PHYSICAL AND CHEMICAL CHARACTERIZATION OF SESAME SEED PROTEIN

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ABSTRACT

PHYSICAL AND CHEMICAL CHARACTERIZATION OF SESAME SEED PROTEIN

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Plant-based food proteins have become very popular and valuable due to increase trend of following a vegan diet and preferring to consume less meat in terms of sustainability reasons. The most popular plant based proteins are soya bean, quinoa, lentil, peanut and chia seed proteins. Sesame seed also has a potential to be an alternative protein source. However, it is a known fact that there is no extended study about sesame seed proteins in the literature although sesame seed contains significant amount of protein which cannot be disregarded. The aim of this study was to perform the characterization of sesame seed proteins obtained by using three different extraction techniques namely alkaline, salt (by NaCl) and enzyme assisted extraction. A comprehensive analysis was done to assess the physicochemical protperties of the extracted proteins. Total protein contenxt by Kjeldahl method, emulsification activity & emulsion stability by turbidimetric method, protein solubility by Lowry method, structural changes via Fourier Transform Infrared (FTIR) spectroscopy, hydration behavior and gelling ability using Time Domain Nuclear Magnetic Resonance (TD-NMR) relaxometry through T_2 relaxation time

measurements were made for all extracted proteins. Also, SDS-PAGE applied to separate the protein subunits in sesame seed by checking their molecular weight. To determine the most efficient method for extraction of protein, yield was also calculated for all extracts. The results showed that extraction type had a significant effect on the yield and properties of the extracted proteins. Salt extracted samples and enzyme assisted alkaline extracted sample had the highest protein contents which were all over 90%, and 1M- NaCl extract had the highest yield among all other extracted protein samples. However, physicochemical properties of salt extracted samples such as solubility, gelling ability, emulsion stability and emulsification activity were found to be the lowest in general. Additionally, TD-NMR results showed that salt extracted samples had the weakest water binding capability with longer T_2 relaxation times (more free water in the environment), confirmed by low solubility results obtained by Lowry method. In summary, depending on the physical property of interest, different extraction methods yielded different properties for the proteins.

Keywords: Sesame seed meal, Protein extraction, SDS-PAGE, TD-NMR Relaxometry, Alternative protein sources

ÖZ

SUSAM TOHUMU PROTEİNİNİN FİZİKSEL VE KİMYASAL KARAKTERİZASYONU

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Günümüzde bitki kökenli besin proteinleri, vegan besinlerin üretimindeki artış ve proteinlerin özelliklerinden dolayı çok popüler ve değerli hale gelmiştir. En popüler bitki kökenli proteinler soya fasulyesi, kinoa, mercimek, yer fıstığı ve chia tohumu proteinleridir. Bununla beraber, literatürde susam tohumu proteinleri hakkında geniş kapsamlı bir çalışma olmadığı bilinen bir gerçektir, fakat susam tohumu göz ardı edilemeyecek kadar önemli miktarda protein içermektedir. Bu çalışmanın amacı, alkali ekstraksiyon, tuz ekstraksiyon ve enzim destekli ekstraksiyon olmak üzere üç farklı ekstraksiyon tekniği kullanılarak elde edilen susam tohumu küspesi proteinlerinin karakterizasyonunu yapmaktır. Bu proteinlerin fizikokimyasal özellikleri, Kjeldahl yöntemi ile toplam protein içeriği, türbidimetrik yöntemle emülsifikasyon aktivitesi ve emülsiyon stabilitesi, Lowry yöntemi ile protein çözünürlüğü, Fourier Transform Infrared (FTIR) spektroskopisi ile moleküler yapı değişiklikleri, T_2 relaksasyon süresi ölçümleri aracılığıyla hidrasyon davranışı ve jelleşme yeteneği dahil olmak üzere analiz edilmiştir. Ayrıca susam tohumundaki protein alt birimlerinin moleküler ağırlıklarına bakılarak ayrıştırılması için SDS-PAGE yöntemi uygulanmıştır. Protein ekstraksiyonu için en verimli yöntemi belirlemek için, tüm ekstraktlar için verim hesaplanmıştır. Sonuçlar, ekstraksiyon

yönteminin ekstrakte edilen proteinlerin verimi ve özellikleri üzerinde önemli bir etkiye sahip olduğunu göstermiştir. Tuz ve enzim kullanılarak ekstrakte edilmiş numunelerin, en yüksek protein içeriğine (% 90'ın üzerinde) sahip olduğu ve 1M-NaCl ile ekstrakte edilen proteinlerin, diğer tüm ekstrakte edilmiş protein numuneleri arasında en yüksek verime sahip olduğu bulunmuştur. Ancak, tuzla ekstrakte edilen numunelerin çözünürlük, jelleşme yeteneği, emülsiyon stabilitesi ve emülsifikasyon aktivitesi gibi fizikokimyasal özelliklerinin genel olarak en düşük olduğu bulunmuştur. Ek olarak, TD-NMR sonuçları, tuzla ekstrakte edilen numunelerin daha uzun T_2 relaksasyon süresine (ortamda daha fazla serbest su), diğer bir deyişle en zayıf su bağlama kabiliyetine sahip olduğunu göstermiştir ve bu sonuçlar Lowry metodu kullanılarak bulunan çözünürlük sonuçları ile desteklenmiştir. Özetle, ilgilenilen fiziksel özelliğe bağlı olarak, farklı ekstraksiyon yöntemlerinin kullanılması farklı özelliklere sahip protein elde edilmesini sağlayabilir.

Anahtar Kelimeler: Susam küspesi, SDS-PAGE, TD-NMR, Protein ekstraksiyonu, Alternatif protein kaynakları

To our gorgeous universe

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LIST OF ABBREVIATIONS

ABBREVIATIONS

E-ACP : Aqueous creamy phase of enzyme extract

E-Precipitate : Precipitate part of enzyme extract

PPC : Pea Protein Concentrate

WPI : Whey Protein Isolate

TD-NMR : Time domain nuclear magnetic resonance

FTIR : Fourier transform infrared

SDS-PAGE : Sodium dodecyl sulfate polyacrylamide gel electrophoresis

EAI : Emulsification activity index

ESI : Emulsion stability index

MW : Molecular weight

BSA : Bovine serum albumin

UV : Ultraviolet

HHP : High hydrostatic pressure

IR : Infrared

RF : Radiofrequency

NMR : Nuclear magnetic resonance

CPMG : Carr-Purcell-Meiboom-Gill

FID : Free induction decay

PAGE : Polyacrylamide gel electrophoresis

SDS : Sodium dodecyl sulfate

IEF : Isoelectric focusing

APS : Ammonium per sulfate

ATR : Attenuated total reflectance

TEMED : Tetramethylethylenediamine

CHAPTER 1

INTRODUCTION

1.1 Sesame Seed

1.1.1 General View

Sesamum indicum L. is one of the world's oldest oil seed crop and it grows widely in tropical and subtropical areas for especially its edible oil (Pusadkar, Kokiladevi, Bonde, & Mohite, 2015). Historical accounts showed that sesame was traded as early as 2000 BC between Mesopotamia and Indian subcontinent. According to the archeological reports, sesame was grown in Turkey at least 2,750 years ago. Sesame can be regarded as a valuable crop grown for the consumption as food (dry seeds) and feed (seeds and leaves). Besides these, other parts of *Sesamum indicum* L. such as flowers can be used in cancer, alopecia, and constipation treatments. Moreover, roots of the crop has antifungal activity and leaves are useful in treating urinary infections and infant cholera (Pusadkar, Kokiladevi, et al., 2015). Sesame seed crop was even mentioned in ancient Egyptian scrolls for being used as a medicinal drug.

Figure 1.1. Sesame seed plant

Sesame seed is used for many food and snack preparations, also in salad dressings. The demand for the sesame crop in the world is increasing these days due to its high copper, calcium, phosphorus, and protein content. Sesame seed contains the highest amount of oil (50% and above) depending on the type of the crop compared to any other oilseeds (Sharaby & Butovchenko, 2019). Sesame seed is generally used for oil production, which is used for cooking purposes extensively. Additionally, it is used in cosmetics, pharmaceuticals, and perfumery industries in small amounts. Sesame seed is known as a rich protein source like soybeans, which makes it an even more valuable crop (Araujo et al., 2018).

According to some Turkish reports published in 2019, Turkey is not a self-sufficient country to produce sesame seed because the crop is difficult to harvest and labor cost is going down day by day. Nevertheless, Turkish sesame is known as one of the best quality sesame seeds in the world which is called as 'golden sesame'. According to export reports, Turkey's main market is Japan (Önel, 2019). Turkey is the 3rd biggest buyer of sesame seed after China and Japan, because sesame seed consumption is quite high due to the production of bakery products such as simit, tahini, halva, and cooking oil (Önel, 2019).

1.1.2 Sesame Seed Oil

Sesame seed is mostly processed for extracting and utilizing its oil for food, pharmaceutical and cosmetic purposes (Carvalho, Galvão, Barros, Conceição, & Sousa, 2012). Sesame seeds have high amount of polyunsaturated fatty acids content after safflower, maize, and soybean. It contains about 47% oleic acid and 39% linolenic acid (Morris, 2002).

Sesame seed oil contains significant amount of tocopherols and lignans which are sesamin and sesamolin (Crews et al., 2006). These two compounds have some beneficial effects on liver function and serum lipid levels. Also, these lignans are the reason why sesame seed oil has remarkably high antioxidant activity (Hirata et al., 1996; Ogawa, Sasagawa, Murakami, & Yoshizumi, 1995). They prevent high blood pressure and increase vitamin E supplies in animals (Yamashita, Nohara, Katayama, & Namiki, 1992).

According to a study, sesame seed oil is found to inhibit the growth of malignant melanoma *in vitro* and proliferation of human cancer cells in the colon (Smith & Salerno, 1992). In human tissues, this oil neutralizes oxygen radicals beneath the skin by penetrating quickly into the skin and afterwards entering blood stream though capillaries. Moreover, sesame seed oil is known as a natural UV protector (Anilakumar, Pal, Khanum & Bawa, 2010). It kills the head lice infestations in the children's hair successfully. Sesamin and sesamolin also have bactericide and insecticide activities (Anilakumar et al., 2010).

The quantity and the quality of the oil extracted from sesame seeds highly depend on ecological, genetics and physiological factors which are soil type, climate, cultivars, and plant maturity. Sesame seed oil is usually extracted by the following procedure in Figure 1.2.

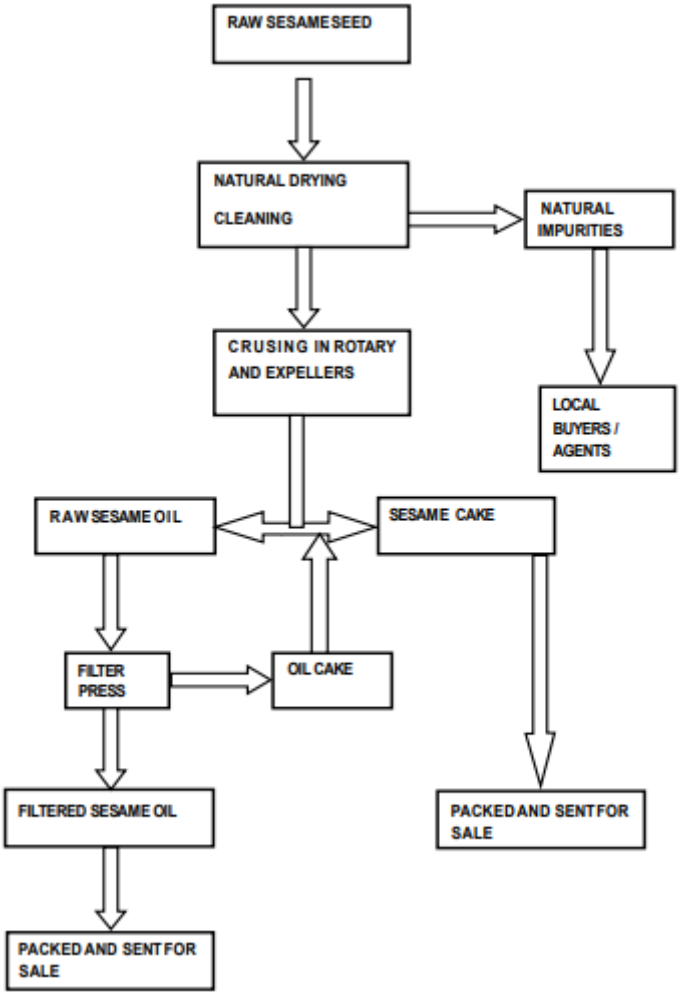

Figure 1.2. Flow chart of sesame seed oil production (Prasadar, Shivananju & Sanjay, 2012)

1.1.3 Sesame seed meal (Sesame Oil Cake) as by-product of sesame seed oil production

During the sesame seed oil production, after extracting the oil from sesame seed by either using organic solvents or mechanical pressing; protein rich sesame meal is left as by-product. This sesame seed meal is generally used as feed for poultry and livestock due to its high protein content (Capellini, Chiavoloni, Giacomini, &

Rodrigues, 2019; Hegde, 2012; Heuzé, Tran, Bastianelli, 2016). Depending on the way oil is extracted, sesame seed meal can be food grade as well. Sesame seed meal is usually obtained by using mechanical extraction only rather than using mechanical extraction followed by solvent extraction. Therefore, its residual oil content is higher than other oil sources(Gharby et al., 2017).

As stated before, following the oil extraction, sesame seed meal contains significant amount of protein (~30-50%) depending on the process (Sari, Mulder, Sanders, & Bruins, 2015). This remaining meal is generally directly fed to the animals, therefore protein is not well utilized (Peter, 2012). Utilizing a valuable by-product like sesame seed meal as a potential protein source might result in a particularly good way of having an alternative protein source for human consumption.

1.1.4 Sesame Seed Proteins

Sesame seed contains 17-32% protein with an average of 25% (Nagaraj, 2009). The seed proteins are generally located in the outer layers of the seed. Sesame proteins can be classified as mostly globulins (67.3%) followed by albumin (8.6%), prolamin (1.3%) and glutelin (6.9%) fractions (Dench, Rivas & Caygill, 1981). In oil seeds, generally globulins are the predominant protein.

When the essential amino acid composition of sesame seed proteins is examined; it was observed that they are rich in sulfur-containing amino acids, especially methionine and tryptophan (Evans & Bandemer, 1967). On the other hand, sesame seed proteins have low content of lysine which is uncommon for oilseed proteins (Cuca & Sunde, 1967).

Researchers suggests that sesame protein should be used widely as a supplement of methionine and tryptophan which is an excellent source for babies and weaning foods. The use of sesame seed protein might eliminate the problems faced when foods are supplemented with free methionine due to its instability (Chatterjee, Dey, Ghosh & Dhar, 2015a; Esssa, Abd, Kassab & Ghazi, 2015). For all these reasons, protein extraction from sesame seed or sesame meal has become crucial and valuable.

1.2 Protein Extraction

Nowadays, the demand for renewable and sustainable sources of especially plant proteins are rising because of the negative impacts of animal protein production on the environment and increasing veganism and vegetarianism trends (Bilek, 2018). Moreover, the production of plant protein isolates or concentrates is one of the greatest interests in the food industry because of their ability to improve the nutritional quality of the foods along with the improvements in their functional properties (Prasad & Prasad, 2012). The method used for the extraction of protein from oilseeds has a significant effect on the functional properties of proteins (*solubility, viscosity, foaming capacity/stability, emulsification activity/emulsion stability, water and oil binding capacity etc.*), quality, and protein composition (Aluko & McIntosh, 2001). There are many factors to concern while developing method for the protein extraction. For example, use of chemicals, which might have some toxic effect, during extraction or precipitation procedure can cause an increase in the cost for removing these chemicals later on (Vergara-Barberán, Lerma-García, Herrero-Martínez & Simó-Alfonso, 2015). Additionally, depending on the part of the plant, extraction procedure might vary. Some parts of the plants may have challenging features such as high fiber content, adhesive structure or hard tissues such as cell wall (Bilek, 2018).

1.2.1 Alkaline Extraction

Alkaline extraction method is one of the most common methods used in food science and industry (Mueller, Eisner & Kirchhoff, 2010). The basic principle of alkaline extraction is solubilizing the proteins by increasing the pH (alkaline pH) at the beginning, then removal of insoluble materials by centrifugation, and finally precipitating the proteins at their isoelectric points (Tirgar, Silcock, Carne & Birch, 2017).

pH is an important factor that influences the structure and functional properties of proteins by changing the denaturation degree and charge of the protein. Due to alkaline pHs, unfolded protein fractions with exposed functional groups such as sulfhydryl and hydrophobic groups are obtained. This situation can facilitate their interaction with water or improve chain to chain protein associations, and formation of new covalent bonds like disulfide bonds (S-S) (Damodaran & Kinsella, 1982; Mauri & Añón, 2008). Depending on the usage area of the protein extracted, unfolding of the proteins might be a desired circumstance. For instance, in the case of forming edible protein films, different sources of proteins are used, and these films generally require unfolded proteins up to some point (Cao & Chang, 2001; Valenzuela, Abugoch, Tapia & Gamboa, 2013).

When proteins are extracted at alkaline pH, majority of the proteins have negative charge due to the ionization of carboxyl groups and deprotonation of amino groups. This condition increases the protein-solvent interaction because of the repulsion between the like-charged proteins, resulting in enhanced protein solubility (Lawal, 2004).

There are some disadvantages of alkaline extraction. In the alkaline medium, some undesirable reactions such as amino acid racemization, lysinoalanine formation, decrease in digestibility and loss of some essential amino acids might occur (Mauri & Añón, 2008). Furthermore, polyphenols might oxidize and dark green/brown color might be observed in the proteins extracted (Xu, 1999).

1.2.2 Salt Extraction

Along with pH, ionic strength of the environment is an important factor affecting the quality and functional properties of the proteins (Oliyaei, Moosavi-Nasab & Ghorbani, 2019; Thawornchinsombut & Park, 2005). Proteins show variation in their solubility behavior depending on the ionic environment of the solution. If the salt in the solution increases the solubility of proteins, this phenomenon is called as '*salting in*' (Duong-Ly & Gabelli, 2014a). On the contrary, if the solubility of proteins decreases with the addition of salt, it is called as '*salting out*' (Duong-Ly & Gabelli, 2014b). Salts reducing the protein solubility also tend to increase the stability of native conformation of proteins. On the contrary, *salting in* ions are known to be denaturants (Wiggins, 1997). Depending on the type and amount of the salt, it can have an effect as '*salting in*' or '*salting out*'. In 1888, Franz Hofmeister firstly reported the capability of different salts to stabilize or destabilize proteins in the water, depending on the type and concentration of the salts as shown in the Figure 1.3 (Hofmeister, 1888; Kang, Tang, Zhao & Song, 2020). At present, this effect has become a widespread phenomenon and called as the Hofmeister series, lyotropic sequences, or ion specificity series (Lee, Choi & Cho, 2017).

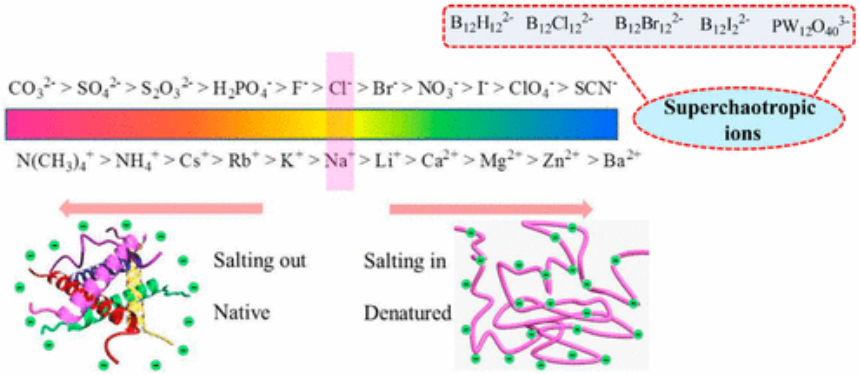

Figure 1.3. Hofmeister series showing the effect of different ions on the solubility and stability of proteins as salting in and salting out (Kang et al., 2020)

Some ions promote protein solubility such as calcium (Ca^{2+}), magnesium (Mg^{2+}), bromide (Br^-), and iodide (I^-) is stated as salting in (E. Lee et al., 2017). On the other hand, some salts such as sulfate (SO_4^{2-}), ammonium (NH_4^+) increase protein-protein interactions and cause proteins to aggregate and precipitate (Aoki, Shiraki & Hattori, 2016).

Often, proteins which contain positively and negatively charged regions on their surface cannot remain in the solution and self-aggregate because of this electrostatic energy between them when there is no salt in the environment (Thawornchinsombut & Park, 2005). During *salting in*, ions in the solution act as a shield and stabilizes the charges on the protein surface and prevent aggregation. However, when there is excess amount of salt in the environment salt molecules compete with protein molecules to bind water molecules. In this case, protein molecules tend to stay together because protein-protein interaction becomes energetically more favorable, called as *salting out* (Oliyaei et al., 2019). Surface of protein is defined as hydration layer, and it plays a critical role in the maintaining solubility and keeping the protein folded in its native conformation. It is possible to have 3 different type of water-protein interactions which are *ion hydration* due to the charged regions, *hydrogen*

bonding (polar groups of protein and water) and *hydrophobic hydration* (Wingfield, 1998).

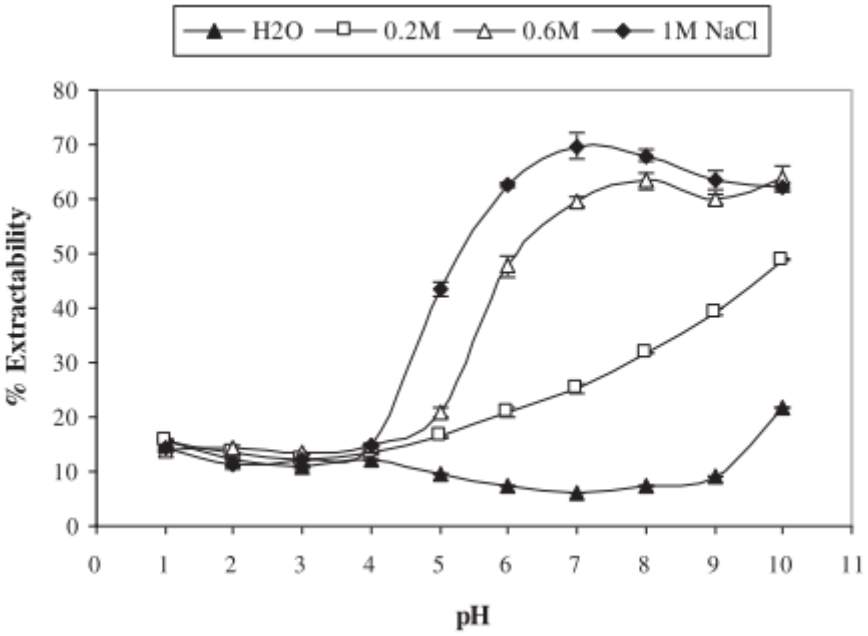

Figure 1.4. Extractability (%) of total sesame proteins as a function of pH and in the presence of NaCl at different concentrations (Onsaard, 2012)

When more salt is added to the solution, surface tension of water increases. Consequently, hydrophobic interaction between water and protein increases which eventually results in a decrease in the surface area of the protein to have minimum contact with water (Wingfield, 1998). Later, protein is folded into itself more and it ends up with self-association and then precipitation.

Salting in phenomenon is utilized during salt extraction of proteins. During salt extraction, instead of increasing the pH like in the case of alkaline extraction, salt solution is used to solubilize the proteins. In Figure 1.4, extractability of sesame seed proteins is shown in the presence of NaCl at different concentrations as a function of

pH. Globulin proteins are sensitive to change in pH and ionic strength of the solutions and dissociate into their subunits (Henning, Mothes, Dudek, Krause & Schwenke, 1997). In situations where protein unfolding in not desired, salt extraction might be preferred.

Generally, higher solubility of proteins is attained when salt extraction is used (Liang & Tang, 2013). According to a study conducted on pea proteins, which is composed of mostly globular proteins, it has been found that salt extracted protein fractions had the highest oil binding and emulsion capacity when compared with alkaline extracted samples (Stone, Karalash, Tyler, Warkentin & Nickerson, 2015). Protein isolates with high oil binding capacity are necessary to be used in meat products, the ones with good emulsifying properties are good for dressings and soups (Ahmedna, Prinyawiwatkul & Rao, 1999). Finally, salt extraction allows producing food-grade protein extracts instead of feed-grade (Stone et al., 2015).

1.2.3 Enzyme Assisted Alkaline Extraction

It is reported that during the extraction process of the proteins, high alkaline conditions might reduce the nutritional value (Ansharullah & Chesterman, 1997). The enzymatic pretreatment method by using carbohydrases is known to improve the extraction of especially plant proteins at moderate or slightly basic pH levels (Wang, Hettiarachchy, Qi, Burks & Siebenmorgen, 1999). Carbohydrases facilitate protein extraction by disintegrating the cell wall. Using enzyme during extraction process is also an environmental-friendly alternative to other methods that includes the usage of some harsh chemicals. This extraction method allows extracting high quality proteins with higher yield and production of food-grade instead of feed-grade protein becomes possible (Latif & Anwar, 2011).

As enzyme, proteases are also widely used for assisting the protein extraction by alkaline method. Protease enzymes help through proteolysis. Combined enzyme mixtures of proteases and carbohydrases might also be used to increase the extraction yield (Le, Parks, Nguyen, & Roach, 2018); however, they are generally used separately. Because it was shown that using proteases alone did not make a huge difference on extraction yield compared to mixture of protease and carbohydrases (Sari et al., 2015). By protease addition, protein size is reduced, and proteins are extracted easily.

Alkaline extraction can be combined with high temperature to increase the protein yield instead of using enzymes, but it is not recommended for some critical reasons (Achouri & Boye, 2013; Long et al., 2015; Saini, Sharma & Sharma, 2018). The most important reasons are denaturation, racemization and lysinoalanine formation (Sari et al., 2015). All these outcomes cause decrease in the protein functionality, in other words; poor properties of the extracted proteins (Sineiro & Domı, 2001).

1.2.4 Ultrasound-assisted extraction

Ultrasound-assisted extraction has the advantage of short operation time, high extraction yield and small amount of solvent usage (Liu et al., 2019). Moreover, it also consumes less energy (green extraction) and produce co-products without contaminants (Li, Chen & Li, 2017).

Ultrasound is defined as high frequency sound wave which exceeds the human hearing limit. Passing of these waves create pressure difference and this pressure difference depends on the quantity of energy applied to system. Material's physicochemical properties might change by the application of ultrasound due to the

induced compression and decompression of the particles (Zou et al., 2017a). Under ultrasonic sound application, the mass transfer rate may be enhanced noticeably which is the basic reason for short operation time (Chemat, Zill-E-Huma & Khan, 2011). Additionally, ultrasound produces microstreaming which has an important mechanical effect on the raw material surface and reduce particle size (Tao & Sun, 2015).

According to a study done on extracting soy proteins, sonication energy was used during the extraction process in a pilot plant, and it was found that protein yield was improved up to 60% from 40% by the application of ultrasound at the solid-solvent ratio of 1:30 (Moulton & Wang, 1982).

1.2.5 Pressure-assisted Extraction

Application of high pressure might be an effective way of extracting proteins from various sources (Khan, Aslam & Makroo, 2019). Pressure promotes the penetration of water into the inner core of especially globular proteins, causing denaturation (Xi et al., 2009). Although proteins are denatured, aggregation is reduced, and size of the protein aggregates decreases because of the increased exposure to water which improves protein solubilization (Khan et al., 2019). Heating causes only protein denaturation, which causes severe aggregation of proteins, and decrease in the solubilization (Li, Zhu, Zhou & Peng, 2012).

High pressure extraction generally operates at low temperatures (up to 60 °C), and high pressures (100-1000 MPa) to extract the proteins quickly with the requirement of less quantities of solvent and other chemicals (Balny, Masson & Heremans, 2002). Moreover, it is proven that high pressure inactivates microbial growth and minimize

the degradation of color, flavor, and texture (Santos et al., 2013). Additionally, pressure induces a reaction in a direction promoting a decrease in volume hence resulting in the improvement of extraction yield (Xi et al., 2009).

In a study, during the production of soybean protein isolate based infant formula, soy proteins were treated by high hydrostatic pressure (HHP), and it was found that application of 300 MPa for 15 minutes decreased the allergenicity of soy protein by 48.6% compared to native soy protein (Li et al., 2012). As a result, it can be concluded that HHP treatment can also modify the properties of the protein to be extracted in a useful way depending on the application.

1.2.6 Reverse micelle extraction

Reverse micelle extraction is a relatively new liquid-liquid extraction technique for isolation of proteins (Naoe, Yoshimoto, Naito, Kawagoe & Imai, 2011a). Surfactants in nonpolar organic solvents form reverse micelles and inner core of the reverse micelle is composed of polar water pools (Naoe, Yoshimoto, Naito, Kawagoe & Imai, 2011b). A representative picture of reverse micelle structure is given in Figure 1.5.

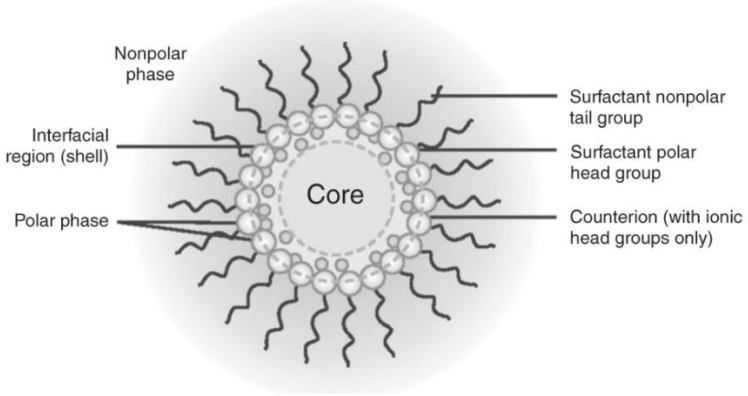

Figure 1.5. Typical structure of reverse micelle (Bagchi, 2013)

Owing to the ability of self-assembly, reverse micelles are thermodynamically stable systems and known as '*dynamic balancing systems*' (Bu et al., 2014). The mechanism of reverse micelle system includes two steps which are forward extraction and backward extraction. In the forward extraction, protein is transported to the inner core, polar water pools and oil is transferred to organic solvent. In the backward extraction, protein is released from the polar water pools and then transferred into an aqueous phase to be recovered (Zhao et al., 2009). The size of polar water pools plays a significant role in the solubility of molecules/macromolecules in the core.

Reverse micelle systems have some advantages over the other extraction methods due to no loss of biological activity and nutritional value of proteins (Guo, Li, Zhang, Meng & Li, 2008). Moreover, other common methods might cause reversible/irreversible denaturation of proteins.

In a study, β-galactosidase enzyme from *Kluyveromyces lactis* was partially purified by using water-in-oil microemulsions which is also called as reversed micellar solutions (Mazı, Hamamcı, Ogrydziak & Dungan, 2016). In the reverse micelle system, water-containing microemulsions can extract protein molecules from water selectively, achieving the removal of nearly 100% of the protein, depending on the pH and salt concentration of the system. In conclusion, the method was found to have a great potential to be applied in a large-scale, continuous process without significant losses of the enzyme (Mazı et al., 2016).

1.3 Technologically Properties of Sesame Seed Proteins

Sesame seed protein mainly contains globular proteins (globulins, 67.3 %) which is a storage protein (Khalid, Babiker & EL Tinay, 2003). Since it is mostly composed of globular proteins, its physicochemical properties are well researched. The 11S globulin and 2S albumin forms almost 80% of the total sesame seed proteins (Tai, Lee, Tsai, Yiu & Tzen, 2001). The main fraction of sesame seed protein which is α-globulin (11S globulin) is ~70% of the total protein in sesame (Onsaard, 2012). According to the food application, different technological properties of proteins might be required. These properties include solubility, gelling ability, emulsification activity, emulsion stability, water absorption and binding, oil and fat binding, foaming capacity and stability, flavor-binding and thermal stability. These properties should be examined properly because they are easily affected by the composition, interaction of proteins with other food components, and conformational changes (Fereidoon, John, Agustin, Glenn & Y., 1998). Additionally, some extrinsic factors such as process conditions used to isolate proteins or treatment (heating, salting) applied on the proteins might also have effect on the functional properties of proteins (Latif & Anwar, 2011).

1.3.1 Solubility

Solubility can be expressed as the amount of protein in a sample that dissolves into solution. As a better explanation, solubility is the thermodynamic equilibrium of proteins in the liquid and solid phases (Hall, 1996). Moreover, solubility of protein is related to its surface protein-protein interactions and protein-water interactions (Chaplin & Andrew, 1989).

The solubility of a protein is the primary parameter that need to be explored because solubility might have a direct effect on other functional properties. Solubility is the result of surface-active properties of proteins and it affects emulsification, foaming and fat binding properties.

According to Inyang and Ekanem the least solubility of sesame seed protein is obtained at pH 4, and then solubility shows an increase at below and above pH 4 (Inyang & Ekanem, 1996). A U-shaped curve is obtained when pH vs solubility graph was plotted, having the lowest solubility value at the isoelectric point (Zayas, 1997). Proteins are generally expected to be least soluble near their isoelectric point as shown in the Figure 1.6 which further supports that sesame seed proteins have the least solubility around pH 4. When there is no net charge on the protein surface, hydrophilic interactions cannot be dominant in the solution and proteins tend to aggregate and precipitate (Xie & Gao, 2013).

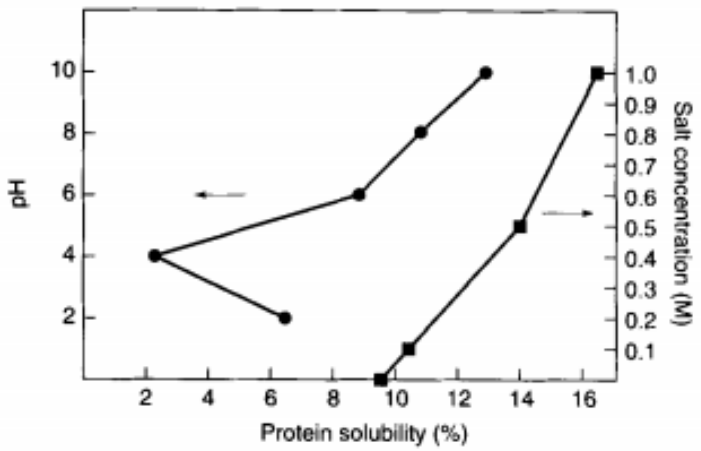

Figure 1.6. Solubility of sesame seed protein concentrate depending on pH and salt (NaCl) concentration (Inyang & Ekanem, 1996)

Another factor affecting protein solubility is ionic strength. Salts increase protein solubility as it was explained previously. To summarize, both salt type and concentration have influence on the protein solubility. Figure 1.6 also shows the effect of salt concentration on the protein solubility. Enhancing the hydrophilic interactions between protein and water by using some salts such as calcium (Ca^{2+}), magnesium (Mg^{2+}), bromide (Br^{-}), and iodide (I^{-}) is stated as *salting in* (E. Lee et al., 2017). On the other hand, some salts such as sulfate (SO_4^{2-}), ammonium (NH_4^{+}) have opposite effect by promoting protein-protein interactions and causing proteins to aggregate and precipitate (Aoki et al., 2016). Moreover, using salts in low concentration in the solution might act as a shield and stabilizes the charges on the protein surface and increase solubility (Oliyaei et al., 2019).

1.3.2 Emulsification Activity and Stability

Emulsions are described as a dispersed systems prepared by mixing two or more immiscible liquids (e.g. oil-water) (Damodaran, Parkin & Fennema, 2011a) . Food emulsions are generally classified as macro-emulsion with size of 0.2 to 50 μm (Zayas, 1997). Proteins can also act as emulsifiers and align at oil/water interface. Hydrophobic regions of proteins protrude into the oil phase while hydrophilic parts remain in the water phase (Hall, 1996). Therefore, an effective protein emulsifier should be able to adsorb to the interface (between oil-water), unfold at the interface, and create a cohesive film through intermolecular interactions (Karaca, Low & Nickerson, 2011). Figure 1.7 shows the representative picture of how proteins are located/aligned at interface.

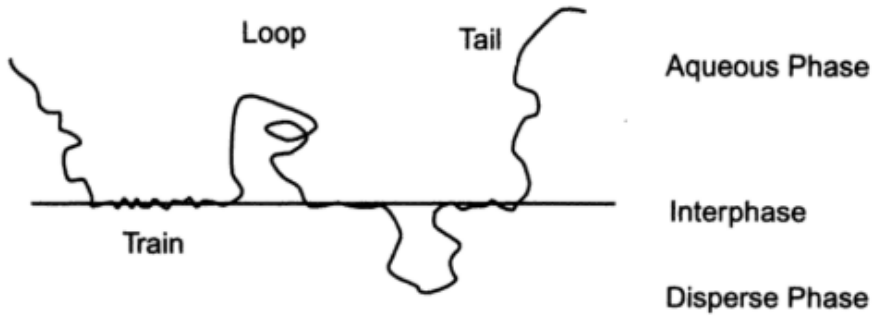

Figure 1.7. Representation of a protein molecule at oil-water interphase (Hall, 1996)

Emulsion stability is an important term that needs to be considered. Stability is related to the free energy of the system. The free energy of an emulsion is generally higher (except micro-emulsions) than the energy of the separate phases because interface created in emulsions increases the energy of the system (Dalgleish, 1997).

Emulsification activity can be described as a measurement of the quantity of oil that can be emulsified per unit of protein while the emulsion stability is the ability of the emulsion to resist changes over a certain period of time (Farooq & Boye, 2011). The highest emulsification activity of sesame seed was found at pH 10 (Chatterjee et al., 2015a). Moreover, same study confirmed that sesame protein had better emulsifying properties than soy proteins. Emulsification activity is related to solubility of proteins, consequently more soluble proteins result in higher emulsifying activity (Karaca et al., 2011). On the contrary, at isoelectric point, which is around pH 4.5 for sesame proteins, emulsifying activity is the lowest. Both emulsification activity and emulsion stability mainly depend on the diffusion of peptides in the oil and water phases. Diffusion of peptides seems to be easier when hydrolase enzymes are used during the extraction of sesame seed protein (Liu, Gasmalla, Li & Yang, 2016). Additionally, presence of larger number of hydrophilic-hydrophobic groups enables to stabilize the emulsion (Chatterjee, Dey, Ghosh & Dhar, 2015b). Therefore, sesame

proteins produced by enzyme extraction were found to have better emulsion formation and stability.

1.3.3 Gelation

Gelation is quite common in the food industry and there are several ways to produce food gels. Proteins generally form strong gels together with pectins, starches and gums (Kavanagh, Clark & Ross-Murphy, 2000). The quality of many food products especially textural properties is determined by the gelling ability of the proteins (Zayas, 1997). During the gel formation, proteins form a special 3-D network and entraps water (Jeantet, Croguennec, Schuck & Brulé, 2016).

Limited information is available for the gelation properties of sesame protein isolate due to the influence of other factors during protein extracted from sesame meal (Kanu et al., 2007). In some of the studies, heat-induced gels of sesame 11S globulin fraction was investigated, and it was found that heating time, temperature, protein extraction type, pH, enzymes, electrostatic interactions, hydrophobic interactions, and disulfide bridges had a great influence on the gel formation (Hasenhuettl & Hartel, 2019; Ohta et al., 2016).

It was also found that some modifications such as acylation might also affect the gelling ability of sesame protein (Hasegawa, Fujino, Okado & Suenobu, 2014). Some denaturation may occur due to acylation and it is followed by deacylation of sesame seed protein. It was observed that, proteins deacylated with maleic and citraconic anhydrides under acidic conditions could not form gel. The study concluded that some amino-acids should be acylated to form proper gels (Hasegawa et al., 2014). As mentioned before, ionic strength and pH are also other factors

affecting gel formation. Addition of salt in small quantities seems to enable gel formation in the form of fine stranded crosslink (Puppo & Añón, 1998). It was also shown that under acidic conditions, sesame protein isolate can form gel above 6% which can be considered as its critical gelation concentration (Inyang & Nwadimkpa, 1992).

1.3.4 Water Adsorption and Binding

Water adsorption and binding, also known as water holding capacity, water hydration and water retention, is the ability of a protein to retain its own and added water during the application of pressing, centrifugation, other forces, or heating (Shevkani, Singh, Kaur & Chand, 2015). The capacity of a food material to hold water is important especially in the texture and color of bakery products, doughnuts, pastas, soups and some meat products (Demirhan & Özbek, 2013). Additionally, it is important for the design of the packaging for a food product (Zayas, 1997). There are several ways of hydration of a protein by water. Ion hydration occurs because of the charged regions of proteins (Wingfield, 1998), hydrogen bonding (polar groups of protein and water) and hydrophobic hydration are other two mechanisms of hydration of proteins (Oliyaei et al., 2019).

Surface of the proteins directly influence water adsorption like in the cases of solubility, emulsifying properties, and oil binding. Protein surface is altered by the treatments (extraction method, modifications etc.) such as ionic strength, pH, and temperature (Damodaran, Parkin, & Fennema, 2011b; Hasenhuettl & Hartel, 2019). It was reported that sesame seed protein isolate's water holding capacity ranged between 1.29 to 3.30 g water/ g protein (Esssa et al., 2015). It is known that hydration of sesame protein isolate is directly affected by the denaturation degree of the protein

(Guerra, Park & Campinas, n.d.). Therefore, it is obvious that process type used to extract protein from the sesame meal affects hydration behavior.

1.3.5 Oil and Fat Binding

The fat binding property of proteins is required for products such as emulsions, dairy foods, sausage products, bread, and dough. This property directly influences the texture of food. Additionally, interaction of oil with proteins directly effects the flavor of foods because oils act as flavor retainer and increase the mouth feel of the food products (Aremu, Olaofe & Akintayo, 2007). Oil absorption capacity of a protein is related to the non-polar side chains and conformation of the proteins (Gbadamosi, Fasuan, Omolayo & Taiwo, 2017; Kinsella, 1979). Fat binding of proteins is affected by a few factors which are protein source, processing conditions, composition, and other components in the environment, particle size, and finally temperature (Zayas, 1997).

The more insoluble and hydrophobic proteins have better fat binding ability. It was found that sesame protein isolate and soy protein isolate had oil absorption capacity of 129% and 134%, respectively (Kanu, Sandy, Kandeh, Bahsoon & Zhou, 2009). Oil holding capacity of sesame seed protein can be also expressed as 1.50 ml oil/ g protein (Khalid et al., 2003).

1.4 Characterization of Sesame Seed Proteins

1.4.1 Fourier Transform Infrared (FTIR) Spectroscopy Analysis

Infrared (IR) light is discovered in 19th century and the first generation IR spectrometer was invented in 1950s to observe the structural changes of molecules (Christy, Ozaki & Gregoriou, 2001). Later, the effects of interactions between radiated energy in the IR range and materials are studied by IR spectroscopy. Since obtained spectrum is in time domain for an IR spectrometer, a transformation is required to observe the results in frequency domain (Kong & Yu, 2007). The reason behind this is to evaluate the specific bonds of the molecules at certain wavelengths (Fatima, 2018). This new version of the IR technique was named as Fourier Transform Infrared Spectroscopy (FTIR) (Anjos, Graça, Contreras & Antunes, 2015).

FTIR system consists of a source, sample compartment, detector, amplifier, interferometer, computer, analog to digital (A/D) convertor and fixed mirror. The working principle is to measure the absorption of IR frequencies by a sample located in the pathway of an IR beam (B. C. Smith, 2011). When the beam of IR radiation passes through the sample, sample either absorbs or transmits the radiation, depending on the molecular structure of the sample and the frequency. Certain molecular groups are excited by the application of IR radiation, then, they produce vibrations from the excited state at certain wavelengths (Anjos, Campos, Ruiz & Antunes, 2015). The Figure 1.8 shows the basic structure of a FTIR spectrometer.

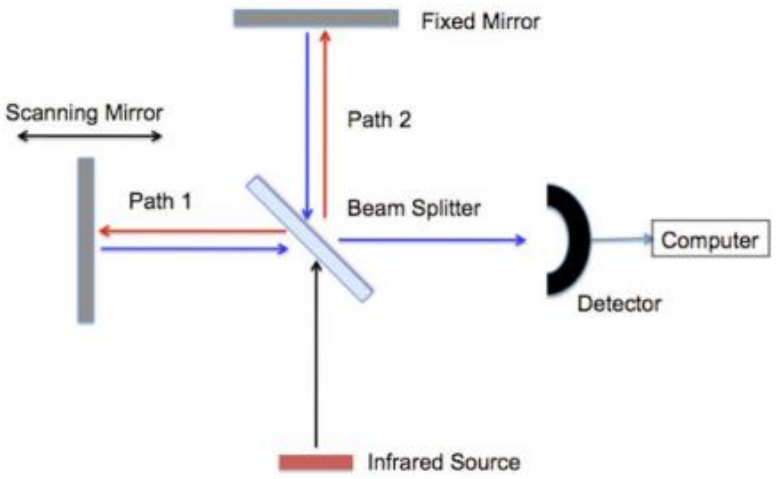

Figure 1.8. Basic structure of FTIR spectrometer (Fatima, 2018)

It is a known fact that the widest range of an IR spectrum is between 12800~10cm^{-1}. However, FTIR mostly works in the mid-IR range of 400-4000 cm^{-1} where the chemical and structural identification of the molecules can be observed (Rahmah et al., 2016). Indeed, there are four different regions of FTIR spectrum which are specified as single bond, double bond, triple bond, and fingerprint regions (Rahmah et al., 2016). In the Table 1.1 below, some of the functional groups and their wavenumber values where the peaks are obtained are listed:

Table 1.1 Functional groups and their frequency values

Functional Group	**Wavenumber (cm^{-1})**
Water OH Stretch	3700-3100
Carboxylic acid OH stretch	3600-2500
N-H stretch	3500-3350
≡C-H stretch	~3300
=C-H stretch	3100-3000
-C-H stretch	2950-2840
-C-H aldehydic	2900-2800
C≡N stretch	~2250
C≡C stretch	2260-2100
C=O aldehyde	1740-1720
C=O ketone	1745-1715
C=O amide	1700-1500
C=C alkene	1680-1600
C=C aromatic	1600-1400
C-O-C stretch	1250-1050
C-OH stretch	1200-1020
NO_2 stretch	1600-1500 and 1400-1300

As can be understood from the Table 1.1, different functional groups have different wavenumber values. Thus, by just looking at the peaks in the certain range of the spectrum in FTIR, chemical composition of samples can be analyzed and evaluated in detail (Naczk & Shahidi, 2004). This is one of the reasons why FTIR technology is commonly used in all chemical areas.

In food applications, FTIR is preferred due to its reliable, accurate, and sensitive analysis on the data obtained (Ulberth & Haider, 1992). Moreover, it analyses the compounds in both qualitative and quantitative ways. Apart from those, FTIR is considered as time saving, precise, basic, efficient, and nondestructive technology (Kong & Yu, 2007; Zou et al., 2017b). For all those reasons, there are lots of organic compounds that are examined by FTIR spectrometer in food area such as proteins, lipids, carbohydrates, and nucleic acids.

1.4.2 Time Domain Nuclear Magnetic Resonance Relaxometry

Cornelis Jacobus Gorter, who was a Dutch experimental and theoretical physicist, discovered paramagnetic relaxation in 1936 (Becker, 1993). However, he missed the discovery of nuclear magnetic resonance. The nuclear magnetic resonance (NMR) phenomenon was discovered in 1945 for the first time by Bloch, Hansen, and Packard at Stanford University by detecting a signal from water (Taveras, 1984). At the same time, Purcell, Torrey, and Pound from Harvard University, detected a signal from the protons of paraffin wax. Bloch and Purcell were awarded Nobel prize this discovery in the area of physics in 1952 (Becker, 1993).

TD-NMR has become a useful and reliable analytical tool used in both research and industry to get detailed information about molecular dynamics and structure, reaction state, and chemical environment of the molecules (Zhou, 2018). TD-NMR is mostly used in low-field systems. This technique is particularly applied on foods as it allows for a nondestructive analysis on their internal structure. The signal obtained from NMR relies on the interaction of electromagnetic radiation in the radio frequency (RF) range and nuclei such as hydrogen and carbon (Aboonajmi & Faridi, 2016). The presence of these nuclei varies in food with different chemical and structural properties, affecting molecular mobility while under the effect of radiation.

The basic principle of TD-NMR is based on nuclear magnetism and relies on a nucleus with an odd number of nucleons, and as hydrogen is abundantly available is highly sensitive to magnetic resonance, it is mostly preferred (Kirtil & Oztop, 2016). On a benchtop NMR system, when a sample is place between the magnetic coil, the protons align their spins in or against the direction of the magnetic field, resulting in a net magnetization in the z-axis (longitudinal magnetization). When an RF pulse is applied to this system, a new magnetic field is generated in the x-y plane while the protons are flipped onto this plane resulting in an increase in the transverse

magnetization. When the RF pulse is removed, the protons return to their original magnetization in the z-axis. The longitudinal relaxation time, also known as spin-lattice or T_1 relaxation time, is the time it takes for z component of the longitudinal magnetization to reach 63% of its initial value while the transverse relaxation time or the spin-spin relaxation time is the time required for the transverse component of the magnetization to decay to 37% of its original value which is also known as T_2 relaxation time (Dale, Brown & Smelka, 2015). Figure 1.9 and Figure 1.10 show example T_2 and T_1 curves, respectively.

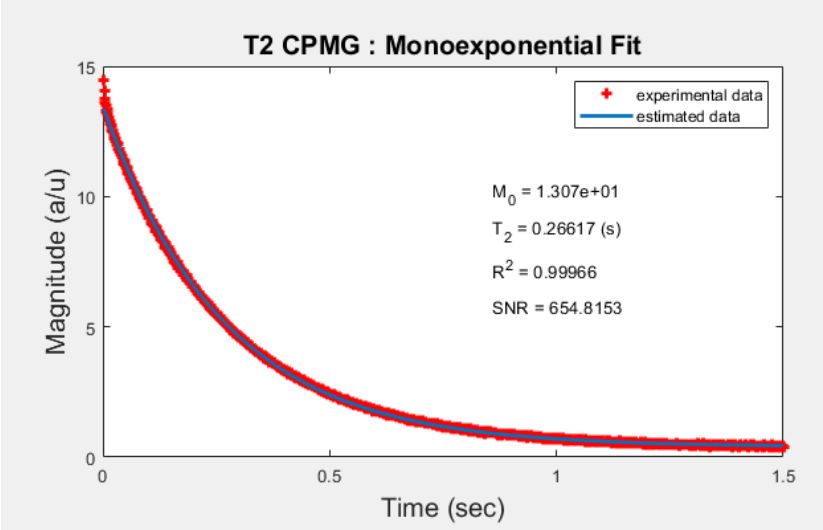

Figure 1.9. Example for T_2 Curve in TD-NMR

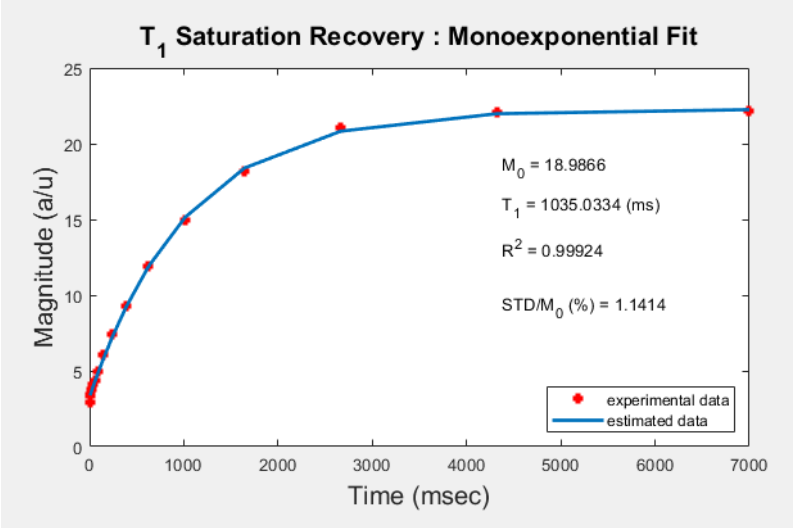

Figure 1.10. Example for T_1 Curve in TD-NMR

Due to the differences in food types, the relaxation times can vary and be used to differentiate between different properties of food. Table 1.2 shows some application of TD-NMR in various food systems(Rodríguez-Alonso, Vergeldt & van der Goot, 2019).

Table 1.2 Main nuclear magnetic resonance applications on food science (Rodríguez-Alonso et al., 2019)

Application		Feedstock
Analysis of food components	Water	Soy protein isolate, vital wheat gluten, pea protein isolate, lupin protein concentrate, vital wheat gluten
	Fat (fatty acids)	Mustard seeds, rapeseeds, beef, chicken, pork meat, milk
	Protein (amino acids)	Mustard seeds, spring rapeseeds, orange juice, grapefruit juice
Processing	Rheology	Wheat doughs
	Salting and storing	Wild and farmed Atlantic cod

1.4.2.1 Hydration Behavior and Gelling Ability by NMR Relaxometry

Differences within a material have been made possible by applying a series of pulses with varying parameters. Different sequences have been used to measure varying properties as well as to differentiate water populations within a sample. It has been known that a spectral analysis for a sample does not provide enough information on the type of water populations present in it as they do not show any chemical differences. However, with NMR relaxometry, different T_2 times can be obtained for both bound and free water within a sample, providing information about the water holding capacity of a proteinaceous sample (Rodríguez-Alonso et al., 2019). Carr-

Purcell-Meiboom-Gill (CPMG) pulse sequence is generally used to obtain T_2 values from different water populations within a sample (Dekkers et al., 2016). The same sequence has been proven TD-NMR method to be an effective method over conventional centrifugation method to analyze water holding capacity of protein gels as it allowed for a more detailed analysis (Peters et al., 2016). Figure 1.11 shows the CPMG pulse sequence below.

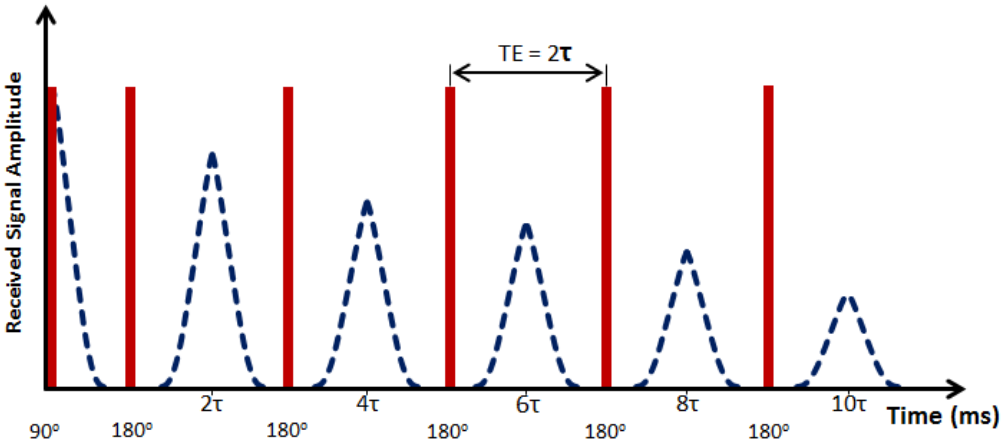

Figure 1.11. CPMG Pulse sequence (Dragan, Crainic, & Fechete, 2018)

1.4.2.2 Determination of Oil Content of the Seed-oils by TD-NMR

Some seeds such as cotton seed, sunflower, soybean, and sesame are generally grown for their oil content. Therefore, accurate and rapid determination of oil content play an important role for the buyers, growers, and breeders. The conventional method for determination of oil and fat content in food samples is Soxhlet extraction. Although, this method is easy to apply and provides excellent results, it requires a long time to carry out the experiment and produces a large amount of toxic waste which is expensive to dispose of and is harmful to the environment (Luque de Castro & García-Ayuso, 1998). Other novel methods are thus sought after. IR spectroscopy was found to be suitable for this, however; there was a struggle with calibrating the

devices. TD-NMR is a common technique applied to calculate oil and fat content in oil seeds. TD-NMR might give a quantitative determination of water and oil fractions due to the bound water found in the cellulose matrix which causes NMR signal coming from the water content to decay quickly in comparison with relatively free oil signal. Traditionally, Free Induction Decay (FID), spin echo and CPMG sequences have been used for this purpose (Gambhir, 1992).

Hahn echo is a type of spin echo sequence and it can be a good alternative way of measuring the oil content (Resonance Systems, 2012). As it can be seen in Figure 1.12, the portion after applying 90° RF pulse shows the signal coming from both water and oil, and echo signal coming after the application of 180° RF pulse shows the signal coming from oil only.

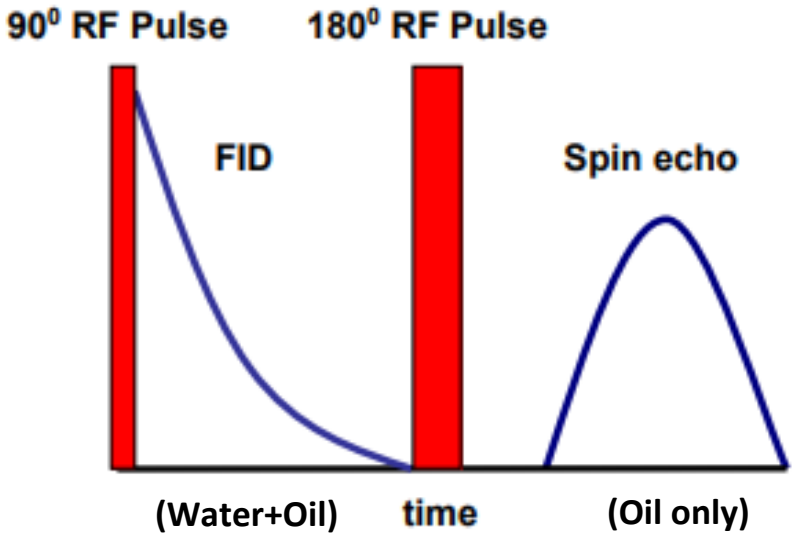

Figure 1.12. Hahn echo sequence (ResonanceSystems, 2012)

1.4.3 Monitoring of Sesame Seed Protein Extracts by Gel Electrophoresis

Electrophoretic procedures involve the migration of electrically charged molecules because of an electric field applied between a cathode and an anode (Vesterberg, 1993). The first steps for the electrophoretic methods have started with Faraday in 1871, after he presented his laws of electrolysis (Michaud & Asselin, 1995). The electrophoretic mobility of individual proteins and nucleic acids depends on various factors including the charge on the protein surface, size, shape, and the strength of electric field applied. Additionally, the pH of the electrophoresis buffer also influences the net charge of the protein molecule. Therefore, separation of proteins with high resolution is possible based on both the charge and size of the protein molecules. There are many different electrophoretic methods to separate proteins according to their charges such as disc electrophoresis, displacement electrophoresis (isotachophoresis), and isoelectric focusing (Andersson, Borg & Mikaelsson, 1972). However, many researchers attempted to eliminate the charge effect and thereby obtained a direct estimate of only the size of the protein molecules (Vesterberg, 1993). Protein separation that is based on only on the size of the protein molecules is obtained by the application of polyacrylamide gel electrophoresis. The combination of isoelectric focusing and polyacrylamide gel electrophoresis (PAGE) in a second-dimensional slab gel results in the highest resolution with up to innumerable spots per gel (Michaud & Asselin, 1995).

PAGE has been widely used as a technique for the characterization and separation of proteins (Andersson et al., 1972). A typical electrophoretic run involves separating of samples on a gel which is immersed in a buffer. Proteins migrate from on electrode to another one. Once separation is done, gel can be stained by a variety of procedures to visualize proteins separated, or it is further processed to detect fluorescent or radioactive tags present, or immunoassays can be applied to characterize the proteins.

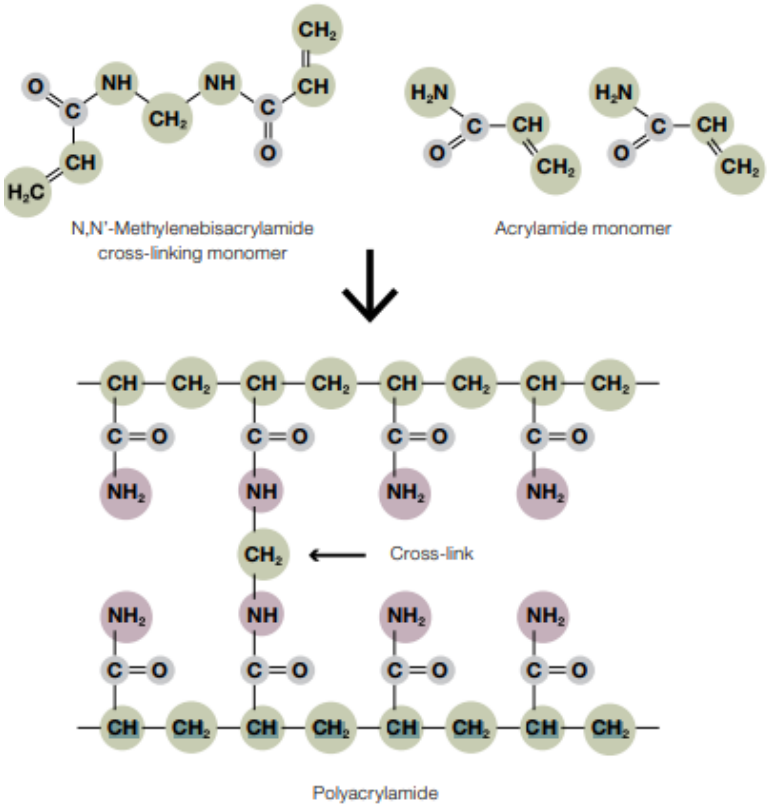

Figure 1.13. Polymerization of acrylamide monomers and bisacrylamide (Laboratories Bio-Rad, 2012)

Polyacrylamide gels are produced by polymerization of acrylamide and N,N'-methylene-bis-acrylamide as shown in Figure 1.13 above. The resulted gel is rigid and stable, having pores in it and molecules are able to move in these pores. Polyacrylamide gel has the advantage of having the lowest level of interaction with migrating molecules (Büyükköroğlu, Dora, Özdemir & Hizel, 2018). A representative Figure 1.14 shows the structure of the polyacrylamide gel and movements of proteins which have different molecular weights.

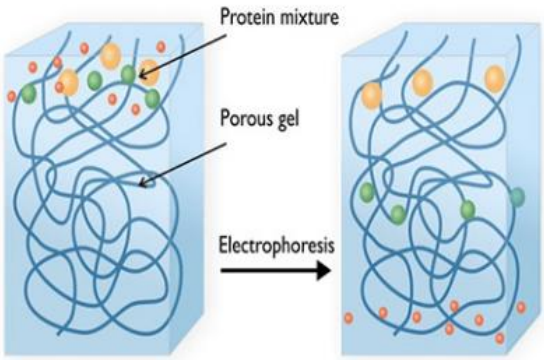

Figure 1.14. Representative picture of polyacrylamide gel and protein movement according to their molecular weight (Hassanzadeh, 2018)

PAGE has been used in food analysis for the separation of proteins and molecular weight determination as a qualitative tool. This method gives the opportunity to investigate the patterns of polypeptides and assay their activities and properties. Enzymes and their activity used in food industry can be examined by using electrophoresis. Also, non-denaturing PAGE (Native-PAGE) gives information about the conformation and state of the protein and its subunits. In food analysis, generally 2 types of polyacrylamide gel electrophoresis are applied which are one-dimension PAGE and two-dimension PAGE (Jada, Soitamo & Lehto, 2013). In one-dimensional PAGE, proteins are separated depending on only size by treated by Sodium dodecyl sulfate (SDS), β- mercaptoethanol, and heating. On the other hand, in two-dimensional electrophoresis, firstly proteins are subjected to denaturing isoelectric focusing (IEF) in a tube gel and separated according to their charge, isoelectric point (pI), later, to SDS-PAGE for further separation depending on their size (Laboratories Bio-Rad, 2012). Two-dimensional PAGE is not frequently used in food analysis because of its complexity, generally one-dimensional, denaturing SDS-PAGE is preferred.

1.4.3.1 Non-denaturing (Native) Polyacrylamide Gel Electrophoresis

The original discontinuous Native-PAGE system was developed by Ornstein and Davis for the separation of human serum proteins in a preserved manner by conserving the native configuration of proteins and preserving the biological activity, and subunit interactions (Davis, 1962; Ornstein, 1962).

In these systems, non-reducing, non-denaturing sample buffers are used to prepare protein samples, and electrophoresis is performed in the absence of any type of denaturing agents. It is difficult to interpret the data obtained from native PAGE due to the preservation of native charge-to-mass ratio of proteins (Poveda, Vilcacundo, Carpio, & Carrillo, 2016). As a result, protein mobility is determined by complex combination of factors. Protein-protein interactions are preserved during this process, therefore some proteins run in the polyacrylamide gel as multi-subunit complexes and their movement becomes unpredictable (Akbar, Yousaf, Rabbani, Shinwari, & Masood, 2012). Another issue is that since native charge is preserved, protein migration can occur toward both cathode and anode, depending on their charge (Laboratories Bio-Rad, 2012).

In conclusion, native PAGE is not a suitable method to predict molecular weight of proteins. However, it allows the separation of proteins in their active state and native configuration. Also, native PAGE resolve proteins of the same molecular weight.

1.4.3.2 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

This protocol was described by Laemmli (1970) for the first time as a denaturing polyacrylamide gel system utilizing SDS to separate protein molecules based on the

size only (Laemmli, 1970). SDS-PAGE can be used for monitoring purification of proteins, and to assess the molecular weights for unknown protein samples. Electrophoretic separation of proteins, and protein bands are shown in the Figure 1.15.

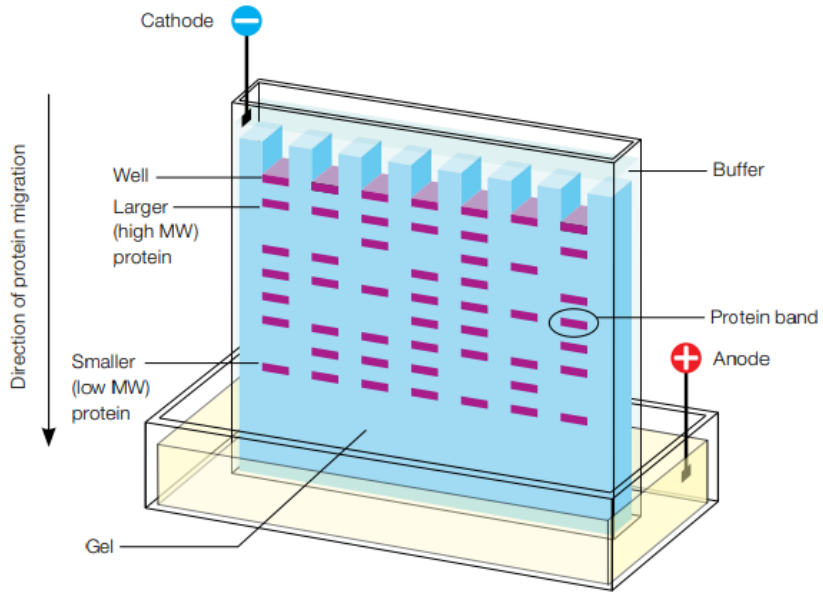

Figure 1.15. Schematic diagram of electrophoretic separation of proteins in polyacrylamide gel. (MW, molecular weight) (Laboratories Bio-Rad, 2012)

The reason behind of including SDS in the electrophoresis was to overcome some limitations caused by Native-PAGE. When protein separation is achieved in the presence of SDS and denaturing agents (heat, β- mercaptoethanol) they become fully separated from each other and denatured. SDS binds to proteins noncovalently and causes and overall net negative charge on the protein surface since its negative charge masks intrinsic charge of the proteins it binds (Beyer, Bardina, Grishina & Sampson, 2002). Moreover, it changes the tertiary conformation of proteins to a long, rod-like shape as shown in the Figure 1.16.

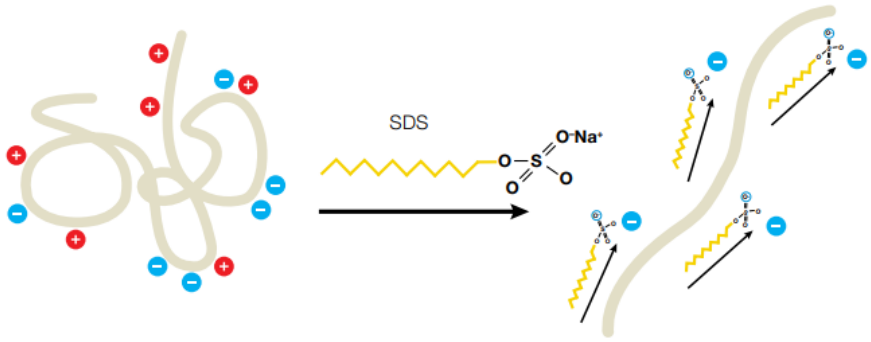

Figure 1.16. Effect of SDS on the charge and conformation of proteins (Laboratories Bio-Rad, 2012)

Also, denaturing agents such as β- mercaptoethanol plays role in the protein denaturation by breaking down the cysteine knots (S-S bonds). As a result, proteins migrate from negative electrode (cathode) to positive electrode (anode) depending on their size only and it enables molecular weight estimation (Aluko & McIntosh, 2001).

1.5 Objectives

Nowadays, plant-based food proteins have become remarkably popular and valuable because of the increase in the production of vegan foods and characteristics of these proteins. In Turkey, sesame seed is consumed in a great amounts because of the production of some products such as simit, tahini, and halva. On the other hand, sesame seed and sesame meal contain significant amount of protein at an average of 25% and 40%, respectively.

However, it is a known fact that there is no extended study about sesame seed proteins in the literature although sesame seed contains significant amount of protein which cannot be disregarded. Sesame seed meal, a byproduct of sesame oil production, is actually a potentially good quality protein, but so far it has only been used as animal feed. There might be various applications of these valuable proteins and depending on the application, different processes might be required to obtain the proteins.

The primary objective of this study is to extract sesame seed protein from sesame seed meal by using different extraction techniques under various conditions and characterize the properties of the proteins. Another objective is to show that TD-NMR can be a good substitute for some conventional methods used for years for characterizing the obtained proteins.

Following the extraction, to observe the differences caused by different extraction methods, some important physicochemical properties were measured such as solubility, denaturation degree, gelling ability, hydration behavior, emulsification activity and emulsion stability. FTIR experiments were also performed to monitor the chemical changes observed on the proteins with different extraction methods.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Sesame seed meal was obtained from a cold-press oil producing company, ONEVA, as a by-product of sesame seed oil production (İstanbul, Turkey). Pea protein concentrate with a 65% protein content was purchased from NorCal Organic (Crescent City, California). Whey protein isolate with 98% protein content was purchased from Nutricost (Utah, USA).

Copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$), sodium potassium tartrate tetrahydrate ($KNaC_4H_4O_6.4H_2O$), sodium hydroxide (NaOH), Folin-Ciocalteau's phenol reagent, Bovine Serum Albumin (BSA), Boric acid (H_3BO_3), hydrochloric acid (HCl), sodium dodecyl sulfate (SDS), sodium carbonate (Na_2CO_3), ethanol (C_2H_5OH), β-mercaptoethanol, glycine, acrylamide, N,N'-methylene-bis-acrylamide, Tetramethylethylenediamine (TEMED), ammonium persulfate (APS) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Alcalase 2.4 L enzyme was purchased from Novozymes (Bagsværd, Denmark).

2.2 Methods

2.2.1 Extraction of Sesame Seed Proteins

Three different extraction techniques were used to extract the protein from sesame seed meal.

2.2.1.1 Alkaline Extraction

Sesame seed proteins were extracted by the alkali method developed by Onsaard et al (2010). Firstly, sesame seed meal was mixed with water at a ratio of 1:10 (w/v). The pH of the sesame seed meal and water mix was adjusted to 12 by using 1 M NaOH. The suspension was continuously stirred with a magnetic stirrer (Daihan Scientific Co., Ltd., Korea) for 1 hour and then centrifuged at 2263 xg for 20 minutes (MF-80, Hanil Science Industrial Co. Ltd., South Korea). Supernatant, which contained the soluble proteins, was taken and its pH was adjusted to 4.5, which is the isoelectric point of the sesame proteins, using 1 M HCl. The suspension was centrifuged again at 2263 xg for 20 minutes. Proteins precipitated because of their isoelectric point. Supernatant was poured away, and precipitate was taken. The pH of the precipitate was adjusted to 7 by using 1 M NaOH to neutralize the proteins and left overnight at 4°C. Finally, dried proteins were obtained after lyophilization (Beijing Songyuan Huaxing Technology Development Co., Ltd., China) for 48 hours and used for further analysis. Extracted samples were kept at 4°C in glass containers wrapped with parafilm.

2.2.1.2 Salt Extraction

Sesame seed meal was weighed and suspended in 0.6 M and 1 M NaCl at pH 6, with a ratio of 1:10 (w/v) at the room temperature and suspension was stirred for 1 hour and then centrifuged at 2263 xg for 20 minutes. Supernatant, which contained the soluble proteins, was taken and its pH was adjusted to 4.5 using 1 M HCl. The suspension was centrifuged again 2263 xg for 20 minutes. Supernatant was poured away, and precipitate, which included the proteins, was taken. Neutralization and storage were done as in the previous section.

2.2.1.3 Enzyme Assisted Alkaline Extraction

Enzyme extraction method developed by previous researchers was modified and used (Latif & Anwar, 2011). Firstly, sesame seed meal was mixed with water at a ratio of 1:10 (w/v). After heating the sample up to 65°C, pH of the suspension was adjusted to 8.5 by using 1 M NaOH to obtain optimum conditions for the enzyme, protease mixture Alcalase 2.4L, and then it was added to the suspension (amount of 2% enzyme by seed weight). In the water bath, sample was constantly shaken for 120 minutes at 80 rpm. Then, the suspension was centrifugated at 2263 xg for 20 minutes which yielded 3 distinct phases: oil phase, creamy & aqueous phase, and finally wet meal (precipitate) part at the bottom. Wet meal was taken, then neutralization and storage were done as in the previous section and this sample was called as E-Precipitate.

Oil and creamy phase which is also called as aqueous creamy phase (ACP) was taken separately and the pH was adjusted to 12 by using 1 M NaOH. The suspension was continuously stirred with a magnetic stirrer for 1 hour and then centrifuged at 2263 xg for 20 minutes. Supernatant, which contained the soluble proteins, was taken and

its pH was adjusted to 4.5, which is the isoelectric point of the sesame proteins, using 1 M HCl. The suspension was centrifuged again at 2263 xg for 20 minutes. Proteins precipitated because of their isoelectric point. Supernatant was poured away, and precipitate was taken. Neutralization and storage were done as in the previous section and this sample was called as E-ACP.

2.2.2 Sample Preparation for Pea Protein Concentrate and Whey Protein Isolate

Pea protein concentrate and whey protein isolate were used in the analysis to compare the results with sesame seed protein extracts obtained by three different extraction methods. Depending on the analysis, they were used directly as powder form or 1% (w/v) protein solutions.

1% (w/v) protein solutions were prepared by weighing 100 mg protein powder and dissolving in 10 ml of distilled water. After dissolving, protein suspension was constantly shaken overnight at the room temperature at 120 rpm . Then, samples were centrifuged at 2263 xg for 20 minutes and supernatant was used for the further analysis.

2.2.3 Characterization of Sesame Seed Proteins

2.2.3.1 Measurement of Moisture Content

Infrared moisture analyzer was used to measure the moisture content of the samples after 48 hours of lyophilization (Radwag MAC 50 Moisture Analyzer, Poland). Data obtained after all the analyses were reported on dry basis.

2.2.3.2 Determination of Hydration Behavior using Nuclear Magnetic Resonance (NMR) Relaxometry

For NMR experiments, 0.15 g of solid sample was put into small tubes and 0.45ml of distilled water was added to it and well mixed. Spin-spin relaxation times (T_2) were measured for these samples by using a CPMG (Carr-Purcell-Meiboom-Gill) sequence on a 0.5 Tesla (20.34 MHz) NMR Relaxometry system (Spin Track, Resonance Systems GmbH, Kirchheim/Teck, Germany). Table 2.1 given below shows the parameters used for NMR relaxometry measurements. Mono-exponential fitting of the T_2 decay data was performed (R2019b, The MathWorks Inc., USA) to collect the data and interpret the results. The mathematical equation used for fitting of the T_2 decay is expressed as :

$$M_{xy}(t) = M_0 * e^{-t/T_2}$$

where M_0 is the initial magnetization, M_{xy} is the magnetization within the x-y plane with the decay rate of T_2.

Table 2.1 NMR Relaxometry System Parameters

Parameters	Values
Echo Time	600 ms
Number of Echo	800-4000
Number of Scans	4
Repetition Time	1000 ms

2.2.3.3 Determination of Gelling Ability using Nuclear Magnetic Resonance (NMR) Relaxometry

Gelling behavior of extracted proteins were examined by preparing protein solutions at different concentrations (20%, 15%, 10%, 5%) with distilled water in NMR tubes directly. Then, T_2 (spin-spin relaxation time) values were measured before heating the solutions. For triggering gel formation, solutions were heated up to 80°C in a water bath (Daihan Scientific Co., Ltd., Korea) and kept at that temperature for 30 minutes. Finally, solutions were cooled down to the room temperature, and T_2 relaxation times were measured. T_2 measurements were conducted as in hydration experiments. However, since samples changed acquisition parameters changed accordingly. Table 2.2 given below shows the parameters used for NMR relaxometry measurements. Data were fitted to a mono exponential model for calculating the T_2s.

Table 2.2 NMR Relaxometry System Parameters

Parameters	Values
Echo Time	1000 ms
Number of Echo	600-3000
Number of Scans	4
Repetition Time	1000 ms

2.2.3.4 Fourier Transform Infrared (FTIR) Spectroscopy Analysis

Sesame seed protein samples were examined with an IR Affinity-1 Spectrometer with Attenuated Total Reflectance (ATR) attachment (Shimadzu Corporation, Kyoto, Japan). The samples were analyzed in the region of 4000-500 cm^{-1} for 32

scans at a resolution of 4 cm^{-1}. The obtained spectra were analyzed by making comparison with each other and the literature.

2.2.3.5 Protein Assays

2.2.3.5.1 Determination of Total Protein Content

Kjeldahl method was followed to determine the total protein content of the samples (Corti, Agnelli & Ugolini, 1999). 1 g sample was used for this method. Kjeldahl method consisted of 3 main steps. The first step was digestion where protein was decomposed by a solution of concentrated sulfuric acid (H_2SO_4). In digestion, 25 ml H_2SO_4 was added into the Kjeldahl tubes along with catalyst tablet and boiling chips. The second step was distillation and neutralization, therefore; samples coming from digestion unit was placed in distillation unit (S3, Behr Labor-Technik GmbH, Germany). Excess base solution was added to the ammonium sulfate salt to convert ammonium (NH_4^+) to ammonia (NH_3). 40% NaOH was used as base solution. For capturing the ammonia gas, 50 ml 4% H_3BO_3 solution, and methyl red indicator was added to the tubes manually to observe the color change in the next step, titration. The amount of ammonia present in the distillate was measured by applying titration and observing a color change. At the end, the total nitrogen content of the sample was calculated and then by using a conversion factor (6.25); crude protein content of the sesame seed protein extracts was calculated.

2.2.3.5.2 Determination of Soluble Protein Content

Lowry method was used for the determination of the soluble protein content.(Lowry et al., 1951). The reagents used to prepare Lowry solution are given in Table 2.1. Lowry solution was prepared by mixing reagents A:B:C at a volume ratio of 100:1:1, respectively.

0.5 mL protein sample (1% (w/v) protein solution) was mixed with 2.5 mL Lowry reagent and incubated 10 min at room temperature. Then, 0.25 mL Folin-Ciocalteu's phenol reagent (diluted with a ratio of 1:1 from 2N stock solution) added to the tubes, mixed, and incubated in the dark for 30 minutes. Finally, the absorbance values were recorded at 680 nm by a UV/VIS Spectrophotometer Optizen Pop Nano Bio (Mecasys Co. LTD, Korea). The calibration curve was obtained by using BSA as the standard in a concentration range of 0.03125-0.5 g/L. Calibration curve was obtained by absorbance vs g/L BSA concentration and given in the Appendix A. The Table 2.3 given below shows the reagents used in the Lowry method.

Table 2.3 Reagents used in the Lowry method

Reagent A	2% (w/v) Na_2CO_3 dissolved in 0.1 N NaOH
Reagent B	2% (w/v) $CuSO_4 \cdot 5H_2O$
Reagent C	2% (w/v) $C_4H_4KNaO_6 \cdot 4H_2O$

2.2.3.6 Oil Content Determination Experiments

2.2.3.6.1 Determination of Oil Content Using Soxhlet Method

Soxhlet extraction was performed by using the Soxhlet apparatus (EFLAB) to determine the oil content of the protein extracts. Hexane was used as the solvent and 5 g sesame seed protein extracts were placed to the column of the soxhlet apparatus. Oil extraction continued for 5-6 hours. Later, the weight of the remaining oil was measured, and oil content was calculated as percentage accordingly.

2.2.3.6.2 Determination of Oil Content Using Nuclear Magnetic Resonance (NMR) Relaxometry

For the oil content measurement, protein extracts in the powder form was directly put in the NMR tubes, and percent oil content for the samples were determined by using Hahn echo sequence on a 0.5 Tesla (20.34 MHz) NMR Relaxometry system (Spin Track, Resonance Systems GmbH, Kirchheim/Teck, Germany). The Table 2.4 given below shows the parameters used for NMR relaxometry measurements. Later, a calibration curve was plotted to find the exact oil content of the samples.

Table 2.4 NMR Relaxometry System Parameters

Parameters	Values
Number of Scans	128
Repetition Time	300 ms

2.2.3.6.3 Monitoring Proteins by Gel Electrophoresis

Firstly, the polyacrylamide gel concentration was decided according to the expected size of the protein to be investigated. Two different gels were prepared, the stacking gel (4%) and the resolving gel (12%). They had different concentrations to align the protein samples first and then separate them at the same time. In this way, only the size was going to be the parameter that separates the proteins. Protein sample were treated by SDS and β- mercaptoethanol. Buffers having different pH values were used as running buffer (6.8) and gel buffer (8.8). Samples were denatured at 95°C for 5 minutes after the chemical treatments and at the same time the gel was formed. Then samples were loaded in the gel and by applying an electrical potential, negatively charged proteins migrated to positive electrode. At the end, the proteins

were separated depending on their size. Larger molecules migrated slower and smaller ones migrated faster (Laemmli, 1970).

2.2.3.7 Determination of Emulsification Activity and Stability Index

For the determination of emulsification activity index (EAI) and emulsification stability index (ESI) of the extracted proteins, modified method of Pearce and Kinsella was used (Nasrollahzadeh et al., 2017; Pearce & Kinsella, 1978). Grinded dry samples were mixed with sodium buffer (pH 7.4) with 10% (w/v) and placed into shaker for 24 hours for complete hydration. Sample solutions were centrifuged at 2688 xg for 5 minutes. 0.9 ml sample was mixed with 0.3 ml corn oil and stirred in silent crusher (60000 rpm, 1 min). 0.1 ml sample-oil mixture were added to 7 ml of sodium dodecyl sulfate solution (SDS solution). Absorbance data was measured at 340 nm. For emulsion stability, second absorbance data was measured after 10 minutes. EAI and ESI values are calculated according to the formula below:

$$EAI\left(\frac{m^2}{g}\right) = \frac{2 \cdot 2.303 \cdot A_0 \cdot N}{c \cdot \varphi \cdot 10000}$$

$$ESI\ (\text{min}) = \frac{A_0}{\Delta A} \cdot t$$

where, A_0 is the absorbance of the diluted emulsion immediately after homogenization, N is the dilution factor (×70), c is the weight of protein per volume (g/mL), φ is the oil volume fraction of the emulsion, ΔA is the change in absorbance between 0 and 10 min (A_0–A_{10}) and t is the time interval, 10 min.

2.2.3.8 Statistical Analysis

Statistical analysis on all the results were conducted by using analysis of variance (ANOVA) (Minitab Version 19, Minitab Inc., Coventry, U`K). Tukey's comparison test was used to compare the results at confidence interval of 95% as a multiple comparison test. In all experiments, three replicates were used. All the data were represented as mean ± standard deviation of the replicates for each experiment by excluding the outliers. The letters in the tables and figures denote significant difference among the samples at 95% CI.

2.2.4 Experimental Design

Experimental design is given below in the Table 2.5

Table 2.5 Experimental design

Extraction Method	Parameters	Analysis
Alkaline Extraction	pH 12	- Emulsification activity and emulsion stability - FTIR (Fourier-transform infrared spectroscopy) - Separation of Proteins by Electrophoresis - Oil content - Protein content (Total & Soluble) - Hydration behavior - Gelling Ability - Statistical analysis
Salt Extraction	0.6 M NaCl 1 M NaCl	
Enzyme Assisted Alkaline Extraction	Protease enzyme mixture (pH 8.5, 65 °C) + pH 12	

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Protein Content & Yield

3.1.1 Protein Yield

Protein yield is one of the most important parameters to be checked following extraction and protein amount was measured by Kjeldahl method. It is expressed as the total amount of protein recovered from the main protein source after the protein extraction. In this study, protein yield results calculated as follows and given in Figure 3.1 :

$$\text{Yield \% (w/w)} = \frac{Amount\ of\ extracted\ protein\ from\ sesame\ meal\ (g)}{Amount\ of\ protein\ present\ in\ sesame\ meal\ (g)} \times 100$$

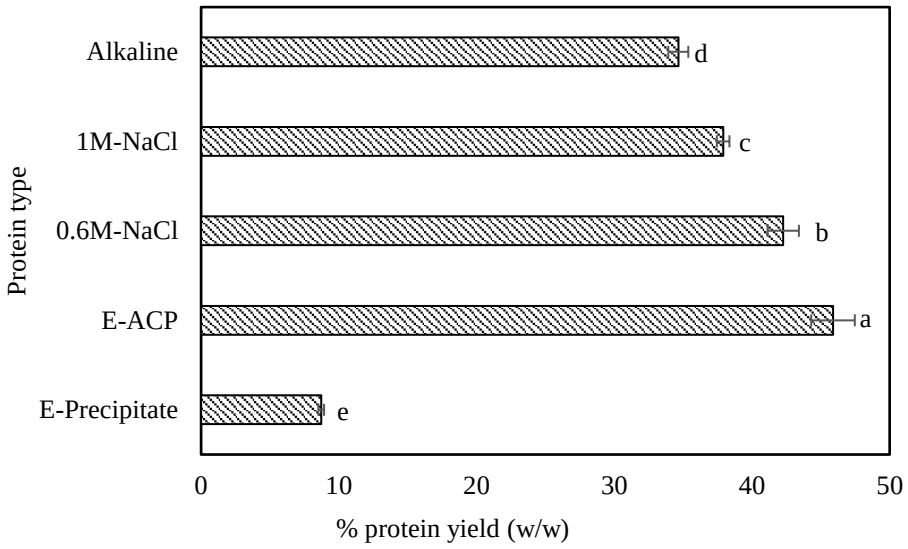

Figure 3.1. Protein yield (%) (w/w) for the extracted sesame seed proteins

According to statistical analysis, it was shown that extraction type had a significant effect on the protein yield ($p<0.05$). The highest yield was obtained by E-ACP part of the enzyme extraction having 45.8% (w/w) ± 1.58 protein yield. On the contrary, the lowest protein yield was found from the precipitate portion of the enzyme extraction, E-Precipitate which was an expected result since it was the remaining part of the extraction.

Alkaline extraction was found to have a lower yield when compared with enzyme and salt extractions. It was found that sesame seed protein fractions had better solubility with the addition of salts (salting in effect) due to the formation of a surface layer around proteins that improved the solvation (Ivanova & Chalova, 2018). According to a study, pH had important effect on the extraction of albumin proteins whereas ionic strength had a more dominant effect than pH while extracting the globulin fractions (Albe Slabi et al., 2020).

Enzyme extraction can be a preferred method over the other methods, yielding high protein amount since it has the advantage of potential stereochemical specificity, and mild process conditions as mentioned in Chapter 1.

3.1.2 Measurement of Soluble Proteins

Functional properties of the proteins are important while formulating and processing foods and one of the most important functional properties is the solubility. In this study, soluble protein amount was measured by using Lowry method and results were given as percent protein solubility (w/w) on dry basis. While evaluating the functional properties results; Whey Protein Isolate (WPI) and Pea Protein Concentrate (PPC) were used as benchmarks and they were also included in the

related figures. Specifications of these commercial protein powders were provided in the *Materials and Methods* Section.

Experimental results are given in the Figure 3.2. According to the statistical analysis, it was found that protein type had a significant effect on the protein solubility ($p<0.05$). The highest solubility was observed in the WPI samples. Solubility of E-ACP powder was slightly lower than pea protein concentrate.

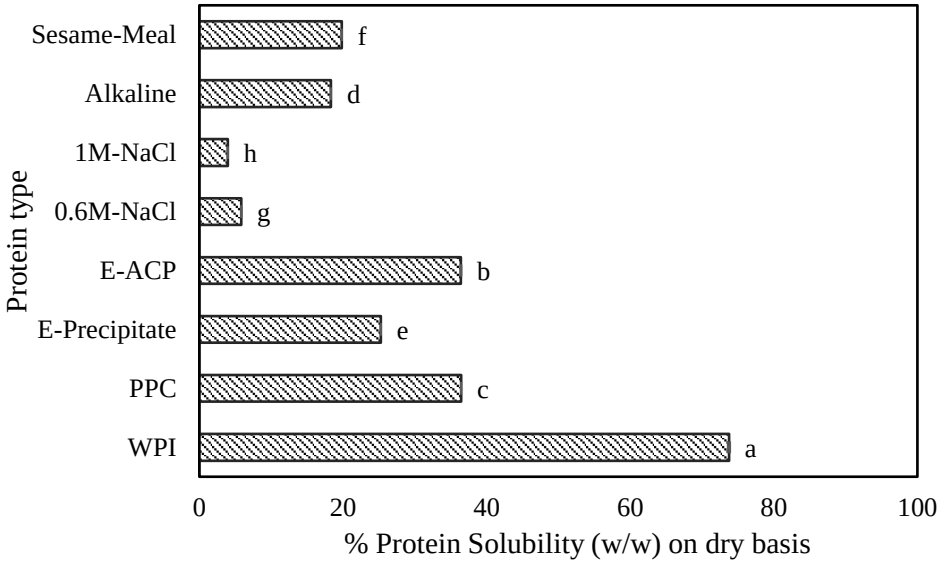

Figure 3.2. Protein solubility (%) (w/w) given on dry basis for extracted sesame seed proteins in comparison with whey protein isolate and pea protein concentrate

When the Sesame-Meal was compared with E-ACP and E-precipitate, it can be concluded that use of the enzyme had a positive effect on the protein solubility. Since enzyme extraction is accepted as a smooth process, the quality of the products is generally higher (Liu et al., 2016). Both E-precipitate part and E-ACP had higher solubility than Sesame-meal.

When the solubility of alkaline extracted proteins and Sesame-meal were compared, it was observed that alkaline extraction caused a slight decrease in the solubility. The major storage proteins which are globulins (named as 7S and 11S) exhibit association/dissociation behavior and this behavior depends on the ionic strength and pH of the solution. Thus the solubilization of these proteins and their ratio in the isolate are affected (Achouri, Nail, & Boye, 2012).

However, salt extraction resulted in a drastic decrease in the solubility of sesame seed proteins. The lowest solubility was observed in the protein samples extracted by using 1M NaCl salt. Protein properties might change because of these structural alterations (Achouri & Boye, 2013). Some salts have a positive effect on the protein solubility depending on the concentration in which they are being used. Therefore, same type of salt might have both positive and negative effect on the solubility of proteins. This mechanism is called as 'salting in and salting out' (Grundl, Müller, Touraud, & Kunz, 2017) as described before.

The salt concentration at which the protein precipitates, differs from one protein to another. For sesame seed protein, the solubility of the proteins was found to be the highest at the salt concentration of 0.25 M (Deak et al., 2006). For the protein extracts of 0.6M-NaCl and 1M-NaCl, since the salt concentrations are high, proteins might have been unfolded and precipitated, resulting in a decrease in the solubility.

3.1.3 Measurement of Total Protein Content

Kjeldahl method is widely used in food science and food industry since it is the officially recognized standard reference method, although it has two main problems which are long process time to carry out the whole assay and not being able to

differentiate between non-protein nitrogen and total protein nitrogen (Shevkani et al., 2015).

According to Kjeldahl experiment results, Sesame-meal which was the control had 36.52% (w/w) ± 0.38 protein content. Total protein contents for all protein samples were given in the Figure 3.3 on dry basis.

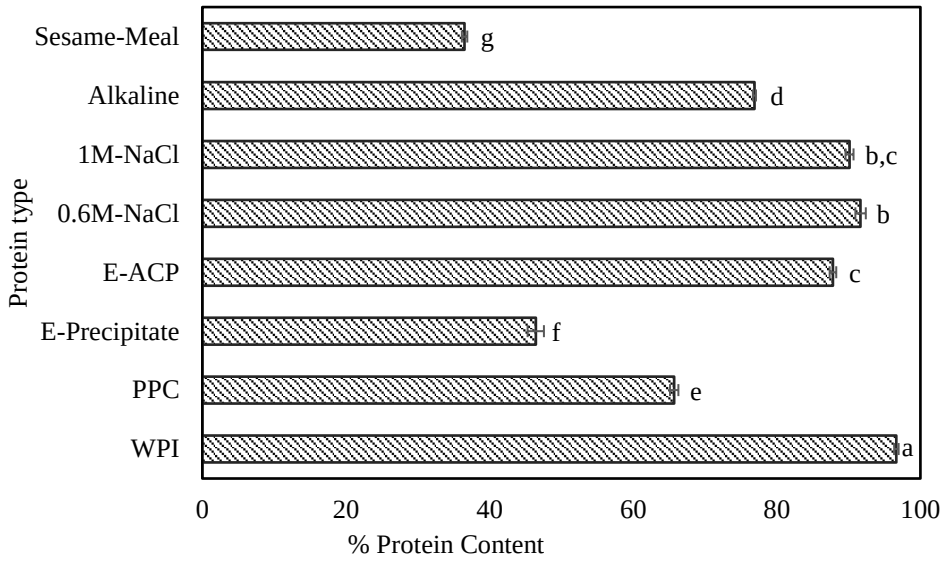

Figure 3.3. Total Protein Content (%) for extracted sesame seed proteins in comparison with whey protein isolate and pea protein concentrate

WPI had the highest protein content among all the samples and it was followed by 0.6M-NaCl, 1M-NaCl and E-ACP protein extracts, respectively. It can be concluded that salt extraction was quite effective and resulting extracts had the highest and similar protein contents which are 91.6% (w/w) ± 0.72 for 0.6M-NaCl and 90.1% (w/w) ± 0.56 for 1M-NaCl. ACP of enzyme extraction method had a similar protein content with 1M-NaCl extract according to statistical analysis. Alkaline extraction was resulted in lower protein content, 76.88% (w/w) ± 0.21

3.2 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR spectroscopy is an important technique for the food industry because of being a quite simple, non-destructive and a rapid method (Rodriguez-Saona & Allendorf, 2011).

In this study, the structural difference between extracted sesame seed proteins, WPI and PPC was examined in the wavenumber range of 600-4000 cm^{-1}. The wavenumbers given in Table 3.1 demonstrates the important peaks observed on the samples. All these peaks in each FTIR spectrum were examined in detail. Each spectrum represented a different protein type including Sesame-Meal as control and given in Figure 3.4.

Table 3.1 FTIR peaks of the protein samples, and corresponding functional groups

Wavenumber (cm^{-1})	**Assignment**	**Functional groups**
1600-1700	C=O Stretching	Amide I
1480-1600	N−H Bending	Amide II
1200-1400	C-N Stretching	Amide III
~ 3300	N-H stretching	Amide A
~ 3100		Amide B
~1050	C-O/C-C Stretching	Carbohydrates

According to studies, one of the most important peaks for the proteins are Amide I and Amide II. As it can be seen from the table, Amide I band shows up in the wavenumber range of 1600-1700 cm^{-1} and it shows the C=O Stretching, Amide II band can be observed between 1480-1600 cm^{-1}, showing N−H Bending (Kong & Yu, 2007). Intensity of these bands might give information about the amount of

proteins in the samples. The highest Amide I and Amide II peaks were observed in WPI, 1M-NaCl and 0.6M-NaCl extracts followed by E-ACP sample. Since WPI was known to be a commercial protein isolate with remarkably high content of protein, this result was expected. The results obtained from FTIR were highly similar with total protein content results as expected.

Amide III band which indicates C-N stretching and N-H bending was observed in the range of 1200-1400 cm^{-1} (Muyonga, Cole, & Duodu, 2004). The highest peaks were obtained for the salt extraction samples again, which confirmed the high protein content in those samples.

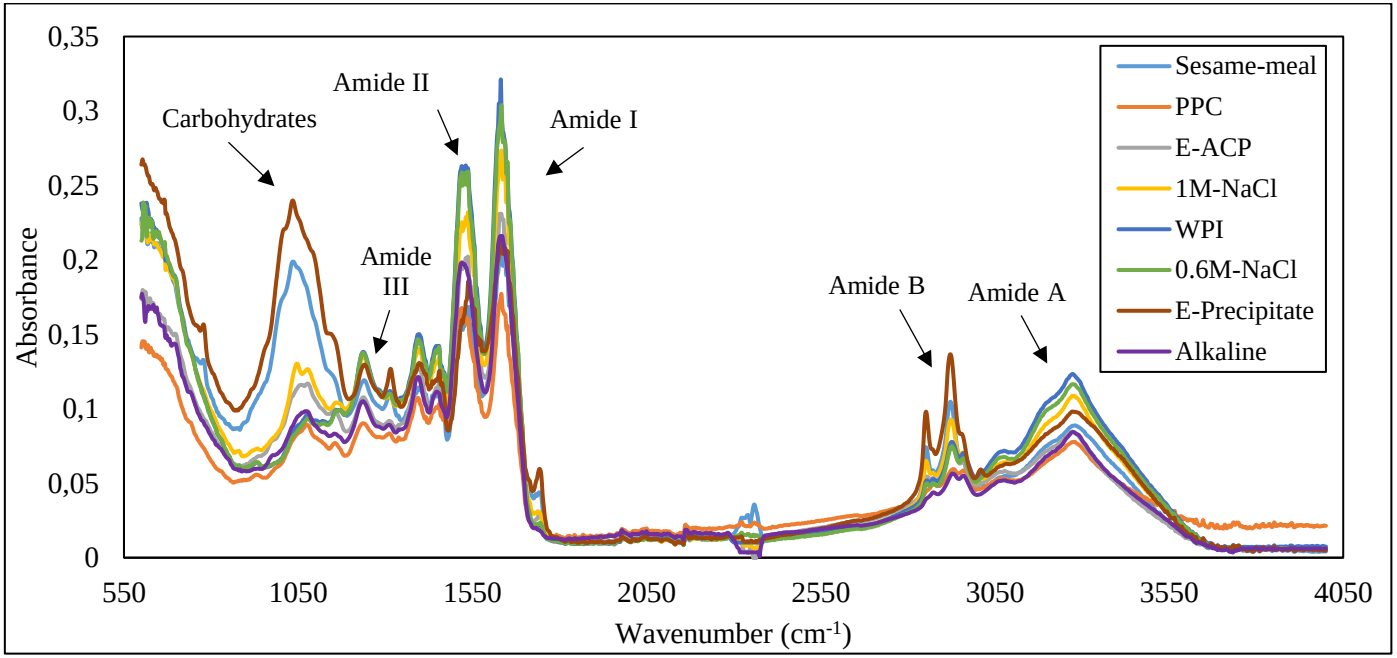

Figure 3.4. FTIR spectra for extracted sesame seed proteins in comparison with whey protein isolate and pea protein concentrate

Carbohydrates were also detected in the samples around the peak at 1050 cm^{-1} (Blanco Pascual, Montero, & Gómez-Guillén, 2014; Ozel, Aydin, Grunin, & Oztop, 2018). This peak shows the stretches of C–O linkages and implies the presence of carboxylic acid units (Simi & Abraham, 2010). This band is common to all polysaccharides. It was obvious that E-Precipitate and Sesame-meal had the highest intensity, meaning that they had the highest carbohydrate content among all the samples. This result was expected since Sesame-meal is the control (non-extracted). E-Precipitate contained more carbohydrates than Sesame-meal because during enzyme extraction, most of the carbohydrates were extracted into precipitate part. Therefore, in the same amount of sample, E-Precipitate had more carbohydrates.

Overall, it was concluded that FTIR results matched with total protein content results. It was possible to get information about the amount of carbohydrates and proteins present in the samples and compare with each other.

3.3 Oil Content by Soxhlet Extraction and TD-NMR

As it is already mentioned in Chapter 1, since sesame seed meal contains unignorable amount of oil, it was important to have information about the final oil content in the protein extracts. Oil content may affect shelf life because of the oxidative stability. Although sesame seed oil contains 85% unsaturated fatty acids, oxidative stability of sesame seed oil is superior to that of other vegetable oils because of the presence of some antioxidants naturally present in sesame seed which prevents the oxidation of unsaturated fatty acids (Abou-Gharbia, Shehata, & Shahidi, 2000).

In this study, oil content of the protein extracts was found by using 2 different methods: Soxhlet Extraction and TD-NMR. Soxhlet extraction is a solid-liquid

extraction technique used as a standard and reference method in food industry for decades (Zhao & Zhang, 2013). However, it has some disadvantages as long process time, usage of large amount of solvent and some other chemicals (Virot, Tomao, Colnagui, Visinoni, & Chemat, 2007). Additionally, to apply Soxhlet method, 3-5 grams of sample is required, and sample cannot be used again for further analysis later. Because of all these disadvantages, an alternative method which is TD-NMR was used and Soxhlet extraction and TD-NMR results were given on dry basis in Figure 3.5 and Figure 3.6.

Soxhlet results showed that sesame seed meal had the highest oil content which is 12.12% (w/w) ± 0.17. According to a study about chemical composition of sesame oil cake, defatted sesame seed meal had 11.9-15% (w/w) of oil (Dimitrov, Georgieva, & Vassilev, 2003).

Alkaline extract and E-Precipitate samples had similar oil contents following Sesame-Meal, 8.72% (w/w) ± 0.36 and 8.11% (w/w) ± 0.17, respectively. E-ACP had the lowest oil content among all other sesame seed protein extracts, 1.61% (w/w) ± 0.06. Both 0.6M-NaCl and 1M-NaCl salt extracts had similar oil content which are 3.75% (w/w) ± 0.05 and 3.60% (w/w) ± 0.05, respectively.

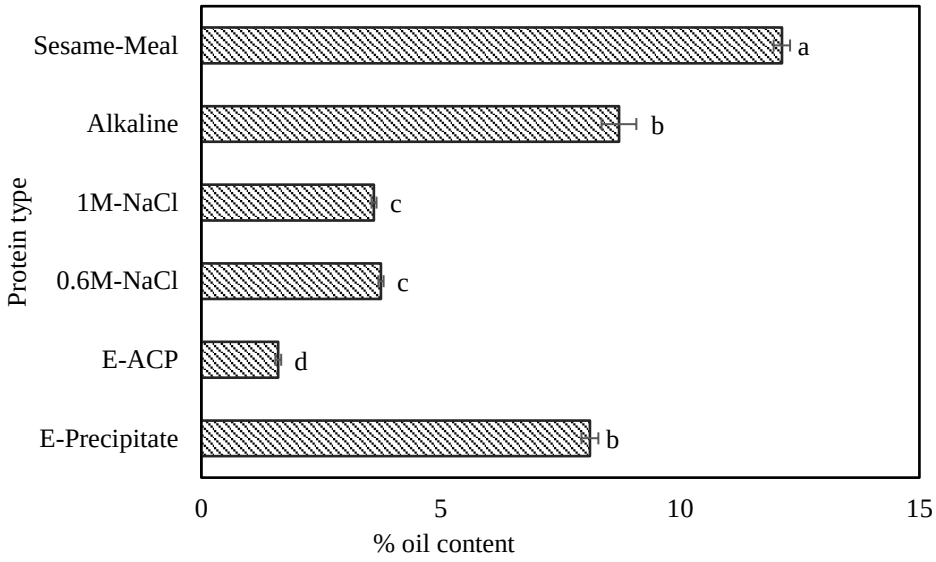

Figure 3.5. Oil content (%) of extracted sesame seed proteins and sesame meal (by using Soxhlet extraction)

TD-NMR could be used as an alternative way to determine the oil content of seeds as it is mentioned in Chapter 1. TD-NMR has several advantages over Soxhlet extraction. Having relatively short operating time, being a non-destructive and chemical-free method, the requirement of less sample can be given as examples to these advantages (Isik, Sahin, & Oztop, 2018). As can be seen from Figure 3.6, % oil content found by using TD-NMR had a similar trend with Soxhlet extraction results. Same as Soxhlet extraction result, sesame meal had the highest oil content followed by Alkaline extract. E-ACP had the lowest oil content among all the other extracts.

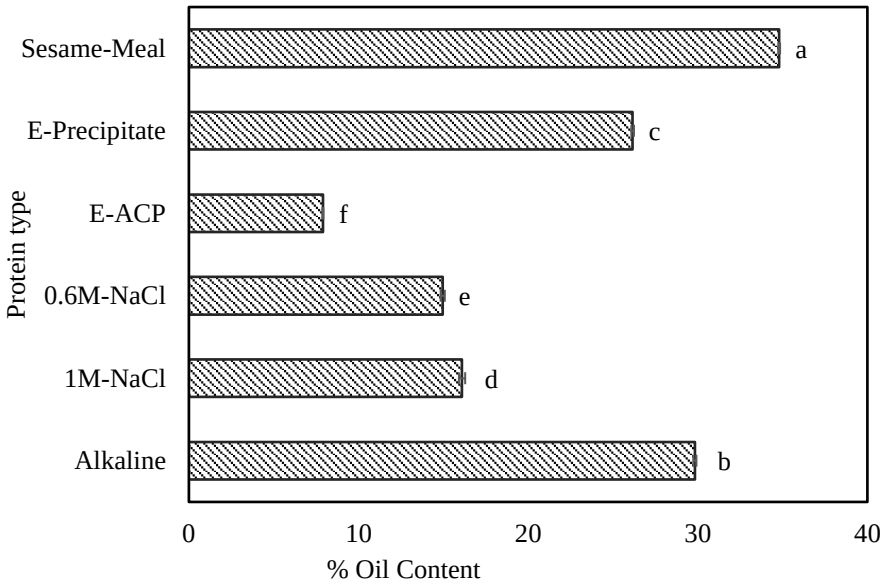

Figure 3.6. Oil content (%) of extracted sesame seed proteins and sesame meal (by using TD-NMR)

A calibration curve with the equation of $y = 0.3833x - 1.9672$ ($R^2 = 0.9774$) was constructed to relate the results for both Soxhlet extraction and TD-NMR. The linear and high relation between TD-NMR and Soxhlet results showed that the power of TD-NMR as an oil content measurement technique.

3.4 Emulsification Activity Index (EAI) and Emulsion Stability Index (ESI)

Emulsifying activity and emulsion stability are two functional properties of proteins which are crucial for the use of various industrial food products (Wagner & Guéguen, 1999). Proteins have unique surface properties due to their amphiphilic nature and large molecular size (Khalid et al., 2003) and they might reduce the surface tension at the water-oil interface and prevent coalescence (Zayas, 1997).

Figure 3.7 and Figure 3.8 show the results for EA (at 340 nm) and ES (after 10 minutes) of extracted sesame seed proteins. Statistical results are given in Appendix. It was found that protein type had a significant effect on the EA and ES ($p<0.05$).

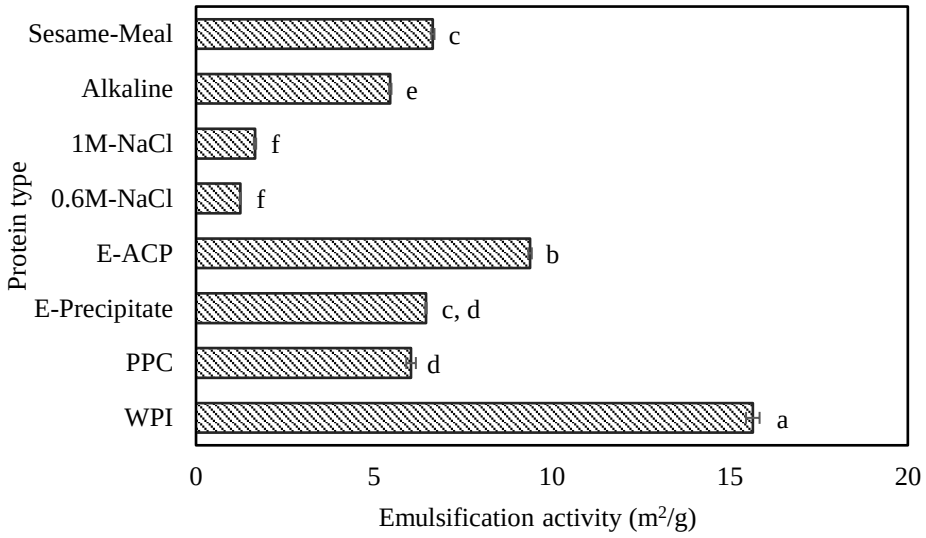

Figure 3.7. Emulsification activity of extracted sesame seed proteins in comparison with whey protein isolate and pea protein concentrate

According to the results in Figure 3.7, it was observed that WPI had the highest EA followed by E-ACP extract. EA basically depends on the diffusion of peptides at oil-water interfaces. The protein extracts with small molecular size and higher solubility might facilitate the diffusion required and improve the interaction between lipids and proteins (Onsaard, 2012). According to Lowry results for the measurement of soluble proteins; since WPI and E-ACP had the highest solubility values among all the others, their emulsifying ability are expected to be higher. On the contrary, 0.6M-NaCl and 1M-NaCl extracts had the lowest solubility and consequently, their EA had the lowest value as well.

When the result of the Alkaline extract was examined, it had the lowest EA value followed by the salt extracts. The reason behind this might be explained by the high oil content already present in the sample. Oil in the sample and oil added to form emulsions may cause molecular crowding, which could result in lower emulsification activity due to the competition between oil and protein (Zheng et al., 2020). A positive correlation was found between EA and Lowry results which further confirmed the relationship between solubility and EA ($p < 0.05$, $R= 0.930$).

Alkaline extract had lower EA when compared with Sesame-Meal. According to some studies, presence of polysaccharides in the environment helps forming emulsion by increasing the viscosity of the environment (Ibanoğlu, 2002). Since Sesame-Meal had higher number of polysaccharides, the increase in the viscosity could result in better EA.

ES of the protein samples were also measured, and results are given in Figure 3.8. As it was mentioned before, in a stable emulsion, phase separation is slow. This slow separation is more likely when there are polysaccharides present which increase the viscosity. Also, these non-adsorbing polysaccharides act as thickening and structuring agents in the aqueous continuous phase (Dalgleish, 1997; Lee, McCarthy, & Dungan, 1998).

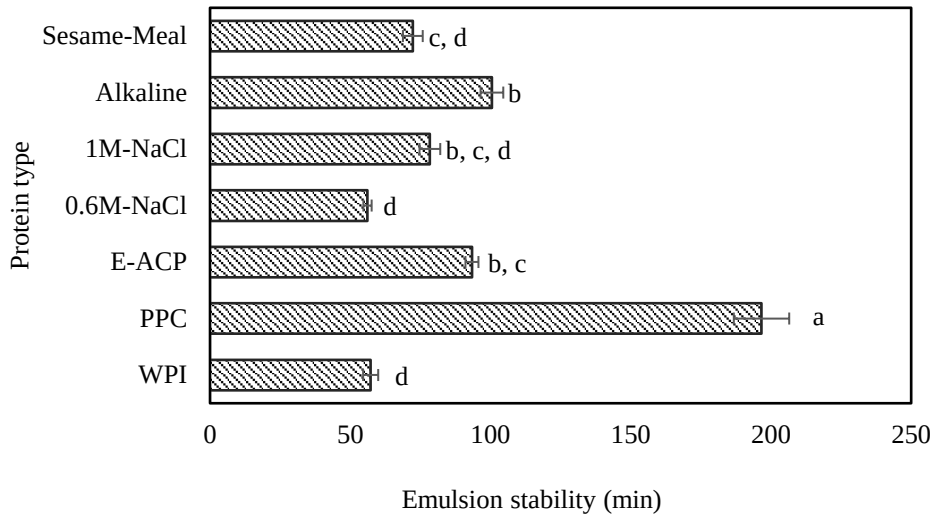

Figure 3.8. Emulsion stability of extracted sesame seed proteins in comparison with whey protein isolate and pea protein concentrate

Proteins form a protective steric barrier around the oil droplets dispersed in the emulsion and this barrier stabilizes the emulsion (H.-Y. Lee et al., 1998). Pea protein concentrate (PPC) emulsion stability was the highest among all the samples. This could be explained by presence of polysaccharides in PPC. Since PPC was not a protein isolate like WPI, polysaccharides might have acted as thickening agent and decreased the mobility of the oil and water molecules and prevented the phase separation.

However, although Sesame-meal also contains polysaccharides, ES of Sesame-meal was not as high as PPC. This was explained by the oil amount in Sesame-meal and PPC. Sesame-meal already contained significant amount of oil (12.12% (w/w)). PPC had only 1.64% (w/w) oil. There was already high amount of oil which was held by the proteins in Sesame-meal, therefore it is highly probable that there was no more protein and polysaccharides to hold more oil and stabilized the emulsion for a longer time.

ES of WPI was too low although the EA of WPI was the highest. This could be explained by WPI being a protein isolate. It means that there was very a smaller number of polysaccharides in the environment to act as thickening agent and increase the emulsion stability.

E-Precipitate data was not considered and discarded from ES analysis. Because it had significantly high stability (>750 min) and this was disturbing the data analysis.

3.5 Determination of Hydration Behavior by NMR Relaxometry

H- bonding between polar groups on the protein surface and water is regarded as the key factor for protein hydration (Mallamace et al., 2015). In this study, NMR relaxometry was used to check the hydration behavior of sesame seed proteins in comparison with WPI and PPC. T_2 relaxation times (spin-spin relaxation or transverse relaxation) were measured to get information about the interaction of proteins with water molecules. This time value is an inherent property of the sample, therefore; it is fixed and specific to the sample (Tas, 2019). The state of water (bound/free, mobile/immobile) can be easily assessed in food systems by measuring T_2 relaxation times. Different methods applied to extract proteins would change some important properties (i.e. water binding, hydrophilicity) of the extracted proteins. More free water or more mobility in the environment could be associated with longer T_2 relaxation times. The results for T_2 relaxation times of the proteins are given in Table 3.2.

Table 3.2 Hydration behavior of extracted sesame seed proteins in comparison with whey protein isolate and pea protein concentrate

Protein source	T_2 Results (ms)
Sesame-Meal	54.19 ± 0.57^{d}
WPI	193.77 ± 3.48^{a}
PPC	41.25 ± 0.09^{e}
Alkaline	52.12 ± 1.07^{d}
E-ACP	39.66 ± 0.34^{e}
E-Precipitate	30.61 ± 0.23^{f}
0.6M-NaCl	75.69 ± 0.50^{c}
1M-NaCl	101.19 ± 1.03^{b}

When protein concentration or water-binding capability of proteins increase, free water in the environment decrease and it causes a decrease in the relaxation time (Dekkers et al., 2016).

T_2 time for control sample which is Sesame-Meal was found as 54.19 ± 0.57 ms which was significantly lower compared to T_2 relaxation time of WPI. Since Sesame-Meal contains significant number of polysaccharides such as cellulose and hemicellulose, it might cause an increase in the water binding capability. The reason for the increase in the water binding capability due to the presence of these polysaccharides, could be their organized structure and their ability to interact with water (Felby, Thygesen, Kristensen, Jørgensen, & Elder, 2008). This conclusion could be further confirmed by the T_2 results of protein extract E-Precipitate. During the extraction of proteins by using protease enzyme, both precipitate part and aqueous phase were kept and freeze-dried. E-Precipitate, contains significant amount of carbohydrates coming from Sesame-Meal. FTIR results also supported that E-Precipitate had the highest peak around the wavenumber 1150 cm^{-1} which belongs to C-O/C-C stretching, corresponding to the carbohydrates. Therefore, T_2 value for

E-Precipitate was found to be the lowest among all the other samples, including control.

It was found that there was a negative correlation between soluble proteins and T_2 relaxation time ($p < 0.05$, $R = -0.824$). This result is predictable since soluble proteins are better at binding water when compared with less soluble ones. As proteins bind more water, there will be less free water in the environment, and it will be resulted in shorter T_2 relaxation times.

T_2 values for the salt extracts of proteins were found much longer than the control and this possibly implied weaker interactions of proteins with water. This result can be explained by considering the solubility of the salt extracts. Soluble proteins results showed that , 1M-NaCl and 0.6M-NaCl extracts had the lowest solubility, caused by weak interactions with water and this was reflected as an increase in T_2 values. The reason for this was the unfolding of the proteins because of the salt and the exposure of the hydrophobic sides of the protein which repelled water (Achouri & Boye, 2013). For the Alkaline extract, there was no significant difference of T_2 values with the control. ($p > 0.05$) . They had similar hydration behaviors.

3.6 Exploring Gelling Behavior by Using TD-NMR

Gel formation by using globular proteins is a quiet complex process including numerous reactions such as protein unfolding, aggregation and association-dissociation (Abaee, Mohammadian, & Mahdi, 2017). Highly unfolded peptide chains must be associated side by side during the formation of globular protein gels (Paula, Sousa, Crespo, & Raymundo, 2005). Heating of protein solution triggers unfolding and disrupts the proteins. As a result, hydrophobic sides are exposed, and

disulfide bonds (S-S) are formed (Mondal, 2019). In the case of whey protein gels, gel network is formed through inter- and intramolecular disulfide bonds (covalent bonds) and later by non-covalent bonds like hydrogen bonds and hydrophobic interactions (Oztop, Mccarthy, Mccarthy, & Rosenberg, 2014; Paula et al., 2005).

In this study, as mentioned in Chapter 2, gel formation was triggered by heating. T_2 relaxation times were measured to obtain information about the interaction of water with protein molecules before and after heat to different protein solutions with different concentrations.

In the Table 3.3, mono-exponential T_2 relaxation time values were given for all protein samples before and after heating, at concentrations of 10, 15, 20 and 25%. Statistical analysis showed that all parameters (concentration, heating treatment and protein type) had a significant effect on the T_2 relaxation times ($p < 0.05$). According to the results, it was found that after heating, there was a significant decrease in the T_2 relaxation times for WPI and E-ACP samples. WPI immediately formed gel even at low concentrations and the sharpest decrease in T_2 relaxation times were observed in this case. It is possible that during the gel formation, water in the solution was trapped in the gel matrix which causing a decrease in the mobility of free protons and resulting in lower T_2 relaxation time values.

It was observed that except WPI and E-ACP extract, there was an increase in the T_2 relaxation time after heat treatment. These results were unexpected since there must have been a decrease in the mobility of protons in the water by formation of gels. This situation might be explained with a different approach. In the literature, it was found that cold-gelation approach works better for globular proteins (Abaee, Mohammadian, & Mahdi, 2017). As mentioned already in Chapter 1, sesame seed proteins are mostly globulins (67.3%) and later albumin (8.6%), prolamin (1.3%)

and glutelin (6.9%) fractions (Pusadkar, Eswaran, et al., 2015). Cold-set gelation occurs in basically two stages which starts with heating the solution to denature and cause unfolding of the proteins (Fazani & Lopes, 2010; Nicolai, Britten, & Schmitt, 2011). Heating stage is followed by acidification (acid-induced gelling) or addition of salts (salt induced gelling). This way, protein repulsion might be decreased and proteins form cross-link with each other and trap water, form gels (Kuhn, Luiz, Cavallieri, & Lopes, 2011). Since there was no use of salt or acid in this study to help the formation of gels, protein repulsion (because of hydrophobic sides) could not have been prevented or decreased, therefore; they could not form gels properly. In the case of WPI, although it was composed of mostly globular proteins, it formed proper gels. The reason behind this might be explained by the ability of globular proteins of WPI to form intermolecular disulfide bonds (S-S linkages) (Abaee, Mohammadian, & Jafari, 2017). The way disulfide bonds is the deprotonation of amino groups which is formed after cooling down the solution. It also favors the cross-linking without the addition of salt and acidification (Abaee, Mohammadian, & Jafari, 2017).

This explanation can be further proved by a study done on perilla globulins and sesame seed globulins (Takenaka, Arii, & Masui, 2011). It was found that perilla globulins were formed in such a way that one basic and one acidic subunits were cross-linked by disulfide bonds which makes this protein to have higher water holding capacity and finer gel microstructure despite of having the similar globular subunit structure with sesame seed (Takenaka et al., 2011). In conclusion, the differences in the order of globulin subunits might have an impact on the gel formation.

Salt extracts which are 1M-NaCl and 0.6M-NaCl had already salts in them but still there was an increase in the T_2 relaxation times. It can be explained by having excessive amount of salt in the environment. Because of solubility results it was

already known that these extracts experienced salting-out effect. Therefore, after heating proteins were unfolded more and aggregation occurred, this might have caused water release to the environment and increased T_2 relaxation times. It is also possible that water release might have occurred after gel formation. During the gel matrix formation and cross-linking, water might have been repelled out of the gel, meaning more free water in the environment (Ma, Cui, Cai, & Shao, 2018).

Overall, TD-NMR was a useful method for exploring the gelling behavior of different proteins at different concentrations. It was important to have information about the free water amount in the environment for interpretation of gelling ability.

Table 3.3 Gelling ability of extracted sesame seed proteins in comparison with whey protein isolate and pea protein concentrate for different concentrations

Protein source	Treatment	T_2 Results (ms)			
		10% (w/v)	15% (w/v)	20% (w/v)	25% (w/v)
WPI	BH	737.00 ± 5.86^{g}	506.53 ± 1.81^{d}	377.93 ± 0.58^{e}	284.75 ± 2.86^{d}
	AH	158.00 ± 1.15^{n}	104.33 ± 0.66^{j}	79.67 ± 0.88^{k}	65.23 ± 1.13^{j}
Sesame-Meal	BH	297.87 ± 5.92^{j}	156.87 ± 2.56^{i}	114.60 ± 3.72^{j}	78.00 ± 0.57^{i}
	AH	270.67 ± 1.45^{k}	228.00 ± 2.08^{h}	136.67 ± 1.20^{i}	86.00 ± 0.57^{i}
1M- NaCl	BH	958.33 ± 3.52^{d}	719.67 ± 4.33^{c}	462.00 ± 2.64^{d}	334.33 ± 3.52^{c}
	AH	1699.00 ± 0.57^{a}	1351.33 ± 2.33^{a}	1065.00 ± 5.77^{a}	853.00 ± 2.08^{a}
0.6M-NaCl	BH	806.40 ± 7.99^{f}	445.83 ± 5.76^{e}	181.10 ± 4.47^{h}	156.50 ± 3.17^{g}
	AH	1505.33 ± 2.60^{b}	1171.00 ± 4.35^{b}	516.67 ± 3.17^{c}	482.00 ± 3.51^{b}
PPC	BH	195.00 ± 2.97^{m}	113.70 ± 1.96^{j}	$68.30 \pm 0.05^{k,l}$	$53.50 \pm 0.45^{k,l}$
	AH	893.00 ± 5.86^{e}	223.00 ± 4.04^{h}	74.33 ± 2.33^{k}	$61.67 \pm 0.33^{j,k}$
Alkaline	BH	372.00 ± 1.53^{i}	345.67 ± 1.85^{f}	317.67 ± 13.83^{f}	220.67 ± 1.20^{e}
	AH	1010.30 ± 20.43^{c}	714.33 ± 3.52^{c}	635.00 ± 4.35^{b}	173.33 ± 1.33^{f}
E-ACP	BH	595.33 ± 3.52^{h}	257.00 ± 7.81^{g}	220.33 ± 1.85^{g}	$63.00 \pm 2.30^{j,k}$
	AH	235.67 ± 2.90^{l}	155.33 ± 3.92^{i}	$125.67 \pm 2.40^{i,j}$	105.33 ± 1.85^{h}
E-Precipitate	BH	130.00 ± 1.53^{o}	64.00 ± 1.53^{k}	54.67 ± 2.02^{l}	$46.00 \pm 1.73^{l,m}$
	AH	215.00 ± 0.57^{m}	74.67 ± 2.02^{k}	55.63 ± 0.08^{l}	40.50 ± 0.28^{m}

BH: Before Heating, AH: After Heating. Letters indicate significant difference in each protein concentration (a-m)

3.7 Monitoring of Proteins by Gel Electrophoresis

SDS-PAGE under reducing conditions (presence of denaturing reagents such as β-mercapthoethanol) showed the differences in the electrophoretic pattern between all types of protein extracts and Sesame-Meal as control. The gel image is given as Figure 3.9.

Sesame seed proteins are mainly composed of 2S albumin (β globulin), 7S and 11S globulins (α globulin) (Asero et al., 2014; Beyer et al., 2002; Beyer, Grishina, Bardina, & Sampson, 2007; Navuluri et al., 2006). The secondary structure of these subunits can be observed by electrophoresis. Water insoluble 11S globulins are hexamers with molecular weights ranging between 300-400 kDa, containing two hexagonal rings, and each rings have three hydrophobically associated pairs of disulfide-linked basic (25-28 kDa), and acidic (30-35 kDa) subunits (Achouri et al., 2012). 7S globulin fraction is found in the range of 40-65 kDa (Orruno & Morgan, 2007). Finally, 2S albumin has the molecular weight between 13-15 kDa (Guerra et al., n.d.).

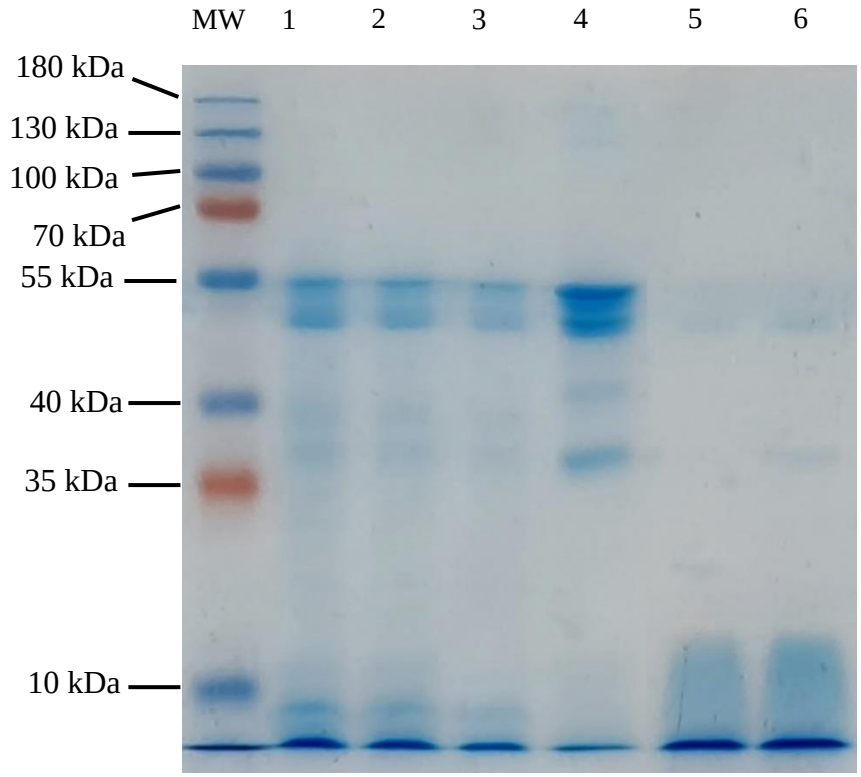

Figure 3.9. Gel result of SDS-PAGE with β-mercaptoethanol of sesame proteins extracted by using different techniques. (MW) molecular weight standard, (1) Sesame-Meal, (2) E-Precipitate, (3) Alkaline, (4) E-ACP, (5) 0.6M-NaCl, (6) 1M-NaCl

SDS-PAGE results were similar with the previous studies in terms of the location of the bands, and molecular weight of the subunits (Akbar et al., 2012, 2012; Beyer et al., 2002; Chatterjee et al., 2015a; Poveda et al., 2016). According to SDS-PAGE results, intense bands show higher ratios of the components/protein subunits. The strongest protein bands were obtained from the sample 4 which is E-ACP extract due to its high solubility in the solution. It was observed that it has 7S globulin fractions between the molecular weights of 40-55 kDa. Moreover, around the molecular weight of 35 kDa, acidic subunit of 11S globulins can be detected. It was not possible to observe 2S albumin fraction for this sample.

The sesame 2S albumin was observed in the first three samples which are Sesame-Meal, E-Precipitate, and Alkaline extracts around the molecular weight of 9 kDa. 2S albumins have two subunits linked by disulfide bond to each other and their molecular weights are 9 and 4 kDa in their cleaved form. According to reports, these insoluble 2S albumins can be described under the category of major food allergens (Poveda et al., 2016).

For both salt extracts, bands were very weak due to their exceptionally low solubility compared with the other samples. However, the smear formation at the end of the gel, which is the low molecular weight region, was noticeable for these two salt extract protein samples. There was no certain separation of the proteins in this region. This situation might be explained by the presence of various low molecular weight unfolded protein fractions (Tianqi et al., 2019). As explained by the other characteristics of these proteins such as poor soluble protein amount, low emulsification activity, and poor gelling behavior; denaturation & unfolding occurred for the salt extracts and it can be further supported by these SDS-PAGE results.

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

In the presented study, a by-product of the sesame seed oil production, which is sesame seed meal was obtained, and proteins were extracted from this sesame meal by using different protein extraction methods. Three different methods were applied with different conditions which were determined by preliminary studies. Protein extraction was performed by alkaline extraction (pH 12), salt extraction using NaCl at concentrations of 0.6 M and 1 M, and finally enzyme assisted alkaline extraction by using protease mixtures. All the results were compared with the sesame seed meal, whey protein isolate, and pea protein concentrate.

It was found that salt extracted samples and enzyme assisted alkaline extracted sample which was E-ACP had the highest protein contents which are all over 90%, and E-ACP extract had the highest yield among all other extracted protein samples. FTIR results confirmed the total protein content results. Solubility of salt extracted samples were the lowest whereas enzyme assisted alkaline extracted protein samples had the highest solubility value. Emulsification activity and emulsion stability were found to be the highest for the enzyme assisted alkaline extracted protein samples. The lowest emulsion stability and emulsification activity were observed in the salt extracted samples. When the hydration behavior of the extracted proteins was compared, it was found that the salt extracted protein samples had poor water binding capacities. All these poor properties of the salt extracted samples showed that there was significant degree of denaturation for these samples which was further confirmed by SDS-PAGE experiments.

The study was successful in showing that that TD-NMR can be a good alternative to Soxhlet method which was the traditional technique used for oil content determination. TD-NMR was also successful for the determination of gelling ability and hydration behavior of the proteins by monitoring the mobility of free protons in the system.

Moreover, the study showed that different extraction methods had a significant effect on the functional properties of proteins. The method of extraction should be chosen by considering the purpose of the protein to be extracted.

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APPENDICES

A. Calibration Curves

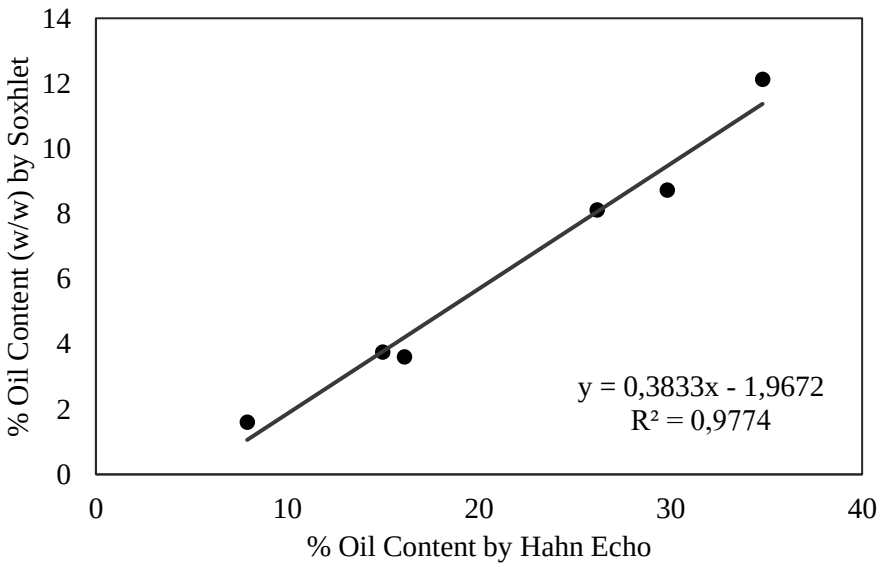


Figure A.1. Calibration curve showing the relationship between Soxhlet results and TD-NMR relaxometry results for determination of oil content

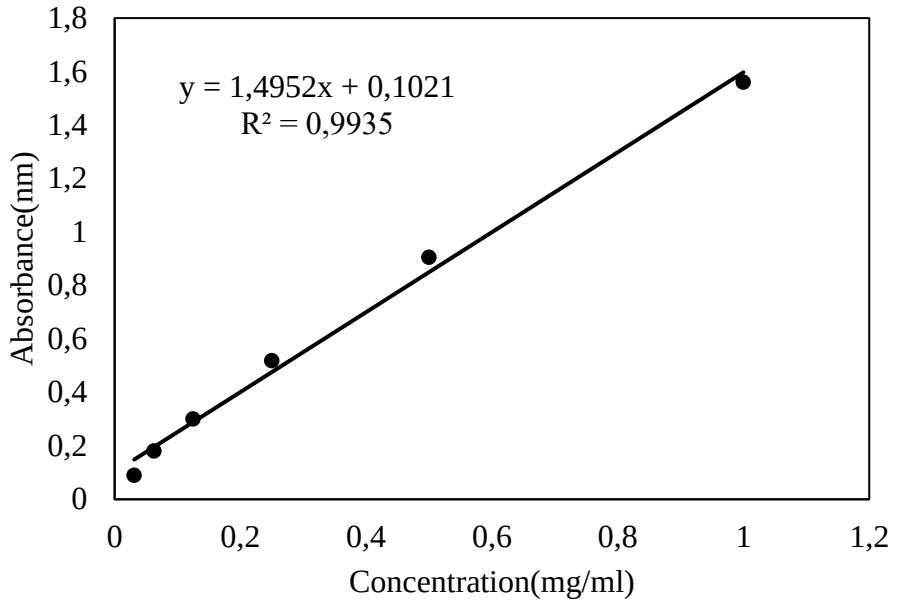


Figure A.2. Calibration curve for Lowry method prepared by Bovine Serum Albumin (BSA) to measure protein concentration

B. Statistical Analyses

Table B1. ANOVA and Tukey's Comparison Test with 95% confidence level for determining total protein content with Kjeldahl method

General Linear Model: Kjeldahl results versus Protein Type

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Protein Type	Fixed	8	0.6M NaCl; 1M NaCl; Alkaline; E+ACP; E+P; Meal; P; W

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	7	10555,8	1507,97	1354,84	0,000
Error	16	17,8	1,11		
Total	23	10573,6			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1,05500	99,83%	99,76%	99,62%

Comparisons for Kjeldahl results

Tukey Pairwise Comparisons: Protein Type

Grouping Information Using the Tukey Method and 95% Confidence

Protein Type	N	Mean	Grouping						
W	3	96,6583	A						
0.6M NaCl	3	91,6708		B					
1M NaCl	3	90,1250		B	C				
E+ACP	3	87,8208			C				
Alkaline	3	76,8833				D			
P	3	65,7125					E		
E+P	3	46,4333						F	
Meal	3	36,5167							G

Means that do not share a letter are significantly different.

Table B2. ANOVA and Tukey's Comparison Test with 95% confidence level for determining oil content by using TD-NMR relaxometry

General Linear Model: Oil Content (HE) versus Protein Type

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein Type	Fixed	6	0.6M-NaCl, 1M-NaCl, Alkaline, E-ACP, E-Precipitate, Sesame-Meal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	1574.24	314.849	9313.52	0.000
Error	12	0.41	0.034		
Total	17	1574.65			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.183863	99.97%	99.96%	99.94%

Comparisons for Oil Content (HE)

Tukey Pairwise Comparisons: Protein Type

Grouping Information Using the Tukey Method and 95% Confidence

Protein Type	N	Mean	Grouping
Sesame-Meal	3	34.8000	A
Alkaline	3	29.8267	B
E-Precipitate	3	26.1600	C
1M-NaCl	3	16.1033	D
0.6M-NaCl	3	14.9633	E
E-ACP	3	7.8967	F

Means that do not share a letter are significantly different.

Table B3. ANOVA and Tukey's Comparison Test with 95% confidence level for determining the protein yield

General Linear Model: yield %(w/w) versus protein type

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
protein type	Fixed	5	0.6M-NaCl, 1M-NaCl, Alkaline, E-ACP, E-Precipitate

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
protein type	4	2585.66	646.414	2098.04	0.000
Error	10	3.08	0.308		
Total	14	2588.74			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.555071	99.88%	99.83%	99.73%

Tukey Pairwise Comparisons: protein type

Grouping Information Using the Tukey Method and 95% Confidence

protein type	N	Mean	Grouping
E-ACP	3	44.7954	A
0.6M-NaCl	3	42.6105	B
1M-NaCl	3	38.0294	C
Alkaline	3	34.8603	D
E-Precipitate	3	8.4156	E

Means that do not share a letter are significantly different.

Table B4. ANOVA and Tukey's Comparison Test with 95% confidence level for determining oil content with Soxhlet method

General Linear Model: Oil content (S) versus Protein Type

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein Type	Fixed	6	0.6M-NaCl, 1M-NaCl, Alkaline, E-ACP, E-Precipitate, Sesame-Meal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	236.661	47.3321	467.55	0.000
Error	12	1.215	0.1012		
Total	17	237.875			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.318172	99.49%	99.28%	98.85%

Comparisons for Oil content (S)

Tukey Pairwise Comparisons: Protein Type

Grouping Information Using the Tukey Method and 95% Confidence

Protein Type	N	Mean	Grouping
Sesame-Meal	3	12.1267	A
Alkaline	3	8.7233	B
E-Precipitate	3	8.1167	B
0.6M-NaCl	3	3.7533	C
1M-NaCl	3	3.6067	C
E-ACP	3	1.6067	D

Means that do not share a letter are significantly different

Table B5. ANOVA and Tukey's Comparison Test with 95% confidence level for determining emulsion stability

General Linear Model: Emulsion stability versus Protein Type

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Protein Type	Fixed	7	0.6M-NaCl; 1M-NaCl; Alkaline; E-ACP; PPC; Sesame-Meal; WPI

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	6	42210,1	7035,02	106,01	0,000
Error	14	929,1	66,36		
Total	20	43139,2			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
8,14624	97,85%	96,92%	95,15%

Comparisons for Emulsion stability

Tukey Pairwise Comparisons: Protein Type

Grouping Information Using the Tukey Method and 95% Confidence

Protein Type	N	Mean	Grouping		
PPC	3	196,634	A		
Alkaline	3	100,511		B	
E-ACP	3	93,394		B	C
1M-NaCl	3	78,392		B	C D
Sesame-Meal	3	72,310			C D
WPI	3	57,276			D
0.6M-NaCl	3	56,152			D

Means that do not share a letter are significantly different.

Table B6. ANOVA and Tukey's Comparison Test with 95% confidence level for determining emulsification activity

General Linear Model: Log(EA) versus Protein Type

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein Type	Fixed	8	0.6M-NaCl, 1M-NaCl, Alkaline, E-ACP, E-Precipitate, PPC, Sesame-Meal, WPI

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	7	2.84862	0.406946	4463.13	0.000
Error	16	0.00146	0.000091		
Total	23	2.85008			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0095488	99.95%	99.93%	99.88%

Comparisons for Log(EA)

Tukey Pairwise Comparisons: Protein Type

Grouping Information Using the Tukey Method and 95% Confidence

Protein Type	N	Mean	Grouping
WPI	3	1.19428	A
E-ACP	3	0.97245	B
Sesame-Meal	3	0.82311	C
E-Precipitate	3	0.81032	C
PPC	3	0.78097	D
Alkaline	3	0.73706	E
1M-NaCl	3	0.21922	F
0.6M-NaCl	3	0.09607	G

Means that do not share a letter are significantly different.

Table B7. ANOVA and Tukey's Comparison Test with 95% confidence level for determining soluble protein content using Lowry method

General Linear Model: Log(lowry) versus Protein type

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein type	Fixed	8	0.6M-NaCl, 1M-NaCl, Alkaline, E-ACP, E-Precipitate, PPC, Sesame-Meal, WPI

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein type	7	3.56825	0.509750	1940.97	0.000
Error	16	0.00420	0.000263		
Total	23	3.57245			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0162058	99.88%	99.83%	99.74%

Comparisons for Log(lowry)

Tukey Pairwise Comparisons: Protein type

Grouping Information Using the Tukey Method and 95% Confidence

Protein type	N	Mean	Grouping
WPI	3	0.773811	A
E-ACP	3	0.504781	B
PPC	3	0.379317	C
Alkaline	3	0.149124	D
E-Precipitate	3	0.069446	E
Sesame-Meal	3	-0.140468	F
0.6M-NaCl	3	-0.272123	G
1M-NaCl	3	-0.447482	H

Means that do not share a letter are significantly different.

Table B8. ANOVA and Tukey's Comparison Test with 95% confidence level for determining hydration behavior by TD-NMR relaxometry

General Linear Model: T2(ms) versus Protein type

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Protein type	Fixed	8	0.6M-NaCl; 1M-NaCl; Alkaline; E-ACP; E-Precipitate; PPC; Sesame-Meal; WPI

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein type	7	62706,9	8958,12	3778,32	0,000
Error	16	37,9	2,37		
Total	23	62744,8			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1,53978	99,94%	99,91%	99,86%

Comparisons for T2(ms)

Tukey Pairwise Comparisons: Protein type

Grouping Information Using the Tukey Method and 95% Confidence

Protein type	N	Mean	Grouping
WPI	3	197,107	A
1M-NaCl	3	101,194	B
0.6M-NaCl	3	75,695	C
Sesame-Meal	3	54,195	D
Alkaline	3	52,116	D
PPC	3	41,257	E
E-ACP	3	39,665	E
E-Precipitate	3	30,612	F

Means that do not share a letter are significantly different.

Table B9. ANOVA and Tukey's Comparison Test with 95% confidence level for determining gelling ability of 10% protein solutions by TD-NMR relaxometry

General Linear Model: Gelling Results versus Protein type, treatment

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein type	Fixed	8	0.6M, 1M, Alkaline, E-ACP, E-Precipitate, Meal, PPC, WPI
treatment	Fixed	2	AH, BH

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein type	7	7.0979	1.01399	22353.49	0.000
treatment	1	0.6613	0.66134	14579.29	0.000
Protein type*treatment	7	2.9130	0.41615	9174.06	0.000
Error	32	0.0015	0.00005		
Total	47	10.6737			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0067351	99.99%	99.98%	99.97%

Comparisons for Gelling Results

Tukey Pairwise Comparisons: Protein type

Grouping Information Using the Tukey Method and 95% Confidence

Protein type	N	Mean	Grouping
1M	6	1.32867	A
0.6M	6	1.15587	B
Alkaline	6	0.68267	C
PPC	6	0.54400	D
WPI	6	0.44750	E
E-ACP	6	0.41550	F
Meal	6	0.28427	G
E-Precipitate	6	0.17250	H

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: treatment

Grouping Information Using the Tukey Method and 95% Confidence

treatment	N	Mean	Grouping
AH	24	0.746250	A
BH	24	0.511492	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Protein type*treatment

Grouping Information Using the Tukey Method and 95% Confidence

Protein type*treatment	N	Mean	Grouping
1M AH	3	1.69900	A
0.6M AH	3	1.50533	B
Alkaline AH	3	0.99333	C
1M BH	3	0.95833	D
PPC AH	3	0.89300	E
0.6M BH	3	0.80640	F
WPI BH	3	0.73700	G
E-ACP BH	3	0.59533	H
Alkaline BH	3	0.37200	I
Meal BH	3	0.29787	J
Meal AH	3	0.27067	K
E-ACP AH	3	0.23567	L
E-Precipitate AH	3	0.21500	M
PPC BH	3	0.19500	M
WPI AH	3	0.15800	N
E-Precipitate BH	3	0.13000	O

Means that do not share a letter are significantly different.

Table B10. ANOVA and Tukey's Comparison Test with 95% confidence level for determining gelling ability of 15% protein solutions by TD-NMR relaxometry

General Linear Model: Gelling Results versus Protein type, treatment

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein type	Fixed	8	0.6M, 1M, Alkaline, E-ACP, E-Precipitate, Meal, PPC, WPI
treatment	Fixed	2	AH, BH

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein type	7	5.03096	0.718709	18245.87	0.000
treatment	1	0.37422	0.374215	9500.21	0.000
Protein type*treatment	7	1.50079	0.214399	5442.95	0.000
Error	32	0.00126	0.000039		
Total	47	6.90723			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0062762	99.98%	99.97%	99.96%

Comparisons for Gelling Results

Tukey Pairwise Comparisons: Protein type

Grouping Information Using the Tukey Method and 95% Confidence

Protein type	N	Mean	Grouping
1M	6	1.03550	A
0.6M	6	0.80842	B
Alkaline	6	0.53000	C
WPI	6	0.30543	D
E-ACP	6	0.20617	E
Meal	6	0.19243	F
PPC	6	0.16835	G
E-Precipitate	6	0.06933	H

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: treatment

Grouping Information Using the Tukey Method and 95% Confidence

treatment	N	Mean	Grouping
AH	24	0.502750	A
BH	24	0.326158	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Protein type*treatment

Grouping Information Using the Tukey Method and 95% Confidence

Protein type*treatment	N	Mean	Grouping
1M AH	3	1.35133	A
0.6M AH	3	1.17100	B
1M BH	3	0.71967	C
Alkaline AH	3	0.71433	C
WPI BH	3	0.50653	D
0.6M BH	3	0.44583	E
Alkaline BH	3	0.34567	F
E-ACP BH	3	0.25700	G
Meal AH	3	0.22800	H
PPC AH	3	0.22300	H
Meal BH	3	0.15687	I
E-ACP AH	3	0.15533	I
PPC BH	3	0.11370	J
WPI AH	3	0.10433	J
E-Precipitate AH	3	0.07467	K
E-Precipitate BH	3	0.06400	K

Means that do not share a letter are significantly different.

Table B11. ANOVA and Tukey's Comparison Test with 95% confidence level for determining gelling ability of 20% protein solutions by TD-NMR relaxometry

General Linear Model: Gelling Results versus Protein type, treatment

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein type	Fixed	8	0.6M, 1M, Alkaline, E-ACP, E-Precipitate, Meal, PPC, WPI
treatment	Fixed	2	AH, BH

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein type	7	2.47033	0.352904	15058.44	0.000
treatment	1	0.14477	0.144771	6177.40	0.000
Protein type*treatment	7	0.85585	0.122264	5217.01	0.000
Error	32	0.00075	0.000023		
Total	47	3.47170			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0048410	99.98%	99.97%	99.95%

Comparisons for Gelling Results

Tukey Pairwise Comparisons: Protein type

Grouping Information Using the Tukey Method and 95% Confidence

Protein type	N	Mean	Grouping
1M	6	0.763500	A
Alkaline	6	0.483000	B
0.6M	6	0.348883	C
WPI	6	0.228800	D
E-ACP	6	0.173000	E
Meal	6	0.125633	F
PPC	6	0.071317	G
E-Precipitate	6	0.055150	H

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: treatment

Grouping Information Using the Tukey Method and 95% Confidence

treatment	N	Mean	Grouping
AH	24	0.336079	A
BH	24	0.226242	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Protein type*treatment

Grouping Information Using the Tukey Method and 95% Confidence

Protein type*treatment	N	Mean	Grouping
1M AH	3	1.06500	A
Alkaline AH	3	0.63500	B
0.6M AH	3	0.51667	C
1M BH	3	0.46200	D
WPI BH	3	0.37793	E
Alkaline BH	3	0.33100	F
E-ACP BH	3	0.22033	G
0.6M BH	3	0.18110	H
Meal AH	3	0.13667	I
E-ACP AH	3	0.12567	I J
Meal BH	3	0.11460	J
WPI AH	3	0.07967	K
PPC AH	3	0.07433	K
PPC BH	3	0.06830	K L
E-Precipitate AH	3	0.05563	L
E-Precipitate BH	3	0.05467	L

Means that do not share a letter are significantly different.

Table B12. ANOVA and Tukey's Comparison Test with 95% confidence level for determining gelling ability of 25% protein solutions by TD-NMR relaxometry

General Linear Model: Gelling Results versus Protein type, treatment

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein type	Fixed	8	0.6M, 1M, Alkaline, E-ACP, E-Precipitate, Meal, PPC, WPI
treatment	Fixed	2	AH, BH

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein type	7	1.45040	0.207200	17061.79	0.000
treatment	1	0.07449	0.074493	6134.07	0.000
Protein type*treatment	7	0.56653	0.080933	6664.36	0.000
Error	32	0.00039	0.000012		
Total	47	2.09181			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0034848	99.98%	99.97%	99.96%

Comparisons for Gelling Results

Tukey Pairwise Comparisons: Protein type

Grouping Information Using the Tukey Method and 95% Confidence

Protein type	N	Mean	Grouping
1M	6	0.593667	A
0.6M	6	0.319250	B
Alkaline	6	0.197000	C
WPI	6	0.174993	D
E-ACP	6	0.084167	E
Meal	6	0.082000	E
PPC	6	0.057583	F
E-Precipitate	6	0.043250	G

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: treatment

Grouping Information Using the Tukey Method and 95% Confidence

treatment	N	Mean	Grouping
AH	24	0.233383	A
BH	24	0.154594	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Protein type*treatment

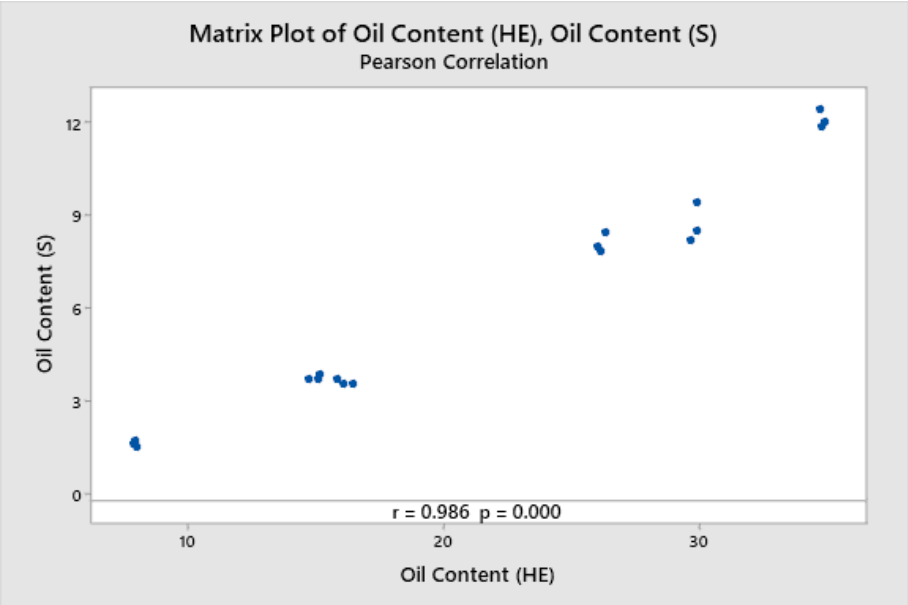
Grouping Information Using the Tukey Method and 95% Confidence

Protein type*treatment	N	Mean	Grouping
1M AH	3	0.853000	A
0.6M AH	3	0.482000	B
1M BH	3	0.334333	C
WPI BH	3	0.284753	D
Alkaline BH	3	0.220667	E
Alkaline AH	3	0.173333	F
0.6M BH	3	0.156500	G
E-ACP AH	3	0.105333	H
Meal AH	3	0.086000	I
Meal BH	3	0.078000	I
WPI AH	3	0.065233	J
E-ACP BH	3	0.063000	J K
PPC AH	3	0.061667	J K
PPC BH	3	0.053500	K L
E-Precipitate BH	3	0.046000	L M
E-Precipitate AH	3	0.040500	M

Means that do not share a letter are significantly different.

Table B13. Correlation results between Soxhlet method and TD-NMR Relaxometry with 95% confidence level

Correlation: Oil Content (HE), Oil Content (S)



Method

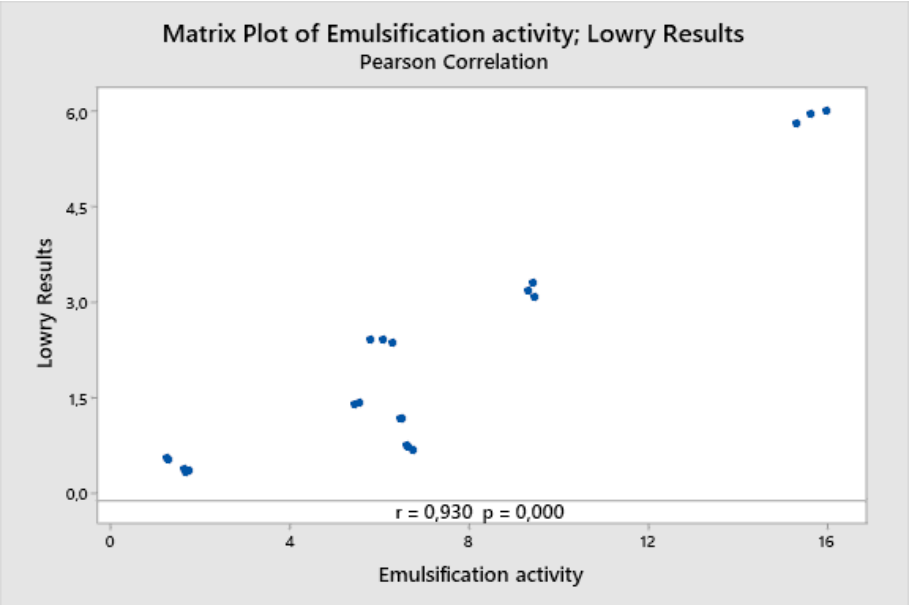
Correlation type	Pearson
Rows used	18

Correlations

	Oil Content (HE)
Oil Content (S)	0.986

Table B14. Correlation results between emulsification activity and protein solubility with 95% confidence level

Correlation: Emulsification activity; Lowry Results



Method

Correlation type	Pearson
Rows used	24

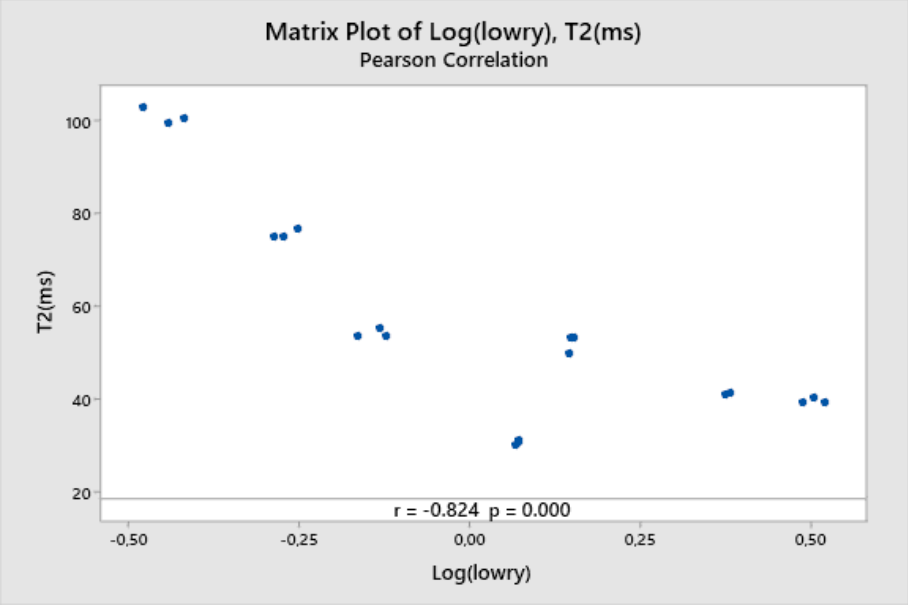
ρ: pairwise Pearson correlation

Correlations

	Emulsification activity
Lowry Results	0,930

Table B.15. Correlation results between hydration behavior and protein solubility with 95% confidence level

Correlation: Log(lowry), T2(ms)



Method

Correlation type	Pearson
Rows used	21

ρ: pairwise Pearson correlation

Correlations

	Log(lowry)
T2(ms)	-0.824