PHYSICOCHEMICAL AND SENSORY EVALUATION OF INVASIVE SILVER CARP (*Hypophthalmichthys molitrix*) FISH NUGGETS

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Dedicated to my Heavenly Father whose grace has led me this far. My father on this earth for his hard work, dedication, and steadfastness. My Mother whose love still showers down even through death. My sister forever and for always my #1 support. My Grandmother, my biggest cheerleader. And to all those who come from lowly beginnings. "With God, all things are possible…"

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ABSTRACT

Silver carp (*Hypophthalmichthys molitrix*) are an underutilized, invasive fish threatening native species throughout major water systems in the United States. The goal of this research was to use silver carp meat to create a value-added product, to analyze the changes in physicochemical structure and consumer liking over time, and to evaluate the benefits of adding soy, pea, and a combination of soy and pea protein isolates to the formulations. Fish nuggets were prepared from minced meat in four treatments consisting of 3% soy protein isolate (SPI), 3% pea protein isolate (PPI), a combination of 1.5% SPI and 1.5% PPI, and a control without plant protein isolate. Nuggets from each treatment were stored frozen for 1.5, 8.5, and 13.5 weeks. Proximate composition, pH, cook loss, textural hardness, expressible moisture, color, microbial counts and lipid oxidation were evaluated. Sensory acceptability was also evaluated for each frozen storage time period and treatment. A descriptive (QDA™) trained sensory panel was also conducted on all treatments independent of the storage testing. Results showed that lipid oxidation and textural hardness significantly ($P < 0.05$) increased with frozen storage time. PPI had significantly lower expressible moisture compared to the control at week 1.5, but there were no statistically significant differences between treatments at weeks 8.5 and 13.5. Similarly, formulations with PPI improved ($p<0.05$) cook loss for week 1.5, but not week 8.5 or 13.5. Overall, sensory acceptability did not change ($P > 0.05$), with the exception of decreased degree of liking scores for SPI aroma ($p=0.03$) and flavor ($p=0.03$)) during the frozen storage period; all degree of liking scores remained above 6.5 throughout analysis, indicating that consumers' acceptability of the sample treatments over time despite the changes in physicochemical structure. The descriptive panel created an attribute lexicon for the aroma, flavor, and mouthfeel of the fish nuggets and did not find significant differences in intensities for those attributes between the treatments. Although there were

measurable changes in oxidation, texture, expressible moisture, and cook loss over 13.5 weeks, these changes did not impact sensory acceptance. The addition of protein isolates improved water holding capacity initially but did not maintain those benefits over extended shelf life and had little impact on consumer liking during any time period. Overall, this study demonstrated that value-added products such as silver carp nuggets can be created using an otherwise under-utilized fish. The fish nuggets had high sensory acceptability, and the addition of protein isolates did not significantly improve their sensory characteristics; therefore, silver carp nuggets can be formulated without the need of additional protein additives .

CHAPTER 1. INTRODUCTION/ LITERATURE REVIEW

Hypotheses:

1. A value-added product in the form of fish nuggets can be successfully formulated using invasive silver carp
2. The sensory and physicochemical qualities of the fish nuggets can be improved by using soy protein isolate or pea protein isolate.

Research Objectives:

1. Assess consumers knowledge about and attitude towards invasive silver carp products.
2. Analyze the physicochemical and sensory characteristics of the treatments with and without the addition of plant proteins.

1.1 The Fishing Industry in the United States of America

The combination of commercial and recreational fisheries makes up some 208 billion dollars in sales annually in the United States alone creating some 1.62 million jobs (Fisheries, 2017). In 2015 the commercial fishing industry was responsible for 1.18 millions of those jobs, and $144.2 billion dollars in sales. (Fisheries, 2017). According to the National Marine Fisheries Service, some 75.3% of fish caught in the United States are sold either fresh or frozen (Fisheries, 2017).

Table 1 "Consumption of Freshwater and Estuarine fish in adults 21+ by demographic characteristics in the United States (in g per day and based on edible portions)" (Estimated Fish Consumption Rates for the U.S. Population and Selected Subpopulations (NHANES 2003-2010), 2014)

Freshwater + Estuarine Finfish and Shellfish	Percentiles (95% CI)					
	50th	75th	90th	95th	97th	99th
Adults (≥21 yrs)	5.0 (4.1,6.0)	11.4 (9.9,13.1)	22.0 (19.1,25.4)	31.8 (26.9,37.6)	40.2 (33.3,48.5)	61.1 (48.7,76.6)
Age						
21 to <35 yrs	3.8 (3.1,4.8)	9.9 (8.3,11.7)	21.1 (17.6,25.1)	32.2 (26.2,39.7)	42.3 (33.3,53.7)	68.1 (50.1,92.5)
35 to <50 yrs	5.2 (4.1,6.6)	11.9 (9.6,14.8)	23.0 (18.4,28.7)	33.0 (26.0,41.8)	41.4 (32.2,53.1)	62.5 (46.8,83.4)
50 to <65 yrs	6.3 (5.0,7.9)	13.2 (10.9,15.9)	23.8 (19.7,28.9)	33.3 (26.9,41.3)	41.4 (32.7,52.4)	60.4 (45.9,79.4)
65+ yrs	4.5 (3.3,6.1)	9.9 (7.9,12.4)	18.7 (15.4,22.7)	26.5 (21.9,32.2)	33.1 (26.9,40.6)	48.8 (38.4,62.1)
Women of childbearing age (13 to 49 yrs)	2.9 (2.3,3.6)	7.6 (6.4,9.1)	15.8 (13.2,19.0)	23.5 (19.2,28.7)	29.9 (24.1,37.0)	46.6 (36.4,59.6)
Gender						
Female	4.1 (3.4,5.0)	9.3 (8.1,10.8)	18.0 (15.4,21.0)	25.7 (21.5,30.7)	32.1 (26.4,39.1)	48.2 (38.0,61.2)
Male	6.2 (5.0,7.6)	13.8 (11.8,16.2)	26.3 (22.4,30.9)	38.0 (31.6,45.6)	47.7 (39.0,58.4)	71.9 (56.4,91.8)
Race/Ethnicity[1]						
Mexican American	6.8 (5.3,8.6)	15.3 (12.4,18.9)	28.7 (23.1,35.8)	40.9 (32.2,51.9)	51.0 (39.6,65.6)	75.7 (56.8,100.8)
Other Hispanic	6.1 (4.4,8.6)	14.1 (10.3,19.3)	27.2 (19.5,37.9)	38.7 (27.5,54.5)	47.8 (33.7,67.6)	69.7 (48.3,100.6)
Non-Hispanic White	4.2 (3.4,5.2)	9.4 (8.0,11.1)	17.9 (15.1,21.1)	25.5 (21.2,30.8)	31.9 (26.0,39.0)	47.9 (37.2,61.6)
Non-Hispanic Black	7.2 (5.8,8.9)	15.4 (13.0,18.1)	28.2 (23.8,33.4)	39.6 (32.7,48.0)	48.8 (39.4,60.3)	70.8 (55.0,91.3)
Other Race	12.6 (9.4,16.9)	25.1 (19.2,32.9)	44.5 (33.3,59.6)	62.3 (45.2,86.1)	78.3 (55.0,111.5)	114.7 (76.4,172.1)
Income						
$0 to <$20K	3.5 (2.8,4.4)	9.1 (7.7,10.7)	19.2 (16.3,22.6)	28.9 (24.2,34.6)	37.4 (30.9,45.4)	59.3 (47.5,74.0)
$20 to <$45K	4.3 (3.5,5.4)	9.9 (8.5,11.5)	19.4 (16.6,22.7)	28.4 (23.7,33.9)	35.9 (29.6,43.6)	55.4 (43.8,70.0)
$40 to <$75K	4.8 (3.8,6.2)	10.8 (9.1,12.9)	20.6 (17.6,24.3)	29.6 (24.6,35.5)	37.3 (30.4,45.9)	56.8 (43.4,74.4)
$75+K	6.6 (5.4,8.1)	13.9 (11.7,16.6)	25.6 (21.3,30.8)	36.2 (29.4,44.5)	45.0 (35.9,56.5)	66.2 (51.1,85.9)
>$20K	5.5 (3.6,8.3)	12.1 (8.5,17.1)	22.3 (16.5,30.2)	30.9 (23.3,40.9)	38.7 (29.2,51.1)	56.1 (41.8,75.4)
Refused/Don't Know Income	5.4 (3.2,9.1)	13.8 (9.2,20.8)	29.0 (19.7,42.6)	43.1 (28.6,65.0)	56.6 (36.3,88.3)	88.6 (54.5,144.1)
Income Missing	1.9 (0.8,4.5)	7.1 (3.6,13.9)	18.9 (10.6,33.7)	31.7 (18.4,54.5)	41.6 (24.5,70.7)	65.9 (39.3,110.5)

[1] Race/ethnicity is as defined by NHANES. Respondents who self-identified as "Mexican American" were coded as such regardless of their other race-ethnicity identities. Otherwise, self-identified "Hispanic" ethnicity was coded as "Other Hispanic." All other non-Hispanic participants were then categorized based on their self-reported races: non-Hispanic white, non-Hispanic black, and other non-Hispanic race including non-Hispanic multiracial (other race).

In table 1 above from a National Health and Nutrition Examination Survey (NHANES) as published by the Environmental Protection Agency (EPA) (*Estimated Fish Consumption Rates for the U.S. Population and Selected Subpopulations (NHANES 2003-2010)*, 2014) information regarding the consumption of freshwater and estuarine fish within different demographics for the United States is documented. Survey data such as the table above is useful in evaluating the existing market of fish consumers, and in coming up with new marketing strategies to entice those not currently consuming fish. The survey data generally indicates that males tend to eat greater quantities of fish than females, and that older populations also tend to consume more fish. Interestingly, the data also indicates that fish consumption increases as income increases. There may be a variety of reasons for this including high costs of fish, perceived value, or familiarity with fish products. This information can be used strategically with marketing and advertising to reach the current consumer base, or as a means of enticing new consumers that might consider purchasing fish at a different price point, or who might be attracted by new forms of marketing. It is possible that value added fish products or lower cost fish products may increase the share of freshwater fish consumers.

1.2 Value Added Products from Fish.

A Value-added product is one which has been transformed such that the monetary value of the final product is higher than the raw material. General food examples include the likes of soy sauce and strawberry jam which transform a raw product or commodity (soybeans or strawberries) into a valuable, marketable product. Value-added products are particularly important as a means of utilizing waste streams and turning them into additional sources of revenue. In the fish industry specifically, a number of value-added products exists for food and non-food uses. Non-food examples include biodiesel and biogas production, as well as chitin and chitosan from crustaceans

(Kim, 2014). Examples for food use include surimi, fish mince, fish nuggets, fish sauces, flavorings, protein hydrolysates, and dietary supplements such as collagen and gelatin. Most of the value-added fish products in food applications rely on or are derived from the fish muscle proteins.

1.3 Fish Muscle Proteins

Fish muscles consist largely of two types of proteins: water-soluble sarcoplasmic proteins (making up 18-20% of the muscle proteins) and the myofibrillar proteins (making up 65-80% of muscle proteins) (Hall, 1997). A third group of proteins called stroma (making up 3-5% of muscle proteins) consists of connective tissues e.g. collagen and is difficult to solubilize (Hall, 1997). Sarcoplasmic proteins have poor water holding capacity and do not form a gel matrix (Paker & Matak, 2015). Myofibrillar proteins form the meat fibers in fish and consists of myosin, actin, tropomyosin, and troponin, all of which can be extracted using salt solutions (Hall, 1997).

In the Surimi making process sarcoplasmic proteins are removed through an extensive washing process. This process is very important as sarcoplasmic proteins interfere with the gel matrix that gives surimi it's characteristic texture. For instance, addition of sarcoplasmic proteins to surimi made with Alaskan pollock (*Theragra chalcogramma*) and also surimi made with silver carp formed weaker gel structures, though it was noted that there was a greater effect on the Alaskan pollock than the carp (Paker & Matak, 2015). Following washing, the minced meat is pressed allowing the myofibrillar proteins to form a gel network.

In addition to surimi, another commonly utilized value-added product is minced fish, hereafter referred to as mince which generally refers to ground fish meat. Mince can be stored up to 6 months, shows improved color upon washing, is generally of higher quality when dark (red) muscle tissues are removed, and requires use of cryoprotectants such as polyphosphates, hydrocolloids, or sucrose-sorbitol (Venugopal & Shahidi, 1995). Once fish is made into mince it

has five main processing applications which include hydrolysis, extrusion, washing (for creation of surimi), traditional products (such as cakes, fish balls, frozen mince blocks, canned products, etc.), and fermented products (Venugopal & Shahidi, 1995).

While there are several advantages to using fish mince, one major disadvantage is the increased risk for denaturation. Denaturation of mince proteins can be the result of degradation of trimethylamine N-oxide (TMAO), a process that is accelerated under frozen conditions (Parkin & Hultin, 1982). TMAO, a constituent found in most fish, is enzymatically broken into dimethylamine and formaldehyde. The formaldehyde is believed to interact with proteins causing cross-linkages resulting in textural changes, muscle gapping, and decreased water holding capacity (Hall, 1997; Parkin & Hultin, 1982; Venugopal & Shahidi, 1995). Further, trimethylamine (TMA) is responsible for the characteristic spoiled fish smell (Landfald, Valeur, Berstad, & Raa, 2017). Despite the increased risk of protein denaturation, fish mince has several uses.

One study evaluated the potential of creating low-salt restructured products using minced silver carp (*Hypophthalmichthys molitrix)* and utilizing microbial transglutaminase to act as a binder. Results indicated that salt is required to create restructured silver carp products, and cannot be replaced by transglutaminase alone; however, it is possible to reduce the amount of sodium in these value added restructured products using microbial transglutaminase (Tellez-Luis, Uresti, Ramirez, & Vazquez, 2002). This study may be useful in the creation of low sodium fish mince gels for consumers that are looking for lower sodium food options.

Elyasi, Zakipour Rahim Abadi, Sahari, and Zare (2010) reported making fish fingers formulated using either mince or surimi of common carp. They found that after 30 s of frying at 180 °C the microbial load was reduced to 0. Fish fingers made from surimi were whiter due to the washing away of sarcoplasmic proteins during the surimi making process resulting in significantly

higher sensory scores for visual appearance. Even so, they found no significant difference in terms of taste or texture. Fish fingers produced from fish mince are more practical from a processing standpoint and result in less waste production due to the large amounts of water required to produce surimi (Elyasi et al., 2010).

Fish sausages are another value-added product that can be made from underutilized fish species. In one study, (Paulo Roberto Campagnoli de Oliveira, Flavia Maria, Kazumi Kawazaki, Marco Antônio, & Elisabete Maria Macedo, 2010), sausages were made using various mixtures of whole Nile tilapia (*Oreochromis nilocticus*) fillets and mechanically separated mince, which utilized the waste of the Nile tilapia fillets. Sensory results indicated that not more than 60% of the formulation should consist of the mechanically separated minced fish as anything above this was considered unacceptable by consumers. This demonstrates that using waste streams, like the meat left behind during the filleting process, can generate valuable products; however, it is important to test such products for sensory acceptability. In a similar study (Oliveira Filho, Fávaro-Trindade, Trindade, Balieiro, & Viegas, 2010) sausages made with Nile Tilapia were evaluated for shelf life over a period of 40 days at 0 °C. Key quality metrics were recorded including tests for lipid oxidation via thiobarbituric acid (TBARS) and microbiological testing including *Salmonella* spp*.,* *Staphylococcus aureus*, and aerobic plate count. Results indicated that increasing the amount of minced fish did not increase the levels of lipid oxidation at 0 °C, though other studies (Cardoso, Mendes, Pedro, & Nunes, 2008; Siddaiah, Reddy, Raju, & Chandrasekhar, 2001; Wenjiao, Yongkui, Yunchuan, Junxiu, & Yuwen, 2014) were referenced as showing differences in lipid oxidation when stored at 2-4 °C. It is hypothesized that increases in lipid oxidation in minced fish products is the result of increased lipid quantities found in minced fish and more interactions between polyunsaturated fatty acids and oxidizing agents released during the mincing process.

Sausages tested negative for *Salmonella* spp. and *Staphylococcus aureus*. There was no clear correlation between the amount of minced meat added to the sausages and the aerobic plate counts, although bacteria were at higher levels in all samples after 40 days of storage compared to 5 days of storage.

Value-added products include but are not restricted to use of byproducts as in the studies above. The function of a value-added product is to raise the value of a naturally present and abundant resource that otherwise has a low value or marketability. For instance, imitation crab meat (surimi) in the United States is made from Alaska Pollock (*Theragra chalcogramma)* and Pacific Whiting (*Merluccius Productus*), and is most commonly used as a cheaper alternative to crab meat in salads and California rolls (Park, 2013). This product meets a unique market demand offering a cheaper and similar tasting alternative to crab meat while adding value to the Alaskan Pollock and Pacific Whiting. With the success of so many value-added products and a large market of fish consumers and potential fish consumers, it makes sense to evaluate sources of underutilized and naturally abundant fish sources such as invasive fish species.

In another study using the Nile Tilapia to create sausages (De Oliveira Filho, Viegas, Kamimura, & Trindade, 2012) a full factorial design was done to evaluate optimum incorporation levels (60 to 100%) of washed or unwashed byproducts. The authors used 4% soy protein isolate (SPI) in each formulation to act as a binder. Other important ingredients in the formulation include cassava starch and sodium tripolyphosphate (both of which may act as cryoprotectants) in addition to other curing additives (0.25% curing salt, 1% sausage seasoning) to impart characteristic sausage flavors. It was noted that the process of washing the fish mince impacted the proximate composition decreasing amounts of protein, lipid, and ash in addition to reduced iron, magnesium, phosphorus, sodium, and potassium and increased water and calcium (De Oliveira Filho et al.,

2012). These results indicated that addition of proteins such as soy protein isolate can improve products produced using fish mince, and thus should be considered when formulating value added products from fish mince. More information of the function of protein additives will be discussed the following section.

1.4 Protein Ingredients Used in Meat Nugget Formulations

1.4.1 Pea Protein Isolate in Literature:

"Yellow peas are readily available and relatively inexpensive"(Marinangeli, Kassis, & Jones, 2009). Pea proteins can replace soy and whey protein supplements and have already found use as a functional ingredient in meat, soups, fish feed, sauces, cereals, and snacks (Sirtori, Isak, Resta, Boschin, & Arnoldi, 2012). In one study, banana bread, biscotti, and spaghetti were made with whole yellow pea flour (WYPF) and sensory scores were compared against samples made with whole wheat flour with the objective of creating low-glycemic index foods using WYPF (Marinangeli et al., 2009). All foods produced with WYPF had similar results as those produced with whole wheat based on appearance, smell, taste, texture, and overall liking on a 5-point hedonic scale. Only pasta produced with WYPF showed significantly different results, having a significantly lower score for aroma ($p<0.05$). All sensory scores for products made with WYPF, with the exception of spaghetti smell were above 3 "neither like nor dislike".

Functional properties including emulsion stability and water absorption capacity in addition to measures of the amino acid score and predicted biological values were conducted on the Beach Pea (*Lathyrus Maritimus* L.)(Chavan, McKenzie, & Shahidi, 2001). A Water binding capacity of 257-288% was reported which may indicate that addition of pea proteins in minced meat products could aid in keeping the mince moist and juicy. It was noted that emulsifying activity decreases as pH decreases, and solubility is maximized away from the isoelectric point (pI

~4.5); addition of salt reduced the amount of proteins that precipitate out of solution at pH near pI. Since low pH is usually desired in food products for food safety purposes, it will be important to monitor the pH of the minced meat+ pea protein emulsion to understand how it may be impacting pea protein functionality in the food matrix. It has been noted that processing conditions can impact the functionality of pea proteins, for instance, it was noted that pea proteins respond better to long time medium temperature heat treatment than high temperature short time (Sirtori et al., 2012) giving further precedence for physicochemical analysis of post processed nuggets. In-vitro digestion revealed digestibility from 80.6-82.6% when using pepsin-trypsin enzymes, and 78.6-79.2% when using pepsin-pancreatic enzymes (Chavan et al., 2001). Pea protein is highly digestible which makes it an excellent way to increase total protein content in addition to increasing overall juiciness of the final products and improving texture of mince products. The precedence for use of peas to formulate novel food and feed products has been established, but there is a gap in the literature for the use of pea proteins in aquatic products for human consumption.

1.4.2 Soy Protein as a Meat Texture Modifier in Literature:

Soy proteins have a long history of use for texture modification and are used in a variety of meat products because they are relatively flavorless, taking on the flavor of the food matrix, and have similar textures to meat products (Riaz, 2006). For instance, textured soy proteins have been used to create meat analogues and as a meat replacement to reduce formulation costs. Textured soy proteins hold water and increase the retention of water through a variety of conditions including freezing, thawing, and cooking (Riaz, 2006). Further, it's noted that in ground minced products like ground beef or turkey, textured soy protein shows an ability to disrupt the orientation of both myofibrillar and collagen matrices reducing shrinkage during cooking. This results in

"rounder and flatter patties" and "more spherical meat balls" (Riaz, 2006). Textured soy protein has several uses in industry; however, it is a derivative product from soy protein isolate, increasing its environmental impacts and costs which is contrary to the goal of this project. Soy protein isolate may offer similar benefits with less environmental impact.

In a study by Chempaka, Yusof, and Babji (1996), soy protein isolate (SPI) and sodium caseinate (SCA) were added to a total of 9 formulations of chicken bologna (a value-added meat product) with variations in ratios from 1:1 to 10:5 for each additive respectively. Sensory data, shear force measurements, emulsion stability, fold testing, and yield were evaluated. The highest emulsion stability was observed in ratios of 5:1 and 5:5 SPI to SCA, which the authors attributed largely to the SPI rather than the SCA. Further, authors suggested that only small quantities of SCA are necessary to help stabilize the emulsion, as was indicated by the fact that large amounts of SCA did not improve bologna texture. Sensory data from the bologna study indicated that the ratio of 5:1 SPI to SCA had the highest acceptability (as measured on a 7-point hedonic scale) with consumers (n=30). Perhaps more interesting is that all formulations with 1% SPI were not significantly different in overall evaluation score than the formulation receiving the highest score. In a different study evaluating the effects of chicken skin and wheat fiber on reduced fat chicken sausages, as little as 0.5% soy protein isolate was used (Choe & Kim, 2019). Since one of the objectives listed in this research proposal is to optimize a formulation according to sensory acceptability, it is reasonable to assume that not more than 5% spi is necessary for maximized sensory acceptance (Chempaka et al., 1996).

1.5 An Invasive Species: Asian and Silver carp in the USA

The Asian carp, as the name would suggest, are a species of carp native to Asia that were introduced into the United States and escaped into the wild sometime in the 1970's. The most

common Asian carp, and the ones considered federally injurious, are the bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*). As federally injurious species, these fish must be killed if captured and cannot be released or transported alive. Black carp (*Mylopharyngodon piceus*) is also classified as Asian carp, however it has not established a population in the Mississippi river system to date. For the purposes of this research proposal the silver carp will be explored in more detail as a candidate for value-added food production.

The silver carp is a planktivore that can survive in a wide variety of conditions and has the ability to consume zooplankton or phytoplankton at a pore size of 20-25 μm and as small as 8 μm (Opuszyński, 1981; Sampson, Chick, & Pegg, 2009), making it a versatile competitor that can consume a wide range of plankton that native fish species depend on. When coupled with the species rapid growth, early maturity rate, and high fertility rate, this efficiency in consuming phytoplankton makes it more competitive than many native species (Pongruktham, Ochs, & Hoover, 2010).

It is believed that the silver carp was introduced to the United States in the 1970's to control blue-green algae in southern ponds and that this species managed to escape during a period of flooding which linked backwater ponds to streams and river systems (Irons, Sass, McClelland, & Stafford, 2007; Monteiro Starling, 1993). The silver carp has since made its way up the Mississippi River Delta. In 2008, silver carp were captured and tagged to estimate population within the La Grange reach, a section of the Illinois river. Researchers estimated some 328,192 fish in this reach alone with a biomass estimate of 705 metric tons (Sass et al., 2010). Further, in a study analyzing body condition of native fishes gizzard shad (*Dorosoma cepedianum)* and bigmouth buffalo (*Ictiobus cyprinellus)* of the Illinois river, it was found that body condition for both significantly

declined after the introduction of the invasive species (Irons et al., 2007; Sampson et al., 2009). While the effects of such competition have been relatively mild in highly productive systems, such as the Mississippi, and Illinois rivers, there is fear that less productive water systems like the Great lakes may suffer from detrimental trophic cascades as native species are outcompeted by the silver carp. (Sampson et al., 2009).

In addition to the threat to native fish species, silver carp pose a threat to motor boaters and watercraft activities. Silver carp have a tendency to jump out of water in response to motors (Vetter, 2017). While other fish species also respond out of fear to sound stimuli, no other fish species in the Mississippi river delta jumps from the water like the silver carp. This tendency becomes dangerous when the size of the fish is considered; according to the U.S. Fish and Wildlife Service silver carp can grow as much as 12 pounds in one year and grow to a maximum of 39 inches and 60 pounds. Fish of such size and weight which jump out to the water have potential to damage property and injure water motorists (*Asian Carp- Aquatic Invasive Species Issues, Program Accomplishments, and Program Needs*, 2004). This behavior may already be negatively impacting recreational usage of some riverways (Garvey, Ickes, & Zigler, 2010; Molly R. Spacapan, 2012).

In order to help eliminate this threat and prevent the invasive species from entering the great lakes region, several preventative measures have been proposed, including use of sound stimuli, bubble walls, and light to manage and deter their proliferation. In a study by Vetter, Cupp, Fredricks, Gaikowski, and Mensinger (2015), a 10 m x 5 m x 1.2 m outdoor concrete flow pond was divided along the middle leaving a 1 m gap between walls for the fish to pass from one side of the pond to the other. The pond was used to measure behavioral response of silver carp to sound stimuli. More specifically, the aim of the study was to evaluate the efficacy of complex sound

tones as a deterrent compared to pure tones which have already demonstrated an ability to deter fish migration. Underwater speakers were used to deliver either pure sound tones ranging from 500-2000 Hz, or a complex sound (a recording of a 100 horsepower Honda 4-stroke engine known to cause the jumping response). The results indicated that, with time, the carp adjusted to pure tones and showed decreased responsiveness (repulsion) to the sound stimuli. It was noted that after the end of the 10 min trial period carp stopped responding to both pure tones and complex tones; however, after a rest period only the complex sound consistently elicited a response, implying that the carp may adapt to simple tones but not complex tones (Vetter et al., 2015).

In a similar study conducted by Murchy et al. (2017) a 100-horse power 4-stroke outboard motor recording was used to monitor behavioral responses of schools of both silver and bighead carp individually and when mixed. Results indicated that schools consisting of only silver carp had an 82.5% repulsion rate (meaning that silver carp did not cross the barrier 82.5% of the time), while schools mixed with bighead and silver carp had a repulsion rate of 90.5% (Murchy et al., 2017).

1.6 Mechanisms Utilizing Silver carp

Various novel uses for the silver carp have been explored as a means of thinning the population including their use as fertilizers, animal feed, fish protein hydrolysates, antioxidant sources, and use of protein hydrolysates as cryoprotectants (Kim, 2014; Malaypally et al., 2015; Mueller & Liceaga, 2014; Thomson, Liceaga, Applegate, & Martyn, 2015)

Mueller and Liceaga (2014) used silver carp protein hydrolysates as cryoprotectants in Atlantic cod (*Gadus Morhua*) fish mince. Antifreeze activity was evaluated using differential scanning calorimetry (DSC). Addition of silver carp protein hydrolysates significantly lowered expressible moisture after six freeze-thaw cycles indicating that silver carp hydrolysates may be

utilized as functional cryoprotectants without many of the negative attributes of the traditional sucrose-sorbitol cryoprotectant, namely the undesirable sweet taste it imparts and addition of sugars. Antioxidant activity for enzymatically hydrolyzed silver carp is also reported as being significant though the results depended largely on the enzyme and conditions used (Dong et al., 2008).

In another study, surimi was produced using silver carp (Barrera, Ramırez, González-Cabriales, & Vázquez, 2002). The effects of pectin addition on gel formation were observed with mixed results. For instance, addition of high methoxyl pectin (HMP) consistently reduced shear stress, while amidated varieties of low methoxyl pectin (LMP) showed an ability to increase gel strength. These results could be due to the nature of pectin gel formation, HMP being largely sugar and pH dependent and LMP being largely calcium dependent. Amidated LMP requires less calcium than non-amidated LMP. There could be a calcium-pectin-myofibrillar protein interaction that impacts the stability of the gel matrix (Barrera et al., 2002). Thus, amidated LMP may require just the right amount of calcium to participate in the formation of a gel network. The addition of $CaCl_2$ in silver carp surimi has a significant impact on gel strength, and is the most important additive when optimized using response surface methodology with the additional variables of whey protein concentrate and setting time (J. Shi, Luo, Shen, & Li, 2014). The addition of soy protein isolate to surimi products is shown to decrease gel strength resulting in the brittle "modori" when added at concentrations greater than 10% and not incubated for 60 mins at 50 °C (Luo, Shen, Pan, & Bu, 2008). It is possible that the soy proteins are interfering with myofibrillar protein network formation much in the way that native sarcoplasmic proteins can disrupt the network if not washed properly.

Frozen minced silver carp meat can also be used to create surimi. A study (Siddaiah et al., 2001) has shown that as frozen silver carp ages, it undergoes the TMAO reaction (as described earlier in Fish Muscle Proteins) resulting in increased total volatile base nitrogen (TVBN) content (TVBN values around 30 mg/100g meat indicate the beginning of spoilage as this is the level at which sensory panelists scores begin decreasing).

According to Barrera et al. (2002), silver carp are considered to be warm water fish. According to Hall (1997) fish caught in colder waters will contain higher levels of polyunsaturated fatty acids. In goldfish (*Carassius auratus*) increasing the temperature by 20 °C halved the quantity of 20:4 and 22:6 unsaturated fatty acids and doubled the quantity of 18:0 fatty acids. Given this information it is important to denote the time of year and region of catch of the silver carp. Since the species has shown a tolerance to a wide variety of conditions (including warmer water in the southern United States and colder water in the norther region of the United States) it is reasonable to suppose that fatty acid composition of the silver carp may vary considerably according to catch location and season. Hall (1997), further indicates that these regional differences in fatty acid content do impact sensory scores within the same species as fish containing higher amounts of polyunsaturated fatty acids were more likely to develop off flavors and odors.

In the United States the carp is generally referred to as a "garbage fish" due to the misconception that all carp are bottom feeders, or because removal of the bones is considered more work than it's worth (Dong et al., 2008). Consequently, there is a limited amount of data on sensory acceptability or perception of value-added silver carp products, which is crucial for investments from industry or government towards these efforts.

To help bridge the gap in information on sensory acceptability of silver carp products in the United States, the research in this paper will focus on the creation of a value-added food product

for consumption in the United States using silver carp. A 1995 study analyzed the acceptability of canned bighead carp when compared with canned tuna and salmon in Arkansas (Engle & Kouka, 1995). In the study, panelists were told they were consuming a freshwater fish so as to eliminate bias associated with the name carp. Results indicate that bighead carp had good acceptability "based on a five-category, modified, staple, interval scale" when compared to tuna, but not salmon (Engle & Kouka, 1995). While this information is helpful in establishing that U.S. populations may be willing to eat carp, the study was limited to canned products, even though fresh and frozen fish make up the majority of the market.

In 2013, Varble and Secchi conducted a national survey which explored key variables such as willingness to try a free sample of Asian carp, and willingness to pay for Asian carp products. Results indicated that customers were willing to pay $4.80 for frozen fish sticks (Varble & Secchi, 2013). This shows that consumers are at least willing to try products such as fish sticks or nuggets made with the silver carp. Based on the data from Table 1 and the above consumer survey data (in which youth were more willing to pay for products made with Asian carp and 73% of survey respondents who had an income of less than $60,000 a year were willing to try a free sample) there is a strong case for new market potential with the younger generations, and low income consumers (Varble & Secchi, 2013). Both of these demographic groups don't currently consume as much fresh water fish as the older 50-65 year old age group and $75K+ income group as indicated by table 1 and the EPA report (*Estimated Fish Consumption Rates for the U.S. Population and Selected Subpopulations (NHANES 2003-2010)*, 2014). Thus, there is a strong case for further research into the sensory acceptability of a value-added fish nugget type product made using the silver carp.

1.7 Example of Value-Added Products: Nuggets

Chicken nuggets are a staple in the United States diet, first gaining popularity with their mainstream introduction by the McDonalds fast food chain. Though the nugget was popularized by the fast food chain, the origin of the chicken nugget is accredited to Cornell University professor Dr. Robert Baker (Rude, 2016).

According to the NPD group (a market research group) in the year from September 2017-September 2018, the food industry served some 2.3 billion servings of chicken nuggets alone. However, nuggets are not limited to chicken, but include a wide range of vegetable-based meat analogues, and even nugget-like products made from fish e.g. fish sticks. While there is limited data on fish nugget formulations, there is a study (Cakli et al., 2005) that analyzed the quality changes of fish sticks made from a variety of fish including whiting, pike perch, and sardines when stored for 8 months at - 18 °C. In this study formulations for fish nuggets consisted of 80% minced meat and 20% added ingredients, including soybean oil (16%) and spices (4%). Changes in microbiological quality (aerobic plate count), quantities of dimethylamine nitrogen (DMA-N), trimethylamine nitrogen (TMA-N), and trimethylamine-n-oxide nitrogen (TMAO-N) (via gas chromatography), and sensory analysis (5 point descriptive scale) were performed on fish fingers for each fish at months 0, 3, and 8. Sensory scores were noted as decreasing with time which the author suggests may be correlated with moisture loss. The decrease in moisture may be a result of TMAO degradation. As TMAO degrades into formaldehyde, the formaldehyde can cause proteins to crosslink resulting in more protein-protein interactions and fewer protein-water interactions that result in an increased expressible moisture. The authors noted that values of TMAO decreased over the 8-month time period for all three fish finger types. As part of the sensory analysis, panelists determined that sardines were no longer suitable for consumption at 8 months due to the

development of rancid flavors. Sardines had the highest amount of crude fat averaging 18.7%, so it is logical to conclude that oxidative rancidity shortened the shelf life of sardines as compared with the other fish in the study. Silver carp are relatively low in fat; however, it is important to analyze the product for signs of oxidative rancidity to ensure adequate shelf life.

Another study aimed to optimize chicken nuggets formulated with fermented cowpea and peanut flour as fillers. The optimization study conducted by (Prinyawiwatkul, McWatters, Beuchat, & Phillips, 1997) utilized a mixture design with the goal of optimizing sensory acceptability as measured by appearance, color, flavor, texture, and overall liking. For this mixture design three ingredients were varied in the formulations namely: chicken (80-100%), flour processed from fermented cowpeas (0-20%), and flour processed from fermented partially defatted peanuts (0-20%). These ingredients made up a total of 100% for the mixtures design, however they totaled 84% of the actual nugget formulations while the other 16% consisted of water (14%), salt (1%), garlic (0.6%), and pepper (0.4%). Results indicated that the flavor acceptability decreased as the fillers increased (both cowpeas, and peanuts), though it is important to note that the formulation containing 2.5% cowpea flour blended with 2.5% peanut flour was not significantly different than the control (100% chicken meat). Nuggets containing 100% chicken meat scored the highest. Once more, the texture acceptance on a 9-point hedonic scale of the formulation containing 2.5% of both fillers received a score of 6.5, which is almost identical to the control score of 6.4. Based on these results, the authors concluded that flavor and texture have the most influential effect on overall sensory acceptability while color and appearance did not have much of an effect on overall acceptability scores (Prinyawiwatkul et al., 1997).

Since texture has been identified as one of the most important factors, methods of measuring texture are important. In a Study (Martínez-Ávila, Vélez-Ruiz, & Sosa-Morales, 2010), evaluating

ways to reduce oil content of chicken nuggets, texture analysis was used as a quality measuerment of the finished products. The method involved the use of a TA.XT2 with a 1/8 inch die and a velocity of 5 mm/s. This method was modified from Rosete-Hidalgo, Sosa-Morales, Cenkowski, & Vélez-Ruiz (2008), who used it to measure texture of chicken nuggets pre-treated with superheated steam to reduce oil uptake. A similar method was used by Cheung, Liceaga, and Li-Chan to analyze texture of frozen cod fillet mince treated with cryoprotectants (Cheung, Liceaga, & Li-Chan, 2009). Now that key quality attributes such as texture and lipid oxidation have been identified, it is important to review the materials and methods neccesary to conduct experments evaluating the physicohcmeical and sensory changes that impact value-added silver carp nuggets.

CHAPTER 2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Fish Mincing Procedure:

Fresh silver carp (*Hypophthalmichtys molitrix*) fillets (81.64 kg) harvested in late April were purchased from a local vendor (Schafer Fisheries, Fulton, IL, USA). Fillets were individually placed in freezer bags (Ziplock®, S.C. Johnson & Son, Inc., Racine, WI) and stored at -20 ºC until used. Individual fillets were thawed in a cold-water bath directly before skinning. Skinned fillets were stored in chafers set on ice to keep them cool. Each fillet weighed between 500 and 700g after being skinned and washed. Skinned and washed fillets were minced twice using an electric meat grinder (Model #8 1/2 HP, Cabela's Inc., Sidney, NE, USA) first with a 7 mm diameter die and then using a 4.5 mm diameter die to remove intramuscular bones (Trindade, King, & Liceaga, 2019). The washed and skinned fillets yielded on average 88% minced meat. The minced meat was divided into four sections: treatment 1 (6,830 g) containing 91.5% fish mince and no additional protein additive (control), treatment 2 (6804g) with 88.5% mince and 3% soy protein isolate (SPI), treatment 3 (6804g) with 88.5% mince and 3% pea protein isolate (PPI), and treatment 4 (6804g), which contained 88.5% mince and a combination of 1.5% soy protein isolate and 1.5% pea protein isolate (SPI+PPI). All four treatments also included (by mass) 6.1% distilled water, 1.5% salt, 0.4% onion powder, 0.3% sodium tripolyphosphate (Pronto Foods, Chicago, IL, USA), and 0.2% garlic powder (see Figure 1). Once weighed, dry ingredients were evenly distributed across the surface of the meat. Water was then poured over the mixture and mixed in a stand mixer (Hobart A 120, Hobart corporation, Troy, OH, USA) for 4 mins until homogenized. Figure 2 is a flow diagram of this process. Soy protein isolate and pea protein isolate were obtained from Bulk Supplements (Henderson, NV, USA). Batter pre-mix, breading, high oleic canola blend liquid fry

shortening and all other ingredients (salt, seasonings, spices, etc.) were obtained at a local grocery store unless otherwise indicated.

Product Name	Control	
Batch ID	1	Total Batch weight
Date	3/20/19	7464.48

Ingredients	Component %	Weight (g)
Meat	91.500%	6830
No protein	0.000%	0
Salt	1.50%	111.97
Onion Powder	0.400%	29.86
Garlic Powder	0.200%	14.93
Sodium Tripolyphosphate	0.300%	22.39
Water	6.100%	455.33
Total	100.000%	7464.48

Product Name	3% Soy Protein isolate fish nuggets	
Batch ID	1	Total Batch weight
Date	3/20/19	7688.14

Ingredients	Component %	Weight (g)
Meat	88.500%	6804
Soy Protein Isolate (SPI)	3.000%	230.64
Salt	1.50%	115.32
Onion Powder	0.400%	30.75
Garlic Powder	0.200%	15.38
Sodium Tripolyphosphate	0.300%	23.06
Water	6.100%	468.98
Total	100.000%	7688.14

Product Name	3% Pea Protein isolate fish nuggets	
Batch ID	1	Total Batch weight
Date	3/20/19	7688.14

Ingredients	Component %	Weight (g)
Meat	88.500%	6804
Pea Protein Isolate (PPI)	3.000%	230.64
Salt	1.50%	115.32
Onion Powder	0.400%	30.75
Garlic Powder	0.200%	15.38
Sodium Tripolyphosphate	0.300%	23.06
Water	6.100%	468.98
Total	100.000%	7688.14

Product Name	1.5% Soy+ 1.5% Pea Protein isolate fish nuggets	
Batch ID	1	Total Batch weight
Date	3/20/19	7688.14

Ingredients	Component %	Weight (g)
Meat	88.500%	6804
Soy Protein Isolate (SPI)	1.500%	115.32
Pea Protein Isolate (PPI)	1.500%	115.32
Salt	1.50%	115.32
Onion Powder	0.400%	30.75
Garlic Powder	0.200%	15.38
Sodium Tripolyphosphate	0.300%	23.06
Water	6.100%	468.98
Total	100.000%	7688.14

Figure 1 Formulation Sheet Examples

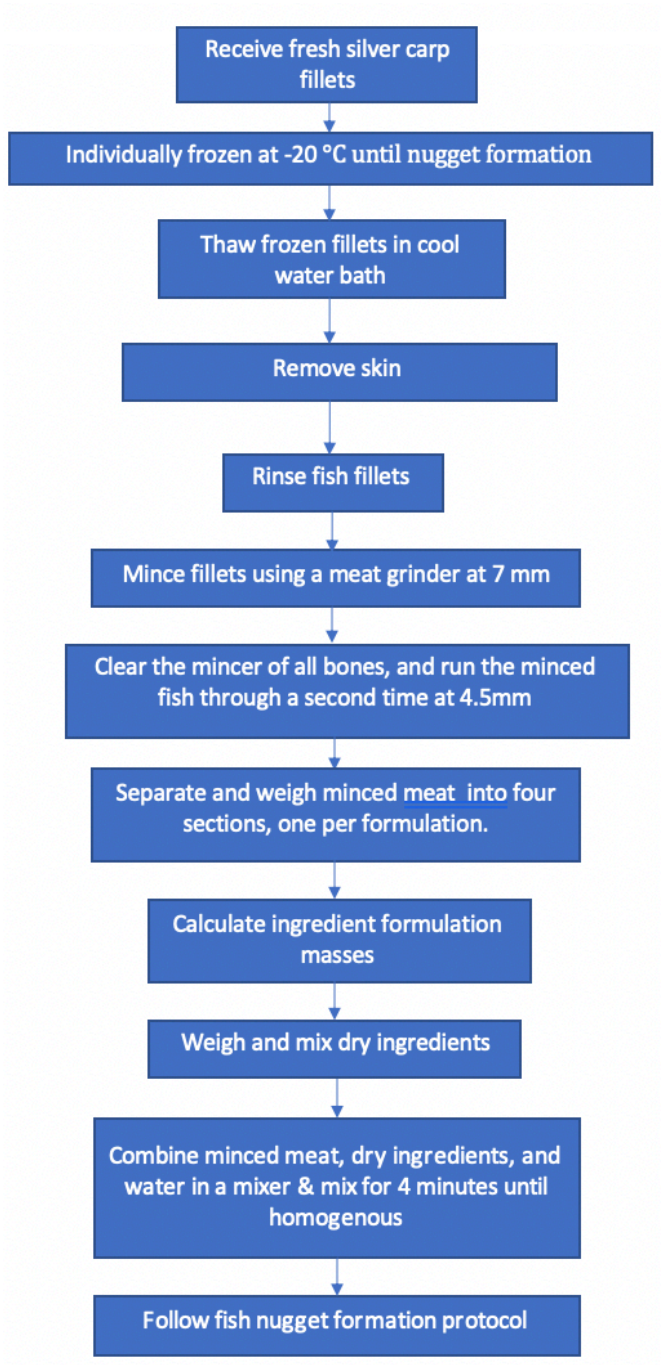

Figure 2 Fish Mincing Procedure

2.1.2 Fish Nugget Formation:

Treatment 1 was the control with no plant protein added. Treatment 2 (referred to as SPI treatment) contains 3% soy protein isolate. Treatment 3 (referred to as PPI treatment) contains 3% pea protein isolate. Finally, treatment 4 (referred to as SPI+PPI treatment) contains a combination of 1.5% soy protein isolate and 1.5% peat protein isolate (see figure 1). The same nugget formation procedure was used for all four treatments. To form the fish nuggets 50 g (threshold of up to +5 g) of minced meat were weighed and placed in a 6.0 x 5.4 x 4.1 cm rectangular patty former. Patties were pressed to a thickness of 1.5 cm, then quick frozen in a blast freezer (Electrolux Air-O-Chill, Augusta, GA, USA) at -35.5 °C for 20 mins. Frozen fish mince squares were cut diagonally in half to form symmetric triangular pieces and placed in the freezer for 12 hrs until breading and frying. Coating batter was made at a 1:1.7 dry batter to water ratio. Frozen fish triangles were dipped in the batter for 5 sec, excess batter was removed by slight shaking, and the battered fish triangles were immediately coated in panko breading. Nuggets were partially fried (par-fried) (All-Clad 3.5 L Deep Fryer, All-Clad Metalcrafters, Canonsburg, PA, USA) in high oleic canola blend liquid fry shortening for 30 sec at 171 ºC. Par-fried nuggets (avg 37.6 ± 4.5 g) were then placed on a sheet tray and transferred to the blast freezer (-35 ºC) for 20 mins. Upon removal, nuggets were placed in labeled freezer bags and stored frozen at -20 ºC until further analysis. Prior to analysis, samples were thawed overnight at 4 ºC. These partially fried (par-fried) nuggets are referred to as “raw” in throughout this study as the par-frying was to set the batter not cook the mince. For cooking, frozen nuggets were evenly spaced on a baking tray and baked at 218 ºC for 7.5 mins, flipped, and baked for 7.5 more mins and checked to ensure a minimum internal temperature of 74 ºC was reached. For the shelf life study, fish nuggets from all four treatments were stored at -20 ºC and analyzed after 1.5, 8.5, and 13.5 weeks of frozen storage. Figure 3 is a flow diagram of this process.

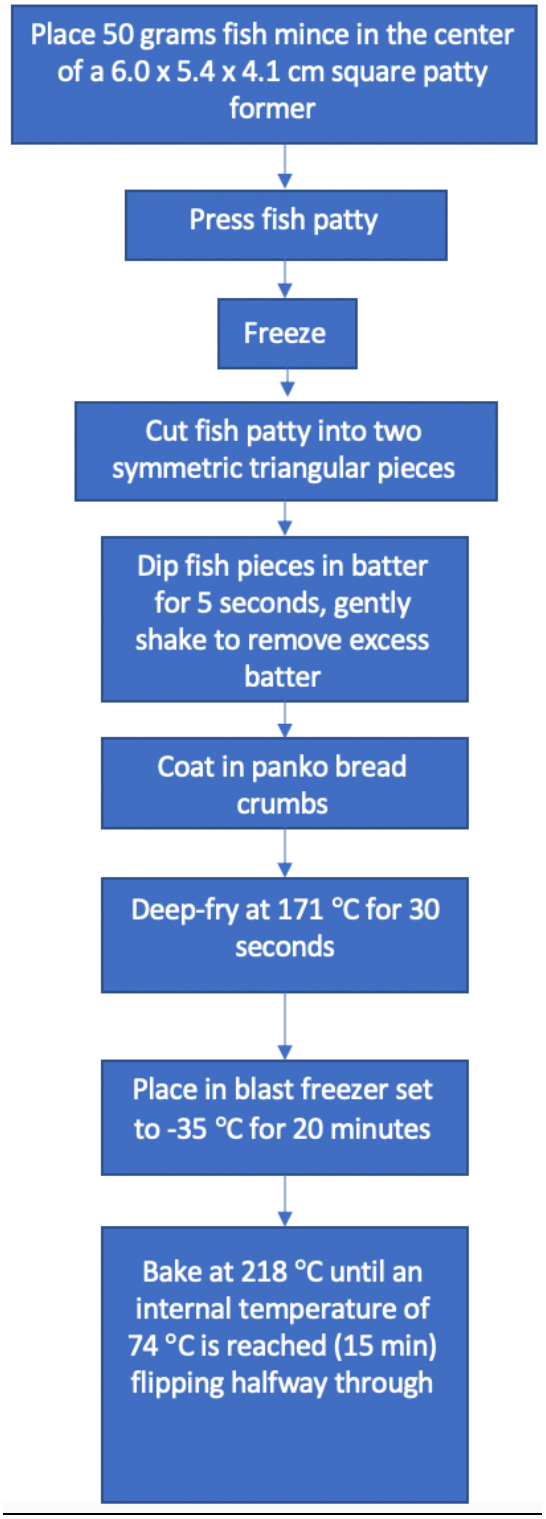

Figure 3 Fish Nugget Formation Procedures

2.2 Methods

2.2.1 Proximate Composition

Proximate composition was determined in duplicate using official methods of analysis from AOAC international on dried fish nuggets for each treatment through a certified food and feed analysis laboratory (A&L Great Lakes Laboratories, Fort Wayne, IN, USA). Nuggets were dried using AOAC method 950.46 before being shipped to A&L Great Lakes Laboratories for further proximate analysis. Results from Great lakes were reported in both wet and dry weight basis. Proximate compositions reported in this thesis are recombined with moisture that was lost during moisture analysis and reported in a wet weight basis.

Total Moisture Analysis:

Moisture analysis was done using AOAC method 950.46 "Loss on Drying (Moisture) in Meat". Aluminum baking boats were weighed and set aside. Duplicate fish nuggets from each treatment were individually blended in a food processor for two mins until homogenous. Nuggets were then weighed in an aluminum baking boat and dried at 105 °C for 16 hrs using a Binder ED series 53 convection oven (Binder Inc., Bohemia, NY, USA). Upon removal from the oven, the samples were placed in a desiccator to cool for 6 hrs before being weighed and packaged in a 15ml Falcon™ tube for transportation to A&L Great Lakes for further analysis. Moisture content was calculated using the equation below:

$$\text{Moisture Content (\%)} = \frac{\text{initial (wet) weight} - \text{final (dry) weight}}{\text{initial (wet) weight}} * 100$$

Dry Ashing:

Dry ashing was completed according to AOAC Method number 923.03 "Ash of flour" by a certified food and feed analysis laboratory (A&L Great Lakes Laboratories, Fort Wayne, IN, USA).

Crude Fat:

Crude fat was completed using AOAC method 960.39 (Soxhlet) by a certified food and feed analysis laboratory (A&L Great Lakes Laboratories, Fort Wayne, IN, USA).

Crude Protein:

Crude protein analysis was completed using AOAC method 954.01 the Kjeldahl method by a certified food and feed analysis laboratory (A&L Great Lakes Laboratories, Fort Wayne, IN, USA).

Total Carbohydrates:

Total carbohydrates were calculated as

$$100\% - (\%\text{Ash} + \%\text{Moisture} + \%\ \text{Crude Fat} + \%\ \text{Crude Protein})$$

2.2.2 Thiobarbituric Acid Reactive Substance Assay (TBARS):

TBARS was determined following a combination of the methods described by (Buege & Aust, 1978) and (Maqsood, Benjakul, & Balange, 2012). TBARS was carried out on nuggets after 1.5, 8.5, and 13.5 weeks of frozen storage. Samples were analyzed in triplicate (3 nuggets per formulation) with each replicate consisting of 3 averaged measurements. Nugget (10 g) was blended in 50 ml DDI water for 2 mins. Nugget slurry (1 ml) was mixed in a tube containing 2ml

of 15% (w/v) Trichloroacetic acid (VWR, Radnor, PA, USA), 2ml of 0.375% (w/v) 2-Thiobarbituric acid (MilliporeSigma, Burlington, MA, USA) and 2ml 0.25N Hydrochloric acid (Fisher Scientific, Waltham, MA, USA) and vortexed. A standard curve was created using 1,1,3,3-tetramethoxypropane (TMP) (MilliporeSigma, Burlington, MA, USA) consisting of 2, 4, 6, 8, and 10 ppm in addition to a blank (0 ppm) which was subject to the same conditions as the treatments. Tubes were vortexed and placed in a boiling water bath for 10 mins, then cooled with cool water. Tubes were then placed in a sonicator bath (FS110H, Fisher Scientific, Waltham, MA, USA) for 30 mins. TBARS solution (2 ml) was taken from each tube and placed in a 2ml microcentrifuge tube and centrifuged (Eppendorf centrifuge 5424, Eppendorf, Hauppauge, NY, USA) for 10 mins at 15,000 rcf. Absorbance was then determined on the supernatant at 532nm, the blank was subtracted, and final results were reported in ppm of TMP equivalents as calculated using the standard curve.

2.2.3 Aerobic Plate Count:

Aerobic Plate count was conducted on raw fish nuggets held under frozen storage conditions for 1.5, 8.5, and 13.5 weeks according to AOAC method 950.46 “Enumeration of Aerobic Bacteria in Food” (*Official Methods of Analysis of AOAC INTERNATIONAL*, 2016). Thawed, par-fired fish nuggets (13.5 g) were aseptically transferred into a stomacher bag. Phosphate buffer (100 ml) was added and stomached on high for 2 mins (Stomacher 400, Seward, Islandia, NY, USA). The nugget slurry was then serially diluted into sterilized phosphate solution. These dilutions were vortexed and 100 µl were aseptically plated using Difco™ Nutrient Agar (Dickinson and Company, Sparks, MD, USA) as the growth medium. Plates were incubated lid side down at 31-33 °C for 22-26 hrs. Plates were then enumerated with those above 300 colonies being listed at too numerous to count. Final counts were converted to CFU/g sample and reported in log CFU/g. Similar methods were

performed on mirror carp (Tokur, Ozkütük, Atici, Ozyurt, & Ozyurt, 2006), and fermented silver carp sausages (Xu, Xia, Yang, Kim, & Nie, 2010).

2.2.4 Expressible Moisture (EM):

A modified version of EM (Das, Anjaneyulu, Gadekar, Singh, & Pragati, 2008; Mueller & Liceaga, 2014) was conducted on raw fish nuggets from each treatment after 1.5, 8.5, and 13.5 weeks of frozen storage. Thawed, raw fish nugget samples (17 mm diameter, 2.5 ± 1 g) were punched out of a whole fish nugget using a 15 ml Falcon™ tube (17 mm diameter, 120 mm length). A total of three samples were removed from each nugget and averaged to make one replicate. Each treatment was measured in triplicate. Samples were placed on two pieces of pre-weighed filter paper (42.5 mm diameter) (Whatman No.1, MilliporeSigma, Burlington, MA, USA) such that the meat contacted the filter paper. Filter papers were then placed in a pre-weighed 50 ml centrifuge tube and centrifuged for 10 mins at 4000 x *g* at 4 °C. The nugget was then removed from the filter paper, and the filter paper was reweighed. EM was determined gravimetrically as seen in the equation below.

$$\%EM = \frac{W_f - W_i}{W_i} * 100$$

2.2.5 Color:

Surface color and internal color were measured with a Hunter Lab colorimeter (HunterLab Miniscan XE, Reston, VA, USA). Illuminant D65, 10° standard observer, 1.75 in area view, and 2.00 in port size were set for color measurements using the CIELAB scale (L*, a*, b*) on cooked nuggets after 1.5, 8.5, and 13.5 weeks of frozen storage. External nugget color had a wider variability in color values due to browning during the cooking process, thus measurements were

taken in triplicate on both the bottom and top sides of the nuggets. The bottom half of the nugget was the portion in contact with the sheet tray at the end of the 15 min baking process. Each replicate consisted of three measurements (rotating the nugget 90° clockwise each time before a new measurement was taken), which were then averaged. Internal color was measured by cutting each nugget horizontally in halves for maximum surface area. Measurements were done in duplicate with each replicate following the procedure used for external color in nuggets. Delta E 75 (ΔE) values were calculated for each treatment with reference to the control at each respective time period according to the following equation where Delta E is the magnitude of color differences between the respective sample (L_2^*, a_2^*, b_2^*) and the control (L_1^*, a_1^*, b_1^*).

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

2.2.6 pH:

The pH of fish nuggets from each treatment were measured after 1.5, 8.5, and 13.5 weeks of frozen storage. Nuggets were measured in triplicate by blending 10 g of fish nugget in 40 ml deionized distilled water for 10 s in a Waring commercial blender model 31BL92 (Conair Corporation, Stamford, CT, USA). The pH of the nugget slurry was then measured using a pH meter model HQ430d (Hach Company, Loveland, CO, USA) (Trindade et al., 2019).

2.2.7 Peak Force Texture Analysis:

Peak force was measured on fully cooked nuggets for each treatment after 1.5, 8.5, and 13.5 weeks of frozen storage using a TA-XT2*i* texture analyzer (Stable Micro System, Godalming, UK). Freshly cooked nuggets were cooled to room temperature (20 °C) in a desiccator before measurements were taken. A puncture test method using a cylindrical 6 mm diameter probe was

set to a velocity of 5 mm s^{-1} and programed to penetrate to 50% thickness (1 cm) (Rosete-Hidalgo, Sosa-Morales, Cenkowski, & Vélez-Ruiz, 2008) as modified by (Martínez-Ávila et al., 2010). A total of 3 measurements were taken per nugget with 6 nuggets being measured per treatment at each respective time period. Final results were expressed in N.

2.2.8 Cook Loss:

Fully cooked nuggets were measured for cook loss after 1.5, 8.5, and 13.5 weeks of frozen storage. Nuggets were weighed before and after cooking (baking, see section 2.1.2 Fish Nugget Formation). Nuggets were then placed in a desiccator for use in color and texture (peak force) analysis. Cook loss (%) was determined according to the following equation:

$$Cook\ loss\ (\%) = \frac{mass\ uncooked\ sample - mass\ cooked\ sample}{mass\ uncooked\ sample} x\ 100$$

2.2.9 Consumer Survey and Sensory Evaluation:

Consumer Survey:

Approval for a consumer survey was obtained from the internal review board IRB# 2019-790, and the survey questionnaire can be found in Appendix B. The survey was created in Qualtrics® (Qualtrics®, Provo, UT, USA) and shared via the Purdue Sensory Evaluation Lab mailing lists. Survey participants were encouraged to share the survey link. The survey ran for a total of 30 days (December 9, 2019- January 7, 2020). Survey data was downloaded and analysed in Excel. Participants that failed the quality control check question, or that did not consume fish at least once a month were screened out and the remaining respondents' results were reported as percentages. Questions in the survey gauged: 1) consumer preference for purchasing fish with bones, 2) willingness to eat freshwater fish from U.S. river systems, 3) knowledge of the invasive

nature of silver carp, and 4) willingness to consume products made with invasive silver carp.

Sensory Acceptability:

Sensory acceptability (certified exempt by IRB) was done using the methodology described by (Trindade et al., 2019) after 1.5, 8.5, and 13.5 weeks of frozen storage. To avoid sensory fatigue, 2 of the 4 treatments were served in one day (session) and testing occurred over a two-session period with new panelists recruited from the sensory mailing list for each session. Cooked nuggets were cut in half vertically into two pieces (5.4 x 3.9 x 3.7 x 1.8 cm and 5.1 x 3.9 x 3.7 x 1.8 cm) and kept warm in a 65 °C oven until served to panelists. Each panelist received two samples (one from each treatment being evaluated) served in random, monadic order along with water and an unsalted cracker to cleanse their palate. Panelists were pre-screened for users and likers of fish. A total of 382 panelists were recruited over 6 test days consisting on average of 57% females and 43% males, age range of 26% (18-24), 52% (25-34), 11% (35-44), 6% (45-54), 3% (55-64), and 3% 65 and older. Panelists determined degree of liking for appearance, aroma, flavor, texture, and overall liking using a 9-point hedonic scale (where 1= dislike extremely, 5= neither like nor dislike, and 9= like extremely). For attribute diagnostics, a 5-point just about right (JAR) scale was used to assess intensity of the fish flavor (1= much too weak, 3= just about right (JAR), 5= much too strong), texture (1= much too soft, 3= JAR, 5= much too firm), and saltiness (1= Definitely not salty enough, 3= JAR, 5= much too salty).

Descriptive Analysis:

Descriptive analysis (certified exempt by IRB) was conducted according to the QDA™ method (Stone, 2012) with some modifications. Nuggets for the descriptive analysis were prepared

one week before training and held in frozen storage (-20 ºC) throughout the training and testing time frame.

Descriptive Panel Pre-screening

An email invitation was sent out to the Purdue Sensory Evaluation Lab mailing list inviting participants to sign up for the pre-screening. Pre-screening lasted a total of 2 hrs and 15 min with 15 min intervals allocated for each panelist (29 total). Panelists were tested for basic taste acuity using a paired comparison test for four of the five basic tastes: sweetness (1.6% (w/v) sugar solution), bitterness (0.0035% (w/v) quinine solution), umami (0.035% (w/v) monosodium glutamate solution), and saltiness (0.2845% (w/v) salt solution). Samples were prepared in 2-quart containers (1892.71 ml) on a weight per volume basis. Additionally, participants were given unlabeled aromas of butter, lemon, vanilla, cinnamon, and mint and asked to identify each aroma. In total, 8 panelists qualified based on the number of correct flavor attributes and aromas identified. For the final panel 6 panelists (3 male, 3 female) were able to commit to training based on schedule availability.

Descriptive Panel Training

Panelists were trained for a total of 8 weeks using nuggets from all four treatments. The descriptive panel had two weekly meetings lasting 60-70 mins per session and trained for a total of 19 hrs before doing the final (individual) analysis in duplicate. During the first couple of weeks of training, panelists collectively identified attributes for aroma, flavor, and mouthfeel. During this phase, standards were used as references that closely matched the attributes identified and further helped panelists to refine, clarify, and distinguish attributes (Table 2). Once the panelists were satisfied with the standards, panelists were asked to mark the intensity of each attribute for each

treatment using a 10 cm line scale with anchors set 1.0 cm from each end and a center anchor (Figure 4). Towards the end of the training panelists measured intensities without reference standards.

Figure 4 QDA™ 10 cm line scale example

Sample Preparation for Descriptive Analysis

For aroma, each panelist was given two quarters (total half) of a fish nugget for evaluation. Whole nuggets were baked and cut in half to form two smaller triangles. Triangles were then cut in half and rolled in a three-digit coded individually labelled aluminum foil packet. Samples to be served immediately where be placed in a 4 oz plastic souffle cup covered with a lid and labeled with the same three-digit code. The remaining samples were held under a heat lamp. Samples held under the heat lamp were distributed after 30 mins during initial training and later, once the judges were more efficient at analyzing the samples, were distributed after panelists analyzed half of the descriptors.

For flavor, each panelist was given two halves (one whole) fish nugget for evaluation. Baked samples were cut in half so as to form two smaller triangles and served using the same procedure and serving temperature as aroma. Sample preparation for mouthfeel was the same as for flavor.

Attribute Analysis Procedures:

Due to the use of a baking sheet some samples were darker on the side that had contact with the baking sheet at the end of cooking which the panelists detected; thus, when evaluating the

fried aroma, panelists smelled the lighter side of the crust. When evaluating fish aroma, panelists broke the sample in half and smelled the meat. When evaluating flavor, panelists ate nuggets with the lighter side facing the tongue. When evaluating mouthfeel, panelists used the definitions in table 2 based on previous literature (Antonova, Mallikarjunan, & Duncan, 2004). Panelists conducted the final, individual, analysis in duplicate during two separate sessions without the use of reference standard or definitions. Panelists were in individual booths with controlled lighting, positive air flow, and at ambient room temperature. Panelists conducted final analysis on a 10 cm line-scale paper ballot and did not use a ruler or straightedge when rating intensities (Figure 4).

2.2.10 Statistical Analysis of Results:

Physicochemical data and differences between frozen time periods for Sensory acceptability were analyzed using Minitab 18® statistical software (State College, PA, USA). Analysis of variance (ANOVA) was used in conjunction with Tukey's pairwise comparison of means with a 5% significance level (95% confidence interval). For all physicochemical experiments one nugget was considered to be one experimental unit, thus triplicate measurements required three full sized nuggets. Collection of sensory acceptability and attribute diagnostic data was done using the sensory software RedJade® (RedJade Software Solutions, LLC). For sensory acceptability tests the nugget was considered the fixed effect and the panelists/consumers were the random effect in the model. Descriptive analysis was performed in duplicate and analyzed using ANOVA in Excel (version 16.33, Microsoft, Redmond, WA, USA) followed by Fishers least significant difference (LSD) test with a 5% significance level.

Table 2 Descriptive analysis Mouthfeel Definitions with Reference Standards and Evaluation Technique

Attribute	**Definition/description**	**Reference Standard**	**Evaluation technique**
Cohesiveness/ springiness	The degree to which sample deforms rather than crumbles, cracks, or breaks	Marshmallow/ Taffy	Place sample between molars and compress fully (can be done with incisors).
Mealy/ Gritty	Feeling of small sand-like particles in the mouth and on the tongue	Nature Valley™ granola bar/ Graham Cracker/ Cornmeal	Chew the sample and evaluate the sensation of tiny particles rubbing against the tongue or other mouth areas,
Fatty/Oily	A mouth-coating sensation associated with fats and oils	Fresh oil (Canola)	Chew the sample and evaluate the sensation of a thick smooth coating around tongue or other mouth areas.
Juicy/ Moist	Feeling of moisture or wetness upon biting induced by saliva or the food itself.	Starbursts® Candy	Place food between the molars and bite down evenly, evaluating the sensation of juice/moisture being released.

CHAPTER 3. RESULTS AND DISCUSSION

3.1 Physicochemical Results

3.1.1 Proximate Composition:

Proximate composition consists of moisture, ash, crude protein, crude fat, and carbohydrates. The proximate composition of the fish nuggets (Table 3) consisted of 60-65% moisture, 2.2-2.4% ash, 7.1-8.4% crude fat, 13.4-14.1% crude protein, and 12.18-14.75% carbohydrates (determined by difference). The control treatment had the highest moisture content and was significantly higher ($p = 0.03$) than the PPI treatment. These results are not surprising because the addition of a protein isolate is replacing 3% of the mince, which is the main source of water in the formulations. The control also had significantly lower carbohydrate composition ($p = 0.00$), than all other treatments, though this is also a result of the increased moisture content. Carbohydrates were determined by the calculation: $100\% - (\%\text{Ash} + \%\text{Moisture} + \%\text{ Crude Fat} + \%\text{ Crude Protein})$; thus, the larger percent moisture resulted in significantly decreased % carbohydrates.

Similar results were obtained in a study analyzing the effects of cooking methods on the chemical composition of oven-baked silver carp harvested during the winter months. Average values reported were 67.57% moisture, 0.99% ash, 7.95% crude fat, and 18.66% crude protein (Naseri, Rezaei, Moieni, Hosseni, & Eskandari, 2010). Additionally, results from chorizo sausages made with silver carp, soy protein isolate, cassava starch, and canola oil added into the formulation were reported at 58.61% moisture, (ash not reported), 4.07% crude fat, and 21.68% crude protein (Trindade et al., 2019). Total moisture contents were slightly lower in the fish nugget compared to the oven baked fillet. This difference is expected since the nuggets were par-fried before baking

which would have resulted in some moisture loss before analysis. Fish nugget moisture content is higher than was reported for the sausages. This is because the percentage of minced meat in the sausage formulation was lower at 74.9% compared to 88.5%-91.5% minced meat found in the nuggets, which is the main source of water in the formulations.

Fish nuggets have a much higher percentage of ash when compared to oven baked fillets. This reflects the addition of the batter and panko breading which are the source of the increased ash contents. Further, the batter and panko breading that coats the fish nuggets is also responsible for the large difference in crude fat. During the frying step of the nugget preparation process, the breading absorbed oil as part of the mass and heat exchange. As water exited the nugget in the fryer, oil and heat were penetrating the nugget and being absorbed into the cavities created by the escaping steam. Seeing as the nuggets had a relatively large surface area, there were relatively large increases in oil absorption. As a result of the dramatically larger percentage of crude fat, crude protein is comparatively low in terms of the total percentage out of 100.

Table 3 Average proximate composition of raw fish nuggets formulated with silver carp and protein isolates

SAMPLE	MOISTURE (%)	ASH (%)	LIPID CONTENT (%)	CRUDE PROTEIN (%)	CARBOHYDRATES (%)
CONTROL	65.33 [a]	2.43 [a]	7.15 [a]	13.45 [a]	12.18 [b]
SPI	61.34 [ab]	2.23 [a]	8.44 [a]	13.88 [a]	13.77 [a]
PPI	60.53 [b]	2.44 [a]	7.97 [a]	13.44 [a]	14.75 [a]
SPI +PPI	62.21 [ab]	2.28 [a]	7.12 [a]	14.15 [a]	14.24 [a]

Control: fish nugget with no protein additive in the formulation. SPI: fish nugget with 3% soy protein isolate in the formulation. PPI: fish nugget with 3% pea protein isolate in the formulation. SPI+PPI: fish nugget with a combination of 1.5% soy protein isolate and 1.5% pea protein isolate in the formulation. Different letters (a, b) in the same column indicate significant differences among treatments ($P < 0.05$).

3.1.2 Thiobarbituric Acid (TBARS):

Levels of 1,1,3,3-tetramethoxypropane (TMP), the more stable diacetyl form of malondialdehyde (a product of secondary oxidative rancidity in oils), were measured. TMP is chemically a 1:1 equivalent to malondialdehyde. Increases in TMP indicate occurrence of lipid oxidation during frozen storage time that can lead to the formation of rancid flavors and aromas (Rodríguez et al., 2007). In this study, TMP values increased from a low at 1.5 weeks of 0.56 ppm to a high after 13.5 weeks of 1.96 ppm (Figure 5). These results are low when compared to those reported for fish sausages stored under refrigerated conditions, which showed initial TMP values of 2-4 ppm (Maqsood et al., 2012). In another study, TBARS results ranging from 0.2-0.6 ppm malondialdehyde were reported for chicken nuggets after 12 days of refrigerated storage (Hwang et al., 2014). These values are lower than the results observed in this study. The lipid oxidation threshold for detection by consumers varies from product to product. Pork sausages are reported to have a threshold of 2 ppm (Wenjiao et al., 2014), whereas a stark increase in rancid aroma has been reported for consumers of fish sausage at around 6 ppm TMP (Maqsood et al., 2012). Nuggets in this experiment approached 2 ppm. Therefore, frozen silver carp products might benefit from the addition of an antioxidant such as sodium erythorbate. TBARS measurements were also conducted on nuggets with the breading removed after 8.5 and 13.5 weeks of frozen storage, results in the appendix (Figure A.1).

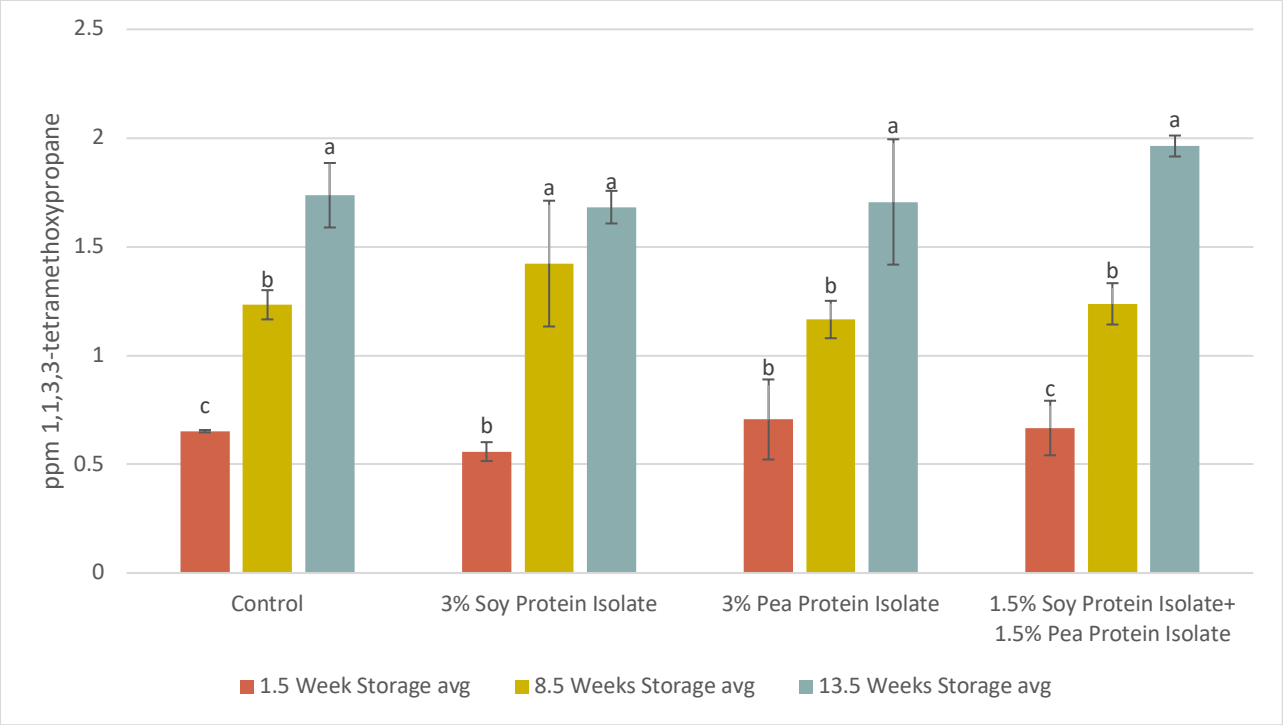

Figure 5 Results of thiobarbituric acid reactive substances reported in parts per million (ppm) of 1,1,3,3-tetramethoxypropane (TMP) for four different formulations of silver carp fish nuggets containing 3% soy protein isolate, 3% pea protein isolate, a blend of 1.5% soy protein isolate and 1.5% pea protein isolate, and a control with no plant protein added.

3.1.3 Aerobic Plate Count:

Microbial counts of raw nuggets were evaluated at each time period for each treatment. Initial microbial counts for the control, SPI and SPI+PPI treatments yielded values below the detection limit (<1 CFU/g), though subsequent counts during later tests showed up to 3.58-3.89 log CFU/g after 13.5 weeks (Table 5). The PPI treatment had its highest total plate count 3.86 log CFU/g at 1.5 weeks of storage. Values between treatments and across storage time periods were relatively close and considered to be low (<4.0 log CFU/g) for all treatments. These microbial counts can be considered low and similar to aerobic plate counts reported for other fish such as salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*), Pacific whiting (*Merlangius merlangus*), plaice (*Pleuronectes platessa*), and mackerel (*Scomber scombrus*) (Hempel et al., 2011).

3.1.4 Expressible Moisture:

Expressible moisture should be lower for formulations containing plant protein additives as they bind water. It was expected that this same trend would continue over the shelf life period, whereas increases in expressible moisture may indicate the breakdown of the proteins resulting in deceased moisture holding capacity and higher expressible moisture. At week 1.5, the control had higher ($p= 0.049$) expressible moisture (77.78%, see Table 4) than treatments containing plant protein. This was expected since plant proteins can form a network capable of forming protein-water bonds (Chavan et al., 2001; Riaz, 2006). At week 8.5 there were no significant differences ($p= 0.183$) though the control still had the greatest expressible moisture at 71.27% compared to other treatments which ranged from 60.73% to 67.45%. The continuation of the trend indicates that the protein isolates continue to bind water even after frozen storage and thawing which explains why there are no significant differences when the same treatment is compared across time.

The protein matrix within the fish nuggets is more stable than that of ribbed muscles (*Aulacomya ater)* which experienced increases from 10% to nearly 25% expressible moisture within weeks (0 – 6) of frozen storage (Paredi, De Vido De Mattio, & Crupkin, 1996). A paper comparing different expressible moisture methods on dark meat poultry reported expressible moisture of 29.93% and 32.14% for turkey and chicken respectively (Earl, King, Fitzpatrick, & Cooper, 1996). The reason these values are substantially lower than those observed for the nuggets is because of the structure of the meat. In the poultry study, 1.5 g of intact un-minced poultry meat was used, meaning the myofibrillar muscle fibers had not been mechanically disturbed. Since the muscle was intact it naturally retained water within the muscle cells. Minced meat, however, breaks apart myofibrillar muscle fibers, and releases intra-muscular water. This water is then available to interact with other components in the food matrices and is consequently detected during expressible moisture measurements.

Results showing that the addition of plant proteins lowers expressible moisture are corroborated by data from other muscle foods such as that reported by Das et al. (2008), who noted that goat meat nuggets containing textured soy maintained lower expressible moisture. The authors also reported that addition of soy paste actually increased expressible moisture potentially as the result of increased protein-protein interactions and less protein-water interactions (Das et al., 2008). This could explain why results observed at weeks 8.5 and 13.5, while following the same trend as week 1.5, were not significantly different between the control and the treatments with plant proteins, as the plant proteins may begin forming bonds with each other during thawing, thus increasing expressible moisture. It is possible that other reactions such as oxidation during frozen storage could also be influencing the proteins causing these differences in protein-water interactions during thawing.

Results from week 13.5 for PPI and SPI+PPI were conducted using a different filter paper which resulted in differences in expressible moisture that were a result of the filter paper rather than the treatments, thus data from those experiments were not included in this analysis (see appendix A Table A.2 for data).

Table 4 Average expressible moisture measurements (mean ± standard deviation) of silver carp nuggets after 1.5 and 8.5 weeks of frozen storage Expressible Moisture

Treatment and frozen storage time		Average % expressible moisture
Week 1.5	Control	77.78 a ± 10.18
	3% SPI	64.89 ab ± 3.08
	3% PPI	60.00 b ± 0.00
	1.5% SPI+ 1.5% PPI	64.44 ab ± 7.70
Week 8.5	Control	71.27 a ± 6.54
	3% SPI	67.45 a ± 5.86
	3% PPI	69.33 a ± 5.96
	1.5% SPI+ 1.5% PPI	60.73 a ± 3.03
Week 13.5	Control	77.16 a ± 3.88
	3% SPI	68.53 a ± 10.61

Values represent mean observations from triplicate determinations of expressible moisture. Control, SPI, PPI, and SPI+PPI abbreviations defined in table 3. There were no significant differences ($P > 0.05$) among storage times for the same treatment. Within columns, different letters (a, ab, b) indicate significant differences ($P < 0.05$) among treatments for the same storage time.

Table 5 Physicochemical analysis summary data table for silver carp nuggets after 1.5, 8.5, and 13.5 weeks of frozen storage

Frozen storage time and treatment		Parameters		
		pH	**Cook-loss (%)**	**Microbial growth (log CFU/g) raw fish nuggets**
Week 1.5	Control	6.38 ± 0.08ax	20.03 ± 0.02ax	< 0.01
	SPI	6.44 ± 0.09ax	19.35 ± 0.03ax	< 0.01
	PPI	6.38 ± 0.06bx	17.30 ± 0.02axy	3.86
	SPI+PPI	6.43 ± 0.04ax	14.58 ± 0.03ay	< 0.01
Week 8.5	Control	6.45 ± 0.08ax	15.55 ± 0.02bx	2.86
	SPI	6.48 ± 0.04ax	13.97 ± 0.03bx	3.04
	PPI	6.51 ± 0.03abx	15.47 ± 0.03ax	3.33
	SPI+PPI	6.52 ± 0.04ax	13.00 ± 0.02ax	3.43
Week 13.5	Control	6.52 ± 0.1axy	14.51 ± 0.02bx	3.59
	SPI	6.35 ± 0.07ay	15.20 ± 0.01bx	3.89
	PPI	6.57 ± 0.09ax	11.26 ± 0.21ax	3.53
	SPI+PPI	5.49 ± 0.08bz	14.14 ±0.10ax	3.58

Values represent mean observations from triplicate determination of pH, log colony forming units (CFU) per g, and 6 replicates for cook-loss. Control, SPI, PPI, and SPI+PPI abbreviations defined in table 3. Within the same column different letters (a, b) indicate significant differences ($p < 0.05$) between storage times for the same treatment. Within the same column different letters (x, y, z) indicate significant differences ($P < 0.05$) between treatments for the same storage time.

3.1.5 Instrumental Color:

Internal and external (bottom) color parameters were measured as L* (lightness) a* (green vs red) and b* (blue vs yellow) values (Table 6). Figure 6 is a picture of the fish nugget and figure 7 shows the internal portion of the nugget; however, for the internal color analysis nuggets were cut horizontally for maximum surface area rather than vertically as shown in the figure. Internal L* values for the control increased from 60.6 to 64.1 between week 8.5 and 13.5 ($p = 0.00$). Within week 13.5 and between treatments, SPI+PPI was significantly ($p = 0.02$) darker with an L* value of 60.8 compared to values of around 64 for other treatments. During week 1.5 internal color values ranged from 60.8 to 64.43. These values are slightly darker than fried chicken nuggets with varying percentages of mechanically deboned chicken meat (68-70) (Perlo, Bonato, Teira, Fabre, & Kueider, 2006) and lighter than silver carp chorizo (39.4 – 41.6) (Trindade et al., 2019). The chorizo sausage was darker due to the addition of dark reddish chorizo seasoning. The lighter color in the chicken nuggets may be a result of the low levels of myoglobin in chicken breast.

The external L* value for both the control ($p = 0.01$) and PPI ($p = 0.00$) at 1.5 weeks was darker than at 13.5 weeks. These differences in lightness are likely a result of contact with the baking sheet during cooking rather than actual color changes in the nugget over time. No significant differences were observed between treatments for external nuggets in the same time period. External (top) color measurements were also recorded (data available in Appendix A Table A.1)

Internal SPI treatment a* values became more positive (red direction) ($p = 0.04$) from 1.5 to 8.5 weeks. While these are instrumentally significant, the nuggets were not visibly red, as values of around 2-3 are not sufficient to be visibly red (see figure 7). Internal delta E values ranged from 1.70 to 4.43, while external delta E values showed more variability and larger magnitudes of

differences between the control and treatments with values ranging from 0.93-8.92. Delta E is a measure of the magnitude of overall color difference between the treatments and the control. Given the definition, the fact that external color had a wider range of delta e values makes sense as some nuggets showed more surface browning than others during the baking process (due to differences in heating based on positioning on the sheet tray, and variation in heat distribution in the oven,) while the internal colors varied less due to indirect contact with the heat.

Figure 6 Intact fish nugget

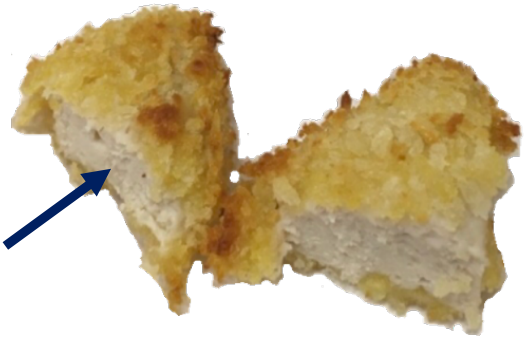

Figure 7 fish nugget cut in half with an arrow pointing to the interior color

Table 6 Instrumental color (mean ± standard deviation) of silver carp nuggets formulated after 1.5, 8.5, and 13 weeks of frozen storage.

Instrumental Color		Parameters							
		Internal color				External color			
		Color L* (lightness)	Color a* (redness)	Color b* (yellowness)	Δ E	Color L* (lightness)	Color a* (redness)	Color b* (yellowness)	Δ E
Week 1.5	Control	62.10 ± 0.80abx	1.96 ± 0.33ax	15.50 ± 0.84ax		42.28 ± 1.71b	14.37 ± 1.73a	30.52 ± 6.02a	-
	SPI	64.17 ± 2.99ax	1.62 ± 0.24bx	17.3 ± 0.27ax	2.77	42.6 ± 2.22a	14.33 ± 2.86a	31.38 ± 5.13a	0.93
	PPI	60.8 ± 1.65ax	2.47 ± 0.56ax	17.00 ± 0.80ax	2.05	38.75 ± 2.24b	11.44 ± 3.68a	28.13 ± 4.14a	5.17
	SPI+PPI	64.43 ± 0.21ax	2.68 ± 0.09ax	17.68 ± 1.42ax	3.27	41.2 ± 9.03a	12.47 ± 0.26	31.72 ± 4.45a	2.49
Week 8.5	Control	60.55 ± 0.38bx	2.28 ± 0.11ax	16.34 ± 0.53ax	-	46.84 ± 2.65a	11.63 ± 1.49ab	31.68 ± 1.49a	-
	SPI	64.34 ± 2.18ax	2.34 ± 0.11ay	18.63 ± 0.8ax	4.43	42.75 ± 2.46a	14.33 ± 0.75a	33.69 ± 1.34a	5.29
	PPI	62.15 ± 0.22ax	2.70 ± 0.12axy	16.72 ± 0.64ax	1.70	44.54 ± 2.36ab	12.42 ± 1.85a	32.82 ± 3.95a	2.68
	SPI+PPI	63.71 ± 0.36ax	3.18 ± 0.3ax	17.72 ± 0.99ax	3.56	45.97 ± 3.7a	11.00 ± 2.52a	31.64 ± 1.42a	1.07
Week 13.5	Control	64.12 ± 0.17ax	1.62 ± 0.08ay	17.27 ± 0.21ax	-	51.00 ±2.73a	9.34 ± 2.89b	33.35 ± 2.1a	-
	SPI	63.94 ± 1.03ax	2.02 ± 0.04aby	18.45 ± 1.32ax	1.26	43.17 ± 4.14a	13.57 ± 2.07a	34.01 ± 2.43a	8.92
	PPI	64.07 ± 0.63ax	2.80 ± 0.11ax	18.59 ± 0.54ax	1.77	48.78 ± 3.8a	10.82 ± 0.96a	31.72 ± 1.92a	3.13
	SPI+PPI	60.79 ± 0.29by	3.19 ± 0.28ax	19.11 ± 1.59ax	4.12	46.36 ± 1.6a	12.22 ± 2.15a	34.54 ± 1.58a	5.59

Values represent mean observations from triplicate determinations of external color and duplicate determinations of internal color. Control, SPI, PPI, and SPI+PPI abbreviations defined in table3. Within columns, different letters (a, ab, b) indicate significant differences ($P < 0.05$) among storage times for the same treatment. Within columns, different letters (x, xy, y) indicate significant differences ($P < 0.05$) among treatments for the same storage time. There were no significant differences between treatments within the same week for external color.

3.1.6 pH:

Among pH measurements (Table 5) both PPI and SPI+PPI treatments showed significant differences between different frozen storage times. PPI showed decreases ($p = 0.02$) in average pH between weeks 1.5 (6.57) and 13.5 (6.37), while SPI+PPI showed decreases ($p = 0.00$) in average pH from week 8.5 (6.42) to week 13.5 (5.49). The average pH for SPI+PPI at week 13.5 (5.49) approached the isoelectric point (5.2-5.5) for myofibrillar fish proteins; however there were no visible differences in the pH slurry (Shiku, Hamaguchi, & Tanaka, 2003). Since myofibrillar proteins make up the majority of the proteins in the mince and are already insoluble in solution, it makes since that approaching the isoelectric point would have little visible impact on the slurry. SPI+PPI at week 13.5 was the only treatment to experience a (relatively) large magnitude change in pH. This was unexpected since pH should not be impacted by frozen storage. It is known that amino acids have the capacity to act as buffers (containing both an acidic carboxylic acid group and a basic amine group). It is possible that changes in protein-protein interactions between the soy, pea, and mince proteins (during frozen storage and subsequent thawing) exposed such amino acids, although the buffering mechanism of such a reaction has not been explored. Regardless, this change in pH did not seem to impact other physicochemical parameters.

3.1.7 Peak Force Texture Analysis:

Texture is an extremely important attribute for consumers of fish products. Previous work showed that texture was the lowest rated sensory attribute for a chorizo-type sausage made using invasive silver carp (Trindade et al., 2019). Figure 8 shows the differences in average peak force of each trial over time. Each treatment showed increased textural hardness with increased time. When each treatment of the same time period was compared, only week 13.5 showed significant differences ($p= 0.00$) with the control being lower than the other treatments. The control showed the smallest difference between weeks 1.5 and 13.5 and was

the only treatment without significantly increase in textural hardness over time, indicating that the protein isolates contribute to the increased textural hardness (Figure 8). It has been noted earlier that lipid oxidation increased during these same time periods, previous literature has reported increased textural hardness with increased oxidation for meats during frozen storage (Decker, Faustman, & Lopez-Bote, 2000). Most fish nuggets showed peak hardness values between 5 and 6 N, which is slightly lower than values reported for fried chicken nuggets (Martínez-Ávila et al., 2010). However, chicken nuggets that were fried and then air-dried had similar (6.1-6.6 N) hardness values as those obtained in this study (Martínez-Ávila et al., 2010). This is likely due to similarities in nugget preparation (both fish and chicken nuggets experienced a partial frying and were then exposed to hot, dry airflow to finish cooking). Frying fish nuggets through until cook completion (rather than par-frying and baking) may result in slightly higher hardness, which could be beneficial in producing a fish nugget with a more chicken-like nugget texture.

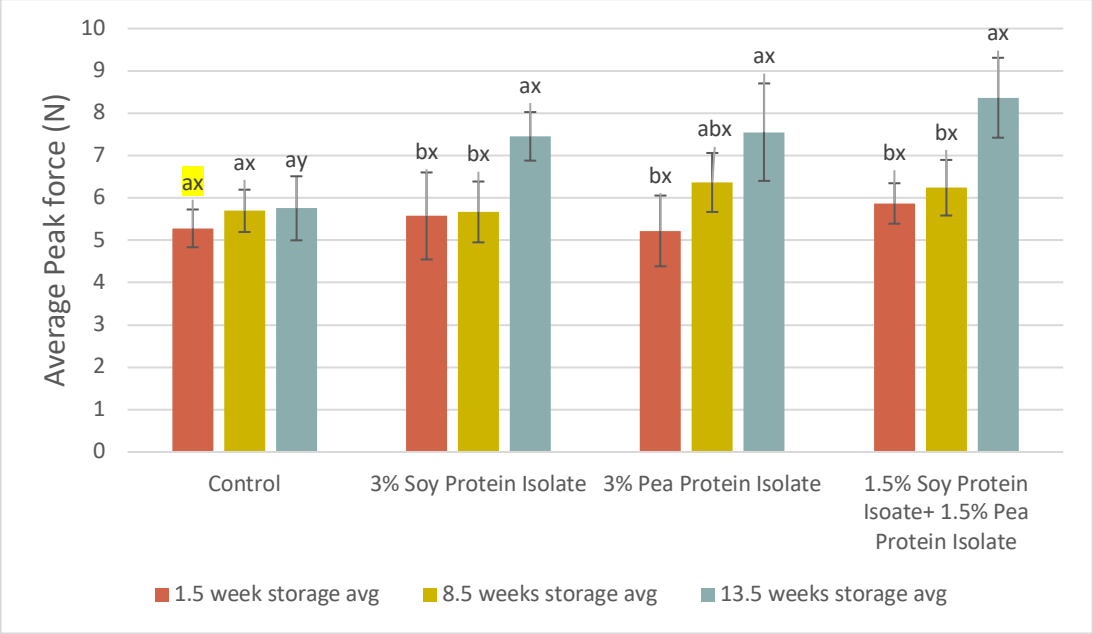

Figure 8 Change in average peak force of cooked silver carp nuggets after 1.5, 8.5, and 13.5 weeks of frozen storage. Average peak force was reported in N required to puncture the fish nugget to 50% thickness. Control, SPI, PPI, and SPI+PPI abbreviations defined in table 3. Different letters (a, ab, b) indicate significant differences ($P < 0.05$) between time periods for the same treatment. Different letters (x, y) indicate significant differences ($p < 0.05$) between different treatments for the same storage time.

3.1.8 Cook Loss:

Cook-loss was measured to test how the addition of different protein isolates (different treatments) would affect the fish nuggets' moisture retention during cooking. Also, differences in cook-loss could be indicative of protein denaturation, which can result in detrimental changes in texture. The increase in hardness due to protein denaturation can be caused by the presence of formaldehyde that can develop from the breakdown of trimethylamine N-oxide, a naturally present compound common in fish, which is known to result in muscle gapping, textural hardness, and can occur during frozen storage (Hall, 1997).

Regarding comparisons of treatments within the same storage time, the combination treatment SPI+PPI was able to decrease ($p= 0.01$) cook loss from 20% to ~15% at the first storage time tested (week 1.5). While not significantly different, nuggets at week 8.5 showed a similar trend with the control having the highest cook-loss and SPI+PPI having the lowest, demonstrating that the addition of protein isolates, in particular the combination of soy and pea protein isolates was able to decrease cook-loss. This reinforces the idea of a plant protein matrix forming with increased water binding capacity as discussed earlier in expressible moisture. In another study fresh silver carp meat was analyzed for cook loss over a 72 hr period (post mortem stored on ice at 0 °C) (C. Shi, Cui, Luo, Zhu, & Zhou, 2015). During the first 12 hrs the cook loss ranged from 21.23% – 23.12% which is similar to the control fish nugget. Because the control nugget was made of minced fish meat only, it was expected that the cook loss would be larger; however, there are two possible explanations for this discrepancy. The first explanation is that any excess water released during mincing is being bound in the protein matrix as the mince protein network congeals during baking. The second explanation is that the batter and breading coating on the exterior of the nugget serves as a protective outer shell, slowing the release of moisture from the nugget.

Within the same treatment and across different time periods there was no significant difference in cook-loss treatments containing PPI and SPI+PPI. However, cook-loss decreased for both the control ($p= 0.00$) and SPI ($p= 0.01$) (Table 5). In a study where changes in cook loss for silver carp were observed over 72 hrs, ~~C.~~ Shi et al. (2015), it was noted that after 12 hrs the cook loss increased up to, on average, 27.31%. This is opposite the trend observed in both the control and SPI treatments, which decreased in percent cook loss when comparing week 1.5 to weeks 8.5 and 13.5.

Large decreases in cook loss were observed for PPI between each time period (though not significant) and SPI+PPI remained relatively consistent decreasing slightly from week 1.5 to 8.5 and increasing slightly from week 8.5 to 13.5. These results indicate trimethylamine N-oxide degradation did not occur in the fish nuggets, as such an event would result in consistent increases in cook loss. The large water binding capacity of pea protein (as much as 257-288% reported in literature) may explain why the PPI treatment underwent large decreases in cook loss between time periods (Chavan et al., 2001). Increases in time and resultant changes in the protein matrix allowed for increased pea protein water binding during the baking process. This data is somewhat contradictory to what was reported earlier in expressible moisture, which showed small increases in expressible moisture over time. Since expressible moisture analysis is done on raw nuggets, these differences between cook loss and expressible moisture reflect a change in structure occurring during the congealing and solidifying of the protein matrix at baking.

There is no clear trend between various treatments with additional plant proteins, and the control. Further, variations between treatment cook loss values over time imply that each plant protein isolate functions differently in the nugget. Plant proteins only made up 3% of the total formulation, therefore, varying the percentage of plant proteins in future studies may help clarify how the plant protein influences the nugget structure and furthermore, how that structure

changes during cooking. Observing different levels of protein may result in clearer data trends emerging.

3.2 Consumer Survey and Sensory Evaluation Results

3.2.1 Consumer Knowledge Survey:

Of the sampled population, 75% (n=126) reported consuming fish at least once a month. Within this portion of the population 58% of respondents indicated that they avoid fish containing bones, 41% of respondents indicated that they never purchase fish that has bones, and 42% indicated that they purchase fish without bones but will occasionally buy fish with bones. This data highlights a general preference by the average consumer in the United States for fish without bones and strengthens the argument for the development of fish products that lack bones, such as fish nuggets. Furthermore, a majority of respondents (87%) reported a willingness to consume freshwater fish from U.S. rivers, and 66% reported having heard of the invasive silver carp prior to the survey.

A small percentage (12%) of respondents reported beliefs about silver carp being unsafe to eat, with the majority (71%) indicating that they had never considered the safety of the fish before taking this survey. A similar survey about Asian carp conducted with Missouri anglers (n=465) reported that top concerns about carp consumption were health risks/poor water quality, the number and structure of the bones, and the taste/flavor/odor (Morgan & Ho, 2018). In another survey (n=202), also for Asian carp, mercury levels were listed as the top concern (Varble & Secchi, 2013). Results from these surveys clearly indicate an opportunity to increase public knowledge via education and extension efforts, and marketing from industry with the goal of improving public understanding on the safety of these fish and the U.S. river systems. A total of 77% of respondents indicated that they were willing to eat food products made with silver carp meat. This percentage is higher than what was reported for Missouri

angles (53%) and Varble and Seechi's findings (73%willing to try a free sample) (Morgan & Ho, 2018; Varble & Secchi, 2013).

Finally, the results of this survey indicate that the sampled population were slightly to moderately educated about the impact of invasive silver carp, with 48% reporting knowledge that silver carp pose a threat to native fish species throughout the Mississippi River and Great Lakes regions, and 45% falsely believing that silver carp are bottom feeders. Similarly, 73% of Missouri anglers were not aware that Asian carp are plankton feeders (Morgan & Ho, 2018). These insights into consumer knowledge provide points of reference for further education efforts and product development endeavors.

3.2.2 Sensory Acceptability:

Sensory acceptance scores by consumers are important to understand how well consumers like/dislike a product and to predict how well it may perform in the market (Stone, 2012). Thus, it was important to measure sensory acceptance scores over time to ensure that the nuggets would be deemed acceptable (scores above 6.5) during the 13.5 weeks of frozen storage. Table 7 shows the results for degree of liking (DOL) for appearance, aroma, flavor, and texture of nuggets made from the four treatments. DOL scores did not significantly differ between treatments, and within treatments, only the SPI treatments showed a significant decrease in DOL for aroma ($p=0.03$) between weeks 1.5 and 8.5, and also in DOL for flavor ($p=0.03$) between weeks 1.5 and 13.5. Nonetheless, DOL scores for all attributes, all treatments, and all times remained acceptable (scores above 6.5-7.0, like slightly/moderately) until the completion of the study. This indicates that consumers liked the appearance, aroma, flavor, and texture of the treatments even after 13.5 weeks of frozen storage. Although instrumental textural hardness as well as levels of lipid oxidation increased with storage time, consumer liking of texture and overall liking did not change and did not seem to be affected by

the magnitude of the changes detected by instrumental texture measurements and oxidation analyses. Similar results were reported for chicken nuggets over a 3-month frozen storage time period, where lipid oxidation differences were detected over time, but no changes in the sensory acceptability were perceived by panelists (DOL scores between 5 and 7) (Jiménez-Martín, Pérez-Palacios, Carrascal, & Rojas, 2016). Previous work showed that a chorizo-type sausage formulated with silver carp received acceptability scores above 6 (Trindade et al., 2019). High sensory acceptance scores of silver carp-containing products coupled with survey results indicating the willingness of fish consumers to try the nuggets are strong evidence that silver carp nuggets could be a viable product for market introduction.

Table 7 Average Sensory acceptance scores for silver carp nuggets formulated with plant protein isolates after 1.5, 8.5, and 13.5 weeks of frozen storage (N=382)

Test Procedure		Control			SPI			PPI			SPI+PPI		
		1.5 weeks	**8.5 weeks**	**13.5 weeks**	**1.5 weeks**	**8.5 weeks**	**13.5 weeks**	**1.5 weeks**	**8.5 weeks**	**13.5 weeks**	**1.5 weeks**	**8.5 weeks**	**13.5 weeks**
DOL	Appearance	7.02 ^{x}	6.97 ^{x}	6.85 ^{x}	7.12 ^{x}	6.94 ^{x}	7.06 ^{x}	7.17 ^{x}	7.22 ^{x}	6.85 ^{x}	7.14 ^{x}	7.00 ^{x}	7.03 ^{x}
	Aroma	7.32 ^{x}	7.14 ^{x}	7.2 ^{x}	7.42 ^{x}	6.88 ^{y}	7.17 ^{xy}	7.28 ^{x}	7.07 ^{x}	7.13 ^{x}	7.32 ^{x}	7.31 ^{x}	7.28 ^{x}
	Flavor	7.11 ^{x}	7.05 ^{x}	6.97 ^{x}	7.43 ^{x}	6.95 ^{xy}	6.85 ^{y}	7.20 ^{x}	7.18 ^{x}	7.15 ^{x}	7.14 ^{x}	6.85 ^{x}	7.25 ^{x}
	Texture	7.14 ^{x}	6.8 ^{x}	6.78 ^{x}	7.17 ^{x}	6.86 ^{x}	6.88 ^{x}	6.74 ^{x}	6.75 ^{x}	6.93 ^{x}	6.86 ^{x}	6.96 ^{x}	6.94 ^{x}
	Overall Liking	7.02 ^{x}	6.88 ^{x}	6.66 ^{x}	7.26 ^{x}	6.92 ^{x}	6.82 ^{x}	7.11 ^{x}	7.00 ^{x}	7.00 ^{x}	7.18 ^{x}	6.91 ^{x}	7.00 ^{x}
JAR	Fish Flavor	2.82	2.65	2.72	2.78	2.71	2.65	2.69	2.64	2.61	2.66	2.55	2.51
		(68%)	(54%)	(52%)	(71%)	(62%)	(58%)	(68%)	(53%)	(64%)	(60%)	(56%)	(51%)
	Texture	2.91	2.95	2.97	3.05	3.14	3.09	2.88	2.98	2.96	2.98	3.22	3.04
		(71%)	(66%)	(62%)	(68%)	(75%)	(63%)	(57%)	(49%)	(61%)	(65%)	(64%)	(58%)
	Saltiness	3.23	3.23	3.17	3.11	3.15	3.11	3.00	3.15	2.9	3.14	3.15	3.01
		(62%)	(51%)	(54%)	(58%)	(63%)	(71%)	(58%)	(64%)	(78%)	(63%)	(65%)	(61%)

Control, SPI, PPI, and SPI+PPI abbreviations defined in table 3. Degree of Liking (DOL) using a 9-point hedonic scale where 1= dislike extremely, 5= neither like nor dislike, and 9= like extremely. Within rows, different letters (x, y) indicate significate differences ($P < 0.05$) among time periods for the same treatment. Average attribute diagnostic scores using a Just about right (JAR) scale where 1= Much too weak/soft/definitely not salty enough, 3= JAR, and 5= Much too strong/firm/salty. Numbers in parenthesis represent percentage of panelists scoring JAR.

3.2.3 Attribute Diagnostic Testing:

The intensity of fish flavor, texture, and saltiness were analyzed across frozen storage time periods with no significant differences between them (Table 7). Saltiness scores for the control were the highest ranging from 3.17-3.23, having slightly too much salt, and the lowest % panelists (51%) scoring JAR (just about right) for all storage time periods. This result was expected, as soy proteins are known to mask flavorings within their protein matrix by binding water and trapping flavor compounds (Riaz, 2006) and pea protein may function in a similar manner. The control formulation did not include plant vegetable proteins; therefore, it is plausible that consumers were able to perceive more saltiness in those samples. Fish flavor varied between treatments from 2.57-2.73 indicating low/ weak fish flavor in the treatments. Texture results ranged from 2.94-3.09 (close to JAR) with average values similar to those reported for dry silver carp chorizo formulations (Trindade et al., 2019).

3.2.4 Quantitative Descriptive Analysis (QDA™):

Figure 9 shows the results from the descriptive analysis. Trained panelists identified the following aromas in silver carp nuggets: lemon, fried, onion/garlic, and fish; the following flavors: fried, salt, spices (the combination of onion and garlic flavors), cornmeal, fish; and the following mouthfeel characteristics: juicy/moist, cohesive/springy, mealy/gritty, and fatty/oily.

Interestingly, panelists identified a lemon aroma in all four treatments. It is possible that the volatile compounds producing the lemon aroma are a result of a combination of the silver carp and the other ingredients in the formulation. While not statistically significant, the lemon aroma for the control was higher than treatments with added vegetable protein. Similarly, the control had higher aroma scores for both fried and onion/garlic aromas and the second highest score (behind PPI) for fish aroma though none of these differences were statistically significant.

All treatments of silver carp nuggets had a mild fish flavor and less intense fish flavor (4.1 to 5.1) than flounder (a reference standard that was given high intensity scores, data not shown). Salt flavor intensity was lowest for PPI (4.2), which was the treatment with the highest JAR percentage (78% at 13.5 weeks). This indicates that a less intense salt flavor was perceived by trained panelists and desired by consumers. Conversely, panelists rated SPI as having the most intense salty flavor while consumers indicated the control as having the lowest JAR percentage (51% at 8.5 weeks). Since the panel was composed of panelists trained with the nuggets, the discrepancy may be due to a difference in exposure to the product. For example, because the panelists consumed the product over multiple weeks of training, they were able to scrutinize the product based on the long-term saltiness rather than a face value or initial observation.

For mouthfeel, the control was listed as generating the least intense mealy/gritty sensation i.e., addition of protein isolates resulted in a more intense sensation of sand-like particles on the tongue. The presence of increased mealy/grainy mouthfeel associated with protein isolates did not negatively impact consumer liking of texture or overall liking. The most intense juicy/moist mouthfeel score was the control sample (5.2) which may have implications for the way that protein in the other treatments is binding water. Juicy/moist results were lower than those reported for chicken nuggets (Pérez-Palacios, Ruiz-Carrascal, Jiménez-Martín, Solomando, & Antequera, 2018).

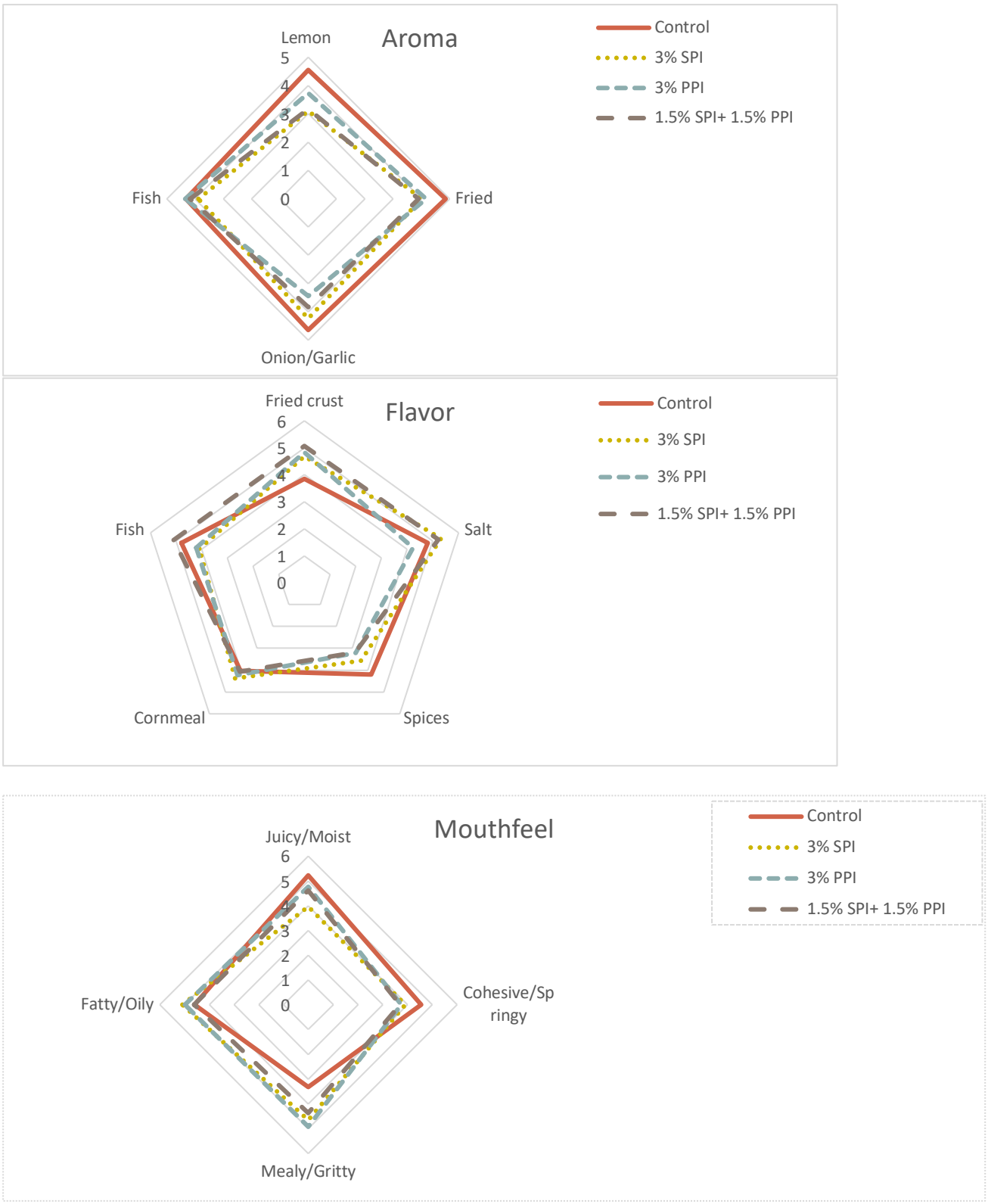

Figure 9 Aroma, flavor, and mouthfeel intensity radar plots for quantitative descriptive analysis of silver carp fish nuggets of four formulations (Control, SPI, PPI, and SPI+PPI abbreviations defined in table 3,) evaluated by a trained panel (n=6) using an attribute lexicon generated by the panel consisting of aroma (lemon, fish, fried, onion/garlic), flavor (fried crust, salt, spices, cornmeal, and fish), and mouthfeel (juicy/moist, cohesive/springy, mealy/gritty, and fatty/oily)

CHAPTER 4. CONCLUSION

Finding ways to turn the invasive sliver carp from a water pest to a value-added commodity is challenging. This research has demonstrated that consumers are willing to eat food products formulated with the invasive fish. Results from this study show that fish nuggets with high sensory acceptability can be successfully prepared using muscle meat from invasive silver carp. Regarding the storage stability, although the fish nuggets showed changes in instrumental textural hardness measurements and oxidation analysis after 1.5-13.5 weeks of frozen storage, the magnitude of such changes did not impact sensory acceptability of the product, as shown by high acceptability scores (6.6-7.4) at the end of the study. Considering the plant protein additives tested (soy and pea protein isolates), except for the cook-loss and expressible moisture parameters in the beginning of storage period (1.5 weeks), both proteins, individually (3%) or in combination (1.5% each), did not bring advantages to the evaluated physicochemical parameters and sensory acceptance of silver carp nuggets. Survey results demonstrated that there is a general lack of consumer knowledge about the safety of silver carp for consumer consumption and indicate an opportunity to educate consumers about this topic. However, despite of this lack of silver carp-safety awareness, consumers are willing to purchase bone-free silver carp products, such as the nuggets prepared in this study.

While a precedent has been set for the development of edible silver carp products, work still needs to be done by industry and government to guarantee a steady supply of fish (removal from water ways and post-harvest storage). In addition, government subsidies may be required as incentive for fishermen until the market can be established. In the meantime, further research studies could be done over longer periods of time to gain insight into long-term frozen shelf life and to develop formulations that guarantee a minimum shelf-life of 6 months. Results from this

study demonstrate that consumption of silver carp containing products is a viable option for removal and better utilization of this invasive species and to improve the overall health of U.S. water ways by promoting non-piscidal options for the control of this invasive fish.

APPENDIX A. ADDITIONAL DATA

Table A.1 Instrumental external top color measurements (mean ± standard deviation) of silver carp nuggets after 1.5, 8.5, and 13.5 weeks of frozen storage

	Instrumental Color	External (top) color			
		Color L* (lightness)	Color a* (redness)	Color b* (yellowness)	Δ E
Week 1.5	Control	48.51 a ± 1.69	12.87 a ± 1.1	36.03 a ± 0.48	-
	SPI	49.5 a ± 2.88	11.22 a ± 2.57	34.36 a ± 2.59	2.55
	PPI	43.26 b ± 3.99	10.67 a ± 2.28	31.29 a ± 3.4	7.41
	SPI+ PPI	49.38 a ± 4.40	9.81 a ± 2.4	33.47 a ± 2.69	4.08
Week 8.5	Control	48.93 a ± 2.92	9.85 a ± 1.23	33.88 a ± 1.41	-
	SPI	50.3 a ± 2.21	9.12 a ± 1.13	33.95 a ± 1.03	1.56
	PPI	48.49 ab ± 3.09	10.19 a ± 1.33	34.03 a ± 2.00	0.58
	SPI+ PPI	49.52 a ± 2.4	10.78 a ± 1.93	36.12 a ± 1.28	2.50
Week 13.5	Control	51.73 a ± 4.71	9.11 a ± 2.99	33.87 a ± 4.12	-
	SPI	47.46 a ± 7.15	11.61 a ± 3.2	34.56 a ± 2.62	5.00
	PPI	51.78 a ± 0.92	9.24 a ± 0.17	33.23 a ± 2.08	0.65
	SPI+ PPI	52.77 a ± 0.73	7.49 a ± 0.68	33.87 a ± 1.63	1.93

Values represent mean observations from triplicate determinations of external top color. Control, SPI, PPI, and SPI+PPI abbreviations defined in table 3. Within columns, different letters (a, ab, b) indicate significant differences ($P < 0.05$) among storage times for the same treatment. There were no significant differences ($P > 0.05$) among treatments for the same storage time.

Figure A.1 TBARS for fish nuggets with breading removed after 8.5 and 13.5 weeks of frozen storage expressed in ppm 1,1,3,3-tetramethoxypropane.

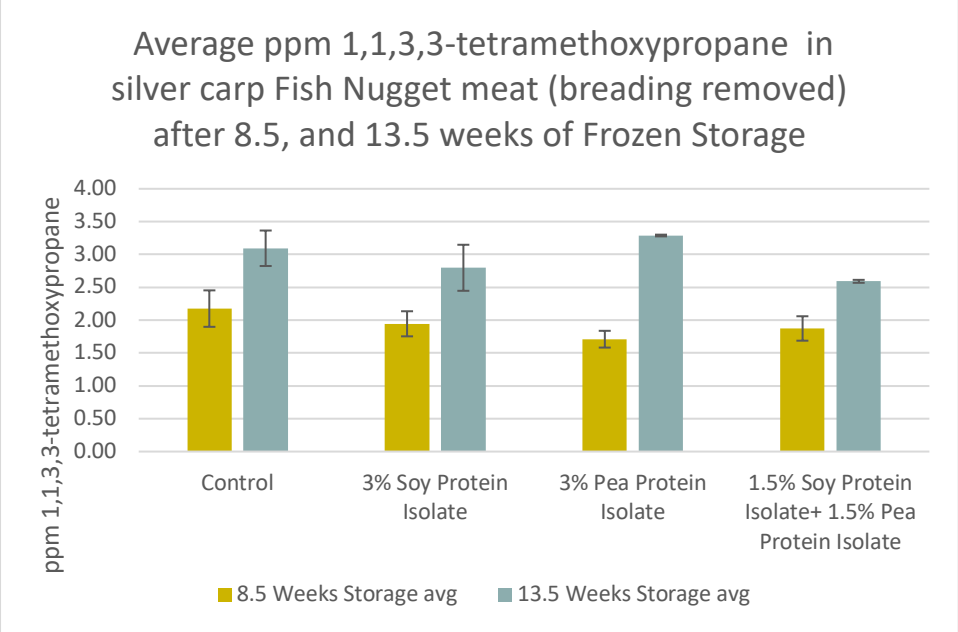

Table A.2 Average expressible moisture measurements (mean ± standard deviation) of silver carp nuggets after 1.5, 8.5, and 13.5 weeks of frozen storage

Treatment and frozen storage time		Average expressible Moisture
Week 1.5	Control	77.78 a ± 10.18
	3% SPI	64.89 ab ± 3.08
	3% PPI	60.00 b ± 0.00
	1.5% SPI+ 1.5% PPI	64.44 ab ± 7.70
Week 7.5	Control	71.27 a ± 6.54
	3% SPI	67.45 a ± 5.86
	3% PPI	69.33 a ± 5.96
	1.5% SPI+ 1.5% PPI	60.73 a ± 3.03
Week 13.5	Control	77.16 a ± 3.88
	3% SPI	68.53 a ± 10.61
	3% PPI	32.12 ± 3.94
	1.5% SPI+ 1.5% PPI	32.89 ± 5.37

Values represent mean observations from triplicate determinations of expressible moisture. Control, SPI, PPI, and SPI+PPI abbreviations defined in table 3. Within columns there are no significant differences ($P > 0.05$) among storage times for the same treatment. Within columns, different letters (a, ab, b) indicate significant differences ($P < 0.05$) among treatments for the same storage time. Values for PPI and SPI+PPI at 13.5 weeks were not included in the statistical analysis.

APPENDIX B. CONSUMER KNOWLEDGE SURVEY QUESTIONS

Q-1 How Often do you Consume fish?
Multiple days per week.
8=4.73%
At least once a week.
28=16.568%
Several times a month.
37=21.89%
At least once a month,
53=31.36%
A few times a year,
38=22.485%
I don't or can't eat fish.
5=2.958%

Total eating at least once a month or more is 126=74.556%
Total 169

Q-2 Are you from the United States? (data shown before removing the QC check fails and those leaving the U.S.)
Yes
153=85.96%
No
25=14.04%
Total 178

Q-3 Do you currently live in the United States?
Yes, and I plan to be here for the next few years.
173=97.74%
Yes, but I plan to leave in the next few years.
4=2.26%
No, I do not currently live in the United States
0=0%
Total 177 (1 response was left blank and their data was removed from analysis)

Q-4 Do you or have you ever lived near a river system?
Question removed because there was no option to select "No/ I have never lived near a river system"

Q-5 do you usually avoid eating fish that contains bones?
Yes
73=57.936%
No
53=42.06%

Total 126

Q-6 When I buy fish I…
Prefer to purchase fish with bones.
3=2.38%
Purchase fish without bones but will buy fish with bones on occasion.
53= 42.06%
Never purchase that have bones
51=40.476%
Don't care if there are bones ore not (I will buy the fish either way/ have no preference).
19=15.079%
Total 126

Q-7 Are you willing to eat freshwater fish from the rivers in the United states?

Yes
109=86.507%
No
17=13.49%
Total 126

Q-8 Have you heard of the silver carp (a fish that is a member of the Asian carp family?)
Yes
77=66.11%
No
49=38.88%
Total 126

Q-9 Quality control check, please select NO
Yes
2=1.12%
Maybe
2=1.12%
No
174=97.75%
Total 178

Q-10 When I think of silver carp I…
Think they are usually safe to eat.
21=16.8%
Have never considered their safety.
89=71.2%
Think they are usually unsafe to eat.
15=12%
Total 125

Q-11 Did you know that silver carp pose a threat to native fish species throughout the Mississippi River and the Great Lakes?
Yes
61=48.41%
No
65=51.587%
Total 126

Q12- Do you believe that silver carp are bottom feeders (fish that eat off the floors of their water bodies)?
Yes
56=45.16%
No
68=54.838%
Total 124

Q-13 Would you be willing to eat silver carp if you knew they are not bottom feeders?
Yes
96=76.8%
No
29=23.2%
Total 125

Q-14 Are you willing to eat food products (fillets, nuggets, etc) made of silver carp?
Yes
96=76.8%
No
29=23.2%
Total 125

Q-15 Would you be willing to try "Rivers Bounty Fresh Water Fish Nuggets" if you knew they were made from Silver Carp and contained no bones?
Yes
101=80.158%
No
25=19.84%
Total 126

Q16 Please indicate your age group
18-24
22=17.46%
25-34
46=36.507%
35-44
23=18.25%
45-54
14=11.11%
55-64
13=10.317%
65-74
7=5.555%
75+
1=0.7936%
Total 126

REFERENCES

Antonova, I., Mallikarjunan, P., & Duncan, S. E. (2004). Sensory Assessment of Crispness in a Breaded Fried Food Held Under a Heat Lamp. *Foodservice Research International, 14*(3), 189-200. doi:10.1111/j.1745-4506.2004.tb00189.x

Asian Carp- Aquatic Invasive Species Issues, Program Accomplishments, and Program Needs. (2004).

Barrera, A., Ramırez, J., González-Cabriales, J., & Vázquez, M. (2002). Effect of Pectins on the Gelling Properties of Surimi from Silver Carp. *Food Hydrocolloids, 16*(5), 441-447.

Buege, J. A., & Aust, S. D. (1978). [30] Microsomal lipid peroxidation. In *Methods in enzymology* (Vol. 52, pp. 302-310): Elsevier.

Cakli, S., Taskaya, L., Kisla, D., Çelik, U., Ataman, C., Cadun, A., . . . Maleki, R. (2005). Production and Quality of Fish Fingers from Different Fish Species. *European Food Research and Technology, 220*(5), 526-530. doi:10.1007/s00217-004-1089-9

Cardoso, C., Mendes, R., Pedro, S., & Nunes, M. L. (2008). Quality Changes During Storage of Fish Sausages Containing Dietary Fiber. *Journal of Aquatic Food Product Technology, 17*(1), 73-95. doi:10.1080/10498850801891249

Chavan, U., McKenzie, D., & Shahidi, F. (2001). Functional Properties of Protein Isolates from Beach Pea (Lathyrus maritimus L.). *Food Chemistry, 74*(2), 177-187.

Chempaka, S., Yusof, M., & Babji, A. S. (1996). Effect of Non-Meat Proteins, Soy Protein Isolate and Sodium Caseinate, on the Textural Properties of Chickn Bologna. *International Journal of Food Sciences and Nutrition, 47*(4), 323-329. doi:10.3109/09637489609041032

Cheung, I. W. Y., Liceaga, A. M., & Li-Chan, E. C. Y. (2009). Pacific Hake (Merluccius Productus) Hydrolysates as Cryoprotective Agents in Frozen Pacific Cod Fillet Mince. *Journal of Food Science, 74*(8), C588-C594. doi:10.1111/j.1750-3841.2009.01307.x

Choe, J., & Kim, H.-Y. (2019). Quality Characteristics of Reduced Fat Emulsion-Type Chicken Sausages Using Chicken Skin and Wheat Fiber Mixture as Fat Replacer. *Poultry Science, 98*(6), 2662-2669. doi:10.3382/ps/pez016

Das, A. K., Anjaneyulu, A. S. R., Gadekar, Y. P., Singh, R. P., & Pragati, H. (2008). Effect of Full-Fat Soy Paste and Textured Soy Granules on Quality and Shelf-Life of Goat Meat Nuggets in Frozen Storage. *Meat Science, 80*(3), 607-614. doi:10.1016/j.meatsci.2008.02.011

De Oliveira Filho, P. R. C., Viegas, E. M. M., Kamimura, E. S., & Trindade, M. A. (2012). Evaluation of Physicochemical and Sensory Properties of Sausages Made with Washed and Unwashed Mince from Nile Tilapia By-products. *Journal of Aquatic Food Product Technology, 21*(3), 222-237. doi:10.1080/10498850.2011.590270

Decker, E., Faustman, C., & Lopez-Bote, C. J. (2000). *Antioxidants in muscle foods : nutritional strategies to improve quality*. New York: Wiley-Interscience.

Dong, S., Zeng, M., Wang, D., Liu, Z., Zhao, Y., & Yang, H. J. F. c. (2008). Antioxidant and Biochemical Properties of Protein Hydrolysates Prepared from Silver Carp (Hypophthalmichthys molitrix). *107*(4), 1485-1493.

Earl, L. A., King, A. J., Fitzpatrick, D. P., & Cooper, J. E. (1996). A modification of a method to determine expressible moisture in ground, dark poultry meat. *A modification of a method to determine expressible moisture in ground, dark poultry meat., 75*(11), 1433-1436. doi:10.3382/ps.0751433

Elyasi, A., Zakipour Rahim Abadi, E., Sahari, M. A., & Zare, P. (2010). Chemical and Microbial Changes of Fish Fingers Made from Mince and Surimi of Common Carp (Cyprinus carpio L., 1758). *International Food Research Journal, 17*(4), 915-920.

Engle, C. R., & Kouka, P.-J. (1995). Potential Consumer Acceptance of Canned Bighead Carp: A Structural Model Analysis. *Marine Resource Economics, 10*(2), 101-116. doi:10.1086/mre.10.2.42629105

Estimated Fish Consumption Rates for the U.S. Population and Selected Subpopulations (NHANES 2003-2010). (2014). Environmental Protection Agency

Fisheries, N. (2017, 05/10/2017). Fisheries Economics of the United States, 2015. Retrieved from https://www.fisheries.noaa.gov/feature-story/fisheries-economics-united-states-2015

Garvey, J., Ickes, B., & Zigler, S. (2010). Challenges in Merging Fisheries Research and Management: the Upper Mississippi River Experience. *The International Journal of Aquatic Sciences, 640*(1), 125-144. doi:10.1007/s10750-009-0061-x

Hall, G. M. (1997). *Fish Processing Technology* (2nd ed.. ed.). London ; New York: London ; New York : Blackie Academic & Professional.

Hempel, A., Borchert, N., Walsh, H., Roy Choudhury, K., Kerry, J. P., & Papkovsky, D. B. (2011). Analysis of Total Aerobic Viable Counts in Raw Fish by High-Throughput Optical Oxygen Respirometry. *Journal of food protection, 74*(5), 776. doi:10.4315/0362-028X.JFP-10-352

Hwang, K., Kim, H., Song, D., Kim, Y., Ham, Y., Choi, Y., . . . Kim, C. J. (2014). Enhanced Antioxidant Activity of Mugwort Herb and Vitamin C in Combination on Shelf-life of Chicken Nuggets. *Korean J. Food Sci. Anim. Resour., 34*(5), 582-590. doi:10.5851/kosfa.2014.34.5.582

Irons, K. S., Sass, G. G., McClelland, M. A., & Stafford, J. D. (2007). Reduced Condition Factor of Two Native Fish Species Coincident with Invasion of Non-Native Asian Carps in the Illinois River, U.S.A. Is This Evidence for Competition and Reduced Fitness? *Journal of Fish Biology, 71*(supplement sd), 258-273. doi:10.1111/j.1095-8649.2007.01670.x

Jiménez-Martín, E., Pérez-Palacios, T., Carrascal, J., & Rojas, T. (2016). Enrichment of Chicken Nuggets with Microencapsulated Omega-3 Fish Oil: Effect of Frozen Storage Time on Oxidative Stability and Sensory Quality. *Food and Bioprocess Technology, 9*(2), 285-297. doi:10.1007/s11947-015-1621-x

Kim, S.-K. (2014). *Seafood Processing By-Products : Trends and Applications*: New York : Springer.

Landfald, B., Valeur, J., Berstad, A., & Raa, J. (2017). Microbial trimethylamine-N-oxide as a Disease Marker: Something Fishy? *Microbial Ecology in Health and Disease, 28*(1), 1327309.

Luo, Y., Shen, H., Pan, D., & Bu, G. J. F. H. (2008). Gel Properties of Surimi From Silver Carp (Hypophthalmichthys molitrix) as Affected by Heat Treatment and Soy Protein Isolate. *22*(8), 1513-1519.

Malaypally, S. P., Liceaga, A. M., Kim, K.-H., Ferruzzi, M., Martin, F. S., & Goforth, R. R. (2015). Influence of Molecular Weight on Intracellular Antioxidant Activity of Invasive Silver Carp (Hypophthalmichthys molitrix) Protein Hydrolysates. *Journal of Functional Foods, 18*, 1158-1166. doi:10.1016/j.jff.2014.06.011

Maqsood, S., Benjakul, S., & Balange, A. K. (2012). Effect of Tannic Acid and Kiam Wood Extract on Lipid Oxidation and Textural Properties of Fish Emulsion Sausages during Refrigerated Storage. *Food Chemistry, 130*(2), 408-416. doi:10.1016/j.foodchem.2011.07.065

Marinangeli, C. P., Kassis, A. N., & Jones, P. J. (2009). Glycemic Responses and Sensory Characteristics of Whole Yellow Pea Flour Added to Novel Functional Foods. *Journal of Food Science, 74*(9), S385-S389.

Martínez-Ávila, M., Vélez-Ruiz, J. F., & Sosa-Morales, M. E. (2010). Combination of Hot Air Drying and Deep-Fat Frying to Reduce the Oil Content in Chicken Nuggets. *International Journal of Food Science & Technology, 45*(10), 2101-2107. doi:10.1111/j.1365-2621.2010.02390.x

Molly R. Spacapan, C. A. M., Greg G. Sass. (2012). *Has River Use In The Mississippi River Basin Changed Following The Invasion Of Asian Carp?*
Paper presented at the Northeastern Recreation Research Symposium.

Monteiro Starling, F. (1993). Control of Eutrophication by Silver Carp (Hypophthalmichthys molitrix) in the Tropical Paranoá Reservoir (Brasília, Brazil): a Mesocosm Experiment. *The International Journal of Aquatic Sciences, 257*(3), 143-152. doi:10.1007/BF00765007

Morgan, M., & Ho, Y. (2018). Perception of Asian carp as a Possible Food Source Among Missouri Anglers. *Human Dimensions of Wildlife, 23*(5), 491-498. doi:10.1080/10871209.2018.1485990

Mueller, J. P., & Liceaga, A. M. (2014). Characterization and Cryoprotection of Invasive Silver Carp (Hypophthalmicthys molitrix) Protein Hydrolysates. *Journal of Aquatic Food Product Technology, 25*(1). doi:10.1080/10498850.2013.832452

Murchy, K. A., Cupp, A. R., Amberg, J. J., Vetter, B. J., Fredricks, K. T., Gaikowski, M. P., & Mensinger, A. F. (2017). Potential Implications of Acoustic Stimuli as a Non-Physical Barrier to Silver Carp and Bighead Carp. *Fisheries Management and Ecology, 24*(3), 208-216. doi:10.1111/fme.12220

Naseri, M., Rezaei, M., Moieni, S., Hosseni, H., & Eskandari, S. (2010). Effect of different precooking methods on chemical composition and lipid damage of silver carp (Hypophthalmichthys molitrix) muscle. *International Journal of Food Science & Technology, 45*(10), 1973-1979. doi:10.1111/j.1365-2621.2010.02349.x

Official Methods of Analysis of AOAC INTERNATIONAL. (2016). (J. Dr. George W. Latimer Ed. 20th ed. Vol. 1). Rockville, Maryland: AOAC INTERNATIONAL.

Oliveira Filho, P. R. C. d., Fávaro-Trindade, C. S., Trindade, M. A., Balieiro, J. C. d. C., & Viegas, E. M. M. (2010). Quality of Sausage Elaborated Using Minced Nile Tilapia Submmitted to Cold Storage. *Scientia Agricola, 67*(2), 183-190. doi:10.1590/S0103-90162010000200009

Opuszyński, K. (1981). Comparison of the Usefulness of the Silver Carp and the Bighead Carp as Additional Fish in Carp Ponds. *Aquaculture, 25*(2), 223-233. doi:10.1016/0044-8486(81)90184-8

Paker, I., & Matak, K. E. (2015). Impact of Sarcoplasmic Proteins on Texture and Color of Silver Carp and Alaska Pollock Protein Gels. *LWT - Food Science and Technology, 63*(2), 985-991.

Paredi, M. E., De Vido De Mattio, N. A., & Crupkin, M. (1996). Biochemical Properties of Actomyosin and Expressible Moisture of Frozen Stored Striated Adductor Muscles of Aulacomya ater ater (Molina): Effects of Polyphosphates. *Journal of Agricultural and Food Chemistry, 44*(10), 3108-3112. doi:10.1021/jf960113v

Park, J. W. (2013). *Surimi and Surimi Seafood* (Third edition.. ed.): Boca Raton : CRC Press, Taylor & Francis Group.

Parkin, K. L., & Hultin, H. O. (1982). Fish Muscle Microsomes Catalyze the Conversion of Trimethylamine Oxide to Dimethylamine and Formaldehyde. *FEBS Letters, 139*(1), 61-64.

Paulo Roberto Campagnoli de Oliveira, F., Flavia Maria, N., Kazumi Kawazaki, R., Marco Antônio, T., & Elisabete Maria Macedo, V. (2010). Elaboration of Sausage Using Minced Fish of Nile Tilapia Filleting Waste. *Brazilian Archives of Biology and Technology, 53*(6), 1383-1391. doi:10.1590/S1516-89132010000600015

Pérez-Palacios, T., Ruiz-Carrascal, J., Jiménez-Martín, E., Solomando, J. C., & Antequera, T. (2018). Improving the Lipid Profile of Ready-To-Cook Meat Products by Addition of Omega-3 Microcapsules: Effect on Oxidation and Sensory Analysis. *Journal of the Science of Food and Agriculture, 98*(14), 5302-5312. doi:10.1002/jsfa.9069

Perlo, F., Bonato, P., Teira, G., Fabre, R., & Kueider, S. (2006). Physicochemical and Sensory Properties of Chicken Nuggets with Washed Mechanically Deboned Chicken Meat. *Meat Science, 72*(4), 785-788. doi:10.1016/j.meatsci.2005.09.007

Pongruktham, O., Ochs, C., & Hoover, J. J. (2010). Observations of Silver Carp (Hypophthalmichthys molitrix) Planktivory in a Floodplain Lake of the Lower Mississippi River Basin. *Journal of Freshwater Ecology, 25*(1), 85-93. doi:10.1080/02705060.2010.9664361

Prinyawiwatkul, W., McWatters, K. H., Beuchat, L. R., & Phillips, R. D. (1997). Optimizing Acceptability of Chicken Nuggets Containing Fermented Cowpea and Peanut Flours. *Journal of Food Science, 62*(4), 889-905. doi:10.1111/j.1365-2621.1997.tb15480.x

Riaz, M. N. (2006). *Soy Applications in Food*. Boca Raton, Fla.: Boca Raton, Fla. : CRC/Taylor & Francis.

Rodríguez, A., Losada, V., Larraín, M. A., Quitral, V., Vinagre, J., & Aubourg, S. P. (2007). Development of Lipid Changes Related to Quality Loss During the Frozen Storage of Farmed Coho Salmon (Oncorhynchus kisutch. *Journal of the American Oil Chemists' Society, 84*(8), 727-734. doi:10.1007/s11746-007-1098-5

Rosete-Hidalgo, J., Sosa-Morales, M. E., Cenkowski, S., & Vélez-Ruiz, J. F. (2008). *Superheated Steam as Pretreatment to Reduce Oil Uptake During Deep Frying of Chicken Nuggets.* Paper presented at the CSBE/SCGAB 2008 Annual Conference, Vancouver, Canada, July.

Rude, E. (2016). Tastes Like Chicken: A History of America's Favorite Bird. In. Beaverton: Pegasus Books.

Sampson, S., Chick, J., & Pegg, M. (2009). Diet Overlap Among two Asian Carp and Three Native Fishes in Backwater Lakes on the Illinois and Mississippi rivers. *Biological Invasions, 11*(3), 483-496. doi:10.1007/s10530-008-9265-7

Sass, G., Cook, T., Irons, K., McClelland, M., Michaels, N., Matthew O'Hara, T., & Stroub, M. (2010). A Mark-Recapture Population Estimate for Invasive Silver Carp (Hypophthalmichthys molitrix) in the La Grange Reach, Illinois River. *Biological Invasions, 12*(3), 433-436. doi:10.1007/s10530-009-9462-z

Shi, C., Cui, J., Luo, Y., Zhu, S., & Zhou, Z. (2015). Post-Mortem Changes of Silver Carp (Hypophthalmichthys Molitrix) Stored at 0°C Assessed by Electrical Conductivity. *International Journal of Food Properties, 18*(2), 415-425. doi:10.1080/10942912.2013.833220

Shi, J., Luo, Y., Shen, H., & Li, Z. J. J. o. a. f. p. t. (2014). Gel Properties of Surimi from Silver Carp (Hypophthalmichthys molitrix): Effects of Whey Protein Concentrate, CaCl2, and Setting Condition. *23*(5), 489-497.

Shiku, Y., Hamaguchi, P. Y., & Tanaka, M. (2003). Effect of pH on the Preparation of Edible Films Based on Fish Myofibrillar Proteins. *Fisheries Science, 69*(5), 1026-1032.

Siddaiah, D., Reddy, G. V. S., Raju, C., & Chandrasekhar, T. (2001). Changes in Lipids, Proteins and Kamaboko Forming Ability of Silver Carp (Hypophthalmichthys molitrix) Mince During Frozen Storage. *Food Research International, 34*(1), 47-53.

Sirtori, E., Isak, I., Resta, D., Boschin, G., & Arnoldi, A. (2012). Mechanical and Thermal Processing Effects on Protein Integrity and Peptide Fingerprint of Pea Protein Isolate. *Food Chemistry, 134*(1), 113-121.

Stone, H. (2012). *Sensory Evaluation Practices* (4th ed. ed.). Burlington: Elsevier Science.

Tellez-Luis, S. J., Uresti, R. M., Ramirez, J. A., & Vazquez, M. (2002). Low-salt Restructured Fish Products Using Microbial Rransglutaminase as Binding Agent. *Journal of the Science of Food and Agriculture, 82*(9), 953-959.

Thomson, S. P., Liceaga, A. M., Applegate, B. M., & Martyn, R. D. (2015). Analysis of Seed Vigor Responses in Soybean to Invasive Silver Carp (Hypophthalmichthys molitrix) Protein Hydrolysate Treatments. *American Journal of Experimental Agriculture, 5*(3), 178-191. doi:10.9734/AJEA/2015/13087

Tokur, B., Ozkütük, S., Atici, E., Ozyurt, G., & Ozyurt, C. E. (2006). Chemical and Sensory Quality Changes of Fish Fingers, Made from Mirror Carp (Cyprinus carpio L., 1758), During Frozen Storage (− 18 C). *Food Chemistry, 99*(2), 335-341.

Trindade, M. A., King, J. L., & Liceaga, A. M. (2019). Production and Evaluation of Mexican-Style Chorizo Sausage Using Invasive Silver Carp (Hypophthalmichthys molitrix) Meat. *Journal of Aquatic Food Product Technology, 28*(5), 531-540. doi:10.1080/10498850.2019.1604600

Varble, S., & Secchi, S. (2013). Human Consumption as an Invasive Species Management Strategy. A Preliminary Assessment of the Marketing Potential of invasive Asian Carp in the US. *Appetite, 65*(C), 58-67. doi:10.1016/j.appet.2013.01.022

Venugopal, V., & Shahidi, F. (1995). Value-Added Products from Underutilized Fish Species. *Critical reviews in food science and nutrition, 35*(5), 431. doi:10.1080/10408399509527708

Vetter, B. J. (2017). Characterization and Management Implications of Silver Carp (Hypophthalmichthys molitrix) Jumping Behavior in Response to Motorized Watercraft. *Management of Biological Invasions, 8*(1), 113-125. doi:10.3391/mbi.2017.8.1.11

Vetter, B. J., Cupp, A. R., Fredricks, K. T., Gaikowski, M. P., & Mensinger, A. F. (2015). Acoustical Deterrence of Silver Carp (Hypophthalmichthys molitrix). *Biological Invasions, 17*(12), 3383-3392. doi:10.1007/s10530-015-0964-6

Wenjiao, F., Yongkui, Z., Yunchuan, C., Junxiu, S., & Yuwen, Y. (2014). TBARS Predictive Models of Pork Sausages Stored at Different Temperatures. *Meat Science, 96*(1), 1-4.

Xu, Y., Xia, W., Yang, F., Kim, J. M., & Nie, X. (2010). Effect of Fermentation Temperature on the Microbial and Physicochemical Properties of Silver Carp Sausages Inoculated with Pediococcus Pentosaceus. *Food Chemistry, 118*(3), 512-518. doi:10.1016/j.foodchem.2009.05.008