

OPTIMIZING EXTRACTION AND GLYCATION OF PUMPKIN SEED
PROTEIN FOR APPLICATION IN TOMATO SAUCE

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OZAN TAŞ

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PROTEIN FOR APPLICATION IN TOMATO SAUCE**

submitted by **OZAN TAŞ** in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Food Engineering, Middle East Technical University
by,

Prof. Dr. Naci Emre Altun
Dean, **Graduate School of Natural and Applied Sciences**

Prof. Dr. Hami Alpas
Head of the Department, **Food Engineering**

Prof. Dr. Mecit Halil Öztop
Supervisor, **Food Engineering, METU**

Prof. Dr. Servet Gülüm Şümnü
Co-Supervisor, **Food Engineering, METU**

Examining Committee Members:

Prof. Dr. Serpil Şahin
Food Engineering, METU

Prof. Dr. Mecit Halil Öztop
Food Engineering, METU

Assoc. Prof. Dr. Işıl Barutçu Mazı
Food Engineering, Ordu University

Prof. Dr. Seda Ersus
Food Engineering, Ege University

Assoc. Prof. Dr. Emin Burçin Özvural
Food Engineering, Çankırı Karatekin University

Date: 29.08.2025

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name Last name : Ozan Taş

Signature :

ABSTRACT

OPTIMIZING EXTRACTION AND GLYCATION OF PUMPKIN SEED PROTEIN FOR APPLICATION IN TOMATO SAUCE

Taş, Ozan
Doctor of Philosophy, Food Engineering
Supervisor: Prof. Dr. Mecit Halil Öztop
Co-Supervisor: Prof. Dr. Servet Gülüm Şümnü

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The growing consumer shift from animal to plant proteins, driven by sustainability goals, affordability, and nutritional value, has highlighted the potential of pumpkin seed protein concentrate (PSPC). This dissertation investigates PSPC extraction, functional modification, and application in tomato sauce as a functional food product. First, PSPC was extracted via alkali, salt, and enzyme-assisted alkali methods combined with microwave or conventional pre-heating. Microwave-assisted alkali extraction (MH-AE) yielded the best results, achieving ~55% yield, 77% protein content, and superior functional properties. Second, PSPC obtained by MH-AE was glycated with fructose or allulose at different protein:sugar ratios (1:1, 3:1). Glycation was assessed by browning index, free amino groups, reducing sugar content, amino acid profile, and FTIR. Conjugates, particularly fructose at 1:1, showed the highest glycation degree and improved solubility, surface hydrophobicity, emulsifying, foaming, antioxidant, and hydration properties. Third, glycated PSPCs were incorporated into tomato sauces enriched with olive and tomato peel powders. Four formulations were tested: control (TS-C), unmodified PSPC (TS-U), allulose-glycated PSPC (TS-A), and fructose-glycated PSPC (TS-F).

Conjugated PSPCs enhanced protein solubility, °Brix, antioxidant activity, and digestibility, with TS-F and TS-A showing the most improvement. Finally, Time-Domain Nuclear Magnetic Resonance (TD-NMR) was used to study hydration and molecular mobility in PSPC and sugars. Results confirmed sucrose's strong and allulose's weak water binding, demonstrating TD-NMR's versatility in food analysis. Overall, optimized extraction and glycation significantly improved PSPC's functionality, supporting its use in plant-based, clean-label, and value-added products.

Keywords: Pumpkin Seed Protein Concentrate, Glycation, Tomato Sauce, TD-NMR Relaxometry

ÖZ

DOMATES SOSUNDA UYGULANACAK KABAK ÇEKİRDEĞİ PROTEİNİNİN EKSTRAKSİYONU VE GLİKASYONUNUN OPTİMİZASYONU

Taş, Ozan
Doktora, Gıda Mühendisliği
Tez Yöneticisi: Prof. Dr. Mecit Halil Öztop
Ortak Tez Yöneticisi: Prof. Dr. Servet Gülüm Şümnü

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Tüketicilerin hayvansal proteinlerden bitkisel proteinlere yönelmesi; sürdürülebilirlik hedefleri, düşük maliyet ve yüksek besin değeri gibi nedenlerle artmaktadır. Bu tezde kabak çekirdeği protein konsantresinin (PSPC) ekstraksiyonu, fonksiyonel modifikasyonu ve domates sosunda kullanımı araştırılmıştır. İlk olarak, PSPC; alkali, tuz ve enzim destekli alkali yöntemleri ile mikrodalga veya konvansiyonel ön ısıtma kullanılarak elde edilmiştir. Mikrodalga destekli alkali ekstraksiyon (MH-AE) en yüksek verimi (~%55), protein içeriğini (%77) ve fonksiyonel özellikleri sağlamıştır. İkinci aşamada, MH-AE ile elde edilen PSPC; fruktoz ve allüloz ile 1:1 ve 3:1 oranlarında glikasyona tabi tutulmuştur. Glikasyon derecesi kahverengileşme indeksi, serbest amino grupları, indirgen şeker miktarı, amino asit profili ve FTIR ile belirlenmiştir. Özellikle 1:1 fruktoz konjugatları en yüksek glikasyon derecesini ve çözünürlük, yüzey hidrofobiklik, emülsiyon, köpürme, antioksidan ve hidrasyon özelliklerinde gelişme göstermiştir. Üçüncü aşamada, glikasyon uygulanmış PSPC; zeytin ve domates kabuğu tozu ile zenginleştirilmiş domates soslarına ilave edilmiştir. Kontrol (TS-C), işlenmemiş

PSPC (TS-U), allüloz-glikasyonlu (TS-A) ve fruktoz-glikasyonlu (TS-F) örnekleri incelenmiştir. Konjugatlar protein çözünürlüğünü, °Brix'i, antioksidan aktiviteyi ve sindirilebilirliği artırmış; en yüksek gelişme TS-F ve TS-A'da gözlenmiştir. Son olarak, Zaman Alanlı Nükleer Manyetik Rezonans (TD-NMR) ile PSPC ve şekerlerde hidrasyon ve moleküler hareketlilik analiz edilmiştir. Bulgular sakkarozun en güçlü, allülozun en zayıf su bağlama kapasitesine sahip olduğunu göstermiştir. Sonuç olarak, optimize ekstraksiyon ve glikasyon PSPC'nin fonksiyonel özelliklerini geliştirmiş, bitkisel, temiz etiketli ve katma değerli ürünlerde kullanım potansiyelini ortaya koymuştur.

Anahtar Kelimeler: Kabak çekirdeği protein konsantresi, glikasyon, domates sosu, TD-NMR Relaksometre

To the ones who never stopped believing in me, especially my family

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LIST OF ABBREVIATIONS

ABBREVIATIONS

1,1-diphenyl-2-picryl hydrazyl	: DPPH
Alkali extracted	: AE
Advanced glycation end-products	: AGEs
Analysis of Variance	: ANOVA
Conventional heated	: CH
Conventional heated alkali extracted	: CH-AE
Conventional heated enzyme-assisted extracted	: CH-EE
Conventional heated salt extracted	: CH-SE
Emulsifying Activity	: EA
Enzyme-assisted alkali extracted	: EE
Emulsifying Stability	: ES
Foaming Capacity	: FC
Foaming Stability	: FS
Fourier transform spectroscopy	: FTIR
Free amino group	: FAG
High-performance liquid chromatography	: HPLC
Microwave-assisted extraction	: MAE
Microwave-heated	: MH
Microwave-heated alkali extracted	: MH-AE

Microwave-heated enzyme-assisted extracted	: MH-EE
Microwave-heated salt extracted	: MH-SE
Multiple Reaction Monitoring	: MRM
o-phthalaldehyde	: OPA
Pumpkin seed flour	: PSF
Pumpkin seed protein concentrate	: PSPC
Pumpkin seed protein isolate	: PSPI
Remaining Reducing Sugar	: RRS
Scavenging activity	: SA
Salt extracted	: SE
Time-Domain Nuclear Magnetic Resonance	: TD-NMR
Tomato sauce with allulose glycated protein	: TS-A
Tomato sauce with fructose glycated protein	: TS-F
Tomato sauce with no additional protein	: TS-C
Tomato sauce with unmodified protein	: TS-U
Untreated	: UT
Untreated alkali extracted	: UT-AE
Untreated enzyme-assisted extracted	: UT-EE
Untreated salt extracted	: UT-SE
Water holding capacity	: WHC
Water solubility index	: WSI

CHAPTER 1

INTRODUCTION

1.1 Pumpkin Seed

1.1.1 General View

Pumpkin seeds, also known as pepitas, are derived from pumpkins, which are members of the Cucurbitaceae family with different varieties such as *Cucurbita pepo*, *Cucurbita maxima*, and *Cucurbita moschata* (Batool, Modassar, et al., 2022). Pumpkin seeds have been used for their nutritional and therapeutic properties throughout history. They were used as food in ancient American civilizations and were also regarded for their medicinal benefits (Yosefina Lewar, 2019). Pumpkin seed production has increased globally over the past few years, with an estimated annual production of approximately 25 million metric tons. Major producer countries are China, India, and the United States. Turkey contributes about 200,000 metric tons of pumpkins annually, making it another major producer in the world (K.O.G. Himani Ruwanthika et al., 2023).

Pumpkin seeds are rich in macro- and micronutrients essential for human health. They contain ~40% oil, mostly in the form of unsaturated fatty acids like oleic and linoleic acids, which are known for their beneficial effects on cardiovascular health (Dotto & Chacha, 2020). Apart from being a great source of oil, pumpkin seeds are an excellent source of high-quality protein. The seeds are also abundant in vitamins and minerals, particularly magnesium, iron, zinc, and vitamin E in the form of tocopherols and other antioxidants, which may reduce oxidative stress and the risk of chronic diseases (Batool, Modassar, et al., 2022; Hussain et al., 2023).

Pumpkin seeds are highly versatile in food applications. They are used in salads, baked dishes, and meals to offer extra flavor and nutrients. They can be consumed raw, roasted, or sprouted. For instance, cold-pressed pumpkin seed oil is preferred to be used in salad dressings and soups with its rich and nutty flavor (Tekin-Cakmak et al., 2021).

1.1.2 Pumpkin Seed Protein

People are becoming more attracted to plant proteins because of the negative environmental implications of animal protein production, as well as expanding veganism and vegetarianism trends in the last few decades (Ertugrul, Namli, Tas, Kocadagli, et al., 2021; Thakur et al., 2024). With the increasing trend towards sustainable food sources, the food industry is particularly interested in producing plant protein concentrates or isolates due to their capacity to enhance both nutritional value and functional properties. Protein sources such as peanuts, peas, pumpkin seeds, sesame, lentils, beans, and chickpeas are under the spotlight (Chandran et al., 2023). Among them, pumpkin seed protein (PSP) is gaining attention as a plant-based protein source in functional foods and dietary supplements. Ongoing research continues to reveal the potential of pumpkin seeds, expanding their position as a functional food (Aziz et al., 2023; Batool, Modassar, et al., 2022; Dotto & Chacha, 2020).

The defatted pumpkin seed flour (PSF) obtained from pumpkin seeds is high in protein content, approximately 29-33%, with an average of 30%. The proteins of pumpkin seeds contain various compartments, including globulins (62%), albumins (12.5%), glutelins (4.5%), and prolamins (1.2%), which are mostly stored in the cotyledons. Like other oilseed proteins, globulins dominate the protein content in pumpkin seeds (Lazos, 1992).

PSP contains all the essential amino acids, particularly rich in tryptophan, leucine, phenylalanine, arginine, and glutamic acid (Singh & Kumar, 2024). The

bioavailability and stability of those amino acids are high in PSP; thus, researchers suggest using PSP as a dietary supplement. Given these nutritional benefits, PSP can be a promising alternative among plant-based proteins. In addition, PSP has low allergenic potential, unlike common plant proteins such as soy or peanuts, which is valuable in developing hypoallergenic food products (Becker et al., 2018; S. Das et al., 2021). Consequently, extracting protein from pumpkin seeds can be considered a valuable process, offering a high-quality protein source for various food applications.

1.2 Protein Extraction

The plant proteins have functional properties such as solubility, water, and oil binding abilities, emulsifying ability and stability, foaming capacity and stability, antioxidant activities, as well as nutritional quality (Ma et al., 2022; Nikbakht Nasrabadi et al., 2021).

The selected process of extraction of protein from plant sources has a significant impact on the protein's composition and quality, in addition to its functional properties (Navaf et al., 2023). For that, many considerations need to be made to develop an efficient protein extraction technique. The use of specific compounds during the precipitation or extraction process may introduce toxicity issues, which may require expensive treatments to eliminate these substances afterward. Furthermore, different plant parts may cause different problems for the extraction process, such as high fiber content, sticky textures, or hard tissues like cell walls (Duistermaat & Kolk, 2000; Nguyen et al., 2023). The existence of these parts highlights how difficult and crucial it is to optimize protein extraction methods to ensure the quality and safety of the final plant protein concentrates or isolates.

The food industry is continuously trying to identify the most effective extraction techniques for plant proteins (Koysuren et al., 2021). Some of the techniques can be

listed as *alkali extraction, salt extraction, enzyme-assisted alkali extraction, solvent extraction, and dry fractionation*.

1.2.1 Alkali Extraction

The method of alkali extraction involves solubilizing plant proteins in an alkaline medium, typically using solutions of sodium hydroxide (NaOH) or potassium hydroxide (KOH) (Momen et al., 2021). In the procedure, plant material is dissolved in water, and then the pH is adjusted to an alkaline range, often between pH 8 and 11. Proteins become soluble in these circumstances and can be extracted from the plant matrix. After that, the pH is adjusted to the isoelectric point (pI) of the target protein in the solution, causing the target protein to precipitate out of the solution. This process is known as isoelectric precipitation. To achieve the final protein isolation, the precipitated proteins are gathered by centrifugation or filtering, followed by washing and drying (Hadidi et al., 2023).

Alkali extraction is affected by several parameters, such as pH, temperature, agitation, and solute-solvent ratio (Hadinoto et al., 2024). The selection of true pH is critical in alkali extraction. Generally, a value between 8 and 11, depending on the specific plant protein being extracted, is essential for solubilization. However, denaturation may result from additional pH rise and lead to undesirable extracted protein (Z. Gao et al., 2020). Besides, the temperature is an effective parameter in the efficiency of extraction (Silva et al., 2023). It is preferred to stay in the 20°C to 60°C range since higher temperatures could again cause denaturation, which may result in lower functionality of the extracted protein (Avula, 2014). Protein solubilization is improved during extraction when there is constant agitation to maintain consistent conditions and improve mass transfer (U. Shah et al., 2024). The protein content in the extract is also influenced by the solute-to-solvent ratio, which is generally used in the range from 1:10 to 1:20 (w/v), balancing the efficiency requirements of processing (Urribarri et al., 2009).

The alkali extraction method has many advantages in the solubilization of plant proteins for several reasons. The method is quite simple and utilizes relatively inexpensive chemicals, which make large-scale production economically feasible (J. Tang et al., 2024). This method can be used in a variety of plant sources, such as oilseeds, including pumpkin seeds, peas, and chickpeas, due to its adaptability (Sari et al., 2015). Furthermore, the proteins obtained through alkali extraction are quite pure, meaning they can be integrated directly into food products, nutraceuticals, and other applications.

There are also some disadvantages associated with the alkali extraction method. In the medium, some undesirable reactions such as amino acid racemization, lysinoalanine formation, decreased digestibility, and loss of essential amino acids may occur (Koysuren et al., 2021). Moreover, polyphenols have the potential to oxidize, resulting in a darker color for the isolated proteins. The strong alkali environment also requires extensive washing to remove any remaining chemicals. If alkaline waste disposal is not handled correctly, it might also cause environmental problems (Zaini et al., 2023; C. Zhang et al., 2014).

1.2.2 Salt Extraction

Salt extraction is a significant approach for isolating plant proteins since the ionic strength of the salt solution affects solubility and functionality (Y. Zhang et al., 2025). Depending on the ionic strength of the environment, proteins display different behaviors in the extraction process (Hewage et al., 2022). It is called ‘salting in’ when the salt solution facilitates the protein solubility. However, if added salt causes proteins to become less soluble, this process is known as ‘salting out’ (C. H. Lee, 2017). These effects depend on the type and concentration of salts used. The hydration layer around proteins, depending on the concentration of the ionic solution, is important for solubility. Three main interactions occur, which are ion hydration, hydrogen bonding, and hydrophobic interactions (Varma et al., 2010). The addition of more salt increases the surface tension of the water, enhancing hydrophobic

interactions. Proteins undergo inward folding and eventually precipitate, resulting in reduced solubility (Dahal & Schmit, 2018; Hadnadjev et al., 2017). The Hofmeister series discovered that certain salts may either stabilize or destabilize proteins (Hofmeister, 1888; Kang et al., 2020). The ions in this series are grouped according to how much they can affect the solubility of proteins. For instance, ions such as calcium and magnesium typically enhance solubility, whereas ions like sulfate and ammonium promote precipitation and protein aggregation (Hyde et al., 2017). Figure 1.1 below shows the Hofmeister series that revealed the effect of varied ions on the solubility and stability of proteins as salting in and salting out.

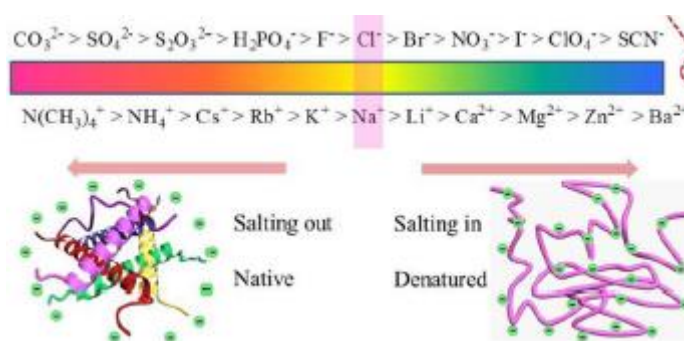


Figure 1.1. The Hofmeister series illustrates how various ions affect the solubility and stability of proteins through salting-in and salting-out (Kang et al., 2020)

To achieve salt extraction, a ‘salting in’ phenomenon is used to dissolve proteins (Vargel, 2020). Globulin proteins, sensitive to pH and ionic strength, break down into subunits under these conditions. A study conducted on pea proteins showed that salt-extracted fractions of the protein had better oil binding and emulsifying capacities than those extracted using alkali (Farshi et al., 2024). Hence, salt-extracted proteins with high oil-binding capacity can be considered advantageous for meat products, while those with good emulsifying properties are ideal for dressings and soups (Maurer et al., 1969; Stone et al., 2015). On the other hand, salt extraction may lead to some undesirable flavors or tastes in the isolated proteins because of the residual salt, which has the potential to affect the sensory qualities of the final product (C. H. Lee, 2017). Moreover, the procedure can involve considerable water

consumption and result in the generation of saline wastewater, which can cause ecological issues if not managed appropriately.

1.2.3 Enzyme-Assisted Alkali Extraction

Enzyme-assisted alkali extraction is a technique that may enhance plant protein extraction by combining the use of enzymes with alkaline conditions (Miranda et al., 2022; Perović et al., 2020). This method is especially beneficial since it can reduce some of the problems associated with high alkaline conditions alone. It has been documented that those proteins extracted by the high-alkaline method lowered nutritional value because of reactions such as amino acid racemization and lysinoalanine production (Caron & Markusen, 2016; Friedman et al., 1984). These outcomes can also cause poor functionality of the extracted proteins. With enzyme-assisted alkali extraction, these problems can be lowered by processing at lower and less severe conditions while achieving preserved nutritional values and improved functional properties (Miranda et al., 2022).

In enzyme-assisted alkali extraction, proteases are frequently employed to facilitate the proteolysis process, which extracts proteins. Protein molecules are reduced in size by these enzymes, which contribute to their extraction (Diasa & de Moura Bell, 2022; Lasrichan et al., 2024). Especially, the high activity of *Alcalase* for enzyme-assisted protein extraction was shown as an effective protease in previous studies (Chatterjee et al., 2015; Ozuna & León-Galván, 2017).

Protease alone has the potential to increase extraction yields, but its combination with carbohydrases is frequently more successful (Le et al., 2018). Carbohydrases facilitate the breakdown of the cell wall, which enables the release of intracellular substances, such as proteins (Rommi et al., 2014). Studies have shown that while proteases alone do not significantly increase yields, combining them with carbohydrases results in a higher extraction yield than using proteases alone (Koysuren et al., 2021; Niemi et al., 2013).

Enzyme-assisted alkali extraction has several advantages. It can improve protein yield and functionality while being more environmentally sustainable. Combining enzyme and alkaline procedures can offer a balanced approach to protein extraction, with its ability to preserve the bioactive compounds, making them more suitable for health-focused food products (Franca-Oliveira et al., 2021; Streimikyte et al., 2022). Although there are several advantages, the procedure can be more expensive since certain enzymes are required, and extreme caution must be taken to avoid excessive protein breakdown, which could reduce the functional properties of the proteins (Streimikyte et al., 2022).

1.3 Pre-treatment Techniques for Enhancing Protein Extraction

Each of the previously discussed techniques, alkali, salt, and enzyme-assisted alkali extraction, has some advantages and disadvantages of its own. Although those methods can sufficiently extract plant proteins, their effectiveness and usefulness may be restricted by issues such as environmental concerns, chemical residues, and denaturation of proteins (Z. Wang & Sun, 2023).

Researchers are trying to find alternative approaches to improve plant protein extraction. Pre-treatment methods are shown as an effective strategy for resolving these problems and enhancing the extraction yield and functionality of protein isolates (Zhu et al., 2024). In the literature, there are some pre-treatment techniques applied and shown for their effects on plant protein extraction, such as microwave preheating, ultrasonication, and pulsed electric field (PEF) treatment (Franca-Oliveira et al., 2021; Navaf et al., 2023; Ramaswamy et al., 2024; Varghese & Pare, 2019).

Ultrasonication is a pre-treatment technology that has attracted attention for its ability to improve plant protein extraction. It works by agitating particles in a liquid medium using high-frequency sound waves (Ojha et al., 2018). The mechanism is based on the phenomenon known as acoustic cavitation, in which localized high

pressures and temperatures are produced by the rapid production and collapse of bubbles within a liquid (Chemat et al., 2017). Proteins and other intracellular components are released when cell walls and membranes are disrupted by this mechanical energy, which increases their accessibility during the extraction process that follows (Chemat et al., 2017).

Pulsed Electric Field (PEF) is a promising non-thermal pre-treatment technique for improving plant protein extraction. It involves applying short bursts of high-voltage electric fields (typically 1–50 kV/cm) to plant materials, which induces electroporation which creating temporary or permanent pores in cell membranes (M. Tiwari et al., n.d.). This enhances the release of intracellular contents, including proteins, especially from tough or fibrous plant tissues. PEF not only improves extraction efficiency and protein yield but also preserves the nutritional and functional properties of proteins, such as solubility, emulsifying ability, and gelation (Patil et al., 2025). Moreover, it is considered environmentally friendly and energy-efficient compared to traditional thermal methods, as it requires no hazardous chemicals and uses less energy. Despite its advantages, PEF does have some limitations. The initial cost and complexity of equipment such as high-voltage pulse generators and treatment chambers can be a barrier to adoption, particularly for small-scale producers. Additionally, ensuring uniform electric field distribution within the treatment chamber can be challenging, potentially leading to inconsistent results (Bocker & Silva, 2022).

Although both ultrasonication and PEF offer notable advantages, microwave preheating has shown even greater potential in terms of enhancing extraction yield, simplifying process scalability, and improving functional properties. Studies have demonstrated that microwave treatment can rapidly heat plant tissues, disrupt cell structures more uniformly, and facilitate solvent penetration, which collectively contribute to higher protein recovery and improved solubility and emulsification properties (Chemat et al., 2017). Furthermore, compared to other techniques, microwave pre-treatment requires shorter processing times and is easier to integrate into continuous processing systems, making it more suitable for industrial

applications (Puligundla et al., 2016). Therefore, the following section will focus more extensively on the microwave pre-treatment in plant protein extraction.

1.3.1 Microwave Pretreatment

Plant protein extraction can be improved by pre-treating with microwave energy, which is a very successful technique (Kadam et al., 2023). Proteins and other intracellular components are more easily accessible during the extraction process thanks to this treatment, which uses microwave radiation to heat plant materials and break down cell structures (Q. Hu et al., 2021; Y. Su et al., 2017).

It was in the middle of the 1940s, when the microwave technique first appeared, that Percy Spencer and his associates at Raytheon Manufacturing Company invented the first microwave oven (Osepchuk, 2009). The concept of using dielectric materials to match electromagnetic radiation dates to before World War II, but it wasn't until the invention of the microwave oven that microwave technology became widely used in food processing. Microwave ovens were initially regarded with mistrust by food makers, but they are now widely used in both home and commercial settings (H. Zhang & Foundation, 2017).

Microwaves function within a particular range of the electromagnetic spectrum, specifically in the 300 MHz to 300000 MHz region. However, in real-world applications, 915 MHz and 2450 MHz are generally utilized in industrial and household settings, respectively (Bhatt et al., 2020; Galema, 1997). The fundamental process of microwave heating is due to the thermal energy created by electromagnetic energy conversion. Ionic conduction and dipolar rotation are the two main processes that cause this creation (Thostenson & Chou, 1999). Dipolar rotation (Figure 1.2A) causes rotational motion and friction that raises the temperature even further as polar molecules in the material try to align themselves with the varying electric field (Galema, 1997). Ionic conduction (Figure 1.2B) involves the oscillation of dissociative ions in the compound due to the alternating electric field produced by

the microwave (J. Sun et al., 2016). The kinetic energy of the ion is increased because of collisions caused by this motion, raising the temperature of the material (Anwar et al., 2015).

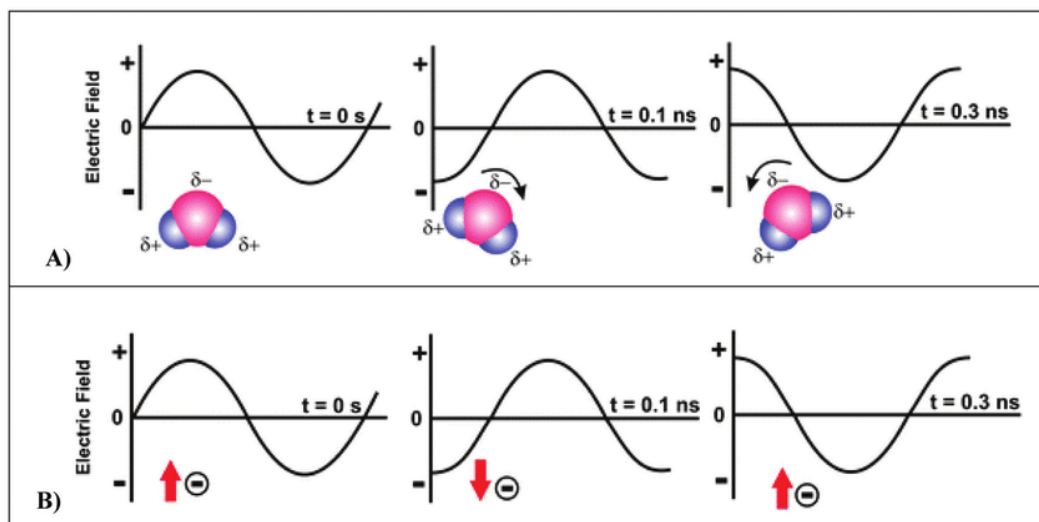


Figure 1.2. Mechanism of heat generation in microwave heating. (A) Dipole rotation, and (B) Ionic conduction (Verma et al., 2020)

Microwave heating is very effective because of these mechanisms, which enable heat to be transported quickly and evenly from the material's interior outward. Microwaves can heat materials quickly and evenly, which allows them to reduce processing times greatly when compared to conventional heating methods (Q. Hu et al., 2021). Because of their effectiveness and capacity to heat ingredients only depending on their dielectric characteristics, microwaves are flexible instruments in the food processing industry (J. Tang, 2015). Moreover, microwave cooking reduces the possibility of overcooking, maintaining the product's desirable texture and fresh flavor while avoiding nutritional loss (Kutlu et al., 2022).

Microwave heating depends on the dielectric properties of the material, which are the dielectric constant (ϵ') and the dielectric loss factor (ϵ'') (Omran et al., 2019). The dielectric constant indicates how well a material can store electrical energy when subjected to an electric field, while the dielectric loss factor reflects how well the material can convert that stored energy into heat. These properties combine to create

complex permittivity ($\epsilon = \epsilon' - j\epsilon''$), which determines the amount of microwave energy absorbed and converted into heat (Z. Li et al., 2025).

Due to its high dipole moment and relatively high dielectric loss factor, water plays a dominant role in microwave heating. Thus, foods that contain more moisture tend to heat up more efficiently and quickly (Zhou et al., 2023). Other components of food, such as fats, carbohydrates, and salts, also affect microwave absorption. Due to their low dielectric loss factor, fats heat less efficiently than water. However, dissolved salts and ions boost ionic conduction, leading to further heating effects (L. Zhang et al., 2007).

Proteins are generally less responsive to microwave heating than water, but microwave applications have been used in protein extraction processes. Numerous investigations have examined the effects of microwave preheating on the extraction of proteins from various plant sources (Choi et al., 2006; Deng et al., 2022; Karabulut et al., 2024). It was revealed that microwave application involves simultaneous heat and mass transfer from the interior part of the solid matrix to the extraction solvent. In this way, the extraction yields of the analyte with microwave are comparable to or even higher than those achieved using traditional methods, but with reduced solvent consumption and shorter extraction times (Xing et al., 2017). Investigations into microwave-assisted extraction (MAE) by Amponsah et al. (2016) revealed that MAE enhanced extraction efficiency and improved the properties of soy proteins more than traditional heating, with less energy consumption and shorter processing times.

The power level and duration of the microwave are important factors that affect the quality and efficiency of protein extraction from plant materials (Bedin et al., 2019; Q. Hu et al., 2021). Microwave power influences the solubility and release of proteins and influences the rates at which heat is generated in the sample matrix (S. Wang et al., 2022). Higher power levels have the potential to accelerate heating and improve protein solubilization by increasing the penetration of microwave energy. However, overpowering can denature the proteins and reduce their functional

properties (X. Zhang et al., 2023). Likewise, the duration of microwave exposure is critical, as insufficient time may result in incomplete protein release, while prolonged treatment could cause thermal degradation or aggregation, which can harm their functionality (Xiang et al., 2020).

Due to its effectiveness and capacity for uniform heating, microwave preheating distinguishes itself from other pre-treatment methods like ultrasonication and high-pressure processing. Plant cell walls can be broken down, and the yield of bioactive compounds increases more with microwave preheating than with ultrasonication, according to comparison research by da Rosa et al., (2019). As this illustrates, the ability to achieve high extraction efficiency in a shorter processing time is one of the special benefits of microwave preheating.

1.4 Functional Properties of Pumpkin Seed Protein

Pumpkin seed proteins (PSPs) are becoming more popular among consumers because of their useful properties, which allow them to be used in a wide range of food applications (Batool, Modassar, et al., 2022; Bedi & Tewari, 2024). Solubility, antioxidant, emulsification, and foaming activities are among the functional properties that are strongly linked to the intrinsic physicochemical properties of proteins, which play a significant role in determining their behavior in food systems during processing, preparation, manufacturing, and storage (Tas, Ertugrul, Oztop, & Mazi, 2021; Y. Zhang et al., 2021). It is essential to understand how these properties are susceptible to changes in the composition, interactions, and structure of proteins with other food components. Moreover, the conditions during extraction treatments and processing methods greatly influence the functional properties.

1.4.1 Protein Solubility

One of the most important functional characteristics of proteins that greatly affects how they are used in different food systems is their solubility (Yousefi & Abbasi,

2022a). It describes the ability of the protein to dissolve in a solvent, usually water. This fundamental property directly affects other functional properties such as emulsification, foaming, and gelation (Haque et al., 2016; Y. Zhang et al., 2021). Hence, the solubility of plant proteins should be well understood to fully understand their beneficial effects in food products. One of the most popular and reliable methods to determine soluble proteins is the Lowry method, developed by Oliver H. Lowry in 1951. Researchers Namli et al., (2021), Waterborg & Matthews, (1994), and many others have confirmed the reliability of this method, which has been widely used in several studies since it was first developed.

A complex interaction of extrinsic factors, including temperature, pH, ionic strength, processing techniques, and intrinsic factors like amino acid composition and structural conformation, determines the protein solubility (K. Gao et al., 2024; Kramer et al., 2012).

One of the most essential extrinsic factors, pH, has a significant impact on protein solubility because it modifies the net charge of the protein (Shaw et al., 2001). Proteins tend to precipitate at the isoelectric point (pI), where the net charge is zero, which reduces their solubility. On the other hand, the positive or negative charge of the proteins increases when the pH moves away from the pI, improving solubility due to the greater interactions between the protein and water (Shaw et al., 2001; Tokmakov et al., 2021). In the case of PSP, studies have shown that solubility is lowest at pH 4.5, corresponding to their pI, and improves significantly at lower and higher pH values (Lazos, 1992). The solubility of proteins from protein isolate, protein concentrate, and PSF was examined in another study at various pH values (Atuonwu & Akobundu, 2010). It was discovered that the pH range of 6 to 8 showed the lowest protein solubility in all samples, whereas pH 10 showed the highest solubility. This shows that the proteins from pumpkin seeds become more soluble under more alkaline circumstances, becoming less soluble at pH values close to neutral. Most foods have a pH range from 3 to 7.5. The products, such as fruit juices and tomato sauces, have a pH of 3-5, while neutral foods like dairy products or cooked vegetables are closer to pH 6-7 (Koutsoumanis et al., 2022). Integrating PSP

into food systems that fall within this pH range could affect its solubility, stability, and other functional properties. Therefore, it is essential to optimize extraction methods or modify the protein to enhance its dispersion and functional properties in the pH environment of the food component.

The solubility of proteins can also be significantly affected by the ionic strength of the solution and the presence of salts (Shire, 2015). As explained before, the ‘salting in’ and ‘salting out’ behavior of salts directly affects protein solubility. Like other plant proteins, PSPs display this behavior based on the type and concentration of salt. According to Pham et al., (2017) salting-in effect increased the solubility of the pumpkin seed albumin, globulin, and glutelin fractions by 33%, 41%, and 62%, respectively, when the salt concentration increased from 0 to 0.2 M NaCl.

The tertiary and quaternary structures of proteins, which monitor the exposure of hydrophobic and hydrophilic groups, play a crucial role in determining solubility associated with intrinsic factors (Terpe, 2003). For instance, the distribution of polar and non-polar amino acids in these structures greatly influences the solubility of plant proteins, including PSPs, which are a mixture of albumins, globulins, and other protein types (Rezig et al., 2013).

The method used to extract and process plant proteins can greatly influence their solubility. Different extraction techniques change the protein structure and the environment in which the proteins are dissolved, thereby affecting their solubility (Chandran et al., 2024; Hadidi et al., 2023). Previous research has shown that extracting plant proteins under various conditions and by different techniques improves the solubility of the protein (Koysuren et al., 2021; Navaf et al., 2023). In addition, by changing the physical structure of the proteins, pre-treatments such as pulsed electric fields (PEF), ultrasonication, and microwave heating can affect protein solubility (Jambrak et al., 2008; Nikbakht Nasrabadi et al., 2021; Taha et al., 2023).

By carefully choosing and refining these parameters, the solubility and other functional properties of PSPs can be enhanced, leading to a greater range of uses in the food industry.

1.4.2 Water Binding and Water Absorption

Water binding and absorption, or water holding capacity (WHC), is an essential functional property of proteins that affects how it is used in different food formulations (Kinsella & Melachouris, 1976). It is one of the properties of proteins that can retain added water during processes such as pressing, centrifugation, and heating (Butler, 1986). This ability is essential for packaging design as well as for the texture and color of baked products, soups, kinds of pasta, and meat products (Q. Cheng & Sun, 2008; Warner, 2017).

The WHC of plant proteins, including PSP, is influenced by several variables, including the surface properties of proteins and environmental factors, including temperature, ionic strength, and pH (Ma et al., 2022). The surface characteristics of the protein affect the hydration mechanisms, which include hydrophobic interactions, hydrogen bonding, and ion hydration (Wiggins, 1997). In one of the studies, the defatted pumpkin seed itself and pumpkin seed protein isolate (PSPI) were compared in terms of WHC (Das et al., 2022). The study found that PSPI showed a slightly greater WHC than the defatted seeds sample. They explained this phenomenon due to the ability of the protein isolate can unfold, dissociate, and expose more water-binding sites.

As in the case of solubility, the WHC of plant proteins is minimal at their pI, where protein-protein interactions are the strongest. However, WHC can be enhanced by the pH variations, which would create more water-binding sites (Cornet et al., 2021). Furthermore, heat treatment has been demonstrated to raise WHC through the unfolding of proteins, which expose additional water-binding sites (Luo et al., 2022).

1.4.3 Oil and Fat Binding

An important characteristic of proteins is their ability to bind fat and oil, especially in food products such as dough, emulsions, dairy products, sausages, and bread. This characteristic greatly affects the mouthfeel and texture of food since fats improve mouthfeel and maintain flavor (Demir et al., 2023).

The primary mechanism of oil binding in proteins is the interaction between the hydrocarbon chains of fats and the nonpolar side chains of amino acids (Aruchunan et al., 2025). Because of the affinity of protein for the hydrophobic parts of fat molecules, oil is retained and stabilized inside the protein matrix by these nonpolar protein regions (Furuhashi & Hotamisligil, 2008). Moreover, the structural conformation of the protein is significant; when the protein unfolds, either on its own or due to processing, more hydrophobic areas become exposed, enhancing the protein's capacity to bind and hold onto oil (G. Wei et al., 2017). In the study by Das et al., (2022), it was discovered that PSPI had a greater capacity for absorbing fat than defatted pumpkin seeds. They explained that due to the presence of non-polar amino acids that bind fat hydrocarbon chains and increase oil absorption, PSPI appeared to have more lipophilic sites in its structure, as indicated by its higher fat absorption.

Particle size, temperature, processing conditions, surface hydrophobicity, and protein source are some of the variables that affect a protein's ability to bind fat (Zayas, 1997). Research on related proteins, like protein isolates from sesame and soy, has demonstrated that these proteins can similarly absorb a large amount of oil, highlighting the role that hydrophobicity plays in fat-binding properties (Koysuren et al., 2021; Yong et al., 2021).

1.4.4 Emulsifying Activity (EA) and Emulsifying Stability (ES)

An emulsion is a mixture of two immiscible liquids, usually water and oil, in which small droplets of one liquid are spread throughout the other (Tadros, 2013). One of the liquids must be separated into droplets and stabilized inside the other liquid to form an emulsion (Evgeny, 1999). This is done by employing emulsifying agents like proteins or surfactants, which lower the interfacial tension between the oil and water phases, as well as mechanical energy applications such as shaking or stirring. Because proteins are amphiphilic, meaning they include both hydrophobic and hydrophilic areas, they are very good emulsifiers (Hoffmann & Reger, 2014). They can interact with the water and oil phases, stabilizing the emulsion by binding themselves to the interface (X. Zhang et al., 2023).

Emulsifying activity refers to the ability of a protein to promote the formation of an emulsion, while emulsifying stability refers to the protein's capacity to maintain that emulsion over time (D. Ding, 2021; X. Zhang et al., 2023). PSP consists of various protein fractions, including albumin and globulin, which play crucial roles in their emulsifying properties (Bučko et al., 2015). Because of its more flexible shape and lower molecular weight, the albumin fraction has been discovered to have a major role in maintaining the stability of emulsions (Jaegers et al., 2022).

PSP, like other plant proteins, has a lower emulsification capability close to its pI because of low solubility (H. N. Liang & Tang, 2013). On the other hand, the protein becomes more soluble as the pH moves away from the pI, improving the protein's ability to form and maintain emulsions. For example, in one study, the highest emulsifying activity was found at an alkaline pH, approximately pH 10, where the protein is more soluble and can interact at the oil-water interface more successfully (Pham et al., 2017). In another study, it was shown that heat-assisted pH shifting treatment, which involves subjecting extreme alkaline conditions with mild heating, played an essential role in the improvement of the emulsifying properties of PSPI (Y. Sun et al., 2024).

The method of extraction used to obtain PSP can affect its emulsifying properties in addition to pH. For instance, it has been discovered that proteins isolated with the help of enzymes have better emulsification activity and stability (Chandran et al., 2024). This improvement was linked to the partial hydrolysis of protein, which increases the quantity of hydrophilic-hydrophobic groups that can stabilize the emulsion. These groups enable the protein to more effectively stabilize the water-oil contact, leading to more stable emulsions.

1.4.5 Foaming Capacity (FC) and Foaming Stability (FS)

When it is related to food applications that require aeration, such as baked products, whipped toppings, and meringues, the ability of proteins to foam and their stability are crucial functional properties (Foegeding et al., 2006). Both intrinsic (such as protein structure and content) and extrinsic (such as pH, temperature, and extraction methods) factors can impact the effectiveness of foaming properties (Amagliani et al., 2021; Phillips et al., 1990).

The ability of a protein to combine with air to create foam is known as foaming capacity, and the ability of that foam's resistance to collapse over time is known as foaming stability (Ivanova et al., 2018). The solubility and capacity of protein to unfold and adsorb at the air-water interface, where it can lower surface tension and trap air bubbles, are major factors affecting these characteristics (Condé et al., 2017). As pointed out earlier, solubility is the main feature that directly affects functional properties, including foaming properties. More soluble proteins can more easily migrate to the interface and stabilize the foam, which leads to a greater foaming capacity (Momen et al., 2021).

Like its effect on emulsifying properties, PSP showed lower foaming properties around pI due to the reduced solubility. According to the studies, PSPs have a greater ability to foam at alkaline pH values (Dawa et al., 2013; Lazos, 1992). This is due to the protein having a higher solubility at these pH levels, which helps to stabilize the

air bubbles within the foam. Furthermore, studies have also revealed that temperature has an impact on the foaming properties (Oetjen et al., 2014). Elevated temperatures have the potential to enhance protein denaturation, thereby facilitating the unfolding of protein at the air-water interface. This unfolding process increases the surface activity of proteins, allowing them to adsorb and rearrange at the interface, forming a viscoelastic film that stabilizes air bubbles within the foam matrix. Moreover, moderate thermal treatment can expose hydrophobic groups and reactive sites, improving interfacial interactions (Nicorescu et al., 2011). On the other hand, overheating could cause aggregation, which would destabilize the foam. Excessive heat may lead to irreversible protein-protein interactions and the formation of large insoluble aggregates, which are known to be less flexible and less capable of forming cohesive interfacial layers (Van der Plancken et al., 2007). As a result, the foam structure may become weak due to insufficient stabilization.

1.4.6 Antioxidant Activity

Antioxidants are substances that inhibit oxidation, a chemical process that can result in free radicals and lead to a chain reaction that could cause cell damage (Lobo et al., 2010). Antioxidants protect the body against oxidative stress in biological processes, which can cause chronic illnesses like cancer, heart disease, and neurological disorders (Sharifi-Rad et al., 2020). By donating an electron, they neutralize free radicals and stop these reactive substances from damaging cells.

The antioxidant activity of compounds can be determined by a variety of approaches, the most widely utilized of which are fluorimetry, DPPH, ORAC, ABTS, FRAP, PFRAP, HORAC, and TRAP, and ferrous reducing power (Munteanu & Apetrei, 2021). The DPPH (2,2-di(4-tert-octyl-phenyl)-1-picrylhydrazyl) radical scavenging activity is one of the most common ones since it is easy to operate, cheap, and fast in determining the antioxidant content of compounds (Shekhar & Anju, 2014).

Because plant proteins contain certain amino acid residues and peptides that can chelate metal ions, scavenge free radicals, and inhibit oxidative enzymes, they can have antioxidant properties (Samaranayaka & Li-Chan, 2011). The structure of the protein, which controls how it interacts with and neutralizes reactive oxygen species (ROS), significantly impacts these characteristics. For example, the antioxidant activity of amino acids like cysteine, tyrosine, and tryptophan is attributed to their ability to neutralize free radicals by donating electrons or hydrogen atoms (N. Xu et al., 2017). Furthermore, the ability of a protein to function as an antioxidant can be affected by several conditions, such as pH, temperature, and the presence of metal ions, which can change the protein structure and how it interacts with free radicals (M. Li et al., 2021).

Natural bioactive chemicals found in pumpkin seeds, such as carotenoids, tocopherols, and sterols, have been shown to have antioxidant effects and may help prevent diseases, including cancer, diabetes, and hypertension (Batool, Ranjha, et al., 2022). In addition, ascorbic acid, an essential vitamin with strong antioxidant properties, is abundant in pumpkin seeds (Kar et al., 2023). In one of the studies on pumpkin seeds, it was revealed that elevated temperatures improved the antioxidant properties, such as total phenols and flavonoids (M. Peng et al., 2021). Another study by Mazloomi et al., (2019) examined the antioxidant properties of the PSP through varied pH and temperature values. The study demonstrated that over a wide pH range, the hydrolyzed protein from pumpkin seeds retained strong antioxidant activity and resisted degradation during the heating process. They suggested that PSP hydrolysates can be considered as alternative natural antioxidants, offering a healthier alternative to synthetic additives.

1.5 Pumpkin Seed Protein Modification Techniques

The use of PSP in the food industry has been growing due to its nutritional and functional properties. However, PSP also has limitations in terms of functionality, like many other plant-based proteins, that may restrict its use in many food

applications (Nikbakht Nasrabadi et al., 2021; Yong et al., 2021). While the choice of extraction method plays a critical role in preserving or enhancing protein functionality, it may not always be sufficient to meet the specific requirements of complex food systems. Therefore, to improve the functional properties of plant proteins, including PSP for broader food applications, it is essential to implement safe and targeted protein modification techniques.

Different modification strategies can be used to improve the functionality of PSP to overcome these challenges. Some of the modification techniques can be listed as acetylation, succinylation, amidation, esterification, and the Maillard reaction at the glycation stage (Kutzli et al., 2021). Among these methods, the Maillard reaction is advantageous thanks to being a chemically free process (Tas, Ertugrul, Oztop, & Mazi, 2021). This reaction not only enhances the solubility, which directly affects other functional properties, but also improves its thermal stability and sensorial properties, such as color and flavor (Zia et al., 2021). A variety of foods, such as fermented products like cheeses and beer, roasted products like coffee beans, and baked products like bread and cookies, are produced by the effects of the Maillard reaction. The development of the unique flavors, colors, and textures that characterize these products depends on this reaction (Lund & Ray, 2017). Thanks to these advantages and features, the Maillard reaction will be discussed in the following sections in detail.

1.5.1 Maillard Reaction

The Maillard reaction, first identified by Louis-Camille Maillard in 1912, involves a condensation reaction between the carbonyl groups of reducing sugars and the amino groups on proteins, peptides, or amino acids (Tessier, 2010). The reaction includes a series of complex chemical changes through four main stages: condensation, Amadori and Heyns rearrangements, decomposition, and polymerization (B. G. Guerrero, 2007). Figure 1.3 shows the overall scheme of the Maillard reaction, which has been prepared by Hodge, (1953):

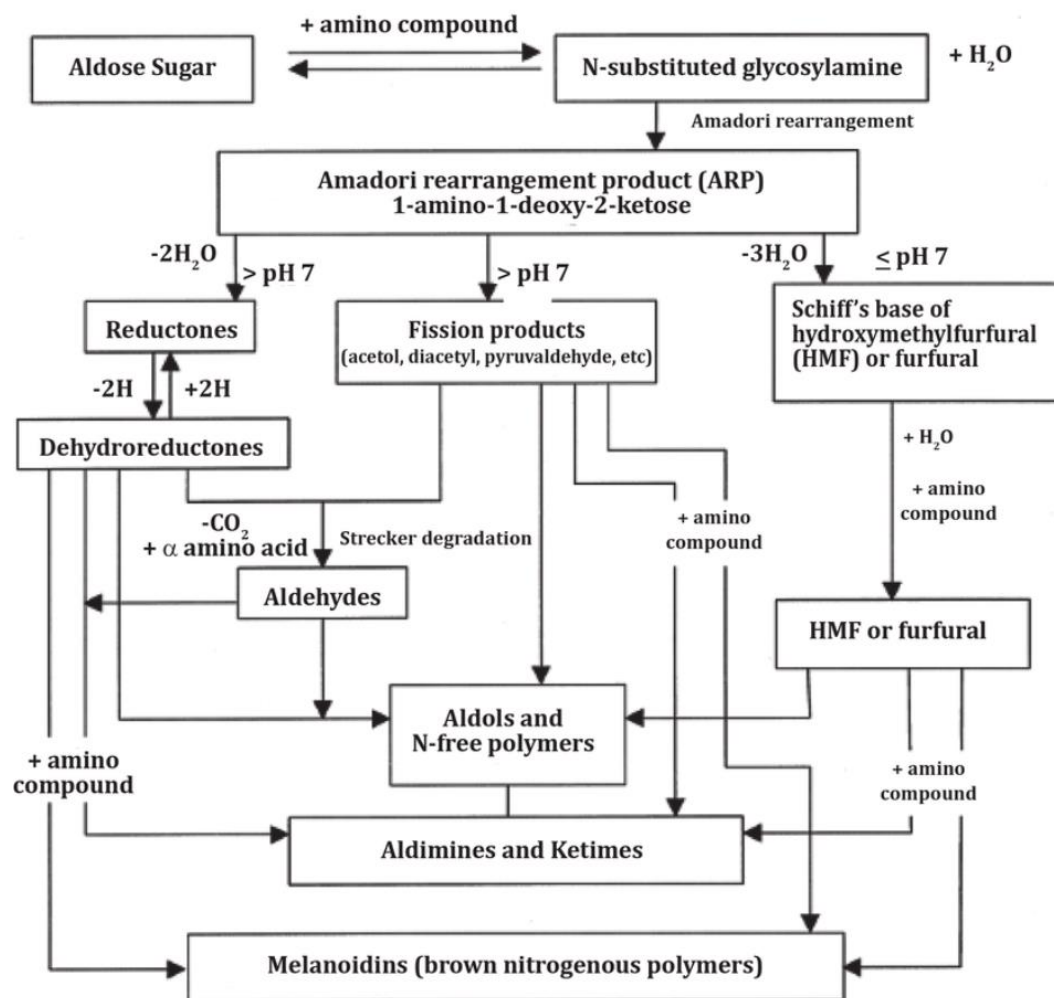


Figure 1.3. The Maillard Reaction scheme of Hodge (Hodge, 1953)

The Maillard reaction starts with a nucleophilic attack where the free amino group of an amino acid, commonly lysine, reacts with the carbonyl group of reducing sugar, forming a glycosylamine, also referred to as a “Schiff’s base,” and releasing water in the process (Horvat & Jakas, 2004).

In the following stage, glycosylamines, being relatively unstable, undergo rearrangement for isomerization to form more stable products. If the substrate is an aldose, Amadori products (ketosamines) are produced, whereas if the substrate is a ketose, Heyns products (aldosamines) are formed (Wrodnigg & Eder, 2001). This stage is characterized by some changes in color and flavor and a reduction in the number of available amino groups (Stadler & Studer, 2016). Despite the stability of

Amadori and Heyns products, they are still susceptible to further reactions such as enolization, dehydration, oxidation, and decarboxylation (Charnock et al., 2022; Reihl et al., 2004). Among these, the formation of highly reactive carbonyl compounds, Strecker degradation, and retro-aldolization, where carbohydrate units break down, are particularly significant (Jeantet et al., 2016). These reactions produce compounds that continue to undergo further interactions.

The final stage involves polymerization, where these reactive compounds interact to form high molecular weight brown pigments known as melanoidins, which contribute to the deep brown color of cooked foods (Martins et al., 2001). Additionally, this stage produces a range of other compounds, including advanced glycation end products (AGEs), aldehydes, ketones, dicarbonyls, acrylamides, and heterocyclic amines. These compounds are the reason for many of the physical and chemical changes observed in food during cooking and processing (Twarda- clapa et al., 2022).

The Maillard reaction has gained attention in food applications due to its ability to enhance the sensory qualities of food, such as color, flavor, and texture, as well as improve properties like protein solubility, hydration, emulsification, foaming, and antioxidant activity (Kutzli et al., 2021). However, the reaction should be carefully controlled since there might be undesirable outcomes if not managed properly. These undesirable outcomes include changes in color and flavor during processing and storage, the loss of essential amino acids (particularly lysine), vitamins (such as vitamin C), and valuable minerals such as copper, zinc, and iron (Ferrer et al., 2000; S. Liu et al., 2022). Thus, it is crucial to carefully regulate the Maillard reaction in food processing to balance improving desirable properties with maintaining nutritional value and safety. In addition, recent studies have shown that the Maillard reaction can have an impact on the bioavailability of nutrients and the overall health benefits of food products, highlighting the importance of control over the reaction (Ames, 2009).

1.5.2 Glycation

The Maillard reaction can enhance the sensory and functional properties of food products; however, if it is not properly regulated, it may result in the production of undesirable and potentially harmful substances. If the reaction goes beyond the glycation stage, many undesirable chemicals may form (Tamanna & Mahmood, 2015).

One example is hydroxymethylfurfural (HMF), an organotoxic and indirect mutagen generated in sugary products at high temperatures and low pH. HMF can interfere with essential enzyme functions and has been linked to a risk of carcinogenesis (Capuano & Fogliano, 2011).

Acrylamide is another substance that is known to be hazardous due to its carcinogenic toxicity, which occurs in the following stages of the Maillard reaction (Adimas et al., 2024). It is a molecule that is produced between asparagine, an amino acid, and reducing sugars at high temperatures, especially in foods rich in carbohydrates. It has been shown that an unstable intermediate known as a Schiff base is created when food is heated above 120°C (248°F), where free amino groups of asparagine combine with carbonyl groups of reducing sugars. After decarboxylating this intermediate, 3-aminopropionamide is created. This compound subsequently dehydrates to make acrylamide (Raffan & Halford, 2019). The temperature, duration, food composition, and pH levels during cooking all affect the development of acrylamide (Pandiselvam et al., 2024). Stopping the process during the glycation stage might effectively reduce these hazards.

Glycation, the initial stage of the Maillard reaction, begins with the nucleophilic attack of a free amino group (typically lysine) on the carbonyl group of reducing sugar, which forms Schiff's base (Figure 1.4) (Q. Zhang et al., 2009).

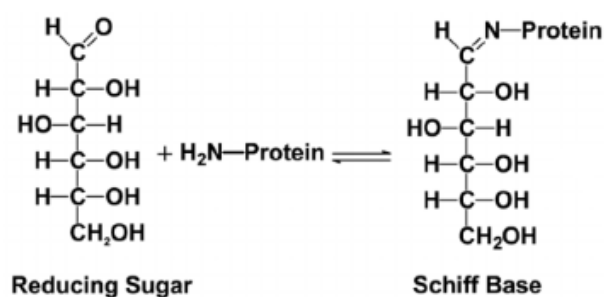


Figure 1.4. The open-chain form of glucose reacts with lysine to form a Schiff base (Maurelli et al., 2023)

The Schiff base can undergo Amadori rearrangement (for aldoses) to form a more stable ketosamine known as the Amadori product (e.g., N- ϵ -fructosyl-lysine) or Heyns rearrangement (for ketoses) (Figure 1.5).

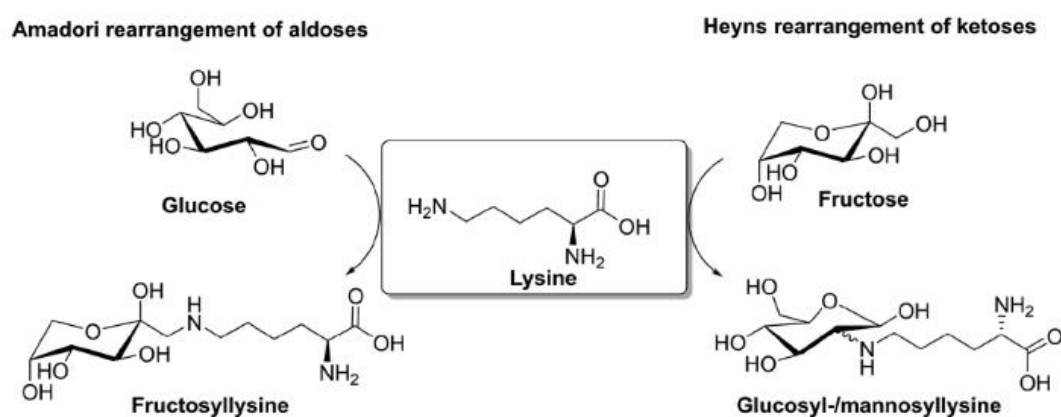


Figure 1.5. Amadori and Heyns rearrangement products during the reaction of glucose and fructose with the ϵ -amino group of lysine (Lassak et al., 2022)

These compounds are known to be relatively stable intermediates and play an essential role in desirable sensory properties in food products, such as flavor and color (Lund & Ray, 2017). For example, it has been documented that these intermediate compounds lead to the browning of bread crusts, the rich flavors in roasted coffee, and the complex aromas in cooked meats (Tamanna & Mahmood, 2015). In addition, studies have pointed out that the antioxidant activity of the protein-sugar conjugates is enhanced significantly during the early stages of the Maillard reaction. The intermediates, which have been demonstrated to scavenge free radicals and protect against oxidative damage, are mostly responsible for this

increased antioxidant activity (J. Liu et al., 2017; Y. Sun et al., 2005). Thus, studies showed that stopping the reactions right after the formation of Amadori and Heyns compounds is a way to improve the functionality and nutritional quality of food products (Troise et al., 2016). However, further progression of these intermediates through additional processes, such as cross-linking with other proteins, can create advanced glycation end products (AGEs). These substances have been linked to several detrimental health outcomes, such as the emergence of long-term conditions like diabetes, heart disease, and neurological illnesses (Poulsen et al., 2013). As a result, even if the glycation stage offers chances to improve food safety and quality, it is crucial to regulate and optimize the reaction strictly.

Several factors affect the extent and rate of glycation. These can be listed as the pH of the solution, the processing time, the temperature, and the type of reducing sugars (B. Wei et al., 2017). For example, the reaction can proceed more quickly to later phases and may generate unwanted compounds if the circumstances are too acidic or alkaline, with pH conditions typically being below pH 4 or above pH 11 (Uceda et al., 2024). Other important variables include temperature and processing time; higher temperatures and longer processing durations raise the possibility of the reaction progressing to the unwanted further stages, with temperatures above 100 °C and prolonged heating (e.g., over 60 minutes) especially increasing the formation of advanced glycation end products (AGEs) (S. Liu et al., 2022.). Similarly, the types of intermediates that occur and the rate of the reaction can be affected by the choice of reducing sugar, such as a ketose or aldose. For instance, ketoses, like fructose, were shown to be more reactive than aldoses such as glucose and galactose, due to their structural differences and higher affinity to form reactive enol intermediates (Gugliucci, 2017). The effect of reducing sugar, as being the main parameter in the glycation reaction, will be explained in detail in the following section.

1.5.2.1 Role of Reducing Sugar in Glycation

Reducing sugars play a central role in the glycation reaction as the carbonyl donor that reacts with the amino groups of proteins (Uceda et al., 2024). The molecular size and structure of reducing sugar strongly influence the rate and extent of the glycation reaction. A fundamental difference can be observed between pentoses and hexoses. Pentoses have been shown to exhibit higher glycation reactivity compared to hexoses, largely due to their shorter carbon chains and reduced steric hindrance, which facilitates easier interaction with free amino groups (Jeantet et al., 2016). Moreover, pentoses tend to exist more frequently in their open-chain form in aqueous solutions, which further increases their chemical availability in glycation reactions (Chevalier et al., 2001). In contrast, hexoses are generally more conformationally stable, which may contribute to a slower rate during glycation (Fournet et al., 2018).

Another parameter that affects glycation is whether the sugar is an aldose or a ketose. While some studies suggest that aldoses such as glucose are more reactive than ketoses like fructose, primarily due to the greater accessibility and electrophilicity of the aldehyde group (Bunn & Higgins, 1981; Uceda et al., 2024). Other research contradicts this by demonstrating higher reactivity for fructose under certain conditions (Gugliucci, 2017). In aldoses, the initial nucleophilic attack by amino groups is facilitated, and the reaction proceeds via Amadori rearrangement at the C1 position, forming 1-amino-1-deoxyketoses. Ketoses like fructose, on the other hand, react at the C2 position and follow the Heyns pathway, which typically results in slower reaction kinetics (Kaufmann et al., 2016). However, fructose has been found in several studies to exhibit higher reactivity than glucose, which has been attributed to its greater availability in open-chain form and its susceptibility to caramelization, a non-enzymatic browning reaction that contributes to overall color development and can occur alongside glycation (Ajandouz et al., 2001). Additionally, certain glycation products formed from glucose may act as reaction inhibitors, and the formation of only a single Amadori compound could limit the extent of glucose reactivity.

In recent years, rare sugars such as allulose (D-psicose) have also gained attention in glycation studies (Ertugrul, Namli, Tas, Kocadagli, et al., 2021; Namli et al., 2021; Zia et al., 2021). Despite being a C-3 epimer of fructose, it exhibits distinct reactivity due to differences in its ring stability and open-chain proportion. Some studies suggest that allulose may participate in glycation more slowly than fructose, potentially forming fewer or structurally different Maillard products (Clarke et al., 2024). However, certain glycation systems involving allulose have also been associated with increased antioxidant activity, suggesting that it may improve the functional properties of the final products (Sun et al., 2006).

Apart from the structural factors, environmental parameters such as pH and temperature are also critical in modulating the reactivity of reducing sugars and must be considered when evaluating glycation efficiency (Laroque et al., 2008; Muñiz-Márquez et al., 2015).

1.6 Incorporating Glycated Plant Proteins in Food Products as Functional Additives

Glycated plant proteins have attracted much interest because they may improve functional properties and nutritional qualities of food products. It has been demonstrated in many studies that glycation enhances the solubility, emulsification, foaming, and antioxidant activity of plant proteins, increasing their suitability for a range of dietary applications (Ertugrul et al., 2021; Tas, Ertugrul, Oztop, & Mazi, 2021; Zia et al., 2021). For instance, studies on soy protein have shown that glycation can greatly enhance the protein's solubility and emulsification capabilities, increasing its usefulness in emulsions and baked goods, among other products (Qiu et al., 2025). In addition, research on rice protein isolate showed that glycation with mono, oligo, and polysaccharides improved its functional qualities, including the ability to hold water and bind oil, which is essential for use in meat analogs and baked goods (Y. Li et al., 2013). Studies also revealed that the antioxidant activity of proteins can be significantly enhanced through protein modification, particularly

via glycation reactions (Hao et al., 2025; Y. Xu et al., 2021). During glycation, reducing sugars interact with free amino groups and form Maillard reaction products (MRPs), which are known to have great antioxidant properties. These MRPs can act as radical scavengers by stabilizing unpaired electrons through their conjugated structures and chelating ability of pro-oxidative metal ions (Kitts, 2021). Numerous studies have revealed that glycation with sugars such as glucose, fructose, and rare sugars like allulose can lead to an increase in DPPH scavenging activity, depending on reaction time, temperature, and the degree of glycation (Y. Sun et al., 2006; Tas, Ertugrul, Oztop, & Mazi, 2021).

There is also interest in healthy and plant-based diets growing around the world in addition to the increased interest in functional ingredients. One of the most well-known of these is the Mediterranean diet, which is rich in vegetables, legumes, whole grains, and olive and olive oil (Fao, 2015; Martínez-González et al., 2017). Following the Mediterranean diet, which includes a variety of functional food compounds, is one of the greatest choices for a healthy lifestyle since it may offer numerous health advantages, such as better weight management, cardiovascular health, and a lower chance of developing chronic illnesses like diabetes and some types of cancer (Dominguez et al., 2023). Despite the potential health benefits, studies show a decline in the consumption of Mediterranean foods, particularly among younger generations in Turkey and other Mediterranean countries (Martínez-González et al., 2017). Therefore, efforts are being made by the food industry and researchers to modify this behavior.

Tomatoes and olives, two well-known Mediterranean diet mainstays, are preferred ingredients when creating functional foods in the related industry. These ingredients are generally chosen due to their rich nutrient profiles and consumer acceptance (Erdem et al., 2024; Naureen et al., 2022).

One of the most fundamental functional foods of a Mediterranean-style diet is tomato sauce (Collins et al., 2022). Appreciated for its taste, health advantages, and versatility, millions of tons of tomato sauce are consumed annually worldwide

(Canene-Adams et al., 2005; Gibson & Newsham, 2018). Within this dietary pattern, tomato-based sauces are frequently consumed, serving as a flavorful base in many dishes like pasta, shakshuka, and vegetable stew. These sauces also offer a good opportunity to enrich foods with functional ingredients. It can be made much more nutritious by adding other nutrients and useful components, like plant-based proteins (Kurek et al., 2022; Langyan et al., 2022). In addition, these proteins provide essential amino acids that are vital for good health. Furthermore, including plant-based proteins aligns with contemporary dietary trends that support enduring, plant-focused eating habits. Plant-based proteins such as pumpkin seeds, chickpeas, and peas are some examples (Gul et al., 2024; Naureen et al., 2022). These components not only enhance the protein content but also bring essential elements, making the resulting tomato sauce more appealing to consumers who have concerns about their health. However, the use of functional plant proteins in Mediterranean-style sauces has not been widely studied.

To improve the applicability of plant proteins in various food systems, glycation has been utilized in some studies. For instance, Coşkun et al. (2020) incorporated glycated seed proteins into gluten-free breads and reported that dough stability and crumb structure were improved in gluten-free breads, supporting the idea that conjugated proteins interact more effectively with the surrounding matrix. Besides, studies on plant protein glycation showed great emulsions, due to improved stability and decreased oxidation, which makes glycated proteins more applicable to customers who are looking for alternatives to conventional dairy-based spreads and sauces (Kutzli et al., 2020). Improvements like this are especially important in Mediterranean cooking, where classic dishes like falafel, hummus, and other plant-based spreads are becoming more and more composed of plant proteins instead of animal proteins (Bal-Prylypko et al., 2024). However, there is still a lack of studies focusing on traditional sauces or Mediterranean-style products that include glycated plant proteins. Some related studies have shown that chickpea protein, after glycation, had better emulsifying and heat resistance properties, making it more suitable for spreads like hummus or other dips (Alshareef, 2018). Similarly, glycated

proteins have been used to stabilize olive oil-based emulsions, helping to improve shelf life and prevent oxidation (Morell et al., 2023). Furthermore, recent studies have explored the pea and lentil protein glycation, which showed improved solubility, foaming ability, and antioxidant activity (H. Khan et al., 2024; Y. Wang et al., 2022; Yousefi & Abbasi, 2022), which are stated to be desirable in oil-rich and pH-sensitive food systems such as tarator, tahini sauces, or olive pastes.

In this context, glycated pumpkin seed protein concentrate (PSPC) stands out as a promising ingredient. PSPC already has a high oil-binding capacity and contains beneficial nutrients and antioxidants. Glycation can further improve its performance in foods, making it more stable and functional in complex systems like sauces. Since no popular Mediterranean sauce or beverage currently includes glycated plant proteins, adding glycated PSPC into tomato sauce represents a new and innovative approach. This approach could meet the increasing demand for plant-based, allergen-friendly, and functionally rich products, while also keeping the traditional structure of the Mediterranean diet. Therefore, this dissertation aims to explore how glycated PSPC can be used in functional tomato sauce and how this application could open new possibilities in the development of functional Mediterranean-based foods.

1.7 TD-NMR Relaxometry as a Non-Destructive Tool for Investigating Structure and Mobility

TD-NMR relaxometry is a flexible method that offers precise, trustworthy, and non-invasive data regarding the characteristics of materials. Finding pore diameters, water intake, water content, and water distribution within a sample are among its most helpful applications. As a result, it is now widely used in areas such as biochemistry, chemistry, food science, and medicine (Ates et al., 2021; Berk et al., 2021; Kirtil et al., 2017).

The magnetic characteristics of atomic nuclei provide the basis of the NMR operating concept. Because of their intrinsic spin, nuclei with an odd number of

protons or neutrons exhibit a magnetic dipole moment, which causes them to act like small magnets (Atta-ur-Rahman, 1986). These nuclei can absorb energy and change between several magnetic states when exposed to radiofrequency (RF) pulses and exposed to a high external magnetic field. The type of nucleus—for example, ^1H , ^{13}C —as well as its chemical surroundings, determines how much energy is absorbed by the nuclei. This absorption produces the NMR signal, and the energy is released when the nuclei return to their initial states. This signal can be measured and examined to reveal important details about the sample.

1.7.1 T_1 and T_2 Relaxation Times

TD-NMR relaxometry uses the two main relaxation times, T_1 and T_2 , to describe the sample (Ozel et al., 2017; Pöcan et al., 2019). These relaxation times are inherent characteristics of the sample and change in response to the environment and composition of the sample.

T_1 is also called spin–lattice relaxation time because it indicates the time necessary for the spins to give the energy they obtained from the radio frequency pulse back to return to their initial equilibrium state (Pöcan, 2021). Despite T_1 being an important relaxation time used to set the parameters in all measurements, it can also be used to characterize the crystal structure of solid phases. Recrystallization of sugar could be detected by the increase in T_1 values (Le Botlan et al., 1998). Thus, at the same moisture content, longer T_1 values could be associated with an increased crystalline region. Moreover, T_1 is highly dependent on the mobility of protons that come from water in the gel systems, which could give an idea about the moisture distribution of confectionery gels (Pöcan et al., 2019). T_1 relaxation time characterizes the rate at which the longitudinal component of the magnetization vector recovers exponentially according to the following equation:

$$M_z(t) = M_0(1 - e^{-t/T_1})$$

where T_1 is the time constant of the magnetization recovery curve, $M_z(t)$ is the component of magnetization along the z-axis and M_0 is the initial magnetization.

T_2 time is known as the time required for transverse magnetization to reach the equilibrium value of zero (Poçan, 2021). It provides information about the mobility of hydrogen molecules by attributing to the state of water (bound/free, mobile/immobile) and the interaction of water with surrounding molecules (Kirtil et al., 2017). Thus, it can be a quick and reliable indicator for understanding the hydration behavior of food products.

The T_2 time to decay to the equilibrium value of zero can be shown with the equation below:

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

where T_2 is the time constant of the magnetization decay curve, $M_{xy}(t)$ is the component of magnetization on the xy plane, and M_0 is the initial magnetization.

The strength of the magnetic field has a significant impact on T_1 , with larger fields producing longer T_1 values. T_2 , on the other hand, is often shorter and less sensitive to the strength of the magnetic field (Traficante, 1991). One of the well-known sequences to determine T_1 relaxation time is stated as ‘Saturation Recovery’ (H. Wang et al., 2017).

When the comparison between those two times was made, it was seen that T_1 times might be 5 to 10 times longer than T_2 times. In that regard, the rate of decrease in T_2 relaxation times can be used to evaluate and compare the hydration behavior of different components, since T_2 times give quicker results (Kirtil et al., 2017). As NMR technology has advanced, numerous approaches designed for applications have been developed. The Carr-Purcell-Meiboom-Gill (CPMG) sequence is one method that is frequently used to calculate T_2 relaxation times (Pell et al., 2006). By focusing spin echoes, the CPMG sequence increases the signal-to-noise ratio even when the magnetic field is subject to changes or inhomogeneities.

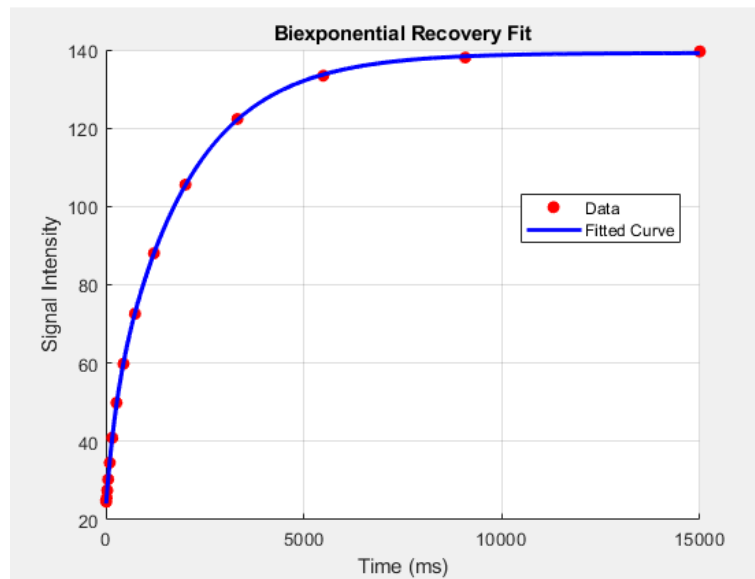


Figure 1.6. A representative T_1 relaxation curve of a sample by the Saturation Recovery (SR) sequence

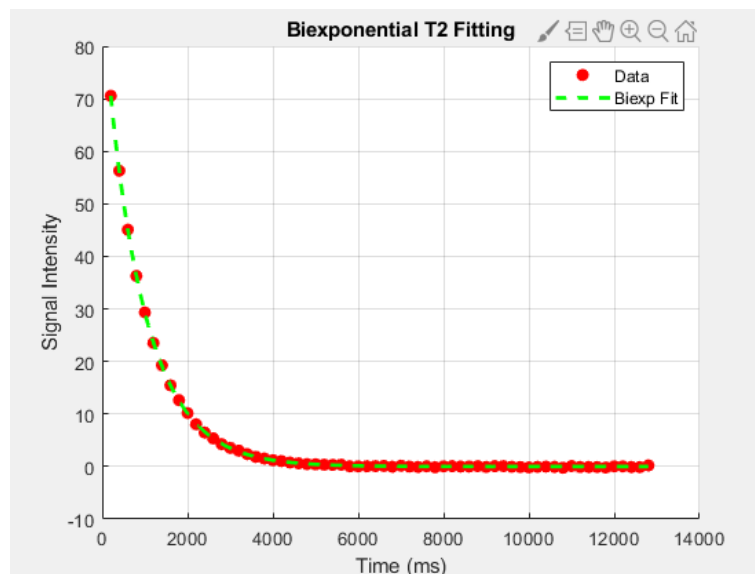


Figure 1.7. A representative T_2 relaxation curve of a sample by the CPMG pulse sequence

Regarding food industry applications, TD-NMR relaxometry is employed to evaluate the behavior, composition, and distribution of water in food products, including fats, carbohydrates, and proteins, providing detailed insights into their hydration dynamics with T_1 and T_2 relaxation times (Ozel et al., 2024; Pocaan et al.,

2019; Tas et al., 2022a; Uguz et al., 2018). Additionally, protein-water interactions are studied using TD-NMR relaxometry with different approaches. Applications of this research range from understanding protein hydration to assessing how protein structure and water absorption are affected by modifications, including heat treatment, pH alterations, and cross-linking activities (Ertugrul, Namli, Tas, Kocadagli, et al., 2021b; Zia et al., 2021).

In addition to these times, some non-conventional NMR experiments, such as diffusion coefficient and interphase layer thickness of the molecules, could also give information about the water interaction of the substances. The self-diffusion coefficient is one of the transport-related properties of water, and it has a crucial role in the physical and chemical explanation of water's interaction with different molecules (Garbacz & Price, 2014). Many studies used the pulsed-field gradient NMR (PFG-NMR) to study the self-diffusion of water in porous food systems. Some of the examples can be given as cheeses, bread matrices, and different types of food gels (Métais & Mariette, 2003).

1.7.2 Solid State NMR Sequences

NMR relaxometry is a highly versatile and non-destructive analytical tool that allows for the investigation of molecular/crystalline information in the solid state. Solid-state NMR sequences, including solid echo (SE), Magic Sandwich Echo (MSE), and spin diffusion, can provide valuable insights into molecular mobility and crystalline structure (Besghini et al., 2019). Some components, such as sugars are used in foods are mostly found in crystalline forms, and the degree of crystallinity is also a parameter that can affect how water interacts with the sugar (S. Tiwari & Talreja, 2020). While sugar hydration has been studied in many studies, conventional techniques still have some limitations in investigating the sugar-water interaction at the molecular level. For instance, techniques such as differential scanning calorimetry (DSC), dielectric spectroscopy, or classical water activity measurements may provide insufficient information at the molecular level.

Free Induction Decay (FID) sequence, which is known as the basic NMR signal obtained by a 90° radiofrequency (RF) pulse, has been used to measure the crystal content of some food products, including sugars such as sucrose, sorbitol, and powdered sugar (Dejong & Hartel, 2016; Porter & Hartel, 2013). However, when using this basic sequence, there is a risk of a loss in the signal coming from the solid part because of the dead time in the sequence. Deadtime is defined as the time that is lost in the RF probe till the first point of the signal is obtained. To eliminate the problem of dead time, alternative sequences such as Solid Echo (SE) and Magic Sandwich Echo (MSE) were developed (Guner et al., 2021). Although the SE sequence is more reliable than the FID sequence, it is not capable of fully refocusing on the multiple dipolar interactions. In that regard, the MSE sequence, which is the modified form of the SE sequence, can be used to determine the crystallinity of a substance since it enables nearly the full refocus on the multiple dipolar interactions with correct parameters (Guner et al., 2021; Hafner et al., 1996). When working with systems that have large line widths or strong dipolar couplings, including solids, membranes, or partly immobilized molecules, these sequences are especially helpful. Spin Diffusion can be determined by the Goldman-Shen sequence. In this sequence, the magnetization of long and amorphous components is starting to diffuse to the short component (L. Y. Grunin et al., 2017). In this way, the signal coming from the crystalline part increases. Hence, the spin diffusion of a substance could be determined by the Goldman-Shen sequence.

Spin diffusion gives a parameter that is related to the domain sizes that can be used as a characterization parameter for different systems. For that, there should be the SE or MSE signal and some fittings as described below.

The 1st parameter that should be found is the 2nd moment, since it is needed for further calculations. Abrahamian- based Time- Domain Free Induction Decay turns out to be as follows (L. Grunin et al., 2019a);

$$s(t) = A_{cr} \exp\left(-\frac{1}{2} a^2 t^2\right) \cdot \sin(bt)/bt + A_{am} \exp\left(-t/T_2^{am}\right)^2 + A_w \exp\left(-t/T_2^{*w}\right)^2$$

where indexes *cr*, *am*, and *w* relate to crystalline, amorphous phases, and water, respectively, T_2 denotes the spin-spin relaxation time, and the second moments are:

$$M_2^{cr} = a^2 + b^2 / 3 \text{ and } M_2^{am} = 2 / (T_2^{am})^2$$

For the fitting of Equation s(t), MSE data is used. Since fitting becomes very hard for the water fraction, that term is subtracted from the signal with a special module in the Relax 8 software. Hence, the fitting is performed to the 1st and 2nd exponents.

M_2^{am} is used for the calculation of the effective spin diffusion coefficient (D_{sd}). For the value of r^2 (*mean square distance between spins*), estimation can be done within a range of 0.22-0.25 nm for sugars that are like cellulose, since the average distance is quite similar in all saccharides (Cheung, 1981).

$$D_{sd} = \frac{\sqrt{\pi}}{6} \langle r^2 \rangle \sqrt{M_2}$$

Finally, the interface layer transfer thickness (*d*) that is between the bounded water and the crystalline is found by using the equation below

$$d = \frac{2\beta t^{0.5}}{\sqrt{\pi}} \sqrt{M_2}$$

with the assumption that the magnetization transfer is in one direction ($\beta = 1$). Also, the value, $t^{0.5}$ is calculated by performing the Goldman-Shen experiment (Fig.1.8) with linear fitting in Origin software.

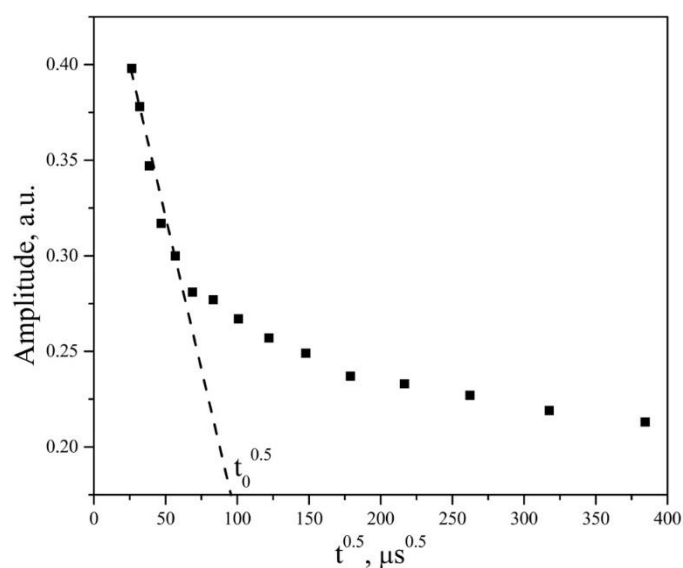


Figure 1.8. An example of the $t^{0.5}$ determination in the Goldman-Shen experiment

In this dissertation, TD-NMR relaxometry was applied to investigate the hydration behavior of extracted and modified PSPC, the molecular mobility of PSPC added tomato sauce samples, and microstructural differences and hydration behavior of different sugars (glucose, fructose, allulose, and sucrose). TD-NMR relaxometry is nondestructive as it does not require any pretreatment or destruction of the samples, practical as it could provide results in a short time, and informative as it could provide information at the molecular level with the obtained data. In this respect, TD-NMR stands out as an effective alternative analysis for evaluating the interactions and structural properties compared to conventional methods.

1.8 Objective of the Study

Plant-based proteins have gained growing interest due to their sustainability, nutritional benefits, and compatibility with various food systems. However, many of the proteins obtained through plants have limitations in terms of functionality, including poor solubility, low emulsifying, foaming, and antioxidant abilities. To address these limitations, this dissertation focused on improving the properties of

pumpkin seed protein (PSP), a promising plant-based protein with high nutritional and functional values.

The main objectives of this dissertation are to extract, modify, and incorporate pumpkin seed protein concentrate (PSPC) into a model Mediterranean food system, a novel tomato sauce, to improve its nutritional and functional values. The study was organized into four parts, including the TD-NMR Relaxometry approach in part IV to analyze hydration behavior, molecular dynamics, and structural properties of the obtained samples.

- **Part I:** Extraction of PSPC from its flour using two different preheating methods (microwave and conventional) and three extraction techniques (alkali, salt, and enzyme-assisted alkali) to determine how the extraction processing influences extraction yield, protein content, and functional properties, especially solubility.
- **Part II:** Modification of the extracted PSPC obtained from the most promising extraction methodology (based on Part I results) through a food-grade glycation reaction using two reducing sugars (fructose and allulose). Furthermore, to show the extensive characterization analyses for observing the extent of glycation reaction and functional properties analyses of the obtained conjugates by the comparison of their unmodified form.
- **Part III:** Inclusion of the modified proteins (conjugated PSPCs) in a reformulated tomato sauce formulation, evaluating its functional attributes inside the sauce and comparing it with the unmodified protein forms, to develop a plant-based and functional food product with the Mediterranean diet.
- **Part IV:** Investigation of structural and dynamic properties of extracted and modified PSPC samples, final tomato sauce formulations, and different sugars by TD-NMR relaxometry. This non-destructive analytical technique was used to demonstrate that it can be a fast, reliable, and alternative method

to conventional methods for understanding the hydration behavior, molecular dynamics, and structural differences of the samples.

CHAPTER 2

MATERIALS AND METHODS

2.1 Extraction of the Pumpkin Seed Protein Concentrate (PSPC) from its Flour with Different Approaches

2.1.1 Extraction Approaches for PSPC

PSF was purchased from Tazemiz (Mersin, Turkey). The enzyme *Alcalase* 2.4 L was purchased from Novozymes (Bagsværd, Denmark). All other chemicals were purchased from Sigma Aldrich Co. (St Louis, MO, USA).

For the extraction of the PSPC from its flour, 3 different extraction techniques (alkali, salt, and enzyme-assisted alkali) with 2 pre-heat treatments (microwave heated and conventional heated) were used. Additionally, untreated flour samples were also extracted with those three extraction techniques.

2.1.1.1 Alkali Extraction

PSPs were extracted by the alkali extraction technique that was developed by Onsaard et al., (2010). First, PSF and distilled water were mixed at a ratio of 1:10 (w/v). The pH of this solution was adjusted to 11 by using 1 M NaOH. For the microwave pre-heating, this solution was put into the microwave oven (Kenwood, New Jersey, USA) with a power of 416 W (determined by IMPI 2-L test) until the temperature reached 50 °C (~50 sec). In the case of conventional pre-heating, the solution was put into the water bath until the temperature reached 50 °C (~15 min). Then, the suspensions were continuously stirred with a magnetic stirrer (Daihan

Scientific Co., Ltd., Korea) for 1 h and then centrifuged at $2263 \times g$ for 15 min (MF-80, Hanil Science Industrial Co. Ltd., South Korea). The supernatant, which contained soluble proteins, was taken, and its pH was adjusted to 5 (the pI of the pumpkin seed proteins), using 1 M HCl. Then, the suspension was centrifuged again at $2263 \times g$ for 15 min. Following centrifugation, the supernatant was poured away, and the 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 36 h. Dried samples were kept at room temperature (25°C) for further analysis. For the extraction of untreated samples, the same procedure was followed except for the pre-heating parts.

2.1.1.2 Salt Extraction

PSF was suspended in 1 M NaCl at pH 7, at a ratio of 1:10 (w/v), and the same pre-heating conditions as stated above were followed, and then the suspension was stirred for 1 h and then centrifuged at $2263 \times g$ for 15 min. The supernatant was removed, and its pH was adjusted to 5 using 1 M HCl. The suspension was centrifuged again at $2263 \times g$ for 15 min. The supernatant was poured away, and the precipitate, which included the proteins, was separated. Proteins were neutralized as before and lyophilized afterward. For the extraction of untreated samples, the established procedure was replicated, omitting only the pre-heating steps.

2.1.1.3 Enzyme-Assisted Alkali Extraction

In this approach, the method of Latif & Anwar, (2011) was followed with a slight modification. Firstly, PSF was mixed with water at a ratio of 1:10 (w/v). The pH of the suspension was adjusted to 8 by using 1 M NaOH. Then, the enzyme (Alcalase 2.4 L) was added to the suspension (amount of 2% enzyme by sample weight). The pre-heating steps were applied in this stage. Then, the solutions were constantly

shaken for 1 hour at 100 rpm. Then, the suspensions were centrifuged at $2263 \times g$ for 15 min. The wet meals were separated, and the pH was adjusted to 5 using 1 M HCl. The suspensions were centrifuged again at $2263 \times g$ for 15 min. The supernatants were poured away, and the precipitates, which included the proteins, were separated. Proteins were neutralized as before and lyophilized afterward. Again, for the extraction of untreated samples, the same procedure was followed except for the preheating parts.

2.1.2 Characterization Methods

2.1.2.1 Extraction Yield

The extraction yield (%) was determined using the following equation:

$$\text{Extraction yield (\%)} = \left(\frac{W_{\text{ext}}}{W_0} \right) * 100 \quad \text{(Eq.1)}$$

where W_{ext} is the weight of the extracted material and W_0 is the weight of the dried raw material before extraction.

2.1.2.2 Proximal Analysis

Proximal analysis of the pumpkin seed flour before and after extractions was carried out for macronutrients (ash, fat, protein, and carbohydrates) and moisture contents by following AACC Methods (AACC, I., 2000).

An infrared moisture analyzer was used for the dried samples for the moisture content analysis (Radwag MAC 50 Moisture Analyzer, Poland). Data were reported as percentages.

The fat content of the samples was determined by the procedure of Zhao & Zhang, (2013). The Soxhlet apparatus (EFLAB) was used for the extraction of the samples in powdered form with a known weight using hexane as the solvent.

For the total protein content measurements, the modified version of the Kjeldahl method was conducted to evaluate the total protein content of the flour and extracted pumpkin seeds by $N \times 6.25$ (ASTM Standard E258, 2007). Finally, the total carbohydrate value was calculated by the following formula:

$$\text{Total carbohydrates} \left(\frac{g}{100 g dw} \right) = 100 - (m_{ash} + m_{protein} + m_{fat}) \quad (\text{Eq.2})$$

2.1.2.3 Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The powder form of samples (control (PSF) and extracts) was examined with an IR Affinity-1 Spectrometer with an Attenuated Total Reflectance (ATR) attachment (Shimadzu Corporation, Kyoto, Japan). The analysis was done in the region of 4000-500 cm^{-1} with 32 scans at a resolution of 16 cm^{-1} . The obtained spectra were compared with each other and with the literature.

The secondary structures of control and extracted PSPC samples were investigated further through quantitative measurement of the Amide I band (1600-1700 cm^{-1}). Using the Savitsky-Golay function, OriginPro (2019b, OriginLab Corporation, Northampton, USA) was utilized to process the spectra. By doing a second derivative spectrum analysis, overlapping components were found. The Gaussian function produced the best fit, and 15 points of the window in the positive direction were chosen (Litvinov et al., 2012).

2.1.3 Physicochemical Properties

2.1.3.1 Protein Solubility by Lowry Method

The Lowry method was used to determine the soluble protein content of the samples (Lowry et al., 1951). 0.5 mL sample (1% (w/v) protein solution) was mixed with 2.5 mL Lowry reagent and waited for 10 min at 25 °C. Next, 0.25 mL of Folin-Ciocalteu's phenol reagent was added to the tubes, mixed, and incubated in the dark

for 30 min. Lastly, the absorbance values were read at 750 nm by a UV/VIS Spectrophotometer (Optizen POP, South Korea). Results were expressed in percentages by dividing the initial protein content in the samples.

2.1.3.2 Water Solubility Index (WSI)

The WSI was performed by the modified version of the method (Yousf et al., 2017). First, the samples were dissolved in distilled water with a 1:4 (w/w) ratio and then put into an orbital shaker (Daihan Scientific Co., Ltd., Korea) at 300 rpm for 1 day to achieve complete hydration. The sample solution was centrifuged at $2263 \times g$ for 20 min. The supernatant and the sediment were separated, and their weights were measured. The following equation was calculated for WSI:

$$WSI = \frac{\text{Weight of the dried solid in supernatant}}{\text{Weight of initial sample}} \quad (\text{Eq. 3})$$

2.1.3.3 Emulsifying Activity (EA) and Emulsifying Stability (ES)

The emulsifying properties of the samples were determined by using the method of D. Gao et al., (2023) with slight modifications. Corn oil (2 mL) and sample solution (8 mL, 0.01 g/mL) were mixed and then homogenized at 20000 rpm for 2 min. Next, 50 μL of emulsion was taken from the bottom part at 0 and 10 min, and diluted with SDS solution (5 mL, 0.1%). The absorbance values were recorded at 500 nm using a UV-VIS spectrophotometer (Optizen POP Nano Bio, Mecasys Co. Ltd., South Korea). The absorbance value (A_0) was immediately measured after emulsification, and the absorbance value (A_{10}) was measured after 10 min of waiting. Finally, the EA and ES values were calculated using the following equations:

$$EA \text{ (m}^2\text{/g)} = (2 \times 2.303 \times A_0 \times N) / (c \times \phi \times 10000) \quad (\text{Eq.4})$$

$$ES \text{ (min)} = (A_0 \times 10) / (A_0 - A_{10}) \quad (\text{Eq.5})$$

where c was the sample concentration (g/mL), ϕ was the oil volume ratio of the emulsion (0.25), and N was a dilution factor (101), A_0 and A_{10} were the absorbance values at 0 and 10 min, respectively.

2.1.3.4 Foaming Capacity (FC) and Foaming Stability (FS)

The foaming properties were evaluated by modifying the method of M. Yang et al., (2014). For the experiment, 1 g of the sample was dispersed in phosphate-buffered solution (0.2 mol/L, pH=7.4). The 20 mL mixture was homogenized at 20000 rpm for 2 min. The volume of the foam was measured at 0 and 30 min after homogenization. FC and FS were calculated by the following equations.

$$FC (\%) = (V_0 - V_i / V_i) \times 100 \quad (\text{Eq.6})$$

$$FS (\%) = (V_{30} - V_i / V_0 - V_i) \times 100 \quad (\text{Eq.7})$$

in which V_0 and V_{30} are the foam volumes at 0 and 30 min after homogenization, and V_i is the initial volume before foaming.

2.1.3.5 Scavenging Activity by DPPH Method

The 1,1-diphenyl-2-picryl hydrazyl (DPPH•) free radical scavenging activities of samples were determined using a modified method of D. O. Kim et al., (2002). 100 μ M DPPH• was dissolved in 80% aqueous methanol. 0.1 mL of the sample solution was added to 2.9 mL of the methanolic DPPH• solution. The mixture was shaken and left in a dark environment for 30 min. The decrease in absorbance values was measured at 517 nm at 30 min. For the control, 0.1 mL of 50% aqueous methanol and 2.9 mL of DPPH• solution were used. The scavenging activity was calculated as:

$$\text{Scavenging activity (\%)} = \left(\frac{A_{517 \text{ of control}} - A_{517 \text{ of sample}}}{A_{517 \text{ of control}}} \right) * 100 \quad (\text{Eq.8})$$

where $A_{517 \text{ of control}}$ is the absorbance containing only methanol and DPPH• solution, and $A_{517 \text{ of sample}}$ is the absorbance of the sample and DPPH• solution.

2.1.3.6 Water Activity and Color Properties

The water activity (a_w) of the extracted proteins was measured using a water activity meter (AQUALAB 4TE; Aqualab, Pullman, WA, USA).

To determine the color of the samples, a portable spectrophotometer (Serlab SL400, İstanbul, Turkey) was used to determine lightness (L^*), red-green (a^*), and blue-yellow (b^*) values.

2.2 Modification of Pumpkin Seed Protein Concentrates (PSPC) through Glycation Reaction

PSPC with 77% protein content (confirmed by the Kjeldahl method) was used, which was produced by the MH-AE method (explained in Section 2.1.1.1) from the purchased PSF. Fructose was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), and allulose was purchased from Keystone Pantry (USA). All other chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2.1 Preparation of PSPC-Fructose and PSPC-Allulose Conjugates

First, reducing sugars (fructose and allulose) were dissolved in distilled water (pH set to 10 by using 0.1 M NaOH). Then, PSPCs were added to these solutions in a Petri dish and well mixed to obtain the selected protein: sugar ratios (1:1 and 3:1) with a 40% (w/v) total solid concentration. These conditions were chosen based on preliminary experiments. A high-shear homogenizer was used at 10,000 rpm for 5 min to ensure the desired hydration and mixing of the components. For the glycation,

the prepared solutions were put in a water bath (Witeg Labortechnik GmbH, Wertheim, Germany) at 90 °C for 30 min and then taken and cooled immediately. In the next step, samples were freeze-dried (Beijing Songyuan Huaxing Technology Development Co., Ltd., Beijing, China) for 48 h. Following, the samples were ground using a grinder and stored at 4 °C for further analysis. The conjugate samples obtained from PSPC glycation with fructose and allulose were named PSPC-Fru and PSPC-All, respectively. Besides, the extracted PSPC was utilized as the control.

2.2.2 Characterization Methods

2.2.2.1 Browning Index Determination

For the browning index, 2.5 mL supernatants of 1% (w/v) sample solutions were taken and measured at 420 nm using a UV-VIS spectrophotometer (Optizen POP Nano Bio, Mecasys Co. Ltd., South Korea). The results were given as absorbance (nm) values (Zia et al., 2021).

2.2.2.2 Free Amino Group Determination by OPA Method

Free amino groups (FAGs) of the PSPC and its conjugates were determined by the method of Diab et al., (2009) with small modifications. First, 80 mg of o-phthalaldehyde (OPA) is dissolved in 2 mL 95% (v/v) ethanol solution. Next, 50 mL 100 mM borax buffer at pH 9.75, 5 mL 20% (w/v) sodium dodecyl sulfate (SDS) solution, 200 μ L β -mercaptoethanol, and OPA reagent solution were combined in a 100 mL volumetric flask. The final volume of the solution was brought to 100 mL. For the analysis, 0.5 mL of sample solution and 1.5 mL of reagent solution were mixed and kept at room temperature for 2 min. The absorbance values were measured at 340 nm using a UV-VIS spectrophotometer (Optizen POP Nano Bio, Mecasys Co. Ltd., South Korea). The glycine solution was used to obtain a

calibration curve under varying conditions. The FAGs results were calculated and given as mg glycine eq./g initial protein.

2.2.2.3 Remaining Reducing Sugar (RRS) Amount Determination by High-Performance Liquid Chromatography (HPLC) Method

To determine the RRS amount, 1 g of the PSPC and its conjugates were dissolved in double-distilled water. To properly hydrate samples, the prepared solutions were put in a shaker (Daihan Scientific Co. Ltd., Korea) overnight. Then, 0.5 mL of Carrez I and Carrez II solutions were added to these solutions and centrifuged at 4000 rpm for 5 min (MF-80, Hanil Science Industrial Co. Ltd., South Korea). The supernatants were collected into vials through 0.45 μm nylon filters. Then, the samples were analysed in the vials using an HPLC system (High-performance liquid chromatography, Shimadzu Scientific Industrial Co. Ltd., South Korea). The chromatographic separations were performed using an inertsil NH_2 column (4.6*250 mm, 5 μm) (Shimadzu Sci. Ins., Japan) with the mobile phase of acetonitrile:water ratio (80:20, v/v) at a flow rate of 1 mL/min at 40 °C. The calibration curve was drawn by reducing sugar solutions at different concentrations (1.25–10 g/L).

2.2.2.4 Amino Acid Composition Analysis

For the analysis, the amount of each sample containing 1 g of protein was mixed with 99 ml of 0.1N HCl and 1 ml of 5-sulfosalicylic acid (5-SSA) solution for 15 min. Then, the mixture was kept at 4°C for 30 min. Next, the centrifugation at 4000 rpm for 10 min was performed, and the supernatant was obtained to analyze the amino acid composition.

To determine the amino acid composition, the LC-ESI-MS/MS analysis was conducted using a Shimadzu HPLC system (Shimadzu Scientific Industrial Co. Ltd., South Korea) coupled with an AB-SCIEX (Framingham, USA) triple quadrupole mass spectrometer (Lane, 2015). The mass spectrometer was operated in positive

ESI mode. The ion spray voltage was set at 4 kV, with nitrogen used as both the sheath gas (25 psi) and the auxiliary gas (1.0 arbitrary units). The ion transfer capillary was heated to 300°C. Injections were performed using an autosampler equipped with a 20- μ l sample loop. Multiple Reaction Monitoring (MRM) was performed for the specific quantification of amino acids and the standard, with a scan time of 0.4 seconds. The standard solutions were infused into the HPLC system under the following conditions:

HPLC system flow rate of 0.94 mL/min; LC separations were conducted using a C18 column at 30 °C. For the elution of amino acids, 0.1% Formic acid in water (solvent A) and 100% acetonitrile (solvent B) were used in gradient mode. With 2 to 20% B over 5 min, 20% to 100% B 20 min, and 100% to 2% B in 5 min (total of 30 min).

2.2.2.5 Structural Analysis by Fourier Transform Infrared (FTIR) Spectroscopy

The analysis of PSPC and its conjugates was conducted following the same procedure described in Section 2.1.2.3. The spectra were recorded using the same instrument and parameters, and the secondary structure analysis of the Amide I region was similarly performed using OriginPro (2019b).

2.2.3 Physicochemical Properties

2.2.3.1 Protein Solubility Determination by the Lowry Method

The solubility of PSPC and its conjugates was determined by using the same procedure explained in Section 2.1.3.1. Since each sample has different protein contents, the solubility results were standardized by dividing the initial protein contents in the sample mixture, which was evaluated by Kjeldahl's method.

2.2.3.2 Surface Hydrophobicity (H_0)

The surface hydrophobicity (H_0) of the PSPC and its conjugates was determined using a modified version of Wang et al., (2025). The protein samples were dissolved in 0.01 M phosphate buffer (pH=7.4) to prepare a series of dilutions ranging from 0.0625 to 0.5 mg/mL. Then, 4 mL of each diluted sample was mixed with 20 μ L of 8 mM ANS (8-anilino-1-naphthalenesulfonic acid) reagent. The mixtures were incubated in the dark for 30 min to allow sufficient binding between ANS and the exposed hydrophobic regions. Fluorescence intensity was measured using an LS-55 Spectrofluorimeter (Perkin-Elmer, USA) with an excitation wavelength of 390 nm and an emission scan range of 400–700 nm. The slit width was set at 10 nm, and the voltage was adjusted to 700 V. The surface hydrophobicity (H_0) values were calculated as the slope of the linear regression curve obtained by plotting fluorescence intensity against sample concentrations.

2.2.3.3 Emulsifying Activity (EA) and Emulsifying Stability (ES) Determination

The emulsifying properties of PSPC and its conjugates were examined using the same procedure explained in Section 2.1.3.4.

2.2.3.4 Foaming Capacity (FC) and Foaming Stability (FS) Determination

The foaming properties of PSPC and its conjugates were measured by the same method described in Section 2.1.3.5.

2.2.3.5 Scavenging Activity by DPPH Method

The scavenging activities of PSPC and its conjugates were determined using the same procedure explained in Section 2.1.3.6.

2.3 Utilization of Conjugated PSPC Proteins in Tomato-Based Sauces

Roma-type tomatoes were purchased from a local market (Ankara, Türkiye). The purchased tomatoes were heated through a hot-break procedure before being put in the reformulated sauce. For that, a 3-minute heating was conducted using Vorwerk's Termomix (Vorwerk & Co. KG, Wuppertal, Germany) at 85°C after peeling. The tomatoes were then cooled and kept in the freezer at -18°C until further processing. Tomato peel powder was produced by peeling tomato skins that were dried for 24 hours at 55°C in a household dehydrator (Klarstein Fruit Jerky 9, Berlin, Germany). The olives from the Marmara area of Turkey were lyophilized following the study of Sinem et al., (2024) The salt was bought from a local market (Ankara, Türkiye). Finally, as the protein sources, PSPC and reducing sugar (fructose and allulose) conjugates at the 1:1 ratio were used, which were obtained as explained in Section 2.2.1. of this dissertation.

2.3.1 Preparation of Reformulated Tomato-Based Sauces

For the reformulated tomato sauce preparation, hot-break tomatoes that were frozen were thawed at room temperature. These samples were sieved twice through 500 µm sieves to exclude the pulp and seeds, resulting in homogeneous tomato juice. After taking 100 grams of this tomato juice, 4% tomato peel powder, 2% olive powder, 1% salt, and 1% conjugated PSPC proteins were added by weight, relative to the weight of the tomato juice. Then this mixture was high-shear homogenized (IKA T18, Staufen, Germany) at 10,000 rpm for 5 min. This mixture was then subjected to a High-Pressure Homogenizer (HPH) at 500 bar (1st stage 500 bar with 2nd stage of 100 bar) to obtain a homogenous and consistent mixture. The samples obtained from HPH were exposed to conventional pasteurization in a water bath at 95°C for 20 minutes. The pasteurized samples were stored at 4°C for further analysis. To see the effect of conjugated proteins, the same formulation with the same steps was followed with PSPC that was not modified. Besides, the formulation without any

protein addition was also prepared. The tomato sauces prepared by conjugated PSPC with fructose were named ‘TS-F’, and the conjugates with allulose were named ‘TS-A’ throughout the text. The tomato sauce enriched with unmodified protein was named ‘TS-U’, and the samples that did not contain additional proteins were the control and named ‘TS-C’ throughout the text. Table 2.1 shows the ingredients:

Table 2.1 The ingredients of the reformulated tomato sauce samples

Ingredients	Amount (g)
Tomato puree	100
Tomato peel powder	4
Proteins (conjugated PSPC, PSPC)	2
Olive powder	2
Salt	1

2.3.2 Methods

2.3.2.1 Moisture Content, Water Activity, pH, °Brix, and Color Measurements

An air oven (Mikrotest, MST-120, Ankara, Türkiye) was used to measure the moisture content of the samples.

The water activity (a_w) of the samples was measured using a water activity meter (AQUALAB 4TE; Aqualab, Pullman, WA, USA).

The pH of the samples was measured by a portable pH meter (FC2022, Hanna Instruments, USA).

The soluble solid content of the products was measured by a hand refractometer at 25°C. (Hanna Instruments, HI96801, George Washington Hwy Smithfield, USA). For the analysis, °Brix values were provided.

The color of the samples was measured using a spectrophotometer (Serlab SL400, İstanbul, Türkiye). Lightness (L*), red-green (a*), and blue-yellow (b*) values were noted.

2.3.2.2 Soluble Protein Content

The soluble protein content of the tomato sauce samples was determined using the procedure explained in Section 2.1.3.1.

2.3.2.3 Lycopene Content

With a few minor adjustments, the lycopene content was measured by following the method of Bal et al., (2024). In summary, 100 g of the sample was high shear homogenized (IKA T18, Staufen, Germany) at 10,000 rpm for 3 min before extraction. In a glass tube, a 100 mg aliquot of the homogenized material was mixed with 4 mL hexane, 2 mL ethanol, and 2 mL acetone. This mixture was vortexed for 30 seconds and then allowed to stand for 30 min. After 30 min, the mixture was mixed with 1 mL of distilled water, vortexed once more for 30 s, and then allowed to sit for an additional 10 min to allow the hexane component to separate. Then, an aliquot of the upper layer was read at 503 nm by a spectrophotometer (Optizen Pop, Mecasys, Daejeon, Republic of Korea). The blank was prepared as a combination of hexane, ethanol, and acetone. The following formula was used to calculate the lycopene content of the samples.

$$Lycopene \left(\frac{mg}{kg} \text{ sample} \right) = \frac{Abs_{503} * MW_{lycopene} * R * V}{m_{sample} * F} \quad (\text{Eq.9})$$

Where; Abs₅₀₃ is the absorbance of the sample at 503 nm, MW_{lycopene} is the molecular weight of lycopene (536.9 g/mol), R is the volumetric ratio of the upper layer compared to all volume, V is the volume hexane-ethanol-acetone mixture, m_{sample} is the weight of sample, and F is the molar constant for lycopene in hexane (172 1/mM).

2.3.2.4 Scavenging Activity by DPPH Method

For the scavenging activity (SA) of the tomato sauce samples, the same procedure described in Section 2.1.3.6 was followed.

2.3.2.5 Total Phenolic Content

The total phenolic content (TPC) of the samples was evaluated by the Folin–Ciocalteu method with slight modifications (Odriozola-Serrano et al., 2008). For the experiment, 500 μ L of sample extract and 500 μ L of Folin-Ciocalteu reagent were mixed and allowed to rest for 3 min. After adding 10 mL of a 20% sodium carbonate solution, the mixture was left to stand in the dark for 1 h. Finally, the absorbance at 725 nm was recorded by the spectrophotometer (Optizen Pop; Mecasys, Daejeon, Republic of Korea). A calibration curve was prepared using six different concentrations of gallic acid, following the equation $y=0.006x+0.0988$ ($R^2=0.998$). The results were expressed as the mg gallic acid equivalent (mg GAE) divided by a 100 ml sample.

2.3.2.6 Flow Behavior

A cup and bob type rheometer (Kinexus Dynamic Rheometer, Malvern, Worcestershire, U.K.) was used to assess the flow behavior of the sauce samples. Curves for shear stress and shear rate were acquired throughout the shear rate range of 100 to 1000 1/s. A steady temperature of 20°C was maintained during the measurement. The rheological parameters of the sauce were determined by fitting shear stress (τ)-shear rate (γ) data to the Herschel-Bulkley model:

$$\tau = y_0 + k\gamma^n \quad (\text{Eq.10})$$

where y_0 is the yield stress (Pa), k is the consistency factor ($\text{Pa}\cdot\text{s}^n$), and n is the flow behavior index.

2.3.2.7 In Vitro Protein Digestibility

This experiment has occurred both for the tomato sauces, including proteins, and the proteins themselves. For the analysis, the samples in distilled water were subjected to an in vitro digestion process based on the INFOGEST 2.0 model described by (Brodkorb et al., 2019) with some modifications. In the experiment, 1 g of the sample was used.

For the oral phase, 1 mL of simulated salivary fluid (pH 7) containing CaCl₂ (1.5 mM final concentration) and α -amylase were mixed with the samples. This mixture was incubated in a shaking water bath (Mikrotest, Ankara, Turkey) at 37 °C for 2 min.

For the gastric phase, 2 mL of simulated gastric fluid with CaCl₂ (0.15 mM final concentration) and pepsin from porcine gastric mucosa were added, and the pH was adjusted to 3 using 2 M HCl. The mixture was incubated at 37 °C in a shaking water bath for 2 h, after which pepsin activity was neutralized by increasing the pH to 7 using 2 M NaOH.

For the intestinal phase, 4 mL of simulated intestinal fluid containing pancreatin from porcine pancreas, bovine bile (10 mM final concentration), and CaCl₂ (0.6 mM final concentration) were added. This mixture was incubated for 2 h at 37 °C in a shaking water bath. After digestion, the samples were immediately placed on ice, and 32 mL of absolute methanol was added to each sample beaker, achieving an 80% (v/v) methanol concentration in the final mixture. The samples were incubated at -20 °C overnight.

To separate supernatants from pellets, the samples were centrifuged at 4000 rpm for 15 min at 4 °C. The supernatants were carefully transferred to new tubes and stored at -20 °C for further analysis. Finally, the OPA assay was conducted to determine the degree of hydrolysis. The results were given as mg eq. glycine/g total protein.

2.3.2.8 Sensory Analysis of Reformulated Sauces

For the sensory analysis of the reformulated sauces, six trained panelists from SELUZ Fragrance & Flavor Company (Turkey) participated. Before the analysis, all samples were stored in a dark environment at 4°C for 24 h. The sensory analysis occurred in a neutral, odor-free room with daylight, maintained at a constant temperature of 22-25°C and relative humidity between 50-55% to ensure a consistent and comfortable environment. The evaluation process followed the Flavor Profile Analysis method (ISO 6564:1985) with two stages. In the first stage, panelists conducted individual assessments, and in the second stage, they discussed their evaluations collectively. In the analysis, they determined the flavor across key attributes, including *sweetness, saltiness, sourness, fresh tomato taste, tomato skin taste, tomato juice taste, paste-like flavor, olive-like taste, astringency, off-taste, protein-related taste, and overall impression.*

2.4 TD-NMR Relaxometry Approaches

2.4.1 Hydration Behavior of Extracted PSPC Samples

For the TD-NMR Relaxometry experiment of extracted PSPC samples, the same sample-distilled water ratio (1:4), which was decided in the WSI experiment (Section 2.1.3.2), was prepared. For the analysis, T_2 relaxation times were measured by a 0.48 T (20.34 MHz) benchtop NMR tool (Spin Track, Resonance Systems GmbH, Kirchheim Teck, Germany). Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence was selected, and the parameters that are echo time, number of echoes, and number of scans were selected as 500 ms, 300–500 ms, and 4, respectively. MATLAB (R2019b, The MathWorks Inc., USA) was operated to find T_2 times by considering monoexponential behavior.

2.4.2 Hydration Behavior of Conjugated PSPC Samples

The samples were prepared by mixing 0.15 g of the ground powder sample with 0.45 mL of distilled water in NMR tubes. The experiment was performed at 298 K by using the 0.48 T (20.34 MHz) NMR system. T_2 times were recorded by using a CPMG pulse sequence with an echo time of 1000 ms, 4 s repetition time, 4 scans, and the number of echoes changed between 300-600. For the analysis, MATLAB (R2019b, The MathWorks Inc., Natick, MA, USA) was used to consider mono-exponential relaxation behavior.

2.4.3 Molecular Mobility of Tomato Sauce Samples

The T_2 relaxation times of the samples were measured by a 0.48 T (20.34 MHz) benchtop NMR. The CPMG pulse sequence was selected for the measurement, and the parameters that are echo time, number of echoes, and number of scans were chosen as 2000 ms, 200–400 ms, and 4, respectively. Lastly, MATLAB (R2019b, The MathWorks Inc., USA) was operated to find T_2 relaxation times by considering both the monoexponential and biexponential behaviors.

2.4.4 Molecular and Structural Insights into Different Sugars

Experiments were performed by using two benchtop TD-NMR systems that are 0.48 T (20.34 MHz) and 0.32 T (13.52 MHz) (Spin Track, Resonance Systems GmbH, Kirchheim/Teck, Germany).

2.4.4.1 Spin-Spin Relaxation Time (T_2)

First, to eliminate the effect of particle size, grinding was applied to all sugars until they became the same size. After grinding the sugars, the experiments were applied at 298 K.

In one approach, according to different concentrations of sugars (2.5%, 5%, 10%, 15%, 20%, 30% and 40%) (g/100 ml), T_2 relaxation times were measured and put in a graph for fitting a model.

In another approach, the effect of time on sugar hydration was evaluated by preparing 3 g of sugar in 1 g of distilled water. Then, T_2 relaxation times were obtained every 15 min for the first 4 hours, 30 min for 2 more hours, and one data for 1 day in total.

The data of T_2 relaxation times were obtained using a CPMG pulse sequence with an echo time of 1,000 ms, 4s repetition time, 16 scans, and a number of echoes changed between 2000-4000. The analysis was conducted using MATLAB (The MathWorks Inc., USA), considering a mono-exponential relaxation behavior.

2.4.4.2 Molecular Diffusion

The diffusion coefficients of the water molecules inside sugar solutions prepared at different concentrations mentioned above were analyzed. For that, a special module in RELAX 8 (Resonance Systems, Kirchheim/Teck Germany) was used. The relaxation period was set as 10^4 ms and the number of scans was set as 64, and the diffusion time was set as 100 ms.

2.4.4.3 Solid Echo (SE) and Magic Sandwich Echo (MSE) Sequences

The NMR line second moment M_2 , which is proportional to crystallinity (L. Grunin et al., 2019) values of the sugars in solid and hydrated (sugars were mixed with water at a ratio of 3/1 of their weight, waited for 1 day) forms were analyzed by FID

recorded by MSE sequences. For this, the relaxation period was set as 100 ms, and the number of scans was set as 16. The data of MSE were analyzed by using the special module in RELAX 8 (Resonance Systems, Kirchheim/Teck, Germany). The analysis was based on the averaging of the three regions of the FID: short (S) (0-2 us), medium (M) (10-16 us), and long (L) (60-80 us). It was supposed that L represented information of residual moisture content. M-L and S-M represent amorphous and crystalline contributions, respectively. Calibration of the method was done with Gaussian and Abrahamian numerical models of FID, and they both showed equal and linear dependence of the value (S-M)/(S-L) on the second moment (M_2) of calibration models in the range of 0 to 30×10^{-8} Tesla², and the M_2 values were calculated as (S-M)/(S-L).

In addition, the relative total crystallinity (%) of the sugars in the solid state was also measured by using X-ray Diffraction (XRD) (Rigaku Ultima-IV X-Ray Diffractometer, WOL, USA) equipment at METU Central Laboratory. The sampling width, scan axis, range, and speed were 0.02° , 2θ , $5-50^\circ$, and $1^\circ/\text{min}$, respectively.

2.4.4.4 Spin Diffusion by Goldman-Shen Sequence

The interface layer thickness of the sugars that occur between bound water and the crystalline part was evaluated by Spin Diffusion. For this, sugars were put in an incubator (Daihan Instruments, Germany) that was set to 298 K and 50% relative humidity (RH) for 14 days to achieve full hydration of the sugars. After samples were obtained, they were analyzed by the Goldman-Shen sequence (Goldman & Shen, 1966) which is used for the Spin Diffusion experiment. The time of observation was set as 1 s, and the relaxation period was set as 10^3 ms. The number of scans was kept at 64. The data was analyzed by using Origin 7 (Version 2017, OriginLab Corporation, Northampton, MA, USA).

2.5 Statistical Analysis

MINITAB (Version 19, Minitab Inc., Coventry, UK) was used for statistical analysis of all the experiments in this study, which were conducted in three replicates. A general linear model regression technique was employed to investigate the impact of variables on the results. Residual normality, constant variance, independence, and transformations were among the ANOVA assumptions that were employed where appropriate. In situations where significance assessment was required, Tukey's comparison test with a 95% confidence interval was employed. A statistically significant difference ($p < 0.05$) between the samples is shown by the different letters in the Figures and Tables.

2.6 Experimental Design

Table 2.2 Experimental design showing the factors, their respective levels, and measured responses

Parts	Factors	Levels	Analytical Responses
1. Extraction	Extraction Method	Alkali, Salt, Enzyme-Assisted	Extraction Yield, Proximate Composition, FTIR Analysis, Protein Solubility, Water Solubility Index, Emulsifying Activity & Stability, Foaming Capacity & Stability, Antioxidant Activity, Color Properties
	Pre-Treatment	Microwave, Water Bath, Non-treated	
2. Modification by Glycation	Sugar Type	Fructose, Allulose	Browning Index, Free Amino Groups, Remaining Reducing Sugar Content, Amino Acid Composition, FTIR, Protein Solubility, Surface Hydrophobicity, Emulsifying & Foaming Properties, Antioxidant Activity,
	Protein:Sugar Ratio	1:1, 3:1	
3. Model Food System	Conjugate Type	PSPC:Fructose, PSPC:Allulose, Unmodified PSPC	Moisture, aw, pH, Brix, Color Properties, Protein Solubility, Lycopene Content, Total Phenolic Content, Flow Behavior, in Vitro Digestibility, Sensory Analysis
4. NMR Characterization	Sugar Type	Glucose, Fructose, Allulose, Sucrose	T ₂ Relaxation Times, Magic Sandwich Echo (MSE), Spin Diffusion, Self-Diffusion
	Sample Type	Extracted PSPCs, Glycated PSPCs, PSPCs added to Tomato Sauces	Hydration Behavior by T ₂ Relaxation Times

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Extraction of the Pumpkin Seed Protein Concentrate (PSPC) from its Flour with Different Approaches

3.1.1 Extraction Yield

Many factors influence protein yield extraction, including the type of protein, sample preparation methods, temperature, pH, and the use of enzymes (Flengsrud, 2019). The temperature of pre-heat treatments was chosen as 50°C by preliminary experiments in the range between 30-60°C. The preliminary results studied for AE samples are shown in the 'Appendix' as Table B.1.

Table 3.1 Extraction yield (% (w/w)) of Pumpkin Seed Protein Concentrate (PSPC) samples

Treatment	Extraction Techniques	Extraction Yield (% (w/w))
UT		34.1±0.05 ^d
CH	Alkali	50.3±0.09 ^b
MH		55.2±0.21 ^a
UT		11.2±0.06 ^h
CH	Salt	14.3±0.07 ^g
MH		16.2±0.05 ^f
UT		26.3±0.06 ^e
CH	Enzyme-Assisted	34.5±0.19 ^d
MH		36.6±0.26 ^c

Upper case superscript letters (a-h) denote a significant difference at 5% (p<0.05).

Table 3.1 displays the results for the extraction yield, and according to the results, the highest yield among the extraction techniques was observed in the AE, followed by EE and SE samples ($p < 0.05$). Each method has its advantages and limitations (Sari et al., 2013). Since proteins are more readily soluble in alkaline environments, alkali extraction frequently yields more protein; however, the high pH can cause the denaturation of proteins and the loss of their functional properties (Chandran et al., 2024). A basic solution, such as sodium hydroxide (NaOH), is commonly used to solubilize proteins in alkali extraction to extract both hydrophobic and hydrophilic proteins (Abdurrhman & Akasha, 2014). Protein functionality is preserved using enzyme-assisted extraction, which works in milder circumstances but may result in a lower yield, and the cost of enzymes can be a limitation (Gouseti et al., 2023). Salt extraction efficiently extracts salt-soluble proteins while maintaining their natural structure without harsh chemical treatments. However, to maximize extraction, precise control over ionic strength may be necessary (Patil et al., 2024). It precipitates and isolates proteins using salt concentration, such as NaCl; nevertheless, salt extraction can precipitate non-target proteins and may be less successful for hydrophobic proteins (Wingfield, 1998). This might be the reason for having the lowest yield of PSPC among the extraction techniques. Enzyme-assisted alkali (EE) protein extraction uses enzymes, such as proteases, and is commonly used for isolating specific proteins or protein subunits (Kamal et al., 2021). When compared with AE samples, EE samples gave lower yields ($p < 0.05$). This could be explained by the fact that the enzyme may not be a target enzyme specifically for PSPC.

When the pre-heat treatments were examined, the highest extraction yield was obtained in MH, followed by CH and UT, respectively ($p < 0.05$). Temperature is a crucial factor that affects the yield of protein extraction. As the temperature increases moderately, the yield generally increases as well (Haberl-Meglič et al., 2016). This fact compiles well with the results of having the lowest yield in UT samples ($p < 0.05$). Besides, the result of MH having the highest extraction yield can be due to the higher interaction of microwaves with the polar molecules in the extraction media and its

working mechanism. In the working principle of the microwave, heat is generated inside the material, and the internal pressure of the solid material is increased spontaneously (Ince et al., 2014). The increased internal pressure may lead to the breakdown of the molecular bonds between the materials, which makes it easier to extract the desired components. In addition, as the material disintegrates, more surface area of the product may be exposed, and this contributes to better contact between the material and surrounding solvent, resulting in higher extraction yields (Choi et al., 2006). A similar trend of higher protein yields in MH extracts compared to CH was reported by Suwannasopon et al., (2022) in their study on soybean protein extraction. Overall, the best combination to get the highest PSPC yield in this experiment design was found to be in the MH-AE samples ($p < 0.05$).

3.1.2 Proximate Composition Analysis

The results are reported in Table 3.2. Also, the purchased PSF was analyzed, and it had 9.41 ± 0.03 (g/100 g dw) moisture, 5.44 ± 0.01 (g/100 g dw) ash, 15.5 ± 0.03 (g/100 g dw) fat, 45.13 ± 0.19 (g/100 g dw) protein, and 34.65 ± 0.23 (g/100 g dw) carbohydrate contents.

Table 3.2 Proximate composition analysis of the extracted Pumpkin Seed Protein Concentrate (PSPC) samples

Treatment	Extraction Technique	Moisture (g/100 g dw)	Ash (g/100 g dw)	Fat (g/100 g dw)	Protein (g/100 g dw)	Carbs (g/100 g dw)
UT		9.24±0.03 ^a	5.37±0.01 ^a bcd	13.41±0.02 c	59.42±0.11 ef	21.82±0.33 b
CH	Alkali	8.67±0.02 ^d	5.60±0.01 ^a	11.61±0.03 f	68.67±0.53 b	14.14±0.14 e
MH		7.95±0.02 ^f	4.95±0.02 ^e	10.63±0.03 g	76.95±0.52 a	7.51±0.06 ^f
UT		8.23±0.03 ^e	5.23±0.02 ^c d	15.19±0.07 ab	55.23±0.84 g	24.44±0.45 a
CH	Salt	8.72±0.03 ^c d	5.12±0.05 ^d e	15.22±0.06 a	61.22±0.59 de	18.44±0.23 c
MH		8.55±0.06 ^d	5.55±0.05 ^a b	14.91±0.06 b	63.55±0.74 cd	15.98±0.41 de
UT		9.13±0.04 ^a b	5.13±0.07 ^d e	13.10±0.06 d	57.13±0.65 fg	24.64±0.44 a
CH	Enzyme-Assisted	9.02±0.03 ^b	5.42±0.09 ^a bc	12.72±0.03 e	63.52±0.50 cd	18.36±0.26 c
MH		8.93±0.04 ^b c	5.3±0.03 ^{bcd}	10.80±0.05 g	66.93±0.63 bc	16.97±0.15 cd

Upper case superscript letters (a-g) denote a significant difference at 5% ($p < 0.05$) in each column.

When comparing these results with all results in Table 3.2, it was shown that the protein content is the lowest, and the carbohydrate content is the highest in the purchased PSF ($p < 0.05$). This demonstrated that regardless of the method or treatment used in the extraction procedure, all samples were successfully extracted.

The moisture content values were found to range from 8.23 to 9.24 (g/100 g dw), indicating that different methods and treatments had affected the moisture content ($p < 0.05$). The determination of moisture content is important in food components because it affects how long the food will last, and what storage conditions should be decided (Hødørugø et al., 2016). Furthermore, it can play a crucial role in the hydration behavior of food components (Tas et al., 2022)

The results for the ash content ranged from 4.95 to 5.60 (g/100 g dw). According to ANOVA results that compared extraction techniques, the results did not show significant differences ($p>0.05$). However, different treatments were shown to be significantly different in the samples ($p<0.05$). When all results were compared, there were some differences in which the highest ash content belonged to the CH-AE sample ($p<0.05$). The higher ash content in the CH-AE sample could be attributed to the effect of conventional heating during alkali extraction, which may enhance the release of minerals and inorganic compounds from the plant matrix due to prolonged heat exposure (Y. Liu et al., 2023)

Table 3.2 shows that the fat contents ranged from 10.64 to 15.22 (g/100 dw). The comparison of the extraction techniques showed that the SE samples had the highest amount of fat content, followed by EE and AE samples ($p<0.05$). Besides, among the treatments, UT samples had the highest fat contents, followed by MH and CH treatments, respectively ($p<0.05$). The fact that the amount of fats in PSPC samples did not decrease significantly following extractions can be evaluated as an advantage since fats are a source of essential or nonessential fatty acids, antioxidants, and energy (Di Pasquale, 2009).

When the total protein contents were examined, the range was found to be from 55.23 to 76.95 (g/100 dw). The purchased PSF had the lowest protein content at 45.13 ± 0.19 (g/100 g dw). Therefore, they were not put into the statistical analysis and Table 3.2 since it would be difficult to see the differences between the data sets of the obtained samples. This outcome was expected since the aim of this study was to achieve the extraction of proteins. Among the extraction techniques, it was seen that AE samples gave the highest protein content, followed by EE and SE samples, respectively ($p<0.05$). In the literature, it was shown that the increase in pH up to a certain value (~11-12) increased the amount diffused into solutions, hence the higher contents of the protein after the extraction (Sari et al., 2015), which was also confirmed in our study. When the effect of pre-heat treatment was compared, MH samples showed the highest protein contents, followed by CH and UT samples, respectively ($p<0.05$). The lowest yield of UT can be linked to the effect of temperature, since a moderate

increase in temperature may contribute to an enhancement of the protein yield (Haberl-Meglič et al., 2016)

In the results, the best combination to get the highest protein content was found to be in the MH-AE samples with a value of 76.95 ± 0.52 ($p < 0.05$). Microwave energy increases the rate of diffusion, allowing for the extraction of proteins from the sample at a faster rate (Ince et al., 2014). Moreover, microwave use can also cause mechanical forces such as pressure to be generated, which can help matrix disruption and protein release (Beejmohun et al., 2007). The overall effect, therefore, might have been a higher protein yield compared to traditional methods with the combination of heat, pressure, and solvent extraction.

The carbohydrate contents were found to range from 7.51 to 24.64 (g/100 dw). The purchased PSF had the highest amount at 34.65 ± 0.23 (g/100 g dw), but they were not evaluated for the statistical analysis, and Table 3.2 was again due to being the outliers for the data set.

The results obtained from carbohydrates are negatively correlated with the protein content results. For instance, the lowest carbohydrate content was seen in the MH-AE samples ($p < 0.05$). Some insoluble components in the carbohydrates, such as dietary fibers and cellulose, can cause molecular crowding in the solution (Tas, Ertugrul, Oztop, & Mazi, 2021) and this can cause lower protein-water interaction and lower solubility of proteins. Therefore, the proteins in the MH-AE samples, having the lowest carbohydrate content, are likely to be more freely available in solutions and, as a result, potentially more functional.

3.1.3 Fourier Transform Infrared (FTIR) Spectroscopy

It is a widely used technique for the identification of functional groups and structural changes of the compounds (Ahmad & Benjakul, 2011). This study demonstrated structural differences in extracted samples. In Figure 3.1, FTIR spectra of AE samples are given. The spectra obtained for EE and SE were supplied separately in

the ‘Appendix’ file (Figure B.1 and Figure B.2). Besides, the purchased PSF was also examined and given as the control in Figure 3.1.

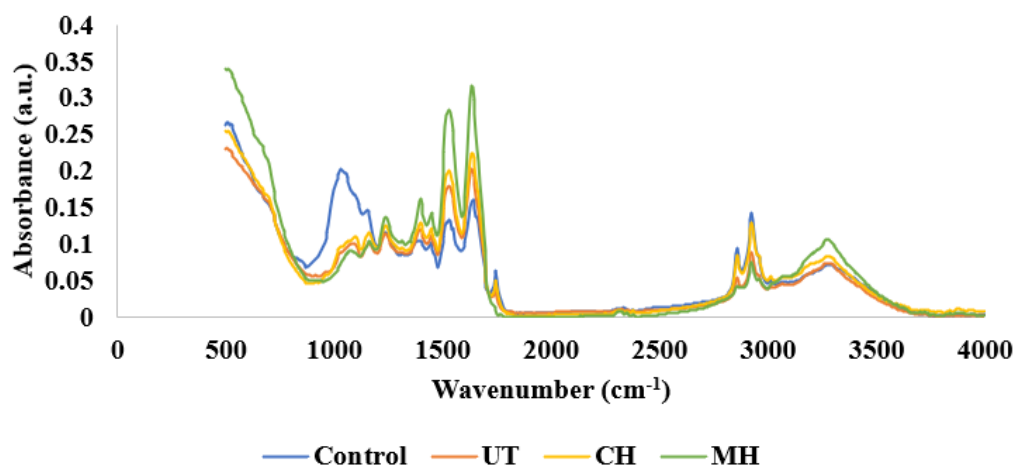


Figure 3.1 FTIR spectra of control (PSF) and alkali-extracted (AE) Pumpkin Seed Protein Concentrate (PSPC) samples

When the spectra were investigated, the peak corresponding to the range of 1000-1100 cm^{-1} was correlated with the coupling of the C-O or the C-C stretching bands (P. Guerrero et al., 2013). The component's relative carbohydrate content may be estimated from the intensity of this peak; thus, the highest intensity can be related to having a high amount of carbohydrates. In the figure, the peak of the control sample (PSF) was much higher compared to the extracted ones. Besides, the decrease in MH samples was much greater than in the other samples. This observation can also be supported by the carbohydrate contents obtained in the proximate composition analysis section.

The observed peaks of 2856 and 2927 cm^{-1} with C-H stretching of $-\text{CH}_3$, $-\text{CH}_2$ provide information about the fat contents of compounds (Araki et al., 2015), and as in the figure, while the control sample had the highest peak, the lowest belonged to the MH samples. Again, the fat contents in the proximate analysis are consistent with these findings.

When it comes to observing the peaks related to proteins, it was seen that the literature is generally focused on two of the most crucial peaks, which are Amide I

(~1700–1600 cm^{-1}) and Amide II (~1585-1480 cm^{-1}) bands (Ertugrul, Namli, Tas, Kocadagli, et al., 2021). In those bands, changes in the C=O stretching of the Amide I band can be used to assess the secondary structures of the proteins, and the C-N stretching vibrations and N-H bending of the Amide II band are utilized to monitor the conformational sensitivity and unfolding of the proteins (J. Kong & Yu, 2007). In the protein extractions, it was expected to observe an increase in those peaks because of the higher protein content in the matrix after the extraction procedure. According to the figure, the highest peaks were observed in MH-AE samples, but the lowest peaks belonged to control samples. These outcomes showed that the indication of extraction and FTIR results are consistent with the other results.

The secondary structures of control (PSF) and extracted PSPC samples were further identified by analyzing the derivative spectra in the Amide I region (1700–1600 cm^{-1}), and the results for AE samples are shown in Table 3.3. The results for SE and EE samples were given in the appendices part as Table B.2 and Table B.3.

Table 3.3 Secondary structures of control (PSF) and alkali-extracted (AE) Pumpkin Seed Protein Concentrate (PSPC) samples

Samples	α-Helix (%)	β-Sheet (%)	β-Turns (%)	Random Coil (%)
Control	36.26 \pm 0.42 ^a	37.46 \pm 0.63 ^d	17.27 \pm 0.12 ^c	8.99 \pm 0.02 ^c
UT	32.41 \pm 0.38 ^b	40.36 \pm 1.13 ^b	17.35 \pm 0.12 ^c	9.87 \pm 0.02 ^b
CH	31.99 \pm 0.34 ^c	39.76 \pm 0.66 ^c	18.22 \pm 0.44 ^b	10.02 \pm 0.12 ^a
MH	29.13 \pm 0.22 ^d	40.81 \pm 0.54 ^a	20.04 \pm 0.47 ^a	10.01 \pm 0.11 ^{ab}

Upper case superscript letters (a-d) denote a significant difference at 5% ($p < 0.05$) in each column.

In the analysis, four peaks were observed as α -Helix (1648-1657 cm^{-1}), β -sheet (1612-1641 cm^{-1}), β -turn (1660-1684 cm^{-1}), and random coil (1640-1650 cm^{-1}), as shown also in other studies (Yan et al., 2017). When these contents were examined, it was seen that α -Helix and β -sheet were the predominant structures, which is consistent with the findings of previous research (Y. Li et al., 2022; Nasrollahzadeh

et al., 2023). Besides, within the different extraction approaches, it was observed that α -Helix contents decreased whereas β -sheet increased independently of the treatments applied ($p < 0.05$). Indeed, the lowest content belonged to the MH-treated samples, followed by CH and UT ($p < 0.05$). Similar results were also obtained for the SE and EE samples. Although the effect of extraction techniques on secondary structures of PSP is not well studied, a study on bovine serum albumin (BSA) found a similar pattern (Nasrollahzadeh et al., 2023). The study showed that the β -sheet content increased while the α -helix content decreased because of the changes in different extraction processes. Also, the changes because of the extraction approaches might have a significant impact on protein-water interactions, primarily due to alterations in hydrogen bonding. It was stated in the studies that the α -Helix structure is often more compact and might form stable structures (Kopeć et al., 2019), which can lower protein-water interaction. In contrast, the β -sheet structure might have more exposed hydrophilic surfaces due to its extended nature (Emberly et al., 2004), which can allow more protein-water interaction.

3.1.4 Protein Solubility and WSI

PSP, like many other plant-based flours, has a lower protein solubility issue due to large and complex molecules that can make it difficult to diffuse proteins into the solution. Besides, protein solubility is affected by several factors, including the structure of the protein, temperature, interaction with other molecules, salts, and extraction methods from their native forms (Ertugrul et al., 2021). Since extraction methodology plays a crucial role in protein-water interaction and, thus, solubility, this study focused on examining this phenomenon. For that, related studies, such as protein solubility and WSI, were performed and are shown in Table 3.4.

Table 3.4 Protein Solubility (PS) (% (w/w)) and Water Solubility Index (WSI) of Pumpkin Seed Protein Concentrate (PSPC) samples

Treatments	Extraction Techniques	PS (% (w/w))	WSI (w/w)
UT		10.32±0.05 ^c	3.31±0.009 ^b
CH	Alkali	9.75±0.02 ^d	2.32±0.03 ^e
MH		15.99±0.04 ^a	3.45±0.005 ^a
UT		7.86±0.01 ^f	2.59±0.02 ^d
CH	Salt	7.97±0.01 ^f	1.99±0.03 ^g
MH		8.04±0.02 ^f	2.75±0.007 ^c
UT		9.88±0.02 ^d	3.29±0.009 ^b
CH	Enzyme-Assisted	9.11±0.03 ^e	2.11±0.009 ^f
MH		13.68±0.03 ^b	3.41±0.005 ^a

Upper case superscript letters (a-g) denote a significant difference at 5% ($p < 0.05$) in each column.

Within the extraction techniques, the highest solubility was found for the samples in the AE, followed by EE and SE ones ($p < 0.05$). The reason for the lowest solubility of the samples in the SE can be explained by the behavior of the used salt as being ‘salting out’ in the solution. It was stated that if the salts are acting as salting out in the solutions, it can affect the stability of the protein-water interactions, leading to the precipitation of proteins and lower solubility (K. H. Lee et al., 2003).

Similar results were reported by Z. Wang et al., (2022) in their study on the influence of ionic strength on soy protein solubility, where they showed that higher salt concentrations led to reduced solubility due to protein aggregation with the ‘salting out’ effect. When the results of different heat treatments were examined, the MH samples were found to be solubilized more than the CH and UT samples, respectively ($p < 0.05$). The reason for the lowest solubility of UT samples can be correlated with the temperature effect. Besides, the reason for MH samples having higher solubility than CH may be that microwave heating can extract proteins under milder conditions, which may help to preserve the native conformation of the proteins and

prevent denaturation and aggregation (De Pomerai et al., 2003). Additionally, microwave energy can damage the cell membrane, and then release intracellular contents, and solubilize the proteins because of the increased internal pressure effect inside the material (Y. H. Cheng et al., 2021). Within the spontaneous increase in internal pressure, the disintegration of the material would be facilitated, leading to higher extraction yields and more interaction with the water, which would also effectively solubilize more of the proteins. A study by Varghese & Pare, (2019) demonstrated similar results in soymilk protein that microwave heating improved protein solubility compared to conventional heating methods, with more extraction yield. Besides, as given in the proximate composition analysis part, MH samples had the lowest carbohydrate contents but the highest protein contents. Having fewer carbohydrates in the solution may lead to more protein-water interaction in the solution, thereby increasing the soluble protein content. Overall, the extraction method employed significantly influenced protein solubility and, by extension, other functional properties.

Analyzing the WSI results is another method to see the water interaction. WSI increases as the soluble contents diffuse into the water (Yousf et al., 2017). Considering the results obtained from proximate composition analyses, it can be stated that the main contributor to the soluble portions is expected to be proteins in the extracted samples. According to the results, the highest WSI values were found for AE, followed by SE, and EE between the techniques, respectively ($p < 0.05$). In addition, for the heat treatments, MH samples had the highest results, followed by CH and UT samples ($p < 0.05$). These outcomes also match the solubility results obtained by the Lowry method. Thus, it can be claimed that both the solubility and WSI results are interrelated, and this claim is corroborated by the Pearson correlation between solubility and WSI with a correlation coefficient of 0.704 ($p < 0.05$).

3.1.5 Emulsifying Activity (EA) and Emulsifying Stability (ES)

The EA and ES of PSPC samples were compared with egg yolk (EY) as a positive control, and the results are given in Figure 3.2.

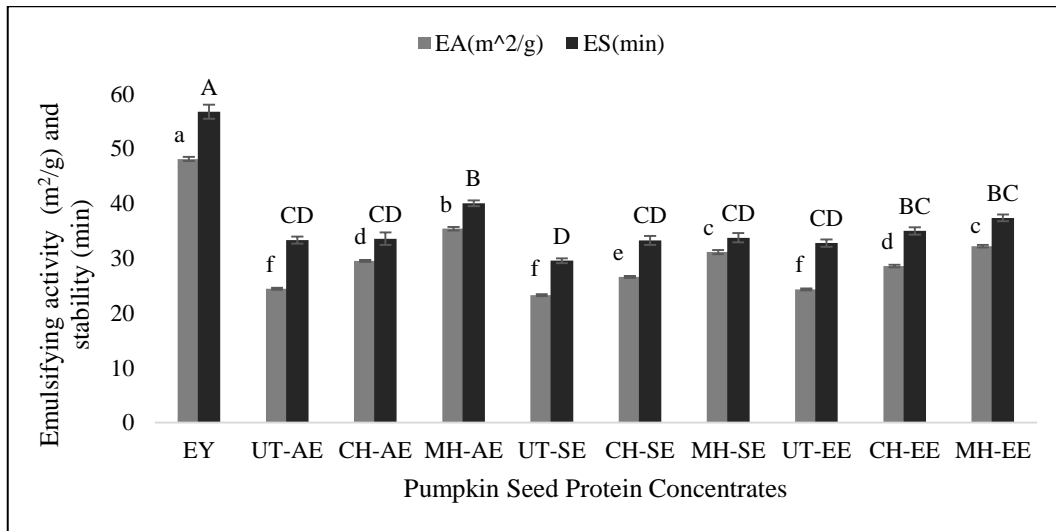


Figure 3.2. Emulsifying activity (EA) (m²/g) and Emulsifying stability (ES) (min) of egg yolk (EY) and Pumpkin Seed Protein Concentrate (PSPC) samples

EY, a well-known emulsifier, contains a phospholipid called lecithin, which improves the interaction between the water and oil phases in emulsions and is primarily responsible for better emulsifying characteristics (Zinia Zaukuu et al., n.d.). Because lecithin is amphiphilic, it can efficiently stabilize emulsions by lowering surface tension and forming a protective layer around oil droplets (M. Wang et al., 2021).

According to the results, EY showed the highest EA and ES significantly among all samples ($p < 0.05$). When the results were compared between PSPC samples, EA increased with the application of pre-heat treatments ($p < 0.05$). The highest EA was observed for MH-treated samples ($p < 0.05$). Between the extraction techniques, the EA of the samples was the highest in AE, followed by EE and SE, respectively ($p < 0.05$). Several factors could support this reason. More soluble proteins in the

solution may lead to the diffusion of more hydrophilic groups, which could enhance the interaction between the proteins and the oil, producing better EA and ES (Q. Zhang et al., 2022). Besides, in the previous studies, it was observed that secondary structures of the proteins play a role in the EA and ES of a protein (Wen et al., 2020; W. Xu & Zhao, 2019). Specifically, higher random coil content in a protein has been linked to improved EA and ES (Wen et al., 2020a). This is because random coils provide more flexibility (M. Wang et al., 2024) that could allow proteins to more easily interact with oil droplets. These outcomes also align with the results obtained through solubility and FTIR results. Although the PSPC samples exhibited lower EA and ES values than EY, the results are still promising, especially for MH-AE. The findings showed potential for developing PSPC as a functional emulsifier in the food industry, providing a beneficial plant-based substitute for egg yolk.

3.1.6 Foaming Capacity (FC) and Foaming Stability (FS)

Egg white was included as a positive control in this study since it has well-established foaming properties, which are frequently cited in the literature (L. Ding et al., 2022; X. Li et al., 2019). The measured values for egg white, with FC of $135.7 \pm 1.75\%$ and FS of $70.06 \pm 0.68\%$, align with the literature (Durakli Velioglu, 2019; X. Li et al., 2021). However, the results were not incorporated into the statistical analysis since the significant difference between the egg white and extracted PSPC samples would have caused data skewing, making the comparison insignificant. Egg white was therefore only employed as a reference point, and its remarkable outcomes confirmed what was expected based on the literature. For the extracted PSPC, the FC and FS were represented in Figure 3.3.

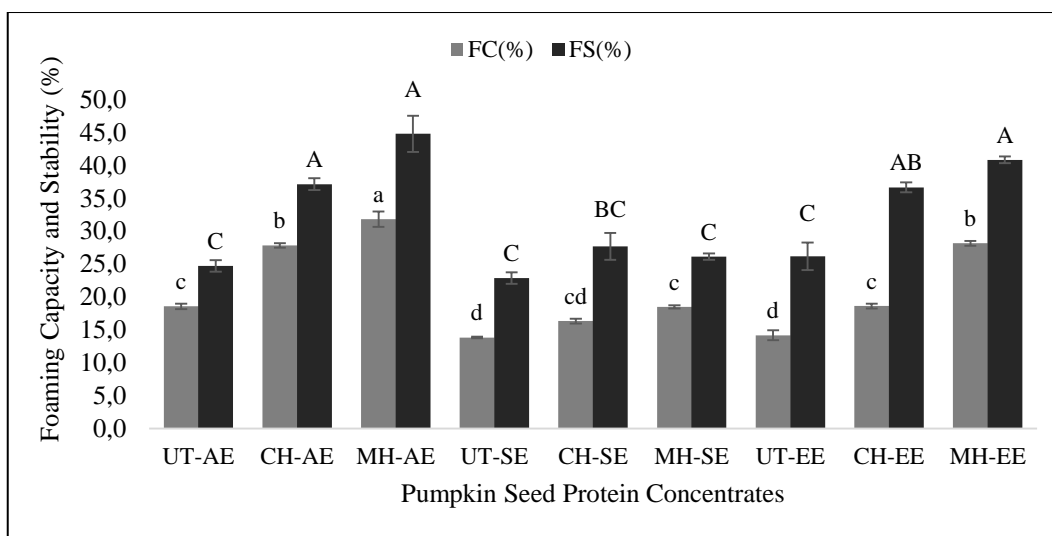


Figure 3.3. Foaming capacity (FC) (%) and Foaming stability (FS) (%) of Pumpkin Seed Protein Concentrate (PSPC) samples

The results showed that pre-treated samples increased both FC and FS, with the highest properties seen in MH, CH, and UT, respectively ($p < 0.05$). Additionally, as seen with emulsifying properties, AE samples gave better results among the extraction techniques ($p < 0.05$). The highest FC and FS (by mass) were obtained from the samples obtained by the combined effect of MH and AE ($p < 0.05$). This can be attributed to both the higher protein content and the improved solubility of the extracted proteins. Increased protein concentration enhances the ability of proteins to form and stabilize foams (Indrawati et al., 2008), while with increased solubility, hydrophilic groups and their diffusion rate to air-water interfaces may be enhanced, which would result in stronger foaming properties (Wouters et al., 2018). In addition, the effect of heat treatment to enhance foaming properties was explained in previous studies that a drop in viscosity of the solution because of heating increases the penetration in the compounds, which could facilitate obtaining more improved foaming properties (Morales et al., 2015). Furthermore, improved alignment of proteins at the air-water interface and increased protein flexibility because of heat treatment and extraction methods can improve the capacity of proteins to form stable foams (Nikbakht Nasrabadi et al., 2021).

3.1.7 DPPH Scavenging Activity

Scavenging activity (SA) (%) of the extracted PSPC samples was determined with the DPPH method, and the results are shown in Table 3.5.

Table 3.5 Scavenging Activity (SA) (%) of Pumpkin Seed Protein Concentrate (PSPC) samples

Treatments	Extraction Techniques	SA (%)
UT		15.24±0.02 ^b
CH	Alkali	14.63±0.07 ^e
MH		15.54±0.08 ^a
UT		14.56±0.02 ^f
CH	Salt	14.12±0.09 ⁱ
MH		14.39±0.06 ^h
UT		15.08±0.03 ^c
CH	Enzyme-Assisted	14.44±0.04 ^g
MH		14.72±0.03 ^d

Upper case superscript letters (a-g) denote a significant difference at 5% ($p < 0.05$) in each column.

It is known that heating could cause a decrease in antioxidant activities (Chua et al., 2015), and with the preheat treatment, there was a chance to degrade the scavenging activities of the extracted PSPC samples. According to the results, it can be said that the samples that were exposed to CH had decreased the SA (%) of samples ($p < 0.05$), which can be linked to the longer duration of heating to obtain the extracted PSPC (Tas, Ertugrul, Oztop, & Mazı, 2021). Besides, the highest SA (%) was obtained for MH-AE samples ($p < 0.05$). This can be seen as the advantage of microwave heating. Due to shorter exposure time, the degradation of SA (%) would be eliminated, or even higher SA (%) could be obtained, thanks to more soluble proteins in the solution (X. Yang et al., 2022). To make the proteins more functional, it is important to preserve their SA (%), and it has been shown in this study that MH pretreatment can be a suitable choice. Proteins with high SA (%) can help prevent lipid oxidation and preserve the quality and shelf life of food products by neutralizing free radicals (Elias

et al., 2008). For this reason, they can be beneficial in meat, dairy, and emulsion products where oxidation can degrade the nutritional value, flavor, and texture (D. Wang et al., 2023). Studies have shown that the MH pretreatment effectively preserved the SA (%) of the proteins (Ketnawa & Liceaga, 2017; X. Yang et al., 2022) as also shown in this study.

3.1.8 Water Activity (a_w) and Color Analysis

The color and water activity analysis of the PSPC samples was measured, and the results for color analysis are given in Table 3.6.

Table 3.6 Color analysis (L^* , a^* , b^*) of Pumpkin Seed Protein Concentrate (PSPC) samples

Treatments	Extraction Techniques	L^*	a^*	b^*
UT		49.57±0.07 ^c	4.13±0.03 ^b	28.77±0.03 ^c
CH	Alkali	45.17±0.03 ^g	4.27±0.03 ^{ab}	27.47±0.06 ^d
MH		48.67±0.07 ^d	4.13±0.02 ^b	28.57±0.02 ^c
UT		51.27±0.07 ^a	3.33±0.03 ^d	30.23±0.03 ^a
CH	Salt	48.77±0.03 ^d	3.73±0.02 ^c	29.80±0.05 ^b
MH		50.37±0.03 ^b	3.43±0.03 ^d	29.97±0.05 ^{ab}
UT		47.57±0.08 ^e	4.27±0.02 ^{ab}	26.73±0.05 ^e
CH	Enzyme-Assisted	46.77±0.06 ^f	4.43±0.03 ^a	26.53±0.03 ^e
MH		47.46±0.02 ^e	4.20±0.05 ^b	26.70±0.05 ^e

Upper case superscript letters (a-g) denote a significant difference at 5% ($p < 0.05$) in each column.

The water activity values of the extracted proteins were statistically insignificant (were not shown in Table 3.6) ($p > 0.05$) and found consistently low, around 0.3, which could indicate good stability. The last stage of the freeze-drying process to obtain extracts in dry form can help maintain stable a_w levels by removing free water,

which would suggest good stability against microbial growth and chemical reactions (Nowak & Jakubczyk, 2020; Srijeeta Saha, 2020).

The color analysis, however, showed slight variations in L*, a*, and b* values across the samples, as shown in Table 3.6. Lightness is represented by the L* value, where higher values denote a lighter hue and lower values a darker product (Ly et al., 2020). When Table 3.6 was examined, it was seen that CH led to darker products with lower L* values, followed by MH and UT, respectively, between the treatments ($p < 0.05$). This suggested that longer heat exposure in CH treatments may contribute to browning reactions and thereby pigment degradation (Enaru et al., 2021). MH, on the other hand, tends to retain the lightness due to shorter exposure times and more localized heating, which can also be confirmed by literature studies that MH is less destructive in food decolorization (Alkanan et al., 2024). Among the extraction methods, SE preserved the most natural color, with higher L* values followed by EE and AE, respectively ($p < 0.05$). SE is generally considered a milder process compared to AE and EE methods since it doesn't involve extreme pH changes or enzyme activity that may disrupt pigment. Hewage et al., (2022) highlighted that at both high and low pH levels, phenolic compounds oxidize into reactive o-quinones or o-dihydroxy structures, which can bind to proteins, leading to the darker coloration of plant protein extracts.

The a* value represents the position on the green-red axis, with positive values denoting redness and negative values denoting greenness (Durmus, 2020). According to the results, CH resulted in higher a* values (more redness), followed by MH, and UT, respectively ($p < 0.05$), which may be again linked to browning reactions with longer exposure to heat treatment. Again, between extraction methods, SE maintained the lowest a* values, followed by EE, and AE ($p < 0.05$), respectively, indicating less change in natural pigments.

The yellow-blue axis is shown by the b values*, where positive values denote yellowness and negative values denote blueness. Positive b values* indicate that all the study's samples are more yellow than blue. Between the treatments, CH caused

the most decrease in b^* values (less yellowness), followed by MH and UT, respectively ($p < 0.05$). In the extraction methods, EE showed the lowest b^* values, possibly due to enzyme-induced exposure of pigments to degradation or oxidation, followed by AE and SE, respectively ($p < 0.05$).

Overall, SE best retained the lightness (L^*) and natural yellow color (b^*) while contributing the smallest increase in redness (a^*), compared to AE and EE methods. Furthermore, CH treatment significantly reduced L^* and b^* values and increased a^* values, while MH had a milder effect, better retaining the natural color. Since color is an important factor in food formulation and consumer acceptance, the selection of the optimum extraction approach should be conducted based on the desired properties (Fiorentini et al., 2020).

3.2 Modification of Pumpkin Seed Protein Concentrates (PSPC) through Glycation Reaction

3.2.1 Browning Index, Free Amino Groups, and Remaining Reducing Sugar Content Analysis

Brown color formation occurs especially in the final stages of Maillard browning. However, it is important to note that even in the early stages, some brown-colored compounds may begin to form (B. G. Guerrero, 2007). Monitoring absorbance values at 420 nm is one of the basic techniques to determine the extent of Maillard browning, and it could also provide valuable information on whether the Maillard reaction occurred or not (Zia et al., 2021). In Table 3.7, the results obtained for the PSPC and its conjugates are shown.

Table 3.7 Browning Index, Free Amino Groups (FAGs) and % Remaining Reducing Sugar Content (% RRS) of Pumpkin Seed Protein Concentrate (PSPC) and its conjugates

Sample Name	PSPC:Sugar Ratio	Browning Index	FAG (mg glycine eq./ g protein)	% RRS Content
PSPC		0.091±0.001 ^e	3.741±0.032 ^a	-
PSPC-Fru	1:1	0.233±0.003 ^a	1.682±0.009 ^e	ND ^d
PSPC-Fru	3:1	0.179±0.002 ^c	2.176±0.006 ^c	4.73 ± 0.21 ^c
PSPC-All	1:1	0.201±0.002 ^b	1.952±0.004 ^d	23.93 ± 0.19 ^b
PSPC-All	3:1	0.141±0.002 ^d	2.991±0.009 ^b	34.66 ± 0.85 ^a

ND: not detected. Upper case superscript letters (a-e) denote a significant difference at 5% ($p < 0.05$) in each column.

According to the table, the highest browning index value was obtained in the PSPC-Fru (1:1) sample, while the lowest value was obtained in the control sample, PSPC that did not participate in the reaction ($p < 0.05$). Considering this comparison, it can be confirmed that the color formation occurred in all samples. The higher affinity of conjugates obtained with fructose than conjugates with allulose in the Maillard reaction was also mentioned in the previous studies (Ertugrul, Namli, Tas, Kocadagli, et al., 2021; Tas, Ertugrul, Oztop, & Mazi, 2021) and the obtained results in this study also support them. Besides, it was seen that the samples with a 1:1 ratio had a higher browning index than the samples with a 3:1 ratio ($p < 0.05$). This could be explained by more water-sample interaction in a 1:1 ratio, which could favor reaction occurrence (Nikbakht Nasrabadi et al., 2021). In other words, since molecular crowding is substantially greater in samples with a 3:1 ratio than in samples with a 1:1 ratio, the reaction may be lower in that case. Molecular crowding refers to the dense environment formed by protein and sugar molecules under limited water availability. This phenomenon can restrict the accessibility of reactive groups, thereby decreasing the extent of the glycation. The Browning index method could supply promising results, but other methods that deal with more specific reagents or

products of the Maillard reaction may be better to identify the reaction occurrence accurately.

FAG determination in glycated conjugates using the OPA method is an accurate and common approach for determining the degree of glycation (Jin et al., 2015; Zia et al., 2021). The decrease in FAG amounts was directly linked to the amino acids that participated in the glycation process (H. Hu et al., 2014). According to Table 3.7, sugar type and protein:sugar ratios were found to have a significant effect on the FAG amounts of samples ($p < 0.05$). Table 3.7 shows the highest FAG amount in PSPC samples with 3.741 ± 0.032 (mg glycine eq./g protein). The amounts in the conjugates were significantly lower than the PSPC, as expected ($p < 0.05$). The results confirm the occurrence of the reaction across all samples, but at different rates. Besides, according to the statistical analysis done between the sugar types, the conjugates glycated with fructose showed lower FAG amounts than the conjugates glycated with allulose samples ($p < 0.05$). This outcome aligns with the idea that the different sugars had different affinities in the reaction (Huang et al., 2016). Moreover, the 1:1 ratio was shown to be much more effective in decreasing FAG amounts compared to 3:1 ($p < 0.05$). This might be explained by the higher content of sugars in the 1:1 ratio, which may contribute to having more carbonyl groups and thereby react and consume more amino groups in the protein (O'Charoen et al., 2015). The results obtained in the OPA method are also negatively correlated with the results of the browning index, with a negative correlation coefficient ($R = -0.987$) ($p < 0.05$). Thus, these two methods show that glycation occurred in all samples, and the best combination of glycated conjugate was found to be PSPC-Fru with the 1:1 ratio sample.

The determination of the RRS content may supply information and insight into the reaction occurrence and its rate from the reducing sugar aspect. Table 3.7 shows that the RRS contents significantly differed between the conjugated samples ($p < 0.05$). The PSPC-Fru conjugates at a 1:1 ratio showed no detectable RRS (ND), indicating a complete reaction; however, the 3:1 ratio had RRS content ($4.73 \pm 0.21\%$), suggesting that at higher sugar concentration, glycation reaction occurred to a higher

extent. These results also supported other outcomes obtained by the OPA results. Besides, PSPC-All samples showed significantly higher RRS contents at both ratios ($p < 0.05$), displaying less glycation rate. The studies in the literature suggested that the type of sugars in the Maillard reaction had an impact on the reaction (Laroque et al., 2008). Although fructose and allulose sugars are epimers of each other, their β -pyranose forms differ in the number of hydroxyl groups in the equatorial position, which would affect their reactivity in the reactions (Fukada et al., 2010). Apart from that, isomerization of the sugars during the reaction, especially in an alkaline environment, can affect the RRS content (Nagasawa et al., 2023). The conversions during isomerization could decrease the overall pool of reducing sugars since some of them may shift away from their original structure (Y. Kong et al., 2023). This can cause less reactivity of the sugars in the reaction, which may affect the RRS content in the sample. In one study, it was revealed that the conjugates obtained via allulose sugar had shown more isomerization compared to fructose and glucose (Ertugrul, Namli, Tas, Kocadagli, et al., 2021). This outcome can support the idea that sugars like allulose, which undergo more isomerization, may lead to a reduced glycation rate due to less availability in the original form of sugar in the solution. Hence, RRS content can significantly give information on the reaction occurrence regarding the sugar side in the reaction.

3.2.2 Amino Acid Composition Analysis

The analysis of amino acid composition offers important information about the efficiency of glycation for various reducing sugars and the reactivity of particular amino acids (Q. Yang et al., 2022). The results were given in Table 3.8.

Table 3.8 Amino Acid contents (g/100g sample) of Pumpkin Seed Protein Concentrate (PSPC) and its conjugates

Amino Acids	PSPC	PSPC:Fru (1:1)	PSPC:Fru (3:1)	PSPC:All (1:1)	PSPC:All (3:1)
Alanine	0.275±0.004 ^a	0.131±0.008 ^d	0.169±0.0008 ^c	0.195±0.016 ^b	0.156±0.004 ^c
Arginine	0.665±0.04 ^a	0.424±0.11 ^c	0.508±0.006 ^b	0.477±0.044 ^{bc}	0.609±0.037 ^a
Aspartic Acid	5.463±0.05 ^a	1.509±0.09 ^e	2.235±0.097 ^d	3.513±0.01 ^c	4.940±0.036 ^b
Cysteine	0.124±0.014 ^a	0.074±0.003 ^b	0.076±0.009 ^b	0.123±0.01 ^a	0.123±0.01 ^a
Glutamic Acid	5.77±0.05 ^a	2.188±0.005 ^e	2.882±0.002 ^d	4.070±0.114 ^c	4.763±0.102 ^b
Glycine	0.17±0.005 ^a	0.112±0.006 ^c	0.117±0.001 ^c	0.120±0.003 ^c	0.145±0.001 ^b
Histidine	0.631±0.009 ^a	0.216±0.002 ^e	0.288±0.003 ^d	0.316±0.003 ^c	0.425±0.005 ^b
Isoleucine	1.963±0.04 ^a	1.32±0.007 ^d	1.045±0.006 ^e	1.464±0.009 ^c	1.778±0.01 ^b
Leucine	2.113±0.03 ^a	1.413±0.05 ^d	1.563±0.04 ^c	2.105±0.03 ^a	1.894±0.04 ^b
Lysine	4.10±0.014 ^a	2.475±0.1 ^d	2.670±0.156 ^{cd}	2.700±0.08 ^c	2.976±0.07 ^b
Methionine	2.303±0.03 ^a	1.650±0.009 ^e	1.78±0.02 ^c	1.72±0.005 ^d	1.930±0.003 ^b
Phenylalanine	5.475±0.115 ^a	4.085±0.03 ^d	4.31±0.05 ^c	5.083±0.05 ^b	5.15±0.07 ^b
Serine	0.054±0.002 ^a	0.028±0.008 ^d	0.031±0.001 ^c	0.034±0.002 ^b	0.035±0.001 ^b
Threonine	4.225±0.123 ^a	3.955±0.065 ^b	4.110±0.036 ^{ab}	4.138±0.103 ^{ab}	4.275±0.112 ^a
Tryptophan	5.867±0.166 ^a	4.925±0.04 ^d	5.270±0.017 ^c	5.60±0.07 ^b	5.75±0.098 ^{ab}
Tyrosine	1.047±0.006 ^a	0.745±0.005 ^d	0.908±0.003 ^b	0.795±0.006 ^c	0.907±0.004 ^b
Valine	2.386±0.011 ^a	1.767±0.009 ^d	2.064±0.017 ^b	2.033±0.02 ^c	2.060±0.003 ^b

Upper case superscript letters (a-e) denote significant difference at 5% ($p < 0.05$) in the same row.

According to the results, PSPC is rich in essential amino acids, including lysine, phenylalanine, and tryptophan. The two most common non-essentials were aspartic acid and glutamic acid. The results also support previous studies in which PSP was analyzed for its amino acid profile (Kim et al., 2012; Singh & Kumar, 2022). These suggest that PSPC has nutritional potential for use in functional foods.

In the case of glycation, significant alterations in the amino acid contents were noted, especially with the reactive side chains ($p < 0.05$). For instance, lysine, which is a key amino acid of glycation, shows significant decreases in all conjugates ($p < 0.05$). In PSPC:Fru with a 1:1 ratio, the lysine content was the lowest ($p < 0.05$). When allulose

is present, lysine is less reactive than fructose ($p < 0.05$), indicating the effect of sugar in the glycation reaction, which is also confirmed by the other findings of this study. Furthermore, among the protein:sugar ratios, the 1:1 ratio showed the most changes compared to the 3:1 ratio, which compiles well with the findings as well. Other common amino acids that are susceptible to glycation are arginine and histidine (Fournet et al., 2018) also showed notable decreases compared to PSPC in their native form. Similar findings were observed in the previous studies, showing that glycation influenced the amino acid content in proteins, including soy, pea, and others (Akharume et al., 2021; Kutzli et al., 2021; Q. Zhang et al., 2022). Hence, the outcomes showed that amino acid analysis can give crucial insights into the degree of glycation, especially in reactive amino acids, in the reaction.

3.2.3 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy is a useful tool for investigating conformational changes caused by the glycation reaction by monitoring molecular changes in functional groups such as amino groups and carbonyl compounds (Zhong et al., 2019). It can also be used to identify key functional groups of proteins, such as amide bonds, and reveal secondary structures, such as α -helix and β -sheet, as well as hydrogen bonding patterns (Sukumaran, 2018).

The changes in protein and carbonyl groups (coming from the sugars) after glycation was given in Fig. 3.4.

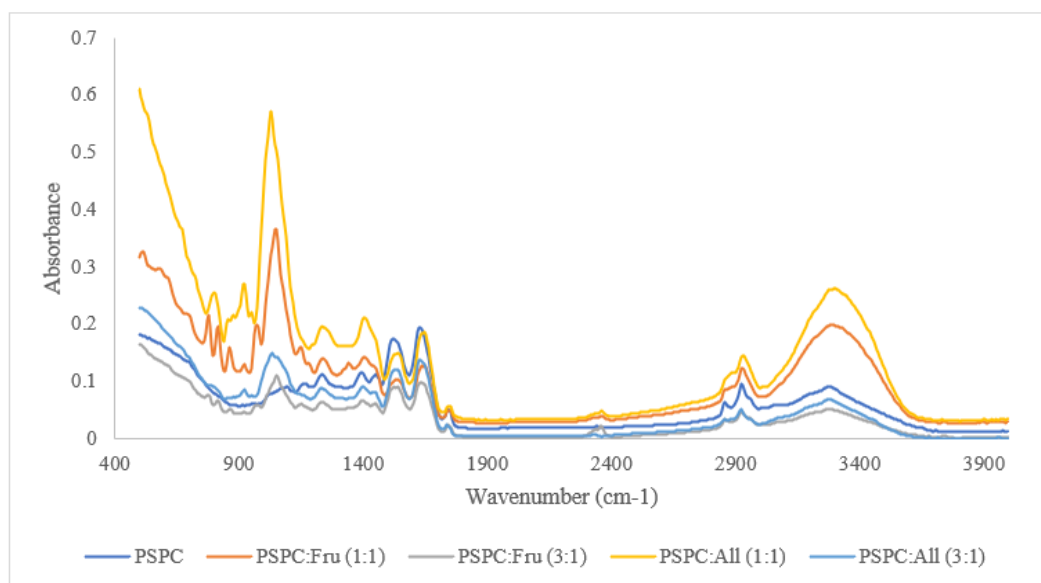


Figure 3.4. Structural characterization of Pumpkin Seed Protein Concentrate (PSPC) and its conjugates by FTIR spectra

When the spectra were investigated, the decrease in the intensities of the bands ($\sim 1600\text{ cm}^{-1}$) and ($\sim 1500\text{ cm}^{-1}$) was seen. These two bands are the most important ones observed in proteins, known as Amide I ($\sim 1600\text{ cm}^{-1}$) and Amide II ($\sim 1500\text{ cm}^{-1}$) bands. The decrease in these bands is expected in glycation since the functional groups containing NH_2 are consumed during the reaction (J. F. Su et al., 2010). When the comparison between the PSPC and its conjugates was made, the highest peaks were observed in PSPC, indicating that in all the conjugates, glycation occurred. The other bands related to proteins are Amide B ($\sim 3100\text{ cm}^{-1}$) and Amide A ($\sim 3300\text{ cm}^{-1}$). Changes in these bands can be used to discuss water interaction by the samples since the vibration mode in these bands is strongly dependent on the strength of hydrogen bonding in the samples (Ji et al., 2020). In the samples, PSPC conjugates showed higher absorbance intensities than PSPC itself. This can be linked to having stronger hydrogen bonds of conjugates, also a higher hydration rate in the solutions (Ertugrul, Namli, Tas, Kocadagli, et al., 2021). Besides, the C-O and C-C stretching bands present in carbohydrates can be detected in an FTIR spectrum within the $950\text{-}1200\text{ cm}^{-1}$ range (Natalello et al., 2005). As shown in Figure 3.4, the

peak obtained from the PSPC sample had the lowest intensity, while the intensity in PSPC conjugates was higher due to more protein-sugar interaction.

FTIR spectroscopy can be used to further examine by evaluating their secondary structures to observe the effect of the glycation reaction on the related structures (Z. Z. Xu et al., 2019). The secondary structures of PSPC and its conjugates were considered by taking the area of α -helix (at 1651 cm^{-1}), β -sheet (at 1627 cm^{-1}), β -turn (at 1666 and 1679 cm^{-1}), and random coil (at 1641 cm^{-1}), and the results are shown in Table 3.9.

Table 3.9 Secondary structure distribution of Pumpkin Seed Protein Concentrate (PSPC) and its conjugates

Sample Name	PSPC:Sugar Ratio	α -Helix (%)	β -Sheet (%)	β -Turns (%)	Random Coil (%)
PSPC		27.72±0.12 ^a	37.41±0.16 ^a	9.11±0.07 ^c	24.22±0.14 ^d
PSPC-Fru	1:1	20.07±0.08 ^e	32.33±0.11 ^e	17.23±0.12 ^a	29.56±0.27 ^a
	3:1	24.67±0.15 ^c	34.16±0.09 ^d	15.06±0.08 ^b	25.69±0.15 ^c
PSPC-All	1:1	22.65±0.21 ^d	34.56±0.18 ^c	15.78±0.14 ^b	26.12±0.19 ^b
	3:1	25.73±0.07 ^b	35.19±0.09 ^b	15.44±0.04 ^b	25.68±0.02 ^c

Upper case superscript letters (a-e) denote significant difference at 5% ($p < 0.05$) in the same column.

According to the results, as glycation progressed, the area of α -helix and β -sheet areas decreased for the conjugates while the β -turn and random coil areas increased ($p < 0.05$). Furthermore, compared to PSPC, the decrease in α -helix and β -sheet areas was greater in PSPC:Fru (1:1) samples compared to other conjugates ($p < 0.05$). The key mechanism can be evaluated by the fact that glycation might disrupt the hydrogen bonds in α -helix and β -sheet structures while it increases β -turn and random coil areas due to the interaction between the protein and reducing sugars (Tas, Ertugrul, Oztop, & Mazi, 2021; Zhong et al., 2019). The related studies explained this by that the interaction between the amino groups and reducing sugars occurs near the α -helix region. In addition, thermal treatment during the reaction causes the unfolding of the protein, leading to an increase in random coil content

since the structure becomes more flexible and disordered (Z. Zhang et al., 2024). Hence, the results of this study also supported the literature findings and the FTIR approach can be shown to be a method to examine the occurrence of glycation and the obtained results are consistent with the FAG and browning index results.

3.2.4 Protein Solubility

Solubility is known to be a common problem among plant proteins, mainly due to their strong intramolecular interactions (Chuang et al., 2021) and it is affected by numerous factors, including pH in the solution (Cao et al., 2023; H. N. Liang & Tang, 2013b). In Fig. 3.5, the solubility of PSPC and its conjugates at pH values of 2, 3, 5, 6.5, 7.5, 8.5, and 9.5 were shown.

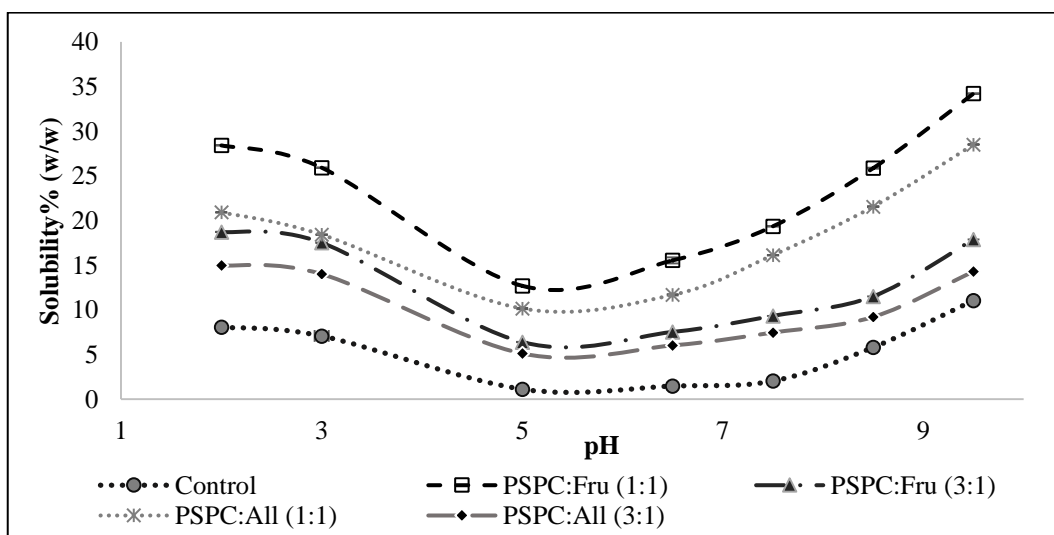


Figure 3.5. Solubility% (w/w) of pumpkin seed protein concentrate (PSPC) and its conjugates at different pH values

According to the results, in all samples, the solubility was the lowest near the isoelectric point (~5), and more soluble proteins were obtained away from the isoelectric point ($p < 0.05$). This trend was observed both in the PSPC and its conjugates ($p < 0.05$). This indicates that the PSPC conjugates are affected similarly by the pH change as PSPC. Besides, it was noted that the solubility at pH 5 was higher in the conjugates compared to the control sample ($p < 0.05$). This fact can

be attributed to the inclusion of sugar molecules that increase the hydrophilicity of the protein surface, thereby reducing protein-protein aggregation tendencies (Wijegunawardhana et al., 2024). Hence, it can be deduced that the glycation resulted in enhanced water-protein interactions in the conjugates while decreasing protein-protein interactions. This demonstrates the effectiveness of conjugation for protein functionality across a wide range of pH values, including challenging conditions like the isoelectric point. Furthermore, PSPC conjugates had higher solubility than PSPC in the given pH range, with PSPC:Fru (1:1) having the highest solubility of 35% at pH 9.5 ($p < 0.05$). This suggests that glycation leads to increased protein solubility. This outcome was also shown in many studies (Garcia-Amezquita et al., 2014; J. Liu et al., 2012; C. H. Tang et al., 2011). Furthermore, the sugar type was seen as an effective parameter in the solubility of the conjugates. This can be explained by the fact that sugars have different reactive hydrophilic groups in the solution, which could also contribute to creating an environment for proteins to bind more water (Tas et al., 2022). Hence, it is also shown in solubility results that the greater the degree of glycation, the more improved the solubility of PSPC.

3.2.5 Surface Hydrophobicity

Surface hydrophobicity (H_0) is a critical functional property that represents the exposure of nonpolar amino acid residues on the protein surface (Q. Wang et al., 2025). The surface hydrophobicity of PSPC and its conjugates is given in Table 3.10.

Table 3.10 Surface Hydrophobicity of Pumpkin Seed Protein Concentrate (PSPC) and its conjugates

Sample Name	PSPC:Sugar Ratio	H ₀ Values
PSPC	-	343.63±0.57 ^e
PSPC-Fru	1:1	665.41±3.52 ^c
	3:1	1049.65±1.15 ^b
PSPC-All	1:1	617.26±0.14 ^d
	3:1	1248.15±5.15 ^a

Upper case superscript letters (a-e) denote significant difference at 5% ($p < 0.05$).

The results showed that compared to PSPC, glycation increased the surface hydrophobicity in all conjugates ($p < 0.05$). This outcome aligns with the previous studies showing that glycation can enhance surface hydrophobicity through partial unfolding and rearrangement of protein structures (Q. Zhang et al., 2022). In native PSPC, hydrophobic residues are likely buried within the compact protein structure; however, the presence of reducing sugar in the glycation reaction may promote conformational loosening, leading to exposure of buried hydrophobic regions on the protein surface (H. Zhang et al., 2023).

When the comparison was made between the conjugates, the highest H₀ values were obtained in the 3:1 ratio conjugated sample ($p < 0.05$). It can be due to the large portion of protein that did not undergo glycation but instead participated in heat-induced protein-protein aggregation. Due to the sugar-limited environment, the reduced availability of the carbonyl group may lead to protein-protein interaction on the surface, thereby increasing surface hydrophobicity. These outcomes highlight the importance of optimizing glycation to achieve desired structural and functional modifications via Maillard conjugation. Although the surface hydrophobicity was higher in the glycated conjugates, their improved functional properties cannot be related to the exposure of hydrophobic residues. The exposure of hydrophilic amino

acids on the surface and the presence of reducing sugars contributed to enhanced functional properties through glycation.

3.2.6 DPPH Scavenging Activity

The DPPH scavenging activity (SA) (%) the results are shown in Figure 3.6.

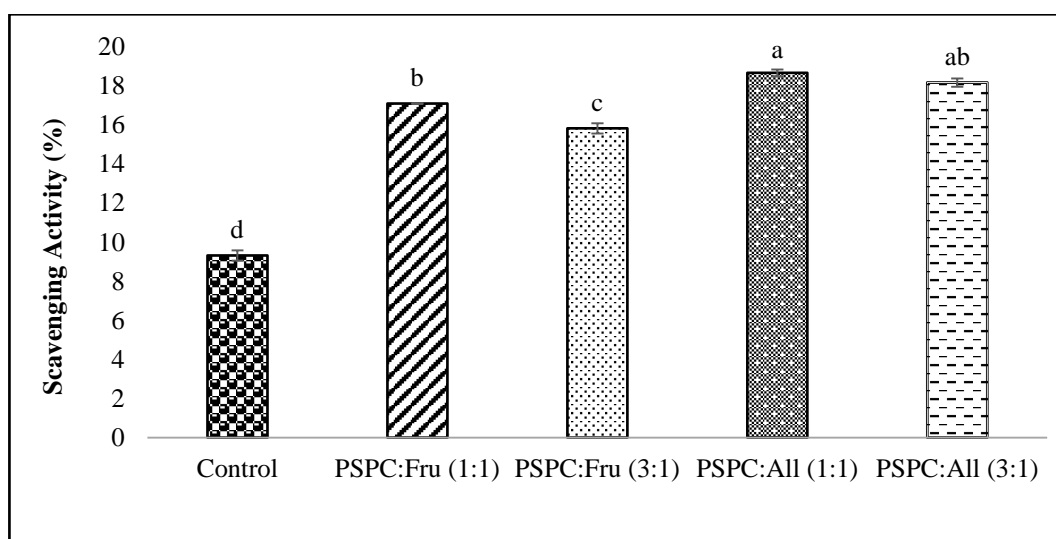


Figure 3.6. DPPH scavenging activity (%) of Pumpkin Seed Protein Concentrate (PSPC) and its conjugates

According to the results, the PSPC (control) itself showed the lowest SA (%), hence antioxidant activity compared to the PSPC conjugates ($p < 0.05$). This indicates that the glycation reaction increased the SA (%) in all samples ($p < 0.05$). It is known that the products of Maillard reactions have the potential to exhibit higher SA (%) due to the various mechanisms occurring, such as radical chain-breaking activity, scavenging of reactive oxygen species, decomposing metal chelation, and hydroperoxides, which collectively may impede the formation of oxidation products (Aminjafari et al., 2016a; Vhangani & Van Wyk, 2016). When the comparison was made between the sugar types, it was seen that the conjugates prepared with allulose had higher scavenging activities than the conjugates prepared with fructose ($p < 0.05$). It was seen that one of the major criteria influencing the results is the type of reducing sugar. This claim was also supported in the literature that SA (%) by DPPH is

facilitated when the solution is more hydrophilic (Gregg et al., 2010). In glycation, since the sugars are introduced in the reaction, a more hydrophilic environment can be achieved thanks to the hydroxyl groups of the sugars (Y. Sun et al., 2007). Although the protein solubility results from conjugates with fructose contributed to having more soluble protein than the conjugates with allulose, scavenging activities by DPPH were vice versa. In that regard, the RRS content after the glycation could also be the reason for the obtained results, which the results of Table 3.7 can confirm. In some of the studies, it was also shown that the conjugates prepared with allulose showed higher DPPH scavenging activities than the conjugates prepared with other sugars, which is explained by that allulose has the remarkable ability to neutralize DPPH radicals by creating a synergistic effect with protein on the reaction (Moriya et al., 2017; Tas, Ertugrul, Oztop, & Mazi, 2021).

3.2.7 Emulsifying Activity (EA) and Emulsifying Stability (ES)

In Figure 3.7, the EA and ES of PSPC and its conjugates were represented.

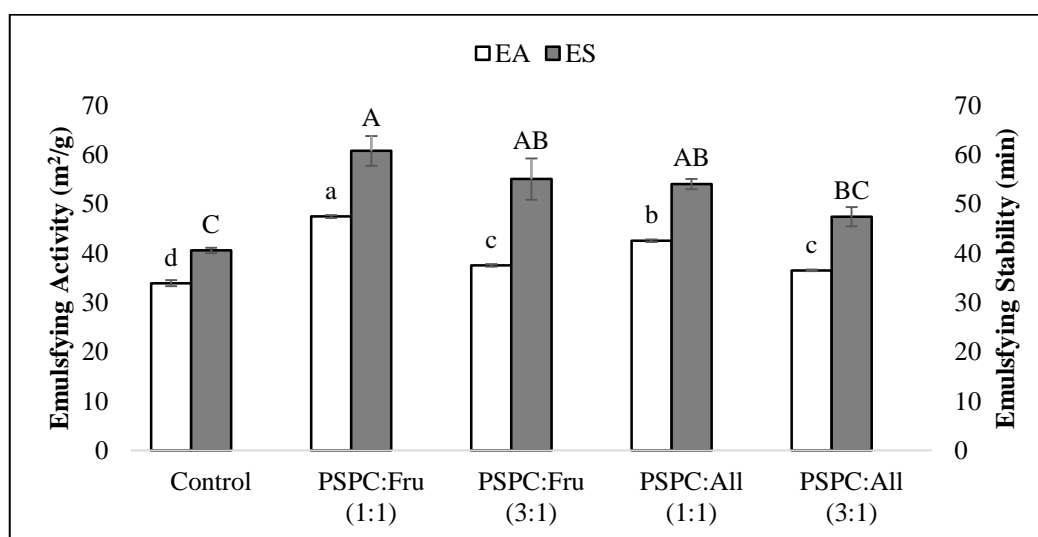


Figure 3.7. Emulsifying Activity (m²/g) and Emulsifying Stability (min) of Pumpkin Seed Protein Concentrate (Control) and its conjugates

According to Figure 3.7, both EA and ES of PSPC were increased after glycation compared to PSPC itself ($p < 0.05$). Furthermore, the highest EA and ES values were obtained in the PSPC:Fru (1:1) conjugate, which was shown to be the best glycated sample ($p < 0.05$).

It is known that proteins have an amphiphilic nature, so the idea here can be explained in two different approaches. First, it was revealed in the solubility experiment that conjugates have more soluble proteins, meaning the hydrophilic nature of the solution increased by the addition of sugar into the solution, which could result in improved EA and ES properties (Pirestani et al., 2017). On the other hand, the bonds between the sugars and the proteins after the glycation could result in the exposure of hydrophobic groups, which would contribute to better adhesion of oil droplets to the conjugates (Hung et al., 2013). This is further supported by the surface hydrophobicity (H_0) results, as the conjugated samples via glycation exhibited increased H_0 values compared to the native PSPC, confirming the enhanced exposure of hydrophobic regions that can promote better emulsifying functionality (H. Zhang et al., 2023). Besides, in the studies, it was revealed that having high random coil content in the proteins can also be another reason for having better emulsifying properties (Wen et al., 2020; Q. Zhang et al., 2022).

3.2.8 Foaming Capacity (FC) and Foaming Stability (FS)

FC and FS are important functional properties of the PSPC that can be incorporated into many commercial food products (Lazos, 1992; Sert et al., 2022). The FC and FS of PSPC and its conjugates are given in Figure 3.8.

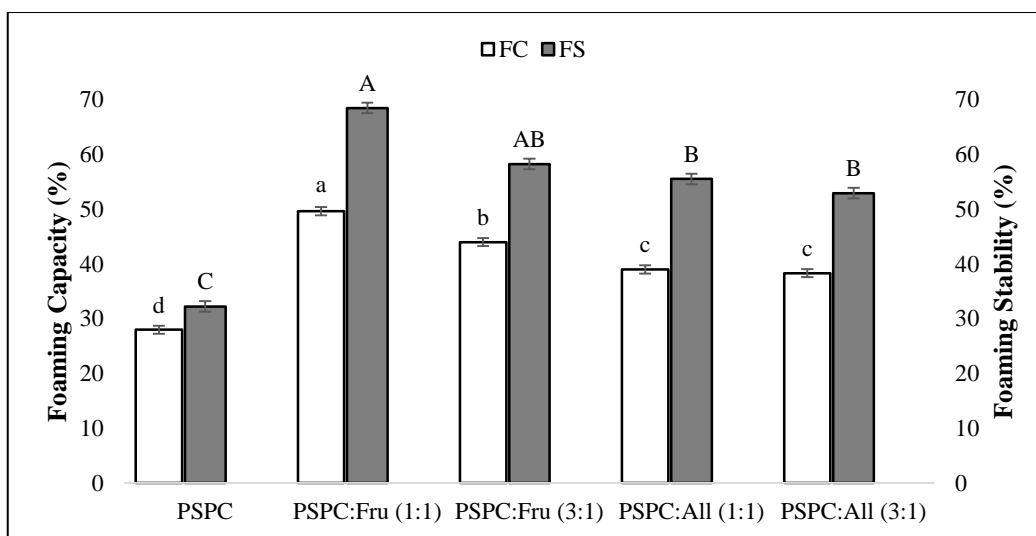


Figure 3.8. Foaming Capacity (%) and Foaming Stability (%) of Pumpkin seed protein concentrate (PSPC) and its conjugates.

The results showed that both functionalities were improved after PSPC was reacted and formed conjugates with sugars ($p < 0.05$). The same approach as in emulsifying properties can explain the phenomenon. The glycation reaction leads to the formation of new compounds and introduces changes in the protein's structure, impacting its hydrophilic and hydrophobic properties (Q. Yang et al., 2022). The glycated conjugates exhibit more complex molecular structures, including cross-linkages, and it has been shown that these substances may develop the overall stability of the foam (Wu et al., 2013). Furthermore, improved alignment of proteins at the air-water interface and increased protein flexibility because of heat treatment during the glycation process may contribute to the capacity of proteins to form stable foams (Nikbakht Nasrabadi et al., 2021).

In Figure 3.8, protein:sugar ratio had a significant impact on the foaming properties of the fructose-conjugated samples ($p < 0.05$), while it was insignificant in the allulose-conjugated samples ($p > 0.05$). This could be due to the different interaction mechanisms between allulose and the protein compared to fructose, suggesting that while fructose might form a more flexible conjugate at an optimal ratio, the interaction of allulose with the protein could be more stable across a wide range of ratios. The study conducted by Ter Haar et al. (2011) discussed the effect of different

sugars on the FS of glycated conjugates, and they suggested that the sugar type is an important parameter playing a role in the foaming properties. Peng et al. (2024) extensively studied the effect of the glycation process on foaming properties, and it was shown that FC and FS of *Trichosanthes kirilowii* seed protein isolate (TPI) were significantly enhanced after the reaction. FC improvement was linked to increased surface activity, faster air/water interfacial adsorption, and thicker interfacial films. Furthermore, the study explained that the FS improved due to the particle size of the bubbles in the TPI-glucose conjugate foams being reduced, resulting in smaller, more uniform, and denser foams, further contributing to their stability. In this study, the glycated PSPC conjugates exhibited notably enhanced FC and FS, indicating their promising potential for broad applications in various food formulations.

3.3 Utilization of Conjugated PSPC Proteins in Tomato-Based Sauces

3.3.1 Moisture Content, Water Activity, pH, °Brix, and Color Determination

The physical properties, such as water activity, moisture content, pH, °Brix, and color, are important to determine, especially in reformulated food products (Gabriel, 2008), and the results are given in Table 3.11 and Table 3.12.

Table 3.11 °Brix, pH, and Color (L*, a*, b*) properties of tomato sauces

Sauce Samples	°Brix	pH	L*	a*	b*
TS-C	9.03±0.07 ^c	4.07±0.007 ^c	38.61±0.05 ^b	19.91±0.08 ^a	15.53±0.05 ^c
TS-U	9.77±0.07 ^b	4.12±0.01 ^b	39.37±0.07 ^a	19.73±0.03 ^{ab}	17.87±0.05 ^a
TS-F	10.46±0.02 ^a	4.16±0.005 ^a	39.57±0.07 ^a	19.53±0.03 ^{bc}	17.93±0.05 ^a
TS-A	10.53±0.03 ^a	4.15±0.01 ^{ab}	38.73±0.03 ^b	19.27±0.05 ^c	16.80±0.04 ^b

Different letters in each column represent significant differences (p<0.05).

Table 3.12 Water Activity and Moisture Content of tomato sauces

Sauce Samples	Water Activity	Moisture Content
TS-C	0.92±0.003 ^a	91.87±0.05 ^a
TS-U	0.88±0.003 ^b	88.17±0.04 ^b
TS-F	0.86±0.003 ^c	87.64±0.11 ^c
TS-A	0.87±0.003 ^{bc}	87.96±0.05 ^b

Different letters in each column represent significant differences ($p < 0.05$).

According to the results of Table 3.12, the moisture contents showed significant differences, with TS-C having the highest content, followed by TS-U, TS-A, and T-F ($p < 0.05$). Furthermore, the water activity results showed the same trend with TS-C having the highest value ($p < 0.05$). Determining moisture content and water activity values is crucial for tomato sauces in terms of quality, consistency, and shelf life (Mohos, 2017). The higher moisture content generally results in a thinner sauce (Bal et al., 2024). Between the samples, the highest value of the TS-C sample was expected since in the other formulations, the addition of extra PSPC proteins, whether unmodified or modified, resulted in more concentrated products. Between the protein-added samples, modifying PSPC with sugars (conjugated PSPCs) resulted in having lower moisture content, which may be attributed to more protein-water interaction of conjugated proteins, which was also stated in the previous studies (Ertugrul et al., 2021; Tas, Ertugrul, Oztop, & Mazi, 2021).

The °Brix value is a measure of soluble solid content in a substance (Jaywant et al., 2022). According to the results, TS-C had the lowest value and was significantly different from TS-F and TS-A, which had the highest values ($p < 0.05$). The differences in the samples were again due to the added PSPC proteins. Besides, in the conjugated samples, the remaining sugars after modifying the protein might be the reason for having the highest °Brix values. It is known that a higher °Brix value typically correlates with sugar content or sweeter tastes (Trumbo et al., 2021). Thus, the obtained results comply with the information obtained from the literature.

The pH values of the samples ranged from 4.07±0.007 in TS-C to 4.16±0.005 in TS-F, with all samples showing a statistically significant difference ($p < 0.05$).

Determining the pH of the tomato sauces is crucial since it affects both the shelf life and the flavor (Tarlak, 2023). The addition of the proteins, with pH values around 7, increased pH slightly ($p < 0.05$). However, the results showed that the pH of all samples was still within the range of the acceptable tomato sauce samples, which can be supported by the information of USDA standards, in which tomato sauce must have a pH below 4.6 to prevent the growth of harmful bacteria (Fda, n.d.).

For the color determination, TS-F and TS-U had the highest L^* value ($p < 0.05$), while TS-A was the lowest ($p < 0.05$), suggesting a darker sauce. The addition of PSPC, whether in the unmodified or conjugated form, seemed to affect the lightness of the sauce matrix. The lighter L^* values in TS-F and TS-U may show that the proteins are possibly interacting with the tomato matrix or pigments, which would result in reflecting the light differently. Moreover, by stabilizing the color components of inhibiting oxidation (Feng et al., 2022), the glycation process in TS-F might affect the lightness of the resulting sauce.

For the a^* values, TS-C had the highest value, indicating a more intense red color, while TS-A had the lowest value, indicating a less intense red color ($p < 0.05$). Since there was no additional protein that might lower the red color intensity, the highest level in the TS-C samples was expected ($p < 0.05$). Besides, the minor decrease in red intensity in TS-F and TS-U samples could be attributed to either the protein-tomato pigment interaction or the dilution impact.

For the b^* values, TS-F had the highest value while TS-C had the lowest value ($p < 0.05$). Having a higher b^* value is attributed to a more yellow hue (Jung & Sato, 2013), which can be due to the modified protein through the Maillard browning reaction, in which the browning reaction occurs (Nursten, 2005). In that reaction, some compounds can enhance yellow hues, particularly when fructose is involved, due to its high reactivity compared to allulose (Tas, Ertugrul, Oztop, & Mazi, 2021). It was important to state the color properties of this study; the results indicated that the incorporation of PSPCs, specifically in glycated form (TS-F), resulted in a lighter and more yellow hue, while not significantly reducing the intensity of the red color.

3.3.2 Soluble Protein Content

Soluble protein content should be defined in protein-added food products since it influences nutritional value, texture, and consumer appeal (Grossmann & McClements, 2023). High protein solubility is often preferred because it results in smoother textures and enhanced sensory properties (Zia et al., 2021). Figure 3.9 represents protein solubility (%) for reformulated sauce products.

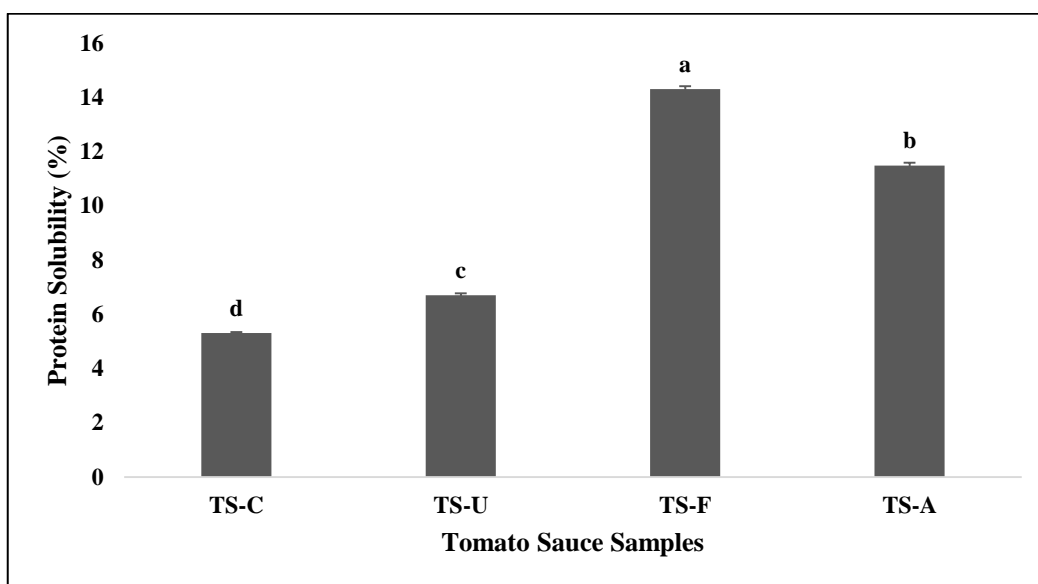


Figure 3.9. Soluble Protein Content (w/w, %) of tomato sauce samples

TS-F showed the highest protein solubility, followed by TS-A, TS-U, and TS-C ($p < 0.05$). TS-C has the lowest protein solubility ($p < 0.05$), indicating the potential benefits of fortifying tomato sauces with additional proteins, either modified or unmodified, to improve their nutritional and sensory properties. Protein solubility results in these reformulated tomato sauces revealing the significance of protein modification techniques, particularly glycation, on functional properties. As shown in previous studies, glycation enhanced the protein solubility of the samples and altered their behaviour through pH by altering the surface hydrophilicity and protein aggregation (Jiang et al., 2010; Martínez-González et al., 2017). Hence, comparing TS-C and TS-U, the higher solubility of TS-F and TS-A aligns with findings in the literature.

As also shown in pH results, the values were relatively close, with a slightly acidic range of typical tomato sauce. This pH range is near the isoelectric point (pI) of PSPC, where the solubility is generally the lowest (Shaw et al., 2001). However, the conjugated proteins obtained through glycation did not show reduced solubility near the pI. It was stated that glycation may prevent typical aggregation seen near pI by introducing additional hydrophilic groups from the reducing sugars, thereby increasing the protein's net charge and solubility (Ertugrul et al., 2021; Kramer et al., 2012). Hence, modified proteins through glycation reaction could be a suitable choice due to their increased solubility, especially in food samples where the pI of the protein is close to the pH of the product, which could otherwise cause solubility issues.

3.3.3 Lycopene Content

Lycopene is a carotenoid pigment mostly present in tomatoes, known for its strong antioxidant properties and other health advantages, such as lowered risk of heart disease and other chronic illnesses like cancer (Agarwal & Rao, 2000). Several variables, such as the processing conditions and the addition of other components, might affect the content of lycopene in tomato products (Akanbi & Oludemi, 2004). Figure 3.10 represents the lycopene content (mg lycopene/kg sample) of different sauce samples.

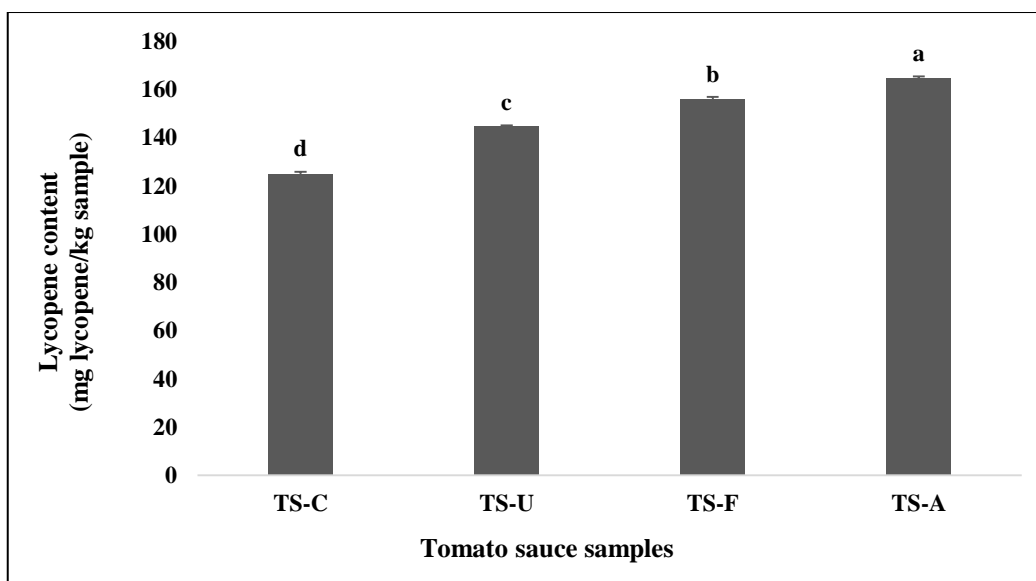


Figure 3.10. Lycopene Content (mg lycopene/kg sample) of tomato sauce samples

According to the results, the highest lycopene was obtained for the TS-A sample, followed by TS-F, TS-U, and TS-C, respectively ($p < 0.05$). The addition of PSPC, whether in unmodified or modified form, enhanced the lycopene content ($p < 0.05$), which may be attributed to the positive impact of lycopene stability. This could be due to the protein's ability to preserve lycopene compounds from oxidative degradation and improve the bioavailability of carotenoids. In the study of Liang et al., (2021), it was shown that the effect of high-pressure homogenization (HPH) in the presence of protein could help stabilize lycopene by forming an emulsion that protects it from oxidative degradation, which may contribute to having higher antioxidant activity and nutritional quality. Besides, the conjugated samples (TS-A and TS-F) showed even higher lycopene content ($p < 0.05$). Indeed, TS-A showed the highest value, and this outcome may be because of allulose as the reducing sugar in the reaction, which was shown to have high antioxidant activity in many studies (Kimoto-Nira et al., 2017; Xia et al., 2021; Xie et al., 2024). Overall, the obtained results revealed a synergistic effect between the lycopene and modified proteins through glycation, which can evaluate the nutritional quality of tomato sauces by preserving the bioactive compounds such as lycopene.

3.3.4 Scavenging Activity (SA), Total Phenolic Content (TPC), and Reducing Power

The SA by DPPH radical scavenging assay, TPC, and reducing power assay of different sauces were evaluated, and the results are given in Table 3.13.

Table 3.13 Scavenging Activity (SA) (%), Total Phenolic Content (TPC) (mg GAE/100 mL sample), and Reducing Power of tomato sauce samples

Sauce Samples	SA (%)	TPC (mg GAE/100mL)	Reducing Power (Absorbance at 700 nm)
TS-C	53.78±0.29 ^c	27.87±0.72 ^c	0.194±0.001 ^d
TS-U	55.09±0.29 ^c	31.42±0.71 ^b	0.307±0.002 ^c
TS-F	58.29±0.39 ^b	34.14±0.52 ^b	0.396±0.004 ^b
TS-A	62.33±0.22 ^a	39.70±0.57 ^a	0.416±0.003 ^a

Different letters in each column represent significant differences (p<0.05).

According to the results, the TS-C sample, which had no additional protein, exhibited the lowest SA (%) (p<0.05). With the addition of protein into the formulation, SA (%) was increased moderately as shown in TS-U samples (p<0.05). This is supported by previous studies showed that proteins have scavenging activities through mechanisms such as hydrogen atom donation and electron transfer (Y. Li et al., 2008). The most significant improvement of SA (%) was observed in conjugated samples (p<0.05). It was shown in many studies that glycation increases the scavenging activity of the obtained conjugates (Aminjafari et al., 2016; Jin et al., 2015). It was explained that introducing reducing sugars in the reaction enhanced the scavenging activity through the inherent properties of sugars and their role in forming conjugated products.

When the results were examined between the conjugates, it was seen that the highest SA (%) was observed in TS-A samples (p<0.05). It was shown that allulose can donate electrons or hydrogen atoms, stabilizing free radicals independently of the glycation process (Y. Sun et al., 2005). This is also supported by Xia et al. (2021)

reported that rare sugars like allulose enhance antioxidant activity more than control sugars like fructose through a combination of direct radical scavenging and the formation of glycated intermediates. The findings of TS-A having the higher SA (%) than TS-F may be attributed to allulose sugar having a more dual role as a direct radical scavenger, in contrast to fructose, which was generally shown to have greater glycation efficiency (Ertugrul, Namli, Tas, Kocadagli, et al., 2021; Tas, Ertugrul, Oztop, & Mazi, 2021).

For TPC values of the samples, like in SA (%) results, the TS-C showed the lowest value, followed by TS-U, TS-F, and TS-A, respectively ($p < 0.05$). With the addition of protein into the matrix, the phenolics can interact with protein and make bonds that contribute to the overall TPC (Schefer et al., 2021). Again, a significant improvement in TPC was observed in the conjugates ($p < 0.05$). It was revealed that in the glycation reaction, the bound phenolics can be released, and the formation of phenolic-conjugate products could be enhanced with more solubility and bioavailability (Gu et al., 2023; Sahraeian et al., 2024). Similar results were reported by C. Zhang et al., (2023) that glycated pea protein isolates showed significant improvements in antioxidant activity and phenolic content compared to non-glycated controls. In this study, a positive correlation between SA (%) and TPC results, with a positive correlation coefficient of $r = 0.940$, was found, which supports the glycation effect on both components. It was also revealed that by introducing reducing sugars (fructose and allulose) to the protein, glycation facilitates the formation of hydrophilic reaction products, improving the solubility, antioxidant activity, and phenolic content of the formulations.

Table 3.13 showed that the TS-A sample had the highest reducing power, followed by TS-F, TS-U, and TS-C, respectively ($p < 0.001$). Previous studies revealed that the reducing power was improved through glycation (Hu et al., 2023; Liu et al., 2022). In the study by Q. Zhang et al., (2022) glycated soy protein isolate demonstrated increased ferrous ion chelating capacity and higher antioxidant activity, which was linked to changes in amino acid composition and the presence of hydroxyl groups

due to the inclusion of reducing sugars during glycation. Our findings also suggest that glycation improved antioxidant functionality when integrated into the tomato sauce matrix, which was confirmed through SA, TPC, and reducing power results.

3.3.5 Flow Behavior

The flow behavior index, apparent viscosity, and yield stress are examples of rheological characteristics that are important in defining the texture, mouthfeel, and spreadability of food products (Fischer et al., 2009).

Table 3.14 Rheological properties of tomato sauce samples

Sauce Samples	Yield Stress (y_0, Pa)	Apparent Viscosity (k, Pa·s)	Flow Behavior Index (n)
TS-C	7.44±0.13 ^c	0.038±0.003 ^d	0.919±0.002 ^a
TS-U	12.40±0.11 ^b	0.104±0.002 ^c	0.758±0.003 ^b
TS-F	17.48±0.12 ^a	0.166±0.003 ^a	0.619±0.005 ^d
TS-A	16.68±0.24 ^a	0.118±0.003 ^b	0.684±0.004 ^c

Different letters in each column represent significant differences ($p < 0.05$).

The Herschel–Bulkley model was used to describe the rheological properties of tomato sauce products by referring to the literature studies (Erdem et al., 2024; Koocheki et al., 2009).

According to results from Table 3.14, the TS-C sample, with no added proteins, resulted in the lowest yield stress, indicating a relatively loose or weak structure, followed by TS-U, TS-A, and TS-F, respectively ($p < 0.05$). This result aligns with the literature, in which tomato matrices with fewer compounds inside tend to exhibit lower yield stress due to limited structural interactions (Sinkora et al., 2024). The reason for TS-F with the highest yield stress could be attributed to improved protein solubility, as also shown in solubility results ($p < 0.05$). With the conjugated protein in the matrix, a more coherent network structure and stronger interactions due to more soluble proteins may be obtained throughout the sauce. Because of the stronger

network, it could require more force to start the flow, which increases the yield stress (A. Sun & Gunasekaran, 2009).

For the apparent viscosity (k) results, which indicate a sample's resistance to flow under steady shear, it was observed that protein addition influenced the viscosity significantly ($p < 0.05$). Samples with higher protein solubility, such as TS-F and TS-A, exhibited higher k values ($p < 0.05$). This can be explained by the easier dispersion of the more soluble proteins throughout the sauce. The more effective dispersion likely strengthens the internal structure of the sauces, leading to greater resistance to flow and, hence, higher apparent viscosity (Farshi et al., 2024b). TS-C had the lowest viscosity value ($p < 0.05$), consistent with its low protein content and structural limitations.

According to the results, all sauces exhibited shear-thinning behaviour, with a flow behaviour index (n) below 1 ($p < 0.05$). The TS-C sample showed a higher flow behavior index closer to 1 (0.919 ± 0.002), suggesting a near Bingham or weakly shear-thinning behaviour (Hollister et al., 2023). This outcome can be linked to the lack of protein structure, leading to minimal shear-thinning, which aligns with typical behaviour in sauces with no added thickeners (Bal et al., 2024). On the other hand, the TS-F sample exhibited strong shear-thinning behaviour, which could make it an ideal candidate for food applications where a thicker and more stable sauce consistency is desired.

3.3.6 In Vitro Protein Digestibility

The protein digestibility in tomato sauces for TS-U, TS-F, and TS-A samples was evaluated via the OPA assay. Furthermore, the proteins (conjugated and non-reacted) that were not included in the sauces were examined (U, F, and A) and given in Figure 3.11.

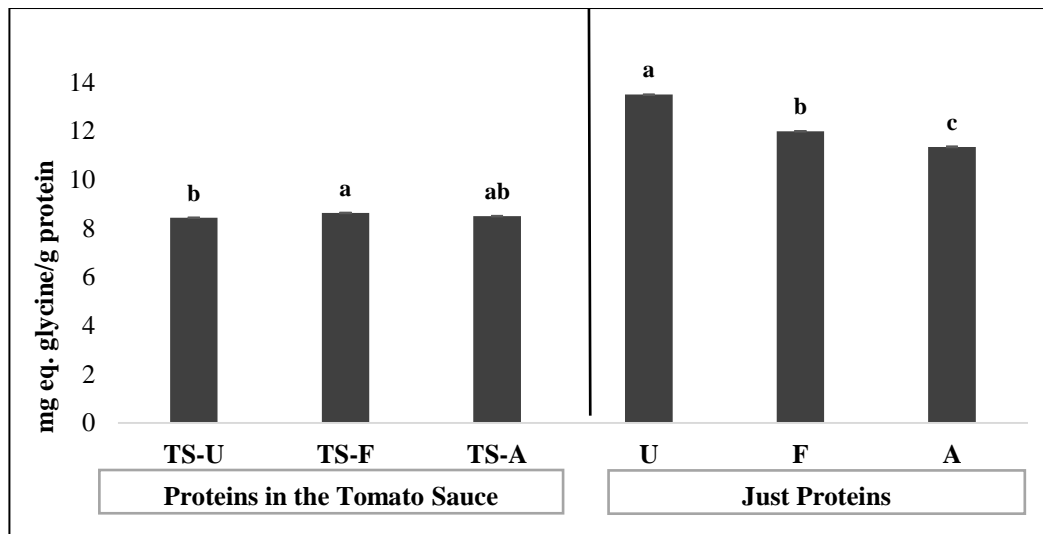


Figure 3.11. In Vitro Protein Digestibility of just proteins and proteins in tomato sauces

According to the results, Unmodified PSPC (U) displayed the highest digestibility among all samples ($p < 0.05$). This is consistent with findings in the literature, where native proteins generally exhibit better accessibility to digestive enzymes (Kaur et al., 2022). Moreover, studies are showing that glycation decreases protein digestibility (Jansen et al., 2023; Ren et al., 2025).

This can be due to the consumption of the reactive amino acids in the glycation reaction, such as lysine, arginine, and cysteine (J. Liu et al., 2022).

When PSPC samples were incorporated into tomato sauces (TS), the digestibility values significantly decreased for all samples compared to their native forms ($p < 0.05$). Tomato sauce is a complex food containing various ingredients that interact with proteins, including fibers, polyphenols, and organic acids (Ali et al., 2021; Szabo et al., 2022). In the organic acid aspect, citric and malic acids are found inside the tomato sauce (Kaya et al., 2024), which may destabilize the protein structure to making it unstable, which can result in partial unfolding and aggregation during the production stages of the sauces. The aggregated structures are more resistant to enzymatic hydrolysis, resulting in reduced digestibility (J. Zhang et al., 2023). Furthermore, polyphenols in tomato sauce are also known to form non-

covalent complexes with proteins, limiting enzyme accessibility (J. Zhang et al., 2023). When the statistical results were evaluated between sauces, TS-F exhibited the highest digestibility, followed by TS-A and TS-U, respectively ($p < 0.05$). This phenomenon can be explained by the solubility effect. As shown also in solubility results and the literature, the solubility is enhanced in glycosylated conjugates (Kutzli et al., 2021). With more homogenized protein inside the sauce, it can be less affected by the processing stages. In this study, protein was incorporated into tomato sauce, involving processes like HPH and heating, significantly lowering digestibility for all samples compared to their native form ($p < 0.05$), likely due to matrix effects of tomato sauce and thermal aggregation. However, the TS-U sample, having the lowest digestibility among the TS-F and TS-A, may be linked to the effect of solubility in the sauces. This effect could be more significant compared to the other effects mentioned, which resulted in higher digestibility of glycosylated conjugates ($p < 0.05$). These findings underscore the importance of balancing protein modification strategies with their nutritional impacts, particularly in complex food systems. Although glycosylation has generally been reported to reduce digestibility (Q. Yang et al., 2022), if the conjugates remain soluble in the matrix they were put, glycosylation can enhance digestibility.

3.3.7 Sensory Evaluation

In the flavor profile analysis, sweetness, saltiness, sourness, fresh tomato taste, tomato skin taste, tomato juice taste, paste-like flavor, olive-like taste, astringency, off-taste, protein-related taste, and overall impression were evaluated and shown in Table 3.15 and Figure 3.12.

Table 3.15 Sensorial attributes of the tomato sauce samples

Attribute	TS-C	TS-U	TS-F	TS-A
Sweetness	2.00	2.16	1.83	1.66
Saltiness	2.83	2.66	3.00	3.00
Sourness	3.00	2.50	3.33	3.16
Fresh Tomato Taste	2.33	3.50	2.50	2.00
Tomato Skin Taste	1.83	2.00	3.00	2.50
Tomato Juice Taste	3.33	3.33	2.50	3.16
Paste-like Flavor	3.16	2.50	4.00	3.50
Olive-like Taste	1.16	1.00	2.00	2.66
Astringency	2.16	1.83	2.66	3.33
Off-taste	1.33	0.50	1.50	2.00
Protein-related Taste	0.00	0.50	1.83	1.83
Overall Impression	3.00	3.16	1.83	1.83

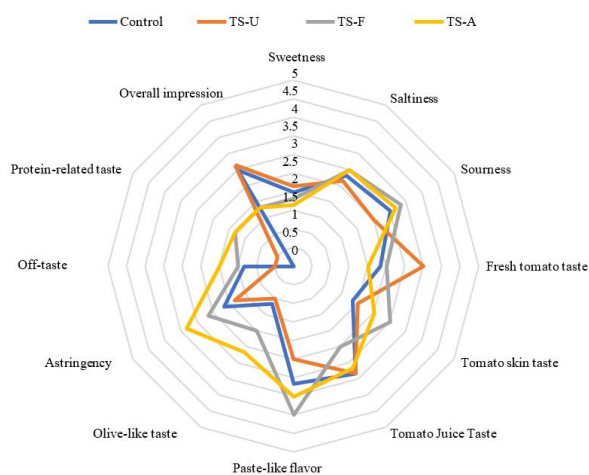


Figure 3.12. Spider Diagram of the tomato sauce samples

According to the sensory panel evaluation, in terms of astringency, off-taste, paste-like flavor, and protein-related flavors, the tomato sauces that included glycosylated conjugates (TS-F and TS-A) had higher scores compared to TS-U and control

samples. These outcomes can be attributed to the flavor development in conjugates that are exposed to the glycation reaction, which may give an intense cooked or bitter taste (S. Liu, Sun, et al., 2022). On the other hand, the TS-U sample showed improved sensorial properties, such as fresh tomato taste and saltiness, which can be linked to the enhancement of the umami and savory dimensions of the product due to the addition of extracted PSPC. These findings align with the previous reported studies showing that seed proteins, such as pumpkin seed protein, may complement savory food systems when used at moderate levels (Adebiyi & Aluko, 2011).

The overall impression results showed that the control sample and TS-U received the highest scores in terms of overall impression (3.00 and 3.16, respectively). This demonstrates that the addition of extracted PSPC did not disrupt the balance of sensory characteristics. Besides, TS-F and TS-A samples, which contained conjugated PSPC, showed notably lower scores (1.83 for both). This decline can be closely linked to the perception of off-taste, as well as astringency and protein-related taste intensities. It has been documented that the conjugates obtained through the Maillard reaction form new volatile compounds or alter the protein surface properties, such that the taste perceptions can be affected negatively in some food products (N. Kumar et al., 2013). For instance, higher reaction times or temperatures can lead to the formation of dark pigments and bitter tastes, which reduce consumer acceptance (Lund & Ray, 2017). In some studies, intense browning or burnt-like aromas have been associated with lower sensory scores compared to moderately reacted or control samples (Starowicz & Zieliński, 2019). Moreover, off-flavors and textural changes caused by volatiles because of the reaction may mask desirable points or create imbalances in flavor profiles (A. Sun et al., 2022). On the other hand, in some food products, it has been shown that Maillard reaction products enhance sensorial properties. In the study of Y. Zhang et al., (2019), the chicken protein hydrolysates were subjected to the Maillard reaction, and it was shown that such as boiled chicken aroma and overall acceptability were increased (Lee et al., 2019). Similarly, in the study of Du et al., (2024), Maillard reaction products demonstrated improved sensory properties by masking undesirable fishy odors and offering a more

pleasant flavor profile than the untreated samples. Like pumpkin seed, another plant-based protein, sunflower protein hydrolysates, were studied through the Maillard reaction with different sugar types, and it was revealed that there were noticeable improvements in taste complexity and browning intensity, resulting in higher sensory scores relative to the control samples (Karangwa et al., 2017).

3.4 TD-NMR Evaluations

3.4.1 Hydration Behavior of Extracted PSPC Samples via T₂ Relaxation Times

Water sorption or hydration is one of the poorly known functional properties since it is difficult to analyze chemical and structural alterations during the process (Fan & Roos, 2017). TD-NMR relaxometry via T₂ relaxation times was used in this study as an alternative way to examine the hydration behavior of the samples. T₂ times supply information regarding the dynamics of water (mobile/immobile or bound/free) in a food system (Kirtil et al., 2017; Ozel et al., 2017b). If it needs to be interpreted, longer T₂ times are associated with more free water in the system. In the case of PSPC samples, we can evaluate the results as if we had more soluble protein in the solution, we would have shorter T₂ times due to protein-water interactions.

Table 3.16 T₂ Relaxation Times of Pumpkin Seed Protein Concentrate (PSPC) samples

Treatments	Extraction Techniques	T ₂ (ms)
UT		127.94±0.774 ^f
CH	Alkali	229.81±1.21 ^d
MH		113.02±1.01 ^g
UT		355.01±2.24 ^a
CH	Salt	319.54±2.87 ^b
MH		274.89±1.91 ^c
UT		164.09±1.19 ^e
CH	Enzyme-Assisted	228.61±4.03 ^d
MH		125.22±2.78 ^{fg}

Different letters represent significant differences (p<0.05).

According to Table 3.16, the T₂ relaxation times of the extracts were found to be significantly different (p<0.05). Moreover, there is a strong negative correlation between the WSI (Section 3.1.4) and T₂ relaxation times, with a correlation coefficient of -0.780 (p<0.05). This is expected since WSI is related to more soluble contents, which would result in less free water in the solution, and thus, shorter T₂ relaxation times. For instance, in the results, the highest WSI value was seen for MH-AE samples (p<0.05). When T₂ relaxation times were looked for MH-AE samples, it was observed that the T₂ times were the shortest (p<0.05). The same negative correlation was also seen for the other results between WSI and T₂ relaxation times. Therefore, it was deduced that TD-NMR relaxometry can be effectively utilized to investigate the extracted protein-water interactions.

3.4.2 Hydration Behavior of Conjugated PSPC Samples via T₂ Relaxation Times

During the glycation reaction, it is expected that protein-water interactions would be changed. Thus, it was thought that T₂ relaxation times obtained through TD-NMR relaxometry can be a good tool to explore the changes that occur as a result of the glycation reaction. The measured values are given in Table 3.17. In this table, in addition to PSPC conjugates, samples that had not been exposed to any glycation (named Control) were evaluated and given. These samples were simply the mixture of the selected ratios of PSPC powders and sugars utilized in the study. This approach enabled a clearer understanding of hydration behavior by examining the effect of the glycation reaction.

Table 3.17 T₂ Relaxation Times of Pumpkin Seed Protein Concentrate (PSPC) and its conjugates

Sample Name	PSPC:Sugar	T ₂ Results (ms)	T ₂ Results (ms)
	Ratio	(Conjugates)	(Control)
PSPC-Fru	1:1	427.03±4.19 ^a	223.65±3.66 ^f
	3:1	351.07±1.02 ^b	258.39±2.89 ^{de}
PSPC-All	1:1	326.07±2.58 ^c	244.16±3.83 ^e
	3:1	306.44±3.64 ^c	271.54±4.22 ^d

Upper case superscript letters (a-f) denote a significant difference at 5% (p<0.05).

According to the results, among the comparisons made for conjugates and control samples, it was seen that the shortest T₂ values were obtained in control samples (p<0.05). This can be explained by the sugar-water interaction in the control samples, where the sugars in their native form readily bind to water (Gharsallaoui et al., 2008), resulting in shorter T₂ values. Furthermore, when comparing the control samples, the 1:1 protein:sugar ratio showed shorter T₂ values than the 3:1 ratio (p<0.05). This may be attributed to a higher likelihood of sugar-water interactions in the 1:1 ratio due to less molecular crowding, allowing for more effective binding. In contrast,

when the comparison was made between the conjugated samples, the PSPC:Fru (1:1) conjugate displayed the longest T_2 values, which were shown to have more glycation, confirmed by the other experiments (Lowry, FAG, and FTIR analysis). The formed complex may not be good at trapping the water, which may be due to reduced protein-protein aggregation, thereby fewer compact structures (Cardoso et al., 2019). Compact structures tend to tightly bind water, leading to more mobile water molecules in the system. Glycated conjugates decrease water entrapment in the matrix, and TD-NMR is a useful tool to explore this behavior.

3.4.3 Evaluation of Tomato Sauce Samples via T_2 Relaxation Times

T_2 relaxation times were measured to determine water mobility in the TS matrix, providing insights into protein interactions in the complex food matrix (Zia et al., 2021). Table 3.18 shows the monoexponential T_2 relaxation time (T_2) and biexponential components (T_{21} and T_{22}). In biexponential components, T_{21} mostly explains solid-water interactions (tightly bonded), whereas T_{22} better explains bulk water mobility (Bal et al., 2024).

Table 3.18 T_2 Relaxation Times (ms) of tomato sauces with mono and biexponential fittings

Sauce Samples	Monoexponential	Biexponential	
	T_2 (ms)	T_{21} (ms)	T_{22} (ms)
TS-C	343.10±0.19 ^a	155.90±2.08 ^a	397.17±1.16 ^a
TS-U	211.94±0.13 ^c	108.47±2.04 ^d	250.83±1.30 ^d
TS-F	216.72±0.34 ^b	121.03±0.60 ^c	261.47±0.51 ^c
TS-A	212.24±0.17 ^c	137.67±0.43 ^b	269.80±0.53 ^b

Different letters in each column represent significant differences ($p < 0.05$).

According to the general results, TS-C showed the longest T_2 values ($p < 0.05$), indicating high water mobility and minimum interaction between water and the matrix. Besides, it was seen that adding unmodified PSPC (TS-U) significantly

reduced T_2 values ($p < 0.05$), suggesting stronger water-protein interactions and reduced water mobility. This is consistent with the protein's ability to create a more rigid network by binding water through hydrophilic amino acid residues. The glycated conjugates showed intermediate T_2 values compared to TS-C and TS-U ($p < 0.05$). In the TS-U sample, the PSPC is in its free form, where it does not undergo glycation or any chemical bonding. This may allow the protein to exhibit a higher potential to entrap water molecules due to its globular molecular structure.

Tomato sauce is composed of various components that may contribute differently to the NMR signal. Thus, biexponential fitting is a more reasonable approach for comparison. Between the samples, both T_{21} and T_{22} had a similar trend ($p < 0.05$). Compared to monoexponential fitting, further discrimination was made between the TS-F and TS-A, in which TS-A had longer T_2 values ($p < 0.05$). The different sugars follow different pathways in the glycation reaction (Ertugrul et al., 2021). Fructose was shown to be more reactive and soluble in the solutions than allulose, which also confirms more glycation occurrence and water interactions (Tas, Ertugrul, Oztop, & Mazi, 2021) which was also confirmed inside the tomato sauce matrix in this study.

3.4.4 Evaluation of Different Sugars with Several TD-NMR Approaches

3.4.4.1 Effect of sugar concentration on the relaxivity of solutions

T_2 relaxation times are expressed in terms of relaxation rates; $1/T_2$ when explaining the changes for concentrations (Counsell et al., 2003). Relaxivity is defined as the change of the relaxation rates of a solution as a function of concentration, and it has been shown to provide meaningful information regarding the hydration of a substance (Liao & Wu, 2017; Zairov et al., 2017). Also, relaxivity is directly proportional to the hydration number since an increase in hydration number causes an increase in relaxivity values (Engelsen & Pérez, 1997).

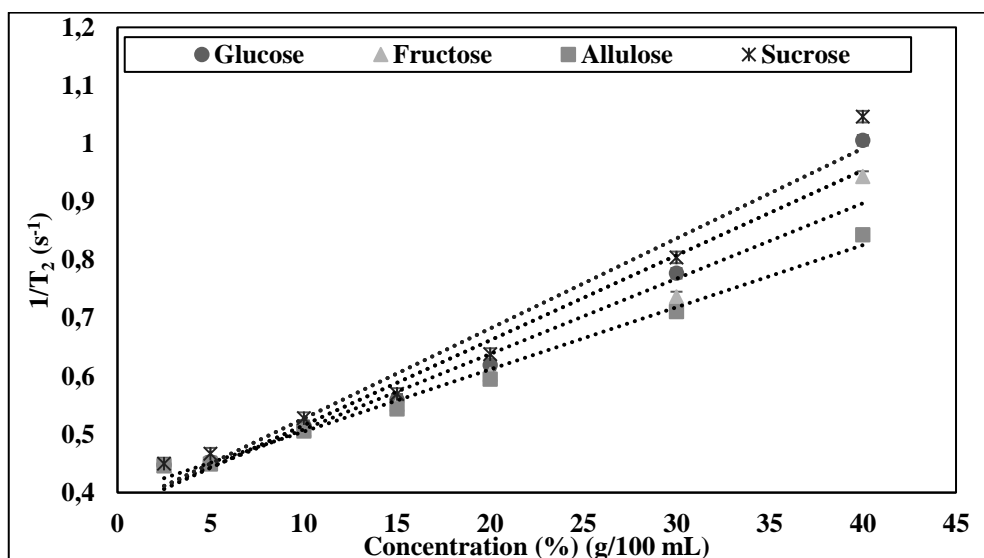


Figure 3.13. Relaxation Rates ($1/T_2$) (s^{-1}) of sugars at different concentrations

Table 3.19 Model Fitting for Relaxation Rates, $1/T_2$ (s^{-1}) for sugar concentration in the solution

Sugar Type	$1/T_2$ (s^{-1})	R^2
Glucose	$1/T_2 = 0.0146x + 0.370^b$	0.967
Fructose	$1/T_2 = 0.0130x + 0.379^c$	0.969
Allulose	$1/T_2 = 0.0107x + 0.398^d$	0.989
Sucrose	$1/T_2 = 0.0155x + 0.373^a$	0.968

Superscript letters (a-d) denote significant difference at 5% ($p < 0.05$).

In this study, the relation between relaxation rate and concentration was fitted to a linear model (Figure 3.13), and the equations of the models were shown in Table 3.19. Relaxivity values were obtained as the slopes of the relaxation rates ($1/T_2$), and they showed significant differences with the values of 0.0146, 0.0130, 0.0107, and 0.0155 for glucose, fructose, allulose, and sucrose, respectively (100 mL/g-s). According to the results, sucrose had the highest relaxivity value, followed by glucose, fructose, and allulose, respectively ($p < 0.05$). Besides, this trend was obtained for all concentrations from 2.5% to 40% ($p < 0.05$). Also, for all sugar types, as the concentration increased, relaxivity increased due to more sugar-water interaction, in which the free water in the system decreased as expected. The

outcome of these results can be interpreted as the sugars showed the same sensitivity to the change in concentrations. Apart from that, sucrose showed the highest interaction with water, namely, it was more hydrated than the other sugars. In some of the studies, the hydration of sucrose was evaluated by considering the molecular dynamics, such as the hydration number. It was shown that sucrose had a relatively large hydrodynamic size due to hydroxylic and acetalic oxygens in the structure, which led to a high tendency to make a hydrogen bond with water (S. A. Shah et al., 2017; Starzak et al., 2000). Furthermore, in the study where glucose and fructose sugars were evaluated and compared in the case of hydration behavior, it was shown that glucose had more tendency to hydrate than fructose which was explained by the molecular ring size and the fractions of axial and equatorial -OH groups in the structures being different (Ikeda et al., 2014). Among the sugar types, the least hydrated sugar was allulose based on relaxivity values obtained ($p < 0.05$). In one study, the hydration behavior of allulose, also known as D-psicose, was determined and compared with other sugars by checking dielectric relaxation (Pocan & Oztop, 2021). The results were determined by using the average hydration number and water activity values. In conclusion of the study, both fructose and D-psicose were shown to interact with water similarly. However, our study results suggested a different behavior for allulose, having less affinity with water, which was also observed in other studies where allulose was used as a sugar substitute in confectionery gels (Kabayama et al., 1958).

Mutarotation also has a significant impact on the hydration behavior of sugars since in solutions, reducing sugars' exchange between their anomers may result in a different hydration behavior (Kabayama et al., 1958). In this study, all the sugars except sucrose (as being non-reducing) can mutarotate in the solution due to the presence of the reducing end. In the native forms, it was shown that D-glucose was found in the α -pyranose crystal form (Srisa-Nga & Flood, 2005) while D-fructose and D-allulose had only one crystal form that was β -pyranose (Fukada et al., 2010). When these sugars are hydrated, they interchange between their tautomers, and the occurrence of different tautomeric ratios significantly affects the hydration behavior

of sugars (Taylor et al., 2013). In aqueous solutions, D-glucose exists in α -pyranose (31.1–37.4%) and β -pyranose (64–67.9%) forms, while D-fructose is in α -pyranose (\approx 4%), β -pyranose (68.4–76.0%), and β -furanose (28.0–31.6%) forms at ambient temperature. Besides, for D-allulose at 27 °C, four different forms could be found, which are α -furanose (39%), β -furanose (15%), α -pyranose (22%), and β -pyranose (24%) (Guner et al., 2021). Studies confirmed that the β anomers showed higher numbers of water oxygens in the first hydration shell compared to the α anomers. This was explained by the improved water interactions of β -anomers having the anomeric oxygen in equatorial form (Fukada et al., 2010).

In this study, solutions of glucose, fructose, and allulose at ambient temperature were investigated, and it was observed that allulose, having more heterogeneous tautomers and less β -pyranose forms, had reduced binding ability towards water molecules compared to glucose and fructose. Besides, although two ketoses (fructose and allulose) are epimers of each other, the number of hydroxyl groups in the equatorial position is different in their β pyranose forms: two (C3 and C4) for fructose and one (C4) for allulose (Fukada et al., 2010). This fact may have also caused allulose to have the least interaction with water compared to other sugars.

3.4.4.2 Change in T_2 Relaxation Times with Time

Hydration is the process by which water penetrates and diffuses within solid molecules. Furthermore, the amount of water absorbed increases as the process time increases (Srisa-Nga & Flood, 2005). Thus, time is one of the most important variables for the hydration of molecules. In this study, the hydration rate of the sugars was observed by TD-NMR relaxometry for 1 day (Figure 3.14). Preliminary experiments showed that after 400 min, there were slight changes in the T_2 relaxation times, so hydration was nearly completed for all sugars by that time. Figure 3.14 displays two different regions (region I and region II) where linear model fittings were applied.

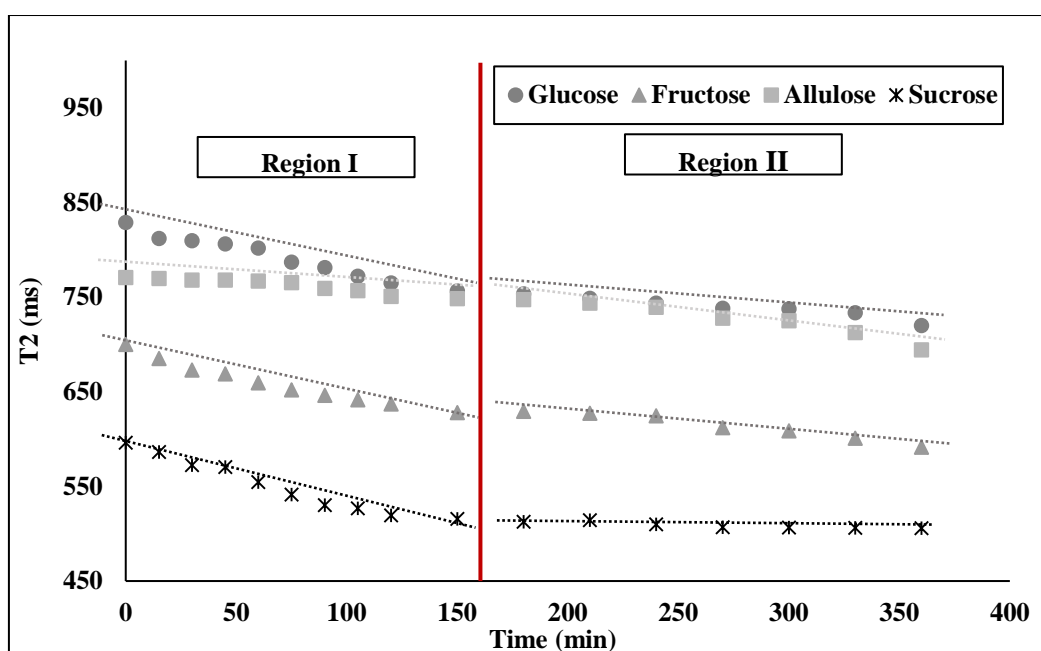


Figure 3.14. T_2 (ms) Relaxation Times of sugars obtained at different times

Table 3.20 Model Fitting of T_2 (ms) for Time Regions I and II

	Region I	R^2	Region II	R^2
Glucose	$T_2 = -0.478x + 824^b$	0.976	$T_2 = -0.163x + 783.341^c$	0.929
Fructose	$T_2 = -0.461x + 690^c$	0.959	$T_2 = -0.217x + 672.072^b$	0.963
Allulose	$T_2 = -0.162x + 773^d$	0.916	$T_2 = -0.280x + 802.703^a$	0.934
Sucrose	$T_2 = -0.587x + 591^a$	0.995	$T_2 = -0.048x + 521.911^d$	0.811

For each column, lower case superscript letters (a-d) denote significant difference at 5% ($p < 0.05$).

To compare the hydration rate of the sugars, the slopes of the curves were taken into consideration (Table 3.20). According to the ANOVA results, the highest hydration rate belonged to sucrose, followed by glucose, fructose, and allulose for region I ($p < 0.05$). Furthermore, sucrose showed the lowest hydration rate in region II ($p < 0.05$). Thus, it can be stated that sucrose was the fastest in water binding since it absorbed the water in 150 min, and there was a little amount of water absorption after that time, as seen in region II. Apart from that, allulose was the only sugar that showed a higher hydration rate in region II, indicating hydration was still ongoing after 150 min. In most of the studies, the structural changes in the sugar molecules,

especially the position and the distance between the -OH groups within the structures, had huge impacts on the hydration behavior (Hishiike et al., 2013). In one study, the effect of intramolecular hydrogen bonds on sucrose hydration was studied, and it was found that sucrose showed rare intramolecular hydrogen bond formation, which indicates more mobility in the aqueous solution (Starzak et al., 2000). Considering the disaccharide nature of sucrose, water molecules might have been bound quickly. Furthermore, glucose was also shown to have higher hydration behavior due to its structural conformation, where -OH groups are positioned to have more water interactions (Fukada et al., 2010). That's why sucrose and glucose could have hydrated quickly than fructose and allulose. Also, the accessibility to -OH groups in ketoses could be harder than the aldoses (Montanuci et al., 2015). Thus, for our ketose sugars (fructose and allulose), the time for complete hydration was longer than the aldoses.

3.4.4.3 Self-Diffusion of Water in Different Sugar Solutions

NMR can be used to calculate the self-diffusion coefficients of water in the range of between 10^{-6} and 10^{-14} m^2s^{-1} , in which the higher coefficient values indicate viscous liquids (Uedaira & Uedaira, 1985). In this study, the self-diffusion coefficient of water in the sugar solutions was found to be significantly different ($p < 0.05$).

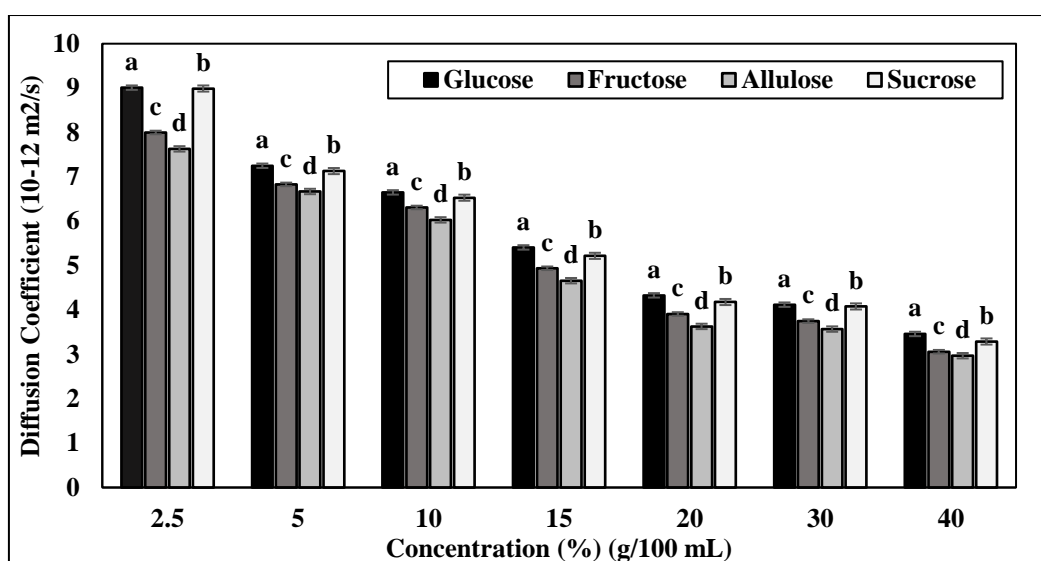


Figure 3.15. Self-diffusion Coefficient of water in sugar solutions at different concentrations (%) (g/100 ml)

By looking at Figure 3.15, the highest self-diffusion coefficient values belonged to glucose, followed by sucrose, fructose, and allulose at ambient temperature (298 K) ($p < 0.05$). In addition, this trend was the same for all concentration values, indicating that increasing concentration did not affect the change in the self-diffusion of water in the sugar solutions. Determining the self-diffusion coefficients based on different sugar solutions was studied mostly in the literature; however, they were mainly for sucrose and glucose (Dashnau et al., 2005). The findings showed that compared to solutions of sucrose, solutions including glucose had a slightly higher self-diffusion coefficient. The reason behind this was explained by the fact that the molecular weight of those two sugars is different. Indeed, sucrose is a disaccharide and may increase the viscosity of a solution, so it can decrease the mobility of water in the solution more compared to glucose, which is a monosaccharide (Dashnau et al., 2005). Nevertheless, results obtained by the NMR showed that the self-diffusion coefficients of water in sucrose and glucose were so close to each other. Apart from that, there were not many studies in literature regarding the self-diffusion coefficient of water in allulose. Our findings concluded that allulose solutions had the lowest self-diffusion coefficient value, although allulose solutions had longer T_2 values at all concentrations compared to other sugars. This finding confirmed that allulose is

likely to form a network in the solution and create a tortuous path for the water molecules to move and restrict the diffusion. Moreover, as also stated in Section 3.4.4.1, allulose had various types of tautomer forms in its solutions at ambient temperature, which might have resulted in a lower self-diffusion coefficient. This can be explained by the fact that in the equilibrium state of the solution, different tautomers might have competed during diffusion; thus, the movement of the water inside the allulose may have been retarded.

3.4.4.4 Crystallinity of Sugars by MSE Sequence

In this study, the Magic Sandwich Echo (MSE) sequence was used to obtain the crystallinity values of the sugars before and after hydration. Sugars were mixed with water at the ratio of 3/1 of their weight and equilibrated for 1 day, so surface water was allowed to form on the sugar crystals. A parameter named as second moment (M_2) is correlated with crystallinity values, and it was calculated as explained in Section 2.4.4.3, and the results were provided in Table 3.21.

Table 3.21 Second Moment (M_2) values by MSE Sequence and Relative Total Crystallinity (%) by XRD of the sugars

Sugar Type	M_2 (10^{-8} Tesla ²) (Solid)	M_2 (10^{-8} Tesla ²) (Hydrated)	Relative Total Crystallinity (%)
Glucose	16.11±0.04 ^c	13.64±0.03 ^c	76.72±0.14 ^c
Fructose	15.18±0.03 ^d	14.24±0.02 ^b	75.73±0.16 ^d
Allulose	16.39±0.04 ^b	14.41±0.04 ^a	79.71±0.11 ^b
Sucrose	16.47±0.06 ^a	13.15±0.02 ^d	81.30±0.21 ^a

For each column, lower-case superscript letters (a-d) denote significant difference at 5% ($p < 0.05$).

When Table 3.21 was examined, after water was added to sugars and waited for 1 day, crystallinity values decreased for all sugars as sugar-water interaction occurred ($p < 0.05$). Non-hydrated (solid) sucrose initially had the highest crystallinity among the other sugars ($p < 0.05$). This was consistent with the fact that sucrose is more crystal in solid form than the other sugars like glucose and fructose (Shin et al.,

2011). Also, the crystallinity of glucose was found to be higher than fructose in the solid form ($p < 0.05$).

To support the results obtained from the MSE sequence, X-ray Diffraction (XRD) analysis was also conducted on the solid forms of the sugars. Relative total crystallinity (%) of sugars was calculated and provided in Table 3.21. According to the results, the highest crystallinity was observed again in sucrose, followed by allulose, glucose, and fructose, respectively ($p < 0.05$). When the results for solid forms of sugars obtained by both the MSE sequence and XRD analysis were statistically compared, the correlation was found to be significant with a value of $+0.828$ ($p < 0.05$). Therefore, these results suggested that these experiments complied well with each other.

When the hydrated results were analyzed, it was seen that the highest crystallinity belonged to allulose, followed by fructose, glucose, and sucrose, respectively ($p < 0.05$). Allulose, having the highest crystallinity following water addition, was an indication that it had the lowest hydration since it did not lose its crystal form as much as the other sugars. In addition, sucrose had the lowest crystallinity value among other sugars in the hydrated form, hence, had the highest sugar-water interaction ($p < 0.05$). In one of the studies, glucose, lactose, and sucrose sugars were processed in freeze and spray dryers to obtain more hygroscopic and amorphous sugars (L. Grunin et al., 2019). According to the crystallinity results, which were measured by MSE sequences, sucrose had the lowest crystallinity values than glucose and lactose in the amorphous state, which confirmed our findings that after the water addition, the lowest crystallinity belonged to sucrose since it bound more water than the other sugars.

3.4.4.5 Spin Diffusion Analysis

In NMR studies, a gradient of magnetization with different mobility between phases is observed by spin diffusion. Spin diffusion measurement can be performed to

characterize the interface layer thickness between amorphous and rigid crystalline phases (L. Y. Grunin et al., 2017; Ziirich, 1985). In spin diffusion experiments, the polarization transfer is observed by the long component reduction and simultaneous short component increase in the overall signal during the spin diffusion time (Schäler et al., 2015). In our case, the long component is water, and the short component is the solid portion of the sugars. The results obtained by the Goldman-Shen pulse sequence are shown in Table 3.22.

Table 3.22 Interface layer thickness (nm) of the hydrated sugars calculated by Goldman-Shen pulse sequence

Sugar Type	Interface layer thickness (nm)
Glucose	52.61±0.38 ^d
Fructose	63.35±0.23 ^b
Allulose	66.85±0.24 ^a
Sucrose	54.15±0.17 ^c

Letters indicate significant differences (p<0.05).

The highest interface layer thickness was obtained in allulose samples, followed by fructose, sucrose, and glucose (p<0.05). The interface layer thickness is defined as the thickness of the layer that is observed between the bounded water and the crystalline. The idea here is that if thickness values are high, the interaction between water and sugar crystals becomes lower. Hence, sugar hydration would be lower as the thickness increases (Demé & Zemb, 2011). The findings of fructose and allulose having lower hydration behavior could also be seen due to having larger thickness values than sucrose and glucose (p<0.05). In the same way, sucrose and glucose, which were shown to have higher hydration behavior than the other two sugars, could also be deduced. As a result, spin diffusion, which is one of the non-conventional NMR approaches, was used for the 1st time to further examination of the hydration behavior of different sugars and provided meaningful information by considering the interface layer thickness, which plays an important role interaction of sugars with water.

CHAPTER 4

CONCLUSION

In the 1st part of the dissertation, an extensive analysis of the optimization of the extraction processes for maximizing the recovery of PSPC from its flour was conducted. The study investigated the effectiveness of three extraction techniques (alkali, salt, and enzyme-assisted alkali) coupled with two different pre-heat treatments (microwave and conventional heating) and compared them to untreated samples to enhance both yield and functional properties of PSPC. Key assessments included extraction yield, proximal analysis, protein solubility, water solubility index (WSI), TD-NMR Relaxometry, emulsification activity and stability, foaming capacity and stability, scavenging activity, and FTIR spectroscopy for structural analysis. Prioritizing a high yield of protein with substantial content, this part of the study revealed that microwave-heated alkali extraction (MH-AE) surpassed conventional and untreated methods in terms of both the protein yield and the functional properties. The conclusions obtained from the experiments were further supported by the FTIR and TD-NMR results. Overall, the MH-AE technique emerged as the most effective, attributed to an accelerated reaction rate under the brief microwave exposure, offering milder extraction conditions while ensuring an enhanced extraction efficiency of PSPC.

In the second part, the extracted PSPC with the most convenient method combination chosen as MH-AE was modified through the glycation reaction. The browning index, FAG amounts, RRS content, amino acid contents, FTIR spectroscopy, and various physicochemical properties have all been used to investigate the effect of different sugars (fructose and allulose) and protein:sugar ratios (1:1 and 3:1) on the modification of PSPC via glycation. The FAG and Browning index results confirmed glycation in all conjugates, and the negative correlation between the methods emphasized the reliability of both methods in confirming glycation. The RRS and

amino acid contents provided valuable insight into the occurrence and progress of the reaction, specifically showing the role and behavior of sugars and reactive amino acids within the reaction mechanism. In addition, FTIR spectroscopy gave detailed insights into conformational changes in proteins, indicating alterations in key functional groups and verifying glycation. This part of the study showed that glycation had a great impact on protein solubility, emulsifying activity (EA), emulsifying stability (ES), foaming capacity (FC), foaming stability (FS), and scavenging activity (SA). The extensive research presented in this part of the study revealed that the modification via glycation influenced the chemical and functional properties of PSPC. The findings showed the impact of glycation on related food products and may suggest potential uses in producing food components with improved nutritional and functional properties.

In the 3rd part, the modified conjugates were incorporated into the reformulated tomato sauce, which also included lyophilized olive powder and valorized tomato peel powder. Incorporating PSPC into reformulated tomato sauce formulations significantly improved functional and nutritional properties. Conjugated PSPC, glycated with reducing sugars, improved protein solubility, preserved lycopene, and influenced rheological behaviour. These modifications also contributed to lower moisture content, higher °Brix values, and acceptable pH levels, maintaining the sensory and microbial safety of the sauces. Due to enhanced protein-water interactions and glycation-induced solubility, tomato sauces with conjugates (TS-F and TS-A) enhanced SA (%) and TPC, which could serve as valuable dietary sources of antioxidants, offering health benefits. Protein digestibility analysis revealed that due to enhanced solubility in glycated samples, digestibility was improved in TS-F and TS-A more compared to TS-U. Overall, these findings emphasize that incorporating glycated PSPC into tomato sauce formulations may lead to the development of more functional, health-promoted, and value-added final products.

In the last part, TD-NMR relaxometry was employed as a powerful tool to explore water interactions, namely hydration behavior of extracted and conjugated PSPC samples. In addition, the molecular mobility of the tomato sauce samples was

analyzed. The results revealed the different interactions and effects of PSPC addition, whether in conjugated or non-reacted form, inside the complex matrix of tomato sauce. These analyses provided valuable information on how water interacts with different components at the molecular level, highlighting the potential of NMR relaxometry as an effective, non-destructive, and informative technique in food science research. Furthermore, the hydration behavior of 4 different sugars (glucose, fructose, allulose, and sucrose) was investigated with several approaches of TD-NMR relaxometry. Different concentrations (2.5%, 5%, 10%, 15%, 20%, 30%, and 40%) (w/vg/100 ml), and time dependency of sugars concerning T_2 relaxation times, self-diffusion coefficients of water, and crystallinity values by MSE, and Spin Diffusion by Goldman Shen sequence experiments were evaluated. The general findings showed that sucrose showed the highest interaction with water, followed by glucose, fructose, and allulose. Results of the study can provide important guidance for the studies that use allulose in different food formulations.

This dissertation highlights how combining extraction optimization, glycation-based modification, and insightful TD-NMR analysis can work together to enhance the functional profile of PSPC and support its use in real food systems such as tomato sauce. Overall, the findings offer a meaningful step forward in the creative and sustainable use of plant proteins in functional food design.

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APPENDICES

A. Calibration Curves

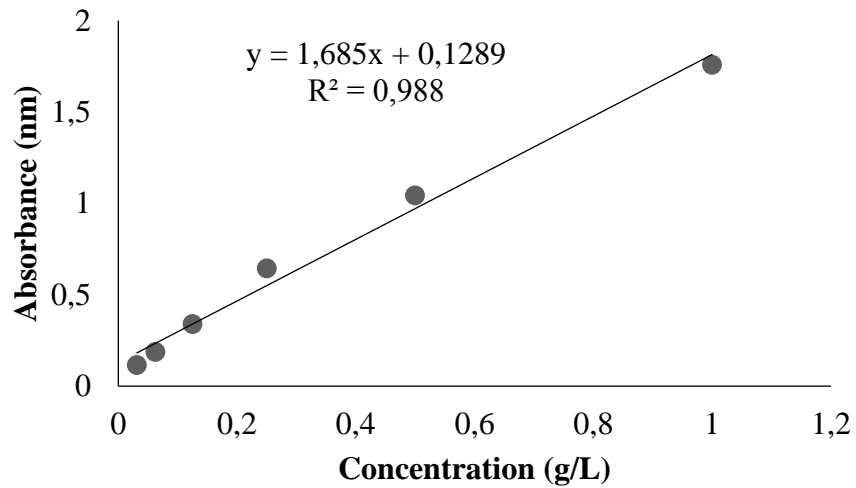


Figure A. 1. Calibration curve of the Lowry method by Bovine Serum Albumin (BSA) to measure soluble protein concentration

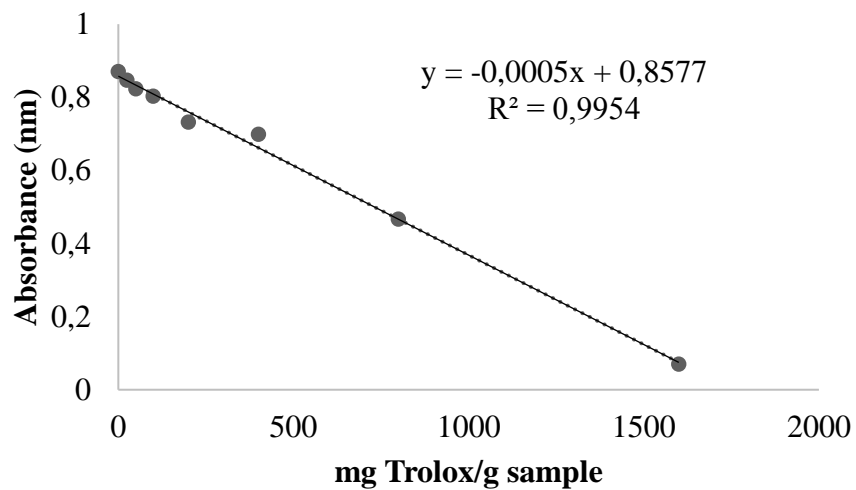


Figure A. 2. Calibration curve of the DPPH method prepared by Trolox to measure antioxidant activity

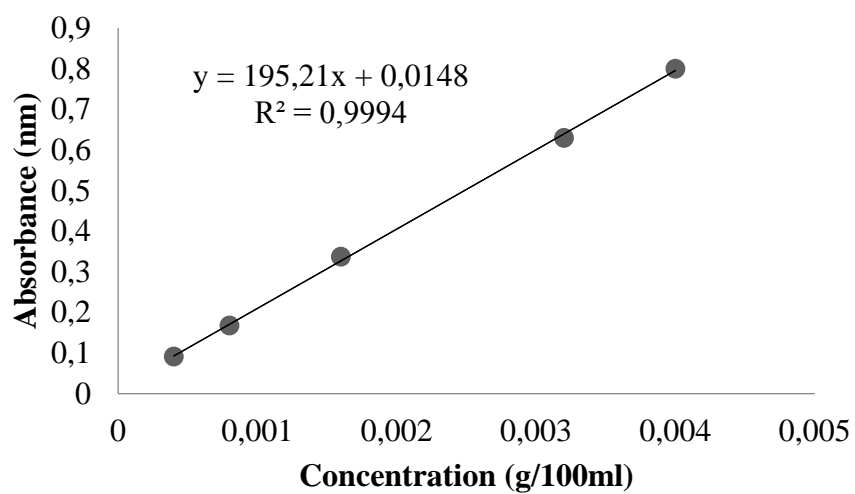


Figure A. 3. Calibration curve of the OPA method prepared by glycine to measure free amino groups

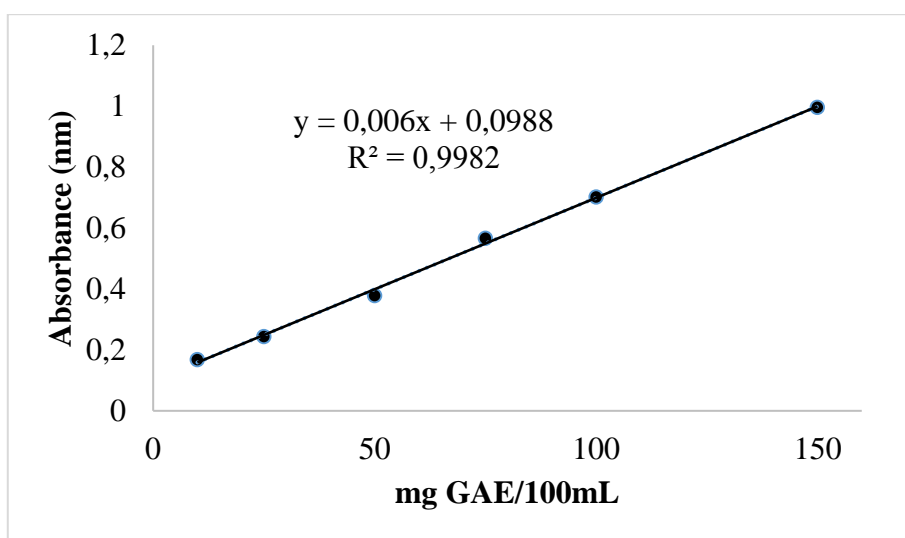


Figure A. 4. Calibration curve of Total Phenolic Content (TPC) prepared by gallic acid equivalent (GAE)

B. Supplementary Figures and Tables

Table B.1 Extraction yield (% (w/w)) of Pumpkin Seed Protein (PSP) samples obtained from the alkali method with different temperature-time combinations

Pre-heating Treatments	Temperatures (°C)	Time (min)	Yields (% w/w)
Water Bath	30 °C	12	31.3±0.14 ^d
	40 °C	13	35.6±0.22 ^c
	50 °C	15	54.4±0.13 ^a
	60 °C	17.5	39.7±0.16 ^b
Microwave	30 °C	0.5	38.3±0.14 ^d
	40 °C	0.7	47.7±0.15 ^c
	50 °C	0.85	66.7±0.23 ^a
	60 °C	1	51.4±0.31 ^b

Upper case superscript letters (a-d) denote a significant difference at 5% ($p < 0.05$) in each treatment separately.

Table B.2 Fourier-transform infrared spectroscopy (FTIR) spectra of control (purchased pumpkin seed flour) and salt extracted (SE) Pumpkin Seed Protein Concentrate (PSPC) samples.

Samples	α -Helix (%)	β -Sheet (%)	β -Turns (%)	Random Coil (%)
Control	36.26±0.42 ^a	37.46±0.63 ^d	17.27±0.12 ^c	8.99±0.02 ^c
UT	35.59±0.27 ^b	40.59±0.44 ^a	17.45±0.22 ^b	9.34±0.32 ^b
CH	34.45±0.37 ^c	38.45±0.56 ^c	18.17±0.35 ^a	8.91±0.25 ^d
MH	31.11±0.24 ^d	39.84±0.43 ^b	18.16±0.22 ^a	10.88±0.11 ^a

Control (Purchased Pumpkin Seed Flour), UT (Untreated samples), CH (Conventional heated), and MH (Microwave heated). Upper case superscript letters (a-d) denote a significant difference at 5% ($P < 0.05$) in the same column. Values are expressed as mean ± SE (n=3).

Table B.3 Fourier-transform infrared spectroscopy (FTIR) spectra of control (purchased pumpkin seed flour) and enzyme assisted-alkali extracted (EE) Pumpkin Seed Protein Concentrate (PSPC) samples.

Samples	α -Helix (%)	β -Sheet (%)	β -Turns (%)	Random Coil (%)
Control	36.26 \pm 0.42 ^a	37.46 \pm 0.63 ^d	17.27 \pm 0.12 ^b	8.99 \pm 0.02 ^d
UT	32.13 \pm 0.31 ^b	40.01 \pm 0.38 ^c	17.21 \pm 0.63 ^b	10.65 \pm 0.13 ^b
CH	31.83 \pm 0.33 ^c	41.11 \pm 0.79 ^b	17.95 \pm 0.23 ^a	9.12 \pm 0.24 ^c
MH	30.74 \pm 0.28 ^d	41.79 \pm 0.93 ^a	16.32 \pm 0.27 ^c	11.14 \pm 0.28 ^a

Control (Purchased Pumpkin Seed Flour), UT (Untreated samples), CH (Conventional heated), and MH (Microwave heated). Upper case superscript letters (a-d) denote a significant difference at 5% ($P < 0.05$) in the same column. Values are expressed as mean \pm SE (n=3).

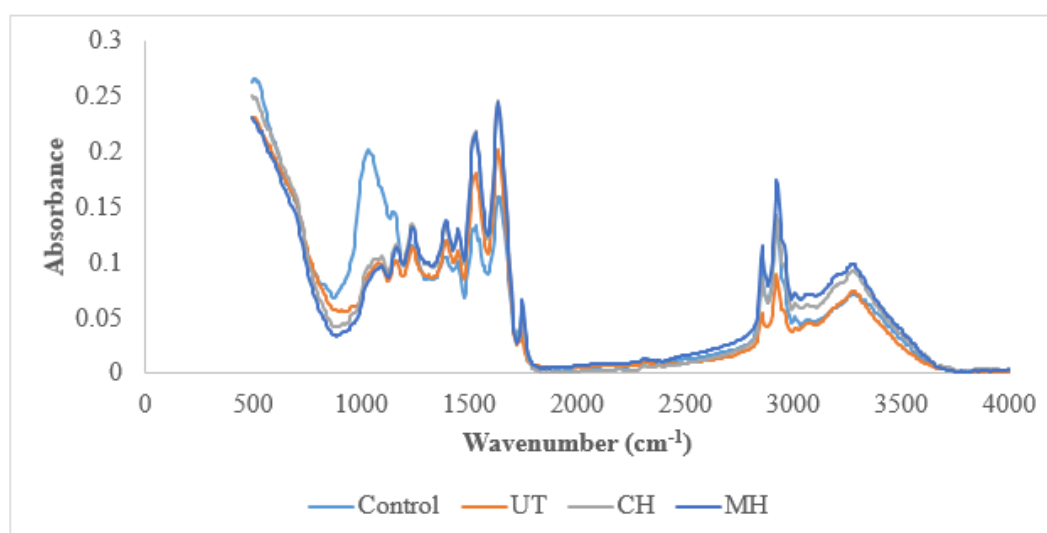


Figure B. 1. Fourier-transform infrared spectroscopy (FTIR) spectra of control and enzyme-assisted alkali extracted (EE) Pumpkin Seed Protein Concentrate (PSPC) samples.

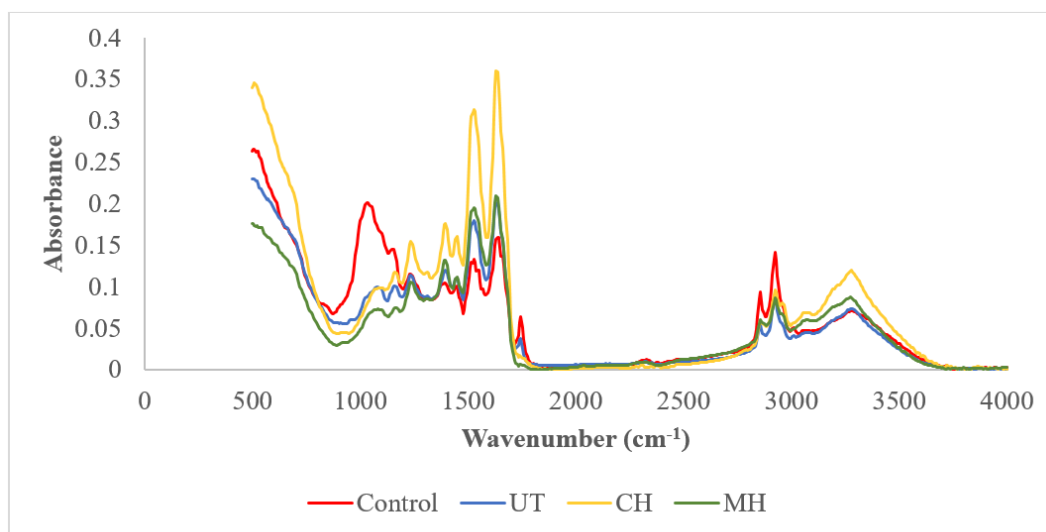


Figure B. 2. Fourier-transform infrared spectroscopy (FTIR) spectra of control and salt extracted (SE) Pumpkin Seed Protein Concentrate (PSPC) samples.

C. Statistical Analyses

1) Extraction of the Pumpkin Seed Protein Concentrate (PSPC) from its Flour with Different Approaches

Table C.1 ANOVA and Tukey's Comparison Test with 95% confidence level for extraction yield

General Linear Model: Extraction Yield Results versus Treatments, Extraction Technique

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Treatments	Fixed	3 CH, MH, UT
Extraction Technique	Fixed	3 Alkali, EnzymeAssisted, Salt

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatments	2	722.00	361.00	4182.19	0.000
Extraction Technique	2	4824.76	2412.38	27947.42	0.000
Treatments*Extraction Technique	4	228.01	57.00	660.37	0.000
Error	18	1.55	0.09		
Total	26	5776.32			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.293800	99.97%	99.96%	99.94%

Comparisons for Extraction Yield Results

Tukey Pairwise Comparisons: Treatments

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping
MH	9	36.0233	A
CH	9	33.0456	B
UT	9	23.8722	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Extraction Technique	N	Mean	Grouping
Alkali	9	46.5522	A
EnzymeAssisted	9	32.4778	B
Salt	9	13.9111	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatments*Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Treatments*Extraction			
Technique	N	Mean	Grouping
MH Alkali	3	55.2433	A
CH Alkali	3	50.3033	B
MH EnzymeAssisted	3	36.6267	C
CH EnzymeAssisted	3	34.5100	D
UT Alkali	3	34.1100	D
UT EnzymeAssisted	3	26.2967	E
MH Salt	3	16.2000	F
CH Salt	3	14.3233	G
UT Salt	3	11.2100	H

Means that do not share a letter are significantly different.

Table C.2 ANOVA and Tukey's Comparison Test with 95% confidence level for proximate composition (moisture, ash, fat, protein, and carbohydrate)

General Linear Model: Moisture Content Results versus Treatments, Extraction Technique

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Treatments	Fixed	3	CH, MH, UT
Extraction Technique	Fixed	3	Alkali, EnzymeAssisted, Salt

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatments	2	0.7891	0.394559	70.35	0.000
Extraction Technique	2	1.3528	0.676400	120.60	0.000
Treatments*Extraction Technique	4	2.1728	0.543201	96.85	0.000
Error	18	0.1010	0.005609		
Total	26	4.4157			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0748908	97.71%	96.70%	94.86%

Comparisons for Moisture Content Results

Tukey Pairwise Comparisons: Treatments

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping
UT	9	8.86811	A
CH	9	8.80122	A
MH	9	8.47667	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Extraction Technique	N	Mean	Grouping
EnzymeAssisted	9	9.02489	A
Alkali	9	8.61789	B
Salt	9	8.50322	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatments*Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Treatments*Extraction				
Technique	N	Mean	Grouping	
UT Alkali	3	9.24267	A	
UT EnzymeAssisted	3	9.12767	A	B
CH EnzymeAssisted	3	9.01600	B	
MH EnzymeAssisted	3	8.93100	B	C
CH Salt	3	8.72200	C	D
CH Alkali	3	8.66567	D	
MH Salt	3	8.55367	D	
UT Salt	3	8.23400	E	
MH Alkali	3	7.94533	F	

Means that do not share a letter are significantly different.

General Linear Model: Ash Content Results versus Treatments, Extraction Technique

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Treatments	Fixed	3 CH, MH, UT
Extraction Technique	Fixed	3 Alkali, EnzymeAssisted, Salt

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatments	2	0.10146	0.050728	5.53	0.013
Extraction Technique	2	0.00314	0.001571	0.17	0.844
Treatments*Extraction Technique	4	0.98337	0.245843	26.78	0.000
Error	18	0.16526	0.009181		
Total	26	1.25323			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0958187	86.81%	80.95%	70.33%

Comparisons for Ash Content Results

Tukey Pairwise Comparisons: Treatments

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping
CH	9	5.38300	A
MH	9	5.26678	B
UT	9	5.24256	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Extraction Technique	N	Mean	Grouping
Alkali	9	5.30933	A

Salt	9	5.29978	A
EnzymeAssisted	9	5.28322	A

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatments*Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Treatments*Extraction Technique	N	Mean	Grouping
CH Alkali	3	5.60400	A
MH Salt	3	5.54667	A B
CH EnzymeAssisted	3	5.42067	A B C
UT Alkali	3	5.37433	A B C D
MH EnzymeAssisted	3	5.30400	B C D
UT Salt	3	5.22833	C D
UT EnzymeAssisted	3	5.12500	D E
CH Salt	3	5.12433	D E
MH Alkali	3	4.94967	E

Means that do not share a letter are significantly different.

General Linear Model: Fat Content Results versus Treatments, Extraction Technique

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Treatments	Fixed	3	CH, MH, UT
Extraction Technique	Fixed	3	Alkali, EnzymeAssisted, Salt

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatments	2	14.6268	7.3134	712.59	0.000
Extraction Technique	2	56.6194	28.3097	2758.39	0.000
Treatments*Extraction Technique	4	6.6748	1.6687	162.59	0.000
Error	18	0.1847	0.0103		
Total	26	78.1058			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.101307	99.76%	99.66%	99.47%

Comparisons for Fat Content Results

Tukey Pairwise Comparisons: Treatments

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping
UT	9	13.9037	A
CH	9	13.1826	B
MH	9	12.1121	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Extraction			
Technique	N	Mean	Grouping
Salt	9	15.1055	A
EnzymeAssisted	9	12.2078	B
Alkali	9	11.8850	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatments*Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Treatments*Extraction			
Technique	N	Mean	Grouping
CH Salt	3	15.2167	A
UT Salt	3	15.1940	A B
MH Salt	3	14.9060	B
UT Alkali	3	13.4137	C
UT EnzymeAssisted	3	13.1033	D
CH EnzymeAssisted	3	12.7243	E
CH Alkali	3	11.6067	F
MH EnzymeAssisted	3	10.7957	G
MH Alkali	3	10.6347	G

Means that do not share a letter are significantly different.

General Linear Model: Protein Content Results versus Treatments, Extraction Technique

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Treatments	Fixed	3 CH, MH, UT
Extraction Technique	Fixed	3 Alkali, EnzymeAssisted, Salt

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatments	2	645.21	322.607	219.15	0.000
Extraction Technique	2	329.41	164.704	111.89	0.000
Treatments*Extraction Technique	4	75.33	18.831	12.79	0.000
Error	18	26.50	1.472		
Total	26	1076.44			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.21329	97.54%	96.44%	94.46%

Comparisons for Protein Content Results

Tukey Pairwise Comparisons: Treatments

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping
MH	9	69.1456	A
CH	9	64.4704	B
UT	9	57.2611	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Extraction Technique	N	Mean	Grouping
Alkali	9	68.3449	A
EnzymeAssisted	9	62.5300	B
Salt	9	60.0022	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatments*Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Treatments*Extraction Technique	N	Mean	Grouping
MH Alkali	3	76.9500	A
CH Alkali	3	68.6647	B
MH EnzymeAssisted	3	66.9333	B C
MH Salt	3	63.5533	C D
CH EnzymeAssisted	3	63.5233	C D
CH Salt	3	61.2233	D E
UT Alkali	3	59.4200	E F
UT EnzymeAssisted	3	57.1333	F G
UT Salt	3	55.2300	G

Means that do not share a letter are significantly different.

General Linear Model: Carbohydrate Content Results versus Treatments, Extraction Technique

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Treatments	Fixed	3 CH, MH, UT
Extraction Technique	Fixed	3 Alkali, EnzymeAssisted, Salt

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatments	2	478.163	239.082	574.53	0.000
Extraction Technique	2	170.072	85.036	204.35	0.000
Treatments*Extraction Technique	4	43.295	10.824	26.01	0.000
Error	18	7.490	0.416		
Total	26	699.021			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.645087	98.93%	98.45%	97.59%

Comparisons for Carbohydrate Content Results

Tukey Pairwise Comparisons: Treatments

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping
UT	9	23.6300	A
CH	9	16.9778	B
MH	9	13.4844	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Extraction			
Technique	N	Mean	Grouping
EnzymeAssisted	9	19.9867	A
Salt	9	19.6178	A
Alkali	9	14.4878	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatments*Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Treatments*Extraction			
Technique	N	Mean	Grouping
UT EnzymeAssisted	3	24.6367	A
UT Salt	3	24.4367	A
UT Alkali	3	21.8167	B
CH Salt	3	18.4367	C
CH EnzymeAssisted	3	18.3567	C
MH EnzymeAssisted	3	16.9667	C D
MH Salt	3	15.9800	D E
CH Alkali	3	14.1400	E
MH Alkali	3	7.5067	F

Means that do not share a letter are significantly differ

Table C.3 ANOVA and Tukey's Comparison Test with 95% confidence level for secondary structure of FTIR spectra

General Linear Model: alpha helix versus AE

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
AE	Fixed	4	CH, Control, MH, UT

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
AE	3	77.2459	25.7486	8630.83	0.000
Error	8	0.0239	0.0030		
Total	11	77.2698			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0546199	99.97%	99.96%	99.93%

Comparisons for alpha helix

Tukey Pairwise Comparisons: AE

Grouping Information Using the Tukey Method and 95% Confidence

AE	N	Mean	Grouping
Control	3	36.2600	A
UT	3	32.4100	B
CH	3	31.9933	C
MH	3	29.1300	D

Means that do not share a letter are significantly different.

General Linear Model: B sheet versus AE

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
AE	Fixed	4	CH, Control, MH, UT

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
AE	3	19.9406	6.64687	1872.36	0.000
Error	8	0.0284	0.00355		
Total	11	19.9690			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0595819	99.86%	99.80%	99.68%

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Comparisons for B sheet

Tukey Pairwise Comparisons: AE

Grouping Information Using the Tukey Method and 95% Confidence

AE	N	Mean	Grouping
MH	3	40.81	A
UT	3	40.36	B
CH	3	39.76	C
Control	3	37.46	D

Means that do not share a letter are significantly different.

General Linear Model: B turn versus AE

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
AE	Fixed	4	CH, Control, MH, UT

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
AE	3	14.7702	4.92340	1852.06	0.000
Error	8	0.0213	0.00266		
Total	11	14.7915			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0515590	99.86%	99.80%	99.68%

Comparisons for B turn

Tukey Pairwise Comparisons: AE

Grouping Information Using the Tukey Method and 95% Confidence

AE	N	Mean	Grouping
MH	3	20.0267	A

CH	3	18.2200	B
UT	3	17.3500	C
Control	3	17.2700	C

Means that do not share a letter are significantly different.

General Linear Model: Random coil versus AE

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
AE	Fixed	4	CH, Control, MH, UT

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
AE	3	2.18843	0.729475	233.43	0.000
Error	8	0.02500	0.003125		
Total	11	2.21343			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0559017	98.87%	98.45%	97.46%

Comparisons for Random coil

Tukey Pairwise Comparisons: AE

Grouping Information Using the Tukey Method and 95% Confidence

AE	N	Mean	Grouping
CH	3	10.02	A
MH	3	10.01	A B
UT	3	9.87	B
Control	3	8.99	C

Means that do not share a letter are significantly different.

General Linear Model: alpha helix versus SE

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
SE	Fixed	4	CH, Control, MH, UT

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SE	3	47.0798	15.6933	16965.70	0.000
Error	8	0.0074	0.0009		
Total	11	47.0872			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0304138	99.98%	99.98%	99.96%

Comparisons for alpha helix

Tukey Pairwise Comparisons: SE

Grouping Information Using the Tukey Method and 95% Confidence

SE	N	Mean	Grouping
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Control	3	36.26	A
UT	3	35.59	B
CH	3	34.45	C
MH	3	31.11	D

Means that do not share a letter are significantly different.

General Linear Model: B sheet versus SE

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
SE	Fixed	4	CH, Control, MH, UT

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SE	3	17.6367	5.87890	3732.63	0.000
Error	8	0.0126	0.00157		
Total	11	17.6493			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0396863	99.93%	99.90%	99.84%

Comparisons for B sheet

Tukey Pairwise Comparisons: SE

Grouping Information Using the Tukey Method and 95% Confidence

SE	N	Mean	Grouping
UT	3	40.59	A
MH	3	39.84	B
CH	3	38.45	C
Control	3	37.46	D

Means that do not share a letter are significantly different.

General Linear Model: B turn versus SE

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
SE	Fixed	4	CH, Control, MH, UT

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SE	3	1.99283	0.664275	781.50	0.000
Error	8	0.00680	0.000850		
Total	11	1.99963			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0291548	99.66%	99.53%	99.23%

Tukey Pairwise Comparisons: SE

Grouping Information Using the Tukey Method and 95% Confidence

SE	N	Mean	Grouping
CH	3	18.17	A
MH	3	18.16	A
UT	3	17.45	B
Control	3	17.27	C

Means that do not share a letter are significantly different.

General Linear Model: Random coil versus SE

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
SE	Fixed	4	CH, Control, MH, UT

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SE	3	7.60380	2.53460	6336.50	0.000
Error	8	0.00320	0.00040		
Total	11	7.60700			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.02	99.96%	99.94%	99.91%

Comparisons for Random coil

Tukey Pairwise Comparisons: SE

Grouping Information Using the Tukey Method and 95% Confidence

SE	N	Mean	Grouping
MH	3	10.88	A
UT	3	9.34	B
Control	3	8.99	C
CH	3	8.91	D

Means that do not share a letter are significantly different.

General Linear Model: alpha helix versus EE

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
EE	Fixed	4	CH, Control, MH, UT

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
EE	3	52.7718	17.5906	17590.60	0.000
Error	8	0.0080	0.0010		
Total	11	52.7798			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0316228	99.98%	99.98%	99.97%

Comparisons for alpha helix

Tukey Pairwise Comparisons: EE

Grouping Information Using the Tukey Method and 95% Confidence

EE	N	Mean	Grouping
Control	3	36.26	A
UT	3	32.13	B
CH	3	31.83	C
MH	3	30.74	D

Means that do not share a letter are significantly different.

General Linear Model: B sheet versus EE

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
EE	Fixed	4	CH, Control, MH, UT

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
EE	3	32.5610	10.8537	7117.16	0.000
Error	8	0.0122	0.0015		
Total	11	32.5732			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0390512	99.96%	99.95%	99.92%

Comparisons for B sheet

Tukey Pairwise Comparisons: EE

Grouping Information Using the Tukey Method and 95% Confidence

EE	N	Mean	Grouping
MH	3	41.79	A
CH	3	41.11	B
UT	3	40.01	C
Control	3	37.46	D

Means that do not share a letter are significantly different.

General Linear Model: B turn versus EE

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
EE	Fixed	4	CH, Control, MH, UT

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
EE	3	4.02382	1.34127	1450.03	0.000
Error	8	0.00740	0.00092		
Total	11	4.03122			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
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0.0304138 99.82% 99.75% 99.59%

Comparisons for B turn

Tukey Pairwise Comparisons: EE

Grouping Information Using the Tukey Method and 95% Confidence

EE	N	Mean	Grouping
CH	3	17.95	A
Control	3	17.27	B
UT	3	17.21	B
MH	3	16.32	C

Means that do not share a letter are significantly different.

General Linear Model: Random coil versus EE

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
EE	Fixed	4	CH, Control, MH, UT

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
EE	3	10.5423	3.51410	10812.62	0.000
Error	8	0.0026	0.00032		
Total	11	10.5449			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0180278	99.98%	99.97%	99.94%

Comparisons for Random coil

Tukey Pairwise Comparisons: EE

Grouping Information Using the Tukey Method and 95% Confidence

EE	N	Mean	Grouping
MH	3	11.14	A
UT	3	10.65	B
CH	3	9.12	C
Control	3	8.99	D

Means that do not share a letter are significantly different.

Table C.4 ANOVA and Tukey's Comparison Test with 95% confidence level for protein solubility (PS)

General Linear Model: PS Results versus Treatments, Extraction Technique

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Treatments	Fixed	3	CH, MH, UT
Extraction Technique	Fixed	3	Alkali, EnzymeAssisted, Salt

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Extraction Technique	2	79.149	39.5746	3937.88	0.000
Treatments	2	70.879	35.4396	3526.42	0.000
Treatments*Extraction Technique	4	36.411	9.1027	905.77	0.000
Error	18	0.181	0.0100		
Total	26	186.620			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.100248	99.90%	99.86%	99.78%

Comparisons for PS Results

Tukey Pairwise Comparisons: Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Extraction			
Technique	N	Mean	Grouping
Alkali	9	12.0195	A
EnzymeAssisted	9	10.8877	B
Salt	9	7.9563	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatments

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping
MH	9	12.5668	A
UT	9	9.3542	B
CH	9	8.9424	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatments*Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Treatments*Extraction			
Technique	N	Mean	Grouping
MH Alkali	3	15.9862	A
MH EnzymeAssisted	3	13.6790	B
UT Alkali	3	10.3224	C
UT EnzymeAssisted	3	9.8767	D
CH Alkali	3	9.7500	D
CH EnzymeAssisted	3	9.1073	E
MH Salt	3	8.0353	F
CH Salt	3	7.9700	F
UT Salt	3	7.8637	F

Means that do not share a letter are significantly different.

Table C.5 ANOVA and Tukey's Comparison Test with 95% confidence level for water solubility index (WSI)

General Linear Model: WSI versus Treatments, Extraction Technique Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Treatments	Fixed	3 CH, MH, UT
Extraction Technique	Fixed	3 Alkali, EnzymeAssisted, Salt

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Extraction Technique	2	1.75603	0.87801	710.32	0.000
Treatments	2	5.99143	2.99572	2423.57	0.000
Treatments*Extraction Technique	4	0.34868	0.08717	70.52	0.000
Error	18	0.02225	0.00124		
Total	26	8.11839			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0351578	99.73%	99.60%	99.38%

Comparisons for WSI

Tukey Pairwise Comparisons: Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Extraction Technique	N	Mean	Grouping
Alkali	9	3.02522	A
Salt	9	2.93556	B
EnzymeAssisted	9	2.44500	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatments

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping
UT	9	3.20189	A
MH	9	3.06333	B
CH	9	2.14056	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatments*Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Treatments*Extraction Technique	N	Mean	Grouping
UT Alkali	3	3.44900	A
UT Salt	3	3.41000	A
MH Alkali	3	3.30667	B
MH Salt	3	3.29333	B
UT EnzymeAssisted	3	2.74667	C
MH EnzymeAssisted	3	2.59000	D
CH Alkali	3	2.32000	E
CH Salt	3	2.10333	F
CH EnzymeAssisted	3	1.99833	G

Means that do not share a letter are significantly different.

Table C.6 ANOVA and Tukey's Comparison Test with 95% confidence level for Hydration Behavior by T₂ Relaxation Times

General Linear Model: T₂ Results versus Treatments, Extraction Technique

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Treatments	Fixed	3	CH, MH, UT
Extraction Technique	Fixed	3	Alkali, EnzymeAssisted, Salt

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Extraction Technique	2	139186	69592.9	3065.48	0.000
Treatments	2	35065	17532.7	772.30	0.000
Treatments*Extraction Technique	4	15201	3800.2	167.39	0.000
Error	18	409	22.7		
Total	26	189861			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
4.76467	99.78%	99.69%	99.52%

Comparisons for T₂ Results

Tukey Pairwise Comparisons: Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Extraction Technique	N	Mean	Grouping
Salt	9	316.479	A
EnzymeAssisted	9	172.639	B
Alkali	9	156.922	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatments

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping
CH	9	259.316	A
UT	9	215.681	B
MH	9	171.043	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatments*Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Treatments*Extraction Technique	N	Mean	Grouping
UT Salt	3	355.007	A
CH Salt	3	319.543	B
MH Salt	3	274.887	C
CH Alkali	3	229.803	D
CH EnzymeAssisted	3	228.600	D
UT EnzymeAssisted	3	164.093	E
UT Alkali	3	127.943	F
MH EnzymeAssisted	3	125.223	F G
MH Alkali	3	113.020	G

Means that do not share a letter are significantly different.

Table C.7 ANOVA and Tukey's Comparison Test with 95% confidence level for Emulsifying Activity (EA)

General Linear Model: EA Results versus Sample, Method

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	3	CH, MH, UT
Method	Fixed	3	AE, EE, SE

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	2	357.632	178.816	460.17	0.000
Method	2	35.065	17.533	45.12	0.000
Sample*Method	4	10.588	2.647	6.81	0.002
Error	18	6.995	0.389		
Total	26	410.280			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.623368	98.30%	97.54%	96.16%

Comparisons for EA Results

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
MH	9	32.9159	A
CH	9	28.2432	B
UT	9	24.0046	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Method

Grouping Information Using the Tukey Method and 95% Confidence

Method	N	Mean	Grouping
AE	9	29.7939	A
EE	9	28.3672	B
SE	9	27.0026	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Sample*Method

Grouping Information Using the Tukey Method and 95% Confidence

Sample*Method	N	Mean	Grouping
MH AE	3	35.4177	A
MH EE	3	32.1923	B
MH SE	3	31.1378	B C
CH AE	3	29.5251	C D
CH EE	3	28.5947	D
CH SE	3	26.6098	E
UT AE	3	24.4388	F
UT EE	3	24.3148	F

UT SE 3 23.2603 F

Means that do not share a letter are significantly different.

Table C.8 ANOVA and Tukey's Comparison Test with 95% confidence level for Emulsifying Stability (ES)

General Linear Model: ES Results versus Sample, Method

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	3	CH, MH, UT
Method	Fixed	3	AE, EE, SE

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	2	122.07	61.033	13.50	0.000
Method	2	61.12	30.560	6.76	0.006
Sample*Method	4	28.75	7.188	1.59	0.220
Error	18	81.35	4.519		
Total	26	293.29			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.12590	92.26%	99.93%	97.59%

Comparisons for ES Results

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
MH	9	37.0584	A
CH	9	33.9501	B
UT	9	31.8851	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Method

Grouping Information Using the Tukey Method and 95% Confidence

Method	N	Mean	Grouping
AE	9	35.6411	A
EE	9	35.0553	A
SE	9	32.1971	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Sample*Method

Grouping Information Using the Tukey Method and 95% Confidence

Sample*Method	N	Mean	Grouping
MH AE	3	40.0472	A
MH EE	3	37.3798	A B
CH EE	3	35.0109	A B C
MH SE	3	33.7482	B C
CH AE	3	33.5589	B C
UT AE	3	33.3173	B C
CH SE	3	33.2804	B C

UT EE	3	32.7752	B	C
UT SE	3	29.5628		C

Means that do not share a letter are significantly different.

Table C.9 ANOVA and Tukey's Comparison Test with 95% confidence level for Foaming Capacity (FC)

General Linear Model: FC(%) Results versus Sample, Method

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	3	CH, MH, UT
Method	Fixed	3	AE, EE, SE

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	2	509.85	254.925	184.67	0.000
Method	2	441.32	220.660	159.84	0.000
Sample*Method	4	107.67	26.917	19.50	0.000
Error	18	24.85	1.380		
Total	26	1083.69			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.17493	97.71%	96.69%	94.84%

Comparisons for FC(%) Results

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
MH	9	26.1667	A
CH	9	20.9278	B
UT	9	15.5229	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Method

Grouping Information Using the Tukey Method and 95% Confidence

Method	N	Mean	Grouping
AE	9	26.0784	A
EE	9	20.3167	B
SE	9	16.2222	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Sample*Method

Grouping Information Using the Tukey Method and 95% Confidence

Sample*Method	N	Mean	Grouping
MH AE	3	31.8333	A
MH EE	3	28.1667	B
CH AE	3	27.8333	B
CH EE	3	18.6167	C

UT AE	3	18.5686	C
MH SE	3	18.5000	C
CH SE	3	16.3333	C D
UT EE	3	14.1667	D
UT SE	3	13.8333	D

Means that do not share a letter are significantly different.

Table C.10 ANOVA and Tukey's Comparison Test with 95% confidence level for Foaming Stability (FS)

General Linear Model: FS(%) Results versus Sample, Method

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	3	CH, MH, UT
Method	Fixed	3	AE, EE, SE

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	2	771.9	385.934	39.05	0.000
Method	2	546.4	273.175	27.64	0.000
Sample*Method	4	222.9	55.723	5.64	0.004
Error	18	177.9	9.884		
Total	26	1719.0			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.14389	89.65%	95.05%	86.71%

Comparisons for FS(%) Results

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
MH	9	37.2567	A
CH	9	33.8369	A
UT	9	24.5981	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Method

Grouping Information Using the Tukey Method and 95% Confidence

Method	N	Mean	Grouping
AE	9	35.5623	A
EE	9	34.5678	A
SE	9	25.5615	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Sample*Method

Grouping Information Using the Tukey Method and 95% Confidence

Sample*Method	N	Mean	Grouping
MH AE	3	44.8074	A
MH EE	3	40.8483	A

CH AE	3	37.1589	A	
CH EE	3	36.6650	A	B
CH SE	3	27.6867	B	C
UT EE	3	26.1901		C
MH SE	3	26.1143		C
UT AE	3	24.7206		C
UT SE	3	22.8836		C

Means that do not share a letter are significantly different.

Table C.11 ANOVA and Tukey's Comparison Test with 95% confidence level for Antioxidant Activity (AA)

General Linear Model: AA Results versus Treatments, Extraction Technique

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Treatments	Fixed	3 CH, MH, UT
Extraction Technique	Fixed	3 Alkali, EnzymeAssisted, Salt

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatments	2	1.68020	0.84010	8401.00	0.000
Extraction Technique	2	2.73780	1.36890	13689.00	0.000
Treatments*Extraction Technique	4	0.52300	0.13075	1307.50	0.000
Error	18	0.00180	0.00010		
Total	26	4.94280			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.01	99.96%	99.95%	99.92%

Comparisons for AA Results

Tukey Pairwise Comparisons: Treatments

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping
UT	9	14.9600	A
MH	9	14.8833	B
CH	9	14.3967	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Extraction Technique	N	Mean	Grouping
Alkali	9	15.1367	A
EnzymeAssisted	9	14.7467	B
Salt	9	14.3567	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatments*Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Treatments*Extraction			
Technique	N	Mean	Grouping
MH Alkali	3	15.54	A
UT Alkali	3	15.24	B
UT EnzymeAssisted	3	15.08	C
MH EnzymeAssisted	3	14.72	D
CH Alkali	3	14.63	E
UT Salt	3	14.56	F
CH EnzymeAssisted	3	14.44	G
MH Salt	3	14.39	H
CH Salt	3	14.12	I

Means that do not share a letter are significantly different.

Table C.12 ANOVA and Tukey's Comparison Test with 95% confidence level for Color Properties (L*, a*, b*)

General Linear Model: L* versus Extraction Technique, Treatments

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Extraction Technique	Fixed	3 Alkali, EnzymeAssisted, Salt
Treatments	Fixed	3 CH, MH, UT

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Extraction Technique	2	41.8400	20.9200	1448.31	0.000
Treatments	2	32.1800	16.0900	1113.92	0.000
Extraction Technique*Treatments	4	11.0000	2.7500	190.38	0.000
Error	18	0.2600	0.0144		
Total	26	85.2800			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.120185	99.70%	99.56%	99.31%

Comparisons for L*

Tukey Pairwise Comparisons: Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Extraction			
Technique	N	Mean	Grouping
Salt	9	50.1333	A
Alkali	9	47.8000	B
EnzymeAssisted	9	47.2667	C

Means that do not share a letter are significantly different

Tukey Pairwise Comparisons: Treatments

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping
UT	9	49.4667	A
MH	9	48.8333	B
CH	9	46.9000	C

Means that do not share a letter are significantly different

Tukey Pairwise Comparisons: Extraction Technique*Treatments

Grouping Information Using the Tukey Method and 95% Confidence

Extraction Technique*Treatments	N	Mean	Grouping
Salt UT	3	51.2667	A
Salt MH	3	50.3667	B
Alkali UT	3	49.5667	C
Salt CH	3	48.7667	D
Alkali MH	3	48.6667	D
EnzymeAssisted UT	3	47.5667	E
EnzymeAssisted MH	3	47.4667	E
EnzymeAssisted CH	3	46.7667	F
Alkali CH	3	45.1667	G

Means that do not share a letter are significantly different.

General Linear Model: a* versus Extraction Technique, Treatments

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Extraction Technique	Fixed	3 Alkali, EnzymeAssisted, Salt
Treatments	Fixed	3 CH, MH, UT

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Extraction Technique	2	3.34296	1.67148	410.27	0.000
Treatments	2	0.31185	0.15593	38.27	0.000
Extraction Technique*Treatments	4	0.07037	0.01759	4.32	0.013
Error	18	0.07333	0.00407		
Total	26	3.79852			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0638285	98.07%	97.21%	95.66%

Comparisons for a*

Tukey Pairwise Comparisons: Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Extraction Technique	N	Mean	Grouping
EnzymeAssisted	9	4.30000	A
Alkali	9	4.17778	B
Salt	9	3.50000	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatments

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping
CH	9	4.14444	A
MH	9	3.92222	B
UT	9	3.91111	B

Means that do not share a letter are significantly different

Tukey Pairwise Comparisons: Extraction Technique*Treatments

Grouping Information Using the Tukey Method and 95% Confidence

Extraction Technique*Treatments	N	Mean	Grouping
EnzymeAssisted CH	3	4.43333	A
Alkali CH	3	4.26667	A B
EnzymeAssisted UT	3	4.26667	A B
EnzymeAssisted MH	3	4.20000	B
Alkali MH	3	4.13333	B
Alkali UT	3	4.13333	B
Salt CH	3	3.73333	C
Salt MH	3	3.43333	D
Salt UT	3	3.33333	D

Means that do not share a letter are significantly different

General Linear Model: b* versus Extraction Technique, Treatments

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Extraction Technique	Fixed	3	Alkali, EnzymeAssisted, Salt
Treatments	Fixed	3	CH, MH, UT

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Extraction Technique	2	50.3563	25.1781	2719.24	0.000
Treatments	2	2.0141	1.0070	108.76	0.000
Extraction Technique*Treatments	4	1.2815	0.3204	34.60	0.000
Error	18	0.1667	0.0093		
Total	26	53.8185			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0962250	99.69%	99.55%	99.30%

Comparisons for b*

Tukey Pairwise Comparisons: Extraction Technique

Grouping Information Using the Tukey Method and 95% Confidence

Extraction Technique	N	Mean	Grouping
Salt	9	30.0000	A
Alkali	9	28.2667	B
EnzymeAssisted	9	26.6556	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatments

Grouping Information Using the Tukey Method and 95% Confidence

Treatments	N	Mean	Grouping
UT	9	28.5778	A
MH	9	28.4111	B

CH 9 27.9333 C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Extraction Technique*Treatments

Grouping Information Using the Tukey Method and 95% Confidence

Extraction Technique*Treatments	N	Mean	Grouping
Salt UT	3	30.2333	A
Salt MH	3	29.9667	A B
Salt CH	3	29.8000	B
Alkali UT	3	28.7667	C
Alkali MH	3	28.5667	C
Alkali CH	3	27.4667	D
EnzymeAssisted UT	3	26.7333	E
EnzymeAssisted MH	3	26.7000	E
EnzymeAssisted CH	3	26.5333	E

Means that do not share a letter are significantly different.

2) Modification of Pumpkin Seed Protein Concentrates (PSPC) through Glycation Reaction

Table C.13 ANOVA and Tukey's Comparison Test with 95% confidence level for Browning Index

General Linear Model: Browning Index Results versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC-All (1:1), PSPC-All (3:1), PSPC-Fru (1:1), PSPC-Fru (3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	0.036334	0.009083	522.04	0.000
Error	10	0.000174	0.000017		
Total	14	0.036508			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0041713	99.52%	99.33%	98.93%

Comparisons for Browning Index Results

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC-Fru (1:1)	3	0.232667	A
PSPC-All (1:1)	3	0.201000	B
PSPC-Fru (3:1)	3	0.179667	C
PSPC-All (3:1)	3	0.141000	D
PSPC	3	0.090667	E

Means that do not share a letter are significantly different.

Table C.14 ANOVA and Tukey's Comparison Test with 95% confidence level for Free Amino Groups by OPA method

General Linear Model: OPA Results versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC-All (1:1), PSPC-All (3:1), PSPC-Fru (1:1), PSPC-Fru (3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	8.56078	2.14019	1500.01	0.000
Error	10	0.01427	0.00143		
Total	14	8.57504			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0377728	99.83%	99.77%	99.63%

Comparisons for OPA Results

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	3.74059	A
PSPC-All (3:1)	3	2.99074	B
PSPC-Fru (3:1)	3	2.17566	C
PSPC-All (1:1)	3	1.95209	D
PSPC-Fru (1:1)	3	1.68229	E

Means that do not share a letter are significantly different.

Table C.15 ANOVA and Tukey's Comparison Test with 95% confidence level for Remaining Reducing Sugar (RRS) Content

General Linear Model: % RRS Results versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	4	PSPC-All (1:1), PSPC-All (3:1), PSPC-Fru (1:1), PSPC-Fru (3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	3	2455.00	818.333	906.57	0.000
Error	8	7.22	0.903		
Total	11	2462.22			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.950088	99.71%	99.60%	99.34%

Comparisons for % RRS Results

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC-All (3:1)	3	35.2950	A
PSPC-All (1:1)	3	23.9352	B
PSPC-Fru (3:1)	3	4.7276	C
PSPC-Fru (1:1)	3	0.0000	D

Means that do not share a letter are significantly different.

Table C.16 ANOVA and Tukey's Comparison Test with 95% confidence level for Amino Acid Profile

General Linear Model: Alanine versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	0.036638	0.009160	129.87	0.000
Error	10	0.000705	0.000071		
Total	14	0.037344			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0083981	98.11%	97.36%	95.75%

Comparisons for Alanine

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	0.275	A
PSPC,All(1:1)	3	0.195	B
PSPC,Fru(3:1)	3	0.169	C
PSPC,All(3:1)	3	0.156	C
PSPC,Fru(1:1)	3	0.131	D

Means that do not share a letter are significantly different.

General Linear Model: Arginine versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	0.116332	0.029083	41.81	0.000
Error	10	0.006956	0.000696		
Total	14	0.123288			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0263742	94.36%	92.10%	87.31%

Comparisons for Arginine

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	0.665	A
PSPC,All(3:1)	3	0.609	A
PSPC,Fru(3:1)	3	0.508	B
PSPC,All(1:1)	3	0.477	B C
PSPC,Fru(1:1)	3	0.424	C

Means that do not share a letter are significantly different.

General Linear Model: Aspartic Acid versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	34.4590	8.61474	3983.88	0.000
Error	10	0.0216	0.00216		
Total	14	34.4806			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0465016	99.94%	99.91%	99.86%

Comparisons for Aspartic Acid

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	5.463	A
PSPC,All(3:1)	3	4.940	B
PSPC,All(1:1)	3	3.513	C
PSPC,Fru(3:1)	3	2.235	D
PSPC,Fru(1:1)	3	1.509	E

Means that do not share a letter are significantly different.

General Linear Model: Cysteine versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	0.008418	0.002104	52.90	0.000
Error	10	0.000398	0.000040		
Total	14	0.008816			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0063071	95.49%	93.68%	89.85%

Comparisons for Cysteine

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	0.124	A
PSPC,All(1:1)	3	0.123	A
PSPC,All(3:1)	3	0.123	A
PSPC,Fru(3:1)	3	0.076	B
PSPC,Fru(1:1)	3	0.074	B

Means that do not share a letter are significantly different.

General Linear Model: Glutamic Acid versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	24.6956	6.17389	1190.54	0.000
Error	10	0.0519	0.00519		
Total	14	24.7474			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0720125	99.79%	99.71%	99.53%

Comparisons for Glutamic Acid

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	5.770	A
PSPC,All(3:1)	3	4.763	B
PSPC,All(1:1)	3	4.070	C
PSPC,Fru(3:1)	3	2.882	D
PSPC,Fru(1:1)	3	2.188	E

Means that do not share a letter are significantly different.

General Linear Model: Glycine versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	0.007136	0.001784	123.90	0.000
Error	10	0.000144	0.000014		
Total	14	0.007280			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0037947	98.02%	97.23%	95.55%

Comparisons for Glycine

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	0.170	A
PSPC,All(3:1)	3	0.145	B
PSPC,All(1:1)	3	0.120	C
PSPC,Fru(3:1)	3	0.117	C
PSPC,Fru(1:1)	3	0.112	C

Means that do not share a letter are significantly different.

General Linear Model: Histidine versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	0.313100	0.078275	3057.62	0.000
Error	10	0.000256	0.000026		
Total	14	0.313356			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0050596	99.92%	99.89%	99.82%

Comparisons for Histidine

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	0.631	A
PSPC,All(3:1)	3	0.425	B

PSPC,All(1:1)	3	0.316	C
PSPC,Fru(3:1)	3	0.288	D
PSPC,Fru(1:1)	3	0.216	E

Means that do not share a letter are significantly different.

General Linear Model: Isoleucine versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	1.59418	0.398546	7066.41	0.000
Error	10	0.00056	0.000056		
Total	14	1.59475			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0075100	99.96%	99.95%	99.92%

Comparisons for Isoleucine

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	1.963	A
PSPC,All(3:1)	3	1.778	B
PSPC,All(1:1)	3	1.464	C
PSPC,Fru(1:1)	3	1.320	D
PSPC,Fru(3:1)	3	1.045	E

Means that do not share a letter are significantly different.

General Linear Model: Leucine versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	1.21266	0.303164	20210.96	0.000
Error	10	0.00015	0.000015		
Total	14	1.21281			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0038730	99.99%	99.98%	99.97%

Comparisons for Leucine

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	2.113	A
PSPC,All(1:1)	3	2.105	A
PSPC,All(3:1)	3	1.894	B
PSPC,Fru(3:1)	3	1.563	C
PSPC,Fru(1:1)	3	1.413	D

Means that do not share a letter are significantly different.

General Linear Model: Lysine versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	5.11707	1.27927	234.91	0.000
Error	10	0.05446	0.00545		
Total	14	5.17152			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0737961	98.95%	98.53%	97.63%

Comparisons for Lysine

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	4.10000	A
PSPC,All(3:1)	3	2.97600	B
PSPC,All(1:1)	3	2.70000	C
PSPC,Fru(3:1)	3	2.63667	C D
PSPC,Fru(1:1)	3	2.47500	D

Means that do not share a letter are significantly different.

General Linear Model: Methionine versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	0.809614	0.202403	715.21	0.000
Error	10	0.002830	0.000283		
Total	14	0.812444			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0168226	99.65%	99.51%	99.22%

Comparisons for methionine

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	2.303	A
PSPC,All(3:1)	3	1.930	B
PSPC,Fru(3:1)	3	1.780	C
PSPC,All(1:1)	3	1.720	D
PSPC,Fru(1:1)	3	1.650	E

Means that do not share a letter are significantly different.

General Linear Model: Phenylalanine versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	4.22225	1.05556	255.47	0.000
Error	10	0.04132	0.00413		
Total	14	4.26357			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0642791	99.03%	98.64%	97.82%

Comparisons for Phenylalanine

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	5.475	A
PSPC,All(3:1)	3	5.150	B
PSPC,All(1:1)	3	5.083	B
PSPC,Fru(3:1)	3	4.310	C
PSPC,Fru(1:1)	3	4.085	D

Means that do not share a letter are significantly different.

General Linear Model: Serine versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
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Sample	4	0.001283	0.000321	2168.07	0.000
Error	10	0.000001	0.000000		
Total	14	0.001285			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0003847	99.88%	99.84%	99.74%

Comparisons for Serine

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	0.0543	A
PSPC,All(3:1)	3	0.0350	B
PSPC,All(1:1)	3	0.0340	B
PSPC,Fru(3:1)	3	0.0310	C
PSPC,Fru(1:1)	3	0.0280	D

Means that do not share a letter are significantly different.

General Linear Model: Threonine versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	0.18007	0.045018	5.28	0.015
Error	10	0.08527	0.008527		
Total	14	0.26534			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0923421	67.86%	55.01%	27.69%

Comparisons for Threonine

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC,All(3:1)	3	4.27500	A
PSPC	3	4.22167	A
PSPC,All(1:1)	3	4.13800	A B
PSPC,Fru(3:1)	3	4.11000	A B
PSPC,Fru(1:1)	3	3.95500	B

Means that do not share a letter are significantly different.

General Linear Model: Tryptophan versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	1.76750	0.441874	50.27	0.000
Error	10	0.08790	0.008790		
Total	14	1.85539			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0937539	95.26%	93.37%	89.34%

Comparisons for Tryptophan

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	5.867	A
PSPC,All(3:1)	3	5.750	A B
PSPC,All(1:1)	3	5.600	B
PSPC,Fru(3:1)	3	5.270	C
PSPC,Fru(1:1)	3	4.925	D

Means that do not share a letter are significantly different.

General Linear Model: Tyrosine versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	0.164554	0.041138	1686.00	0.000
Error	10	0.000244	0.000024		
Total	14	0.164798			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0049396	99.85%	99.79%	99.67%

Comparisons for Tyrosine

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	1.047	A
PSPC,Fru(3:1)	3	0.908	B
PSPC,All(3:1)	3	0.907	B
PSPC,All(1:1)	3	0.795	C
PSPC,Fru(1:1)	3	0.745	D

Means that do not share a letter are significantly different.

General Linear Model: Valine versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC,All(1:1), PSPC,All(3:1), PSPC,Fru(1:1), PSPC,Fru(3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	0.578550	0.144637	1434.90	0.000
Error	10	0.001008	0.000101		
Total	14	0.579558			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0100399	99.83%	99.76%	99.61%

Comparisons for Valine

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	2.386	A
PSPC,Fru(3:1)	3	2.064	B
PSPC,All(3:1)	3	2.060	B
PSPC,All(1:1)	3	2.033	C
PSPC,Fru(1:1)	3	1.767	D

Means that do not share a letter are significantly different.

Table C.17 ANOVA and Tukey's Comparison Test with 95% confidence level for Secondary Structure of PSPC Conjugates

General Linear Model: α -Helix versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC-All (1:1), PSPC-All (3:1), PSPC-Fru (1:1), PSPC-Fru (3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	103.627	25.9067	1612.81	0.000
Error	10	0.161	0.0161		
Total	14	103.787			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.126740	99.85%	99.78%	99.65%

Comparisons for α -Helix

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	27.7267	A
PSPC-All (3:1)	3	25.7310	B
PSPC-Fru (3:1)	3	24.7060	C
PSPC-All (1:1)	3	22.6477	D
PSPC-Fru (1:1)	3	20.0650	E

Means that do not share a letter are significantly different.

General Linear Model: B sheet versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC-All (1:1), PSPC-All (3:1), PSPC-Fru (1:1), PSPC-Fru (3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	40.5136	10.1284	1577.32	0.000
Error	10	0.0642	0.0064		
Total	14	40.5778			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0801328	99.84%	99.78%	99.64%

Comparisons for B sheet

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC	3	37.4103	A
PSPC-All (3:1)	3	35.1907	B
PSPC-All (1:1)	3	34.5603	C
PSPC-Fru (3:1)	3	34.1607	D
PSPC-Fru (1:1)	3	32.3310	E

Means that do not share a letter are significantly different.

General Linear Model: B Turns versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC-All (1:1), PSPC-All (3:1), PSPC-Fru (1:1), PSPC-Fru (3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	119.312	29.8279	489.40	0.000
Error	10	0.609	0.0609		

Total 14 119.921

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.246877	99.49%	99.29%	98.86%

Comparisons for B Turns

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC-Fru (1:1)	3	17.2297	A
PSPC-All (1:1)	3	15.7810	B
PSPC-All (3:1)	3	15.4403	B
PSPC-Fru (3:1)	3	15.3933	B
PSPC	3	9.1113	C

Means that do not share a letter are significantly different.

General Linear Model: Random Coil versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC-All (1:1), PSPC-All (3:1), PSPC-Fru (1:1), PSPC-Fru (3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	47.2565	11.8141	2005.59	0.000
Error	10	0.0589	0.0059		
Total	14	47.3154			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0767502	99.88%	99.83%	99.72%

Comparisons for Random Coil

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC-Fru (1:1)	3	29.5607	A
PSPC-All (1:1)	3	26.1203	B
PSPC-Fru (3:1)	3	25.6897	C
PSPC-All (3:1)	3	25.6810	C
PSPC	3	24.2160	D

Means that do not share a letter are significantly different.

Table C.18 ANOVA and Tukey's Comparison Test with 95% confidence level for Antioxidant Activity by DPPH Method

General Linear Model: DPPH Results versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC-All (1:1), PSPC-All (3:1), PSPC-Fru (1:1), PSPC-Fru (3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	172.507	43.1268	225.67	0.000
Error	10	1.911	0.1911		
Total	14	174.418			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.437155	98.90%	98.47%	97.53%

Comparisons for DPPH Results

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC-All (1:1)	3	18.6656	A
PSPC-All (3:1)	3	18.1693	A B
PSPC-Fru (1:1)	3	17.0940	B
PSPC-Fru (3:1)	3	15.8258	C
PSPC	3	9.3190	D

Means that do not share a letter are significantly different.

Table C.19 ANOVA and Tukey's Comparison Test with 95% confidence level for Emulsifying Activity (EA) of PSPC Conjugates

General Linear Model: EA Results versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC-All (1:1), PSPC-All (3:1), PSPC-Fru (1:1), PSPC-Fru (3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	32.7235	8.18088	665.40	0.000
Error	10	0.1229	0.01229		
Total	14	32.8465			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)

Comparisons for EA Results

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC-Fru (1:1)	3	6.12168	A
PSPC-All (1:1)	3	5.81216	B
PSPC-Fru (3:1)	3	3.40476	C

PSPC-All (3:1)	3	3.37610	C
PSPC	3	2.38161	D

Means that do not share a letter are significantly different.

Table C.20 ANOVA and Tukey's Comparison Test with 95% confidence level for Emulsifying Stability (ES) of PSPC Conjugates

General Linear Model: ES Results versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC-All (1:1), PSPC-All (3:1), PSPC-Fru (1:1), PSPC-Fru (3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	1030.7	257.68	11.60	0.001
Error	10	222.1	22.21		
Total	14	1252.8			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
4.71257	82.27%	75.18%	60.11%

Comparisons for ES Results

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC-Fru (1:1)	3	62.1707	A
PSPC-Fru (3:1)	3	59.0340	A B
PSPC-All (1:1)	3	58.4014	A B
PSPC-All (3:1)	3	47.3880	B C
PSPC	3	40.2183	C

Means that do not share a letter are significantly different.

Table C.21 ANOVA and Tukey's Comparison Test with 95% confidence level for Foaming Capacity (FC) of PSPC Conjugates

General Linear Model: FC (%) versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC-All (1:1), PSPC-All (3:1), PSPC-Fru (1:1), PSPC-Fru (3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	771.07	192.767	70.52	0.000
Error	10	27.33	2.733		
Total	14	798.40			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.65328	96.58%	95.21%	92.30%

Comparisons for FC (%)

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC-Fru (1:1)	3	49.6667	A
PSPC-Fru (3:1)	3	44.0000	B
PSPC-All (1:1)	3	39.0000	C
PSPC-All (3:1)	3	38.3333	C
PSPC	3	28.0000	D

Means that do not share a letter are significantly different.

Table C.22 ANOVA and Tukey's Comparison Test with 95% confidence level for Foaming Stability (FS) of PSPC Conjugates

General Linear Model: FS (%) versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	5	PSPC, PSPC-All (1:1), PSPC-All (3:1), PSPC-Fru (1:1), PSPC-Fru (3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	2110.3	527.57	30.07	0.000
Error	10	175.4	17.54		
Total	14	2285.7			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
4.18864	92.32%	89.25%	82.73%

Comparisons for FS (%)

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC-Fru (1:1)	3	68.4960	A
PSPC-Fru (3:1)	3	58.2680	A B
PSPC-All (1:1)	3	55.5364	B
PSPC-All (3:1)	3	52.9252	B
PSPC	3	32.2554	C

Means that do not share a letter are significantly different.

Table C.23 ANOVA and Tukey's Comparison Test with 95% confidence level for Hydration Behavior via T₂ Relaxation Times of PSPC Conjugates

General Linear Model: T2_Results versus Sample

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample	Fixed	8	C_PSPC-All (1:1), C_PSPC-All (3:1), C_PSPC-Fru (1:1), C_PSPC-Fru (3:1), PSPC-All (1:1), PSPC-All (3:1), PSPC-Fru (1:1), PSPC-Fru (3:1)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	7	92834.6	13262.1	254.14	0.000
Error	16	834.9	52.2		
Total	23	93669.5			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
7.22380	99.11%	98.72%	97.99%

Comparisons for T2_Results

Tukey Pairwise Comparisons: Sample

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping
PSPC-Fru (1:1)	3	427.027	A
PSPC-Fru (3:1)	3	351.067	B
PSPC-All (1:1)	3	326.067	C
PSPC-All (3:1)	3	306.443	C
C_PSPC-All (3:1)	3	271.537	D
C_PSPC-Fru (3:1)	3	258.390	D E
C_PSPC-All (1:1)	3	244.160	E
C_PSPC-Fru (1:1)	3	223.650	F

Means that do not share a letter are significantly different.

3) Utilization of Conjugated PSPC Proteins in Tomato-Based Sauces

Table C.24 ANOVA and Tukey's Comparison Test with 95% confidence level for Water Activity of Tomato Sauce Samples

General Linear Model: Water activity versus Sample Names

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample Names	Fixed	4	TS-A, TS-C, TS-F, TS-U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Names	3	0.004825	0.001608	48.25	0.000
Error	8	0.000267	0.000033		
Total	11	0.005092			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0057735	94.76%	92.80%	88.22%

Comparisons for Water activity

Tukey Pairwise Comparisons: Sample Names

Grouping Information Using the Tukey Method and 95% Confidence

Sample Names	N	Mean	Grouping
TS-C	3	0.916667	A
TS-U	3	0.883333	B
TS-A	3	0.873333	B C
TS-F	3	0.863333	C

Means that do not share a letter are significantly different.

Table C.25 ANOVA and Tukey's Comparison Test with 95% confidence level for Moisture Content of Tomato Sauce Samples

General Linear Model: Moisture Content (MC) versus Sample Names

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample Names	Fixed	4	TS-A, TS-C, TS-F, TS-U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Names	3	35.5247	11.8416	1300.08	0.000
Error	8	0.0729	0.0091		
Total	11	35.5976			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0954376	99.80%	99.72%	99.54%

Comparisons for Moisture Content (MC)

Tukey Pairwise Comparisons: Sample Names

Grouping Information Using the Tukey Method and 95% Confidence

Sample Names	N	Mean	Grouping
TS-C	3	91.8733	A
TS-U	3	88.1733	B
TS-A	3	87.9633	B
TS-F	3	87.6367	C

Means that do not share a letter are significantly different.

Table C.26 ANOVA and Tukey's Comparison Test with 95% confidence level for pH of Tomato Sauce Samples

General Linear Model: pH versus Sample Names

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Sample Names	Fixed	4 TS-A, TS-C, TS-F, TS-U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Names	3	0.012492	0.004164	35.69	0.000
Error	8	0.000933	0.000117		
Total	11	0.013425			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0108012	93.05%	90.44%	84.36%

Comparisons for pH

Tukey Pairwise Comparisons: Sample Names

Grouping Information Using the Tukey Method and 95% Confidence

Sample Names	N	Mean	Grouping
TS-F	3	4.16000	A
TS-A	3	4.15000	A B
TS-U	3	4.12333	B
TS-C	3	4.07667	C

Means that do not share a letter are significantly different.

Table C.27 ANOVA and Tukey's Comparison Test with 95% confidence level for Brix Value of Tomato Sauce Samples

General Linear Model: Brix versus Sample Names

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Sample Names	Fixed	4 TS-A, TS-C, TS-F, TS-U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Names	3	4.4433	1.48111	111.08	0.000
Error	8	0.1067	0.01333		
Total	11	4.5500			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.115470	97.66%	96.78%	94.73%

Comparisons for Brix

Tukey Pairwise Comparisons: Sample Names

Grouping Information Using the Tukey Method and 95% Confidence

Sample Names	N	Mean	Grouping
TS-A	3	10.5333	A
TS-F	3	10.4667	A
TS-U	3	9.7667	B
TS-C	3	9.0333	C

Means that do not share a letter are significantly different.

Table C.28 ANOVA and Tukey's Comparison Test with 95% confidence level for Color Properties (L*, a*, b*) of Tomato Sauce Samples

General Linear Model: L* versus Sample Names

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample Names	Fixed	4	TS-A, TS-C, TS-F, TS-U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Names	3	1.9697	0.65658	46.46	0.000
Error	8	0.1131	0.01413		
Total	11	2.0828			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.118884	94.57%	92.54%	87.79%

Comparisons for L*

Tukey Pairwise Comparisons: Sample Names

Grouping Information Using the Tukey Method and 95% Confidence

Sample Names	N	Mean	Grouping
TS-F	3	39.5667	A
TS-U	3	39.3667	A
TS-A	3	38.7333	B
TS-C	3	38.6133	B

Means that do not share a letter are significantly different.

General Linear Model: a* versus Sample Names

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample Names	Fixed	4	TS-A, TS-C, TS-F, TS-U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Names	3	0.6692	0.22306	17.84	0.001
Error	8	0.1000	0.01250		
Total	11	0.7692			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.111803	87.00%	82.12%	70.75%

Comparisons for a*

Tukey Pairwise Comparisons: Sample Names

Grouping Information Using the Tukey Method and 95% Confidence

Sample Names	N	Mean	Grouping
TS-C	3	19.9000	A
TS-U	3	19.7333	A B
TS-F	3	19.5333	B C
TS-A	3	19.2667	C

Means that do not share a letter are significantly different.

General Linear Model: b* versus Sample Names

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample Names	Fixed	4	TS-A, TS-C, TS-F, TS-U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Names	3	11.4267	3.80889	304.71	0.000
Error	8	0.1000	0.01250		
Total	11	11.5267			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.111803	99.13%	98.81%	98.05%

Comparisons for b*

Tukey Pairwise Comparisons: Sample Names

Grouping Information Using the Tukey Method and 95% Confidence

Sample Names	N	Mean	Grouping
TS-F	3	17.9333	A
TS-U	3	17.8667	A
TS-A	3	16.8000	B
TS-C	3	15.5333	C

Means that do not share a letter are significantly different.

Table C.29 ANOVA and Tukey's Comparison Test with 95% confidence level for Soluble Protein Content of Tomato Sauce Samples

General Linear Model: Soluble Protein Content versus Sample Names

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample Names	Fixed	4	TS-A, TS-C, TS-F, TS-U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Names	3	157.091	52.3637	1713.64	0.000
Error	8	0.244	0.0306		
Total	11	157.335			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.174806	99.84%	99.79%	99.65%

Comparisons for Soluble Protein Content

Tukey Pairwise Comparisons: Sample Names

Grouping Information Using the Tukey Method and 95% Confidence

Sample Names	N	Mean	Grouping
TS-F	3	14.3045	A
TS-A	3	11.4855	B
TS-U	3	6.7074	C
TS-C	3	5.3108	D

Means that do not share a letter are significantly different.

Table C.30 ANOVA and Tukey's Comparison Test with 95% confidence level for Lycopene Content of Tomato Sauce Samples

General Linear Model: Lycopene Content versus Sample Names

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample Names	Fixed	4	TS-A, TS-C, TS-F, TS-U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Names	3	2625.41	875.137	305.35	0.000
Error	8	22.93	2.866		

Total 11 2648.34

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.69294	99.13%	98.81%	98.05%

Comparisons for Lycopene Content

Tukey Pairwise Comparisons: Sample Names

Grouping Information Using the Tukey Method and 95% Confidence

Sample Names	N	Mean	Grouping
TS-A	3	164.640	A
TS-F	3	156.055	B
TS-U	3	144.584	C
TS-C	3	125.146	D

Means that do not share a letter are significantly different.

Table C.31 ANOVA and Tukey's Comparison Test with 95% confidence level for Scavenging Capacity (SC) of Tomato Sauce Samples

General Linear Model: SC (%) versus Sample Name

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Sample Name	Fixed	4 TS-A, TS-C, TS-F, TS-U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Name	3	130.825	43.6083	102.84	0.000
Error	8	3.392	0.4240		
Total	11	134.217			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.651170	97.47%	96.52%	94.31%

Comparisons for SC (%)

Tukey Pairwise Comparisons: Sample Name

Grouping Information Using the Tukey Method and 95% Confidence

Sample Name	N	Mean	Grouping
TS-A	3	62.3337	A
TS-F	3	58.2884	B
TS-U	3	55.0881	C
TS-C	3	53.7756	C

Means that do not share a letter are significantly different.

Table C.32 ANOVA and Tukey's Comparison Test with 95% confidence level for Total Phenolic Content (TPC) of Tomato Sauce Samples

General Linear Model: TPC versus Sample Name

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample Name	Fixed	4	TS-A, TS-C, TS-F, TS-U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Name	3	224.16	74.719	41.12	0.000
Error	8	14.54	1.817		
Total	11	238.69			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.34801	93.91%	91.63%	86.30%

Comparisons for TPC

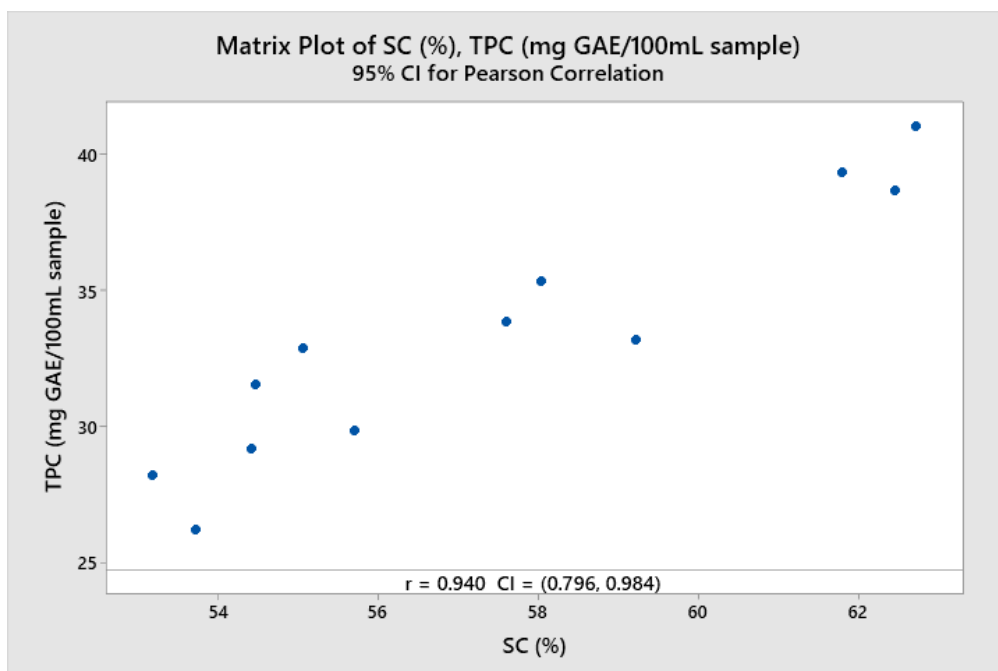
Tukey Pairwise Comparisons: Sample Name

Grouping Information Using the Tukey Method and 95% Confidence

Sample Name	N	Mean	Grouping
TS-A	3	39.7000	A
TS-F	3	34.1444	B
TS-U	3	31.4222	B
TS-C	3	27.8667	C

Means that do not share a letter are significantly different.

Correlation: SC (%), TPC (mg GAE/100mL sample)



Method

Correlation type Pearson
Rows used 12

Correlations

	SC (%)
TPC	0.940

Table C.33 ANOVA and Tukey's Comparison Test with 95% confidence level for Rheological Properties (y_0 , k , n) of Tomato Sauce Samples

General Linear Model: y_0 versus Samples

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Samples	Fixed	4	TS-A, TS-C, TS-F, TS-U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Samples	3	191.574	63.8579	502.36	0.000
Error	8	1.017	0.1271		
Total	11	192.591			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.356534	99.47%	99.27%	98.81%

Comparisons for y_0

Tukey Pairwise Comparisons: Samples

Grouping Information Using the Tukey Method and 95% Confidence

Samples	N	Mean	Grouping
TS-F	3	17.4800	A
TS-A	3	16.6767	A
TS-U	3	12.4033	B
TS-C	3	7.4400	C

Means that do not share a letter are significantly different.

General Linear Model: k versus Samples

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Samples	Fixed	4	TS-A, TS-C, TS-F, TS-U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Samples	3	0.025104	0.008368	392.25	0.000
Error	8	0.000171	0.000021		
Total	11	0.025275			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0046188	99.32%	99.07%	98.48%

Comparisons for k

Tukey Pairwise Comparisons: Samples

Grouping Information Using the Tukey Method and 95% Confidence

Samples	N	Mean	Grouping
TS-F	3	0.165667	A
TS-A	3	0.117667	B
TS-U	3	0.104333	C
TS-C	3	0.037667	D

Means that do not share a letter are significantly different.

General Linear Model: n versus Samples

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Samples	Fixed	4	TS-A, TS-C, TS-F, TS-U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Samples	3	0.150242	0.050081	899.08	0.000
Error	8	0.000446	0.000056		
Total	11	0.150688			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0074634	99.70%	99.59%	99.33%

Comparisons for n

Tukey Pairwise Comparisons: Samples

Grouping Information Using the Tukey Method and 95% Confidence

Samples	N	Mean	Grouping
TS-C	3	0.919200	A
TS-U	3	0.758467	B
TS-A	3	0.683667	C
TS-F	3	0.619333	D

Means that do not share a letter are significantly different.

Table C.34 ANOVA and Tukey's Comparison Test with 95% confidence level for T₂ Relaxation Times of Tomato Sauce Samples

General Linear Model: T2 Results versus Sample Names

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
--------	------	--------	--------

Sample Names Fixed 4 TS-A, TS-C, TS-F, TS-U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Names	3	37757.8	12585.9	57540.11	0.000
Error	8	1.7	0.2		
Total	11	37759.6			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.467689	100.00%	99.99%	99.99%

Comparisons for T2 Results

Tukey Pairwise Comparisons: Sample Names

Grouping Information Using the Tukey Method and 95% Confidence

Sample Names	N	Mean	Grouping
TS-C	3	343.103	A
TS-F	3	216.717	B
TS-A	3	212.243	C
TS-U	3	211.943	C

Means that do not share a letter are significantly different.

Table C.35 ANOVA and Tukey's Comparison Test with 95% confidence level for Digestibility of Tomato Sauce Samples

General Linear Model: Digestibility Results versus Sample Name

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sample Name	Fixed	3	TS-A, TS-F, TS-U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Name	2	0.06243	0.031217	5.21	0.049
Error	6	0.03597	0.005996		
Total	8	0.09841			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0774310	63.44%	51.26%	17.75%

Comparisons for Digestibility Results

Tukey Pairwise Comparisons: Sample Name

Grouping Information Using the Tukey Method and 95% Confidence

Sample Name	N	Mean	Grouping
TS-F	3	8.63429	A
TS-A	3	8.50196	B

TS-U 3 8.43365 B

Means that do not share a letter are significantly different.

General Linear Model: Digestibility Results versus Sample Name

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Sample Name	Fixed	3 A, F, U

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Name	2	7.3018	3.65088	65.62	0.000
Error	6	0.3338	0.05563		
Total	8	7.6356			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.235866	95.63%	94.17%	90.16%

Comparisons for Digestibility Results

Tukey Pairwise Comparisons: Sample Name

Grouping Information Using the Tukey Method and 95% Confidence

Sample Name	N	Mean	Grouping
U	3	13.4952	A
F	3	11.9830	B
A	3	11.3478	C

Means that do not share a letter are significantly different.

4) Evaluation of Different Sugars with Several TD-NMR Approaches

Table C.36 ANOVA and Tukey's Comparison Test with 95% confidence level for 1/T₂ (s⁻¹) of Different Sugars

D: Glucose, F: Fructose, A: Allulose, and S: Sucrose

General Linear Model: 1/T₂ (s⁻¹) versus Sugar Types

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sugar Types	Fixed	4	A, D, F, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sugar Types	3	0.000027	0.000009	253.14	0.000
Error	4	0.000000	0.000000		
Total	7	0.000027			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0001871	99.48%	99.08%	97.90%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.013450	0.000066	203.34	0.000	
Sugar Types					
A	-0.002750	0.000115	-24.00	0.000	1.50
D	0.001150	0.000115	10.04	0.001	1.50
F	-0.000450	0.000115	-3.93	0.017	1.50

Regression Equation

$$1/T_2 (s^{-1}) = 0.013450 - 0.002750 \text{ Sugar Types}_A + 0.001150 \text{ Sugar Types}_D - 0.000450 \text{ Sugar Types}_F + 0.002050 \text{ Sugar Types}_S$$

Comparisons for 1/T₂ (s⁻¹)

Tukey Pairwise Comparisons: Sugar Types

Grouping Information Using the Tukey Method and 95% Confidence

Sugar Types	N	Mean	Grouping
S	2	0.0155	A
D	2	0.0146	B
F	2	0.0130	C
A	2	0.0107	D

Means that do not share a letter are significantly different.

Table C.37 ANOVA and Tukey's Comparison Test with 95% confidence level for T₂ Relaxation Times for Regions I and II of Different Sugars

General Linear Model: Region I versus Sugar Types

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Sugar Types	Fixed	4 A, D, F, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sugar Types	3	0.198964	0.066321	33160.67	0.000
Error	4	0.000008	0.000002		
Total	7	0.198972			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0014142	100.00%	99.99%	99.98%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.422000	0.000500	844.00	0.000	
Sugar Types					
A	-0.260000	0.000866	-300.22	0.000	1.50
D	0.056000	0.000866	64.66	0.000	1.50
F	0.039000	0.000866	45.03	0.000	1.50

Regression Equation

$$\text{Region I} = 0.422000 - 0.260000 \text{ Sugar Types}_A + 0.056000 \text{ Sugar Types}_D + 0.039000 \text{ Sugar Types}_F + 0.165000 \text{ Sugar Types}_S$$

Comparisons for Region I

Tukey Pairwise Comparisons: Sugar Types

Grouping Information Using the Tukey Method and 95% Confidence

Sugar Types	N	Mean	Grouping
S	2	0.587	A
D	2	0.478	B
F	2	0.461	C
A	2	0.162	D

Means that do not share a letter are significantly different.

General Linear Model: Region II versus Sugar Types

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Sugar Types	Fixed	4 A, D, F, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sugar Types	3	0.058092	0.019364	9682.00	0.000
Error	4	0.000008	0.000002		
Total	7	0.058100			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0014142	99.99%	99.98%	99.94%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.177000	0.000500	354.00	0.000	
Sugar Types					

A	0.103000	0.000866	118.93	0.000	1.50
D	-0.014000	0.000866	-16.17	0.000	1.50
F	0.040000	0.000866	46.19	0.000	1.50

Regression Equation

$$\text{Region II} = 0.177000 + 0.103000 \text{ Sugar Types}_A - 0.014000 \text{ Sugar Types}_D + 0.040000 \text{ Sugar Types}_F - 0.129000 \text{ Sugar Types}_S$$

Comparisons for Region II

Tukey Pairwise Comparisons: Sugar Types

Grouping Information Using the Tukey Method and 95% Confidence

Sugar Types	N	Mean	Grouping
A	2	0.280	A
F	2	0.217	B
D	2	0.163	C
S	2	0.048	D

Means that do not share a letter are significantly different.

Table C.38 ANOVA and Tukey's Comparison Test with 95% confidence level for Second Moment (M_2) (Solid) and (Hydrated) of Different Sugars

General Linear Model: M2 Solid versus Sugar Types

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Sugar Types	Fixed	4 A, D, F, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sugar Types	3	2.10680	0.702267	200.65	0.000
Error	4	0.01400	0.003500		
Total	7	2.12080			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0591608	99.34%	98.84%	97.36%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	16.0400	0.0209	766.86	0.000	
Sugar Types					
A	0.3500	0.0362	9.66	0.001	1.50
D	0.0800	0.0362	2.21	0.092	1.50
F	-0.8600	0.0362	-23.74	0.000	1.50

Regression Equation

$$\text{M2 Solid} = 16.0400 + 0.3500 \text{ Sugar Types}_A + 0.0800 \text{ Sugar Types}_D - 0.8600 \text{ Sugar Types}_F + 0.4300 \text{ Sugar Types}_S$$

Comparisons for M2 Solid

Tukey Pairwise Comparisons: Sugar Types

Grouping Information Using the Tukey Method and 95% Confidence

Sugar Types	N	Mean	Grouping
S	2	16.47	A
A	2	16.39	A
D	2	16.12	B
F	2	15.18	C

Means that do not share a letter are significantly different.

General Linear Model: M2 Hydrated versus Sugar Types

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sugar Types	Fixed	4	A, D, F, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sugar Types	3	1.99880	0.666267	403.80	0.000
Error	4	0.00660	0.001650		
Total	7	2.00540			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0406202	99.67%	99.42%	98.68%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	13.8600	0.0144	965.09	0.000	
Sugar Types					
A	0.5500	0.0249	22.11	0.000	1.50
D	-0.2200	0.0249	-8.84	0.001	1.50
F	0.3800	0.0249	15.28	0.000	1.50

Regression Equation

$$\text{M2 Hydrated} = 13.8600 + 0.5500 \text{ Sugar Types}_A - 0.2200 \text{ Sugar Types}_D + 0.3800 \text{ Sugar Types}_F - 0.7100 \text{ Sugar Types}_S$$

Comparisons for M2 Hydrated

Tukey Pairwise Comparisons: Sugar Types

Grouping Information Using the Tukey Method and 95% Confidence

Sugar Types	N	Mean	Grouping
A	2	14.41	A
F	2	14.24	B
D	2	13.64	C
S	2	13.15	D

Means that do not share a letter are significantly different.

Table C.39 ANOVA and Tukey's Comparison Test with 95% confidence level for Total Relative Crystallinity obtained by XRD Analysis of Different Sugars

General Linear Model: Relative Total Crystallinity versus Sugar Types

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Sugar Types	Fixed	4 A, D, F, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sugar Types	3	40.1450	13.3817	263.94	0.000
Error	4	0.2028	0.0507		
Total	7	40.3478			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.225167	99.50%	99.12%	97.99%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	78.3650	0.0796	984.38	0.000	
Sugar Types					
A	1.345	0.138	9.75	0.001	1.50
D	-1.645	0.138	-11.93	0.000	1.50
F	-2.635	0.138	-19.11	0.000	1.50

Regression Equation

$$\text{Relative Total Crystallinity} = 78.3650 + 1.345 \text{ Sugar Types}_A - 1.645 \text{ Sugar Types}_D - 2.635 \text{ Sugar Types}_F + 2.935 \text{ Sugar Types}_S$$

Comparisons for Relative Total Crystallinity

Tukey Pairwise Comparisons: Sugar Types

Grouping Information Using the Tukey Method and 95% Confidence

Sugar Types	N	Mean	Grouping
S	2	81.30	A
A	2	79.71	B
D	2	76.72	C
F	2	75.73	D

Means that do not share a letter are significantly different.

Table C.40 ANOVA and Tukey's Comparison Test with 95% confidence level for Self Diffusion Coefficient of Different Sugars at Different Concentrations

General Linear Model: DiffCoeff_1 versus Sugar Type_1

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels Values
Sugar Type_1	Fixed	4 A; D; F; S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sugar Type_1	3	0,640800	0,213600	2136,00	0,000
Error	8	0,000800	0,000100		
Total	11	0,641600			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,01	99,88%	99,83%	99,72%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6,97000	0,00289	2414,48	0,000	
Sugar Type_1					
A	-0,30000	0,00500	-60,00	0,000	1,50
D	0,28000	0,00500	56,00	0,000	1,50
F	-0,14000	0,00500	-28,00	0,000	1,50

Regression Equation

$$\text{DiffCoef}_1 = 6,97000 - 0,30000 \text{ Sugar Type}_1\text{A} + 0,28000 \text{ Sugar Type}_1\text{D} - 0,14000 \text{ Sugar Type}_1\text{F} + 0,16000 \text{ Sugar Type}_1\text{S}$$

General Linear Model: Diffcoef versus Sugar Type

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Sugar Type	Fixed	4	A, D, F, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sugar Type	3	4.41652	1.47217	24367.02	0.000
Error	8	0.00048	0.00006		
Total	11	4.41701			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0077728	99.99%	99.98%	99.98%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	8.40875	0.00224	3747.52	0.000	
Sugar Type					
A	-0.77875	0.00389	-200.38	0.000	1.50
D	0.60292	0.00389	155.13	0.000	1.50
F	-0.40542	0.00389	-104.32	0.000	1.50

Regression Equation

$$\text{Diffcoef} = 8.40875 - 0.77875 \text{ Sugar Type}_1\text{A} + 0.60292 \text{ Sugar Type}_1\text{D} - 0.40542 \text{ Sugar Type}_1\text{F} + 0.58125 \text{ Sugar Type}_1\text{S}$$

Comparisons for Diffcoef

Tukey Pairwise Comparisons: Sugar Type

Grouping Information Using the Tukey Method and 95% Confidence

Sugar Type	N	Mean	Grouping
D	3	9.01167	A
S	3	8.99000	B
F	3	8.00333	C
A	3	7.63000	D

Means that do not share a letter are significantly different.

Comparisons for DiffCoef_1

Tukey Pairwise Comparisons: Sugar Type_1

Grouping Information Using the Tukey Method and 95% Confidence

Sugar			
Type 1	N	Mean	Grouping
D	3	7,25	A
S	3	7,13	B
F	3	6,83	C
A	3	6,67	D

Means that do not share a letter are significantly different.

General Linear Model: DiffCoeff2 versus Sugar Type2

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Sugar Type2	Fixed	4	A; D; F; S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sugar Type2	3	0,668400	0,222800	2228,00	0,000
Error	8	0,000800	0,000100		
Total	11	0,669200			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,01	99,88%	99,84%	99,73%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6,38000	0,00289	2210,10	0,000	
Sugar Type2					
A	-0,35000	0,00500	-70,00	0,000	1,50
D	0,27000	0,00500	54,00	0,000	1,50
F	-0,07000	0,00500	-14,00	0,000	1,50

Regression Equation

$$\text{DiffCoeff2} = 6,38000 - 0,35000 \text{ Sugar Type2_A} + 0,27000 \text{ Sugar Type2_D} - 0,07000 \text{ Sugar Type2_F} + 0,15000 \text{ Sugar Type2_S}$$

Comparisons for DiffCoeff2

Tukey Pairwise Comparisons: Sugar Type2

Grouping Information Using the Tukey Method and 95% Confidence

Sugar			
Type2	N	Mean	Grouping
D	3	6,65	A
S	3	6,53	B
F	3	6,31	C
A	3	6,03	D

Means that do not share a letter are significantly different.

General Linear Model: DiffCoeff3 versus Sugar Type3

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Sugar Type3	Fixed	4	A; D; F; S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sugar Type3	3	0,967425	0,322475	3224,75	0,000
Error	8	0,000800	0,000100		
Total	11	0,968225			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,01	99,92%	99,89%	99,81%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5,05750	0,00289	1751,97	0,000	
Sugar Type3					
A	-0,39750	0,00500	-79,50	0,000	1,50
D	0,35250	0,00500	70,50	0,000	1,50
F	-0,11750	0,00500	-23,50	0,000	1,50

Regression Equation

$$\text{DiffCoeff3} = 5,05750 - 0,39750 \text{ Sugar Type3}_A + 0,35250 \text{ Sugar Type3}_D - 0,11750 \text{ Sugar Type3}_F + 0,16250 \text{ Sugar Type3}_S$$

Comparisons for DiffCoeff3

Tukey Pairwise Comparisons: Sugar Type3

Grouping Information Using the Tukey Method and 95% Confidence

Sugar Type3	N	Mean	Grouping
D	3	5,41	A
S	3	5,22	B
F	3	4,94	C
A	3	4,66	D

Means that do not share a letter are significantly different.

General Linear Model: DiffCoeff4 versus Sugar Type4

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Sugar Type4	Fixed	4	A; D; F; S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sugar Type4	3	0,857025	0,285675	2856,75	0,000
Error	8	0,000800	0,000100		
Total	11	0,857825			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,01	99,91%	99,87%	99,79%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	4,01250	0,00289	1389,97	0,000	
Sugar Type4					
A	-0,38250	0,00500	-76,50	0,000	1,50
D	0,31750	0,00500	63,50	0,000	1,50
F	-0,10250	0,00500	-20,50	0,000	1,50

Regression Equation

$$\text{DiffCoeff4} = 4,01250 - 0,38250 \text{ Sugar Type4_A} + 0,31750 \text{ Sugar Type4_D} - 0,10250 \text{ Sugar Type4_F} + 0,16750 \text{ Sugar Type4_S}$$

Comparisons for DiffCoeff4

Tukey Pairwise Comparisons: Sugar Type4

Grouping Information Using the Tukey Method and 95% Confidence

Sugar Type4	N	Mean	Grouping
D	3	4,33	A
S	3	4,18	B
F	3	3,91	C
A	3	3,63	D

Means that do not share a letter are significantly different.

General Linear Model: DiffCoeff5 versus Sugar Type5

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels Values
Sugar Type5	Fixed	4 A; D; F; S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sugar Type5	3	0,631800	0,210600	2106,00	0,000
Error	8	0,000800	0,000100		
Total	11	0,632600			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,01	99,87%	99,83%	99,72%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	3,88000	0,00289	1344,07	0,000	
Sugar Type5					
A	-0,31000	0,00500	-62,00	0,000	1,50
D	0,24000	0,00500	48,00	0,000	1,50
F	-0,13000	0,00500	-26,00	0,000	1,50

Regression Equation

$$\text{DiffCoeff5} = 3,88000 - 0,31000 \text{ Sugar Type5_A} + 0,24000 \text{ Sugar Type5_D} - 0,13000 \text{ Sugar Type5_F} + 0,20000 \text{ Sugar Type5_S}$$

Comparisons for DiffCoeff5

Tukey Pairwise Comparisons: Sugar Type5

Grouping Information Using the Tukey Method and 95% Confidence

Sugar Type5	N	Mean	Grouping
D	3	4,12	A
S	3	4,08	B
F	3	3,75	C
A	3	3,57	D

Means that do not share a letter are significantly different.

General Linear Model: DiffCoeff6 versus Sugar Type6

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels Values
Sugar Type6	Fixed	4 A; D; F; S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sugar Type6	3	0,444300	0,148100	1481,00	0,000
Error	8	0,000800	0,000100		
Total	11	0,445100			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,01	99,82%	99,75%	99,60%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	3,19500	0,00289	1106,78	0,000	
Sugar Type6					
A	-0,22500	0,00500	-45,00	0,000	1,50
D	0,26500	0,00500	53,00	0,000	1,50
F	-0,13500	0,00500	-27,00	0,000	1,50

Regression Equation

$$\text{DiffCoeff6} = 3,19500 - 0,22500 \text{ Sugar Type6}_A + 0,26500 \text{ Sugar Type6}_D - 0,13500 \text{ Sugar Type6}_F + 0,09500 \text{ Sugar Type6}_S$$

Comparisons for DiffCoeff6

Tukey Pairwise Comparisons: Sugar Type6

Grouping Information Using the Tukey Method and 95% Confidence

Sugar Type6	N	Mean	Grouping
D	3	3,46	A
S	3	3,29	B
F	3	3,06	C
A	3	2,97	D

Means that do not share a letter are significantly different

Table C.41 ANOVA and Tukey's Comparison Test with 95% confidence level for d (thickness) obtained by Spin Diffusion of Different Sugars

General Linear Model: d(thickness)(A) versus Sugar Types

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels Values
Sugar Types	Fixed	4 A; D; F; S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sugar Types	3	289,338	96,4461	128594,84	0,000
Error	4	0,003	0,0008		
Total	7	289,341			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0273861	100,00%	100,00%	100,00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	59,2400	0,0097	6118,28	0,000	
Sugar Types					
A	4,1100	0,0168	245,07	0,000	1,50
D	-6,6300	0,0168	-395,34	0,000	1,50
F	7,6100	0,0168	453,77	0,000	1,50

Regression Equation

$$d(\text{thickness})(A) = 59,2400 + 4,1100 \text{ Sugar Types}_A - 6,6300 \text{ Sugar Types}_D + 7,6100 \text{ Sugar Types}_F - 5,0900 \text{ Sugar Types}_S$$

Grouping Information Using the Tukey Method and 95% Confidence

Sugar Types	N	Mean	Grouping
F	2	66,85	A
A	2	63,35	B
S	2	54,15	C
D	2	52,61	D

Means that do not share a letter are significantly different.

CURRICULUM VITAE (ONLY FOR DOCTORAL THESIS)

Surname, Name: Taş Ozan

EDUCATION

Degree	Institution	Year of Graduation
MS	METU Food Engineering	2019
BS	METU Food Engineering	2017
High School	İnönü High School, Ankara	2011

FOREIGN LANGUAGES

Advanced English, Beginner French

PUBLICATIONS

Ates, E. G., Bal, M., Cetin Karasu, M., Cifte, N. E., Erdem, F., Gul, M. R., Tas, O., Tonyali Karsli, G., Pleslić, S., Smokrović, K., Maltar-Strmečki, N., Abiad, M. G., Dukić, J., Režek Jambrak, A., Tchonkouang, R. D., C. Vieira, M., Antunes, M. D., Mert, B., Sumnu, G., ... Oztop, M. (2025). Reformulation and Characterization of Mediterranean Ingredients by Novel Technologies. *Food Engineering Reviews*. <https://doi.org/10.1007/s12393-025-09401-0>

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