000

Least square means for Pressure :

Group Mean

0.1000 -0.275

400.000 4.135

Std Err of LS Mean = 0.000

Least square means for Temperature :

Group Mean

30.000 4.935

10.000 -1.075

Std Err of LS Mean = 0.000

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Pressure**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
400.000 vs. 0.100	4.410	2	(+inf)	1.000	No

Comparisons for factor: **Temperature**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
30.000 vs. 10.000	**6.010**	**2**	**(+inf)**	**1.000**	**No**

General Full Factorial Regressions: G'' of Fish Gelatin for HHP Treatment;Pressure,Temperature

Two Way Analysis of Variance

Data source: Data 1 in Notebook1

General Linear Model (No Interactions)

Dependent Variable: G"

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Pressure	1	1.140	1.140	(+inf)	<0.001
Temperature	1	1.075	1.075	(+inf)	<0.001
Residual	0	8.382E-031	0.000		
Total	2	1.477	0.739		

The difference in the mean values among the different levels of Pressure is greater than would be expected by chance after allowing for effects of differences in Temperature. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature is greater than would be expected by chance after allowing for effects of differences in Pressure. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.0500: for Pressure : 1.000

Power of performed test with alpha = 0.0500: for Temperature : 1.000

Least square means for Pressure :

Group	**Mean**
0.1000	0.247
400.000	1.757

Std Err of LS Mean = 0.000

Least square means for Temperature :

Group	**Mean**
30.000	1.735
10.000	0.269

Std Err of LS Mean = 0.000

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Pressure**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
400.000 vs. 0.100	1.510	2	(+inf)	1.000	No

Comparisons for factor: **Temperature**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
30.000 vs. 10.000	1.466	2	(+inf)	1.000	No

General Full Factorial Regressions: T_m of Fish Gelatin for HHP Treatment;Pressure,Temperature

Two Way Analysis of Variance

Data source: Data 1 in Notebook1

General Linear Model (No Interactions)

Dependent Variable: Tm

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Pressure	1	0.281	0.281	(+inf)	<0.001
Temperature	1	0.0300	0.0300	(+inf)	<0.001
Residual	0	1.136E-028	0.000		
Total	2	0.293	0.146		

The difference in the mean values among the different levels of Pressure is greater than would be expected by chance after allowing for effects of differences in Temperature. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Temperature is greater than would be expected by chance after allowing for effects of differences in Pressure. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.0500: for Pressure : 1.000

Power of performed test with alpha = 0.0500: for Temperature : 1.000

Least square means for Pressure :

Group	**Mean**
0.1000	23.012
400.000	22.262

Std Err of LS Mean = 0.000

Least square means for Temperature :

Group	**Mean**
30.000	22.515
10.000	22.760

Std Err of LS Mean = 0.000

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Pressure**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
0.100 vs. 400.000	0.750	2	(+inf)	1.000	No

Comparisons for factor: **Temperature**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**	
10.000 vs. 30.000	0.245	2	(+inf)	1.000	No	No

General Full Factorial Regressions: T_2 of Bovine Gelatin for HHP Treatment;Pressure,Temperature

Two Way Analysis of Variance

Data source: Data 1 in Bovinepressure

General Linear Model (No Interactions)

Dependent Variable: T2

Normality Test (Shapiro-Wilk) Passed (P = 0.352)

Equal Variance Test: Failed (P < 0.050)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Pressure	1	42.120	42.120	4.361	0.128
Temperature	1	1974.914	1974.914	204.485	<0.001
Residual	3	28.974	9.658		
Total	5	3102.906	620.581		

The difference in the mean values among the different levels of Pressure is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Temperature. There is not a statistically significant difference (P = 0.128).

The difference in the mean values among the different levels of Temperature is greater than would be expected by chance after allowing for effects of differences in Pressure. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.0500: for Pressure : 0.250

Power of performed test with alpha = 0.0500: for Temperature : 1.000

Least square means for Pressure :

Group	**Mean**	**SEM**
0.1000	736.120	2.691
400.000	742.610	1.554

Least square means for Temperature :

Group	**Mean**	**SEM**
30.000	717.145	1.554
10.000	761.585	2.691

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Temperature**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
10.000 vs. 30.000	44.440	2	20.223	0.001	Yes

General Full Factorial Regressions: T_2 of Bovine Gelatin for US Treatment;Amplitude,Time

Two Way Analysis of Variance

Data source: Data 1 in BovineUltrasound

Balanced Design

Dependent Variable: T2

Normality Test (Shapiro-Wilk) Passed (P = 1.000)

Equal Variance Test: Failed (P < 0.050)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Amplitude	1	15391.351	15391.351	1483.325	<0.001
Time	1	697.511	697.511	67.222	0.001
Amplitude x Time	1	4273.501	4273.501	411.854	<0.001
Residual	4	41.505	10.376		
Total	7	20403.869	2914.838		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of Amplitude depends on what level of Time is present. There is a statistically significant interaction between Amplitude and Time. (P = <0.001)

Power of performed test with alpha = 0.0500: for Amplitude : 1.000

Power of performed test with alpha = 0.0500: for Time : 1.000

Power of performed test with alpha = 0.0500: for Amplitude x Time : 1.000

Least square means for Amplitude :

Group Mean

60.000 751.825

100.000 839.550

Std Err of LS Mean = 1.611

Least square means for Time :

Group Mean

5.000 805.025

10.000 786.350

Std Err of LS Mean = 1.611

Least square means for Amplitude x Time :

Group Mean

60.000 x 5.000 738.050

60.000 x 10.000 765.600

100.000 x 5.000 872.000

100.000 x 10.000 807.100

Std Err of LS Mean = 2.278

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Time within 60**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
10.000 vs. 5.000	27.550	2	12.095	0.001	Yes

Comparisons for factor: **Time within 100**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
5.000 vs. 10.000	64.900	2	28.493	<0.001	Yes

Comparisons for factor: **Amplitude within 5**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
100.000 vs. 60.000	133.950	2	58.808	<0.001	Yes

Comparisons for factor: **Amplitude within 10**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
100.000 vs. 60.000	41.500	2	18.220	<0.001	Yes

General Full Factorial Regressions: T_2 of Fish Gelatin for HHP Treatment;Pressure,Temperature

Two Way Analysis of Variance

Data source: Data 1 in FishHHP

General Linear Model (No Interactions)

Dependent Variable: T2

Normality Test (Shapiro-Wilk) Passed (P = 0.992)

Equal Variance Test: Failed (P < 0.050)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Pressure	1	117.723	117.723	0.578	0.503
Temperature	1	51.123	51.123	0.251	0.651
Residual	3	611.405	203.802		
Total	5	733.095	146.619		

The difference in the mean values among the different levels of Pressure is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Temperature. There is not a statistically significant difference (P = 0.503).

The difference in the mean values among the different levels of Temperature is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Pressure. There is not a statistically significant difference (P = 0.651).

Power of performed test with alpha = 0.0500: for Pressure : 0.0521

Power of performed test with alpha = 0.0500: for Temperature : 0.0521

Least square means for Pressure :

Group	Mean	SEM
0.1000	815.275	12.363
400.000	804.425	7.138

Least square means for Temperature :

Group	Mean	SEM
30.000	806.275	7.138
10.000	813.425	12.363

General Full Factorial Regressions: T_2 of Fish Gelatin for US Treatment;Amplitude,Time

Two Way Analysis of Variance

Data source: Data 1 in FishUltrasound

Balanced Design

Dependent Variable: T2

Normality Test (Shapiro-Wilk) Passed (P = 0.995)

Equal Variance Test: Failed (P < 0.050)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Amplitude	1	748.845	748.845	51.379	0.002
Time	1	954.845	954.845	65.513	0.001
Amplitude x Time	1	1008.005	1008.005	69.160	0.001
Residual	4	58.300	14.575		
Total	7	2769.995	395.714		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of Amplitude depends on what level of Time is present. There is a statistically significant interaction between Amplitude and Time. (P = 0.001)

Power of performed test with alpha = 0.0500: for Amplitude : 0.999

Power of performed test with alpha = 0.0500: for Time : 1.000

Power of performed test with alpha = 0.0500: for Amplitude x Time : 1.000

Least square means for Amplitude :

Group Mean

60.000 824.100

100.000 843.450

Std Err of LS Mean = 1.909

Least square means for Time :

Group Mean

5.000 844.700

10.000 822.850

Std Err of LS Mean = 1.909

Least square means for Amplitude x Time :

Group Mean

60.000 x 5.000 846.250

60.000 x 10.000 801.950

100.000 x 5.000 843.150

100.000 x 10.000 843.750

Std Err of LS Mean = 2.700

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Time within 60**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
5.000 vs. 10.000	44.300	2	16.410	<0.001	Yes

Comparisons for factor: **Time within 100**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
10.000 vs. 5.000	0.600	2	0.222	0.883	No

Comparisons for factor: **Amplitude within 5**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
60.000 vs. 100.000	3.100	2	1.148	0.463	No

Comparisons for factor: **Amplitude within 10**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
100.000 vs. 60.000	41.800	2	15.484	<0.001	Yes

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CURRICULUM VITAE

PERSONAL INFORMATION

Surname, Name	: Sezer, Pürlen
Nationality	: Turkish (TC)
Date and Place of Birth	: 5 April 1994, İzmir
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EDUCATION

Degree	Institution	Year of Graduation
MS	METU Food Engineering	2019
BS	METU Food Engineering	2017
High School	Erol Altaca High School, İstabul	2012

WORK EXPERIENCE

Year	Place	Enrollment
2016-July	Dardanel	Intern Engineering Student
2015-August	Talay Wine	Intern Engineering Student

FOREIGN LANGUAGES

Advanced English, B2.1. German