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Proximate Composition, Retained Water, and Bacterial Load for Two Sizes of Hybrid Catfish (Ictalurus Furcatus X Ictalurus Punctatus) Fillets at Different Process Steps

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Proximate composition, retained water, and bacterial load for two sizes of hybrid catfish (*Ictalurus furcatus* x *Ictalurus punctatus*) fillets at different process steps

By

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The overall baseline (as received) moisture, protein and fat content of hybrid catfish (*Ictalurus furcatus* × *Ictalurus punctatus*) fillets were 77.8 ±1.38%, 16.7±0.50% and, 5.7±1.6%, respectively. Small fillets (111±19 g) had higher ($P \leq 0.05$) baseline moisture (78.6±0.87% vs 76.8±1.15%) and lower (P≤0.05) fat content (4.7±0.64% vs 6.8±1.72%) than large fillets (247±62 g), whereas protein content was similar ($P > 0.05$) for both sizes. Retained water of the final fresh and frozen fillets was 1.2±2.03% and 3.1±1.02%, respectively, irrespective of fillet size. Psychrotrophic (PPC) and total coliform plate counts (TCC) of the baseline fillets were ~4 log CFU/g and 1.6 log CFU/g, respectively and were not different between the process steps, except after injection which were higher (P>0.05) than baseline. Moisture-protein ratio and fat content were good ($P \leq 0.05$) predictors for retained water in catfish fillets during processing.

DEDICATION

I would like to dedicate this manuscript to my revered parents, Mr. Shamsuddin Ahmed and Mrs. Hasna Hena. Their unconditional love and support are my motivation and inspiration.

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CHAPTER I

INTRODUCTION

Catfish (*Ictalurus punctatus*) is the most prevalent aquaculture species in the United States, accounting for over 60% of all US aquaculture production. This is due to their high fecundity, artificial spawning, adaptability to earthen ponds for culture, high tolerance to low dissolved oxygen, high resistance against infectious diseases, and high feed conversion efficiency (Jin et al., 2016; Hargreaves and Tucker, 2004). Farm-raised channel catfish (*Ictalurus punctatus*) aquaculture was initiated in the 1960s along the Mississippi delta area (Mack 1971) and currently, 94% of all U.S. farm-raised catfish is cultured in Mississippi, Arkansas, Texas, and Alabama (USDA-NASS, 2018; Liu, 2011). The water surface used for catfish production in the United States was about 25 thousand hectares in 2016 (USDA-NASS, 2018). In 2015, per capita consumption of catfish in the United States was 0.24 kg (NFI, 2018). In the interest of increasing the efficiency of catfish production, female channel catfish (*Ictalurus punctatus*) was crossed with male blue (*Ictalurus furcatus*) catfish to produce hybrid offspring (Li et al., 2004). The hybrid catfish [Blue (*Ictalurus furcatus*) × channel (*Ictalurus punctatus*)] are now grown more often than channel catfish for their faster growth, greater feed conversion efficiency, resistance to major bacterial diseases, and moreover, greater fillet yield during processing (Dunham and Masser, 2012).

The farm-raised catfish industry in the United States employed approximately 10,000 people, which contributed around $4 billion to the US economy each year from

2010 to 2016 (USDA-NASS, 2018; TCI, 2018). Catfish growers sold approximately 96.4% of food-size (weighted 0.3 to 1.5 kg) catfish directly to processors in 2016 (USDA-NASS, 2018). There are currently 16 "The Catfish Institute" (TCI) "certified" catfish processing plants in the USA with a maximum process capacity of 4.5 million kg per week (TCI, 2018). Processed farmed raised catfish production in the US amounted to 13.6 million kg in 2013 (USDA-NASS, 2014). Fresh catfish (fish intended for immediate consumption, also referred to as ice-packed) accounted for approximately 36% of total sales during 2013. Fillets (deboned sides of the fish, includes regular, shank, and strip fillets; excludes any breaded products) accounted for 60%, whole fish (fish with no processing done or viscera only removed; only head, viscera, and skin removed), 20%, and the remaining 20% were mostly steaks (cross-section cuts from larger dressed fish), nuggets (small fillets cuts from below the rib section of the fish and usually includes weight of breading and added ingredients), and value-added products (USDA-NASS, 2014).

The proximate composition of catfish (*Ictalurus punctatus*) includes moisture (70 to 80%), protein (14 to 19%), fat (2 to 11%) and ash (1 to 2%) (Robinson and Oberle, 2001). Moisture content is an important measure of seafood quality, as the flesh naturally has a high-water content. Moisture content also has the functional relationship with protein, fat, and glycogen of the muscle (Ward, 1963). An inverse correlation between fat and moisture content of fish was reported in several studies (Linhartová et al., 2018; Karl et al., 2018; Yeannes & Almandos, 2003). Water-related adulteration (added water by immersion chilling during processing) of seafood could be determined using moisture-protein ratio as protein content usually remained similar with the process steps (Breck, 2014; Yennes et al., 2003; Botta and Cahil, 1992). This relationship could be worthwhile approximating fat

or protein content based on the estimation of moisture content of fish (Lupin, 1980). Thus, it is important to discern the natural moisture content of the catfish fillets as received in the processing plant and its relationship to the protein and fat content. However, proximate composition of fish differs from species to species, individual to individual considering size, sex, season, feeding habit and processing stress (Emre et al., 2015; FAO, 2016; Huss, 1988; 1995).

Indicator bacterial counts (aerobic plate counts, psychrotrophic counts, total coliform counts and *E. coli*) could reveal temperature abuse, cross contamination, and mishandling during fish processing (Huss, 1995; Gould, 1990). The maximum acceptable limits of Aerobic plate counts (APC) at 20 to 25^0 C and *E. coli* in the fresh and frozen fish are 5.7 log CFU/g and 1.0 log CFU/g, respectively specified by ICMSF (International Commission on Microbiological Specifications for Foods) (Gould, 1990). Fish with microbiological load exceeding these limits are considered as spoiled or unacceptable. However, Watchalotone et al. (2001) suggested that psychrotrophic counts (PPC) and Total Coliform counts (TCC) of the catfish fillets during processing should be <3-4 log CFU/g and <2 log CFU/g), respectively. Initial microbial load (at receiving in the processing plants) of the fish, temperature abuse and cross-contamination during fish handling and storing, dictate the quantity of final fish products' bacterial load during processing (Nunez, 1995; Fapohunda et al., 1994; Huang and Leung, 1993; Mayer & Ward, 1991). Bacterial counts (APC, PPC, TCC and *E.coli*) of catfish differ for different harvesting season, size of the processing plant, and processing methods (Marroquin et al., 2004; Fernandes et al., 1997). Previous studies reported bacterial load of channel catfish (*Ictalurus punctatus*) fillets for different season, different sizes of the processing plants and different methods of

the processing (Marroquin et al., 2004; Fernandes et al., 1997; Nunez, 1995; Watchalotone et al., 2001). However, bacterial load (PPC, TCC and *E. coli*) of hybrid catfish fillets at each process steps has not been reported yet.

The USDA-Food Safety Inspection Service (FSIS) is now inspecting Siluriformes (the scientific order which contains all families of catfish) including both channel and hybrid catfish from September 2017 with full enforcement (USDA, 2017). The agency adopted existing meat and poultry net weight and retained water (water that remains in the raw product after it undergoes immersion chilling or a similar process) regulations (9 CFR Parts 381 and 441) without changes for labeling the net weight and retained water of Siluriformes products (USDA, 2001). Fresh or fresh-frozen packages of catfish or parts must be labeled to reflect 100% net weight after thawing. The processor is required to state the maximum percentage of retained water on the product label (USDA, 2015).

Several studies (Bigbee and Dawson, 1963; Young & Smith, 2004; James et al., 2006; Jeong et al., 2011) of poultry processing reported that poultry carcass retained 4 to 11% water after immersion chilling. The amount of water absorption of poultry carcass depends on water temperature, hydrostatic pressure, water stirring conditions and immersion time during chilling/cooling (Carciofi & Laurindo, 2007). Some studies (James et al., 2006; Carciofi & Laurindo, 2007) also established models for the prediction of retained water of poultry carcass during processing. It is also essential for the catfish industry to identify the main variables that affect the water uptake or loss of catfish products during processing. This might improve the process control of the catfish. The natural composition (moisture) of catfish products prior to and during processing can provide information to both processors and inspection authorities with respect to regulatory

compliance and labeling requirements. Surprisingly, no reports are available surveying the proximate composition and actual contents of retained water of catfish fillets during processing. There is no officially approved Near-Infrared (NIR) method for the determination of proximate composition of fish and fish products although NIR spectroscopy is faster, noninvasive and more economical in comparison to other conventional methods (Hirose et al., 2016; Xiccato et al., 2004). A prediction model could be established using NIR spectroscopic data of proximate composition to predict the retained water of catfish products at a fast space (Khodabux et al., 2007; Majolini et al., 2009).

The objectives of the study were:

i. To determine proximate composition and retained water of the two sizes (small= 50 to 150 g; large=150 to 450 g) of hybrid catfish (*Ictalurus furcatus* × *Ictalurus punctatus*) fillets as received (baseline) and at different process steps,

ii. To determine the microbial load of the two sizes (small= 50 to 150 g; large= >150 to 450 g) of hybrid catfish (*Ictalurus furcatus* × *Ictalurus punctatus*) fillets at different process steps, and

iii. Establish models for the prediction of retained water of the processed hybrid catfish (*Ictalurus furcatus* × *Ictalurus punctatus*) fillets.

CHAPTER II

LITERATURE REVIEW

2.1 Moisture and proximate composition of aquaculture finfish and factors that affect it

Fish flesh composition includes water (66-81%), protein (16-21%), carbohydrates (<0.5%), lipids (0.2-25%) and ash (1.2 to 1.5%) (FAO, 2016). Muscle of fish also contains essential amino acids (Hatae et al., 1990), micronutrients (Luten et al., 2008 and McManus and Newton, 2011) and essential fatty acids (omega-3 and omega-6) (Gjedrem et al., 2012).

The proximate composition of fish may differ from species to species, individual to individual considering age, sex, environment and season (Emre et al., 2015; FAO, 2016; Huss, 1988; 1995). The proximate composition varies due to spawning season, nutrition , fishing ground and the movement pattern of fish (Shearer, 1994; Stansby, 1976).

Linhartová et al. (2018) reported that moisture, protein and fat composition varied due to the different culture systems, species and size of the fish. They analyzed the proximate composition of thirteen commercially important freshwater fish (African catfish, rainbow trout, Wels catfish, Nile tilapia, brook trout, northern whitefish, pikeperch, common carp, northern pike, grass carp, European perch, trench, silver carp) from different culture systems (Intensive, semi-intensive, extensive) in Czech Republic (Table 2.1).

Boran & Karaçam (2011) reported that protein and fat content of the fish flesh (goldel mullet, horse mackerel) increased during heavy feeding periods but decreased during the shortage of food and starvation. This is because fish utilize reserved lipids and occasionally protein as an energy source for the synthesis of Adenosine triphosphate (ATP) during starvation (Huss, 1988; 1995). Hirano et al. (1980) reported that protein content of fish flesh decreased from summer to autumn. Protein content were not different between farmed and wild fish in their study. Karl et al. (2018) reported that protein content was not different (18–19%) in different areas (anterior ventral/dorsal, medial dorsal/ventral and posterior dorsal/ventral) of the reported fish fillets (Table 3.1). They also established an inverse correlation between water and fat content. Shearer (1994) reported an inverse relationship between body weight and moisture content, a direct relationship between lipid and protein where protein and lipid typically increased within the increase of body weight of fish.

Manthey-Karl et al. (2016) reported that skinning and trimming technique reduced the lipid content of the fillets during processing of pangasius (*Pangasius hypophthalmus*). Kristoffersen et al. (2007) reported the loss of weight and protein content of the fish during subsequent storage due to pre-rigor filleting.

Among all proximate components, fat content varies in greatest extent in all fish (Stansby, 1976). A negative inverse correlation was reported between fat and moisture content for several species of fish flesh (Linhartová et al., 2018; Karl et al., 2018; Yeannes & Almandos, 2003). This relationship may be worthwhile in approximating moisture or fat content of fish (Lupin, 1980). Moisture, protein, fat and ash content of selected finfish are reported in Appendix Table B.1.

2.2 Siluriformes including catfish

Siluriformes is one of the largest orders of teleost. They represent about 12% of all teleost and 6.3% of all vertebrate fish species (Eschmeyer and Fong, 2014; Wilson and Reeder, 2005). Catfish are highly diverse and distributed worldwide and most abundantly distributed in the tropics of South America, Africa Asia, North America and in Europe (Lundberg and Friel, 2003).

The US Government's Interagency Taxonomic Information System (ITIS) defines *Siluriformes* order as "catfishes and silures". This order comprises 36 Families, 22 subfamilies, 447 Genus, 2970 Species and 2 subspecies (ITIS, 2017). The Siluriformes order comprises the *Ictaluridae* family that includes channel catfish (*Ictalurus punctatus*), blue catfish (*Ictalurus furcatus*), and the flathead catfish (*Pylodictis olivaris*). Other species include white catfish (*Ameiurus catus*), black (*Ameiuru melas*), brown (*Ameiuru nebulosus*) and yellow bullhead (*Ameiuru natalis*) (ITIS, 2017). Another family of the Siluriformes order includes Pangasiidae (the giant catfishes) that comprises the species basa (*Pangasius bocourti*), tra (*Pangasius. hypophthalmus*,) or swai (*Pangasius* sutchi) These Pangasiidae are commercially farmed and raised in Southeast Asia for both export and domestic consumption. Other farm-raised catfish in this region includes hybrid *Clarias macrocephalus* and channel catfish (*Ictalurus punctatus*) (ITIS, 2017).

USDA-FSIS regulates labeling to use the term ``catfish'' only to the species comprises the *Ictaluridae* family. Siluriformes fish, rather than Ictaluridae, need to be labeled with the appropriate common or usual name. (USDA, 2015). In the United States, channel *Ictalurus punctatus*), blue (*Ictalurus furcatus)* and their hybrid (*Ictalurus*

furcatus×*Ictalurus punctatus*) catfish are the most commercially important species (NASS, 2017). The hybrid catfish species yield higher fillet percentage compare to blue and channel catfish (Argue et al., 2003).

2.3 Proximate composition of Siluriformes including channel and hybrid catfish

Siluriformes (several species: *Clarias gariepinus Ictalurus punctatus, Pseudoplatystoma fasciatum, Pseudoplatystoma corruscans, Pangasius gigas, Pangasianodon hypophthalmus, and Rhamdia quelen*) flesh composed of 74 to 85% of moisture, 12 to 22% of protein, 0.4 to 5.7% of lipid, and 0.8 to 2% of ash. Fish flesh generally comprises of 66 to 81% moisture, 16 to 21% protein, 0.2 to 2.5% lipid, and 1.2 to 1.5% ash content (Casallas et al., 2012) (Table 2.2). Catfish (*Ictalurus punctatus*) contained several fatty acids: saturated fatty acids (23.2±0.37 % of total fatty acid content), monounsaturated fatty acids (46.8±1.56%), polyunsaturated fatty acids (6.3±0.78), omega-6 fatty acids (18.6±0.45), omega-3 fatty acid (2.7±0.55), eicosapentaenoic acids (1.2±0.1) and docosahexaenoic acids (2.0±0.2) (Li et al., 2009). Catfish (*Clarias gariepinus*) flesh also contains several minerals: potassium (1.8 ± 132.4 mg/kg), sodium (308 ± 0.35 mg/kg), magnesium (184 ± 18.5 mg/ kg) and calcium (40.1 ± 0.08 mg kg-1) (Ersoy & Özeren, 2009).

Olaniyi et al. (2017) reported that moisture content of whole *Clarias gariepinus, Heterobranchus bidorsalis*, and their hybrids (*Clarias gariepinus* × *Heterobranchus bidorsalis*) was 73.7±2.02%, 76.3±12.7%, and 77.3±6.03% respectively. The moisture content was different between parent species and the hybrids. Guimarães et al. (2016) reported 83.8 to 85.6% of moisture, 12.5 to 14.5% of protein, 1.1 to1.7% of lipid, and 0.8

to 2.4% of ash content for Vietnamese frozen catfish (*Pangasius hypophthalmus)* fillet. Pongpet et al. (2015) and Orban et al. (2008) reported similar ranges of moisture, protein and fat content for *Pangasianodon hypophthalmus* and *Pangasius bocourti* fillets. Karl et al. (2010) also reported similar moisture and protein but lower fat content (1.4 to 3.2%) for farmed raised *Pangasius* fillets. Mushahida et al. (2012) reported 74.1 to 79.15% of moisture, 15.50 to 16.60% of protein, 4.08 to 8.08% of lipid, and 1.20–1.24% of ash conten for *Pangasius hypophthalamus* fillets.

Robinson and Oberle (2001) studied large sizes (440 to 1098 g) of channel catfish (*Ictalurus punctatus*) fillet at three seasons (May and October 1998 and February 1999). They stated the overall crude protein (16.3±0.4 % with a range of 14.1% to 18.7%), fat (5.4±0.3% with a range of 1.9% to 10.9%) and moisture (77.3±0.4 % with a range of 70.9% to 80.4%) were not different for reported seasons. Tidwell & Robinette (1990) reported that moisture (81.4%), protein (14.0%) and fillet lipid (overall means 1.8%), were not different among blue, channel and hybrid catfish. Proximate composition of channel catfish varied for different sizes of fillet (Tidwell & Robinette 1990; Robinson & Robinette 1994). Silva and Ammerman (1993) reported that moisture content was higher for small, whole and dressed frozen channel catfish fillet (70.8% vs 68.1%) but fat content was lower (10.8% vs 13.2%) than larger fillet. Protein content (17.1% vs 17.0) was similar for two sizes of the fillet. Nettleton (1990) reported average moisture, protein and fat content of the channel catfish fillet were 76.4 %, 15.6% and 6.9%, respectively at four seasons (Fall, winter, spring, and summer). Moisture content (74.4%, 77.4%, 77.8%, and 76.0% in the fall, winter, spring and summer respectively) of the fillet was higher in the winter and spring season and lower in the fall and summer season.

Bosworth et al. (1998) reported average moisture, protein and fat content of the juvenile hybrid catfish [Blue (*Ictalurus furcatus)* × Channel *(Ictalurus punctatus*)] was 80.2%, 15% and 2.4%, respectively. Li et al. (2007) reported 73.2 % moisture, 17.3% protein and 8.59% fat content for marketable size (680 to 1150 g) hybrid catfish flesh. Bosworth et al. (2001) reported that whole hybrid catfish had lower moisture (71.4±1.02% with a range of 69.5 to 73.6% vs 77.7±2.12% with a range of 73.7 to 80.9%) but higher fat content (11±1.44% with a range of 9.2 to 14.2% vs 6.9±1.70 with a range of 4.6 to 14.2%) in comparison to fillets.

Proximate composition of selected Siluriformes, channel and hybrid catfish are reported in Appendix Table B.2 and Appendix Table B.3, respectively.

2.4 Catfish Processing

Processed catfish products include eviscerated whole fish, eviscerated dressed fish, fillets (with or without belly flap), shank fillets, fillet strips (with belly flap), nuggets (belly flap), and steaks (Silva and Dean, 2001). These products are usually sold as either iced, frozen, battered and breaded or fresh (Ammerman, 1985; Silva et al., 2001). Sales of fresh catfish in the United States accounted for 36% of total sales in 2013 (USDA-NASS, 2014). Catfish processing consists of holding, stunning, deheading, skinning, eviscerating, filleting, grading, chilling/freezing, packaging and storing procedures (Ammerman 1985). Silva et al. (2001) reported the following steps in the automatic processing line of channel catfish (*Ictalurus punctatus)* : receiving the live fish at the processing plant premises, holding the fish in the transporting truck tank, stunning (stun the fish by a low voltage alternative electric current to render the fish less dangerous to workers and easily handled

in further operations), deheading (remove the head of fish from the carcass by a band saw or other means of deheaders), eviscerating (draw the viscera from the body through the opening of body cavity), skinning (separate the skin from the flesh manually or mechanically, chilling (immersed in a mixture of ice and water or cold water less than 5^0C), size grading (manually or electronically based on weight and size), injecting (catfish products are injected with polyphosphate solution) before freezing, freezing or ice packing (Individual Quick Freezing where temperature is below 9^0C) , packaging (coating of ice glazed over the fish), ware- housing, icing, and shipping the finished product.

2.5 Microbiology of Catfish

Bacteria are naturally found in the outer slime/skin (ranges 10^2- 10^7CFUu/g), gills and the intestine (up to 10^8CFUu/g) of fish (Jay, 1990) but a natural defensive system protect flesh free from bacteria. Temperate fish possess mostly psychrophilic bacterial species (Shewan, 1977), whereas, in tropical fish, the predominant bacterial species are mesophilic (Huss, 1995). Several bacterial florae in processed fish have been isolated and reported in previous studies (Fernandes et al., 1997; Kim et al., 2000; Nunez et al., 2003; Marroquin et al., 2004). In fish processing plants, several factors such as temperature abuse (Mayer & Ward, 1991) during fish handling and storage, cross contamination (Fapohunda et al., 1994), and fish cultural environment (Huang and Leung, 1993), dictate the microbiological load in the final product. Bacterial counts on catfish also differ with the season, size of the processing plant (Fernandes et al., 1997), and processing methods (Marroquin et al., 2004). The microorganisms found in the catfish are typically spoilage indicator bacteria such as aerobic (Andrews et al., 1977; Kim et al., 1995; Fernandes et al., 1997), psychrotrophic (Andrews et al., 1977; Huang and Leung, 1993), *Escherichia coli,*

total coliform, and *Staphylococcus aureus* (Fernandes et al., 1997) which postulate an understanding into the microbiological quality of the processed catfish products. Fernandes et al., (1997) reported significant quantitative differences in the aerobic, psychrotrophic, total coliform, *E. coli*, and *S. aureus* counts in catfish fillet due to temperature variation during production and differences in processing protocols of different processing plants. They reported that catfish fillets which were collected in summer had higher counts of *E. coli* and *S. aureus* in comparison to fillets collected in winter.

Huang and Leung (1993) reported that psychrotrophic bacterial counts was 2.8 to 3 log CFU/ml in whole, deheaded, eviscerated, and skinned aquacultured channel catfish and fecal coliform counts was 1.48 log CFU/ml in deheaded and eviscerated catfish and less than 1.0 log CFU/ml in skinned channel catfish which were harvested from southern Georgia during spring season. Martin and Hearnsberger (1994) estimated psychrotrophic counts of catfish fillets ranging from 10^4 to 10^7 CFU/g. Watchalotone et al. (2001) stated that total coliform counts should not be over 2 log CFU/g, and psychrotrophic counts should be within 3-4 log CFU/g for good quality catfish products.

Nunez et al. (1995) conducted a study on channel catfish product, contact equipment, and personal utensils, from the receiving point to the packaging end in three different catfish processing plants during fall, winter, and spring. The highest aerobic plate counts (APC) and psychrotrophic plate counts (PPC) were reported in the evisceration place ($\geq$ 5 log CFU/cm^2) and lowest in the skinned/dressed channel catfish fish fillets (~2.63 log CFU/cm^2). TCC were greater in fish processed in the spring (0.8 log CFU/cm^2) than those processed in the fall or winter. However, Watchalotone et al. (2001) found no effect of different processing flows on the microbial load for channel catfish fillets. They

isolated *Acinetobacter, Flavobacterium, Aeromonas, Pseudomonas, Pasteurella, Agrobacterium, Plesiomonas, Oligella, Weeksella, Alcaligenes, Staphylococcu,* and *Stomatococcus* from the channel catfish fillet processed in five different ways during the fall season.

The predominant microorganisms in the catfish fillets, processing equipments, and environments were reported by several authors. Andrews et al. (1977) reported that APC in the fresh (93.0%) and frozen (94.5%) channel catfish samples were 7 log CFU/g whereas, fecal coliform MPN counts in the 70.7% of the fresh and 92.4% of the frozen samples were 4 log CFU/g. The prevalent bacteria found in the catfish processing plant were *Acinetobacter, Aeromonas, Pseudomonas, Flavobacterium, Alcaligenes, Moraxella, Xanthomonas, Sphingobacterium, Pasteurella, Weeksella, Comamonas, Micrococcus, Staphylococcus, and Flavimonas* (Kim et al., 2000). Chen et al. (2010) isolated *Listeria monocytogenes (*21.6%*), Listeria innocua (*13.0%*) and a group of Listeria seeligeri, Listeria welshimeri and Listeria ivanovii* (29.5%,) from fresh catfish fillets, different food contact surfaces (deheading machine, trimming board, chiller water, conveyor belts at different stages, and fillet weighing table) and non-food contact surfaces. In their study, 76.7% of *L. monocytogenes* was isolated from chilled fresh catfish fillets and 43.3% from unchilled fillets. However, no *L. monocytogenes* strains were isolated from catfish skins or intestines in this study.

Fernandes et al. (1997) isolated *Campylobacter jejuni/coli, Escherichia coli OI57:H7, Klebsiella pneumoniae subsp. pneumoniae, Plesiomonas shigelloides, and Vibrio cholerae* from catfish fillets processed in several processing plants in the southeastern United States during four annual seasons (summer, fall, winter, and spring).

The reported average PPC in the small, medium and large size catfish processing plant were 4, 5.8, and 6.1 log CFU/g, respectively during summer, 4.2, 5 and 4 log CFU/g, respectively during spring, 4.6, 5.4 and 5 log CFU/g, respectively during fall and, 3.1, 5.3 and 4.7 log CFU/g, respectively during winter. C*ampylobactor jejuni/coli, E. coli 0157 :H7* and *K. pneumoniae subsp. Pneumoniae* were not detected in the catfish processing plant in their study.

2.6 Retained water/moisture of muscle food in the process steps

Process step such as immersion in the chiller water (at 4ºC) of poultry carcass is a common practice in the poultry processing industry. The main purpose of chilling is to reduce carcass temperature below the minimum growth temperature of most foodborne pathogens and spoilage microorganisms (Thompson et al., 1975; James et al., 2006). Poultry carcasses retain water during the immersion process that passthrough the intercellular spaces of the muscle at rigor mortis. In 2001, the USDA-FSIS restricted the moisture retention in post eviscerated poultry. The regulations required processors to provide documentation of retained water of chilled poultry carcass and parts of it. Processors should reveal the amount of water on the label due to any processing. USDA-FSIS also stated that "retained moisture should be documented to provide consumers with the information necessary to make adequate purchase decisions" (USDA, 2001**).**

The amount of water absorption of poultry carcass during chilling depends on water temperature, hydrostatic pressure, water stirring conditions and immersion time (Carciofi & Laurindo, 2007). Among these variables, immersion time has the most influence on water uptake by poultry carcass. Water absorption differed for different sizes of the poultry carcass. Smaller carcasses absorbed more water than larger ones (Young & Smith, 2004).

James et al. (2006) reported that immersion time and water agitation intensity regulate the water uptake by poultry carcasses.

Different chilling practices such as water-chillling, evaporative air chillling and water spray chilling also impacted the poultry carcass water uptake/losses throughout the process. Young & Smith (2004) observed that the water-chilled poultry carcasses absorbed 11.7% moisture in chilling but retained 7.0% through precutting storage, 6.0% through cutting and 3.9% through post-cutting storage. Jeong et al. (2011) reported both water chilled, and evaporative air-chilled poultry carcasses gained up to 4.6 and 1.0% of their weights respectively, whereas, air chilled carcasses lost 1.5% of their weight.

James et al. (2006) reviewed the influence of chilling process on product safety (microbiology), product quality (flavor, appearance and texture), and the chilling parameters (operating costs, weight loss and chilling time) and chilling methods (immersion, spray/evaporative, air and deep/super chilling). They reported that poultry carcasses lost 1 to 3% of their body during air chilling process but gained 2% during water spray chilling, 4 to 8% during immersion chilling, 12% during slush ice immersion for 30 minutes. Huezo et al. (2007) reported that poultry carcass lost 2 to 4% of their body weight during 150 min air chilling but retained 3.4 to 14.7% of water during immersion chilling.

However, moisture absorption by the poultry carcasses differed for ice-water ratio in the chiller. About 35% of ice (in relation to the water mass in the chiller) contributed the highest weight gain (12%) of the poultry carcass in comparison to lower ice-water ratio.

Savell et al. (2005) reported that chilling systems particularly cooling time also affect pork meat quality (tenderness, color, and shrinkage). Rapid cooling affected carcass

by cold-induced shortening and toughening but delayed chilling exhibited positive influence on postmortem tenderness of the pork meat.

2.7 Models to calculate/predict water/moisture uptake/loss

Few studies have investigated and modeled poultry carcass water retention (James et al., 2006; Carciofi & Laurindo, 2007). Non-linearity, the influence of many variables and parameters hampered the analytical solution of convoluted models; however, simple mathematical models exclude some important aspects that influence the process (Carciofi & Lurindo, 2007).

Martins et al, (2011) developed a model using Artificial Neural Networks (ANNs) to predict the water uptake of the chicken carcass during immersion chilling. ANNs are the mathematical algorithms that have the capacity to relate input (independent variables) and output parameters (dependent variables) learning from given examples, without requesting any knowledge about the variables relation that interferes on the studied process (Hornik et al.,1989). In their study, water retention by the poultry carcasses in the chilling process was modeled using several ANN structures with one hidden layer, besides the input and output layers. They considered ambient temperature, ice quantity in three chillers, air bubbling intensity in three chillers, slaughter speed, water temperature at the exit and entrance of chillers, ice quantity in chiller, residence time in chillers, renewal water flow in chiller, scalding temperature, jacket temperature in chillers, initial carcass mass and initial carcass temperature for modeling of the retained water. The correlation coefficient (r^2) in this model ranged from 0.65 to 0.87 with the same neuron numbers in several layers.

Khodabux (2007) established regression models for the prediction of moisture, protein and fat determined by NIR based on reference method (combustion, oven dry and lyophilization) in skipjack (*Katsuwonus pelamis*) and yellow fin (*Thu nus albacares)* tuna. Coefficient (R^2) of the prediction values against reference values of the constructed models were 0.98, 0.99, 0.95 and 0.96 for moisture, protein, total fat and free fat, respectively. Breck (2014) reported a strong inverse relationship between water and protein mass of bluegill, common carp, trout, and salmon. Botta and Cahill (1992) used moisture-protein ratio for the determination of added water of the scallop meat.

2.8 Determination methods of moisture and proximate composition in muscle foods/fish and factors that affect it

Precise determination of the proximate composition of muscle foods is necessary as moisture content affects the stability, inherent quality, processing potential and retail value of the products. Water content also has the functional relationship with proteins, fat, and glycogen of the muscle (Ward,1963). Several conventional moisture determination methods along with near-infrared technology (NIR) are readily used but most broadly used methods involve thermal drying because of the minimum loss of other volatile components during heating (Woyewoda et al., 1996). Windsor (1981) suggested convection type oven for the uniform distribution of the heat in all samples. Uneven heat distribution can be minimized by altering heating element placement. Woyewoda et el. (1996) suggested using a small number of samples (spread thinly) to minimize the effect of crusting (trap moisture).

Moisture determination methods of muscle food also vary due to the form of the water present in a food (Nielsen, 2010). The tightly bound water in fresh fish muscle cannot

readily be expelled even under high pressure. Physically or chemically bound water takes on varying physicochemical properties, making it very challenging to accurately measure the moisture content. So, official methods (AOAC 950.46,1990) and procedures are important for moisture determination.

Previous studies (Manthey-Karl et al., 2016; Olaniyi et al., 2017; Karl et al., 2018) used gravimetric method (AOAC 950.46,1990) for the determination of moisture content of fish. Sample collection and homogenizations processes are analogous in all methods except drying time in the oven.

Karl et al. (2018) followed gravimetric method for moisture determination of redfish (*Sebastes mentella*) and Greenland halibut (*Reinhardtius hippoglossoides*). They dried the homogenate for 12 h at 105 °C. Similarly, Manthey-Karl et al. (2016), determined percent moisture of *Pangasius* by drying at 105 ◦C for 12 h to a constant weight taking 5 g of homogenate. Other studies (Chijan et al., 2010; Boran and Karaçam, 2011; Njinkoue *et al.*, 2016) also followed gravitational method by oven drying the homogenized samples at 105 ± 2^0 until a constant weight was obtained to determine the moisture content of catfish (*Pangasianodon gigas)* and marine fish. Guimarães et al. (2016) determined moisture of Vietnamese *Pangasius hypophthalmus* fillets by using a drying oven at 100–105°C until constant weight was obtained. However, Olaniyi et al. (2017) estimated moisture content by drying the Clariid catfish species samples in the hot air oven at 70°C to a constant weight. Kim, et al. (2016) placed the homogenate of jack mackerel (*Trachurus japonicus*) in an oven at 65◦C and dried for approximately 24 h until it reached a constant weight. The water content was determined by the weight difference between the dried and original homogenate.

Near-infrared (NIR) spectrometer (Food Scan Lab Analyzer Model 78,800, Foss Analytical, Eden Prairie, MN) is an AOAC approved proximate analyzer, has been used for the analysis of proximate composition (protein, fat, collagen, and moisture) of meat and meat products (Cai et al., 2018). Khodabux et al. (2007) stated that NIR spectroscopy has the prospective for rapid, accurate and non-destructive determination of fish proximate composition. They analyzed proximate composition (moisture, protein, fat, ash) both chemically and using NIR method of 20 skipjack tunas (*Katsuwonus pelamis*) and 18 yellow fin tunas (*Thunnus albacares*) and established a correlation between conventional and NIR assessed value. The correlation coefficient (R^2) was 0.98, 0.99, 0.95 and 0.96 for moisture, protein, total fat and free fat, respectively.

Near Infrared Reflectance (NIR) and Transmission (NIT) Spectroscopy technology was also used for the analyses of fish flesh (Gjerde and Mathias. 1987; Rasco et al., 1991; Downey. 1995). Near-Infrared (NIR) technology has been used to analyze the proximate composition of fish muscle: trout (Rasco et al., 1991), sea bass (Xiccato et al., 2004; Majolini et al., 2009), pacific bluefin tuna (Hirose et al., 2016). The standard error of prediction was 1.1%, 3.1% and 5.4% for the moisture, lipid, and protein content. However, their method required no homogenization, drying, or extraction of fish muscle before analysis. In another study, Valdes et al. (1997) determined percent protein, fat and moisture of 68 fish samples (herring, whitebait, capelin, mackerel, squid, trout, Pollock, and sardines) using NIR spectrophotometer model 6500 (Perstorp Analytical, Maryland, USA).

CHAPTER III

MATERIALS AND METHODS

3.1 Sample collection and treatment

A total of 228 hybrid catfish [Blue (*Ictalurus furcatus)* × Channel *(Ictalurus punctatus*)] fillets were collected from a local catfish processing plant in Mississippi during February to June 2018. Three fillet samples (one for microbiological analysis and two for proximate analysis) of two sizes (small : 50g to 150g and large: 150g to 450g) from seven process steps [before trimming (BT): assumed to have the similar proximate composition as received fish in the processing plant, after trimming/before water chilling (BC), after water chilling (AC), after ice slush chilling (AS), before ice Packing (BIP): fresh fillets, after injection (AI), after freezing (AF): frozen fillets] from automatic processing lines based on the availability of the fillets at each process step (Figure 3.1) were randomly picked and placed into quart size ziplock bags (GreatValue^{TM} Slide Zipper 7in×8 in). The temperature of the BT, BC, AC, AS, BIP, AI and AF fillets during sampling was 21^{0}C, 20.6 ^{0}C , 6.2^{0}C , 0^{0}C, 3.7^{0}C, 4.6^{0}C and -2.6^{0}C. The sampled catfish fillets were kept in an ice chest with ice and transported within 40 min to the Food Safety and Processing Laboratory of the department of Food Science, Nutrition and Health Promotion at Mississippi State University. Microbiological analysis was performed within 5 to 7 h of sampling. Collected catfish fillets (placed in ziplock bag) kept in the ice chest covered with

ice were placed at 4°C in a refrigerator (Isotemp Plus Laboratory Refrigerator, Fisher Scientific, Pittsburg, USA) for 22 to 24 h prior to proximate analysis.

3.2 Proximate analysis

The weight and length of fillets were measured prior to proximate analysis. Ice glazes of the frozen fillets were removed by spraying of cold water and drained the water for 2 min and immediately transferred to the refrigerator (4^0C) for further proximate analysis (AOAC 963.18). The whole fillet was homogenized with a food chopper (Black & Decker@ Handy Choper Plus™ , Towson, MD, USA) by homogenizing for 15 to 20 sec. The homogenized sample was transferred to large (150×15 mm) petri dish (Falcon 35 1058 PetriDish Style Sterile, Oxnard, Calif.) with a cover to protect the moisture evaporation of the sample.

The 42-mL aluminum weighing dishes (without sample) (Fisher Scientific, 08732101, Houston, Texas, USA)) were also dried for 24 h in the ISOTEM OVEN (300 series Model 318, Fisher Scientific, Houston, TX) prior to analyzing and weighed with analytical balance (Denver Instrument APX-100, Denver, CO, USA). An aliquot of 5g homogenized sample was taken from the petri dish and evenly distributed into a 42-mL aluminum weighing dish (Fisher Scientific, 08732101, Houston, Texas, USA). Dishes (with sample) were weighed and dried at 105±2^0C in an ISOTEM OVEN 300 series Model 318 (Fisher Scientific, Houston, TX) for 5±2 h or until a constant weight was achieved (AOAC 950.46,1990). After drying, the dishes (with sample) were placed in a desiccator (Sanplatec Corporation, Japan) for 15±5 min to cool. After cooling, the dishes (with sample) were weighed again. Moisture content was calculated on wet basis as follows:

$$\text{Moisture content (\%)} = \frac{(W2 - W3)}{(W2 - W1)} \times 100$$

Where,
W1 = weight of dish (without sample);
W2 = weight of dish (with sample) before drying
W3 = weight of dish (with sample) after drying

Proximate composition (protein, fat, collagen, moisture) of the fish fillets were analyzed on a wet basis using a Near-infrared (NIR) spectrometer (Food Scan Lab Analyzer Model 78,800, Foss Analytical, Eden Prairie, MN). NIR transmittance range was 850-1048 nm on a rotating sample. The NIR spectrometer was calibrated by the artificial neural network (ANN) that covers all types of muscle food products. The homogenized sample (180 g) was taken from petri dish into the FoodScan sample cup. The sample cup was placed in the holder of the instrument and analysis was conducted.

3.3 Models to predict retained water

A significant correlation (r=0.90, $P \leq 0.05$) was obtained between moisture determined by NIR and oven method (Appendix Table A.18). For the establishment of a prediction model, moisture content determined by NIR was fitted using simple linear regression. The moisture-protein ratio (wet basis) was calculated as follows:

$$\text{Moisture-protein ratio (M:P)} = \frac{\text{Fitted moisture content}}{\text{Protein content determined by NIR}}$$

Retained water (%) was calculated based on the moisture retention/loss in each point of the processing as follows:

Retained water (%) = Moisture at any process point (e.g. AC, BIP, AF) –moisture (%) at baseline (BT)

3.4 Microbiological analysis

A 25g fillet sample was aseptically cut with a sterile stainless-steel knife, weighed and placed in a stomacher bag (Nasco, Whirl-pak, 19 × 30 cm; Fort Atkinson, WI, USA). A 225 ml of 0.1% sterilized buffer peptone water (BPW) solution (Difico, Detroit, MI) was added and stomached for two min in a laboratory Blender stomacher 400 (A. J. Seward and Co. Ltd., London, England). Dilutions were made by transferring 1 ml of the homogenate into dilution tubes with 9 ml of 0.1% sterilized peptone solution. Plating was conducted on aerobic (APC) count Petrifilm™ (3M Co., St. Paul, MN, USA) in duplicate and these were incubated for 72 h at 20±2°C (Dormedy et al., 2000; Marroquin et al., 2004; Kim et al., 2000) for psychrotrophic counts (PPC). *E. coli* plates were incubated for 24 to 48 h at 35±2°C (Swanson et al., 1992; Fernandes et al., 1997) on 3M Petrifilm *E. coli* (3M Co., St. Paul, MN, USA) in duplicate for the enumeration of *E. coli* and total coliform counts. Colonies were identified and enumerated using 3M™ Petrifilm™ Plate Reader (3M Company, Technopath, St. Paul, MN, USA) as per manufacturer's instructions. Selected plates counting was verified by conventional (visual) counting method.

3.5 Experimental design and statistical analysis

Data were arranged in a 2-way factorial [2 sizes of the fillets (small- 50 to 150 g; large:150g to 450g) × 7 process points] randomized complete block (RCB) design with 7 replications (blocks) based on the availability of the fillets from each process point [BT: 15 fillets (small=7, large=8); BC:16 fillets (small=9, large=6); AC= 10 fillets (small=5,

large=5); AS: 14 fillets (small=8, large=6); BIP: 9 (small=3, large=6); AI: 9 fillets (small=5, large=4); AF: 7 fillets (small=3, large=4)]. Data were unbalanced in the blocks due to the unavailability of the fillets for some replications. The General Linear Model (GLM) procedure of the Statistical Analysis System (SAS universal edition, 2018) was used to examine the interaction of sizes and process steps. Tukey's honestly significant difference (HSD) was used for the mean separation of the measurements of the fillets (α=0.05). Pearson's correlation coefficient was used to determine the multiple correlations among the variables (Freud and Wilson, 1997). Simple linear regression (SLR) (Kenney and Keeping, 1962) and multiple linear regression (MLR) (Lai et al., 1979) models were used to calculate the correlation of the variables. All Statistical analysis were performed using SAS universal edition (2018) on significance ($P \leq 0.05$).

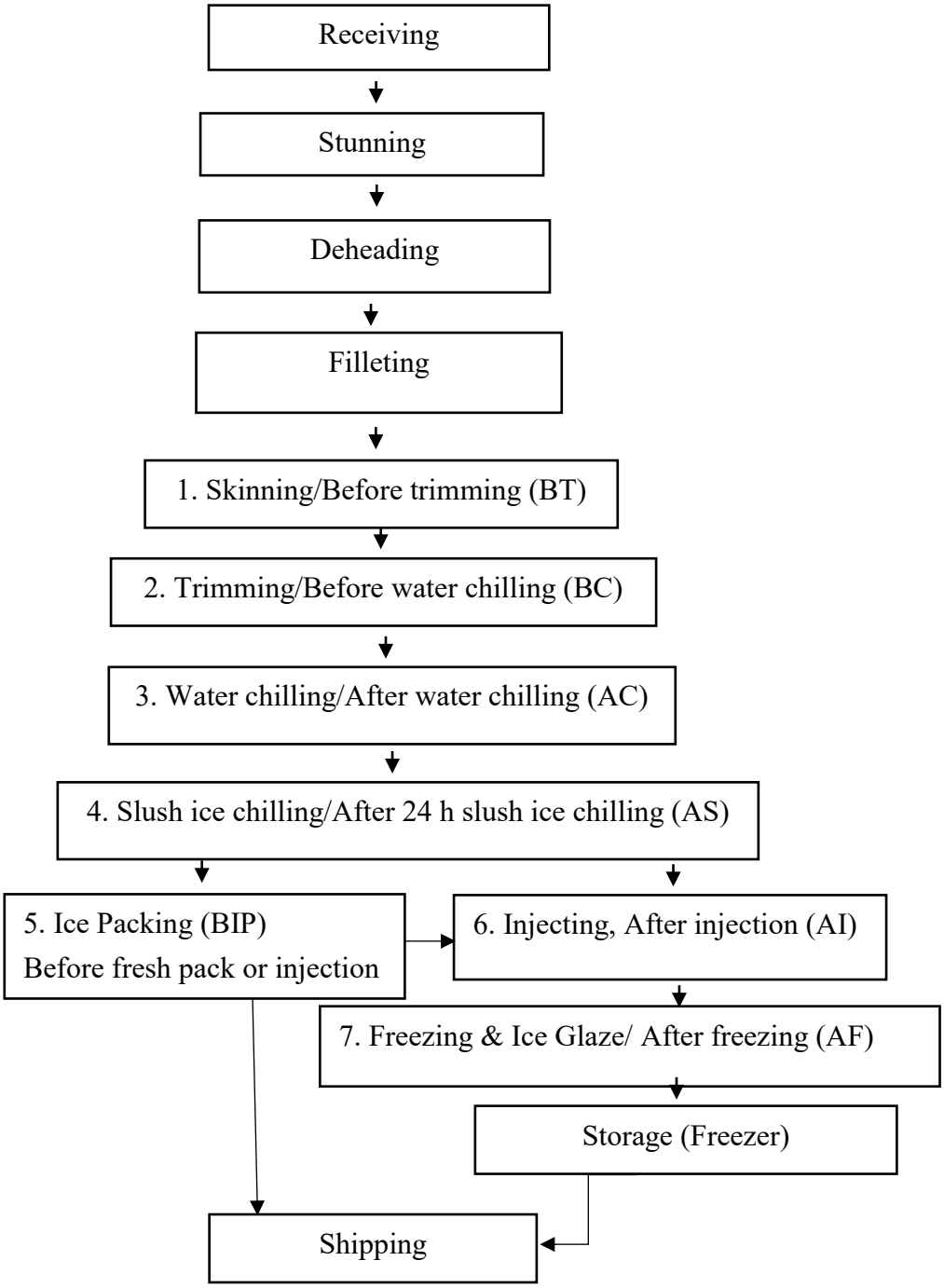

Figure 3.1 Typical process flow for catfish fillet showing sampling points (numbered and abbreviated)

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Baseline proximate composition of the hybrid catfish fillet

There was no interaction (*P>0.05*) between sizes [small fillets (SF)=111±19 g; large fillets (LF)= 247±62 g) and process steps [Before trimming (BT); after water chilling (AC); after slush ice chilling (AS): fillets kept for 24 h covered with slush ice; before ice packing (BIP): final fresh fillet; after polyphosphate injection (AI) and after freezing (AF): frozen fillet] for the proximate composition (moisture, protein, fat) and retained water (Appendix Table A.1 to A.5). Moisture content determined by oven method was reported in this section.

The overall (all sizes) baseline (fillets collected before trimming points; BT-assumed to have the same proximate composition as received fish in the processing plant) moisture was 77.8 ±1.38%, with a range of 74.5 to 80.0%; fat was 5.7±1.6% with a range of 4.0 to 10.3%; and protein content was 16.7±0.50% with a range of 15.5 to 17.4% (Figure 4.1, Appendix Table A.4). Bosworth et al. (2001) reported similar moisture (77.7±2.12% with a range of 73.7 to 81.0%) and fat content (7.0±1.69%) and Li et al. (2007) reported similar protein content (17.3%) for manually filleted hybrid catfish (*Ictalurus furcatus*×*Ictalurus punctatus*). Similar ranges of moisture (73 to 81%), fat (5.4% to 8.4%) and protein (16 to 19%) content were also reported for manually filleted channel catfish (*Ictalurus punctatus*) (Robinson & Oberle, 2001; Nettleton et al., 1990; Mustafa and

Medeiros, 1985; Fisher and Ammerman, 1983). Manthey-Karl et al. (2016) also reported similar mean moisture (79.0±1.10%) and protein (18.7±1.1%) for whole and skinned Vietnamese catfish (*Pangasius hypothalmus*). Several studies (Chomnawang et al., 2007; Manthey-Karl et al., 2016; Linhartová et al., 2018) reported lower ranges of fat content (2.3% to 3.0%) for undressed and whole Pangasius (*Pangasius hypophthalmus*), hybrid Thai catfish (*Clarias macrocephalus* × *Clarias gariepinus*) and wels catfish (*Silurus glanis*) flesh.

However, the baseline moisture content (78.6±0.87% vs 76.8±1.15%) was greater (*P≤0.05*) and fat content (4.7±0.64% vs 6.8±1.87%) was less (P≤0.05) for small fillets (LF=111±19 g) in comparison to large fillets (LF=247±62 g), whereas protein content was similar (*P*>0.05) for both sizes of fillets (Table 4.1). This is due to the conversion of the moisture into fat over the growing of fish (Boggess et al., 1971). This result is in accordance with Silva and Ammerman (1993). They reported greater (*P*≤0.05) moisture content (70.8% vs 68.1%) and less (*P*≤0.05) fat content (10.8% vs 13.2%) for small (0.3 kg) channel catfish (*Ictalurus punctatus*) in comparison to larger (1.0 kg) one.

4.2 Moisture and Retained water content of the hybrid catfish fillet at several process steps

Fillets' moisture and retained water (moisture difference from baseline; BT) differed (*P≤0.05*) in some process steps (Figure 4.1; Figure 4.2; Appendix Table A.1 and Appendix Table A.2. However, retained water content was not different (*P*>0.05) for size differences (large and small) of the fillets at any process step (Appendix Table A.2).

Moisture content of BC fillets was similar ($P>0.05$) to that of BT fillets (Figure 4.1). After water chilling, fillets absorbed 2.4±1.53% (with a range of -0.7 to 4.4%) of water (Figure 4.1; Appendix B.4). James et al., (2006) reported that during chilling poultry carcasses absorbed these water in the subcutaneous layer of the muscle tissue. After 24-hour ice slush chilling, fillets' retained water (4.0±1.74%, with a range of 0.3 to 6.3%) was highest ($P\leq0.05$) in comparison to other process step's fillets (Figure 4.3; Appendix B.4). This is due to the immersion of the fillets for a longer period in the slush ice, where fillets trap more water in the intercellular space of the muscle tissues that caused more water absorption by the fillets (James et al., 2006; Young and Smith, 2004). Carciofi and Laurindo (2007) reported that water absorption of poultry depends on immersion time, water temperature and water stirring conditions during chilling. However, fillets lost around 2.8% of this moisture before ice packing (BIP). Retained water of the BIP fillets was 1.2±2.03% (with a range of -2.1 to 5.0%) (Figure 4.2; Appendix Table B.4). Klose et al. (1960) reported that most of the absorbed water loosely held (unbound water) in pockets between the tissues of the muscle during immersion chilling and most of these waters could be lost when taking out the fillets from the water. Silva et al. (2001) support these results stating that fillets could gained weight due to water absorption during chilling but lost most of it before ice packing. The reported (Young & Smith, 2004; James et al., 2006) retained water (6 to 12%) of poultry carcass after immersion chilling was greater in comparison to retained water (-0.7 to 6.3%) of hybrid catfish fillets after water and ice slush chilling in this study. Retained water was not different ($P>0.05$) for AI fillets (2.6±1.63%, with a range of 0.3 to 5.4%) in comparison to AF fillets (3.1±1.02%, with a range of 1.8 to

5.0%) (Figure 4.2; Appendix Table B.4). This result might be due to the injection of polyphosphate in the fillets prior to freezing that increased the water binding capacity of the myofibrillar protein of the muscle and protect moisture loss during freezing (Kin et al., 2009; McCormick, 1983). However, Kin et al. (2009) reported 8 to 9% solution (water, salt and phosphate) pick up for channel catfish fillets (collected from after chilling points and marinated with solution).

4.3 Proximate compositions of the hybrid catfish fillet at several process steps

Moisture and fat content of the AC, AS, BIP and frozen fillets were not different ($P>0.05$) due to size differences (small and large) of the fillets at any process (Figure 4.4 and Figure 4.5). This result indicated that when fillets were chilled (both water and slush ice), and frozen, moisture and fat percentages were not fillet's size dependent.

BT and BC fillets' fat content were similar regardless of fillets' sizes (Figure 4.5, Appendix A.4). AS fillets had less ($P\leq0.05$) fat (3.8±0.92) but greater ($P\leq0.05$) moisture content (82.0±1.40) in comparison to BT and BC fillets (Figure 4.1; figure 4.3; Appendix Table B.4). This is because of the increased moisture content of the AS fillets after slush ice chilling. Several studies reported inverse correlation between fat and moisture content of the fish flesh (Linhartová et al., 2018; Karl et al., 2018; Yeannes & Almandos, 2003). Moreover, fat content of the fillets were not different ($P>0.05$) with the process steps, when measured on dry basis (Appendix Table A.4).

Protein content of the fillets were not different ($P>0.05$) for two sizes (large and small fillet) at any process steps (Appendix A.5). Protein content was not different ($P>0.05$) for BT (16.7±0.50%), BC (16.8±0.49%) and BIP (16.3±0.61%) fillets regardless

of sizes (Figure 4.5; Appendix B.4). However, when AC and AS fillets' moisture content increased (2 to 4%) due to water absorption during chilling (both water and slush ice), the percentage of protein content was less for these (AC:16.1±0.51% and AS:15.0±0.71%) fillets in comparison to protein content of BT, BC and BIP fillets (Figure 4.5; Appendix B.4). AI and AF fillets also resulted in less protein percentage (AI: 14.7±0.61% and AF: 14.8±0.38%), where moisture of these (AI and AF) fillets was higher ($P \leq 0.05$) in comparison to BT, BC and BIP fillets (Figure 4.1; Figure 4.5). These are in accordance with a report by Breck (2014). He reported that protein mass (g/100g) decreased with the increase of water mass per unit in the bluegill, common carp, trout, and salmon fish. However, fillets' protein percentage on dry basis differed ($P \leq 0.05$) with the process steps. (Appendix A.6).

4.4 Bacterial load of the hybrid catfish fillets at several process steps

There was no interaction ($P>0.05$) between sizes [small fillets (SF)=111±19 g; large fillets (LF)= 247±62 g) and process steps for psychrotrophic plate counts (PPC) and total coliform plate counts (TCC) (Appendix Table A.7; Appendix Table A.8). PPC and TCC were not different (P>0.05) due to the size differences of the fillets at any process steps (Appendix Table A.7; Appendix Table A.8). This result indicated that bacterial counts of the fillets were fillets' size independent.

PPC of the BT fillets were ~4 log CFU/g, with a range of 3.2 to ~5 log CFU/g (Figure 4.7; Appendix B.4). Fernandes (1997) and Watchalotone (1996) reported similar PPC (3.5 to 5.5 log CFU/g) whereas Huang (1993) and Nunez (1995) reported less PPC (2 to 3 log CFU/g) for channel catfish (*Ictalurus punctatus*) fillets collected from catfish

processing plants. Total coliform counts (TCC) of the BT fillets were 1.6 log CFU/g, with a range of 0 to 3 log CFU/g (Figure 4.8; Appendix A.4). Nunez (1995) and Fernandes (1997) reported similar TCC (0.8 to ~2 log CFU/g) whereas Watchalotone (1996) reported greater TCC (2.66 log CFU/g) for channel catfish (*Ictalurus punctatus*) fillets collcted from catfish processing plants. The reported higher TCC was resulted due to temperature abuse and mishandling as these fillets were collected from manual catfish processing scheme (Watchalotone, 1996). *E. coli* was not detected in this study at any process step. The maximum acceptable limits of Aerobic plate counts (APC) at 20 to 25^0C and *E. coli* in the fresh and frozen fish are 5.7 log CFU/g and 1.0 log CFU/g, respectively specified by ICMSF (International Commission on Microbiological Specifications for Foods) (Gould, 1990). However, Watchalotone et al. (2001) suggested that PPC and TCC of the catfish fillets during processing should not be more than 3-4 log CFU/g and 2 log CFU/g, respectively.

PPC and TCC were not different ($P>0.05$) for AC and AS fillets (Figure 4.7; Figure 4.8). This result indicated that 24 h slush ice chilling could not reduce the bacterial load (PPC and TCC) in comparison to water chilling. Fillets' PPC and TCC were not different ($P>0.05$) with the process steps except for AI fillets which had greater ($P\leq0.05$) PPC (5 log CFU/g, with a range of 4 to 6 log CFU/g) and TCC (~3 log CFU/g, with a range of 2.3 to 4.3 log CFU/g) in comparison to BT and AC fillets (Figure 4.7; Figure 4.8; Appendix Table B.4). AI fillets' greater PPC and TCC may have resulted from the additional handling of the fillets after injection (Fernandes, 1997). PPC were not different ($P>0.05$) for AI and AF fillets (Figure 4.7). However, TCC of the fillets was reduced ($P\leq0.05$) by 2.4 log CFU/g

after freezing. This result is in accordance with a report by Nunez (1995). They reported 2.6 log CFU/cm^2 reduction of TCC of the channel catfish fillets due to rapid freezing.

4.5 Modeling of retained water of catfish fillets

Multiple linear regression analysis was used to develop models for predicting retained water (%) of the hybrid catfish fillets during processing. Several studies (Breck, 2014; Ruth et al., 2014) reported that moisture-protein ratio could be used for determining added water during processing of the seafood. Breck (2014) reported that relationship of moisture and protein is size dependent and fat content inversely correlated to moisture content of the fish. Thus, moisture-protein ratio, weight (g) and fat content of the catfish was examined by multiple linear regression analysis to predict the retained water of the catfish fillets during processing.

NIR spectroscopy is fast, noninvasive and more economical to determine the proximate composition of the muscle food in comparison to other conventional (oven dry, kjeldahl) methods (Hirose et al., 2016; Xiccato et al., 2004). Data of the proximate composition determined by NIR spectrometer was used to predict the retained water at a fast space. Moisture determined by NIR spectrometer was fitted based on moisture determined by oven method (AOAC approved method) using simple linear regression model (Kutner and Neter, 2004). A significant correlation ($F(1.74) = 513.97$, $P < .0001$), $R^2 = 0.87$) was obtained between moisture determined by NIR and moisture determined by oven method (Figure 4.9; Appendix Table A.12). Fitted moisture was equal to 14.7 +0.80 (moisture determined by oven method) % (Figure 4.9).

The retained water calculated from moisture determined by NIR was fitted based on retained water calculated from moisture determined by oven method using simple linear regression model (Figure 4.10). The fitted retained water was equal to 3.0 +1.10 (calculated retained water from moisture determined by oven) % (Figure 4.10), [(F (1.56) = 255.93, p < .0001), R^2 =0.82) (Appendix A.14).

This fitted retained water were used as dependent variable (Y) and moisture-protein ratio (M:P), fat content (%) and weight (g) of the catfish were used as independent variables (X) in the prediction models.

A stepwise regression analysis was conducted with backward elimination of the independent variables to fit the models. At first, all the independent variables (M:P, fat content and weight) were used for the model establishment. The descriptive statistics of this model was shown in Appendix Table A.14. The regression equation of this model (F (3, 57) = 419.36, p < .0001, R^2 = 0.96) was as follows

Retained water (%) = -5.6+2.1 (M:P) -0.13 (Fat)+ 0.0004 (weight) (Model 1)

Both M:P and fat were significant ($P \leq 0.05$) predictors for retained water, however, weight was not a significant (P>0.05) predictor for retained water in this model (Appendix Table A.14).

Thus, weight was excluded from the model and a reduced model (F (2, 58) = 635.59, p < .0001), R^2=0.96) was established (Figure 4.11). Adjusted R-square was not different (P>0.05) for this reduced model (Appendix A.15) after excluding weight. This also indicated that weight was not a significant predictor along with moisture-protein ratio and fat content. The regression equation of this reduced model (Figure 4.11) was as follows:

Retained water (%)= -5.6+2.13 (M:P)-0.70 (Fat) (Model 2)

Both M:P and fat were significant predictors of retained water in this model 2 (Appendix A.15). However, when fat content was excluded from this model 2, adjusted R^2 (0.58) was different ($P \leq 0.05$) for the reduced model 3 (Appendix A.16). This indicated that fat content was a significant predictor for retained water in model 2. The regression equation of this reduced model (F (1, 59) = 84.84, $P < .0001$) was as follows

Retained water (%) = -12.2+2.8 (M:P) (Model 3)

However, when weight (g) was added excluding fat content in this reduced model 3, adjusted R^2 (0.73) increased ($P \leq 0.05$), which indicated that weight was a significant predictor for retained water excluding fat in model 4 (Appendix Table A.17). The regression equation of this model (F (2, 58) =79.78, $P < .0001$, R^2 =0.73) was as follows

Retained water (%) = -12.3+3.0 (M:P)-0.007 (weight) (Model 4)

Both M:P and weight were significant predictors of this model 4 (Appendix Table A.17)

Model 2 (Figure 4.11; Appendix Table A.17) fulfilled the goodness of fit criteria of a multiple linear regression model (Kutner and Neter, 2004). The model contained 76 observations and 3 parameters. The coefficient of multiple determination (R^2) was 0.96, indicating the greater proportion of variation was accounted for by this model. The residual of both M:P and fat, was distributed randomly (Figure 4.16). The value of residual degrees of freedom adjusted R square (Adj. R^2=0.96) and means square error (MSE=0.104) also exhibited a good fit of this model for the prediction of retained water based on moisture-protein ratio and fat content of the hybrid catfish fillets during processing.

Table 4.1 Proximate composition of baseline (BT) hybrid catfish fillets

Fillet Size	Moisture (%) (Oven)		Moisture (%) (NIR)		Fat (%) (NIR)		Protein (%) (NIR)		Psychrotrophic counts (log CFU/g)		Total Coliform counts (log CFU/g)	
	(mean±SD)	Range	(mean±SD)	Range	(mean±SD)	Range	(mean±SD)	Range	(mean±SD)	Range	(mean±SD)	Range
Small	78.6±0.87[a]	77.5-80.0	76.3±1.47[a]	74.4-78.6	4.7±0.64[a]	4.0-6.2	16.7±0.47[a]	16.0-17.4	4.1±0.48[a]	3.2-4.5	1.6±1.33[a]	0.0- 3.1
Large	76.8±1.15[b]	74.5-78.0	75.3±1.82[b]	73.5-78.0	6.8±1.87[b]	5.3-10.3	16.7±0.57[a]	15.5-17.2	4.0±0.61[a]	3.5 -4.8	1.7±1.5[a]	0.0-3.1
CV	1.38		0.85		23.60		3.13		10.58		80.30	
MSE	1.16		0.42		1.80		0.27		0.18		1.73	
HSD	1.32		0.80		1.64		0.64		0.72		2.21	

[a b] Means in same column not followed by same letter differ ($P \leq 0.05$); Small (111±19 g, n=44), Large (247±62 g, n=32); SD= Standard Deviation

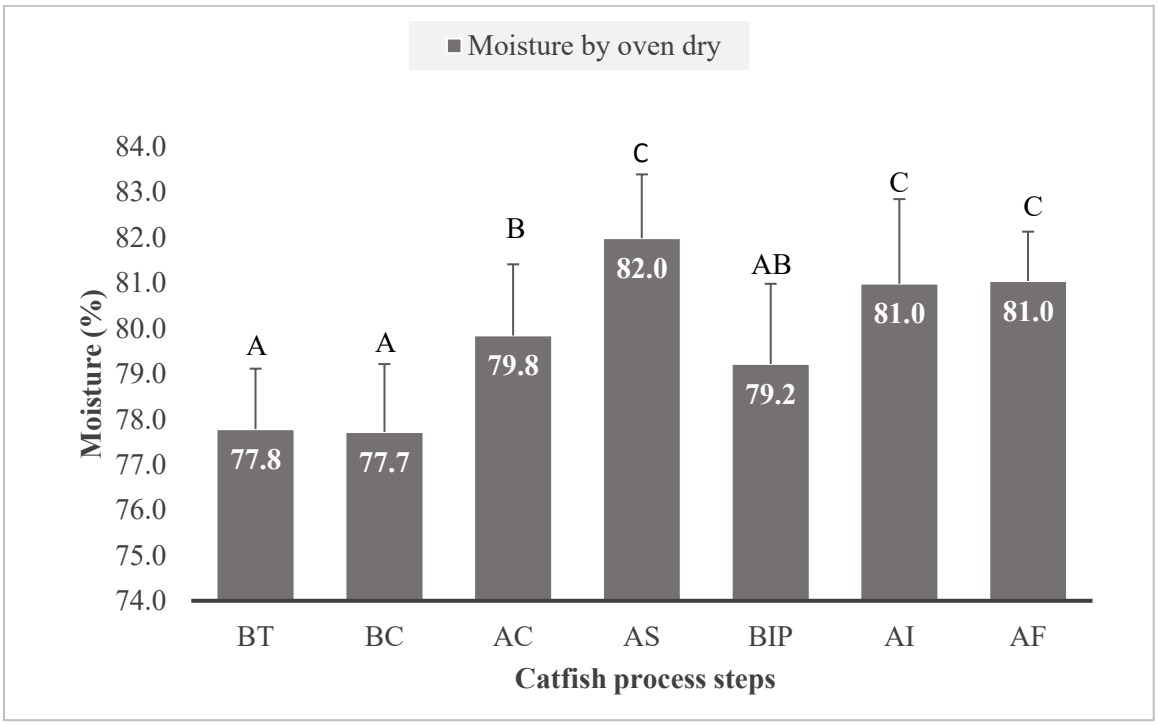

Figure 4.1 Moisture content (%) (oven method) of hybrid catfish fillets at different catfish process steps regardless of sizes (small fillets=111±19 g; large fillets=247±62 g)

$^{A\ B\ C}$ Means not followed by the same letter differ ($P \leq 0.05$);
BT=Before Trimming (Baseline; assumed to have the same proximate composition as received fish at processing plant); BC= After trimming/before chilling; AC=After water chilling; AS= After slush ice chilling; BIP= Before ice packing (Fresh fillets); AI= After injecting (polyphosphate injection), AF=After freezing (Frozen fillets)

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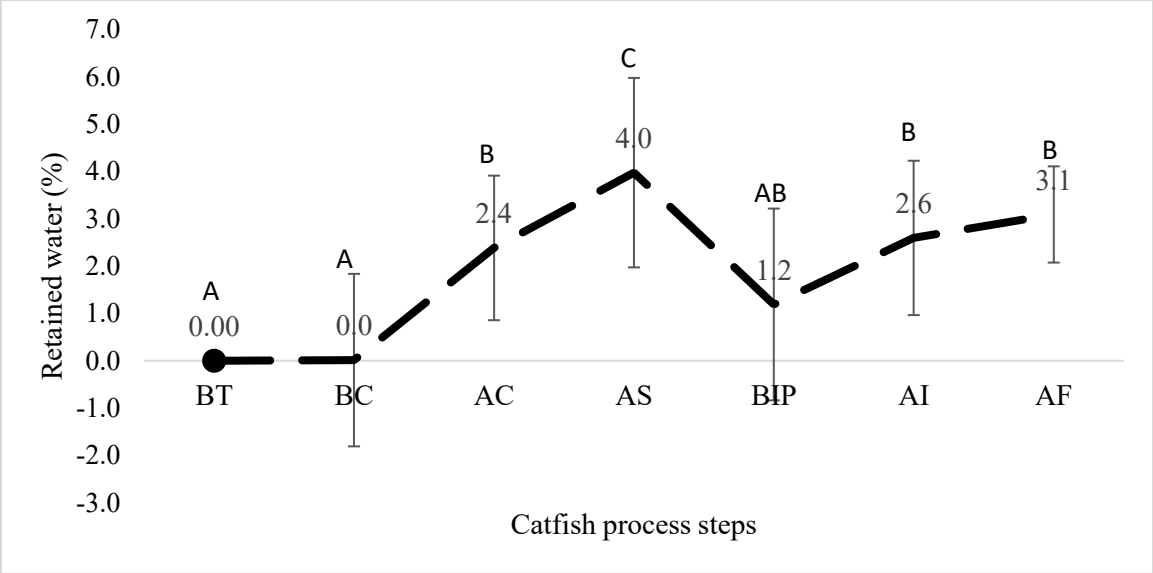

Figure 4.2 Retained water (%) of hybrid catfish fillets at different catfish process steps regardless of sizes (small fillets=111±19 g; large fillets=247±62 g)

[A B C] Means not followed by the same letter differ ($P \leq 0.05$);
BT=Before Trimming (Baseline; assumed to have the same proximate composition as received fish at processing plant); BC= After trimming/before chilling; AC=After water chilling; AS= After slush ice chilling; BIP= Before ice packing (Fresh fillets); AI= After injecting (polyphosphate injection), AF=After freezing (Frozen fillets)

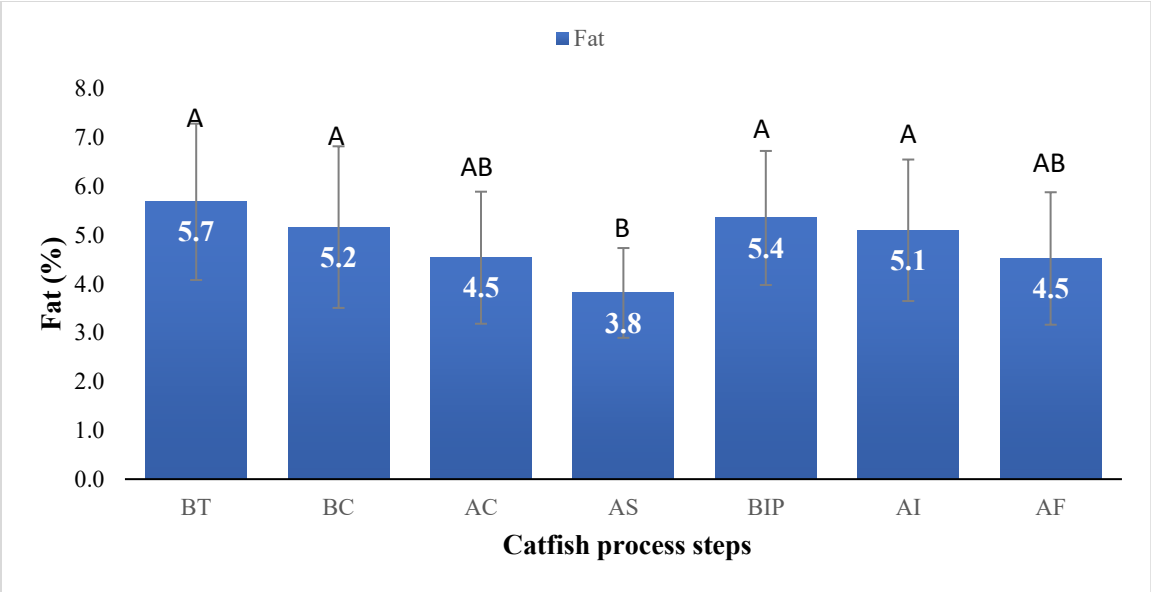

Figure 4.3 Fat content (%) (NIR) of hybrid catfish fillets at different catfish process steps regardless of sizes (small fillets=111±19 g; large fillets=247±62 g)

[A B] Means not followed by the same letter differ ($P \leq 0.05$);
BT=Before Trimming (Baseline; assumed to have the same proximate composition as received fish at processing plant); BC= After trimming/before chilling; AC=After water chilling; AS= After slush ice chilling; BIP= Before ice packing (Fresh fillets); AI= After injecting (polyphosphate injection), AF=After freezing (Frozen fillets)

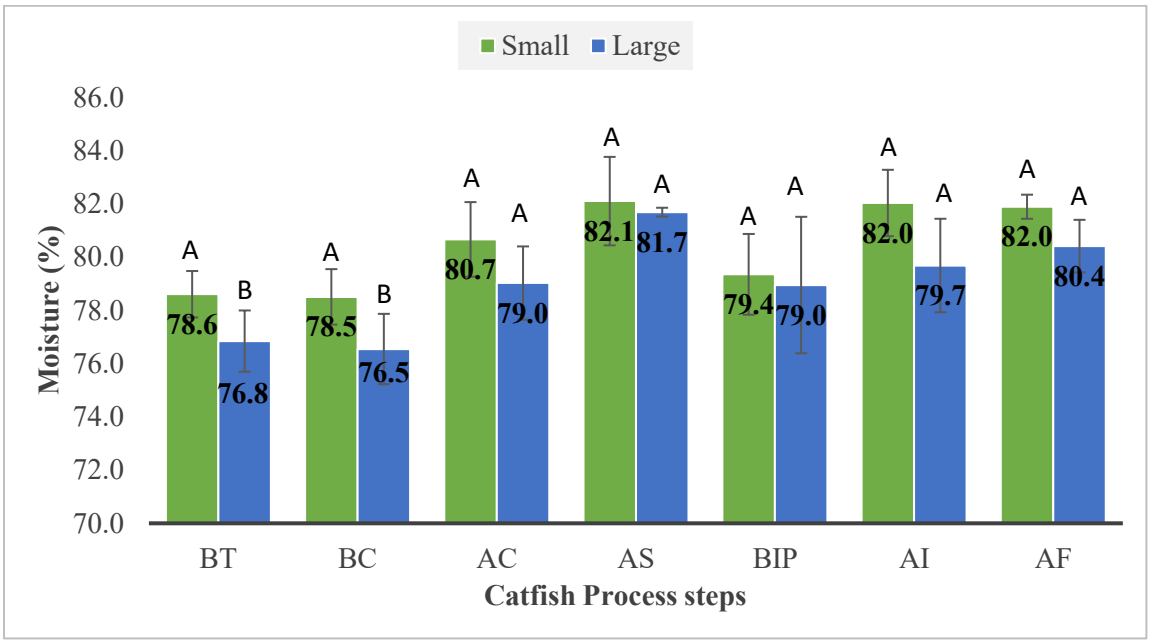

Figure 4.4 Moisture content (%) (oven method) of hybrid catfish fillets by size (small fillets=111±19 g; large fillets=247±62 g) at different catfish process steps

[A B] Means within fillet size not followed by the same letter differ ($P \leq 0.05$); BT=Before Trimming (Baseline; assumed to have the same proximate composition as received fish at processing plant); BC= After trimming/before chilling; AC=After water chilling; AS= After slush ice chilling; BIP= Before ice packing (Fresh fillets); AI= After injecting (polyphosphate injection), AF=After freezing (Frozen fillets)

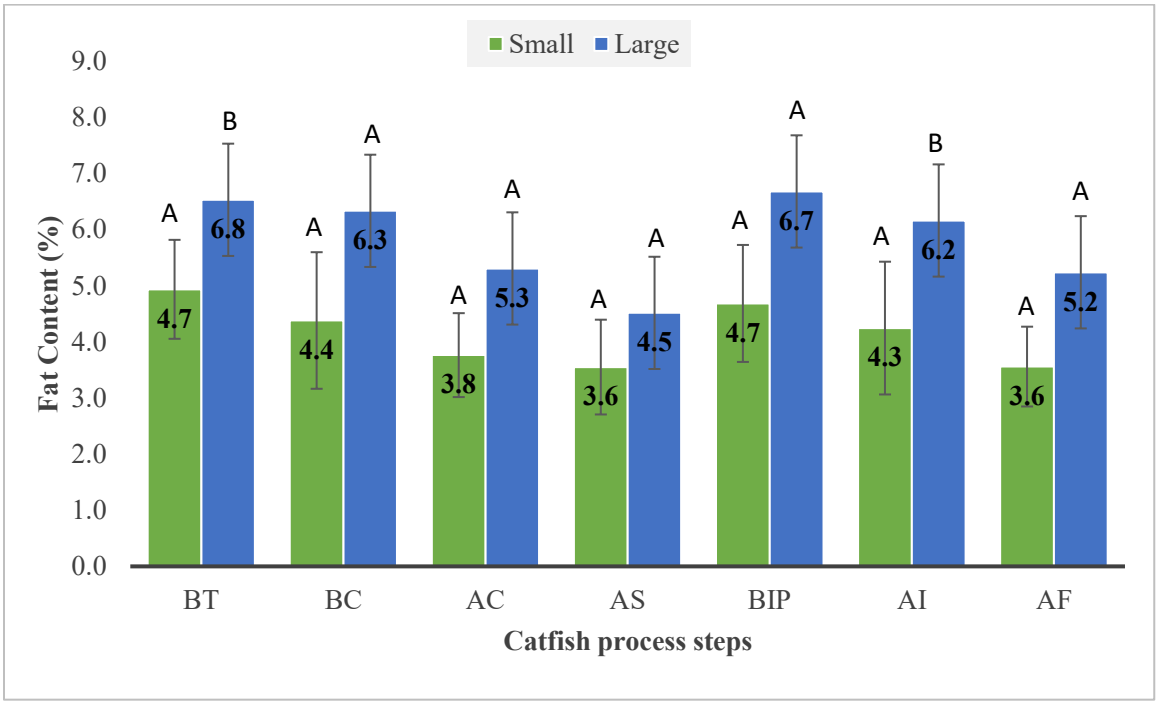

Figure 4.5 Fat content (% wet basis) (NIR) of hybrid catfish fillets by size (small fillets=111±19 g; large fillets=247±62 g) at different process steps

[A B] Means within fillet size not followed by the same letter differ ($P \leq 0.05$); BT=Before Trimming (Baseline; assumed to have the same proximate composition as received fish at processing plant); BC= After trimming/before chilling; AC=After water chilling; AS= After slush ice chilling; BIP= Before ice packing (Fresh fillets); AI= After injecting (polyphosphate injection), AF=After freezing (Frozen fillets)

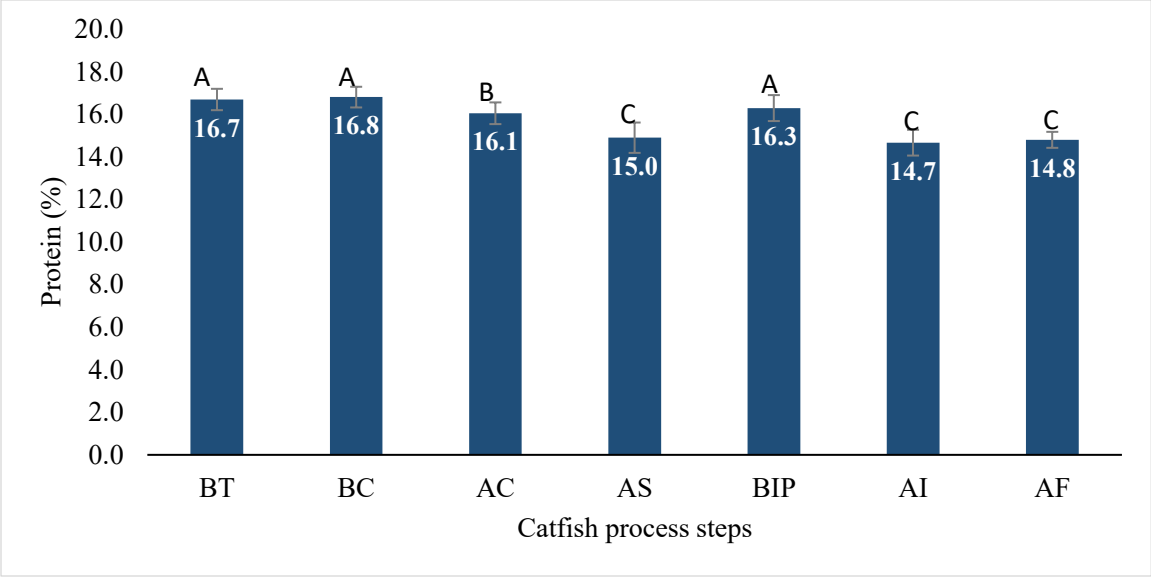

Figure 4.6 Protein content (%) (NIR) of hybrid catfish fillets at different catfish process steps regardless of size (small fillets=111±19 g; large fillets=247±62 g)

[A B C] Means within fillet size not followed by the same letter differ ($P \leq 0.05$); BT=Before Trimming (Baseline; assumed to have the same proximate composition as received fish at processing plant); BC= After trimming/before chilling; AC=After water chilling; AS= After slush ice chilling; BIP= Before ice packing (Fresh fillets); AI= After injecting (polyphosphate injection), AF=After freezing (Frozen fillets)

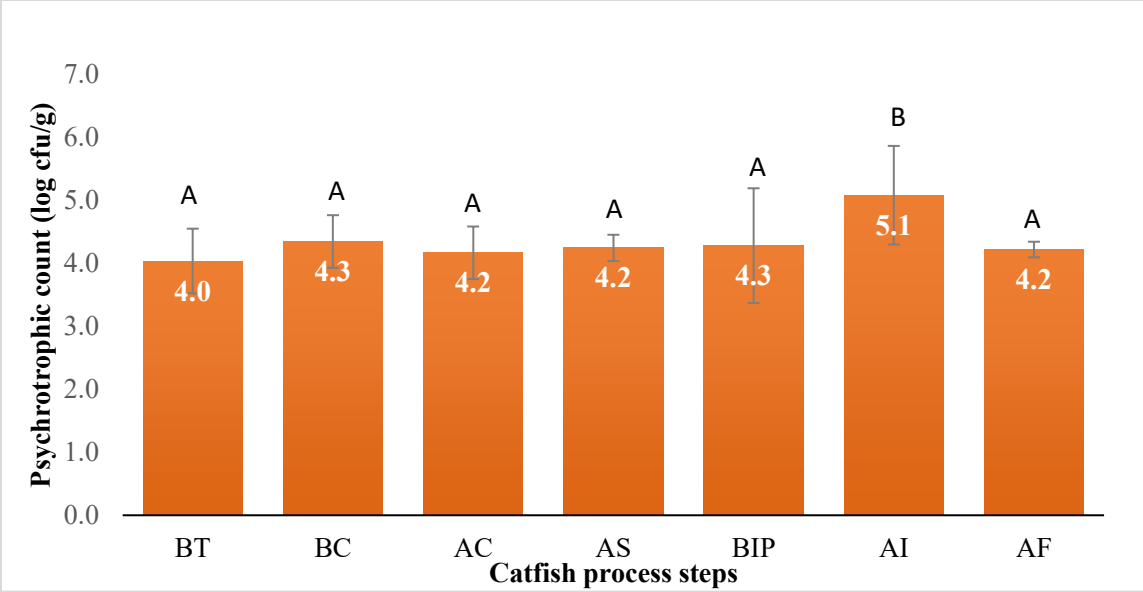

Figure 4.7 Psychrotrophic plate counts (PPC) (log CFU/g) of hybrid catfish fillets at different catfish process steps regardless of sizes (small fillets=111±19 g; large fillets=247±62 g)

[A B C] Means within fillet size not followed by the same letter differ ($P \leq 0.05$); BT=Before Trimming (Baseline; assumed to have the same proximate composition as received fish at processing plant); BC= After trimming/before chilling; AC=After water chilling; AS= After slush ice chilling; BIP= Before ice packing (Fresh fillets); AI= After injecting (polyphosphate injection), AF=After freezing (Frozen fillets)

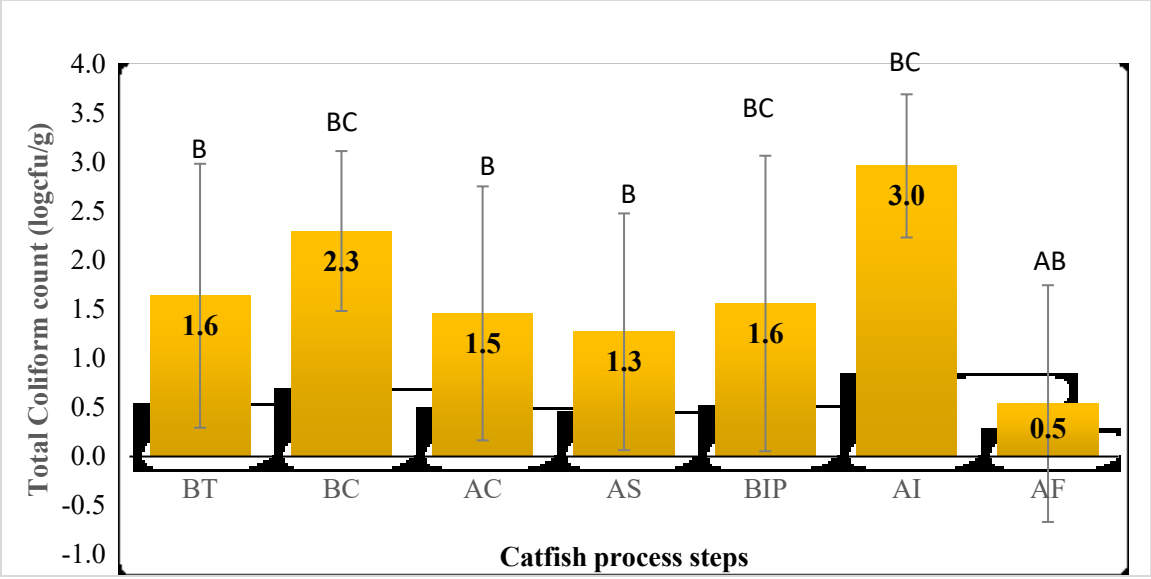

Figure 4.8 Total Coliform plate counts (TCC) of hybrid catfish fillets at different catfish process steps regardless of sizes (small fillets=111±19 g; large fillets=247±62 g)

$^{A\ B\ C}$ Means within fillet size not followed by the same letter differ ($P \leq 0.05$); BT=Before Trimming (Baseline; assumed to have the same proximate composition as received fish at processing plant); BC= After trimming/before chilling; AC=After water chilling; AS= After slush ice chilling; BIP= Before ice packing (Fresh fillets); AI= After injecting (polyphosphate injection), AF=After freezing (Frozen fillets)

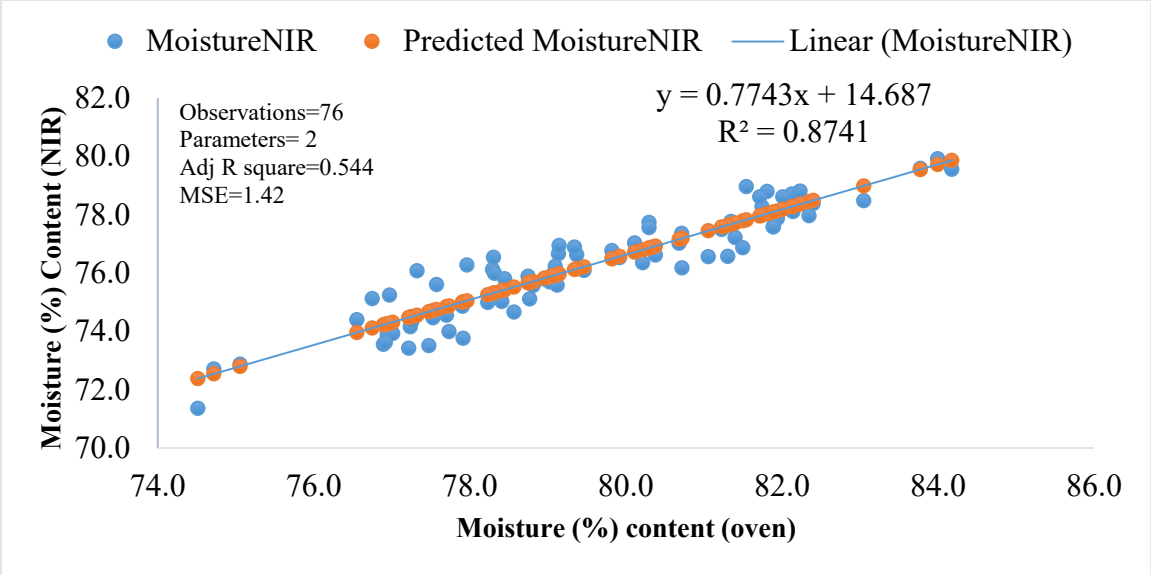

Figure 4.9 Correlation between moisture (%) content determined by NIR and moisture (%) content determined by oven method of the hybrid catfish fillets

Moisture (%) content (NIR)= moisture content determined by NIR spectrometer
Moisture (%) content (oven)= moisture content determined by oven dry method (AOAC approved method)

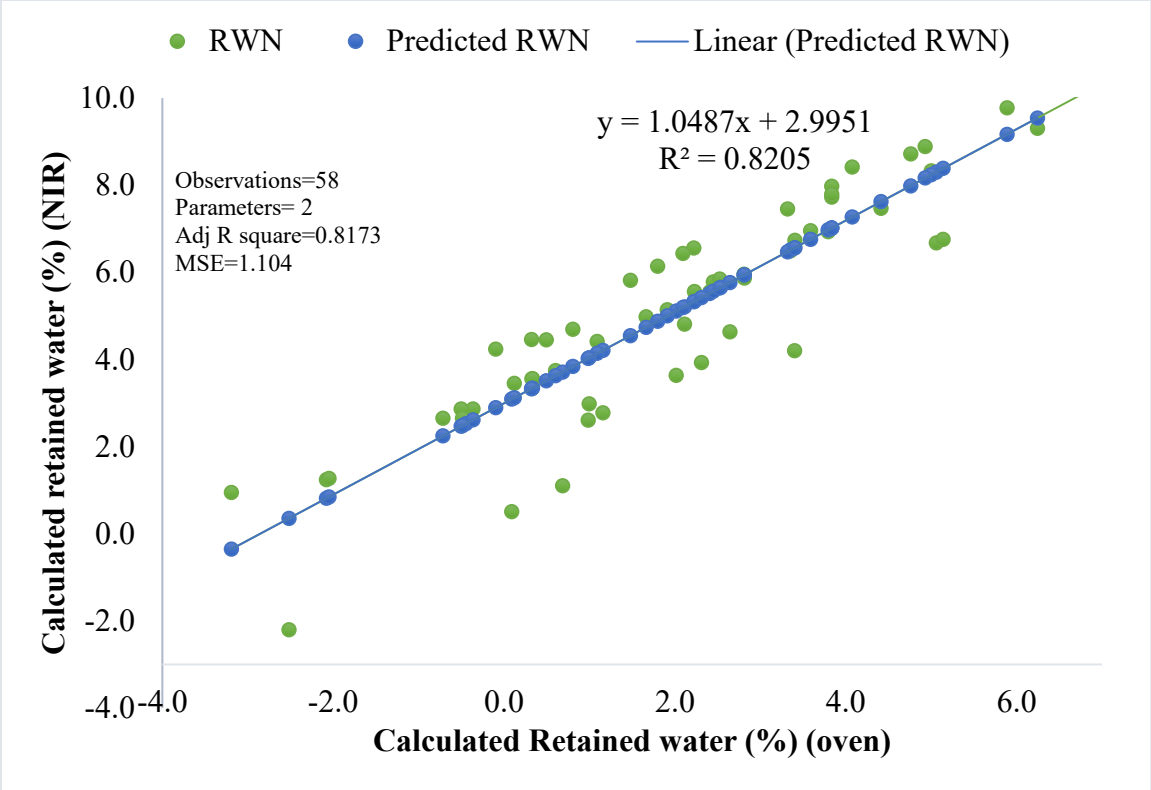

Figure 4.10 Correlation between calculated retained water (%) from moisture determined by NIR spectrometer and calculated retained water (%) from moisture determined by oven method of the hybrid catfish fillets

Calculated retained water (%) (NIR) (RWN)= Retained water (%) calculated from moisture content (%) of the hybrid catfish fillets determined by NIR spectrometer
Calculated retained water (%) (oven)= Retained water (%) calculated from moisture content (%) of the hybrid catfish fillets determined by oven dry method (AOAC approved method)

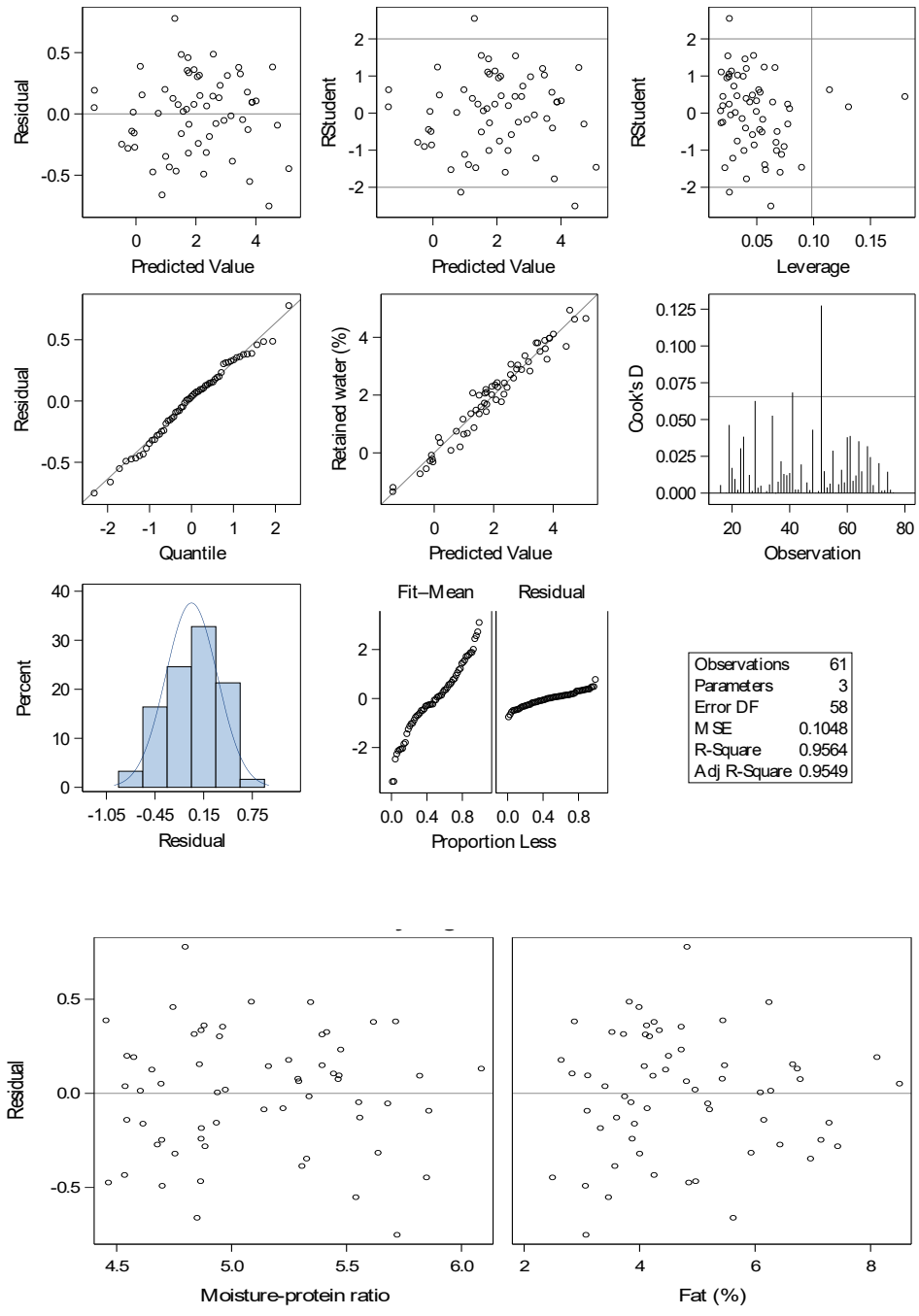

Figure 4.11 Fit diagnostic and residual distribution of model 2 for the prediction of retained water (%) of hybrid catfish fillets during processing

Dependent variable= Fitted retained water (%) (Calculated from regression analysis between retained water from moisture determined by oven and NIR method.
Dependent variable=moisture-protein ratio, weight (g) and fat content (%) of the catfish fillets

CHAPTER V

SUMMARY AND CONCLUSIONS

There was no interaction between size and process steps for all measurements. The baseline moisture of the hybrid catfish fillets ranged from 75 to 80% but varied due to fillets' size differences. Baseline moisture content was significantly greater for small fillets (SF) whereas fat was less in comparison to large fillets (LF) and protein content was similar for both sizes. After slush ice chilling fillets retained more water (up to 6.5%) in comparison to water chilling. However, most of this moisture was lost before ice packing. Retained water, protein content, psychrotrophic plate counts (PPC) and total coliform counts (TCC) of the fillets were not different due to size differences of the fillets at any process step. Only baseline and trimmed fillets' moisture and fat content differed due to size differences of the fillets. Chilled, fresh and frozen fillets' proximate composition and bacterial load were not size dependent. Fillets' PPC and TCC were not different with process steps except for injected fillets which had greater bacterial load in comparison to other fillets. Slush ice chilling for 24 h could not reduce bacterial counts of the fillets in comparison to water chilling. Moisture-protein ratio and fat content were significant predictors for retained water during processing of the hybrid catfish fillets.

In conclusion, baseline moisture content dictated the amount of retained water of the catfish fillets with the process steps. Final fresh and frozen fillets' retained water could be predicted using moisture-protein ratio and fat content of the fillets. This study would

provide information to both processors and inspection authorities with respect to regulatory compliance of correct labeling of retained water and microbiological quality of the hybrid catfish fillets at several process steps.

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APPENDIX A

ANALYSIS OF VARIANCE (ANOVA) AND REGRESSION ANALYSIS TABLES

Table A.1 Analysis of variance for moisture (%) (Oven) of hybrid catfish fillets

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Size	1	38.0273741	38.0273741	23.25*	<.0001
Process Steps	6	184.8339972	30.8056662	18.84*	<.0001
Block	6	17.3231425	2.8871904	1.77	0.1230
Size*Process Steps	6	8.6779725	1.4463288	0.88	0.5125
Error	56	91.5786697	1.6353334		
Corrected Total	75	355.9535643			

*Means significantly different at $P \leq 0.05$

Table A.2 Analysis of Variance of Retained water (%) of hybrid catfish fillets

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Size	1	0.0283935	0.0283935	0.01	0.9181
Process Steps	5	108.4665463	21.6933093	8.17*	<.0001
Block	6	32.4963200	5.4160533	2.04	0.0809
Size* Process Steps	5	7.3137107	1.4627421	0.55	0.7368
Error	43	114.1911538	2.6556082		
Corrected Total	60	265.9488538			

*Means significantly different at $P \leq 0.05$

Table A.3 Analysis of variance for fat content (%) of hybrid catfish fillets

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Size	1	51.26570654	51.26570654	36.49*	<.0001
Process Steps	6	26.13226838	4.35537806	3.10*	0.0108
Block	6	11.26867584	1.87811264	1.34	0.2564
Size* Process Steps	6	3.48513328	0.58085555	0.41	0.8670
Error	56	78.6721354	1.4048596		
Corrected Total	75	169.6472737			

*Means significantly different at $P \leq 0.0$

Table A.4 Analysis of variance for fat content (dry basis) of hybrid catfish fillets

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Process Steps	6	410.0171318	68.3361886	1.92	0.0913
Block	6	149.0900529	24.8483421	0.70	0.6520
Error	63	2242.255032	35.591350		
Corrected Total	75	2787.655855			

*Means significantly different at $P \leq 0.05$

Table A.5 Analysis of variance for protein content (%) of hybrid catfish fillets

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Size	1	1.11738565	1.11738565	3.75	0.0579
Process Steps	6	54.87891220	9.14648537	30.69*	<.0001
Block	6	3.37433678	0.56238946	1.89	0.0992
Size* Process Steps	6	0.77940434	0.12990072	0.44	0.8518
Error	56	16.69223377	0.29807560		
Corrected Total	75	77.67636842			

*Means significantly different at $P \leq 0.05$

Table A.6 Analysis of variance for protein content (dry basis) of hybrid catfish fillets

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Process Steps	6	379.5083871	63.2513979	2.41	0.0358
Block	1	0.0016698	0.0016698	0.00	0.9937
Error	68	1783.125571	26.222435		
Corrected Total	75	2163.894418			

*Means significantly different at $P \leq 0.05$

Table A.7 Analysis of Variance for Psychrotrophic counts (PPC) (log CFU/g) of hybrid catfish fillets

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Size	1	0.02668571	0.02668571	0.12	0.7335
Process Steps	6	3.28321657	0.54720276	2.41*	0.0449
Block	5	2.45166017	0.49033203	2.16	0.0788
Size*Process Steps	6	1.43605136	0.23934189	1.06	0.4059
Error	38	8.61718199	0.22676795		
Corrected Total	56	17.93470282			

*Means significantly different at $P \leq 0.05$

Table A.8 Analysis of Variance for Total Coliform Counts (TCC) (log CFU/g) of hybrid catfish fillets

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Size	1	0.61035628	0.61035628	0.50	0.4851
Process Steps	6	24.03869306	4.00644884	3.26*	0.0110
Block	5	13.70392075	2.74078415	2.23	0.0709
Size*Process Steps	6	8.29258480	1.38209747	1.13	0.3662
Error	38	46.66616157	1.22805688		
Corrected Total	56	91.86899747			

*Means significantly different at $P \leq 0.05$

Table A.9 Analysis of variance for moisture (%) (NIR) of hybrid catfish fillets

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Size	1	12.15080094	12.15080094	4.01*	0.0502
Process Steps	6	22.27328049	3.71221341	1.22	0.3081
Block	6	32.04920467	5.34153411	1.76	0.1240
Size*Process Steps	6	13.82191384	2.30365231	0.76	0.6048
Error	56	169.8832332	3.0336292		
Corrected Total	75	245.2391421			

*Means significantly different at $P \leq 0.05$

Table A.10 Analysis of variance for moisture content (oven) of before chilling (BC) fillets by sizes

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Size	1	10.58900297	10.58900297	5.79*	0.0470
Block	6	4.61930491	0.76988415	0.42	0.8442
Error	7	12.80414883	1.82916412		
Corrected Total	14	31.23153535			

*Means significantly different at $P \leq 0.05$ CV=1.74; HSD=1.69

Table A.11 Analysis of variance for moisture content (oven) of after injected (BC) fillets by sizes

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Size	1	8.16216056	8.16216056	21.56*	0.0434
Block	5	7.88332833	1.57666567	4.16	0.2049
Error	2	2.46203669	1.23101835		
Corrected Total	8	27.73177034			

*Means significantly different at $P \leq 0.05$ CV=1.37; HSD=1.78

Table A.12 Regression analysis for correlation between moisture (%) determined by NIR and oven method (AOAC approved method) of hybrid catfish fillets

Variable	Coefficient	Std. Error	t-statistic	Pr > |t|
Intercept	14.68689	2.71620	5.41	<.0001
Moisture (Oven)	0.77430	0.03415	22.67	<.0001
R-Squared	0.8741	**MSE**	0.41522	
Adjusted R-Squared	0.8724	**F-statistics**	513.97	
No. of observations	76	**Pr (F-statistics)**	<.0001	

Table A.13 Regression analysis for correlation between calculated retained water (%) from moisture determined by NIR and oven method (AOAC approved method) of hybrid catfish fillets

Variable	Coefficient	Std. Error	t-statistic	Pr > |t|
Intercept	2.99511	0.18682	16.03	<.0001
Retained water (%) (oven)	1.04874	0.06556	16.00	<.0001
R-Squared	0.8205	**MSE**	1.10355	
Adjusted R-Squared	0.8173	**F-statistics**	255.93	
No. of observations	58	**Pr (F-statistics)**	<.0001	

Table A.14 Regression analysis of model l for predicting retained water of hybrid catfish fillets during processing

Variable	Coefficient	Std. Error	t-statistic	Pr > |t|
Intercept	-5.57298	0.64447	-8.65	<.0001
Moisture-protein ratio	2.10743	0.11482	18.35	<.0001
Fat (%)	-0.68568	0.04010	-17.10	<.0001
weight (g)	0.00042745	0.00068984	0.62	0.5380
R-Squared	0.9567	**MSE**	0.10589	
Adjusted R-Squared	0.9544	**F-statistics**	419.36	
No. of observations	61	**Pr (F-statistics)**	<.0001	

Table A.15 Regression analysis of model 2 for predicting retained water of hybrid catfish fillets during processing

Variable	Coefficient	Std. Error	t-statistic	Pr > |t|
Intercept	-5.73469	0.58613	-9.78	<.0001
Moisture-protein ratio	2.13779	0.10329	20.70	<.0001
Fat (%)	-0.66955	0.03033	-22.07	<.0001
R-Squared	0.9564	**MSE**	0.10477	
Adjusted R-Squared	0.9549	**F-statistics**	635.59	
No. of observations	61	**Pr (F-statistics)**	<.0001	

Table A.16 Regression analysis for model 3 for predicting retained water of hybrid catfish fillets during processing

Variable	Coefficient	Std. Error	t-statistic	Pr > |t|
Intercept	-12.18393	1.54456	-7.89	<.0001
Moisture-protein ratio	2.77637	0.30142	9.21	<.0001
R-Squared	0.5898	**MSE**	0.96811	
Adjusted R-Squared	0.5829	**F-statistics**	84.84	
No. of observations	61	**Pr (F-statistics)**	<.0001	

Table A.17 Regression analysis for model 4 for predicting retained water of hybrid catfish fillets during processing

Variable	Coefficient	Std. Error	t-statistic	Pr > |t|
Intercept	-12.26687	1.25489	-9.78	<.0001
Moisture-protein ratio	3.02419	0.24893	12.15	<.0001
Weight (g)	-0.00718	0.00129	-5.57	<.0001
R-Squared	0.7334	**MSE**	0.64006	
Adjusted R-Squared	0.7242	**F-statistics**	79.78	
No. of observations	61	**Pr (F-statistics)**	<.0001	

Table A.18 Pearson Correlation of Coefficients of proximate composition of hybrid catfish fillets during processing

Pearson Correlation Coefficients, N = 76 Prob > |r| under H0: Rho=0						
	Moisture Oven	**Moisture NIR**	**Protein**	**Fat**	Predicted Moisture by NIR	**Moisture: Protein**
Moisture oven	1.00000	0.90767 <.0001	-0.63380 <.0001	-0.71116 <.0001	0.90767 <.0001	0.77873 <.0001
Moisture NIR	0.90767 <.0001	1.00000	-0.58927 <.0001	-0.82400 <.0001	1.00000 <.0001	0.76966 <.0001
Protein	-0.63380 <.0001	-0.58927 <.0001	1.00000	0.09050 0.4369	-0.58927 <.0001	-0.96697 <.0001
Fat	-0.71116 <.0001	-0.82400 <.0001	0.09050 0.4369	1.00000	-0.82400 <.0001	-0.32188 0.0046
Predicted Moisture by NIR	0.90767 <.0001	1.00000 <.0001	-0.58927 <.0001	-0.82400 <.0001	1.00000	0.76966 <.0001
Moisture: Protein	0.77873 <.0001	0.76966 <.0001	-0.96697 <.0001	-0.32188 0.0046	0.76966 <.0001	1.00000

APPENDIX B

PROXIMATE COMPOSITION, RETAINED WATER, AND BACTERIAL

LOAD TABLES

Table B.1 Proximate composition of selected finfish (both wild and cultured) other than Siluriformes

Species	Scientific name	Proximate composition				Culture/product	Origin	Ref
		Moisture	Protein	Fat	Ash			
Golden mullet	*Liza aurata*	76.7± 2.01	16.2±1.44	4.8±1.52	2.0±0.21	Raw Fish (350 g in weight) Mechanically Filleted	Black Sea, Turkey	Boran and Karaçam (2011)
Shad	*Alosa sapidissima*	61.5±4.98	16.4±2.46	19.7±8.30	2.0±0.71	Raw Fish Mechanically Filleted	Black Sea, Turkey	Boran and Karaçam (2011)
Horse mackrel	*Trachurus trachurus*	72.5±3.60	14.8±2.12	10.5±1.82	1.8±0.48	Raw Fish (50 g in weight) Mechanically Filleted	Black Sea, Turkey	Boran and Karaçam (2011)
Garfish	Belone belone	76.0±2.89	16.9±1.64	5.0±1.05	1.7±0.54	Raw Fish (90 g in weight) Mechanically Filleted	Black Sea, Turkey	Boran and Karaçam (2011)
Bobo croaker	*Pseudotolithus typus*	76.2 ± 0.57	16.2 ± 0.31	0.5 ± 0.05	7.3 ± 0.25	Raw Fish	Cameroonian coast	Njinkoue, et al. (2016)
Rainbow trout	*Oncorhynchus mykiss*	69.4 ± 0.17	1645 ± 0.13	11.4 ± 0.33	2.1 ± 0.25	Intensive Culture Raw Fish	Czech Republic	Linhartová et al. (2018)
Nile tilapia	*Oreochromis niloticus*	73.8± 1.65	18.0 ± 0.53	4.8 ± 0.85	1.6 ± 0.10	Intensive Culture Raw Fish	Czech Republic	Linhartová et al. (2018)

Table B.1 (Continued)

Species	Scientific name	Proximate composition				Culture/product	Origin	Ref
		Moisture	Protein	Fat	Ash			
Brook trout	*Salvelinus fontinalis*	67.± 1.95	18.5 ± 0.16	9.7 ± 1.81	2.8 ± 0.29	Intensive Culture Raw Fish	Czech Republic	Linhartová et al. (2018)
European perch	*Perca fluviatilis*	79.6 ± 0.61	16.6 ± 0.27	0.8 ± 0.05	1.7 ± 0.46	Semi-intensive Culture Raw Fish	Czech Republic	Linhartová et al. (2018)
Common carp	*Cyprinus carpio*	75.8 ± 1.81	17.6 ± 0.58	6.5 ± 1.30	1.8 ± 0.36	Semi-intensive Culture Raw Fish	Czech Republic	Linhartová et al. (2018)
Northern pike	*Esox lucius*	77.5 ± 0.25	18.6 ± 0.23	0.8 ± 0.13	2.2 ± 0.05	Semi-intensive Culture Raw Fish	Czech Republic	Linhartová et al. (2018)
Tench	*Tinca tinca*	76.0± 0.99	17.3 ± 0.50	4.2 ± 0.18	2.2 ± 0.53	Semi-intensive Culture Raw Fish	Czech Republic	Linhartová et al. (2018)
Grass carp	*Ctenopharyngodon idella*	74.1 ± 0.95	17.7 ± 0.41	5.1 ± 0.86	2.0 ± 0.15	Semi-intensive Culture Raw Fish	Czech Republic	Linhartová et al. (2018)
Silver carp	*Hypophthalmichthys molitrix*	73.5 ± 1.27	17.6 ± 0.11	5.8 ± 1.47	1.6 ± 0.22	Semi-intensive Culture Raw Fish	Czech Republic	Linhartová et al. (2018)

Table B.1 (Continued)

Species	Scientific name	Proximate composition				Culture/product	Origin	Ref
		Moisture	Protein	Fat	Ash			
Pikeperch	*Sander lucioperca*	78.8 ± 0.93	17.3 ± 0.95	0.8 ± 0.08	1.4 ± 0.11	Semi-intensive Culture Raw Fish	Czech Republic	Linhartová et al. (2018)
Rainbow trout	*Oncorhynchus mykiss*	74.0± 0.92	19.7 ± 0.84	2.6 ± 0.04	1.8 ± 0.51	Extensive Culture Raw Fish	Czech Republic	Linhartová et al. (2018)
Tench	*Tinca tinca*	75.8± 1.66	17.2 ± 1.63	3.7 ± 1.35	2.1 ± 0.71	Extensive Culture Raw Fish	Czech Republic	Linhartová et al. (2018)
Common carp	*Cyprinus carpio*	77.0 ± 1.22	17.6± 0.58	3.0 ± 0.73	1.6 ± 0.23	Extensive Culture Raw Fish	Czech Republic	Linhartová et al. (2018)

ND=Not determined

Table B.2 Proximate composition of selected Siluriformes

Species	Scientific name	Proximate composition				Culture/product	Origin	Ref
		Moisture	Protein	Fat	Ash			
African catfish	*Clarias gariepinus*	71.9 ± 0.07	19.5 ± 0.18	14.3 ± 0.19	3.1± 0.04	Raw fish (Mean weight-278.34±2.21g)	Minna, Nigeria	Chukwu & Shaba (2009)
Hybrid catfish	*(Clarias macrocephalus × Clarias gariepinus)*	75.7 ± 1.09	18.7 ± 0.32[a]	3.0 ± 0.07[a]	1.2 ± 0.01	Fillet (Store at 4 ^{0}C at Day 0)	Thailand	Chomnawang et al., 2007
Hybrid catfish	*(Clarias macrocephalus × Clarias gariepinus)*	75.2 ± 1.00	18.4 ± 0.50	3.0± 0.02	1.2 ± 0.03	Fillet (Store at 4 ^{0}C at Day 3)	Thailand	Chomnawang et al., 2007
Hybrid catfish	*(Clarias macrocephalus × Clarias gariepinus)*	75.1 ± 1.11	18.4± 0.65	3.0 ± 0.02	1.2± 0.01	Fillet (Store at 4 ^{0}C at Day 6)	Thailand	Chomnawang et al., 2007
Hybrid catfish	*(Clarias macrocephalus × Clarias gariepinus)*	74.1 ± 1.36	17.7 ± 0.40	3.0 ± 0.03	1.2 ± 0.01	Fillet (Store at 4 ^{0}C at Day 9)	Thailand	Chomnawang et al., 2007
Hybrid catfish	*(Clarias macrocephalus × Clarias gariepinus)*	74.1 ± 1.36	17.4 ± 0.46	3.0 ± 0.03	1.2± 0.02	Fillet (Store at 4 ^{0}C at Day 12)	Thailand	Chomnawang et al., 2007

Table B.2 (Continued)

Species	Scientific name	Proximate composition				Culture/product	Origin	Ref
		Moisture	Protein	Fat	Ash			
Pangasius	*Pangasius hypophthalmus*	79.0±1.1	18.8±1.1	2.7±0.9	0.9 ± 0.1	Culture (Whole skin)	Vietnam	Manthey-Karl et al., 2016
Hybrid catfish	*(Clarias macrocephalus × Clarias gariepinus)*	73.7 ± 1.08	17.3 ± 0.55	3.0 ± 0.03	1.2 ± 0.04	Fillet (Store at 4 ^{0}C at Day 15)	Thailand	Chomnawang et al., 2007
Pangasius	*Pangasius hypophthalmus*	83.8±85.59	12.5±14.52	1.1±1.65	0.8±2.38	Frozen Fillet	Vietnam	Guimarães et al. (2016)
African catfish	*Clarias gariepinus*	73.7 ± 2.08	ND	ND	ND	Raw Fish	Nigeria	Olaniyi et al. (2017)
African catfish	*Heterobranchus bidorsalis*	76.3 ± 12.74	ND	ND	ND	Raw Fish	Nigeria	Olaniyi et al. (2017)
Hybrid catfish	*Clarias gariepinus× Heterobranchus bidorsalis*	77.3 ± 6.03	ND	ND	ND	Raw Fish	Nigeria	Olaniyi et al. (2017)

Table B.2 (Continued)

Species	Scientific name	Proximate composition				Culture/product	Origin	Ref
		Moisture	Protein	Fat	Ash			
Reciprocal hybrid catfish	*Clarias gariepinus*× *Heterobranchus bidorsalis*	77.7 ± 4.16	ND	ND	ND	Raw Fish	Nigeria	Olaniyi *et al.* (2017)
African catfish	*Clarias gariepinus*	69.3 ± 0.52	17.5 ± 1.18	9.7 ± 1.93	1.1 ± 0.08	Intensive Culture Raw Fish	Czech Republic	Linhartová et al. (2018)
Wels catfish	*Silurus glanis*	74.6 ± 0.15	16.0 ± 0.17	3.3 ± 1.08	2.2 ± 0.05	Intensive Culture Raw Fish	Czech Republic	Linhartová et al. (2018)
Wels catfish	*Silurus glanis*	79.2± 1.00	16.8 ± 1.03	0.8 ± 0.06	1.6 ± 0.45	Semi-intensive Culture Raw Fish	Czech Republic	Linhartová et al. (2018)

ND=Not determined

Table B.3 Proximate composition of selected channel (*Ictalurus punctatus*) and hybrid (*Ictalurus furcatus*× *Ictalurus punctatus*) catfish

Species	Scientific name	Proximate composition				Culture/product	Origin	Ref
		Moisture	Protein	Fat	Ash			
Channel catfish	*Ictalurus punctatus*	75.0	21.0	3.0	1.22	Canned, tuna-style	Mississippi	Drake et al. (1971)
Channel catfish	*Ictalurus punctatus*	74.3-77.0	15.4-16.8	6.9-8.4	0.9-0.94	Dressed (150 sample)	Mississippi	Fisher and Ammerman (1983)
Channel catfish	*Ictalurus punctatus*	77.8	15.4	4.5	1.2	Cultured whole Raw fish	Mississippi	Mustafa and Medeiros (1985)
Channel catfish	*Ictalurus punctatus*	71.1	20.4	6.3	1.4	0ven cooked/baked	Mississippi	Mustafa and Medeiros (1985)
Channel catfish	*Ictalurus punctatus*	62.7	19.0	7.9	1.6	Breaded and Fried	Mississippi	Mustafa and Medeiros (1985)
Channel catfish	*Ictalurus punctatus*	80.3	16.1	3.6	1.0	Raw wild fish July 1985	Charleston, North Carolina Coast	Gooch et al. (1987)
Channel catfish	*Ictalurus punctatus*	83.7	16.2	1.3	1.0	Raw wild fish September 1985	Charleston, North Carolina Coast	Gooch et al. (1987)
Channel catfish	*Ictalurus punctatus*	82.4	16.2	2.3	0.9	Raw wild fish November 1985	Charleston, North Carolina Coast	Gooch et al. (1987)
Channel catfish	*Ictalurus punctatus*	76.4 ± 0.37	18.2 ± 0.44	4.3 ± 0.41	1.3± .05	Raw, edible portion	US	Exler (1987).
Channel catfish	*Ictalurus punctatus*	76.4 (Average)	15.6 (Average)	6.9 (Average)	1.0 (Average)	Fillet (size 0.45-0.68 kg)	Mississippi	Nettleton et al. (1990)

Table B.3 (Continued)

Species	Scientific name	Proximate composition				Culture/product	Origin	Ref
		Moisture	Protein	Fat	Ash			
Channel catfish	*Ictalurus punctatus*	74.4	16.1	7.7	1.1	Fillet (size 0.45-0.68 kg) Fall	Mississippi	Nettleton et al. (1990)
Channel catfish	*Ictalurus punctatus*	77.4	15.4	6.2	0.8	Fillet (size 0.45-0.68 kg) Winter	Mississippi	Nettleton et al. (1990)
Channel catfish	*Ictalurus punctatus*	77.8	15.4	6.4	0.9	Fillet (size 0.45-0.68 kg) Spring	Mississippi	Nettleton et al. (1990)
Channel catfish	*Ictalurus punctatus*	76.0	15.7	7.4	0.9	Fillet (size 0.45-0.68 kg) Summer	Mississippi	Nettleton et al. (1990)
Channel catfish	*Ictalurus punctatus*	70.7	14.9	13.7	1.2	Fillet Lateral side	Mississippi	Freeman (1990)
Channel catfish	*Ictalurus punctatus*	70.5	16.1	12.1	1.0	Fillet Skin side	Mississippi	Freeman (1990)
Channel catfish	*Ictalurus punctatus*	82.5	15.6	1.1	0.99	Fillet Visceral side	Mississippi	Freeman (1990)
Channel catfish	*Ictalurus punctatus*	68.1	17.0	13.2	1.9	Whole, dressed, Frozen fillet (small size .30kg)	Stoneville, Mississippi	Silva and Ammerman (1993)
Channel catfish	*Ictalurus punctatus*	70.8	17.1	10.8	1.8	Whole, dressed, Frozen fillet (large size 1.0 kg)	Stoneville, Mississippi	Silva and Ammerman (1993)

Table B.3 (Continued)

Species	Scientific name	Proximate composition				Culture/product	Origin	Ref
		Moisture	Protein	Fat	Ash			
Channel catfish	*Ictalurus punctatus*	76.4	18.2	4.3	1.3	Cultured whole fish	Mississippi	Silva and Chamul (2000).
Channel catfish	*Ictalurus punctatus*	77.3±0.4	16.3±0.4	5.4±0.3	1.1±0.03	Manually fillet	Mississippi	Robinson & Oberle (2001).
Hybrid Catfish	(*Ictalurus furcatus*) × *Ictalurus punctatus*)	80.2	15.0	2.4	ND	Juvenile, raw fish	Mississippi	Bosworth et al. (1998)
Hybrid Catfish	(*Ictalurus furcatus*) × *Ictalurus punctatus*)	77.7±2.12	ND	7.0±1.69	ND	Raw. Dressed, manually fillet	Mississippi	Bosworth et al. (2001)
Hybrid Catfish	(*Ictalurus furcatus*) × *Ictalurus punctatus*)	71.4±1.02	ND	11.0±1.44	ND	Whole undressed fish	Mississippi	Bosworth et al. (2001)
Hybrid Catfish	(*Ictalurus furcatus*) × *Ictalurus punctatus*)	73.2	17.3	8.59	ND	Raw. Dressed, frozen, manually fillet	Mississippi	Li and Robinson (2007)

ND=Not determined

Table B.4 Mean proximate composition and bacterial load of hybrid catfish fillet regardless of size

Process Steps	N	Label	N	Mean	Std Dev	Minimum	Maximum
BT	15	Protein	15	16.70	0.50	15.54	17.42
		Fat	15	5.68	1.60	3.97	10.30
		MoistureOven	15	77.78	1.33	74.51	79.92
		MoistureNIR	15	74.83	1.37	71.36	76.53
		RetainedWaterOven	0	.	.	.	.
		RetainedWaterNIR	0	.	.	.	.
		Predicted retained water	0	.	.	.	.
		Moisture:Protein	15	4.67	0.14	4.39	4.98
		Psychrotrophic Count (logcfu/g)	11	4.03	0.51	3.18	4.80
		Total Coliform (logcfu/g)	11	1.64	1.35	0.00	3.08
BC	15	Protein	15	16.81	0.49	15.95	17.52
		Fat	15	5.16	1.66	3.06	8.50
		MoistureOven	15	77.72	1.49	74.72	80.34
		MoistureNIR	15	75.03	1.34	72.71	76.85
		RetainedWaterOven	15	0.01	1.69	-3.19	2.81
		RetainedWaterNIR	15	0.43	1.86	-1.97	4.72
		Predicted retained water	15	0.68	1.17	-1.34	2.27
		Moisture: Protein	15	4.66	0.15	4.46	4.98
		Psychrotrophic Count (logcfu/g)	12	4.34	0.42	3.72	5.35
		Total Coliform (logcfu/g)	12	2.30	0.82	0.00	3.11

Table B.4 (continued)

Process Steps	N	Label	N	Mean	Std Dev	Minimum	Maximum
AC	10	Protein	10	16.06	0.51	15.24	16.66
		Fat	10	4.54	1.35	2.64	7.43
		MoistureOven	10	79.84	1.57	76.92	82.13
		MoistureNIR	10	76.58	1.44	73.62	78.72
		RetainedWaterOven	10	2.38	1.53	-0.72	4.42
		RetainedWaterNIR	10	1.92	1.46	-0.20	4.32
		Predicted retained water	10	2.03	1.25	-0.55	3.90
		Moisture: Protein	10	4.98	0.20	4.76	5.34
		Psychrotrophic Count (logcfu/g)	10	4.17	0.42	3.38	4.66
		Total Coliform (logcfu/g)	10	1.46	1.29	0.00	3.04
AS	11	Protein	11	14.91	0.71	14.16	16.23
		Fat	11	3.81	0.92	2.49	5.47
		MoistureOven	11	81.99	1.40	79.14	84.18
		MoistureNIR	11	78.11	1.10	76.18	79.59
		RetainedWaterOven	11	3.82	1.74	0.33	6.25
		RetainedWaterNIR	11	2.89	2.04	-0.83	5.30
		Predicted retained water	11	3.37	0.96	1.68	4.65
		Moisture: Protein	11	5.49	0.32	4.95	5.85
		Psychrotrophic Count (logcfu/g)	7	4.24	0.21	3.90	4.43
		Total Coliform (logcfu/g)	7	1.27	1.21	0.00	2.60
BIP	9	Protein	9	16.30	0.61	15.43	17.33
		Fat	9	5.35	1.37	3.87	7.28
		MoistureOven	9	79.21	1.77	76.97	81.89
		MoistureNIR	9	75.31	1.29	73.42	77.22
		RetainedWaterOven	9	1.19	2.03	-2.05	5.00
		RetainedWaterNIR	9	0.33	1.24	-1.14	2.03
		Predicted retained water	9	0.92	1.13	-0.72	2.59
		Moisture: Protein	9	4.82	0.20	4.55	5.22
		Psychrotrophic Count (logcfu/g)	7	4.28	0.91	3.14	6.15
		Total Coliform (logcfu/g)	7	1.56	1.51	0.00	3.15

Table B.4 (continued)

Process Steps	N	Label	N	Mean	Std Dev	Minimum	Maximum
AI	9	Protein	9	14.67	0.61	13.31	15.19
		Fat	9	5.10	1.45	2.87	6.96
		MoistureOven	9	80.98	1.86	78.23	84.00
		MoistureNIR	9	77.46	1.41	74.99	79.92
		RetainedWaterOven	9	2.60	1.63	0.32	5.14
		RetainedWaterNIR	9	2.68	1.11	1.22	4.34
		Predicted retained water	9	2.79	1.23	0.64	4.94
		Moisture: Protein	9	5.52	0.25	5.29	6.09
		Psychrotrophic Count (logcfu/g)	5	5.08	0.78	4.18	6.10
		Total Coliform (logcfu/g)	5	2.96	0.73	2.30	4.15
AF	7	Protein	7	14.81	0.38	14.26	15.25
		Fat	7	4.52	1.36	2.83	6.78
		MoistureOven	7	81.04	1.09	79.06	82.40
		MoistureNIR	7	77.65	1.11	75.95	78.97
		RetainedWaterOven	7	3.09	1.02	1.80	4.93
		RetainedWaterNIR	7	2.74	1.40	0.82	4.88
		Predicted retained water	7	2.96	0.96	1.48	4.11
		Moisture: Protein	7	5.48	0.15	5.29	5.68
		Psychrotrophic Count (logcfu/g)	5	4.22	0.12	4.05	4.40
		Total Coliform (logcfu/g)	5	0.54	1.21	0.00	2.70

Table B.5 Mean proximate composition and bacterial load of hybrid catfish fillets by size and process steps

Process Steps	Size	N Obs	Variable	Label	N	Mean	Std Dev	Minimum	Maximum
BT	Large	7	Protein	Protein	7	16.72	0.57	15.54	17.17
			Fat	Fat	7	6.76	1.72	5.26	10.30
			MoistureOven	MoistureOven	7	76.84	1.15	74.51	77.91
			RetainedWaterOven	RetainedWaterOven	0	.	.	.	.
			PPC	Psychrotrophic Count (logcfu/g)	5	4.00	0.61	3.48	4.80
			TCC	Total Coliform (logcfu/g)	5	1.65	1.52	0.00	3.08
	Small	8	Protein	Protein	8	16.69	0.47	16.04	17.42
			Fat	Fat	8	4.73	0.64	4.0	6.20
			MoistureOven	MoistureOven	8	78.60	0.87	77.47	79.92
			RetainedWaterOven	RetainedWaterOven	0	.	.	.	.
			PPC	Psychrotrophic Count (logcfu/g)	6	4.06	0.48	3.18	4.46
			TCC	Total Coliform (logcfu/g)	6	1.63	1.33	0.00	3.08
BC	Large	6	Protein	Protein	6	16.72	0.64	15.95	17.50
			Fat	Fat	6	6.34	1.60	4.85	8.50
			MoistureOven	MoistureOven	6	76.54	1.32	74.72	77.91
			RetainedWaterOven	RetainedWaterOven	6	-0.34	2.25	-3.19	2.81
			PPC	Psychrotrophic Count (logcfu/g)	5	4.46	0.61	3.72	5.35
			TCC	Total Coliform (logcfu/g)	5	1.98	1.18	0.00	3.11
	Small	9	Protein	Protein	9	16.87	0.39	16.43	17.52
			Fat	Fat	9	4.38	1.22	3.06	6.43
			MoistureOven	MoistureOven	9	78.50	1.04	76.94	80.34
			RetainedWaterOven	RetainedWaterOven	9	0.25	1.30	-2.08	2.81
			PPC	Psychrotrophic Count (logcfu/g)	7	4.26	0.22	4.04	4.72
			TCC	Total Coliform (logcfu/g)	7	2.53	0.38	2.00	3.11

Table B.5 (continued)

Process Steps	Size	N Obs	Variable	Label	N	Mean	Std Dev	Minimum	Maximum
AC	Large	5	Protein	Protein	5	16.05	0.47	15.65	16.66
			Fat	Fat	5	5.31	1.44	4.08	7.43
			MoistureOven	MoistureOven	5	79.02	1.38	76.92	80.71
			RetainedWaterOven	RetainedWaterOven	5	2.63	0.88	2.02	4.16
			PPC	Psychrotrophic Count (logcfu/g)	5	3.95	0.47	3.38	4.53
			TCC	Total Coliform (logcfu/g)	5	0.90	1.24	0.00	2.48
	Small	5	Protein	Protein	5	16.06	0.61	15.24	16.64
			Fat	Fat	5	3.76	0.75	2.64	4.72
			MoistureOven	MoistureOven	5	80.66	1.40	78.74	82.13
			RetainedWaterOven	RetainedWaterOven	5	2.14	2.08	-0.72	4.42
			PPC	Psychrotrophic Count (logcfu/g)	5	4.38	0.24	4.06	4.66
			TCC	Total Coliform (logcfu/g)	5	2.02	1.20	0.00	3.04
AS	Large	3	Protein	Protein	3	14.59	0.38	14.16	14.87
			Fat	Fat	3	4.52	0.85	3.85	5.47
			MoistureOven	MoistureOven	3	81.68	0.17	81.50	81.81
			RetainedWaterOven	RetainedWaterOven	3	4.23	0.72	3.80	5.06
			PPC	Psychrotrophic Count (logcfu/g)	2	4.26	0.21	4.11	4.41
			TCC	Total Coliform (logcfu/g)	2	0.00	0.00	0.00	0.00
	Small	8	Protein	Protein	8	15.03	0.79	14.21	16.23
			Fat	Fat	8	3.55	0.84	2.49	5.21
			MoistureOven	MoistureOven	8	82.10	1.66	79.14	84.18
			RetainedWaterOven	RetainedWaterOven	8	3.66	2.01	0.33	6.25
			PPC	Psychrotrophic Count (logcfu/g)	5	4.23	0.23	3.90	4.43
			TCC	Total Coliform (logcfu/g)	5	1.78	1.03	0.00	2.60

Table B.5 (Continued)

Process Steps	Size	N Obs	Variable	Label	N	Mean	Std Dev	Minimum	Maximum
BIP	Large	3	Protein	Protein	3	16.20	0.57	15.60	16.74
			Fat	Fat	3	6.68	0.92	5.62	7.28
			MoistureOven	MoistureOven	3	78.95	2.56	77.22	81.89
			RetainedWaterOven	RetainedWaterOven	3	1.83	2.84	-0.49	5.00
			PPC	Psychrotrophic Count (logcfu/g)	3	4.45	1.54	3.14	6.15
			TCC	Total Coliform (logcfu/g)	3	1.95	1.70	0.00	3.15
	Small	6	Protein	Protein	6	16.35	0.67	15.43	17.33
			Fat	Fat	6	4.69	1.04	3.87	6.65
			MoistureOven	MoistureOven	6	79.35	1.52	76.97	81.40
			RetainedWaterOven	RetainedWaterOven	6	0.87	1.72	-2.05	2.65
			PPC	Psychrotrophic Count (logcfu/g)	4	4.15	0.17	4.03	4.39
			TCC	Total Coliform (logcfu/g)	4	1.27	1.53	0.00	3.08
AI	Large	4	Protein	Protein	4	14.44	0.76	13.31	14.94
			Fat	Fat	4	6.16	1.01	4.72	6.96
			MoistureOven	MoistureOven	4	79.68	1.76	78.23	81.89
			RetainedWaterOven	RetainedWaterOven	4	2.37	2.31	0.32	5.14
			PPC	Psychrotrophic Count (logcfu/g)	3	5.60	0.44	5.32	6.10
			TCC	Total Coliform (logcfu/g)	3	3.08	0.96	2.30	4.15
	Small	5	Protein	Protein	5	14.86	0.45	14.17	15.19
			Fat	Fat	5	4.25	1.18	2.87	5.93
			MoistureOven	MoistureOven	5	82.03	1.25	80.68	84.00
			RetainedWaterOven	RetainedWaterOven	5	2.78	1.10	1.66	4.08
			PPC	Psychrotrophic Count (logcfu/g)	2	4.30	0.17	4.18	4.41
			TCC	Total Coliform (logcfu/g)	2	2.80	0.45	2.48	3.11

Table B.5 (continued)

Process Steps	Size	N Obs	Variable	Label	N	Mean	Std Dev	Minimum	Maximum
AF	Large	4	Protein	Protein	4	14.78	0.48	14.26	15.25
			Fat	Fat	4	5.24	1.32	3.57	6.78
			MoistureOven	MoistureOven	4	80.41	0.99	79.06	81.23
			RetainedWaterOven	RetainedWaterOven	4	3.10	0.52	2.31	3.40
			PPC	Psychrotrophic Count (logcfu/g)	3	4.27	0.11	4.20	4.40
			TCC	Total Coliform (logcfu/g)	3	0.90	1.56	0.00	2.70
	Small	3	Protein	Protein	3	14.85	0.29	14.63	15.17
			Fat	Fat	3	3.56	0.71	2.83	4.25
			MoistureOven	MoistureOven	3	81.89	0.45	81.55	82.40
			RetainedWaterOven	RetainedWaterOven	3	3.08	1.64	1.80	4.93
			PPC	Psychrotrophic Count (logcfu/g)	2	4.14	0.12	4.05	4.23
			TCC	Total Coliform (logcfu/g)	2	0.00	0.00	0.00	0.00