THE FEASIBILITY OF PRODUCING PROTEIN HYDROLYSATE POWDERS FROM UNDERUTILIZED GEORGIA-CAUGHT BROWN SHRIMP (*Farfantepenaeus aztecus*) MEAT

by

EMMA ISABEL MOORE

(Under the Direction of Kevin E. Mis Solval)

ABSTRACT

Underutilized Georgia-caught brown shrimp (GABS) meat is a good source of protein and essential amino acids. Therefore, it could be used as raw material to produce high-quality protein-based food ingredients. The overall objective of this research was to investigate the feasibility of producing protein hydrolysate powders from GABS meat. Protein hydrolysates often exhibit enhanced functional and antioxidant properties compared to their native proteins. The proximate and selected nutritional composition of GABS meat was characterized. Then, shrimp protein hydrolysate powders were produced via enzymatic hydrolysis under optimal pH and temperature and spray drying under mixed-flow (MX) and concurrent (CC) contact configurations. In general, MX powders showed higher moisture content, protein solubility, emulsification properties, and agglomeration. Meanwhile, CC powders showed higher foaming properties, antioxidant activities, and particle sizes. The resultant hydrolysate powders from GABS meat can be potentially used in different food and beverage applications.

INDEX WORDS: brown shrimp, hydrolysis, alkaline protease, spray drying, functional properties

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DEDICATION

I would like to dedicate this thesis to my dad, Dr. Robert Michael Moore, Jr., who ensured I understood the importance of education at such a young age. Without his extensive planning, I would not be where I am today. Little did I know I would follow closely in his footsteps. I am forever grateful for this unexpected opportunity to keep his memory alive by studying a similar chemical reaction.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

In Georgia, the shrimp industry had a market value of $12.4 million in 2019, which was $3.3 million lower than in 2000 (Southern Shrimp Alliance, 2020). Nevertheless, the demand for seafood and shrimp-based products has steadily increased in the U.S., driven by health-conscious consumers looking for tasty and nutritious foods. In addition, current global events, including the COVID-19 pandemic, are shifting the purchasing behavior of Americans who are buying more locally sourced foods (including seafood). Currently, approximately 70-85% of the seafood (mainly shrimp, salmon, and tuna) consumed in the U.S. is imported from other countries, including India, Indonesia, China, and Ecuador (National Oceanic Atmospheric Association [NOAA], 2021b), which is threatening the long-term viability of the local seafood industry (University of Georgia [UGA] Marine Extension and Georgia Sea Grant, 2021a). In 2017, U.S. seafood imports were valued at $21.5 billion, while U.S. seafood landings and aquaculture had a value of $5.4 and $1.5 billion, respectively (NOAA, 2018). Food innovation can help develop and introduce safe, nutritious, and high-value food ingredients from underutilized seafood commodities such as brown shrimp into the domestic market. Currently, two shrimp species are harvested from Georgia's coast: white shrimp (*Litopenaeus setiferus*) and brown shrimp (*Farfantepenaeus aztecu*s). Georgia harvests between 4.5 and 9.5 million pounds of shrimp annually with white shrimp contributing to approximately two-thirds of the harvest (Rawson, 2003). White shrimp is more valuable and demanded, while brown shrimp is less known due to its smaller size and sweeter taste (Rawson, 2003; UGA Marine Extension and Georgia Sea

Grant, 2021b). Georgia shrimpers have stated (by word of mouth) that brown shrimp's smaller size often contributes to a lower commercial value. Additionally, because the brown shrimp season (June-August) corresponds with part of the spring white shrimp season (Rawson, 2003), it presents an additional reason for not always harvesting the brown shrimp if there is a max processing capability. Therefore, Georgia-caught brown shrimp (GABS) is an underutilized seafood commodity that is typically discarded (often considered a by-catch) due to the higher commercial value of the larger white shrimp and limited processing capability.

Wild-caught shrimp is an excellent source of high-quality protein that can be potentially used to develop several protein-based ingredients (Halim et al., 2016). In the U.S., the protein ingredients market is constantly growing due to the increased demands from health-conscious consumers looking for a wide range of amino acids that perform specific functions in the body, such as providing satiety, energy balance, and lean muscle mass (Grand View Research, 2021a). According to Grand View Research (2021a), the protein ingredients market is projected to reach approximately $85.5 billion by 2028. Additionally, the protein supplements market is projected to reach $36.05 billion by 2028 with protein powders dominating this market (Grand View Research, 2021b). The predicted growth in the market size for protein ingredients and supplements may open opportunities for GABS.

Protein hydrolysates are pre-digested proteins that are rapidly absorbed by the human gastrointestinal (GI) tract and can provide enhanced functional food properties compared to the native protein (Venugopal, 2009). Production of protein hydrolysate powders from several shrimp species, including *Penaeus monodon, Metapenaeus dobsoni,* and *Litopenaeus vannamei* and their by-products have been reported (Dey & Dora, 2014; Gunasekaran et al., 2015; Latorres et al., 2018). These shrimp protein hydrolysates (SPH) powders exhibited enhanced functional

and antioxidant properties that may interest the food industry due to their potential applications in foods (Halim et al., 2016). However, no scientific studies have been reported with GABS. Hence, the overall objective of this research was to investigate the feasibility of developing protein hydrolysate powders from GABS meat. This project is important because it could lead to the development of a safe product that could be accessible to a broad range of consumers. Additionally, it could help increase the Georgia shrimp industry's profits.

This thesis is divided into four chapters. The first chapter comprises the introduction and the literature review of related topics. The second chapter focuses on the characterization of GABS meat, including the proximate composition and the amino acid, fatty acid, and mineral profiles. The third chapter describes the production of SPH powders from GABS meat, which includes the enzymatic hydrolysis procedures, development of the powders via spray drying, and characterization of the resultant powders (proximate composition, functional and antioxidant properties, particle size, and scanning electron microscopy). Finally, the conclusions of this work and recommended future research are presented in the fourth chapter.

The objectives of this research were:

- To characterize the proximate and selected nutritional composition of GABS meat.
- To develop protein hydrolysate powders from GABS meat and to characterize the physicochemical, functional, and antioxidant properties of the powders.

Georgia's shrimp industry

White shrimp is one of Georgia's most valuable seafood commodities. It is more demanded and valuable than brown shrimp which is often smaller (**Figure 1.1**) (Rawson, 2003; UGA Marine Extension and Georgia Sea Grant, 2021b). Georgia shrimpers typically discard brown shrimp (often considered a by-catch) because of the max processing capability and

knowing that they will not contribute to as big a profit as white shrimp. Although brown shrimp is an underutilized seafood commodity in Georgia, it is one of the most popular shrimp species in other parts of the U.S., including Texas and Louisiana (NOAA, 2021a). According to the NOAA (2021a), the U.S. brown shrimp landings were approximately 67 million pounds in 2020, valued at ~$151 million, with most landings coming from the Gulf of Mexico. This presents an additional economic challenge because they are profitable in states near Georgia.

Figure 1.1. Georgia-caught white shrimp (left) and brown shrimp (right) (Anchored Shrimp Co., 2021).

Additionally, shrimping on Georgia's coast has suffered an overall economic decline in the last decades (UGA Marine Extension and Georgia Sea Grant, 2021a). The Georgia shrimp industry had a market value of ~$15.7 million in 2000, which fell to ~ $12.4 million by 2019 (Southern Shrimp Alliance, 2020). Despite fluctuations in these numbers over the past two decades, the overall economic decline is part of a long-term trend for the Georgia shrimp industry. One contributing factor is competition with imported seafood (UGA Marine Extension and Georgia Sea Grant, 2021a). According to the NOAA (2021b), approximately 70-85% of the seafood consumed in the U.S. is imported from other countries, including India, Indonesia, China, and Ecuador. The high consumption of imported seafood in the U.S. undermines

competitiveness and threatens the long-term viability of the local seafood industry (UGA Marine Extension and Georgia Sea Grant, 2021a).

Due to the current market opportunities for powdered protein ingredients and supplements, we hypothesize that GABS meat can be used as raw material for producing protein hydrolysate powders, which may add more value for consumers than whole fresh brown shrimp. Additionally, SPH powders can potentially increase the Georgia shrimp industry's profits due to the expected growth of the protein ingredient and supplement markets.

Brown shrimp nutritional composition

The characterization of the nutritional composition of GABS meat is limited (U.S. Department of Agriculture [USDA], 2019). Hence, there is an opportunity to determine GABS meat's physicochemical and nutritional properties. This research focused on determining GABS meat's proximate and selected nutritional composition. Brown shrimp can also be found in the Gulf of Mexico (NOAA, 2021a). In Louisiana, brown shrimp is widely used as an ingredient in several dishes, including gumbo and jambalaya, due to its unique flavor and texture profile. It has been reported that Gulf brown shrimp is low in calories, carbohydrates, and saturated fats and high in protein (Dupont et al., 2011; USDA, 2019). Brown shrimp is also known for its high contents of vitamin B12, selenium, omega-3 fatty acids, and astaxanthin (Venugopal, 2009). A 100-g serving of Gulf brown shrimp provides 80 calories, 17.86 g protein, 0.89 g unsaturated fat, 66 mg calcium, 197 mg potassium, 446 mg sodium, and 152 mg cholesterol (USDA, 2019).

Proteins

Proteins are made up of amino acids linked together by peptide bonds. Most proteins consist of the same primary 20 amino acids. However, their structures and functions vary due to their amino acid sequence (Alberts et al., 2002). According to Fennema (1996), amino acids can

be classified according to their side chain's degree of interaction with water. Amino acids with aliphatic and aromatic side chains are hydrophobic, limiting their water solubility. While hydrophilic amino acids can be charged (positive or negative) or uncharged (neutral), promoting their water solubility.

According to Sun et al. (2004), protein structure can consist of four levels: primary, secondary, tertiary, and quaternary (**Figure 1.2**). The primary structure (a linear sequence of amino acids linked by peptide bonds) determines the formation of the secondary and tertiary structures and the protein's biological functionality (Alberts et al., 2002; Sun et al., 2004). Meanwhile, the secondary structure consists of repeating arrangements of amino acids within the polypeptide chain that are stabilized by hydrogen bonds between amide hydrogens and carbonyl oxygens of the peptide backbone (Gromiha, 2010). The two common types of secondary structures are alpha helices and beta sheets (Gromiha, 2010; Sun et al., 2004).

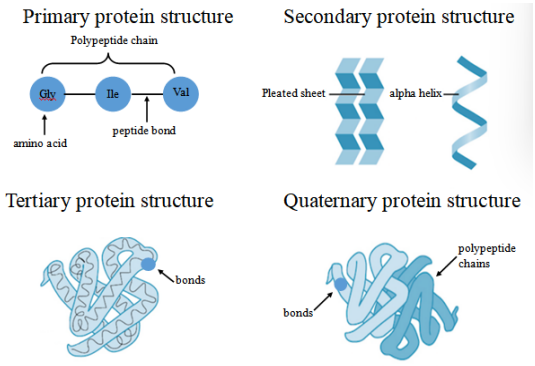

Figure 1.2. Orders of protein structure
(Khan Academy, 2022 with modifications).

The tertiary structure is the three-dimensional form of the linear peptide chain with secondary structure arrangements (Sun et al., 2004). The formation of the tertiary structure is a complex process involving hydrophobic, electrostatic, van der Waals interactions and hydrogen bonding between amino acid side chains. However, the most critical rearrangement that reduces

the free energy is when most of the hydrophobic residues are buried in the interior of the protein, and most of the hydrophilic residues are exposed to the exterior of the protein at the protein-water interface. It should be noted that not all the interior of the protein is hydrophobic, nor is the exterior completely hydrophilic (Fennema, 1996). The quaternary structure consists of two or more folded polypeptide chains (Sun et al., 2004).

A protein's functional properties are dependent on its structure, size, shape, amino acid composition and sequence, net charge and distribution of charges, hydrophobicity/hydrophilicity ratio, molecular flexibility/rigidity, and interactions with other components. A functional property is a physiochemical property that influences protein behavior in food systems during processing and storage due to interactions between protein structure, conformation, and composition. Some protein functional properties include solubility, emulsification, foaming, gelation, water-holding capacity, and water binding. Consumers choose foods based on sensory attributes they enjoy, such as texture, flavor, color, and appearance. Proteins contribute to these sensory attributes by possessing multiple functions. Animal proteins are widely used as additives in foods due to their wide range of functional properties such as gelation, emulsification, foaming, and water-binding (Fennema, 1996). In a later section, functional properties will be discussed more in detail.

Shrimp proteins

Animal proteins include egg, milk, and muscle proteins (Fennema, 1996). Shrimp is considered a muscle protein, consisting of water-soluble sarcoplasmic proteins, salt-soluble myofibrillar proteins, and insoluble stroma proteins (Hashimoto et al., 1979; Ochiai & Ozawa, 2020). Laly et al. (2019) reported myofibrillar protein as the predominant protein component in Flower tail shrimp meat (*Metapenaeus dobsonii*), comprising 76.67% of the total protein content.

Sarcoplasmic protein was the second major protein component comprising 16.82% of the total protein content. Meanwhile, alkali-soluble and stroma proteins comprise 1.52% and 1.02% of the total proteins, respectively (Laly et al., 2019).

Amino acid profile and protein quality of shrimp

Amino acids are divided into essential and non-essential. Essential amino acids are those that the human body cannot synthesize on its own and must be consumed through dietary sources of protein to perform essential functions in the body, such as muscle growth and repair, energy balance, and immune cell function (Dayal et al., 2013; Smith, 2017; Wu, 2009). Therefore, one indicator of dietary protein quality is based on the protein's essential amino acid composition, which is associated with the protein's digestibility, absorption, and bioavailability (Berrazaga et al., 2019; Moore & Soeters, 2015). Furthermore, animal proteins such as shrimp proteins are complete and high-quality proteins because they contain all nine essential amino acids. Meanwhile, plant proteins often lack one or more essential amino acids (Berrazaga et al., 2019; Ensle, 2011). Shrimp is a rich source of essential amino acids (Turan et al., 2011). Turan et al. (2011) reported leucine, lysine, valine, and isoleucine as the primary essential amino acids in Black Sea brown shrimp (*Crangon crangon* L. 1758). For example, leucine, isoleucine, and valine are branched-chain amino acids that regulate muscle and tissue protein synthesis as well as protein degradation (Monirujjaman & Ferdouse, 2014). Lysine is a precursor for synthesizing glutamate, a non-essential amino acid and neurotransmitter (Papes et al., 2001). The amino acid composition of Black Sea brown shrimp is shown in **Table 1.1** (Turan et al., 2011).

Table 1.1. Amino acid composition (g/100 g, d.b.) of brown shrimp (*Crangon crangon* L. 1758).

Essential AA	(g/100g, d.b.)	Non-essential AA	(g/100g, d.b.)
Valine	0.883 ± 0.00	Alanine	1.433 ± 0.00
Leucine	1.483 ± 0.01	Glycine	1.677 ± 0.00
Isoleucine	0.724 ± 0.01	Serine	0.538 ± 0.01
Lysine	1.039 ± 0.02	Proline	0.980 ± 0.00
Methionine	0.455 ± 0.01	Aspartic acid	3.890 ± 0.01
Threonine	0.587 ± 0.01	Hidoksil-L	0.185 ± 0.01
Phenylalanine	0.601 ± 0.01	Glutamic acid	1.956 ± 0.05
Histidine	0.163 ± 0.00	Tyrosine	0.433 ± 0.00
Tryptophan	N.D.		
Arginine	N.D.		
Essential/Non-essential ratio			0.535

Results are mean value of three replicates ± standard error.
d.b. = dry weight basis; AA = amino acid; N.D. = not determined.
Table and data from Turan et al., 2011.

Protein hydrolysis

Hydrolysis involves alteration in the protein's primary amino acid sequence by cleaving peptide bonds (Kilara & Vaghela, 2004). Hence, a hydrolysate is a protein that is broken down into smaller peptides of various sizes (Halim et al., 2016; Venugopal, 2009). Protein hydrolysates are often preferred over their native protein due to being pre-digested and having enhanced functional properties such as solubility, emulsification, and foaming properties (Kilara & Vaghela, 2004; Venugopal, 2009), which will be discussed in more detail in a later section. Many hydrolysis procedures begin with heat treatments, which often result in protein denaturation (Halim et al., 2016; Khan Academy, 2022; Kilara & Vaghela, 2004). Protein denaturation unfolds the protein's globular structure, which exposes previously buried hydrophobic amino acids to the surface (Fennema, 1996; Kilara & Vaghela, 2004). Hydrolysis involves cleaving peptide bonds, which alters the primary amino acid sequence. Additionally, hydrolysis decreases the protein's molecular weight and increases the number of ionizable groups available (Kilara & Vaghela, 2004).

Furthermore, hydrolysis may enhance the antioxidant properties such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and reducing power activity of the resultant products (Intarasirisawat et al., 2012). Lassoued et al. (2015) suggested that the amino acid composition, sequence, and hydrophobicity are associated with a hydrolysate's antioxidant activity. The DPPH radical is oil-soluble, which requires hydrophobic regions that can react with the hydrophobic peroxyl radicals to terminate the chain reaction (Intarasirisawat et al., 2012). Moreover, longer peptides have more hydrophobic groups and are thus more effective at scavenging the DPPH radical. The reducing power activity determines the ability of the protein hydrolysate to reduce ferric to ferrous ions, which seems to be more effective in longer peptides

as well (Intarasirisawat et al., 2012). The recent interest in protein hydrolysates is due to their enhanced functional and antioxidant properties and improved digestibility compared to their native proteins. Therefore, protein hydrolysates with desirable functional properties may have various food applications (Tacias-Pascacio et al., 2020).

Different techniques including enzymatic, chemical, and thermal hydrolysis, can produce protein hydrolysates (Chalamaiah et al., 2010; Halim et al., 2016). Chemical hydrolysis involves the addition of an acid or alkaline reagent to hydrolyze the native proteins (Wisuthiphaet et al., 2015). Acid hydrolysis is a fast and cost-effective hydrolysis method that cannot be easily controlled due to the unspecified cleavage of the peptide bonds. Additionally, acid hydrolysis can result in the production of D-amino acids, which cannot be utilized by humans and animals (Wisuthiphaet et al., 2015). Lastly, essential amino acids such as tryptophan and cysteine may be destroyed during the acid hydrolysis (Jaswal, 1990; Wisuthiphaet et al., 2015).

According to Kilara and Vaghela (2004), enzymatic hydrolysis is a controlled process where enzymes can target specific peptide bonds within a protein, and it is often the preferred method to produce high-quality protein hydrolysates (Chalamaiah et al., 2010; Halim et al., 2016). Enzymes can be isolated from various sources such as plants (papain, ficin), animals (trypsin, pancreatin), or microorganisms (Alcalase, Pronase) (Venugopal, 2009). However, enzyme specificity, enzyme concentration, pH, temperature, and reaction time often affect the yield and quality of the resultant protein hydrolysates (Kilara & Vaghela, 2004).

Enzymes

Enzymes are proteins that catalyze chemical reactions with high selectivity for a limited number of substrates. Enzymes have active sites that are highly specific to the substrate's structure, which allows the reaction to occur (Fennema, 1996; Kristinsson & Rasco, 2000).

According to Fennema (1996), the enzyme binds to the substrate to convert it into a new product, and there are six groups of enzymes (classified based on the reaction that they catalyze). The groups are:

1. Oxidoreductases catalyze oxidation-reduction (redox) reactions.
2. Transferases catalyze group transfer reactions.
3. Hydrolases catalyze hydrolysis reactions using water.
4. Lyases catalyze the breakdown of a chemical bond without using hydrolysis or oxidation. As a result, they often add a group to a double bond or form a double bond.
5. Isomerases catalyze isomerization reactions; and
6. Ligases catalyze condensation reactions.

According to Mótyán et al. (2013), proteolytic enzymes or proteases belong to the hydrolase group and are further classified as peptide hydrolases or peptidases, which can be further divided into exopeptidases and endopeptidases. Exopeptidases cleave peptide bonds near the N- or C-terminal ends of the protein, while endopeptidases cleave peptide bonds of nonterminal amino acids within the polypeptide chain (Mótyán et al., 2013).

As mentioned previously, enzymes are highly specific to a limited number of substrates, which indicates that variation in the enzyme used in an enzymatic hydrolysis procedure can result in a different final product (Tacias-Pascacio et al., 2020). Therefore, choosing an appropriate protease is essential for producing the desired product. Alcalase is one of the most effective proteases for the hydrolysis of various proteins, resulting in products with enhanced functional and antioxidant properties (Tacias-Pascacio et al., 2020). It has been successfully used to produce seafood hydrolysates (Chalamaiah et al., 2010; Intarasirisawat et al., 2012), specifically a white shrimp hydrolysate (Latorres et al., 2018). Alcalase is an alkaline

endopeptidase obtained from the fermentation of *Bacillus licheniformis*. It requires alkaline pH conditions to gain activity and stability. Additionally, Alcalase preferentially cleaves hydrophobic amino acid residues. Alcalase is Novozymes Corporation's registered trademark name (Tacias-Pascacio et al., 2020). Therefore, the Enzyme Development Corporation (EDC)'s alkaline-protease L-660 (equivalent to Novozyme's Alcalase) was used in this study due to previous successful studies.

Production of seafood protein hydrolysates

The production of seafood protein hydrolysates has been recently investigated to add value to underutilized seafood commodities as well as seafood by-products (Halim et al., 2016). Interestingly, by-products from the processing of catfish, tuna, salmon, and shrimp have been used to produce high-quality protein hydrolysates (Randriamahatody et al., 2011; Unnikrishnan et al., 2020; Vázquez et al., 2019; Yin et al., 2010). However, limited studies are available on the production of protein hydrolysates from shrimp meat, and no studies have been conducted on GABS meat. Hence, this research investigated the feasibility of producing SPH powders from GABS meat.

The hydrolysis process must be carefully controlled to produce products with the desired functional and antioxidant properties (Venugopal, 2009). Thus, choosing the appropriate proteolytic enzyme and reaction conditions (pH, temperature, etc.) is critical to reaching the targeted degree of protein hydrolysis (DH) (Venugopal, 2009), which may vary depending on the protein substrate at certain reaction conditions. DH is the percentage of available peptide bonds for hydrolysis, which influences functional and antioxidant properties of the resultant protein hydrolysates (Himonides et al., 2011).

According to Halim et al. (2016), enzymatic hydrolysis generally begins with homogenizing the sample (protein source) and distilled or deionized (DI) water in the desired ratio (e.g. 1:2) and heating for 10-30 min at 80-90°C to inactivate any endogenous enzymes. Then, exogenous proteolytic enzymes are added to the mixture to initiate the hydrolysis reaction at optimum conditions. Enzymes require specific conditions such as enzyme concentrations (0.02-10.00%); pH levels (2.0-10); reaction times (0.5 - >24 h); and incubation temperatures (37-65°C) (**Table 1.2**). Enzymatic hydrolysis is often terminated by inactivating the enzymes via heating between 85-95°C for 5-20 min (Chi et al., 2014; Halim et al., 2016; Latorres et al., 2018; Parvathy et al., 2018).

Functional properties of protein hydrolysates

This study investigated three main functional properties of protein hydrolysates: protein solubility, emulsification, and foaming properties. Protein solubility is the most important functional property as it dramatically influences the emulsification and foaming properties (Parvathy et al., 2018). Protein solubility (in water) is enhanced during hydrolysis because more hydrophilic groups become exposed due to the conversion of some hydrophobic to hydrophilic groups via the formation of two-end carbonyl and amino groups (Ghelichi et al., 2018). Additionally, protein solubility of protein hydrolysates is affected by DH and pH conditions (Ghelichi et al., 2018). Latorres et al. (2018) reported higher protein solubility for white shrimp hydrolysates with 20% DH than 10% DH due to smaller peptides with more ionizable polar groups that can form hydrogen bonds with water. Furthermore, protein solubility in water increases as the pH moves further from the protein's isoelectric point (pI), where there is no net charge. Thus, as pH moves further from the pI, protein-water interactions dominate increasing protein solubility

Table 1.2. Example parameters for enzymatic hydrolysis.

Enzyme	Protein source	Enzyme activity	Enzyme concentration (%, w/w or v/w)	pH	Time (min)	Temperature (°C)	Reference
Alcalase	Herring by-products	2.4 AU/g	0.5	8.0	75	50	Sathivel et al. (2003)
Trypsin	Shrimp head	439.8 U/mg	0.1	8.0	60	50	Limam et al. (2008)
Alcalase Papain	Meriga egg	2.4 AU/g 6000 NF	0.5 0.5	8.0-8.5 6.0-6.5	90	50-55 60-65	Chalamaiah et al. (2010)
Alcalase	Catfish skin	> 0.24 U/g	0.5	8.0	45	50	Yin et al. (2010)
Pepsin Novozym 37020 Protex 6L Devolase	Shrimp by-products	N/A	0.05 0.05 0.10 0.20	2.0 3.0 9.5 10.0	1320	40 50 60 60	Randriamahatody et al. (2011)
Alcalase	Skipjack roe	2.4 units/mL	1 2 5 6 10	8.0	Up to 300	50	Intarasirisawat et al. (2012)
Pepsin	Mackerel skin	2500-3500 U/mg	N/A	2.5	300	37	Chi et al. (2014)

Alcalase	Catfish roe	2.4 units/mL	0.5	8.0	120	50	Binsi et al. (2016)
Papain	Fish frame waste	N/A	0.02 0.13 0.70	6.8	60	50	Gajanan et al. (2016)
Bromelain		3-7 U/mg	0.22 0.91 3.79	6.7	60	50	
Pepsin	Tilapia waste	≥ 250 units/mg	1	7.0	180	37	Tejpal et al. (2017)
Papain	Yellowfin tuna by-products	≥ 4.5 mL of 0.1M NaOH	1	6.5	60	60	Parvathy et al. (2018)
Alcalase Protamex	White shrimp	N/A	1 1	8.0 7.0	N/A N/A	50 50	Latorres et al. (2018)
Papain	Yellowfin tuna by-product	~30,000 USP units/mg	0.35	6.5	30	60	Unnikrishnan et al. (2020)
Alcalase	Different fish species' by-products	N/A	N/A	8.0	30	55	Zamorano-Apodaca et al. (2020)

(Fennema, 1996; Latorres et al., 2018). High protein solubility over a wide range of pH values is essential and desired because it allows for various food and beverage applications (Siddik et al., 2021). Many studies have reported increased protein solubility for protein hydrolysates compared to their native proteins (Binsi et al., 2016; Chalamaiah et al., 2010; Sathivel et al., 2003; Yin et al., 2010). For example, Chalamaiah et al. (2010) reported that meriga egg hydrolysates had at least 72% protein solubility, while the untreated control had 60% or less protein solubility at pH values between 2 and 12. Different protein solubility ranges reported in various studies can be found in **Table 1.3**.

Furthermore, hydrolysis can enhance the emulsification properties of the resultant protein hydrolysates, which can promote higher oil-water interactions due to their high hydrophilic groups that can remain in the water phase and hydrophobic groups that can interact with the oil phase, resulting in a reduced interfacial tension (Binsi et al., 2016; Chalamaiah et al., 2010; Fennema, 1996). The DH of the resultant protein hydrolysates plays an essential role in promoting emulsification properties. For example, a higher DH often decreases emulsification properties (Intarasirisawat et al., 2012). Extensive hydrolysis yields smaller, predominantly hydrophilic peptides that cannot adsorb at the surface (Intarasirisawat et al., 2012). It has been reported that peptides with a minimum length of 20 residues are generally required to exhibit enhanced emulsification properties by lowering interfacial tension between immiscible liquids (Binsi et al., 2016; Chalamaiah et al., 2010; Intarasirisawat et al., 2012). According to Unnikrishnan et al. (2020), peptides of this size exhibit better emulsification activity than native proteins due to the unfolding of the globular structure, increasing the hydrophobicity and subsequent interaction with oil droplets. Latorres et al. (2018) reported that white shrimp hydrolysates with 10% DH had higher emulsification properties than those with a 20% DH.

Table 1.3. Functional properties of seafood by-product hydrolysates.

Seafood species	Body part	Functional properties					Reference
		Protein solubility (%)	EAI (m^2/g)	ESI	FC (%)	FS (%)	
Herring (*Clupea harengus*)	Head, gonad	56.0-85.1	-	48.6-54.2%	-	-	Sathivel et al. (2003)
Shrimp (*Penaeus kerathurus*)	Head	-	-	-	1-1.2	-	Limam et al. (2008)
Meriga (*Cirrhinus mrigala*)	Roe	72-~88	-	-	25, 70	40, 50	Chalamaiah et al. (2010)
Channel Catfish (*Ictalurus punctus*)	Skin	99.8	-	79-99%	-	-	Yin et al. (2010)
Skipjack (*Katsuwonous pelamis*)	Roe	82-99	5.1-25.16	14.2-24.3 min	-	-	Intarasirisawat et al. (2012)
Spanish mackerel (*Scomberomorous niphonius*)	Skin	65.98-99	32.87-97.44	-	32.76-65.27	26.72-54.16	Chi et al. (2014)
Engraved catfish (*Nemapteryx caelata)*	Roe	97.64-99.15	-	0.28-0.41 min	30	16.7-83.3	Binsi et al. (2016)
Threadfin breams (*Nemipterus japonicus*)	Fish frame waste	87.64-97.35	~15-32	~35-90 min	~50-105	~38-100	Gajanan et al. (2016)

Tilapia (*Oreochromis niloticus*)	Fish waste	-	12-14	65-70%	76.36-94.61	58.36-66.15	Tejpal et al. (2017)
White shrimp	Meat	~45-95	9.3-81.5	0.2-9.9 min	6.5-28.8	63.6-88.3	Latorres et al. (2018)
Yellowfin tuna (*Thunnus albacares*)	White /red meat	86.53-88.74	13.85-15.04	31.39-38.71 min	126.7-150	36.7-40	Parvathy et al. (2018)
Yellowfin tuna (*Thunnus albacares*)	Red meat	-	-	98.65-99.75%	-	-	Unnikrishnan et al. (2020)
Mixed fish species	Skins, heads, skeletons	~75-99	70-130	10-50 min	42-100	10-78	Zamorano-Apodaca et al. (2020)

EAI = emulsifying activity index; ESI = emulsion stability index; FC = foaming capacity; FS = foaming stability.

Many studies measure emulsification properties by determining the emulsifying activity index (EAI) and emulsion stability index (ESI) (Gajanan et al., 2016; Intarasirisawat et al., 2012; Latorres et al., 2018; Parvathy et al., 2018; Tejpal et al., 2017; Zamorano-Apodaca et al., 2020). Ranges for EAI and ESI reported in various studies are summarized in **Table 1.3**.

Lastly, hydrolysis can also improve foaming properties. Foam is an immiscible system where water is the continuous phase and air/gas is the dispersed phase (Fennema, 1996). Proteins can reduce interfacial tension at the air/gas-water interface due to their ability to form films (Tejpal et al., 2017). In addition, hydrolysis exposes more hydrophobic groups of peptides, which can orient towards the air/gas phase while hydrophilic regions remain in the water phase, stabilizing the system (Limam et al., 2008). Latorres et al. (2018) reported an increase in foaming properties for white shrimp hydrolysates with 10% DH compared to 20% DH. Protein hydrolysates with lower DH contain higher molecular weight peptides and can envelop and retain air via forming a cohesive interfacial film (Latorres et al., 2018; Villamil et al., 2017). Many studies measure foaming properties by determining the foaming capacity (FC) and foaming stability (FS) (Binsi et al., 2016; Chalamaiah et al., 2010; Chi et al., 2014; Gajanan et al., 2016; Latorres et al., 2018; Parvathy et al., 2018; Tejpal et al., 2017; Zamorano-Apodaca et al., 2020). Ranges for FC and FS reported in various studies are summarized in **Table 1.3**.

Production of hydrolysate powders via spray drying

According to Haque and Adhikari (2015), hydrolysate solutions can be kept as a liquid or transformed into powder. Drying of liquid protein hydrolysates promotes ease of handling and increases stability. Spray drying and freeze drying are among the most popular methods to produce protein hydrolysate powders. However, spray drying is more cost-effective than freeze drying to produce dry powdered ingredients.

Spray drying allows the liquid feed to be rapidly transformed into dried particles via moisture evaporation. Generally, the spray drying of a liquid feed involves pumping the liquid feed through an atomizer, which breaks it up into a mist of fine droplets. Then, the fine droplets enter the drying chamber containing hot air, which evaporates the liquid and produces dried particles within seconds. These dry particles are then carried into the cyclone, and the powder can be collected in the collector. Additionally, the inlet and outlet temperatures, flow rate, and airflow pattern are essential process parameters in spray drying. However, the most critical factor for controlling the drying rate and particle characteristics (particle shape and residual moisture content) is the drying temperatures (Haque & Adhikari, 2015).

Recent studies reported the production of fish protein hydrolysate (FPH) powders from red and white tuna meat and tilapia waste via spray drying (Parvathy et al., 2018; Tejpal et al., 2017; Unnikrishnan et al., 2020). Interestingly, these FPH powders exhibited enhanced functional and antioxidant properties compared to their native proteins (Parvathy et al., 2018; Tejpal et al., 2017; Unnikrishnan et al., 2020). For example, Unnikrishnan et al. (2020) reported that tuna protein hydrolysate may potentially be used to replace egg yolk in mayonnaise and serve as an emulsifier (Unnikrishnan et al., 2020).

Because proteins can be affected when exposed to high temperatures, this study investigated the effect of different spray drying contact configurations (mixed-flow vs. concurrent) on the quality of the resultant protein hydrolysate powders. The main difference between the two configurations is when the droplets are exposed to hot air (Jiang et al., 2020). Mixed-flow (MX) spray drying configuration is when the liquid feed is introduced from the bottom while the drying air is introduced from the top of the drying chamber which takes longer for the droplets to be exposed to the hot air (Jiang et al., 2020). Concurrent (CC) spray drying

configuration is when the liquid feed and drying air are both introduced from the top of the drying chamber, which causes the droplets to be immediately exposed to the hot air (Jiang et al., 2020). Jiang et al. (2020) demonstrated that MX configuration was a more gentle and effective drying process to produce microencapsulated probiotic powders coated with fish gelatin, which are sensitive to heat. However, since there are limited studies on the production of SPH powders, this study investigated which configuration was more effective for drying GABS meat protein hydrolysates.

Food applications of protein hydrolysates

According to Grand View Research (2020), applications for FPH powders include pharmaceuticals, food, cosmetics, and animal feed and pet food. The current predominant market for FPH powders is for animal feed and pet food, which have a low market value (Chalamaiah et al., 2012; Grand View Research, 2020). Nevertheless, the FPH powders market for human consumption is projected to grow due to an increased demand for protein-based products driven by the expected increase in the world population as well as health-conscious consumers becoming more aware of the role protein plays in their health and well-being (Henchion et al., 2017). According to Grand View Research (2020), FPH powders could also be utilized in infant formulations, sports nutrition, protein ingredients, and protein supplements.

Furthermore, many researchers have begun investigating FPH powders for human consumption to increase the market value of underutilized seafood commodities. Some recently investigated food applications for FPH powders include fish tofu, extruded snack, biscuit, and mayonnaise (discussed in the previous section) (Jeyakumari et al., 2016; Ketnawa et al., 2016; Sinthusamran et al., 2019; Unnikrishnan et al., 2020). For example, Ketnawa et al. (2016) investigated SPH powders in fish tofu, an emulsion-type meat product. Tofu is susceptible to

lipid oxidation because it contains oil and is typically fried before packing and storing. Therefore, tofu requires an antioxidant to prevent lipid oxidation from deteriorating nutritional and sensory quality (Ketnawa et al., 2016). In addition, proteins and peptides may exhibit antioxidant activity that is enhanced during the hydrolysis procedure. Hence, Ketnawa et al. (2016) added SPH powder to fish tofu to serve as a natural antioxidant and discovered that 2% (w/w) SPH powder helped improve the toughness and extended the shelf-life of fish tofu. Moreover, Sinthusamran et al. (2019) evaluated the chemical, physical, rheological, and sensory properties of a biscuit fortified with different amounts of SPH powder. They observed that when 5% SPH powder was used to substitute flour in a biscuit, it had increased sensory properties and higher nutritional value, suggesting that SPH powders could successfully be used for biscuit fortification (Sinthusamran et al., 2019). The potential applications of SPH powders in consumer goods (especially foods) are endless but require extensive research (Intarasirisawat et al., 2012; Kilara & Vaghela, 2004; Venugopal, 2009).

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CHAPTER 2

CHARACTERIZATION OF GEORGIA-CAUGHT BROWN SHRIMP (*Farfantepenaeus aztecus*) MEAT

Introduction

Shrimp is the most consumed seafood in the U.S. (National Oceanic Administration Association [NOAA], 2020). However, approximately 70-85% of the shrimp consumed in the U.S. is imported from other countries (NOAA, 2021b). High seafood imports threaten the long-term viability of the domestic seafood industry (University of Georgia [UGA] Marine Extension and Georgia Sea Grant, 2021a). In Georgia, two shrimp species are harvested: white shrimp (*Litopenaeus setiferus*) and brown shrimp (*Farfantepenaeus aztecu*s). However, white shrimp is more valuable and demanded (Rawson, 2003; UGA Marine Extension and Georgia Sea Grant, 2021b). In contrast, brown shrimp often has a lower commercial value than white shrimp due to its smaller size. Therefore, Georgia shrimpers regularly discard brown shrimp (often considered a by-catch). Since Georgia-caught brown shrimp (GABS) is an underutilized seafood commodity with potential food uses, it is necessary to understand its nutritional properties. Nevertheless, the scientific literature regarding its nutritional characterization is minimal. Hence, our team hypothesized that GABS meat is a good source of protein that can be used to develop high-quality protein-based ingredients.

Although brown shrimp are underutilized in Georiga, Gulf brown shrimp are one of the most popular shrimp species in Texas and Louisiana (NOAA, 2021a). Gulf brown shrimp is appreciated for its unique texture, flavor profiles, and perceived nutritional value. The nutritional

composition of Gulf brown shrimp has been characterized (Menard et al., 2015; U.S. Department of Agriculture [USDA], 2019). According to the USDA (2019), Gulf brown shrimp is high in protein but low in calories, carbohydrates, and saturated fats. According to Menard et al. (2015), Louisiana's brown shrimp contain a crude protein of 19.23% (wet basis, w.b.). This may indicate that GABS meat is a good source of protein that could be used as raw material to produce protein hydrolysate powders, which have been recently investigated to add value to underutilized seafood commodities and their by-products (Halim et al., 2016). Ideal raw materials for fish protein hydrolysates (FPH) include lean seafood species or by-products (Venugopal, 2016). According to Venugopal (2016), FPH contain 81-93% (dry basis, d.b.) crude protein and <5% (d.b.) crude fat. Menard et al. (2015) reported that Gulf brown shrimp meat had 76.81% (w.b.) moisture, which indicates that the crude protein and fat were 82.92% (d.b.) and 4.05% (d.b.), respectively. Based on these results, GABS meat has the potential to serve as a suitable raw material for producing protein hydrolysate powders. In general, shrimp is high in cholesterol, which concerns many consumers who believe there is a direct link between dietary cholesterol and cardiovascular disease (CVD) (Dayal et al., 2013). However, it is more important to consider the food's overall lipid and fatty acid profiles when concerned about dietary cholesterol and its effects on our overall health (Dayal et al., 2013). Foods with high amounts of saturated fats are more likely to raise low-density lipoprotein (LDL) cholesterol, associated with the increased risk for diseases such as CVD (Fielding et al., 1995). Shrimp has been reported as being low in saturated fats and rich in polyunsaturated fatty acids (PUFAs), linked to promoting health (Dayal et al., 2013; Pires et al., 2018).

The exact nutritional composition of wild-caught shrimp may be affected by several factors, including the diet, environment/habitat, harvesting season, water salinity and

temperature, and life stage of the same and/or different harvested shrimp species (Ockerman, 1992; Tropea et al., 2015). Hence, it is essential to characterize the nutritional composition of GABS meat to understand how this underutilized seafood commodity could be utilized as an ingredient in foods. Therefore, the objective of this study was to characterize GABS meat's proximate and selected nutritional (amino acid, fatty acid, and mineral profiles) composition.

Materials and Methods

Materials

GABS was supplied by Georgia shrimpers in Brunswick, GA. Fresh brown shrimp were caught in the Summer of 2021 and immediately frozen. Then, the fresh-frozen brown shrimp was shipped to the UGA Food Science and Technology facilities in Griffin, GA, where it was kept frozen until needed for analyses.

Before being used for analyses, fresh-frozen shrimp were thawed overnight in the refrigerator or under running tap water. Then, the heads, shells, and intestines were manually removed to obtain the GABS meat, which was then washed under running tap water and patted dry before analyses. Shrimp were kept on ice during the preparation step.

Prior to measuring water activity, moisture, and ash content analyses, shrimp meats were chopped and homogenized. Before determining crude protein and fat, amino acid, fatty acid, and mineral profiles, GABS meat was freeze-dried in a pilot-scale freeze-dryer (Virtis, The Virtis Company, Gardiner, NY, USA) and ground into a powder. L-aspartic acid and petroleum ether were purchased from Millipore Sigma (St. Louis, MO, USA).

Proximate composition

Water activity and moisture content

The water activity of GABS meat was determined by using an Aqualab water activity meter (Model Series 3 T.E., Decagon Devices, Inc., Pullman, WA, USA). Moisture content was determined by following the Association of Official Analytical Collaboration (AOAC) International Official Method 934.01 (oven drying) (AOAC International, 2019a; AOAC International, 2020) using an Isotemp® vacuum oven (Model 281A, Thermo Fisher Scientific, Waltham, MA, USA). Briefly, samples were weighed (~2 g), placed in the vacuum oven, and left overnight for 24 h at 70°C. Then, the dried sample and container were weighed, and the moisture content was calculated using Eq. (1) and (2).

$$\mathrm{MW} = [\mathrm{SW} - (\mathrm{DC} - \mathrm{EC})] \quad (1)$$

$$\text{Moisture content } (\%) = \frac{\mathrm{MW}}{\mathrm{SW}} * 100 \quad (2)$$

where MW = moisture weight, SW = sample weight, DC = dried sample + container weight, and EC = empty container weight.

Ash content

Ash content was determined by following the AOAC International Official Method 938.08 (furnace combustion) (AOAC International, 2019b) using a muffle furnace (Model F-A170, Thermolyne, Dubuque, IA, USA). Briefly, ~2 g of sample was weighed in ceramic crucibles and placed into the muffle furnace set to 550°C for 12-18 h. The next day, samples were cooled in a desiccator, weighed, and the ash content was calculated using Eq. (3).

$$\text{Ash content } (\%) = \frac{\text{weight of sample after ashing}}{\text{weight of original sample}} * 100 \quad (3)$$

Crude protein content

The crude protein of powdered samples was determined by following the AOAC International Official Method 993.13 (AOAC International, 2019c). An automated nitrogen and protein analyzer (Rapid MAX N Exceed, Elementar, Langenselbold, Germany) was used to determine the total nitrogen content by the Dumas method using dry combustion described by Jung et al. (2003). A conversion factor of 6.25 was used to determine the crude protein content (Pires et al., 2018) then converted to w.b. using the moisture content. L-aspartic acid was used as the nitrogen calibration standard.

Crude fat content

Crude fat was determined following the American Oil Chemists' Society (AOCS) Am 5-04 Official Crude Fat Extraction Method, which uses the Soxhlet principle (AOCS, 2017). Approximately ~1.5-2 g of powdered sample was placed into a fat extraction pouch and baked in a Thelco laboratory oven (Model No. 31620 Jouan, Inc., Saint-Herblain, France) at 105°C for 3 h. After 3 h, samples and pouches were weighed again. Samples were then placed in the ANKOM^{XT15} extractor (XT15, ANKOM Technology, Macedon, NY, USA) using petroleum ether as the extraction solvent for 1 h at 90°C. After 1 h, samples were baked at 105°C for 20 min, re-weighed, and the crude fat content was calculated using Eq. (4) then converted to w.b. using the moisture content.

$$\text{Crude fat (\%)} = \frac{100 * (W2 - W3)}{W1} \quad (4)$$

where W1 = original weight of the sample, W2 = weight of the pre-extraction dried sample + pouch, and W3 = weight of the post-extraction dried sample + pouch.

Other nutrient analysis

Amino acid analysis

Amino acid analysis was conducted at the Louisiana State University (LSU) Agcenter's Biotechnology Lab in Baton Rouge, LA using High-Performance Liquid Chromatography (HPLC) (Ultimate 3000 system, Dionex, Sunnyvale, California). Briefly, 1 g of powdered sample was hydrolyzed in 7 mL 6N HCl containing 0.25% phenol to hydrolze the protein into its individual amino acids. After the 24-h hydrolysis at 110°C, 10 µL of hydrolysate solution was mixed with 20 µL of the internal standard, norleucine, and dried down. Then, 100 µL of PITC solution (EtOH: water: Phenyl isothiocyanate: triethylamine = 7:1:1:1) was added to the residue and mixed for pre-column derivatization then freeze-dried. The derivatized residue was dissolved into 1 ml of buffer (140 mM sodium acetate, 0.05% triethylamine, titrated to pH 6.40 with glacial acetic acid with the addition of 60 ml/L acetonitrile) and filtered with 0.2 µm filter to obtain the injection sample for HPLC, which was used to separate the free amino acids so that they could be detected and quantified (Smith, 2017). The results were reported in g/100 g sample.

Fatty acid analysis

The fatty acid profile of GABS meat was determined at the UGA Soil, Plant, and Water Lab in Athens, GA, following the method described by Saha and Jackson (2017). Briefly, 0.88-0.96 g of oil was extracted from 50 g of a powdered sample via the Soxhlet procedure. The fatty acids were methylated using methanolic potassium hydroxide. Fatty acids were determined with the upper organic layer using an Agilent 7890 gas chromatograph equipped with an SGE BPX70 capillary column and a flame ionization detector. The results are reported as % of the total area covered by individual fatty acids in the chromatograph.

Mineral analysis

Mineral analysis of GABS meat was determined by the UGA Soil, Plant, and Water Lab in Athens, GA using a Spectro Arcos inductively coupled plasma mass spectrometer (Model FHS16, Ametek Inc., Berwyn, PA, USA) via the inductively coupled plasma – mass spectrometry (ICP – M.S.) method. A CEM Mars5 microwave-acid (HNO_3) digestion system (Model 61E ICP, Thermo Jarrell-Ash, Franklin, MA, USA) was used to quantify the minerals Ca, K, Mg, P, N, S, Al, B, Cd, Cr, Cu, Fe, Mn, Mo, Na, Ni, Pb, and Zn.

Statistical analysis

All analyses were conducted at least in triplicate determinations. In addition, the mean and standard deviations were calculated in a Microsoft Excel® Spreadsheet and reported.

Results and Discussion

Proximate composition

Water activity, moisture, and ash contents

The proximate composition of GABS meat (**Table 2.1**) showed that it had a high a_w (0.99), which is common in fresh seafood (USDA, 1995). Foods with high a_w values are prone to a quick deterioration. They may require freezing, dehydration, cooking, or other processing steps to extend their shelf life (Mauer & Bradley, 2017; USDA, 1995). Additionally, fresh fish and shrimp have a high moisture content (Pires et al., 2018). In this study, GABS meat had a moisture content of 76.76% (w.b.) (**Table 2.1**). Menard et al. (2015) reported similar results (76.81%, w.b.) for Gulf brown shrimp. Additionally, Pires et al. (2018) reported moisture contents of 75.65% and 77.95% (w.b.) for Atlantic white shrimp. Another essential component for a complete proximate composition analysis is the ash content, which accounts for the inorganic compounds present in foods (Mauer & Bradley, 2017). The typical ash content

Table 2.1. Proximate composition of GABS meat[†].

Description	Values (w.b.)	Values (d.b.)
Water activity (a_w)	0.99 ± 0.00	N.D.
Moisture content (%)	76.76 ± 0.43	330.51 ± 7.32*
Ash content (%)	1.55 ± 0.09	6.67 ± 0.38
Crude protein (%)	21.26 ± 0.11	91.49 ± 0.49
Crude fat (%)	0.64 ± 0.16	2.77 ± 0.48

[†]Values are reported as mean ± standard deviation of at least triplicate determinations.
GABS = Georgia-caught brown shrimp; w.b. = wet weight basis; d.b. = dry weight basis; N.D. = not determined.

*$\text{Moisture content (d.b.)} = \frac{\%\ \text{moisture content (w.b.)}}{(100 - \%\text{moisture content (w.b.)})} * 100$ (Singh & Heldman, 2009).

in foods varies from 0-12% (w.b.). More specifically, the ash content is typically <5% (w.b.) in fresh foods (Mauer & Bradley, 2017). The ash content of GABS meat was 1.55% (w.b.) (**Table 2.1**). These results agree with those reported by Menard et al., (2015), who reported an ash content of 1.69 % (w.b.) for the Gulf brown shrimp. Meanwhile, Pires et al. (2018) reported ash contents between 1.24% and 1.93% (w.b.) for Atlantic white shrimp.

Crude protein content

GABS meat had a crude protein content of 21.26% (w.b.) (**Table 2.1**). Wild-caught shrimp is an excellent source of high-quality protein. Dietary protein is essential for a well-balanced and healthy diet (U.S. Food and Drug Administration, 2021). Menard et al. (2015) reported crude protein content of 19.23% (w.b.) for Gulf brown shrimp. Additionally, Pires et al. (2018) determined that Atlantic white shrimp had crude protein contents between 17.74% and 18.99% (w.b.). Not surprisingly, the results obtained in this study suggest that GABS meat is an excellent source of protein. It is common to observe minor differences in the proximate composition of shrimp (within the same species and in different species) due to differences in diet, quality, temperature and salinity of the water, season, and growth stage (Ockerman, 1992; Pires et al., 2018; Turan et al., 2011). These differences may influence the nutritional value, texture, sensory attributes, and shelf-life of the shrimp (Pires et al., 2018; Saldanha et al., 2008; Sriket et al., 2007). It is important to note that crude protein differs from the true protein content of a sample. The main difference is that crude protein measures all nitrogen in a sample, which may not all be from protein and is then multiplied by a protein conversion factor. While true protein is a measure of the difference between the total nitrogen and non-protein nitrogen multiplied by a protein conversion factor (Dupont et al., 2011). This suggests that the crude protein content reported in this study and other studies may be higher than the true protein

content of shrimp. Nevertheless, shrimp is considered an excellent protein source due to its high crude protein content (Liu et al., 2021).The primary purpose for determining the proximate composition of GABS meat in this study was to understand the nutritional properties of GABS meat better and design a way to add value to this underutilized seafood commodity. Therefore, GABS meat could be used to produce value-added products such as protein hydrolysates and bioactive peptides (Kristinsson & Rasco, 2000).

Crude fat content

Shrimp are conventionally low in fat, specifically saturated fat (Dayal et al., 2013). GABS meat had a 0.65% (w.b.) crude fat content (**Table 2.1**), which is low compared to other seafood commodities such as catfish (2.02%, w.b.) (Binsi et al., 2016). The crude fat content found in this study was also slightly lower than what Menard et al. (2015) reported for Gulf brown shrimp (0.95%, w.b.). It has been reported that Atlantic white shrimp has crude fat contents between 0.86-1.40% (w.b.) (Pires et al., 2018). As previously mentioned, it is common to observe variations in the proximate composition between the same shrimp species as well as different shrimp species due to various factors previously mentioned (Ockerman, 1992; Pires et al., 2015; Turan et al., 2011). Nevertheless, the low crude fat content makes GABS meat an ideal raw material for producing protein hydrolysates (Kristinsson & Rasco, 2000).

Other nutrient analysis

Amino acid analysis

The amino acid composition of GABS meat is presented in **Table 2.2**. GABS meat's most abundant amino acids were asparagine/aspartic acid, glutamine/glutamic acid, glycine, arginine, leucine, and lysine, which comprise around half of the total amino acids. Turan et al. (2011) reported similar abundant amino acids in Black Sea brown shrimp (*Crangon crangon* L. 1758).

Table 2.2. Amino acid composition (g/100 g, d.b.) of GABS meat[†].

Amino Acid	**Quantity (g/100 g)**
Asparagine and aspartic acid (Asx)*	7.24 ± 0.49
Glutamine and glutamic acid (Glx)*	12.77 ± 0.31
Serine (Ser)	2.88 ± 0.14
Glycine (Gly)	7.38 ± 0.12
Histidine (His)	1.11 ± 0.02
Arginine (Arg)	9.39 ± 0.30
Threonine (Thr)	2.72 ± 0.08
Alanine (Ala)	4.13 ± 0.22
Proline (Pro)	2.57 ± 0.10
Tyrosine (Tyr)	2.50 ± 0.07
Valine (Val)	3.30 ± 0.10
Methionine (Met)	2.29 ± 0.09
Cysteine (Cys)	0.26 ± 0.03
Isoleucine (Ile)	2.78 ± 0.10
Leucine (Leu)	5.76 ± 0.17
Phenylalanine (Phe)	3.32 ± 0.12
Lysine (Lys)	6.19 ± 0.23
Tryptophan (Trp)	N.D.**
Total	76.60 ± 1.78
Total essential amino acids	27.47
Total non-essential amino acids	49.12
Essential/non-essential ratio	0.56

[†]Values are reported as mean ± standard deviation of triplicate determinations.
GABS = Georgia-caught brown shrimp; d.b. = dry weight basis.
*Asparagine and aspartic acid were determined together; glutamine and glutamic acid were determined together.
**Tryptophan was N.D. (not determined) due to being destroyed during acid hydrolysis.
Bold type indicates essential amino acid.

According to Sikorski et al. (1990), glycine, alanine, serine, and threonine contribute to the sweetness found in some crustaceans. Meanwhile, arginine, leucine, valine, methionine, phenylalanine, histidine, and isoleucine are associated with bitter taste. GABS meat has been reported as being sweet in taste, which may be due to the relatively high content of glycine in comparison to other amino acids (Rawson, 2003; Sikorski et al., 1990; UGA Marine Extension and Georgia Sea Grant, 2021b).

Shrimp is an excellent source of complete protein because it contains all nine essential amino acids (Ensle, 2011). Essential amino acids must be consumed through food because the human body cannot synthesize them on its own, while non-essential amino acids can be synthesized by the human body (Dayal et al., 2013; Smith, 2017). Therefore, it is crucial to determine the essential and non-essential amino acids in a protein source. GABS meat contained eight of the nine essential amino acids (**Table 2.2**). Tryptophan, an essential amino acid, was not determined in this study due to being destroyed during acid hydrolysis. Acid hydrolysis is commonly used to hydrolyze the protein into its individual amino acids, which is necessary for their quantification (Smith, 2017). According to Dayal et al. (2013), the mean tryptophan content in two shrimp species (*Penaeus monodon* and *Fenneropenaeus indicus*) was 0.223 g/100 g (d.b.).

Protein quality is influenced by multiple factors that have been extensively debated for decades. Two important factors of protein quality are the protein's characteristics and the food matrix that the protein is consumed as well as the metabolic demands of the individual (Millward et al., 2008). The body requires adequate amounts of essential amino acids as well as non-essential amino acids per day to function properly and maintain nitrogen balance (World Health Organization [WHO], 2007). Nitrogen balance occurs when nitrogen intake is equivalent to nitrogen output. The intake of essential amino acids determines the minimum nitrogen intake for

nitrogen balance (WHO, 2007). Therefore, it is important to determine if a protein source supplies adequate amounts of essential amino acids. WHO joined with the Food and Agriculture Organization (FAO) and United Nations University (UNU) to publish the essential amino acid requirements for different age groups, one being healthy adults (WHO, 2007). These requirements are based on nitrogen balance studies conducted on men and women (Hegsted, 1963; Irwin & Hegsted, 1971; Rose, 1957). The amino acid reference scores can then be used to calculate the Protein Digestibility-Corrected Amino Acid Score (PDCAAS) or Digestible Indispensable Amino Acid Score (DIAAS) of dietary protein, which are based on fecal or ileal digestibility, respectively (FAO, 2013; WHO, 2007). PDCAAS is based on human requirements and estimates of the protein's digestibility of an amino acid reference pattern depending on age group. It helps predict net protein utilization, which is predicted by the protein's digestibility and amino acid profile. These characteristics can then be used to determine if minimum amino acid requirements have been met for nitrogen balance in humans, which indicates the dietary protein's nutritional quality (Millward et al., 2008; WHO, 2007). Hence, it is difficult to measure GABS meat's protein quality without measuring the digestibility of each of its essential amino acids, which is outside the scope of this study. However, Dayal et al. (2013) reported a PDCAAS of one (the highest value) for shrimp. This indicates that shrimp has superior protein quality because it can provide 100% of the demand for essential amino acids in the human body (Dayal et al., 2013; FAO, 2013). Milk and egg proteins also have a PDCAAS of one. Animal proteins such as milk, egg, and shrimp have high PDCAAS because they are complete proteins that contain enough of all nine essential amino acids. While plant proteins typically have a PDCAAS less than one because they lack one or more essential amino acids. For example, black beans have a PDCAAS of 0.75 (Hoffman & Falvo, 2004). The exception is the plant protein, soy,

which has a PDCAAS of one because it contains all essential amino acids. However, there are limitations with PDCAAS for determining protein quality since it does not consider ileal digestibility, which is why the newer method, DIAAS, was developed (Hoffman & Falvo, 2004). Limited dietary protein qualities have been determined with DIAAS because it is a relatively newer method for measuring protein quality based on the ileal digestibility of pigs, which is why PDCAAS was discussed.

Furthermore, GABS meat's essential/non-essential amino acid ratio was 0.56 (total essential amino acids divided by total non-essential amino acids) (**Table 2.2**). Similarly, Turan et al. (2011) determined the essential/non-essential amino acid ratio of Black Sea brown shrimp to be 0.535. Yanar and Celik (2006) reported green tiger shrimp (*Penaeus semisulcatus*) and speckled shrimp (*Metapenaeus monoceros*) essential/non-essential amino acid ratios between 0.58-0.63 and 0.57-0.62, respectively. In comparison, Sriket et al. (2007) reported ratios for white shrimp (*Penaeus vannamei*) and black tiger shrimp (*Penaeus monodon*) to be 0.67 and 0.70, respectively. These ratios may vary slightly depending on the harvest season (Yanar & Celik, 2006). Additionally, the essential/non-essential amino acid ratios found in different shrimp species were typically lower than other dietary proteins. Wu et al. (2016) reported the amino acid composition of beef rounds, and their essential/non-essential amino acid ratio was calculated and found to be 0.83. Meanwhile, Iwasaki and Harada (1985) reported ratios of 0.76 and 0.93 for crab and squid, respectively. Nevertheless, shrimp contain a well-balanced amino acid composition and are an excellent source of amino acids (Iwasaki & Harada, 1985).

Fatty acid analysis

The fatty acid composition of GABS meat is summarized in **Table 2.3**. GABS meat contained fatty acids with medium and long carbon chain lengths that ranged from C12 to C24.

Table 2.3. Fatty acid composition (%) of GABS meat[†].

Fatty Acid Methyl Esters	**Quantity**
Lauric Acid (C12:0)	0.02 ± 0.00
Myristic Acid (C14:0)	1.07 ± 0.03
Palmitic Acid (C16:0)	18.64 ± 0.42
Palmitoleic Acid (C16:1)	6.11 ± 0.11
Heptadecanoic Acid (C17:0)	2.92 ± 0.08
Heptadecenoic Acid (C17:1)	1.50 ± 0.10
Stearic Acid (C18:0)	12.98 ± 0.06
Oleic Acid (C18:1)	7.35 ± 0.08
Linoleic Acid (C18:2n6)*	1.40 ± 0.05
α-Linolenic Acid (C18:3n3)*	0.39 ± 0.02
Arachidic Acid (C20:0)	0.19 ± 0.02
Eicosadienoic Acid (C20:2)	0.29 ± 0.02
Erucic Acid (C22:1)	1.05 ± 0.15
Lignoceric Acid (C24:0)	0.75 ± 0.02

Total SAFA	36.57
Total USFA	
Likely USFA**	45.34
Total MUFA	16.01
Total PUFA	2.08
Total ω-3*	0.39
Total ω-6*	1.69
ω-6/ω-3	4.33
Total identified	54.66
Total	100

[†]Values are reported as mean ± standard deviation of six replicates.
Results are reported as a percentage of total fatty acids.
GABS = Georgia-caught brown shrimp; SAFA = saturated fatty acids; USFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.
*= essential fatty acid.
**Most of the unsaturated fatty acids were unable to be identified. Based on experience and literature, it is likely that the rest of the fatty acids in GABS meat are unsaturated fatty acids (Yerlikaya et al., 2013).

Interestingly, the most abundant saturated fatty acids were palmitic acid (C16:0) and stearic acid (C18:0). The most abundant monounsaturated fatty acids were oleic acid (C18:1) and palmitoleic acid (C16:1). Meanwhile, the most abundant PUFA was linoleic acid (C18:2n6). Overall, GABS meat contained 34 fatty acids with more unsaturated than saturated fatty acids. However, most of the unsaturated fatty acids could not be identified and are therefore not included in **Table 2.3**. Turan et al. (2011) reported similar fatty acid results for Black Sea brown shrimp. Additionally, Yerlikaya et al. (2013), reported similar findings for nine shrimp species from deep and shallow waters of the Gulf of Antalya (Turkey's Western Mediterranean coast). They reported ~29-36% saturated fatty acids and ~52-79% unsaturated fatty acids for the nine shrimp species from deep and shallow waters (Yerlikaya et al., 2013), which is why it is highly likely that the rest of the unidentified fatty acids are unsaturated in GABS meat. Meanwhile, the fatty acid profile of GABS meat varies slightly from those reported in Menard et al. (2015) for Gulf brown shrimp. It has been reported that the fatty acid profile of shrimp can vary due to the depth of the water that the shrimp originates (Menard et al., 2015; Yerlikaya et al., 2013). According to Yerlikaya et al. (2013), higher concentrations of PUFAs such as linoleic acid, α-linolenic acid, and eicosadienoic acid may be seen in shrimp harvested from shallower waters, which may be the reason Gulf brown shrimp had higher concentrations of PUFAs compared to GABS meat.

Additionally, GABS meat contains the two essential fatty acids, α-linolenic acid, an ω-3 fatty acid, and linoleic acid, an ω-6 fatty acid. These fatty acids must be consumed through our food because the human body cannot synthesize them (Simopoulos, 2002). Additionally, an adequate ω-6/ω-3 ratio is critical in the diet for optimal health and prevention of various diseases such as cardiovascular disease (CVD) (Pires et al., 2018; Simopoulos, 2002). The recommended ratio for ω-6/ω-3 varies between 1/1 and 4/1 depending on the disease in consideration.

Nevertheless, increased intakes of ω-3 fatty acids (or a low ω-6/ω-3 ratio) are always recommended as it has been shown to reduce the pathogenesis of many diseases such as CVD and cancer as well as inflammatory and autoimmune diseases (Simopoulos, 2002). Based on the reported fatty acids, GABS meat had an ω-6/ω-3 ratio of 4.33, which is within the recommended values. However, since all of the unsaturated fatty acids could not be identified, this ratio could be lower. For example, Pires et al. (2018) obtained values between 0.34 and 0.36 for Atlantic white shrimp. Thus, GABS meat is a source of essential fatty acids in addition to protein. However, unsaturated fatty acids are highly prone to lipid oxidation. Nevertheless, GABS meat is considered lean meat due to its low lipid content. Therefore, it can effectively be used as a substrate for enzymatic hydrolysis (Kristinsson & Rasco, 2000).

Mineral analysis

The mineral composition of GABS meat is shown in **Table 2.4**. The most abundant elements in GABS meat were sodium (Na), Zinc (Zn), Iron (Fe), aluminum (Al), and copper (Cu), with Na being the most predominant. The Na content of GABS meat is approximately 2044 ppm or mg/kg (w.b.), equivalent to about 204 mg/100 g (w.b.). This Na content is relatively higher than what has been reported for the same and different shrimp species. For example, Menard et al. (2015) found the Na content of Gulf brown shrimp to be 416.12 ppm (w.b.). Additionally, Yanar and Celik (2006) reported the average Na content for green tiger shrimp and speckled shrimp to be 147 mg/100 g and 139 mg/100 g (w.b.), respectively. Differences in Na content may be due to the different shrimp species and/or seasonal variations (Yanar & Celik, 2006). Furthermore, Georgia shrimpers often put freshly caught shrimp in onion sacks. The sacks are then dipped in a brine solution to create a glaze around the shrimp while they are in the freezer, which may be why there was a higher Na content in GABS meat. Na plays a role in

Table 2.4. Mineral composition of GABS meat[†].

Mineral	Quantity (d.b.)	Quantity (w.b.)
Calcium (Ca)	0.28 ± 0.02 (%)	0.07 ± 0.00 (%)
Potassium (K)	1.54 ± 0.05 (%)	0.36 ± 0.01 (%)
Magnesium (Mg)	0.21 ± 0.01 (%)	0.05 ± 0.00 (%)
Phosphorus (P)	1.21 ± 0.02 (%)	0.28 ± 0.00 (%)
Nitrogen (N)	14.63 ± 0.12 (%)	3.40 ± 0.03 (%)
Sulfur (S)	1.25 ± 0.03 (%)	0.29 ± 0.01 (%)
Aluminum (Al)	29.97 ± 12.40 (ppm)	6.96 ± 2.88 (ppm)
Boron (B)	2.98 ± 0.79 (ppm)	0.69 ± 0.18 (ppm)
Cadmium (Cd)	<0.80 (ppm)	CBD
Chromium (Cr)	2.64 ± 0.57 (ppm)	0.49 ± 0.24 (ppm)
Copper (Cu)	23.80 ± 1.00 (ppm)	5.53 ± 0.23 (ppm)
Iron (Fe)	40.90 ± 11.34 (ppm)	9.50 ± 2.64 (ppm)
Manganese (Mg)	<2.00 (ppm)	CBD
Molybdenum (Mo)	<1.00 (ppm)	CBD
Sodium (Na)	8799 ± 256 (ppm)	2044 ± 59 (ppm)
Nickel (Ni)	<1.00 (ppm)	CBD
Lead (Pb)	3.22 ± 0.93 (ppm)	0.65 ± 0.22 (ppm)
Zinc (Zn)	55.07 ± 1.53 (ppm)	12.79 ± 0.35 (ppm)

[†]Values are reported as mean ± standard deviation of triplicate determinations.
GABS = Georgia-caught brown shrimp; d.b. = dry weight basis; w.b. = wet weight basis; CBD = could not be determined; ppm = parts per million.
Values with < symbol indicate that the mineral was not within detectable limits.

the preservation and flavor enhancement of many foods, especially in seafood (Garrido & Otwell, 2008). Additionally, Na is an essential mineral required for acid-base balance, blood pressure regulation, fluid balance, muscle contraction, and nervous system function (Strazzullo & Leclercq, 2014). However, excessive intakes of Na are associated with hypertension, which can cause heart disease and strokes (Strazzullo & Leclercq, 2014). It is recommended that children (+14 years old) and adults consume less than 2300 mg of Na per day (Strazzullo & Leclercq, 2014).

According to the FAO (1987), another essential trace mineral for humans is Zn. Zn has many essential biological functions, such as serving as a cofactor for many enzymes, aiding in carbohydrate, protein, and lipid metabolism, and healing wounds (FAO, 1987.). Furthermore, most foods contain approximately 2 to 29 ppm of Zn (Agency for Toxic Substances and Disease Registry, 2005). GABS meat had a Zn content of 12.79 ppm (w.b.), which is higher than what Oksuz et al. (2009) reported for deep seawater rose shrimp and golden shrimp (6.1 ppm and 5.87 ppm, respectively). Marine animals obtain their minerals from seawater and their feed (Sriket et al., 2007). Therefore, it is common to observe differences in mineral content, especially if the shrimp are from different waters.

According to Oksuz et al. (2009), Fe is also an essential trace mineral because it carries oxygen from the lungs to the tissues via red blood cells. Therefore, an adequate amount of Fe is required in the diet to prevent iron deficiency anemia (Oksuz et al., 2009). GABS meat had a Fe content of 0.95 mg/100 g (w.b.), which is similar to what Yanar & Celik (2006) reported for green tiger shrimp and speckled shrimp (1.48 mg/100 g and 1.55 mg/100 g, respectively). However, Fe, as well as Cu (also present in GABS meat), are transition metals that are known to promote lipid oxidation (Sriket et al., 2007; Thanonkaew et al., 2006). Hemocyanin, a blood

pigment found in crustaceans, contains a Cu ion at the active site (Decker & Tuczek, 2000; Sriket et al., 2007). Therefore, Fe and Cu may induce lipid oxidation during handling, processing, and storage, significantly reducing the nutritional and sensory quality of shrimp meat (Sriket et al., 2007). Nevertheless, there are preservation methods and processing techniques that can be used to preserve shrimp meat quality (Samples, 2013).

According to the National Organization for Rare Disorders (2006), lead (Pb), mercury (Hg), arsenic (As), and cadmium (Cd) are most associated with heavy metal positioning. Only Pb was detectable via ICP-M.S. in GABS meat (**Table 2.4**). A small amount of Pb (0.65 ppm, w.b.) was detected in GABS meat, which is within the permissible Pb limit of 0.1-3 ppm for foods (Monchanin et al., 2021). This study could not determine the contents of other heavy metals. Nevertheless, high heavy metal contents are not a significant concern in shrimp if consumed in moderation (Djedjibegovic et al., 2020).

Conclusion

The current scientific literature regarding the nutritional characterization of Georgia-caught brown shrimp (GABS) meat is limited. This study characterized the proximate composition and selected nutritional profiles of GABS meat. The nutritional characterization of GABS meat is essential to explore alternatives for value addition. Not surprisingly, high water activity and moisture content were observed in GABS meat. Additionally, GABS meat was high in crude protein and contained all evaluated essential amino acids (since tryptophan was unable to be determined). Moreover, GABS meat is considered lean meat due to its low-fat content. Palmitic and stearic acids were the main fatty acids detected in GABS meat. This study demonstrated that GABS meat is a good source of high-quality protein, which can be used to produce protein hydrolysate powders.

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CHAPTER 3

PRODUCING PROTEIN HYDROLYSATE POWDERS FROM UNDERUTILIZED GEORGIA-CAUGHT BROWN SHRIMP (*Farfantepenaeus aztecus*) MEAT

Introduction

White shrimp (*Litopenaeus setiferus*) and brown shrimp (*Farfantepenaeus aztecus*) are the main shrimp species harvested in Georgia (Rawson, 2003; University of Georgia [UGA] Marine Extension and Georgia Sea Grant, 2021). Georgia harvests between 4.5 and 9.5 million pounds of shrimp annually with white shrimp contributing to approximately two-thirds of the harvest (Rawson, 2003). Often, brown shrimp has a lower commercial value than white shrimp due to its smaller size. As a result, Georgia shrimpers regularly discard brown shrimp (often considered a by-catch). However, the previous characterization of Georgia-caught brown shrimp (GABS) meat revealed that it is a good source of protein and essential amino acids; therefore, it may be used to develop high-value protein-based ingredients (Grand View Research, 2021). Novel protein hydrolysate powders derived from GABS meat may provide an alternative use to this underutilized seafood commodity.

Numerous studies have recently investigated the production of protein hydrolysate powders from underutilized seafood commodities and seafood by-products (Chalamaiah et al., 2010; Dhanabalan et al., 2020; Halim et al., 2016; Parvathy, Binsi et al., 2018; Tejpal et al., 2017). A hydrolysate is a protein broken down into smaller peptides of various sizes. Protein hydrolysates often exhibit better functional and antioxidant properties than their native proteins (Halim et al., 2016; Venugopal, 2009). The hydrolysis procedure generally begins with a pre-

heat treatment, which often results in protein denaturation (Halim et al., 2016; Khan Academy, 2022; Kilara & Vaghela, 2004). Protein denaturation unfolds the protein's globular structure, which exposes previously buried hydrophobic amino acids to the surface (Fennema, 1996; Kilara & Vaghela, 2004). Gao et al. (2016) reported that white shrimp (*Litopenaeus vannamei*) began to denature after 10 min of heating at 50°C, suggesting that temperatures greater than or equal to 50°C result in shrimp protein denaturation. Hydrolysis involves cleaving peptide bonds, which then alters the primary amino acid sequence (Kilara & Vaghela, 2004). Therefore, the hydrolysis procedure may alter the protein's molecular weight, amino acid composition and sequence, net charge and distribution of charges, hydrophobicity/hydrophilicity ratio, and/or molecular flexibility/rigidity, which consequently modifies or enhances protein functional properties (Fennema, 1996; Kilara & Vaghela, 2004). Enhanced functional properties observed in protein hydrolysates may be of interest to the food industry due to the high potential for these hydrolysates to serve in various food and beverage applications (Halim et al., 2016).

Currently, enzymatic hydrolysis is one of the most effective methods to produce seafood hydrolysates (Chalamaiah et al., 2010; Halim et al., 2016). Different types of proteolytic enzymes such as alkaline protease (Alcalase), Protamex, Flavourzyme, pepsin, trypsin, papain, and bromelain have been successfully used to produce fish and shrimp protein hydrolysates (Chalamaiah et al., 2012; Halim et al., 2016). According to Dhanabalan et al. (2020), enzyme selection depends on its efficacy and cost. Previous studies demonstrated the effectiveness of using Alcalase for producing fish and shrimp protein hydrolysates with enhanced functional and antioxidant properties (Binsi et al., 2016; Chalamaiah et al., 2010; Intarasirisawat et al., 2012; Latorres et al., 2018). Alcalase (obtained from the fermentation of *Bacillus licheniformis*) is an alkaline endopeptidase that requires alkaline pH conditions to gain activity and stability and

preferentially cleaves hydrophobic amino acids residues (Intarasirisawat et al., 2012; Tacias-Pascacio et al., 2020). We previously reported that GABS meat contained at least eight of the nine hydrophobic amino acids, comprising 31.53 g/100g of the total 76.60 g/100 g (d.b.) of GABS meat, suggesting Alcalase would be highly specific for GABS meat since almost half of its total amino acids were hydrophobic. Additionally, Alcalase's optimal pH and incubation temperatures are between 7-9 and 30-65°C, respectively (Tacias-Pascacio et al., 2020). However, Alcalase was unable to be delivered by the start of this study. Therefore, for this study, Enzyme Development Corporation (EDC) provided their equivalent enzyme to Alcalase, alkaline protease L-660 (APL-660). EDC claims the functionality to be similar to Alcalase and reports APL-660's optimal pH and incubation temperature to be 9 and 60°C, respectively (EDC, personal communication, July 9, 2021). Because of the success with Alcalase and APL-660's similarity in function, APL-660 was chosen as the enzyme for hydrolyzing GABS meat.

Liquid protein hydrolysate solutions are generally transformed into powder. Freeze drying and spray drying are the most common methods for drying liquid protein hydrolysate solutions (Haque & Adhikari, 2015). Previous studies reported using freeze drying to effectively dry fish protein hydrolysate (FPH) solutions (Ben Slama-Ben Salem et al., 2017; Binsi et al., 2016; Jemil et al., 2014; Latorres et al., 2018). However, freeze drying is more time-consuming and less cost-effective than spray drying (Haque & Adhikari, 2015). Hence, this study selected spray drying to dry the resultant shrimp protein hydrolysate (SPH) mixtures. Spray drying has been reported for successfully drying other marine protein hydrolysate solutions (Dhanabalan et al., 2020; Tejpal et al., 2017). However, the high temperatures and the feed droplet air contact configurations used in spray drying may reduce some functional properties in the resultant protein hydrolysates. Therefore, because there are limited studies on the production of shrimp

protein hydrolysate powders using different spray drying contact configurations, this study investigated the effect of two spray drying contact configurations (mixed-flow vs. concurrent). Mixed-flow (MX) and concurrent (CC) spray drying expose the feed droplets to the hot air at different speeds and positions. In MX, the feed droplets are not immediately exposed to the hot air because they are introduced from the bottom while the hot drying air is introduced from the top of the drying chamber. Whereas CC does immediately expose the feed droplets to the hot drying air because both are introduced from the top of the drying chamber (Jiang et al., 2020).

Many studies have successfully produced several high-value marine protein hydrolysate powders to valorize underutilized seafood commodities (otherwise used to make feed or fertilizer) (Chalamaiah et al., 2010). To date, there are no current reports on the production of protein hydrolysate powders from GABS meat. Therefore, the objective of this study was to determine the feasibility of producing shelf-stable protein hydrolysate powders with enhanced nutritional and functional properties (compared to the native protein) from GABS meat via enzymatic hydrolysis and spray drying.

Materials and Methods

Materials

Fresh-frozen brown shrimp were supplied by shrimpers located in Brunswick, GA. Fresh shrimp were caught in the Summer of 2021 and immediately frozen until pick up. The fresh-frozen shrimp was shipped to the UGA Food Science and Technology facilities located in Griffin, GA, where it was kept frozen until needed for analyses.

Food-grade APL-660 was provided by EDC (Scranton, PA, USA) with an activity of 739 kilo detergent alkaline protease units (KDAPU)/g. The chemicals used in this study included disodium tetraborate decahydrate, sodium dodecyl sulfate (SDS), o-phthalaldehyde (OPA),

dithiothreitol (DTT), L-serine, L-aspartic acid, and petroleum ether and were all obtained from Millipore Sigma (St. Louis, MO, USA). Also, sodium hydroxide (NaOH) pellets, hydrochloric acid (HCl), 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, and all the other chemicals were purchased from Fisher Scientific (Fair Lawn, NJ, USA) or Millipore Sigma (St. Louis, MO, USA).

Production of shrimp protein hydrolysate (SPH) powders

SPH powders were produced following the modified methods of Latorres et al., (2018) and Dhanabalan et al. (2020). Fresh-frozen shrimp (~1550 g) were thawed at room temperature under running tap water for 8 min before the heads, shells, veins, and intestines were manually removed. GABS meat was then rinsed with tap water and patted dry (GABS meat was kept on ice during sample preparation). Then, GABS meat was finely chopped in a commercial blender (Model BL610, NINJA, SharkNinja Operating LLC, Needham, MA, USA) for 20 s. Afterward, 750 g of chopped GABS meat was homogenized with 3 L of deionized (DI) water (w/v, ratio 1:4; shrimp meat: DI water) in an ultra-shearing homogenizer (Homogenizer 850, Fisherbrand, Fisher Scientific UK Ltd, Loughborough, UK) at 6000 rpm for 5 min then 8000 rpm for 15 min (in 5 min intervals). The shrimp meat: DI water ratio was based on Dhanabalan et al. (2020) and needed for preliminary studies to more accurately measure the degree of protein hydrolysis (DH). Homogenization conditions were determined through trial and error after obtaining a homogenous solution with minimal bubble formation, which was observed at speeds higher than 10,000 rpm. The homogenized samples were pre-incubated at 60°C for 15 min before hydrolysis. The samples' pH was then adjusted once with a 1 M NaOH solution to a pH of 9.5 for optimal enzyme activity and to avoid further pH adjustments. Enzymatic hydrolysis was initiated by the addition of APL-660 at 0.1% (v/w) enzyme to substrate concentration (% enzyme to the mass of

protein in the diluted sample) per EDC recommendation. The protein content of GABS meat was previously determined to be 21.26% (wet basis, w.b.). The hydrolysis reaction was carried out in a shaking water bath (Model 2872, Precision, Thermo Electron Corporation, Waltham, MA, USA) with constant agitation (105 rpm) by maintaining the temperature at 60°C. A DH study was carried out prior to obtaining hydrolyzed samples to determine the extent of GABS meat protein hydrolysis over 24 h. The resultant information from this preliminary study was used to select the hydrolysis time for the treatments (3 and 10 min). The hydrolysis procedure was run for 3 min or 10 min to produce SPH-1 and SPH-2 liquid samples, respectively. Based on preliminary studies, it was estimated that the DH of SPH-1 was ~13%, while the DH of SPH-2 was ~15%. Low DHs of <15% were desired based on the literature review and being achieved in a short time frame. Hydrolysis was terminated by inactivating the enzyme at 85°C for 3 min per EDC guidelines. After inactivation, the samples were cooled down to room temperature before centrifuging at 12,429 x *g* for 10 min at 4°C. The supernatant was collected and refrigerated overnight until spray drying (**Figure 3.1**). Prior to spray drying, the supernatant was filtered through a Büchner funnel to ensure only the supernatant was collected after centrifugation. Alternatively, SPH control samples (SPHC) were prepared by following the procedure described above without the addition of APL-660 (SPHC-1 and SPHC-2). Additional unhydrolyzed control samples (SUHC) containing only GABS meat and DI water were also prepared. Then, SPH-1, SPH-2, SPHC-1, SPHC-2, and SUHC were spray- dried under mixed-flow (MX) and/or concurrent (CC) contact configurations in a pilot-scale spray dryer (Anhydro, PSD 52, Denmark) at the UGA Food Product Innovation and Commercialization Center (Food PIC) in Griffin, GA. The inlet air temperature was set to 170°C. Meanwhile, the outlet temperatures were kept between 70-76 °C for MX and between 76-96 °C for CC by adjusting the feed flow rate that

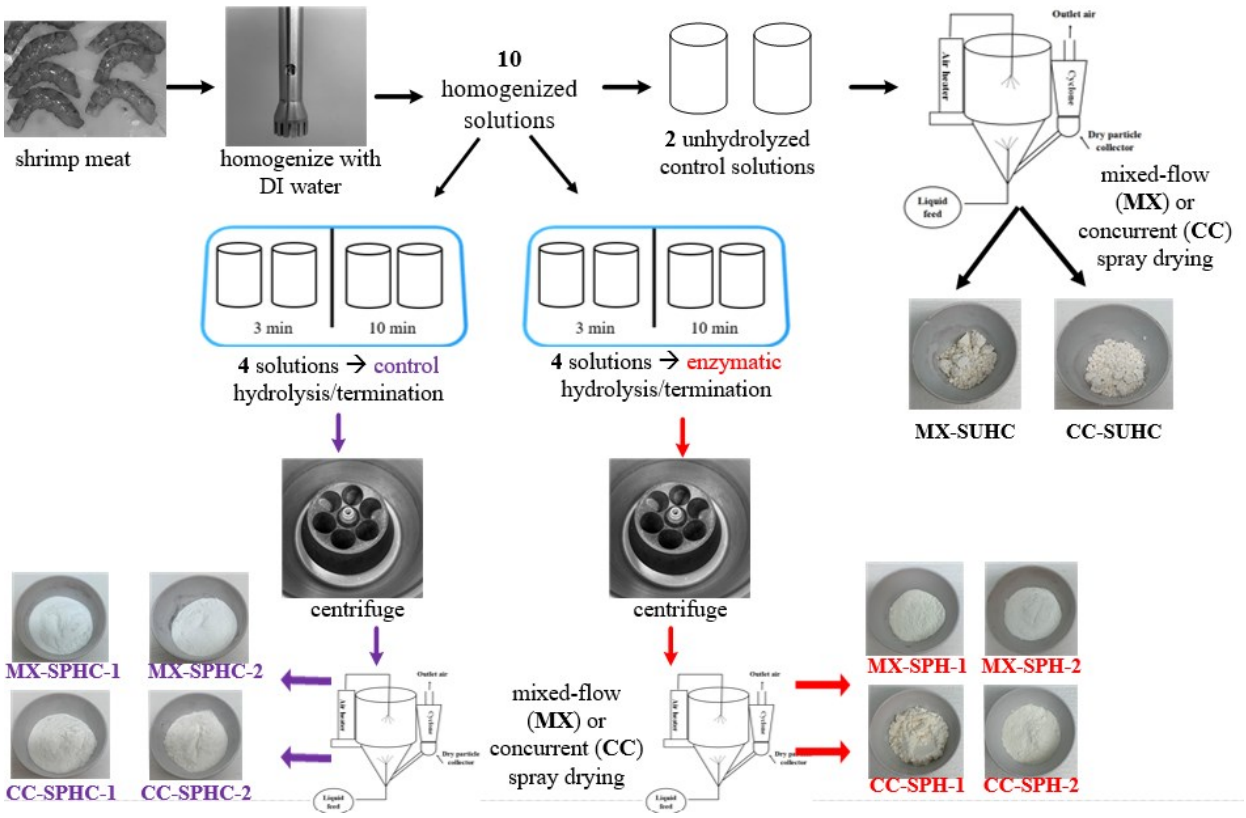


Figure 3.1. Process diagram to produce SPH powders.
APL-660 = alkaline protease L-660; SPH = shrimp protein hydrolysate; SPHC = shrimp protein hydrolysate control; SUHC = shrimp unhydrolyzed control; MX = mixed-flow spray drying configuration; CC = concurrent spray drying configuration.
Made with Microsoft shapes on Microsoft Visio 2012.

was set between 2-2.5 L/h. These spray drying parameters were based on preliminary studies done in our lab (Jiang et al., 2020; Yang et al., 2022). Then, the powders were collected and stored at room temperature in a desiccator with low relative humidity (<10%) until further analysis. In total, ten powders were produced and analyzed in this study including MX-SPH-1, MX-SPH-2, MX-SPHC-1, MX-SPHC-2, MX-SUHC, CC-SPH-1, CC-SPH-2, CC-SPHC-1, CC-SPHC-2, and CC-SUHC. Powder yields ranged from 50-100 g depending on the amount of solids in the SPH, SPHC, and SUHC solutions. These powder yields were required to have enough powder for analyses. Powder yields were determined by measuring the amount of solids in each solution and multiplying by the spray drying yield (~60%), which is how 750 g of GABS meat was determined.

Determination of DH

A DH study was carried out prior to obtaining hydrolyzed samples to determine the extent of GABS meat protein hydrolysis over 24 h. The resultant information from this preliminary study was used to select the hydrolysis time for the treatments (3 and 10 min). DH was measured according to the OPA method described by Nielsen et al. (2001) with slight modifications. An aliquot of 5 mL was taken prior to enzyme addition (time 0 h). Then, 5 mL aliquots were taken again after 0.5, 1, 2, 3, 4, 5, 6, 12, and 24 h of hydrolysis. The aliquots were placed in a shaking water bath at 85°C for 3 min to terminate hydrolysis and stored in the freezer until further analysis. The OPA reagent, serine solution, and hydrolysate samples were prepared according to Nielsen et al. (2001). A total of 3 mL of OPA reagent was added to test tubes labeled standard, blank, and sample. Then, a total of 400 µL of serine standard, DI water, or protein hydrolysate was added to their respective tubes. Each tube was vortexed for 5 s and then stood at room temperature for 2 min before the absorbance at 340 nm was measured using a

Genesys 30™ spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The spectrophotometer was blanked before two standards were measured. The sample values were measured after the first two standards followed by the blanks. The last standard was measured after all sample values and blanks were measured. The means of the standards and blanks were used for calculations. DH was calculated using Eqs. (1) and (2):

$$\text{Serine} - \text{NH2} = \left(\frac{(\text{ODsample} - \text{ODblank})}{(\text{ODstandard} - \text{ODblank})}\right) * 0.9516 * 0.1 * 100/(\text{X} * \text{P}) \quad (1)$$

where serine-NH2 = meqv serine NH2/g protein; X = g sample (influenced by the DH); P = protein % (w.b.) in sample; 0.1 is the sample volume in liters (L). h = (Serine-NH2 – β) / α meqv/g protein, where α is given as 1, β is given as 0.4, and htot is given as 8.6, according to Nielsen et al. (2001).

$$\text{DH}\ (\%) = \frac{\text{h}}{\text{htot}} * 100 \quad (2)$$

Proximate composition of SPH powders

Moisture content and water activity

Moisture content was determined following the Association of Official Analytical Collaboration (AOAC) International Official Method 934.01 (oven drying) (AOAC International, 2019a; AOAC International, 2020) using an Isotemp® vacuum oven (Model 281A, Thermo Fisher Scientific, Waltham, MA, USA). The water activity of SPH powders was determined using an Aqualab water activity meter (Model Series 3 TE, Decagon Devices, Inc., Pullman, WA, USA).

Ash content

Ash content was determined following the AOAC International Official Method 938.08 (furnace combustion) (AOAC International, 2019b) using a muffle furnace (Model F-A170,

Thermolyne, Dubuque, IA, USA). Briefly, ~2 g of powdered sample was weighed in ceramic crucibles and placed into the muffle furnace set to 550°C for 12-18 h.

Crude protein content

The crude protein of powders was determined following the AOAC International Official Method 993.13 (AOAC International, 2019c). An automated nitrogen and protein analyzer (Rapid MAX N Exceed, Elementar, Langenselbold, Germany) was used to determine the total nitrogen content by the Dumas method using dry combustion described by Jung et al. (2003). A conversion factor of 6.25 was used to determine the crude protein content (Pires et al., 2018). L-aspartic acid was used as the nitrogen calibration standard.

Crude fat content

Crude fat was determined following the American Oil Chemists' Society (AOCS) Am 5-04 Official Crude Fat Extraction Method, which uses the Soxhlet principle (AOCS, 2017). Approximately 1.5-2 g of SPH powder was weighed and placed into a fat extraction pouch then baked in a Thelco laboratory oven (Model No. 31620 Jouan, Inc., Saint-Herblain, France) at 105°C for 3 h. After 3 h, samples and pouches were weighed again. Then, samples were placed in the ANKOM^{XT15} extractor (XT15, ANKOM Technology, Macedon, NY, USA) using petroleum ether as the extraction solvent for 1 h at 90°C. After 1 h, samples were baked at 105°C for 20 min and re-weighed.

Mineral analysis

Mineral analysis of SPH powders was determined by the UGA Soil, Plant, and Water Lab in Athens, GA with a Spectro Arcos inductively coupled plasma mass spectrometer (Model FHS16, Ametek Inc., Berwyn, PA, USA) via the inductively coupled plasma – mass spectrometry (ICP – M.S.) method. A CEM Mars5 microwave-acid (HNO_3) digestion system

(Model 61E ICP, Thermo Jarrell-Ash, Franklin, MA, USA) was used to quantify the minerals Ca, K, Mg, P, N, S, Al, B, Cd, Cr, Cu, Fe, Mn, Mo, Na, Ni, Pb, and Zn.

Functional properties of SPH powders

Protein solubility

The protein solubility of SPH powders was evaluated at different pH values following the method described by Latorres et al. (2018) and Dhanabalan et al. (2020) with modifications. Briefly, 2 g of powder was dissolved in 200 mL DI water, and the pH was adjusted to 2, 4, 7, and 10 with 1 M HCl or 1 M NaOH solutions. Then, the solutions were stirred at room temperature for 30 min before being centrifuged at 1381 x *g* for 15 min. The supernatant was collected and freeze-dried in a pilot-scale freeze-dryer (Virtis, The Virtis Company, Gardiner, NY, USA). The protein content in the supernatant was determined using a nitrogen and protein analyzer. The protein solubility was calculated using Eq. (3) (Dhanabalan et al., 2020):

$$\text{Protein solubility (\%)} = \frac{\text{protein content in supernatant}}{\text{initial protein content in sample}} * 100 \quad (3)$$

Emulsification properties

Emulsifying activity index (EAI) and emulsion stability index (ESI) were measured following the modified method of Pearce and Kinsella (1978) described by Latorres et al. (2018). Briefly, 60 mL of dissolved SPH powders in DI water with a protein concentration of 2 mg/mL was homogenized with 20 mL of soybean or vegetable oil in an ultra-shearing homogenizer at 10,000 rpm for 1 min at room temperature. Then, the pH of the emulsions was adjusted to 2, 4, 7, or 10 after homogenization with 1 M HCl or 1 M NaOH solutions. The emulsions were homogenized again at 10,000 rpm for 1 min and a 50 µL aliquot was taken at 0 min and 10 min after emulsion formation. The aliquot of the emulsion was diluted with 5 mL of 0.1% (w/v) SDS and vortexed for 10 s. The diluted emulsion's absorbance was measured in a Genesys 30™

spectrophotometer at a wavelength of 500 nm. EAI and ESI were calculated using Eqs. (4) and (5):

$$\text{EAI } (\frac{\text{m}^2}{\text{g}}) = \frac{(2*2.303*100*\text{A})}{(\text{c}*0.25*10000)} \quad (4)$$

$$\text{ESI (min)} = \frac{(A_0*10)}{(A_0-A_{10})} \quad (5)$$

where A is the absorbance at 500 nm and c is the protein concentration (g/mL), A_0 and A_{10} are the absorbance of the diluted emulsions at 0 and 10 min after emulsion formation, respectively.

Foaming properties

Foaming capacity (FC) and foaming stability (FS) were measured following the modified method of Sathe and Salunkhe (1981) described by Latorres et al. (2018). First, 1 g of SPH powder was dispersed in 100 mL of DI water. Then, the solution was adjusted to pH values of 2, 4, 7, and 10 with 1 M HCl or 1 M NaOH solutions followed by homogenization in an ultra-shearing homogenizer at 10,000 rpm for 1 min at room temperature. The whipped samples were transferred to a 250 mL graduated cylinder and the total volume was immediately recorded. The FC was calculated using Eq. (6):

$$\text{FC (\%)} = \left(\frac{(\text{A}-\text{B})}{\text{B}}\right) * 100 \quad (6)$$

where A is the volume after whipping (mL) and B is the volume before whipping (mL).
The whipped sample stood at room temperature for 20 min and the volume of the whipped sample was then recorded. FS was calculated using Eq. (7) as reported by Intarasirisawat et al. (2012):

$$\text{FS (\%)} = \left(\frac{(\text{A20 min} - \text{B})}{(\text{A0 min} - \text{B})}\right) * 100 \quad (7)$$

where A is the volume (mL) after standing 0 or 20 min at room temperature (mL) and B is the volume (mL) before whipping.

Antioxidant properties

DPPH radical scavenging activity (RSA)

The DPPH RSA of the SPH powders was evaluated following the modified method of Shimada et al. (1992) and Centenaro et al. (2011) described by Latorres et al. (2018). First, aliquots of 1 mL of dissolved SPH powders in DI water prepared at two concentrations (5.0 and 7.5 mg/mL) were added to 1 mL of 0.1 mM/L DPPH in 95% ethanol. The mixture was vortexed for 10 s. Then, the solution stood in the dark for 30 min before its absorbance was measured at 517 nm in a Genesys 30™ spectrophotometer. The control followed the same procedure without the SPH powders. The DPPH RSA was calculated using Eq. (8):

$$\text{DPPH RSA (\%)} = \frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrol}} * 100 \quad (8)$$

Reducing power assay

The reducing power of the SPH powders was evaluated following the modified method of Zhang et al. (2008) described by Latorres et al. (2018). A 500 µL aliquot of the dissolved SPH powders in DI water prepared at two concentrations (5.0 mg/mL and 7.5 mg/mL) was mixed with 500 µL of 0.02 mol/L phosphate buffer (pH 6.6) and 4 mL of 1% potassium ferricyanide (w/v) in a test tube. The mixture was incubated at 50°C in a shaking water bath for 20 min. Then, 4 mL of 10% TCA (w/v) was added followed by vortexing for 2 s. In a new test tube, a 1000 µL aliquot of the vortexed mixture was added to 800 µL of DI water and 200 µL of 0.1% ferric chloride (w/v). The absorbance of the resulting solution was measured at 700 nm after 10 min of the reaction in a Genesys 30™ spectrophotometer. A high absorbance was indicative of strong reducing power.

Particle size distribution

The particle size distribution of powdered samples was determined using a particle size analyzer (Model PSA 1190, Anton Paar, Austria) equipped with laser diffraction. SPH powders were shaken through a fine sieve (mesh size 1/32 inch) before entering the unit's hopper. The powders entered the unit's hopper and were transferred to the analytical area via Venturi/free fall, where the powder was illuminated with three lasers and the diffracting light was analyzed. All samples followed parameters of 40% vibrator duty cycle, 30 Hz vibrator frequency, and 1200 mBar of air pressure with a 10 s run time. The D_{10}, D_{50}, and D_{90} (diameter of the particles at 10%, 50%, and 90% cumulative volume) and the span value (spread of particles) was determined by following the method referred to Mis Solval, Bankston, Bechtel, and Sathivel (2016).

Particle morphology

Particle morphologies of each powder were observed using a scanning electron microscope (1450 EP, Carl Zeiss MicroImaging, Thornwood, NY, USA) at the UGA Georgia Electron Microscopy Lab in Athens, GA. First, powdered samples were sputter-coated with gold before being imaged with an acceleration potential of two kV and systematically captured at a magnification of 800x and 3000x.

Statistical analysis

Experiments and analyses were performed in triplicate determinations, and then the mean values and standard deviations were calculated from the raw data. Next, the data were analyzed using analysis of variance (ANOVA), and the differences between the means were evaluated by a post-hoc Tukey's studentized range test ($\alpha = 0.05$). All statistical analyses were analyzed with RStudio statistical software (RStudio Inc., Boston, MA, USA) and SAS Studio (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

The effect of time on DH

A preliminary study was conducted to understand the effect of time on the DH of the resultant SPH. Therefore, the DH of the resultant SPH and a control sample (without the addition of APL-660) was monitored over 24 h (**Figure 3.2**). According to Nielsen et al. (2001), the DH is the percentage of cleaved peptide bonds. It is measured via the OPA method, which is based on OPA's reaction with free amino groups (Nielsen et al., 2001). The initial DH was ~11% which may be due to endogenous enzyme activity (Tejpal et al., 2017), then there was a gradual increase in the DH as time increased. This effect was more evident when APL-660 was used to hydrolyze GABS meat. The DH of the enzymatic hydrolysates increased rapidly during the first 6 h of hydrolysis, then the rate of reaction was reduced until reaching a final DH of ~31% after 24 h. Similar hydrolysis curves have been reported for FPH (Chalamaiah et al., 2010; Intarasirisawat et al., 2012; Sathivel et al., 2005). Conversely, the DH of the control sample only increased 5% over the 24 h of incubation. The enzymatic hydrolysate exhibited a rapid initial phase that was followed by a decrease in the hydrolysis rate as the hydrolysis time increased, which was also reported in previous studies for enzymatic hydrolysis of fish proteins (Chalamaiah et al., 2010; Intarasirisawat et al., 2012; Sathivel, et al., 2005). The rapid initial phase can be attributed to the large number of peptide bonds available for cleavage. As hydrolysis time increases, less peptide bonds become available for cleavage, which may result in the decrease in the hydrolysis rate (Intarasirisawat et al., 2012; Safari et al., 2009). Another possible explanation is a decrease in the enzyme activity rate (Intarasirisawat et al., 2012; Safari et al., 2009). The optimal pH for the enzyme APL-660 is 9, with activity between pH values of 7-10. However, in this study, the pH was initially adjusted to 9.5 to avoid further pH adjustments

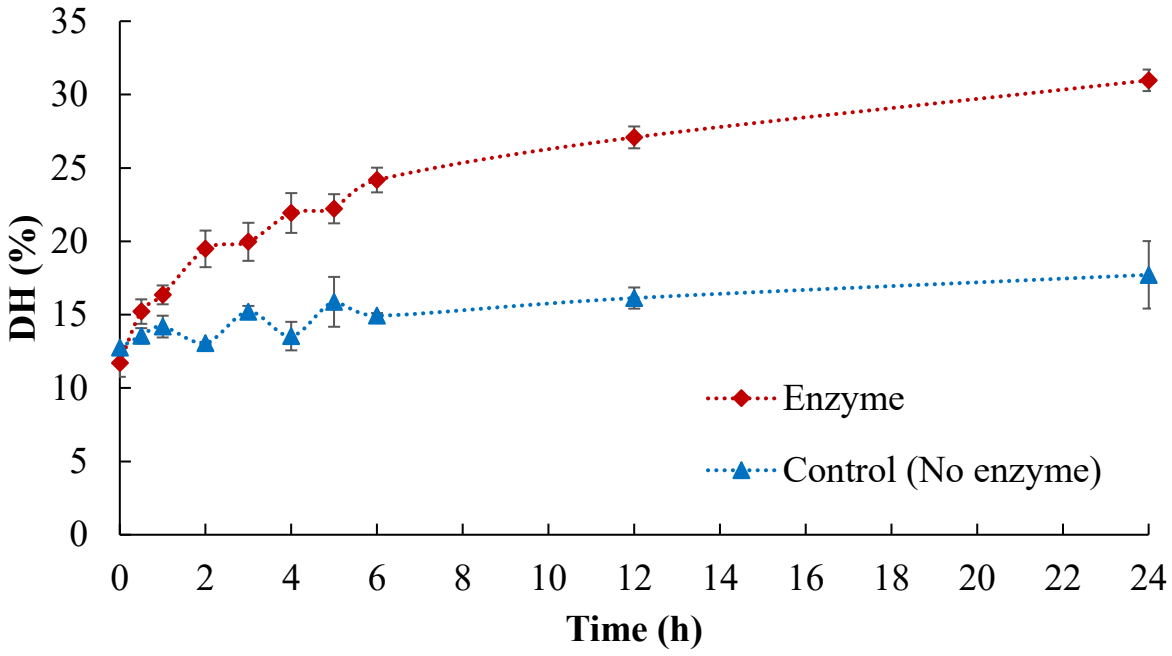


Figure 3.2. Effect of time on the DH of SPH over 24 h of enzymatic hydrolysis.
DH = degree of hydrolysis; SPH = shrimp protein hydrolysate; enzyme = alkaline protease L-660.

and use the least amount of NaOH solution possible. The pH of the samples decreased to approximately 9 after 2 h of hydrolysis. Thus, it is possible that enzyme activity decreased due to changes in pH.

DH is an important factor that influences the functional and antioxidant properties of the resultant hydrolysates (Kristinsson & Rasco, 2000). It has been reported that protein hydrolysates with low DHs show better functional characteristics (e.g. emulsification, foaming and antioxidant properties) than hydrolysates with high DHs (Binsi et al., 2016; Intarasirisawat et al., 2012; Latorres et al., 2018). Binsi et al. (2016) investigated the functional properties of hydrolysates prepared from fish frame waste with 5%, 10%, and 15% DH and reported that the EAI, ESI, FC and FS significantly decreased as the DH increased. The initial DH of the substrate (GABS meat) was ~11%. Bitterness is often reported in protein hydrolysates with extensive hydrolysis and peptides with molecular weights (MW) < 6 kDa as higher DHs often result in lower MW peptides as well as increased exposure of previously buried hydrophobic amino acids that are responsible for contributing a bitter taste (Idowu & Benjakul, 2019; Yarnpakdee et al., 2015). According to Lee (2007), extensive hydrolysis includes DHs >40% which often results in increased bitterness intensity. Therefore, developing protein hydrolysates with less than 15% DH was aimed in this study.

The DH in the resultant protein hydrolysates depends on the enzyme to substrate concentration, enzyme activity, pH, temperature, and reaction time (Intarasirisawat et al., 2012; Ishak & Sarbon, 2018). For example, Intarasirisawat et al. (2012) monitored the DH of skipjack roe hydrolysates over 5 h of hydrolysis with 1% and 5% Alcalase, and the final DH after 5 h was ~60% and 70%, respectively. Protein hydrolysis with higher enzyme concentration results in hydrolysates with higher DH (Intarasirisawat et al., 2012). However, lower enzyme

concentration is often recommended for producing protein hydrolysates with good functional properties due to the high cost of enzymes (Kristinsson & Rasco, 2000). Hence, 0.1% enzyme to substrate concentration was used in this study per EDC recommendations.

Proximate composition of SPH powders

Moisture content and water activity

SPH powders had moisture contents <9% (w.b.) (**Table 3.1**). These results are consistent with Binsi et al. (2016), who reported moisture contents of 5.88% and 7.23% for catfish roe hydrolysate powders. Additionally, studies reported that FPH powders should contain <10% moisture to preserve their quality (Chalamaiah et al., 2010; Siddik et al., 2021). However, hygroscopicity has been reported in FPH powders (Kristinsson & Rasco, 2000). Therefore, SPH powders were kept in a desiccator with a relative humidity <10%. All SPH powders had water activities of <0.3 (**Table 3.1**), which is the typical value of commercial spray dried powders (Jayasundera et al., 2011; Wang et al., 2020). Both, moisture content and water activity influence the shelf-life and microbial stability of powdered products (Wang et al., 2020). The CC powders generally had significantly (P<0.05) lower moisture and water activities than the MX powders. According to Jiang et al. (2020), spray dried particles have a longer residence time in the drying chamber during CC than in MX, which results in drier particles. Additionally, unhydrolyzed and non-heat-treated control powders (MX-SUHC and CC-SUHC) had significantly (P<0.05) lower water activities and moisture contents than the rest of MX and CC powders. These findings suggest that MX-SUHC and CC-SUHC powders may have had less water-binding capacity due to their higher content of native proteins compared to the other SPH powders. According to Jay, Loessner, and Golden (2005), the resultant spray dried powders developed in this study would be

Table 3.1. Proximate composition of SPH powders[†].

SPH powder	Water activity (a_w)	Moisture (%, w.b.)	Ash (%, d.b.)	Crude protein (%, d.b.)	Fat (%, d.b.)
MX-SPH-1	0.28 ± 0.00^{a}	8.68 ± 0.44^{a}	10.93 ± 1.35^{c}	86.83 ± 0.23^{d}	0.27 ± 0.05^{a}
MX-SPH-2	0.22 ± 0.00^{d}	7.06 ± 0.57^{b}	11.13 ± 0.83^{bc}	87.43 ± 0.48^{d}	0.48 ± 0.29^{a}
MX-SPHC-1	0.26 ± 0.00^{b}	8.26 ± 0.24^{a}	12.39 ± 0.11^{abc}	84.71 ± 0.13^{e}	0.60 ± 0.51^{a}
MX-SPHC-2	0.27 ± 0.00^{ab}	8.89 ± 0.34^{a}	13.08 ± 0.04^{a}	84.70 ± 0.08^{e}	0.28 ± 0.20^{a}
MX-SUHC	0.24 ± 0.01^{c}	5.65 ± 0.08^{cd}	6.16 ± 0.02^{e}	90.32 ± 0.13^{b}	0.55 ± 0.09^{a}
CC-SPH-1	0.13 ± 0.00^{g}	5.12 ± 0.24^{d}	5.90 ± 0.53^{e}	90.14 ± 0.48^{b}	0.37 ± 0.08^{a}
CC-SPH-2	0.14 ± 0.00^{g}	5.12 ± 0.37^{d}	8.75 ± 0.29^{d}	90.52 ± 0.32^{b}	0.48 ± 0.11^{a}
CC-SPHC-1	0.15 ± 0.00^{f}	5.43 ± 0.41^{d}	12.87 ± 0.15^{ab}	89.12 ± 0.34^{c}	0.40 ± 0.18^{a}
CC-SPHC-2	0.18 ± 0.00^{e}	6.58 ± 0.12^{bc}	11.76 ± 0.96^{abc}	87.62 ± 0.14^{d}	0.42 ± 0.02^{a}
CC-SUHC	0.11 ± 0.00^{h}	3.41 ± 0.13^{e}	6.07 ± 0.10^{e}	92.64 ± 0.15^{a}	0.54 ± 0.03^{a}

[†]Values are reported as mean ± standard deviation of triplicate determinations.
SPH = shrimp protein hydrolysate; w.b. = wet weight basis; d.b. = dry weight basis.
Different superscripts (a-h) within the same column indicate a significant ($P<0.05$) difference.
MX-SPH-1 and MX-SPH-2 = shrimp protein hydrolysate powders produced by 3 or 10-min enzymatic hydrolysis and spray-dried under MX configuration; MX-SPHC-1 and MX-SPHC-2 = shrimp protein hydrolysate powders produced as MX-SPH-1 and MX-SPH-2 without the addition of enzymes and used as controls; MX-SUHC = GABS meat dried via MX.
CC-SPH-1 and CC-SPH-2 = shrimp protein hydrolysate powders produced with 3 or 10-min enzymatic hydrolysis and spray-dried with CC configuration; CC-SPHC-1 and CC-SPHC-2 = shrimp protein hydrolysate powders produced as CC-SPH-1 and CC-SPH-2 without the addition of enzymes and used as controls; CC-SUHC = GABS meat dried via CC.

considered microbiologically stable due to having water activities <0.6 and moisture contents contents <25% (w.b.).

Ash content

All SPH powders had ash contents between 5.90-13.08% (dry basis, d.b.) (**Table 3.1**). Chalamaiah et al. (2012) reported ash contents of FPH powders between 0.45-27% (d.b.). Interestingly, the unhydrolyzed and non-heat-treated control powders (MX-SUHC and CC-SUHC) had significantly (P<0.05) lower ash contents compared to most of the other SPH powders. The significantly (P<0.05) higher ash contents found in the other SPH powders may be due to the addition of NaOH for the pH adjustment in the hydrolysis procedure. Additionally, CC-SPH-1 and CC-SPH-2 had significantly (P<0.05) lower ash contents compared to their counterpart treatments produced via MX configuration (MX-SPH-1 and MX-SPH-2). This may be due to the variation in the ash content of GABS meat used to produce these powders. Additionally, GABS were provided on three separate occasions throughout this study and were not obtained from the same source. Differences in the proximate composition are common among the same shrimp species, especially if not obtained from the same source (Ockerman, 1992; Turan et al., 2011). In the future, GABS should be obtained from the same source to reduce the significant variations in the ash contents. According to Venugopal (2016), FPH powders typically have an ash content between 3-8% (d.b). Therefore, some SPH powders may be within higher ash content limits (Thiansilakul et al., 2007; Venugopal, 2016). Future studies may investigate lowering the ash content of SPH powders for improving functional properties. A possible way to lower the ash content of SPH powders would be to demineralize them by dialysis or electrodialysis (Roblet et al., 2012).

Crude protein content

SPH powders had protein contents between 84.70-92.64% (d.b.) (**Table 3.1**), which is within the recommended protein content (81-93%, d.b.) for FPH powders (Venugopal, 2016). MX-SPH-1 and MX-SPH-2 had significantly (P<0.05) higher protein contents compared to their controls, MX-SPHC-1 and MX-SPHC-2, respectively. The same trend was observed with CC-SPH-1 and CC-SPH-2 and their controls, CC-SPHC-1 and CC-SPH-2, respectively. It has been reported that enzymatic hydrolysis increases protein solubility; meanwhile, centrifugation removes insoluble solids (Dhanabalan et al., 2020). Since no enzymes were added to the MX-SPHC-1/CC-SPHC-1 and MX-SPHC-2/CC-SPHC-2 powders, it is possible that more insoluble solids were removed during the centrifugation procedure. Furthermore, significantly (P<0.05) higher protein contents (~up to 3%) were observed in the unhydrolyzed and non-heat-treated control powders (MX-SUHC and CC-SUHC) compared to the rest of their respective MX and CC powders. One explanation is that significant quantities of insoluble protein were removed during centrifugation of enzymatic SPH and their controls. Additionally, the initial protein content may have been lower in some of the samples as evidenced by CC-SUHC (unhydrolyzed and non-heat-treated control) containing significantly (P<0.05) higher protein content than MX-SUHC since it is common to observe slight variation in the proximate composition among the same shrimp species (Ockerman, 1992; Turan et al., 2011).

Crude fat content

SPH powders had crude fat contents between 0.28-0.60% (d.b.) and did not significantly (P>0.05) differ from each other (**Table 3.1**). GABS meat had a low-fat content (**Table 2.1**). According to Venugopal (2016), FPH powders typically have fat contents <5%. This statement agrees with Chalamaiah et al. (2012), who reviewed several studies that reported FPH powders

with fat contents <5%. According to He et al. (2013), FPH powders' fat content must be <0.5% for human consumption. This fat content is recommended to prevent FPH powders from lipid oxidation (He et al., 2013). Most of the SPH powders produced in this study, including the enzymatic SPH powders, meet this recommended guideline.

Mineral analysis

As expected, the enzymatic SPH powders had similar (P>0.05) mineral compositions (**Table 3.2**). The most abundant mineral in the SPH powders was Na, which was similar to GABS meat (**Table 2.4**). The increased concentrations of Na in the SPH powders compared to GABS meat were expected since NaOH was added during the enzymatic hydrolysis procedure for the pH adjustment. Sathivel et al. (2003) also detected increased concentrations of Na (9321-23811 ppm) in their herring hydrolysate powders. Additionally, lower concentrations of Ca, Mg, P, S, Al, Cr, and Pb were detected in the SPH powders than GABS meat. The high concentrations of Na in SPH powders may limit their applications in foods (Doyle & Glass, 2010). Therefore, future studies should investigate demineralizing SPH powders to reduce the Na content (Roblet et al., 2012). It is recommended that children (+14 years old) and adults consume less than 2300 mg of Na per day (Strazzullo & Leclercq, 2014).

Table 3.2. Mineral composition of enzymatic SPH powders[†].

Mineral	MX-SPH-1	MX-SPH-2	CC-SPH-1	CC-SPH-2
Ca (%, d.b.)	0.02 ± 0.00[a]	0.02 ± 0.00[a]	0.03 ± 0.00[a]	0.02 ± 0.00[a]
K (%, d.b.)	1.77 ± 0.08[a]	1.83 ± 0.10[a]	1.82 ± 0.1[a]	1.87 ± 0.12[a]
Mg (%, d.b.)	0.06 ± 0.00[a]	0.04 ± 0.00[a]	0.02 ± 0.00[a]	0.03 ± 0.00[a]
P (%, d.b.)	0.98 ± 0.01[a]	1.08 ± 0.02[a]	0.92 ± 0.04[a]	1.08 ± 0.01[a]
N (%, d.b.)	14.70 ± 0.21[a]	14.80 ± 0.30[a]	14.90 ± 0.31[a]	14.80 ± 0.30[a]
S (%, d.b.)	1.11 ± 0.05[a]	1.15 ± 0.07[a]	1.15 ± 0.06[a]	1.11 ± 0.08[a]
Al (ppm, d.b.)	<10.00	<10.00	<10.00	<10.00
B (ppm, d.b.)	12.30 ± 0.36[a]	12.40 ± 1.11[a]	17.10 ± 0.76[a]	17.20 ± 0.46[a]
Cd (ppm, d.b.)	<0.80	<0.80	<0.80	<0.80
Cr (ppm, d.b.)	<1.00	<1.00	<1.00	1.17 ± 0.02
Cu (ppm, d.b.)	20.80 ± 0.66[a]	20.40 ± 1.31[a]	24.00 ± 0.78[a]	23.30 ± 1.73[a]
Fe (ppm, d.b.)	<6.00	<6.00	<6.00	<6.00
Mg (ppm, d.b.)	<2.00	<2.00	<2.00	<2.00
Mo (ppm, d.b.)	<1.00	<1.00	<1.00	<1.00
Na (ppm, d.b.)	35,089 ± 4912[a]	34,338 ± 4640[a]	35,535 ± 5094[a]	34,513 ± 5048[a]
Ni (ppm, d.b.)	<1.00	<1.00	<1.00	<1.00
Pb (ppm, d.b.)	<2.00	<2.00	<2.00	<2.00
Zn (ppm, d.b.)	48.60 ± 0.16[a]	48.30 ± 3.02[a]	50.10 ± 1.61[a]	50.00 ± 2.83[a]

[†]Values are reported as mean ± standard deviation of triplicate determinations.
Values with < symbol indicates that the mineral was not within detectable limits.
d.b. = dry weight basis.
Means with the same superscript indicate no significant (P>0.05) difference within the same row.
See Table 3.1 for the description of MX-SPH-1, MX-SPH-2, CC-SPH-1, CC-SPH-2.

Functional properties

Protein solubility

In this study, all SPH powders showed significant protein solubility at pH values of 2, 4, 7, and 10, with values between 84.62-100.00% (**Figure 3.3**). Interestingly, lower protein solubility values were observed at pH values of 2 and 4 with minimal solubility at pH 2 (**Figure 3.3A and B**). Meanwhile, higher protein solubility values were observed at pH of 7 and 10, with maximum values observed at pH 7 (**Figure 3.3C and D**). It has been reported that enzymatic hydrolysis alters protein solubility by modifying the net load of amino acid residues (Latorres et al., 2018), which explains why enzymatic SPH powders exhibited high protein solubility. Additionally, solubility increases as the pH moves further away from the isoelectric point (pI) because positive or negative net charges dominate, promoting protein-water interactions (Dhanabalan et al., 2020; Latorres et al., 2018). These findings are in close agreement with those reported by Dhanabalan et al. (2020), who reported the lowest protein solubility at pH 2 and 3 in their SPH powders. According to Abreu et al. (2019), pIs of 4.0-5.5 have been reported for fish proteins. Hoffman et al. (1981) found the pI of shrimp meat to be around 4.5. Meanwhile, Shanti et al. (1993) reported the pI of tropomyosin (the main protein in shrimp meat) to be between 4.8-5.2. Interestingly, unhydrolyzed control powders (MX-SUHC and CC-SUHC) showed their lowest protein solubility at pH 4 which was significantly ($P<0.05$) lower than the rest of the SPH powders indicating that the pI of the native protein could be around pH 4. Since the pI can vary depending on the exact protein composition, it is likely that the pI for the other SPH powders is below pH 4, which suggests that the hydrolysis procedure may have shifted the pI. An observation such as this has been reported by León-López et al. (2019) who observed a pI shift from 4.61 (in the native protein) to 3.68 (in the sheepskin collagen).

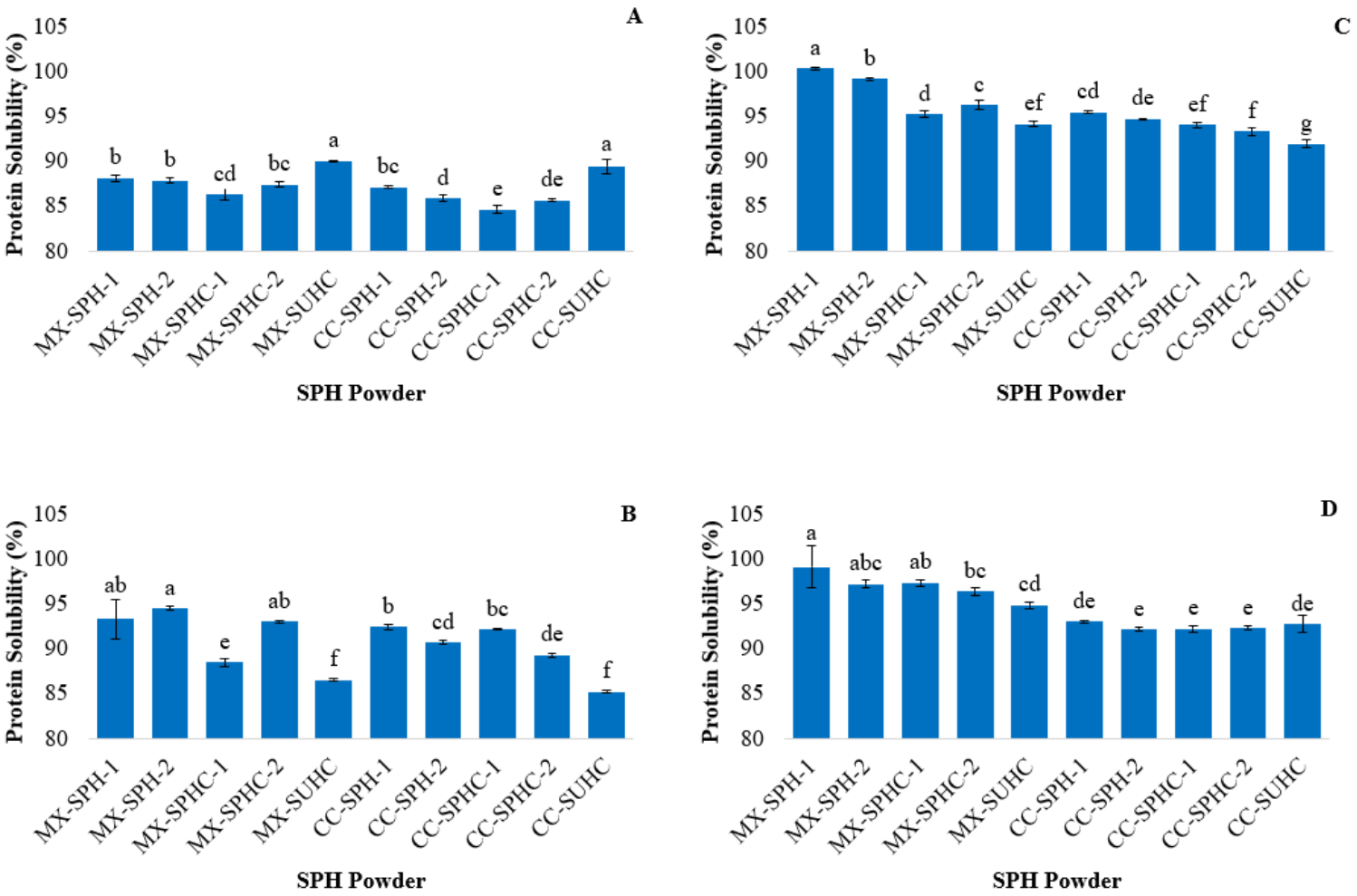


Figure 3.3. Protein solubility (%) of SPH powders at different pH conditions **A)** 2, **B)** 4, **C)** 7, and **D)** 10.
Different superscripts (a-g) among SPH powders indicate a significant (P<0.05) difference.
See Table 3.1 for the description of MX-SPH-1, MX-SPH-2, MX-SPHC-1, MX-SPHC-2, MX-SUHC, CC-SPH-1, CC-SPH-2, CC-SPHC-1, CC-SPHC-2, and CC-SUHC.

According to León-López (2019), the high temperature used to hydrolyze their collagen led to the transformation of asparagine groups to aspartic acid and glutamine groups to glutamic acid, which results in a decrease in the amino groups and an increase in the carboxyl groups (or an increase in the acidic amino acids). This indicates that acidic amino acids predominate, shifting the pI to a lower value (León-López, 2019), which is a possible explanation for why the other SPH powders exhibited their lowest solubility at pH 2 instead of pH 4.

Furthermore, higher protein solubility values were generally observed in the powders with lower DH. For example, MX-SPH-1 (13% DH) had a significantly ($P<0.05$) higher solubility value than MX-SPH-2 (15% DH) at pH 7. The same trend was observed for CC-SPH-1 (13% DH) and CC-SPH-2 (15% DH) where a significantly ($P<0.05$) higher solubility value was observed at pH values of 2 and 7. The DH affects protein MW and hydrophobicity as well as polar and ionizable peptide residues. Additionally, protein solubility is influenced by the ratio of hydrophilic to hydrophobic residues (Dhanabalan et al., 2020; Gbogouri et al., 2004). According to Gbogouri et al. (2004), smaller peptides (typically in hydrolysates with higher DHs) obtained from myofibrillar proteins contain more polar residues that can form hydrogen bonds with water; therefore, increasing protein solubility. However, it is possible that there are more polar residues in MX-SPH-1 and CC-SPH-1 with 13% DH compared to MX-SPH-2 and CC-SPH-2 with 15% DH, which would explain why the lower DHs had higher solubility values and could eventually be confirmed by the amino acid composition. In addition to the influence of DH and pH on protein solubility, the enzyme used for the hydrolysis may also affect functional properties (Kristinsson & Rasco, 2000; Latorres et al., 2018).

Although the SPH control (MX/CC-SPHC-1 and -2) and unhydrolyzed control (MX/CC-SUHC) powders were not exposed to the enzyme, they showed high protein solubility in water.

These findings suggest that the high temperatures used to spray dry may have unfolded the protein globular structure exposing some previously buried hydrophobic amino acids, which may have resulted in a greater ratio of hydrophilic to hydrophobic residues that contributes to higher protein solubility (Dhanabalan et al., 2020; Gbogouri et al., 2004). Additionally, MX spray drying led to particle agglomeration, which contributes to increased solubility (Jinapong et al., 2008). This is because particle agglomeration is when multiple particles clump together via bridges, which allows for more water that can flow in between, contributing to a more uniform distribution of the particles in the water (Innovative Food Processors, Inc. [IFP, Inc.], 2013). MX powders generally showed significantly (P<0.05) higher solubility than CC powders in all evaluated pH values. This suggests that MX was a gentler spray drying configuration than CC. These results may be related to the moisture contents (CC powders were drier than MX powders). Protein solubility is an important functional property that influences other functional properties in protein hydrolysates (Zamorano-Apodaca et al., 2020). The high protein solubility observed in SPH powders over a wide pH range indicates the potential for these SPH powders to have various food and beverage applications.

Emulsification properties

The emulsification properties of SPH powders were evaluated by measuring the EAI and ESI at pH values of 2, 4, 7, and 10 (**Figure 3.4**). EAI measures the ability of protein hydrolysates to emulsify oils, while ESI indicates the ability of emulsions to resist emulsion structural changes such as coalescence, creaming, and separation over time (Thiansilakul et al., 2007). SPH powders exhibited EAIs between 3.36-90.88 m^2/g and ESIs between 10.55-190.18 min. The highest EAI (90.88 m^2/g) was observed in MX-SPH-2 at pH 10. Whereas the highest ESI (190.18 min) was observed in CC-SPH-2 at pH 10, which was significantly (P<0.05) higher than

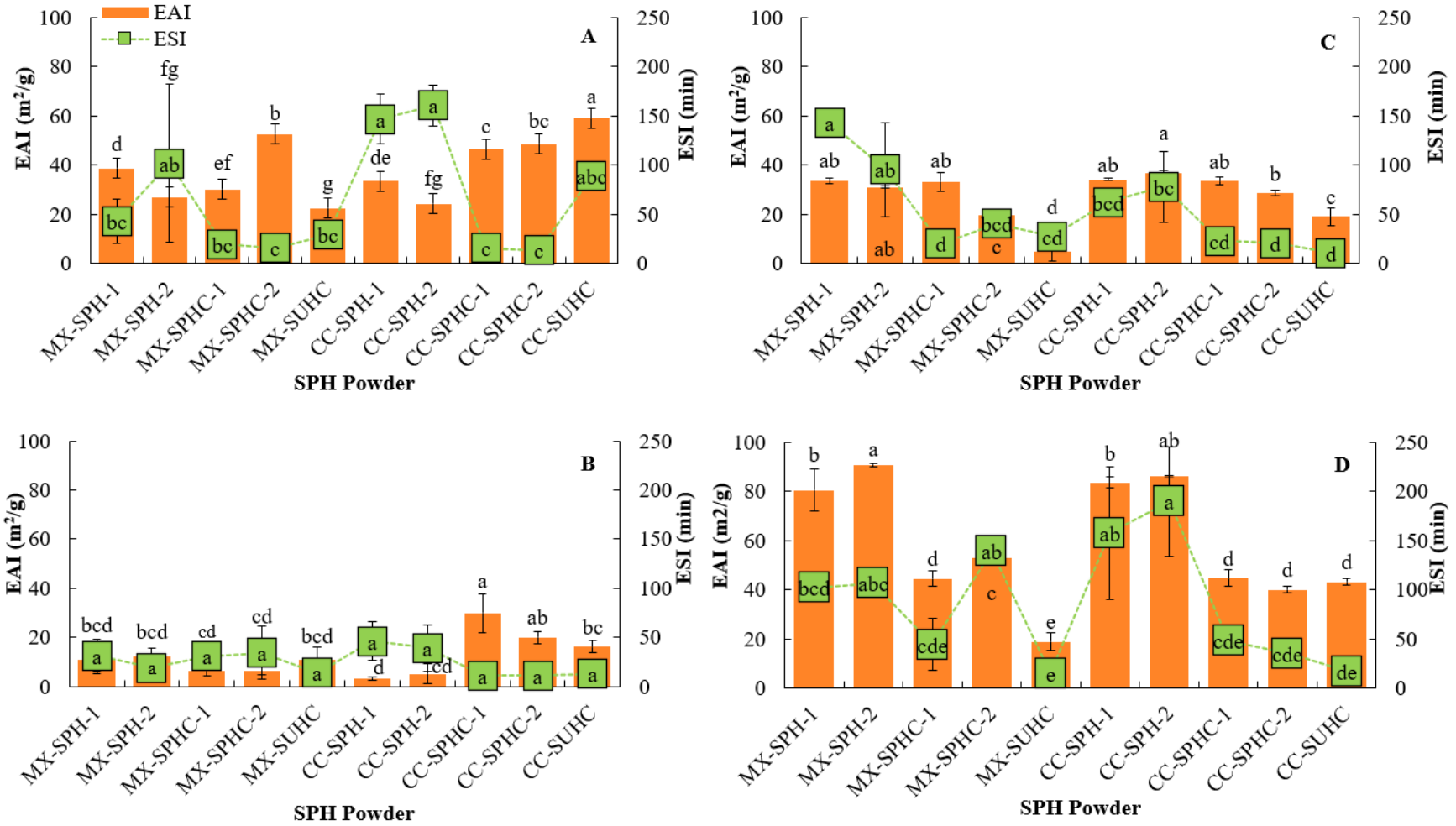


Figure 3.4. Emulsifying activity index (EAI) and emulsion stability index (ESI) of SPH powders at different pH conditions **A)** 2, **B)** 4, **C)** 7, and **D)** 10.
SPH = shrimp protein hydrolysate.
Different superscripts (a-g) between SPH powders of the same EAI or ESI bar indicate a significant (P<0.05) difference.
See Table 3.1 for the description of MX-SPH-1, MX-SPH-2, MX-SPHC-1, MX-SPHC-2, MX-SUHC, CC-SPH-1, CC-SPH-2, CC-SPHC-1, CC-SPHC-2, and CC-SUHC.

the other SPH powders (**Figure 3.4D**). Similar trends were observed in SPH and FPH powders reported by Latorres et al. (2018) and Taheri et al. (2013), respectively. The emulsification properties of protein hydrolysates are influenced by environmental pH. It modifies the protein's solubility, surface hydrophobicity, and protective layer that surrounds the lipid globules (Taheri et al., 2013). According to Taheri et al. (2013), alkaline pH results in the unfolding of polypeptides, which exposes previously buried hydrophobic residues and modifies the electric charge of peptides. This results in repulsion among the protein molecules allowing for greater orientation at the interface because hydrophilic and hydrophobic residues are exposed, promoting important interactions that enhance emulsification properties (Latorres et al., 2018; Taheri et al., 2013). Protein hydrolysates can form oil-in-water emulsions due to containing hydrophilic groups that can remain in the water phase and hydrophobic groups that can interact with the oil phase, reducing interfacial tension (Binsi et al., 2016; Chalamaiah et al., 2010).

Additionally, some studies have reported that protein hydrolysates with lower DHs and larger peptides are required to exhibit enhanced emulsification properties (Latorres et al., 2018). However, this relationship was not observed in this study. In fact, no correlation between DH and emulsification properties can be inferred from this study. Kristinsson and Rasco (2000) reported that there is no clear association between peptide size and/or DH of protein hydrolysates and emulsification properties. Several studies have reported that both smaller and larger peptides exhibited enhanced emulsification properties (Zamorano-Apodaca et al., 2020). Therefore, the amino acid composition and sequence, as well as the amphiphilic character, are considered more relevant factors that contribute to enhanced emulsification properties of protein hydrolysates than peptide length/DH (Parvathy et al., 2018; Taheri et al., 2013; Tejpal et al., 2017). In general, SPH powders had the lowest EAI and ESI values at pH of 4; while the highest EAI and ESI

values were observed at pH 10. Furthermore, CC-SUHC had significantly (P<0.05) higher EAI compared to the rest of the SPH powders at pH 2. In comparison, CC-SPH-1 and CC-SPH-2 had significantly (P<0.05) higher ESI compared to most of the other SPH control powders at pH 2 (**Figure 3.4A**). At pH 7, MX-SPH-1 and CC-SPH-2 exhibited significantly (P<0.05) higher EAI and ESI compared to most of the SPH control powders (**Figure 3.4C**). These differences could be due to the differences in pH, which alter the solubility and surface hydrophobicity of the peptides as well as the protective layer that surrounds the lipid globules, as mentioned previously (Taheri et al., 2013).

Lastly, minimal EAI and ESI values were generally observed at pH 4 compared to the other pH values (**Figure 3.4B**). According to Latorres et al. (2018), a pH of 4 is near the pI of fish proteins, where there is no net charge resulting in the precipitation of proteins, which would reduce emulsification properties. MX and CC powders had similar emulsification properties, and it seemed that the spray drying configuration did not significantly affect the emulsification properties of the resultant SPH powders. Therefore, the present study revealed that SPH powders could stabilize oil-in-water emulsions at different pH values (mainly at pH values of 7 and 10) and thus have the potential to serve as an emulsifier in foods. Nevertheless, it is possible that a different SPH powder could be used as an emulsifier depending on the pH of the desired food application.

Foaming properties

The foaming properties of SPH powders were evaluated by measuring the FC and FS at pH values of 2, 4, 7, and 10 (**Figure 3.5**). FC measures the percent volume increase generated from whipping the protein in liquid compared to the liquid's initial volume (Kristinsson & Rasco, 2000; Latorres et al., 2018), whereas FS measures the amount of time required for the

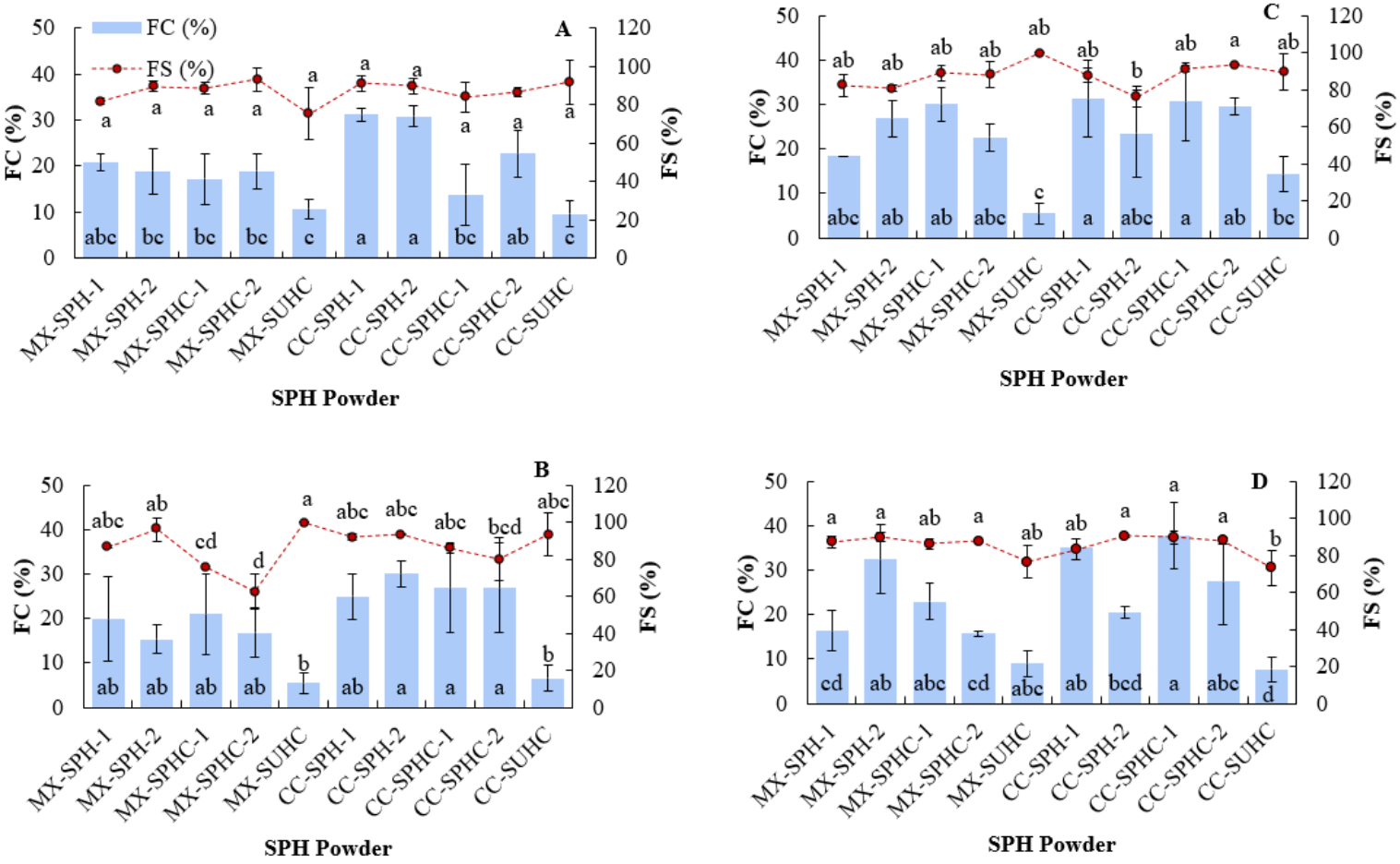


Figure 3.5. Foaming capacity (FC) and foaming stability (FS) of SPH powders at different pH conditions **A)** 2, **B)** 4, **C)** 7, and **D)** 10. Different superscripts (a-e) between SPH powders of the same FC or FS bar/point indicate a significant (P<0.05) difference. See Table 3.1 for the description of MX-SPH-1, MX-SPH-2, MX-SPHC-1, MX-SPHC-2, MX-SUHC, CC-SPH-1, CC-SPH-2, CC-SPHC-1, and CC-SPHC-2, and CC-SUHC.

foam to lose half its volume (Kristinsson & Rasco, 2000). SPH powders showed FC values between 5.46-37.91% and FS values between 58.73-100.00%. The highest FC (37.91%) was observed in CC-SPHC-1 at pH 10 (**Figure 3.5D**). While the highest FS (100%) was observed in MX-SUHC at pH 4 (**Figure 3.5B**). Overall, the unhydrolyzed controls (MX-SUHC and CC-SUHC) exhibited the lowest FCs at the four pH values evaluated in this study. Similar results were reported by Ben Slama-Ben Salem et al. (2017) for their undigested octopus protein hydrolysates. Low FC is expected in the native protein because foam formation is governed by transportation, penetration, and rearrangement of molecules at the air-water interface (Binsi et al., 2016; Intarasirisawat et al., 2012). Foams require hydrophobic groups to orient towards the air phase and hydrophilic regions to remain in the water phase, which stabilizes the system (Limam et al., 2008). Hydrophobic residues are generally buried in the interior of the native protein (Fennema, 1996), which suggests that the native protein does not have enough hydrophobic residues exposed to decrease the surface tension of the foam (Intarasirisawat et al., 2012). Additionally, CC powders had higher FC compared to the MX powders. A possible explanation is that more particle agglomeration was observed in the MX powders (confirmed by the higher span values in **Table 3.3** and SEM in **Figure 3.8A, C, E, G, and I**), which affects powder functionality (Rodríguez-Díaz et al., 2014). In this case, it caused the decrease in the FC for MX powders.

According to Siddik et al. (2021), DH is positively associated with the FC and FS in FPH powders. Alternatively, many studies report that DH is positively associated with FS and inversely associated with FC (Binsi et al., 2016; Tejpal et al., 2017). These studies argue that smaller peptides (in hydrolysates with higher DH) can rapidly migrate to the air-water interface. Whereas larger peptides (in hydrolysates with lower DH) can surround the air bubbles better by

forming thicker and stronger films (Binsi et al., 2016; Latorres et al., 2018; Tejpal et al., 2017). In this study, neither relationship was clearly identified, which is in accordance with the results reported for emulsification properties. One speculation is that the two DHs (13% and 15%) investigated in this study were too close to observe a clear relationship. Studies reported this relationship by evaluating hydrolysates with DH with at least a 5% difference (Binsi et al., 2016; Intarasirisawat et al., 2012; Latorres et al., 2018). For most of the SPH powders, minimal FC values were observed at pH 2 and 4, which similarly follows the results of solubility and emulsification properties further confirming the possibility of the pI to be near pH 2 and 4.

Lastly, the FS of all SPH powders were similar ($P>0.05$) at their respective pH value. A similar trend was described by Latorres et al. (2018), who did not observe many differences between their enzymatic hydrolysates with 10% and 20% DHs at their respective pH value. Latorres et al. (2018) reported production of protein hydrolysates from white shrimp meat, and this study similarly evaluated the FS after 20 min. However, other studies have evaluated the FS after 30 min and observed lower FS values, which could explain the relatively higher FS values compared to those studies (Ben Slama-Ben Salem et al., 2017; Binsi et al., 2016; Intarasirisawat et al., 2012). According to Taheri et al. (2013), a protein may not necessarily have the best foaming ability, but the foam it creates may exhibit superior foaming stability and vice versa. Thus, SPH powders from this study could be further investigated in foods to determine if they are an ideal foaming agent.

Antioxidant properties

DPPH RSA

The DPPH RSA of SPH powders ranged from 26.84-57.65% at protein concentrations of 5 and 7.5 mg/mL with most SPH powders exhibiting activities greater than 50% (**Figure 3.6**).

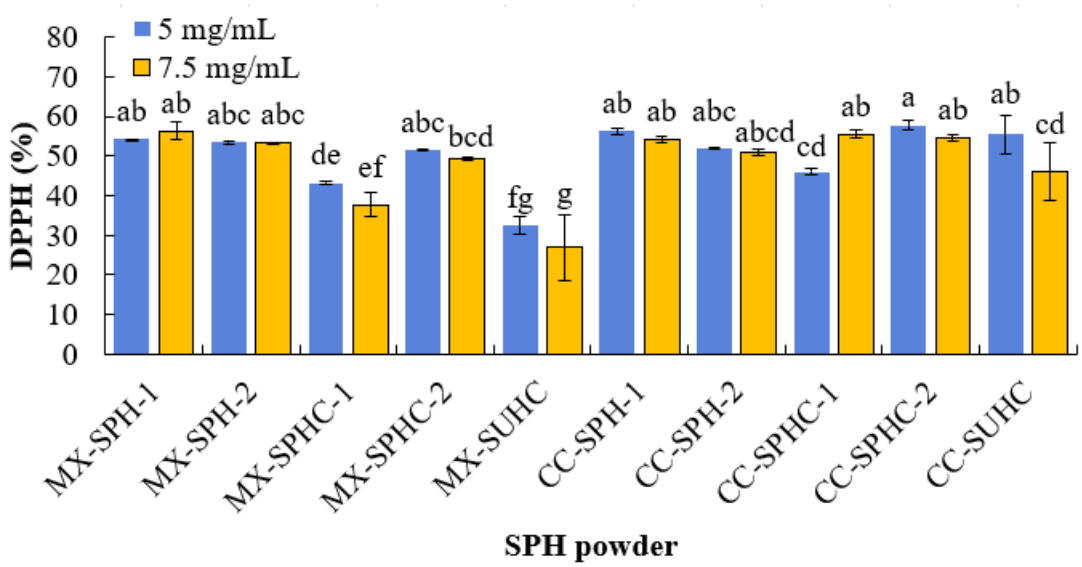


Figure 3.6. DPPH RSA (%) of SPH powders at two concentrations (5 and 7.5 mg/mL). Different superscripts (a-g) among SPH powders indicate a significant (P<0.05) difference between powders and concentrations.
See Table 3.1 for the description of MX-SPH-1, MX-SPH-2, MX-SPHC-1, MX-SPHC-2, MX-SUHC, CC-SPH-1, CC-SPH-2, CC-SPHC-1, and CC-SPHC-2, and CC-SUHC.

These results are comparable with those of Latorres et al. (2018), who reported DPPH RSA of FPH powders between 5-70% at concentrations of 2.5 mg/mL, 5 mg/mL, and 7.5 mg/mL using 0.1 mM/L DPPH in 95% ethanol (the same as this study). These observations suggest that SPH powders can stabilize DPPH, thus terminating the radical chain reaction (Latorres et al., 2018). Protein concentration influences the DPPH RSA (Ben Slama-Ben Salem et al., 2017; Latorres et al., 2018). Latorres et al. (2018) reported higher DPPH RSA at 7.5 mg/mL compared to 2.5 mg/mL and 5 mg/mL. However, in this study, as concentration of SPH powders increased to 7.5 mg/mL, the DPPH RSA generally stayed the same or decreased, suggesting that it is ineffective to use SPH powders at concentrations higher than 5 mg/mL to observe the highest antioxidant activities.

Furthermore, two control powders (MX-SPHC-1 and MX-SUHC) had significantly ($P<0.05$) lower DPPH RSA values compared to the enzymatic SPH powders. Similarly, Ben Slama-Ben Salem et al. (2017) reported a lower DPPH RSA of 33% for undigested octopus protein at 6 mg/mL using 0.02% DPPH in 99.5% ethanol compared to their octopus hydrolysates. Non-surprisingly, SPH powders with lower DHs (MX-SPH-1 and CC-SPH-1) exhibited higher DPPH RSA values. According to Intarasirisawat et al. (2012), DH plays a role in determining the antioxidant properties of protein hydrolysates due to its ability to influence the peptide chain length. Intarasirisawat et al. (2012) and Binsi et al. (2016) suggested that hydrolysates with higher DHs (containing shorter peptides with more hydrophilic groups) are less efficient at scavenging the DPPH radical. Also, shorter peptides are unable to interact with the hydrophobic peroxyl radicals. In general, peptide chain length, hydrophobicity, amino acid composition, and amino acid sequence affect the DPPH RSA of protein hydrolysates (Ben Slama-Ben Salem et al., 2017). Interestingly, enzymatic SPH powders generally had

significantly (P<0.05) higher DPPH RSA activities than the unhydrolyzed control samples (MX/CC-SUHC). Enzymatic hydrolysis releases active peptides with antioxidant activity via peptide bond cleavage (You et al., 2010). Surprisingly, control samples including CC-SPHC-1, CC-SPHC-2, and CC-SUHC also exhibited DPPH RSA activity. This effect may be due to the high heat used in CC that may have unfolded the protein globular structure releasing peptides with antioxidant activity. Overall, non-enzymatic SPH powders produced via CC had higher DPPH SRA values than those non-enzymatic produced MX powders (**Figure 3.6**). It has been reported that the amino acids tyrosine, phenylalanine, histidine, lysine, proline, methionine, and arginine (present in GABS meat) contribute to higher DPPH RSA activities (You et al., 2010), which is a possible reason why most SPH powders exhibited DPPH activities >50%.

Tejpal et al. (2017) reported the synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), to have DPPH RSA of 90.38% and 79.17%, respectively, at 200 ppm (0.2 mg/mL) using 0.1 mM/L DPPH in 99.5% ethanol. Additionally, Ben Slama-Ben Salem et al. (2017) reported BHA as having 100% DPPH RSA activity at concentrations of 2-6 mg/mL using 0.02% DPPH in 99.5% ethanol. The differences in BHA's DPPH activity may be due using different BHA and/or DPPH concentrations. Despite SPH powders having lower DPPH RSAs than synthetic antioxidants, SPH powders are still effective antioxidants. Hence, these SPH powders could potentially be used as a source of natural antioxidants to prevent lipid oxidation in foods.

Reducing power assay

Reducing power of SPH powders exhibited a concentration-dependent increase (**Figure 3.7**). Higher absorbance at 700 nm indicated a higher reducing power (Ben Slama-Ben Salem et al., 2017). As expected, significantly (P<0.05) higher reducing power was observed when SPH

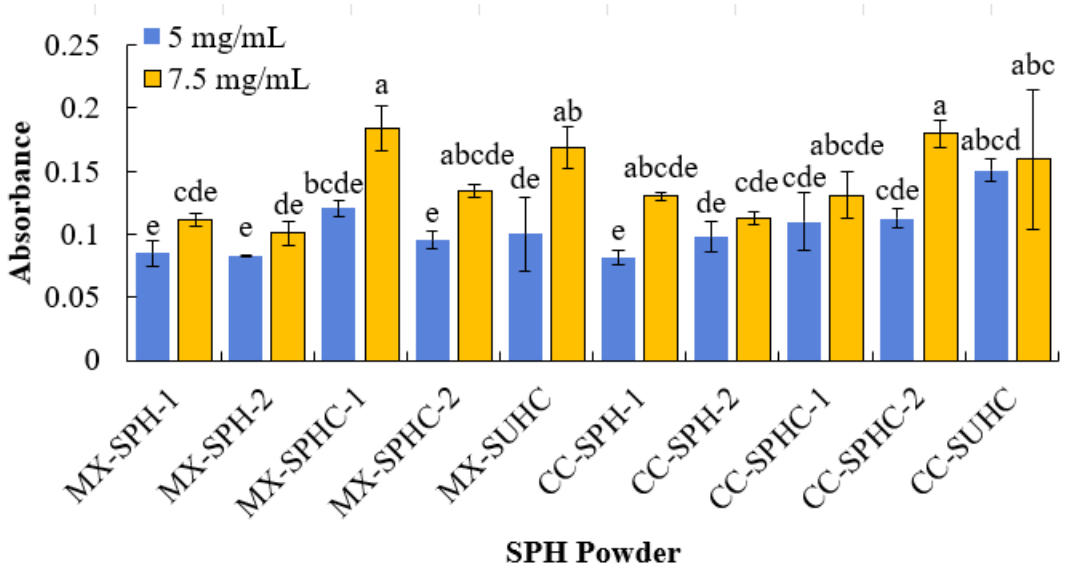


Figure 3.7. Reducing power of SPH powders at two concentrations (5 and 7.5 mg/mL). Different superscripts (a-e) among SPH powders indicate a significant (P<0.05) difference between powders and concentrations.
See Table 3.1 for the description of MX-SPH-1, MX-SPH-2, MX-SPHC-1, MX-SPHC-2, MX-SUHC, CC-SPH-1, CC-SPH-2, CC-SPHC-1, and CC-SPHC-2, and CC-SUHC.

powders were used at concentrations of 7.5 mg/mL than at 5 mg/mL (**Figure 3.7**). Several studies also reported observing an increase in the reducing power with an increasing concentration of protein hydrolysate (Ben Slama-Ben Salem et al., 2017; Dhanabalan et al., 2017; Latorres et al., 2018). Interestingly, enzymatic SPH powders generally had significantly (P<0.05) lower reducing powder than non-enzymatic control (MX/CC-SPHC-1 and -2) and unhydrolyzed control (MX/CC-SUHC) powders at both concentrations. According to Pownall et al. (2010), the reducing power of protein hydrolysates is associated with their total amount of hydrophobic amino acids. Additionally, Pownall et al. (2010) reported a high reducing power (~2.5) for glutathione which may be associated with its high cysteine content. This suggests the importance of the sulfhydryl group in cysteine as a reducing agent. The results obtained in this study suggests that enzymatic hydrolysis may have destroyed some amino acids (specially sulfur-containing ones such as methionine and cysteine). Overall, SPH powders exhibited weaker reducing power compared to protein hydrolysates reported in other studies such as Latorres et al. (2018) who observed reducing power in their SPH powders in the range of approximately 0.2-0.5 at concentrations of 2.5 mg/mL, 5 mg/mL, and 7.5 mg/mL. GABS meat contained lower amounts of cysteine and methionine (**Table 2.2**), which would explain the lower reducing power of SPH powders produced in this study.

Particle size distribution

CC powders had significantly (P<0.05) higher particle size distribution values compared to MX powders (**Table 3.3**). Jiang et al. (2020) similarly observed significantly (P<0.05) higher particle sizes for CC powders compared to MX powders. Additionally, CC powders had median particle size values (D_{50}) > 5.00 μm, while MX powders had D_{50} values < 5.00 μm. In spray drying, the final product's particle size is influenced by the atomization conditions, the feed flow

Table 3.3. Particle size distribution values of SPH powders[†].

SPH powder	*D_{10} (µm)	*D_{50} (µm)	*D_{90} (µm)	Mean (µm)	Span
MX-SPH-1	0.18 ± 0.03^{d}	4.35 ± 0.07^{d}	17.09 ± 0.18^{e}	7.38 ± 0.13^{c}	3.89 ± 0.04^{d}
MX-SPH-2	0.56 ± 0.33^{c}	3.80 ± 0.30^{de}	22.31 ± 0.07^{bc}	9.58 ± 0.55^{b}	5.75 ± 0.54^{c}
MX-SPHC-1	0.09 ± 0.00^{d}	1.52 ± 0.98^{f}	21.58 ± 0.13^{c}	5.60 ± 0.15^{d}	10.34 ± 0.33^{a}
MX-SPHC-2	0.24 ± 0.01^{cd}	3.28 ± 0.04^{e}	22.98 ± 0.51^{b}	8.05 ± 0.40^{c}	6.94 ± 0.21^{b}
MX-SUHC	0.27 ± 0.17^{cd}	4.59 ± 0.42^{d}	13.27 ± 0.96^{f}	6.28 ± 0.58^{d}	2.84 ± 0.22^{e}
CC-SPH-1	2.93 ± 0.03^{ab}	8.58 ± 0.08^{a}	24.70 ± 0.79^{a}	12.19 ± 0.22^{a}	2.54 ± 0.09^{e}
CC-SPH-2	2.79 ± 0.01^{b}	7.03 ± 0.07^{b}	19.92 ± 0.28^{d}	9.96 ± 0.03^{b}	2.44 ± 0.06^{ef}
CC-SPHC-1	3.15 ± 0.05^{a}	9.08 ± 0.15^{a}	25.04 ± 0.36^{a}	12.57 ± 0.18^{a}	2.41 ± 0.03^{ef}
CC-SPHC-2	3.10 ± 0.01^{ab}	9.05 ± 0.10^{a}	25.10 ± 0.34^{a}	12.56 ± 0.15^{a}	2.43 ± 0.01^{ef}
CC-SUHC	2.76 ± 0.06^{b}	5.92 ± 0.04^{c}	13.87 ± 0.46^{f}	7.81 ± 0.11^{c}	1.88 ± 0.08^{f}

[†]Values are reported as mean ± standard deviation of triplicate determinations.
*D_{10}, D_{50}, and D_{90} indicate 10%, 50%, or 90% of the total particles are smaller/larger than the reported value.
Different superscripts (a-f) within the same column indicate a significant ($P<0.05$) difference.
See Table 3.1 for the description of MX-SPH-1, MX-SPH-2, MX-SPHC-1, MX-SPHC-2, MX-SUHC, CC-SPH-1, CC-SPH-2, CC-SPHC-1, and CC-SPHC-2, and CC-SUHC.

rate, the feed's solid content, and the particle residence time (Jiang et al., 2020; Pinto et al., 2014). Additionally, the shear applied by the atomizer impacts the feed droplet size and, ultimately the particle size of the powder particles (Santos et al., 2017). For example, higher shear results in smaller feed droplets and powder particles (Jiang et al., 2020; Santos et al., 2017). Therefore, the atomizer used in MX may exert higher shear, which results in smaller droplet sizes and thus smaller powder particles.

Furthermore, SPH powders generally had span values greater than two. CC powders typically had significantly ($P<0.05$) lower span values than MX powders. Particle span is calculated using the D_{10}, D_{50}, and D_{90} values to determine the spread of the particles or particle agglomeration (Jiang et al., 2020; Microtrac, 2022). Jiang et al. (2020) also observed lower span values in CC powders compared to MX powders. This effect may be due to more particle agglomeration in MX, which is further supported by Tonon et al. (2011) who suggested that particle agglomeration is associated with span values greater than two, while span values less than two indicate homogenous distribution. Therefore, particle agglomeration occurs more in MX (Cal & Sollohub, 2010; Jiang et al., 2020). In MX spray drying, particles have shorter residence times in the drying chamber compared to CC spray drying, which results in particles with higher moisture contents. Because of the higher moisture contents, the particles are able to stick to eachother or the walls of the drying chamber and form aggregates (Francia et al., 2016). In general, agglomerated particles have higher water solubility compared to non-agglomerated particles, and it is desirable in instant powders (IFP, Inc., 2013).

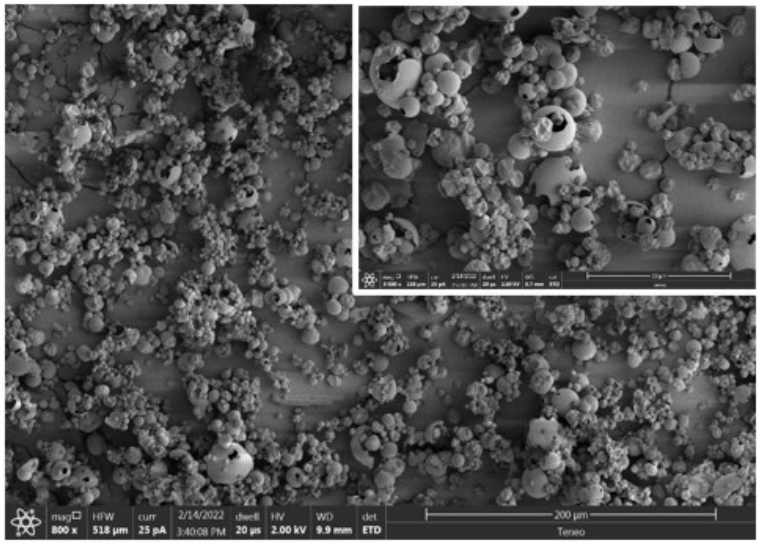
Because particle size affects the production and handling of ingredients, formulation, processing, and quality control of foods, it is important to understand the particle size of powdered products. More specifically, powdered products also affect the reactivity, solubility,

and flowability as well as the texture and mouthfeel of the ingredients they are incorporated into (Mermelstein, 2016). According to Agboola et al., (2005), 0.1-10 μm are in the low particle size range, which was observed in all SPH powders for their D_{50} values. These small particle size values may have contributed to a low product yield (between 50-100 g of powder from 750 g of shrimp meat), which might be problematic for the scale-up of these powders. Smaller particles may also contribute to decreased flowability, which may limit the storage and application of SPH powders (Hazlett et al., 2021). Jiang et al. (2020) reported larger D_{50} values for their MX and CC fish gelatin powders containing probiotics, which could be due to an increased feed solids content compared to this study. In the future, carrier agents such as maltodextrin, gum Arabic, or inulin could be added to help increase the feed solids content and thus the particle size values, while also increasing product yield and glass transition temperature to reduce the stickiness of the final powders (Etzbach et al., 2020; Xu et al., 2018). Optimal SPH powder particle size values could also be investigated for better incorporation of these powders into food and beverage applications.

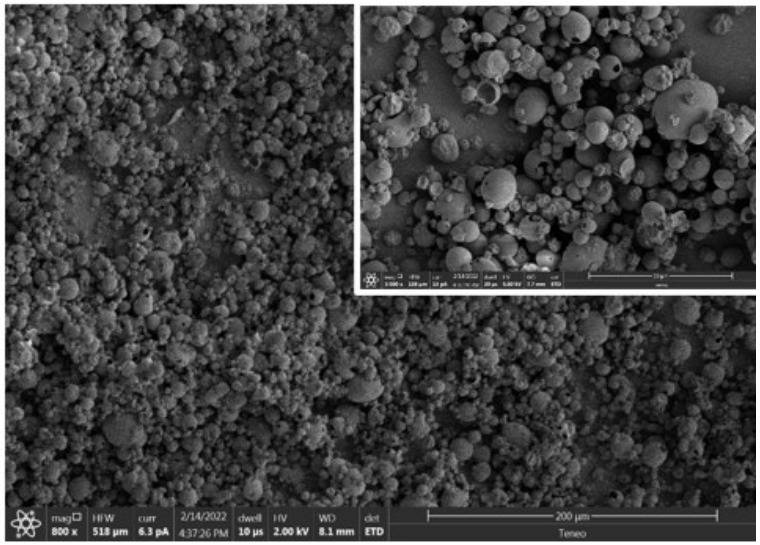
Particle morphology

Micrographs of SPH powders are illustrated in **Figure 3.8**. MX powders are depicted on the left (**Figures 3.8 A, C, E, G, I**) and CC powders on the right (**Figures 3.8 B, D, F, H, J**). All SPH powders exhibited irregular spherical shapes with hollow structures. However, more hollow structures can generally be observed in CC powders, indicating a harsher drying condition than MX powders. During spray drying, moisture evaporation causes the feed droplets to shrink and the solute concentration to continuously rise at the surface of the feed droplet. Then, a solid shell is formed around the free droplet, and crystallization occurs due to high solute concentration at

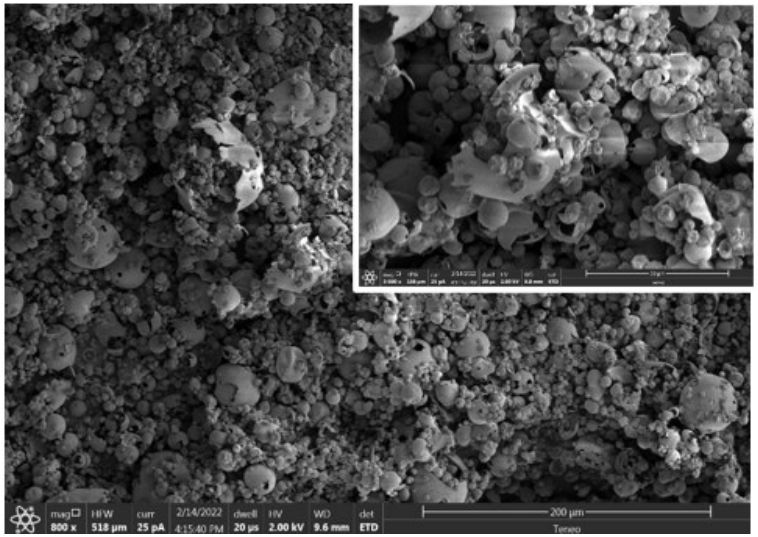
A)


mag HFW curr 2/14/2022 dwell HV WD det
800 x 518 µm 25 pA 3:40:08 PM 20 µs 2.00 kV 9.9 mm ETD
200 µm
Tenero

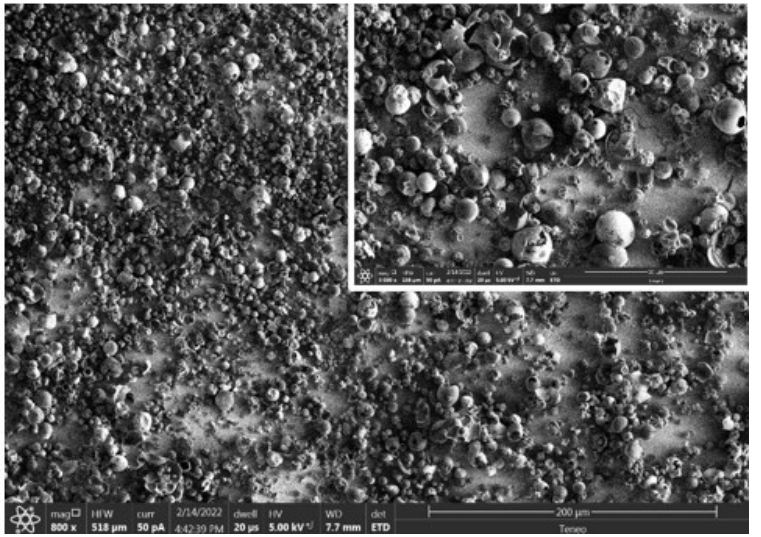
B)


mag HFW curr 2/14/2022 dwell HV WD det
800 x 518 µm 6.3 pA 4:37:26 PM 10 µs 2.00 kV 8.1 mm ETD
200 µm
Tenero

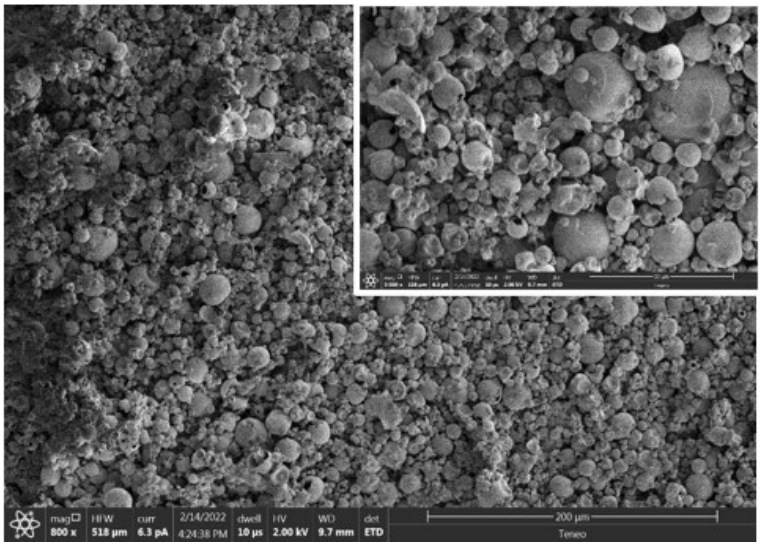
C)


mag HFW curr 2/14/2022 dwell HV WD det
800 x 518 µm 25 pA 4:15:40 PM 20 µs 2.00 kV 9.6 mm ETD
200 µm
Tenero

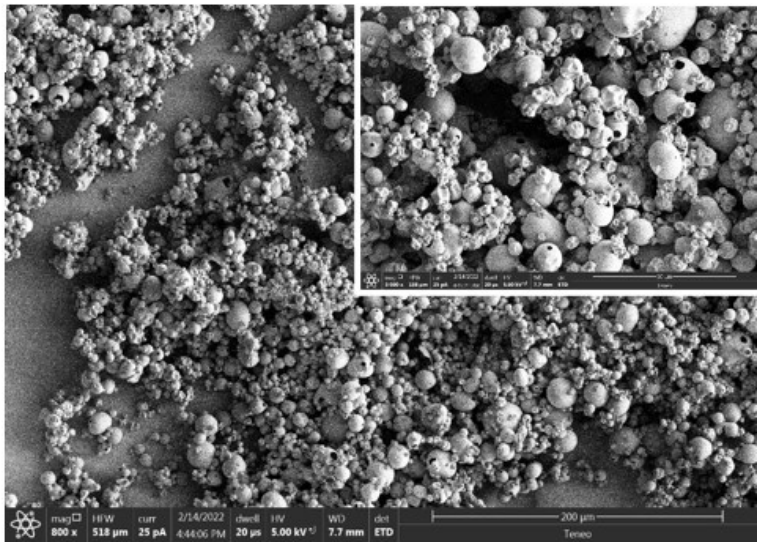
D)


mag HFW curr 2/14/2022 dwell HV WD det
800 x 518 µm 50 pA 4:42:39 PM 20 µs 5.00 kV 7.7 mm ETD
200 µm
Tenero

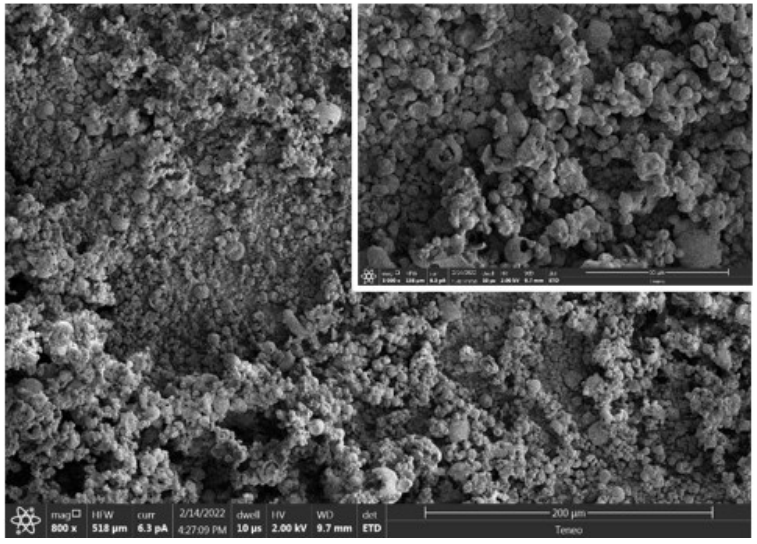
E)


mag 800 x
HFW 518 µm
curr 6.3 pA
2/14/2022 4:24:38 PM
dwell 10 µs
HV 2.00 kV
WD 9.7 mm
det ETD
200 µm
Teneo

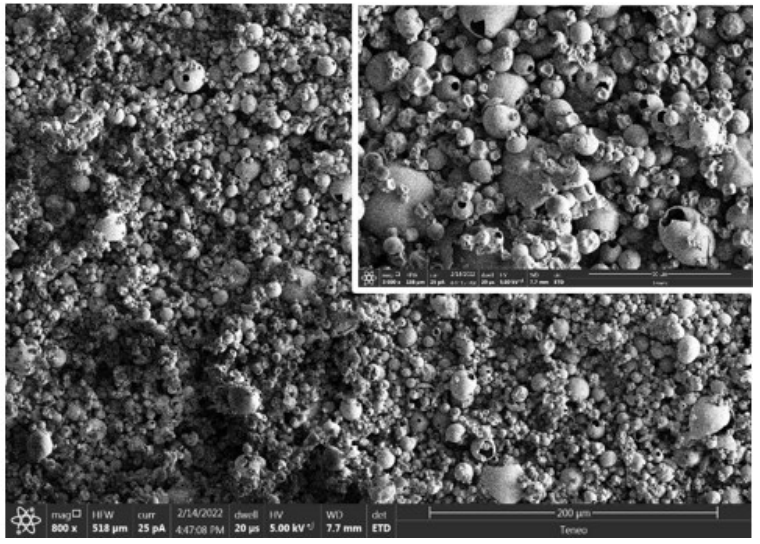
F)


mag 800 x
HFW 518 µm
curr 25 pA
2/14/2022 4:44:06 PM
dwell 20 µs
HV 5.00 kV
WD 7.7 mm
det ETD
200 µm
Teneo

G)


mag 800 x
HFW 518 µm
curr 6.3 pA
2/14/2022 4:27:09 PM
dwell 10 µs
HV 2.00 kV
WD 9.7 mm
det ETD
200 µm
Teneo

H)


mag 800 x
HFW 518 µm
curr 25 pA
2/14/2022 4:47:08 PM
dwell 20 µs
HV 5.00 kV
WD 7.7 mm
det ETD
200 µm
Teneo

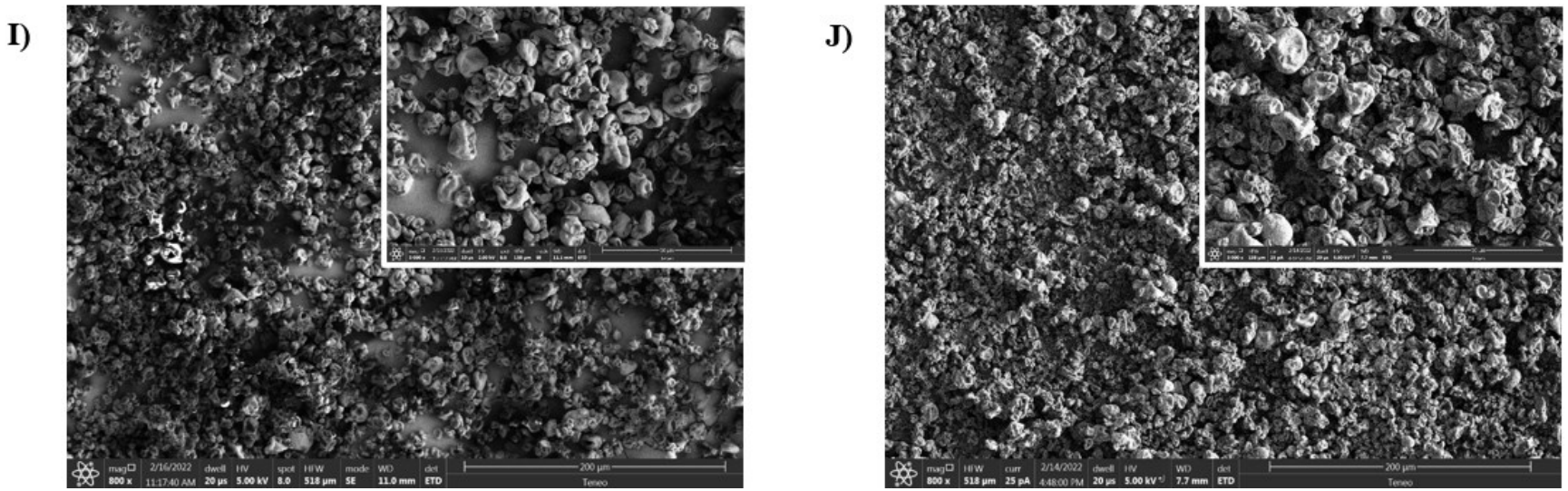


Figure 3.8. Scanning electron micrographs of **A)** mixed-flow shrimp protein hydrolysate 1 (MX-SPH-1) **B)** concurrent shrimp protein hydrolysate 1 (CC-SPH-1) **C)** mixed-flow shrimp protein hydrolysate 2 (MX-SPH-2) **D)** concurrent shrimp protein hydrolysate 2 (CC-SPH-2) **E)** mixed-flow shrimp protein hydrolysate control 1 (MX-SPHC-1) **F)** concurrent shrimp protein hydrolysate control 1 (CC-SPHC-1) **G)** mixed-flow shrimp protein hydrolysate control 2 (MX-SPHC-2) **H)** concurrent shrimp protein hydrolysate control 2 (CC-SPHC-2) **I)** mixed-flow shrimp unhydrolyzed control (MX-SUHC) **J)** concurrent shrimp unhydrolyzed control (CC-SUHC). See Table 3.1 for description of MX-SPH-1, MX-SPH-2, MX-SPHC-1, MX-SPHC-2, MX-SUHC, CC-SPH-1, CC-SPH-2, CC-SPHC-1, and CC-SPHC-2, and CC-SUHC.

the surface. Heat and mass transfer rates (from hot drying air to liquid feed and vice versa) influence how fast or slow this solid shell is formed around the droplet. Once the droplet shell is formed, internal vapor flow becomes constricted by the shell, which increases droplet pressure leading to the formation of internal droplet gas bubbles resulting in the formation of hollow structures in the spray-dried particles (Re', 1998; Rodríguez-Díaz et al., 2014; Seydel et al., 2006). Furthermore, MX powders generally showed fewer hollow structures and more particle agglomeration. Jiang et al. (2020) reported similar observations for MX powders. Lastly, **Figures 3.8 I and J** show severe shrinkage with minimal observations of hollow structures in the unhydrolyzed control powders, MX-SUHC and CC-SUHC. Particle shrinkage results from the rapid evaporation of water from the sample during spray drying (Wang et al., 2020). Additionally, particles may shrink when internal droplet pressure exceeds the shell strength or due to slow shell formation during drying of the solution droplet (Re', 1998; Rodríguez-Díaz et al., 2014; Seydel et al., 2006). Particle morphology is highly associated with powder functionality, stability, and flowability (Rodríguez-Díaz et al., 2014). Both CC and MX powders could be utilized in different food and beverage applications.

Conclusion

This study revealed that shrimp protein hydrolysate (SPH) powders can be effectively produced from Georgia-caught brown shrimp (GABS) meat via enzymatic hydrolysis with an alkaline protease under mixed-flow (MX) and/or concurrent (CC) spray drying. Enzymatic SPH powders had a degree of protein hydrolysis (DH) of 13% and 15% and are considered microbiologically-stable products because they had water activities <0.3 and moisture contents <9% (wet basis, w.b.). SPH powders were high in crude protein (84.70-92.64%, dry basis, d.b.) and generally exhibited enhanced protein solubility compared to the unhydrolyzed control

powders. Moreover, enzymatic SPH powders exhibited high emulsification properties at pH values of 7 and 10, which suggests that SPH powders can be potentially used in neutral and alkaline food applications. Also, SPH powders had antioxidant activities (DPPH RSA > 50% at 5 mg/mL). Therefore, they could serve as natural emulsifiers with antioxidant properties to prevent lipid oxidation. SPH powders produced via MX generally had higher moisture content, protein solubility, emulsification properties, and particle agglomeration. Meanwhile, SPH powders produced via CC generally showed higher foaming properties, antioxidant activities and particle sizes. Therefore, both CC and MX powders could be utilized in different foods and beverages. The nutritional and functional properties found in the resultant SPH powders indicate the potential for them to have various food applications, which would ultimately help increase the commercial value of an underutilized seafood commodity in Georgia.

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CHAPTER 4

CONCLUSIONS AND FUTURE STUDIES

Before this research, GABS meat's proximate and selected nutritional characterization was limited. Therefore, a comprehensive understanding of GABS meat's nutritional composition is required to explore alternative uses for this underutilized seafood commodity. Not surprisingly, GABS meat had a high water activity, moisture content, crude protein, and essential amino acids. Moreover, GABS meat is considered lean meat due to its low-fat content. Palmitic and stearic acids were the main fatty acids detected in GABS meat. Thus, this study demonstrated that GABS meat is an excellent raw material for producing protein hydrolysate powders, which may add more value for consumers than whole fresh brown shrimp.

SPH powders were effectively produced from GABS meat via enzymatic hydrolysis with an alkaline protease and spray drying. The effect of spray drying configurations including mixed-flow (MX) and concurrent (CC) on the properties of the SPH powders were evaluated. Microbiologically-stable, enzymatic SPH powders with two degrees of protein hydrolysis (%, DH) (13 and 15) were produced. Furthermore, SPH powders had water activities <0.3, moisture contents <9% (w.b.), were high in protein (84.70-92.64%, d.b.), and exhibited enhanced protein solubility compared to unhydrolyzed control powders. In addition, enzymatic SPH powders exhibited high emulsification properties at pH values of 7 and 10, which suggests that these powders can be effectively used in neutral and alkaline food applications. Also, enzymatic SPH powders had high antioxidant activities. Therefore, they could serve as emulsifiers with antioxidant properties to prevent lipid oxidation. Generally, SPH powders produced via MX had

higher moisture content, protein solubility, emulsification properties, and agglomeration. While, CC powders showed higher foaming properties, antioxidant activities, and particle sizes. This research revealed that SPH powders have the potential to have various applications in foods and beverages due to their enhanced solubility, emulsification properties, and antioxidant activity, which could ultimately help increase the commercial value of an underutilized seafood commodity in Georgia.

Future studies are recommended because this process was found to be feasible on a lab-scale. For example, a shelf-life study is needed to confirm SPH powders' microbiological stability. Also, SPH powders could be evaluated in different food/beverage applications, such as soups and smoothies. If successfully incorporated in the food/beverage application, a sensory evaluation could be conducted to determine if any bitterness and/or off-odors need to be masked or reduced. If unsuccessful, different DH and hydrolysis times could be assessed using the hydrolysis method from this study. Furthermore, the feasibility of an industrial scale-up for SPH powders would require consideration of various factors. The hydrolysis method in this study is less labor intensive than some methods (ex: adjusting the pH of the sample once) and uses very short hydrolysis times that resulted in high protein solubility, which would contribute to the feasibility of scaling up. However, the low powder yield and limited availability and quantities of GABS meat year-round may limit the feasibility of scaling up.

Other future studies include the potential to use the hydrolysis method from this study for hydrolyzing GABS by-products or similar underutilized raw materials. Thus, future studies could investigate the feasibility of producing hydrolysate powders from GABS by-products and other underutilized seafood commodities. Lastly, future research could investigate if SPH powders'

allergenicity has been reduced since shrimp is an allergen. GABS may be small in size, but they have enormous potential to be utilized in various food and beverage applications.