Doctoral Dissertation

Development of Dried Tigertooth Croaker (*Otolithes ruber*) Obtained from Different Salting and Drying Conditions in San Miguel Bay, Philippines

フィリピンサンミゲル湾で漁獲されるTigertooth Croakerの干物の開発のための塩漬および乾燥条件の検討

by

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Additional papers

1. Takahiro Taguchi, Erika Tagami, Takuma Mezaki, Joshua Magnaye Vacarizas, Katrina Lasalita Canon, Teresa Narvaez Avila, Dan Anthony Uy Bataan, Akira Tominaga, Satoshi Kubota (2020). Karyotypic mosaicism and molecular cytogenetic markers in the scleractinian coral *Acropora pruinosa* Brook, 1982 (Hexacorallia, Anthozoa, Cnidaria). Coral Reefs, 39: 1415–1425.

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List of Conference Presentations

1.Teresa N. Avila, Raul G. Bradecina, and Satoshi Kubota, 13th International Kuroshio Science Symposium / Tuguegarao city, Cagayan, Philippines, November 19 – 21, 2019

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Chapter 1: General Introduction

1.1 Bicol region and its fisheries

The Bicol region is situated in the southern part of Luzon, the largest island in the Philippine archipelago. It is composed of four mainland provinces (Camarines Norte, Camarines Sur, Albay, and Sorsogon) and two island provinces (Catanduanes and Masbate). Having a large amount of rich flatland, agriculture is the largest component of the economy, followed by commercial fishing. The major industries of the Bicol region are the abaca (Bicol hemp), coconut, and pili. The area is also known for dishes cooked in coconut milk or garnished with small pepper, which are often associated with the region (Gilbas, 2015).

Fishing activities in the Bicol region is mainly municipal fishing, including fishing for sustenance and fishing in shallow areas. Bicol was the second leading contributor in municipal fisheries in the country in 2018 (Bureau of Fisheries and Aquatic Resources, 2019). However, small to medium-scale commercial fishing are also conducted in coastal waters. Municipal fishing in the region accounts for 126,771 metric tons (MT), while commercial fishing only produced 58,543 MT and aquaculture production was 71,275 MT in 2018. Fisheries production in the region contributes 5.9% of the total fisheries production in the Philippines. This production is translated to PhP 10.6 billion in terms of value for municipal fisheries and PhP 3.3 million for commercial fisheries. These values show the significance of municipal fishing in the region, compared to other regions in the Philippines (DA-BFAR, 2019).

Bicol region is surrounded by sixteen (16) major fishing grounds, seven of which are among the richest in the country, namely: Lagonoy Gulf, Sorsogon Bay,

Albay Gulf, San Miguel Bay, Lamon Bay, Ragay Gulf and Asid Gulf (in the Visayan Sea) (Santos et al., 2017). Fishing operations are affected by the southwest monsoon (November to March) and northeast monsoon (June to October). Different fishing gears are used by fishers to exploit the multispecies resources in these fishing grounds (Silvestre et al., 1995). Status of fisheries in the Bicol region are characterized by declining fish catch and higher fishing efforts (Nieves et al., 2009). Lagonoy Gulf is overfished due to heavy fishing pressure and growth overfishing. Unsustainable fishing practices due to unregulated use of fishing gears are continuously depleting the fishery resources in Lagonoy Gulf (Olaño et al., 2018). In San Miguel Bay, annual catch was dominated by anchovies, scads, sardines, slipmouths, and mackerels. The increase in the proportion of pelagic families, squids and pricanthids, and the decline of sciaenids in the catch reflect the heavy fishing pressure in the bay (Silvestre and Hilomen, 2004). These may have led to negative implications on the resource base and living conditions of the community.

The top 10 landed aquatic species in Bicol region by volume are the following: yellowfin tuna (*Thunnus albacares*), albacore tuna (*Thunnus alalunga*), bigeye scad (*Selar crumenopthalmus*), white sardines (*Escualosa thoracata*), bay sillago (*Sillago ingenuua*), short mackerel (*Rastrelliger brachysoma*), island mackerel (*Rastrelliger faughni*), bullet tuna (*Auxis rochei),* tigertooth croaker (*Otolithes ruber*), largehead hairtail (*Trichiurus lepturus*) (Santos et al., 2017). Focus on these fishery resources should be directed toward sustainable management and optimizing production through fish processing innovations.

1.2 Fish processing activities

In Lagonoy Gulf, there are livelihood activities based on fish processing to add value to fish catch. These include fermentation of siganids and anchovies, hot smoking or broiling of tuna and tuna-like fishes (Pelea, 2008). In San Miguel Bay, approximately 30% of all fish landed in SMB are processed before being sold to consumers. The most common form of fish processing is salting, usually in a brine solution, and sun-drying. Products include dried croaker, smoked round scad, herring, salted and dried sergestid shrimp (*Acetes* sp.) and anchovies (Esporlas, 1982). Fish drying activities during March through October when large volumes of fish and weather conditions are favorable. The marketing channels for processed fish are different than for fresh fish. Generally, middlemen specialize in one or the other. Products are marketed at a more leisurely fashion due to its storable condition (Esporlas, 1982).

1.3 Salting and drying processes

Of the various fish processing technologies available, salting and drying are widely adopted in the Bicol region. Drying is a process which involves simultaneous heat and mass transfer. The heat causes the evaporation of water from the surface and mass transfer of water from the interior to the surface of the fish (Jain, 2006). Drying works as a preservation technique due to the decrease of water and thereby the water activity. During natural drying, the fish is exposed to air and natural light. Natural drying can dry fish faster and the flesh becomes hard even though some moisture is still inside, slowing down the drying process and promoting protein

degradation. Exposure of fish for a long period of time to sunlight can oxidize the lipids, which can reduce nutritional quality (Smida et al., 2014).

Dry salting, brine salting and mixed salting are the commonly used methods in salting of fish. Salting is a process where the common salt, sodium chloride (NaCl), is used as a preservative that penetrates the fish tissues. The main purpose of salting is to separate the water from the fish and replace it with salt. Thus, the water concentration in the fish decreases. Chlorine and sodium ions are carried from brine to fish, and water dipoles are carried from fish to the environment. The rate during this process is high, and it slows during ripening (Binici & Kaya, 2018). The quality of the final product is influenced by many factors. This include salting techniques, ripening and drying time conditions, size and thickness of fish, and salt composition (Binici & Kaya, 2018; Sampels, 2015).

1.4 Salt reduction approach

The interest for highly salted dried products is gaining popularity among consumers despite the risk of consumption of high salt diets. The salt intake of adults is higher than the recommended amount by the World Health Organization (WHO) (DOST-FNRI, 2016; Lasco et al., 2020), which could be attributed to the dietary habits of the Filipinos. The major source of sodium in the diet of Filipinos are contributed by the condiments added during cooking (salt, soy sauce, fish sauce, monosodium glutamate, etc.), accounting for 76.3% of the total sodium intake, whereas whole foods contributed only 23.7% (Batcagan-Abueg et al., 2013, Lee, 2009). It has been reported that the prevalence of elevated blood pressure among Filipino adults has increased to 23.9% in 2015, wherein Bicol region exhibited

higher prevalence (26.2%) than the national estimate (DOST-FNRI, 2016). Hypertension is one of the risk factors for cardiovascular diseases, that is, as the blood pressure increases, the risk of heart attack, heart failure, stroke and kidney diseases also increases (DOST-FNRI, 2016). However, as health promotion campaigns focused on the modifiable risk factors of hypertension, such as improving diet and exercise become more popular, consumers' health consciousness also increases; therefore, increasing demand for low-salt food is expected. It is also speculated that dried fish, a source of dietary sodium in the Filipino diet, could be a potential vehicle for salt reduction hence, the knowledge of the chemical composition of a fish is essential for its product development.

1.5 Dissertation outline

One of the abundant fishery species at San Miguel Bay, Philippines, is a croaker locally known as “abo.” This croaker is popular and marketed as fresh fish and processed dried salted products. However, there has been limited information available on this important bioresource and products. The potential of the processed dried abo could be explored for sustainable fishery resource management and product development, therefore, characterization of abo and dried abo products were investigated.

This dissertation is composed of several studies that are arranged as follows:

Chapter 2 introduces the situation of the dried abo processing industry in the study site. By conducting interviews and review of secondary data, status of abo fishing, demographic profile of dried fish processors, and the present dried fish processing conditions were examined.

Chapter 3 presents the species identification of collected abo from San Miguel Bay. By means of molecular analysis of two mitochondrial genes, the species identification of abo was confirmed.

Chapter 4 highlights the proximate composition and salt content of laboratory-produced dried abo. The process flow gathered from the interview of dried fish processors was used as basis for the laboratory production of dried salted abo. Six groups of dried abo products with combination of salt concentration and drying time were investigated for its proximate composition and salt content.

Chapter 5 presents the protein profile of dried abo. The protein identification and biochemical changes as influenced by the different salt concentrations and drying times were evaluated. Characterization of the myofibrillar proteins, actin and myosin heavy chain, has also been studied.

Chapter 2: Dried abo processing situation in Calabanga, Camarines Sur, San Miguel Bay, Philippines

Abstract

San Miguel Bay (SMB), Philippines, is known for the production of dried salted abo products, as abo is an abundant fishery species in the bay regarded to be commercially important. Previous and present fish processing procedures in SMB have shown differences in terms of salting and drying conditions resulting in two different types of products. However, there have been no recent studies conducted on dried fish processing in SMB. Hence, this study focused on the evaluation of the current situation of abo fishing and dried abo processing, particularly in Calabanga, Camarines Sur. Data collection involved three sets of interviews conducted to dried fish processors, small-scale fisherman, and key informants, and review of secondary data. Gillnet is the main fishing gear used for abo. Landed catch of abo increased from 2016 – 2018 which was in contrast to the declining fish catch claimed by the respondents. Peak and lean months of abo production varied every year as influenced by monsoon seasons and heavy rains. Dried salted abo was found to be available year-round. The market price of dried abo products also varied depending on the season and size of fish. There was weak enforcement of the fishery rules and regulations, specifically on the closed fishing season of abo. The lack of formal organization among dried fish processors was also noted from the independent efforts of processors. Majority of dried fish processors are women and fish processing is their main source of income. Generally, dried fish production in Calabanga, Camarines Sur, are small-scale operations. The general process flow of

dried abo production involves initial washing of abo, removing the gut, splitting into butterfly fillet, soaking in brine, washing followed by sun-drying, packaging, and marketing. The standard salt concentration was found to be 8% salt concentration (w/w), whereas standard drying time was 12 h. Hence, highly salted and highly dried abo are the major products in SMB. This is despite awareness of the increasing preference of consumers for low-salt dried fish products. The lack of proper storage of raw materials and products, low income from processing, and declining fish stock were the main problems raised by the processors. Thus, the present situation of the dried fish processing industry in SMB necessitates the improvement of the processing conditions of abo to boost its potential as a high market value product and eventually help address the challenges of this industry.

2.1 Introduction

Dried fish products are widely accepted for their taste, nutritional quality, availability, and low cost. In the typical Filipino diet, fish is considered the third major component. The mean one-day household food consumption of fish and fish products was 392 g contributing 11.5% of the total food intake. The country's per capita consumption of fish and fish products in 2015 was estimated at 36.8 kg/year, wherein dried fish consumption was at 4.2 kg/year ([DOST-FNRI] Department of Science and Technology - Food and Nutrition Research Institute, 2016).

Among the major species inhabiting San Miguel Bay (SMB) (Fig. 2.1) is the fish species locally known as "abo" (Lim et al., 1995) (Fig. 2.2a). Production of dried-salted abo (Fig. 2.2b) is one of the fishing-associated economic activities in SMB. Hence, abo fisheries are among the leading industries in the bay (Lanzuela et al., 2020). At present, the commercially available dried abo is produced with a long drying time and brining at high concentration of salt, as highly dried and highly salty abo products are popular and preferred by the market. However, Yater et al. (1982) previously reported that traditional drying procedures in SMB follow a long soaking period in a salt solution and a short drying time. Therefore, dried abo can be categorized into two types according to the previous and recently adopted dried fish processing conditions. One type of dried abo has low moisture content, hence, it is highly dried. The other type, on the other hand, is the traditional one with high moisture content. However, both types have high salt content. To date, there have been no studies conducted on dried abo production in SMB.

The fish processing industry in the Philippines are faced with some issues such as losses in value of fish and fishery products due to poor handling practices,

sanitation and hygiene, which result in inferior quality of fishery products (Guevara and Camu, 1984). Thus, to maximize the potential of a fishery resource and product, postharvest processing methods should be examined to suit consumers' demands. The improvement of the quality of fishery products could help meet the demand of the domestic market and expand to the export market. Hence, this study aims to evaluate the situation of dried abo processing in Calabanga, Camarines Sur in SMB. Specifically, it aims to describe the status of abo fishing and fisheries management in the area, characterize the demographic profile of dried fish processors, and document the process flow of dried abo production.

2.2 Methods

2.2.1 Study area

The study was carried out in Calabanga, Camarines Sur, Philippines, one of the seven coastal municipalities bordering San Miguel Bay. It is the selected study site since one of the major fish landing sites of abo, Sabang fishing port, is found at Calabanga, Camarines Sur.

2.2.2 Data collection

Three sets of face-to-face interviews were conducted for this study. The first interview was conducted to nine dried fish processors who were purposively selected in February 2019. A questionnaire was designed to obtain the respondents' demographic information and their existing dried abo processing practices. Key informants such as dried fish processors and small-scale fisherman were also interviewed in May 2019 and September 2019. The interview guide covered

questions related to their knowledge on the status of abo fishing, fisheries management in SMB, fish processing, and marketing.

In addition, a key informant interview (KII) was also conducted to an officer from the Municipal Agriculture Office of the local government unit (LGU) of Calabanga, Camarines Sur in September 2019. This is to confirm the processors' responses and obtain information about the regulations and projects of the local government intended for the dried fish processing industry. Secondary data (unpublished) was obtained from the National Stock Assessment Program of the Bureau of Fisheries and Aquatic Resources (NSAP-BFAR) Region 5 through email communication. Survey data were collated and analyzed using simple frequency count and percentage technique.

2.3 Results

2.3.1 Status of abo fishing

2.3.1.1 Landed catch of abo in landing centers around SMB

Figure 2.3 presents the contribution of landed catch of abo from the two provinces surrounding SMB. Of the 14 landing centers, ten landing centers are found in Camarines Sur and four in Camarines Norte. NSAP-BFAR data (unpublished) indicated that the landing centers in Camarines Sur contributed the biggest share to the total landed catch of abo from 2015 – 2018 compared to the landing centers in Camarines Norte (Fig. 2.3). The three major landing centers with the highest contribution of the landed catch of abo with 40.56%, 15.25%, and 12.39% (data not shown) were found in Camarines Sur: Sabang, Calabanga; Castillo, Cabusao; and Sugod, Tinambac, respectively.

2.3.1.2 Seasonal variation of production of abo

According to the respondents, the occurrence of abo is whole year-round, but the peak months of abo could be observed between August and September while the lean months could be observed during January to March. On the other hand, the spawning season could fall from November to December.

Based on the collected NSAP-BFAR data (unpublished), the overall catch of abo in SMB reached 104.78 metric tons in 2018 (Fig. 2.4). Data indicated an increase in the landed catch of abo from 2016 – 2018. The monthly landed catch data (Fig. 2.5) exhibited the peak months and lean months. Peak months (months with the highest landed catch) were varying during the four years. In 2015, peak months were observed in May and August while March and May in 2016. The highest landed catch was recorded only in March 2017, whereas May and June in 2018. On the other hand, the lean months (months with the lowest landed catch) of abo were recorded in June and October (2015), July and December (2016), October to December (2017) and July to December (2018).

In addition, the fisherman respondent claimed that catch of abo is declining over the years. Low fish catch was attributed to competition with other fishing vessels, bad weather conditions, and the influence of monsoons, southwest monsoon (from November to April) and northeast monsoon (from May to October).

2.3.1.3 Fishing gears used for abo

Different types of gears exploit the fishery resources of SMB. In the case of abo, gillnet is the main fishing gear used by small-scale fishermen. Fig. 2.6 presents the landed catch of abo by fishing gear (BFAR, unpublished). Landed catch were

highest with drift gillnet, followed by trawl and bottom set gillnet. Drift gillnet and bottom set gill net are considered municipal fishing gears. Trawl, on the other hand, is among the commercial fishing gears. From 2016 to 2018, increasing landed catch of abo was exhibited using drift gillnet. In contrast, decreasing landed catch of abo were observed with bottom set gillnet.

2.3.1.4 Identification of abo

Respondents claimed that they could easily identify abo by its physical appearance. They can also distinguish abo from other related species such as "pagotpot" and "arakaak." Abo was described to be longer and without scales, whereas pagotpot was described as round and shorter in size than abo.

2.3.1.5 Selling price of raw and processed products

The selling price of fresh and processed abo products differ according to fish body size and season (Table 2.1). These prices were obtained from the dried fish processors based on the highest production months (peak months) and lowest production months (lean months). The reported prices of fresh fish were the prices bought by processors from the fish port. After processing, dried products command a higher price per kilogram than fresh fish. As expected, the price increased with increasing size of abo. During peak months, prices of dried abo ranged from Php 200.00 – 400.00 which was lower compared to the prices during the lean months.

2.3.2 Fisheries management

Closed season is the period during which the taking of specified fishery species by specified fishing gear is prohibited in a specified area or areas in Philippine waters (DA-BFAR, 1998). According to the respondents, they are not aware of a closed season for abo in SMB. On the other hand, the NSAP-BFAR data (unpublished) (R. Intia, personal communication, September 12, 2019) indicated that July to September is the closed season for abo fishing using trawls. Commercial trawls are prohibited in the municipal waters with depth less than 7 fathoms in the Philippines' Fisheries Code. However, respondents have claimed that illegal fishing still existed. This is despite the boat patrolling conducted to monitor the commercial waters and guard the fish sanctuaries in the area.

In addition, respondents have also reported that there is no active association among dried fish processors. Although fisherfolk organizations are well represented in the established Fisheries and Aquatic Resources Management Council (FARMC) at the municipal level, dried fish processors' organization needs to be established yet.

2.3.3 Dried abo processing conditions in Calabanga, Camarines Sur

2.3.3.1 Demographic profile of the dried fish processors

The demographic profile of the nine dried fish processors is presented in Table 2.2. The age distribution of dried fish processors ranged from 29 – 65 years old. The majority (44.44%) of them are 50 – 60 years old. Most of them are female (55.66%) and married (77.78%). 66.67% of the processors have more than five years of experience in dried fish processing, with fish processing as their main occupation (100%). Processors can be classified according to the volume of dried fish

production. The majority (66.67%) are small-scale processors, employing only a few workers in their operation.

2.3.3.2 Handling of collected fresh abo

Table 2.3 presents the handling of fresh abo before processing. The majority (88.9%) of the dried fish processors obtain their supply of abo directly from the fish port. *Bañera* is a conical plastic tub (approximately 35 – 40 kg capacity) commonly used for carrying the collected fish which is then filled with ice. The purchased fish are then transported to their own processing facility located short distances away from the Sabang fish port via tricycle. The majority (44.44%) of the processors do not have chilling equipment in their fish processing facility, while 33.33% have cold storage or chiller. Alternatively, they use styrofoam fish boxes for cold storage which are often refilled with ice. Processors refer to this as the "re-ice technique." Fresh and dried fish are packed during the rainy season and placed in the iced fish boxes with holes to serve as cold storage.

Based on the observation, ice are often discarded when fish are unloaded from the fishing boat at the landing port. The fish are either wrapped in plastic bags or placed directly in the *bañera.* Whisper bidding or "*bulungan*" (Fig. 2.7a) is a popular fish marketing practice participated by fisherfolk, fish traders, and processors wherein bids are whispered in secret to the broker. The bulk of fish will then be awarded to the highest bidder. The processors who could not win in the *bulungan* will have to purchase either from the winner or other sellers.

According to the processors during the second interview, about one-half to two *bañera* of fish are purchased by a processor. The cost of ice spent for

one *bañera* is around Php 20.00. On the other hand, depending on the number of *bañera,* transportation cost via tricycle is around Php 10.00 to 100.00.

2.3.3.3 Duration of the dried fish processing

Respondents pointed out that purchased fresh abo are processed into dried fish by one processing batch only. The duration of dried abo processing depends on the timing of obtaining fresh abo. If the fish were purchased early in the morning, processing would start upon arrival at their facility, while in case of processors purchased the fish during mid-day, processing can be started in the afternoon; thus, they will extend drying time the following day. On the other hand, if the fish is bought in the late afternoon, the fish will be subjected to the initial steps of processing including brining and will be continued for drying the next day. In addition, most of the processors do not practice sorting fish according to size and weighing before processing. According to them, all sizes of abo are acceptable for drying. Smaller fish are dried whole while medium-sized, and large-sized abo are butterfly-filleted before drying. However, respondents estimated that the best marketable size of dried abo could be between 15 – 18 cm.

2.3.3.4 Cleaning of abo

The butterfly-filleted or "*badi*" is the most produced dried abo (Table 2.4). Upon arrival at the fish processing facility, abo is transferred to another container and then washed with tap water. The fish are degutted, cut by splitting into butterfly fillet then transferred to another perforated plastic basket (Fig. 2.8a). Based on

observation, ice was no longer used at this point. The swim bladder or *"biti"* were also collected from the degutted abo and placed in another container for drying.

2.3.3.5 Brining conditions

The butterfly-filleted abo are then subjected to brine salting (Table 2.4). Fish are submerged in a prepared brine (Fig. 2.8b) known as "*birok*" placed in a plastic basin or box. The salt concentration of the brine solution ranged from 13.8 – 50%. Including the fish weight, the estimated salt concentration ranged from 7.1 to 12.5% (w/w) (data not shown). Brining time takes about one-half to two hours with majority of them (55.56%) practiced one-half hour brining time. After the brining period, fish are then rinsed in tap water wherein the majority (55.56%) of them practiced two times washing.

2.3.3.6 Drying conditions

Sun-drying is the traditional method of drying fish in SMB, wherein drying usually takes about 6 h to 12 h and majority of the processors practiced 12 h of drying (Table 2.5). Sun-drying (Fig. 2.8c) is done on "*kapin"* or bamboo racks which are placed in poles above the ground. The brined fish are put first with the flesh-side up on the racks. The endpoint of drying is determined by visual observation and with the hourly turning of the dried fish (Fig. 2.8d). The flesh is touched to monitor dryness.

2.3.3.7 Packaging and marketing

The packaging material being used by processors is presented in Table 2.5. The majority of the processors used polyethylene plastic (55.56%) as packaging material for the dried product, while only 22.22% of the processors used vacuum packaging.

The packaging and marketing system was clarified from the three processors. The dried products are covered with newspaper and stored in boxes at room temperature. These dried fish are then sold via house-to-house marketing or sold to the retailers in the local market. Other marketing outlets are the urban centers in the Bicol region, such as Naga City and Legazpi City. Some of the processors also supply their processed fish products to Metro Manila.

Generally, the process flow involves initial washing of abo, removal of the gut, splitting into butterfly fillet, soaking in brine solution, washing followed by sun-drying, packaging, and marketing (Fig. 2.9).

2.3.4 Cooking practices and product preferences among consumers

Frying is the cooking method for dried salted abo as recommended by all of the respondents. Rehydration of the dried fish prior to cooking is also recommended. On the other hand, 66.67% of the respondents perceive that low salt dried fish products are preferred by the consumers.

2.3.5 Problems encountered by the respondents

Among the issues raised by the processors related to fish processing include the lack of proper storage of raw materials and products. Processors also indicated

low income from fish processing, especially during unfavorable weather conditions. Moreover, declining fish stock are also one of their main concerns. Boat trawls that operate within the bay are regarded as a competition of the small-scale fishermen.

2.4 Discussion

2.4.1 Status of abo fishing

Sabang, Calabanga is the landing center that recorded the highest landed catch of abo since it is the main fishing port where a large number of people participate in the "*bulungan"* marketing system. The overall landed catch recorded the highest in 2018 during the four-year period which was in contrast to the respondents' observations that the fish stock is declining. This information can be confirmed with further assessment. Lanzuela et al. (2020) reported that the spawning potential ratio of abo in SMB is still above the limit reference points, which indicated that the stock of abo is still in good condition despite the fishing pressures experienced by this species. In addition, the recorded peak and lean months of abo production varied yearly. This can be attributed to the influence of monsoon seasons and heavy rains. Thus, the production of dried abo can be observed year-round. Depending on the season and size of fish, the price of dried fish products also varied. During the lean months, when the supply of abo is low, the cost of fresh and dried fish are expected to be higher. This suggests that in the case that fresh abo production would be sustained, and the value of dried abo products would be improved in terms of quality, hence, income of dried fish processors may be improved.

2.4.2 Fisheries management

Respondents have claimed that illegal fishing still existed; hence, the weak enforcement of the government fishery rules and regulations require attention. The closed season indicated in the unpublished NSAP-BFAR data (R. Intia, personal communication, September 12, 2019) is in agreement with the estimated spawning season of abo in the bay from July to November (Lanzuela et al., 2020). The closed season should be short but enforced during particular periods when a substantial number of adults can be guaranteed to spawn and a high probability of survival for the young. The three months closed fishing season would assure the stable supply of fishery products by allowing the fish species to reproduce and the juveniles to mature and eventually restore their valuable fish stocks. Furthermore, the establishment of a formal organization is not yet realized among dried fish processors; hence, they tend to have independent efforts when it comes to fish processing. The participation of processors would be also essential to facilitate the management of fishery resources and products in SMB.

2.4.3 Dried abo processing conditions in Calabanga, Camarines Sur

Demographic profile of respondents indicated that the prevailing respondents were women who play an important role in fish processing. The majority of the dried fish processors are 50 – 60 years old which may imply that processing activities are managed by active individuals who possesses strength and a reasonable level of maturity. The fish drying operation is labor-intensive in nature. The knowledge of the age structures of the dried fish processors is necessary for estimating the potential productive human resources (Hossen et al., 2020).

Small-scale processors tend to have only a small number of workers for the operation, whereas large-scale processors could employ several workers. Most fish processing facilities were operated by members of the same household. Since fish processing is the main source of income of the respondents, reliance on income earned from fish processing could present a major problem during seasons when the fish supply is poor. Engagement in other occupations is necessary to augment their income during the period of low catch and unsteady market prices (Omoruyi & Eronmhonbor, 2017).

Generally, dried fish production in Calabanga, Camarines Sur, are small-scale operations. Dried fish processing facilities in the study site were observed to be lacking with proper facilities for icing, transportation, and cold storage for handling raw materials and processed products. This might cause fish spoilage during distribution and processing and result in inferior quality of products or product rejects.

It is also interesting to note that processors commonly prepare brine solutions with high salt concentration (Table 4). The estimated salt concentration including the fish weight utilized by the processors ranged from 7.1 to 12.5% (w/w) wherein the standard salt concentration used was 8%. Consequently, highly salted products are supplied to the market. This could be attributed to the current demand and preference of consumers.

There was also no processing facility that uses modernized equipment for drying since the traditional sun-drying is still employed by all respondents. Sun-drying is a method of preservation that remains popular in the country due to low-capital investment and other advantages (Guevara and Camu, 1988). Long drying

time for about 12 h is the standard practice at Calabanga, Camarines Sur. In addition, although dried products are sorted according to size, products are not sorted in terms of quality. Dried fish could be less attractive to consumers, in cases of poor product handling and unsanitary processing than undamaged dried fish. This could result in lower market prices leading to more economic losses. In terms of packaging, only a few processors utilized vacuum packaging. The use of innovative packaging could also help in marketing high graded or quality dried fish. This might also be useful for logistic solutions where cold storage is not available.

2.4.4 Cooking practices and product preferences among consumers

The majority of the processors produced highly salted and highly dried abo. This is despite the processors' awareness that low salt dried fish are also now being preferred by most consumers. Rehydration of dried fish is being recommended to lessen the salty taste before frying. Low-salted dried abo, also known as *"padagos",* are produced by some processors only when there are extra fish on hand that needs to be processed immediately. However, the highly salted products are still the major products being supplied in the market. Production of dried fish with reduced level of salt could be an attractive alternative to improve the value of the existing dried abo products in SMB. In this way, it would also cater the demand of other consumers who prefer less salty dried fish. A high level of dietary sodium has been associated with raised blood pressure and risks to cardiovascular diseases (Brown et al., 2009). Hence, reformulation of the salt content could be one of the strategies that can be adopted to address the health concerns related to high salt consumption.

2.4.5 Problems encountered by the dried abo processing industry

The dried fish processing industry in Calabanga, Camarines Sur is faced with many problems which are either associated to fish processing or supply of raw materials. Issues that are related to fish processing could affect the quality of the dried products. Due to the lack of proper storage of raw materials and products, there is a high risk of contamination that could lead to poor quality of the products. Improvement of the sanitary conditions of the processing facilities needs to be highlighted. This would prevent insect infestation and nutritional losses. Thus, good manufacturing practices should be adopted. Guevara and Camu (1988) and Espejo-Hermes (2004) also presented similar issues and problems in the industry, including shortage of raw materials, poor quality of raw materials, inconsistent quality of products, lack of appropriate safety standards, and lack of infrastructure for the products. Moreover, low income from processing was also a leading concern by the processors. This could be addressed by improving the processing conditions to produce highly graded dried products. On the other hand, the declining fish stock that the respondents associated to the operation of commercial trawls was also one of the reported concerns. Since trawling could scrape the bottom part of the bay, fish cannot escape, and even juveniles are exploited. Hence, reduced income is one of the consequences experienced by municipal fishers. Intensifying the implementation of existing laws and regulations by the government could help manage the declining fish catch in SMB.

The local government unit of Calabanga, Camarines Sur, supports the dried fish processing industry through implementation of projects intended for the development of this sector. These include the construction of a fish processing

facility and provision of fish carts for the registered fisherfolk. However, the government should facilitate capacity development trainings and provide logistical support in order to address the challenges being faced by the processors.

The current scenario of the dried abo processing in Calabanga, Camarines Sur necessitates the improvement of the dried abo products to boost and maximize its potential as an important fishery product in San Miguel Bay, and eventually increase its market value. Thus, investigating the chemical composition of dried abo through food analytical methods would be essential for its development.

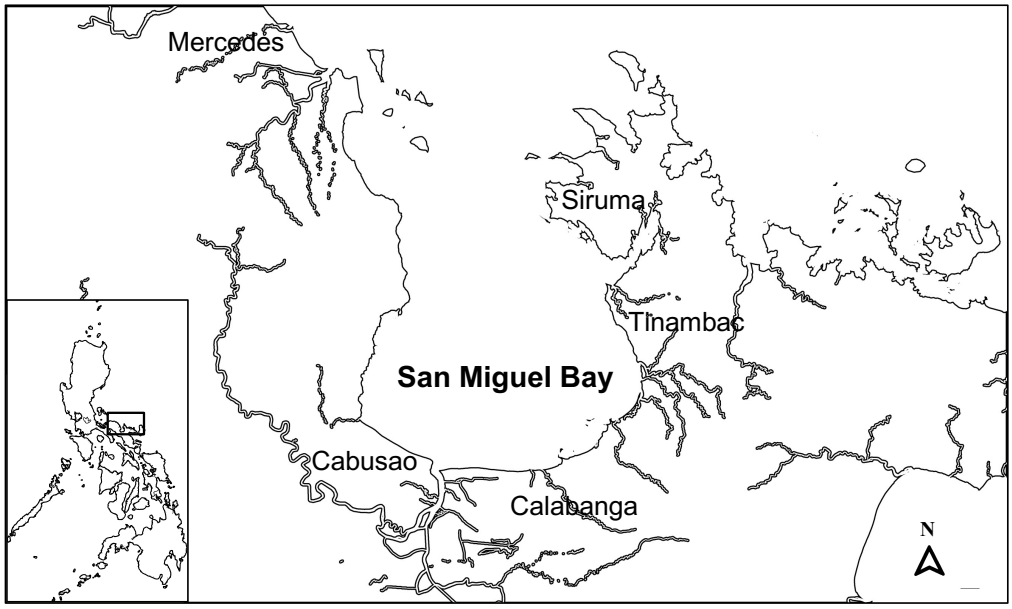


Figure 2.1 Map of San Miguel Bay, Philippines

(**a**) (**b**)

Figure 2.2 Target fishery resource and product in the study

(a) fresh abo as marketed in the local market; **(b)** commercially available dried abo

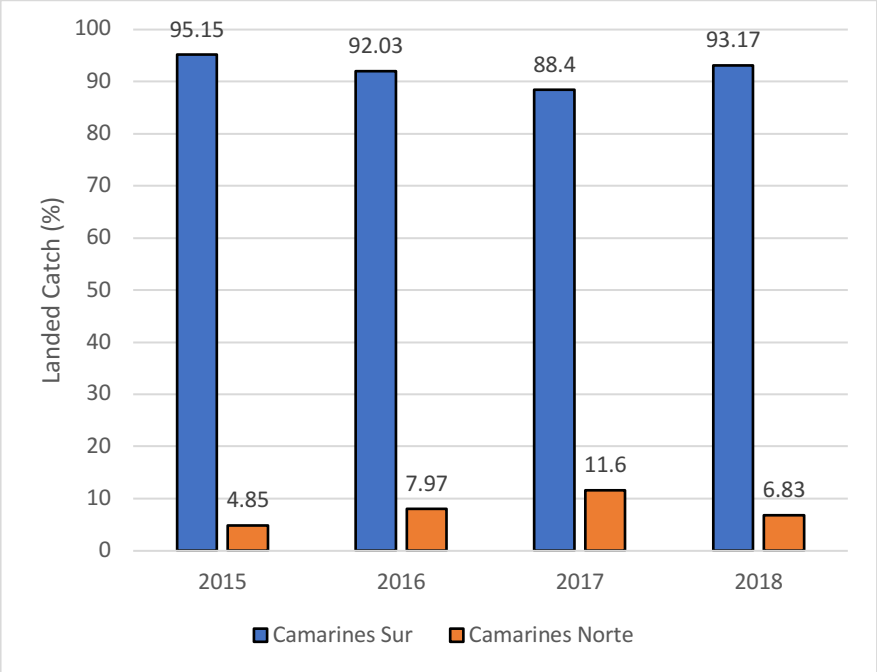


Figure 2.3 Contribution of landed catch of abo by province from 2015 – 2018 (NSAP-BFAR, 2019, unpublished)

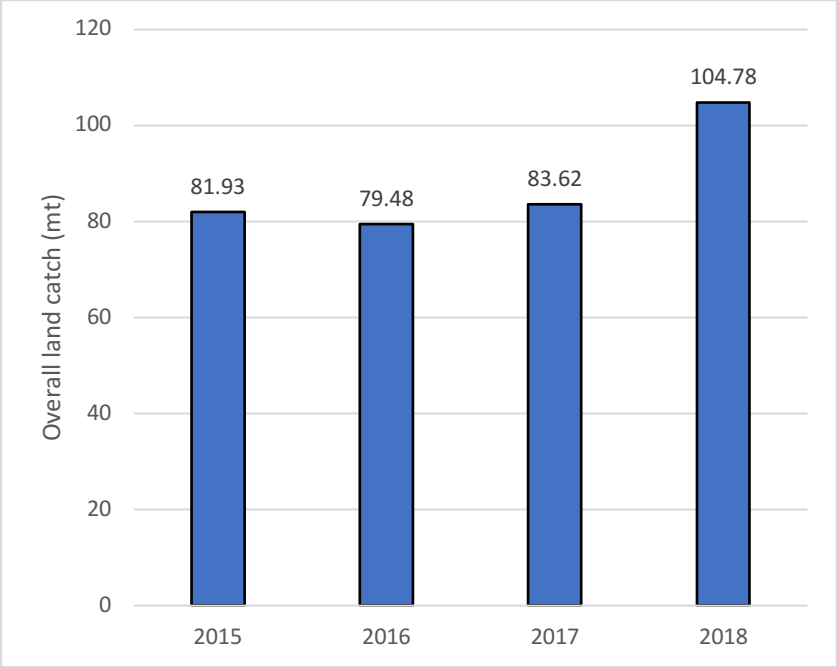


Figure 2.4 Overall landed catch of abo (in mt) from 2015 – 2018 (NSAP-BFAR, 2019, unpublished)

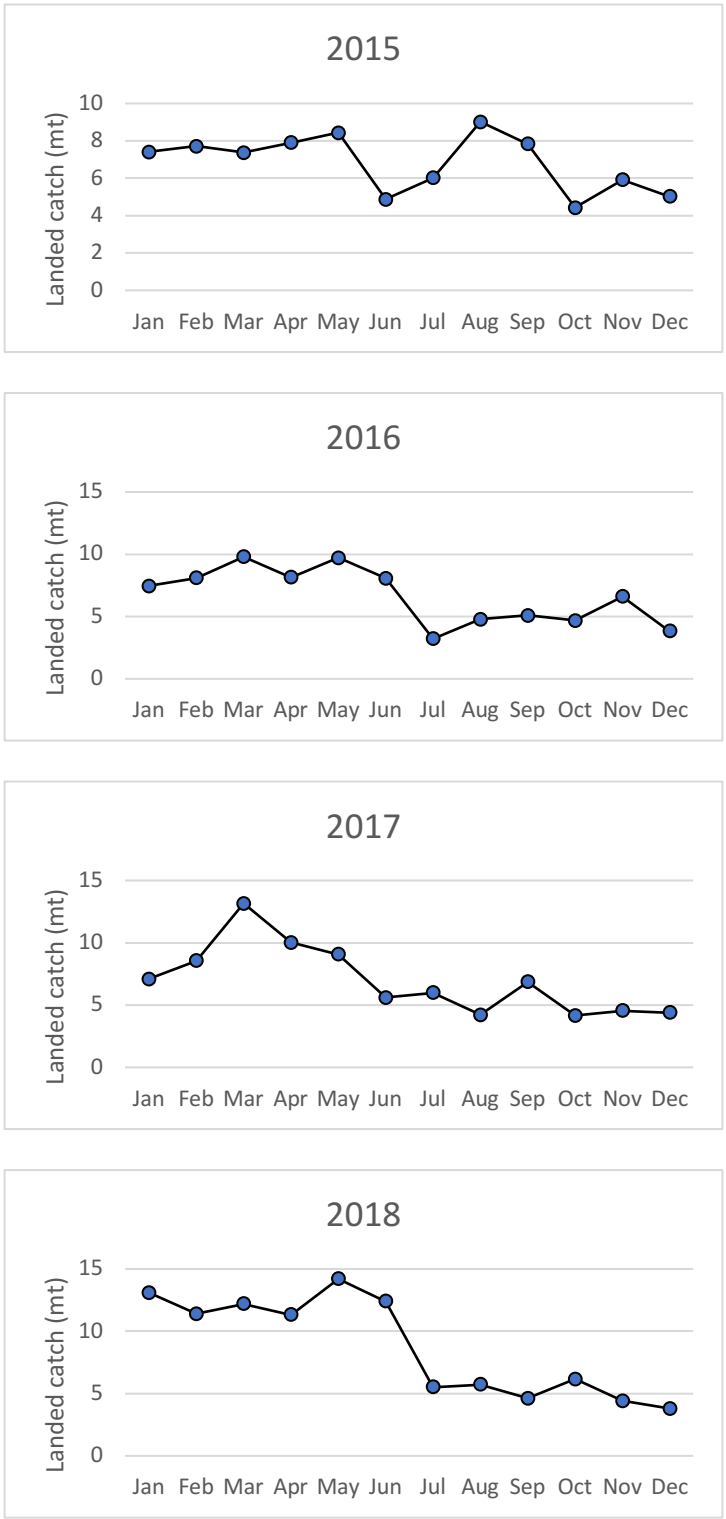


Figure 2.5 Landed catch trend of abo from 2015 – 2018 (NSAP-BFAR, 2019, unpublished)

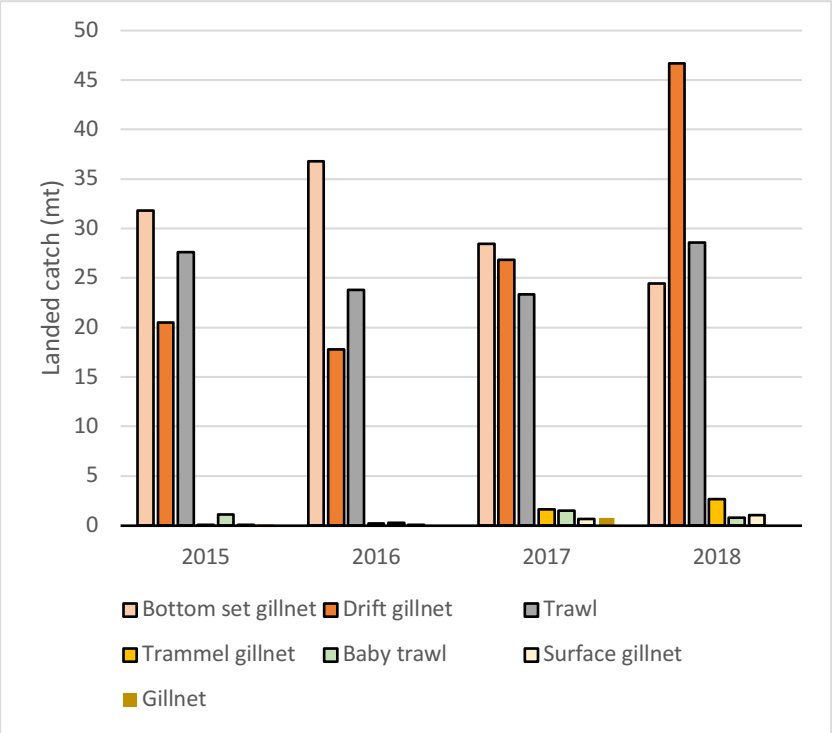


Figure 2.6 Landed catch of abo by fishing gear from 2015 – 2018 (NSAP-BFAR, 2019, unpublished)

(a) **(b)**

Figure 2.7 Data gathering procedures employed in the study

(**a**) observation during the whisper bidding or "*bulungan*" at Sabang port (**b**) interview to one of the small-scale dried abo processors

(a)

(b)

(c)

(d)

Figure 2.8 Traditional dried fish processing by processors

(**a**) degutting and splitting; (**b**) soaking in brine solution; (**c**) traditional sun-drying; (**d**) monitoring of dryness of fish

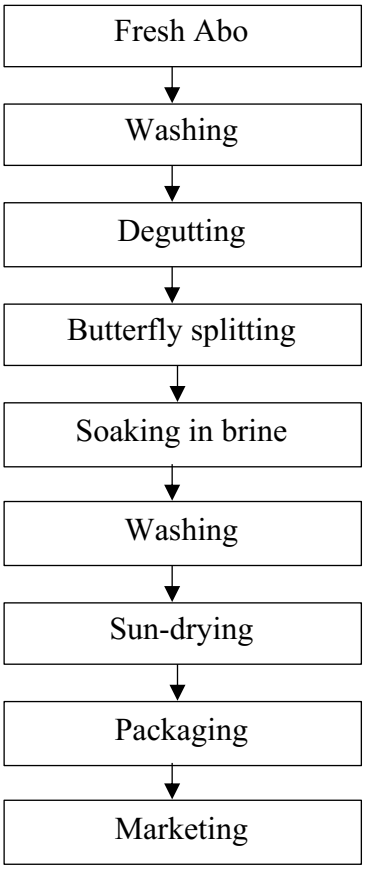


Figure 2.9 Process flow of dried abo adopted by processors in Calabanga fishing village

Table 2.1 Selling price of abo in Philippine pesos (Php) per kilogram based on body size and season

Size of Abo	Selling Price (Php)			
	Fresh		Dried	
	Peak months	Lean months	Peak months	Lean months
Small	120.00	*No data*	200.00	*No data*
Medium	240.00	320.00	320.00	400.00
Large	240.00	350.00	320.00 – 400.00	< 500.00

Table 2.2 Demographic profile of the respondents

Parameter	n	%
Age		
20 – 30	1	11.11
30 – 40	2	22.22
40 – 50	1	11.11
50 – 60	4	44.44
60 – 70	1	11.11
Gender		
Male	4	44.44
Female	5	55.56
Civil Status		
Married	7	77.78
Single	0	0
Widowed	2	22.22
Main occupation		
Fishing	0	0
Farming	0	0
Fish processing	9	100
Trading	0	0
Others	0	0
No. of years in fish processing		
0 – 5 y	3	33.33
More than 5 y	6	66.67
Classification as processor		
Small – scale	6	66.67
Medium – scale	1	11.11
Large – scale	2	22.22

Table 2.3 Handling of abo during fish collection

Parameter	n	%
Purchasing of raw materials		
Direct buying in fish port	8	88.90
Local market	1	11.11
Means of transportation		
Tricycle	9	100
Jeep	0	0
Refrigerated truck	0	0
Container used for transporting		
Bañera	9	100
Others	0	0
Material used for chilling of abo before drying		
Styro box	2	22.22
Chiller	3	33.33
None	4	44.44

Table 2.4 Brining conditions

Parameter	n	%
Type of dried Abo produced		
Whole	0	0
Splitted	9	100
Fillet	0	0
Salting of Abo		
Yes	9	100
No	0	0
Salting method		
Dry salting	0	0
Brine salting	9	100
Kench salting	0	0
Container for salting		
Brining tank	0	0
Plastic basin	8	88.89
Stainless basin	0	0
Brine salt concentration (w/w)		
10 – 20	4	44.44
20 – 30	1	11.11
30 – 40	3	33.33
40 – 50	1	11.11
Brining time		
0.5 h	5	55.56
1 h	2	22.22
2 h	2	22.22
No. of washing after brining		
Once	4	44.44
Two times	5	55.56
Three times	0	0

Table 2.5 Drying and packaging conditions

Parameter	n	%
Method of drying		
Sun-drying	9	100
Artificial drying	0	0
Cabinet drying	0	0
Hours of drying		
6 h	3	33.33
12 h	5	55.56
More than 12 h	1	11.11
Material for drying		
Bamboo rack	9	100
Aluminum rack	0	0
Stainless rack	0	0
Packaging material		
Vacuum packaging	2	22.22
Polyethylene plastic	5	55.56
Box	2	22.22
Paper	0	0
Others	0	0

Chapter 3: Species identification of abo

Abstract

San Miguel Bay is an important fishing ground in the Bicol region, Philippines, inhabited by croakers such as abo, pagotpot and arakaak. Although there was a reported massive decrease in abundance of these croakers, abo remained to be a commercially important fishery resource, marketed as fresh and dried salted products. By morphometry and meristic characterization, abo has been identified as a tigertooth croaker (*Otolithes ruber*). However, the similarity of morphological characteristics of these croakers in the bay could pose confusion in the recognition of these fish species. In addition, no molecular studies have been conducted yet in SMB despite being rich in fishery resources. Therefore, to clarify the species identification of abo, molecular analysis was conducted. Abo specimens were procured at the local market of Calabanga, Camarines Sur. The mitochondrial 16S ribosomal RNA and cytochrome c oxidase subunit I (COI) genes were amplified and products were bidirectionally sequenced. Obtained sequences were aligned with other published sequences of related genera by ClustalW. Neighbor-joining trees were constructed using MEGA 7.0. Both the COI and 16S rRNA sequences of three abo specimens have 100% nucleotide identity. The nucleotide differences between abo and *O. ruber* sequences were estimated to be 13.1% and 0.9% in COI and 16S rRNA sequences, respectively. The NJ tree-based on COI sequences revealed that abo formed a separate subclade with the clustered *O. ruber* and *Otolithes cuvieri* with 98% bootstrap support, whereas the *Otolithoides* species formed a separate group. On the other hand, the NJ tree of 16S rRNA sequences clearly showed that abo is

clustered with *O. ruber* with 100% bootstrap support. Therefore, DNA sequence analysis of COI genes confirmed that abo might belong to Otolithes genus, and analysis of the 16S ribosomal RNA genes suggested that abo in SMB is likely to be *Otolithes ruber*. The correct identification of fishery resources in SMB may be essential for the efficient assessment and management of the stocks, to assure their availability for domestic consumption, processing of products and marketing.

3.1 Introduction

San Miguel Bay is a shallow estuarine fishery with 80% of the bay less than seven fathoms (12.81 metres) deep, and 95% of the bay's bottom is composed of sand, mud and sandy-muddy substrate (Pomeroy & Pido, 1995). It is an important fishing ground for shrimps and a variety of other finfish species such as croakers, herrings, mullets, juvenile Spanish mackerels, anchovies, and crevalles (NSAP-BFAR, 2017). Croakers, belonging to the Sciaenidae family, are abundant in estuarine areas and prefer to occupy the soft substrata (Lanzuela et al., 2020). Of the several species of croakers in the bay, "abo" was the most abundant species, where it is considered as a first-class fish, commanding a high market price (Navaluna, 1982). Other sciaenids in SMB are locally known as "pagotpot" or "arakaak or alakaak" (Pauly et al., 1982; Lim et al., 1995). Assessment of catch composition conducted in 2001 – 2002 reported the massive decrease in abo, pagotpot, and arakaak, which were formerly the dominant species in trawl catches (Silvestre & Hilomen, 2004). Despite the observed decrease in abundance, landed catch of abo indicated an increasing trend in 2016 – 2018 (Chapter 2). Abo remains to be a commercially important fishery resource in SMB, marketed as fresh and dried-salted products.

Previous study in SMB had identified abo as a tigertooth croaker (*Otolithes ruber*) by morphometry and meristic characterization (Navaluna, 1982). Although there was no information on the morphological features of pagotpot and arakaak found in SMB, it is speculated that these croaker species could pose confusion due to the similarity of morphological characteristics of croakers. Among these species, abo is the most expensive in the market. Possible misidentification of fish species could occur during sample collection, which may lead to incorrect marketing, presentation

or labeling of products. Authentication studies and market monitoring reported that fish products are especially vulnerable to mislabeling than other consumers' goods (Chang et al., 2016). Commercial profit may then be affected when the nutritional composition of the expected and substituting fish differs from each other. Thus, a reliable identification that is independent of morphological characteristics would be necessary. Therefore, species identification of abo needs to be confirmed via molecular approaches.

No molecular studies have been conducted on the existing fishery species found in San Miguel Bay despite being rich in fishery resources. Previous works had focused only on the assessment of fisheries and socioecological studies (Palomares et al., 2003; Lim et al., 1995; Pauly, 1982; Navaluna, 1982). Moreover, only a few molecular studies have been carried out on *Otolithes ruber*. Lakra et al. (2009) reported the phylogenetic relationship and genetic relatedness among seven commercially important Indian sciaenids, including *O. ruber* using 16S rRNA and cytochrome c oxidase I gene (COI) genes. Zemlak et al. (2009) found deep divergences in the COI gene between *O. ruber* populations from South African and Australian waters that exceeded species-level divergence threshold value. Lo et al. (2017) investigated the evolutionary relationships, species diversity, and taxonomic status of Sciaenidae within the Indo-West Pacific region using multiple genetic markers and concluded that the taxonomy of *O. ruber* requires further investigation to identify potential cryptic and new species. Lin et al. (2019) proposed a new species named *Otolithes arabicus* sp. nov from the collected specimens of *Otolithes* sp. from evidences found in the integrative approach which combined mitochondrial COI gene, morphological characteristics, and otolith-shape analyses.

In this study, abo collected from San Miguel Bay, Philippines, were molecularly identified through the mitochondrial COI and 16S rRNA as molecular markers.

3.2 Methods

3.2.1 Sample collection

Abo individuals were procured from the local fish market in Calabanga, Camarines Sur, one of the fish landing centers of San Miguel Bay, Philippines, in October 2018. A total of three abo individuals were randomly selected. A small piece of pectoral fin tissue (approximately 100 mg) was excised from each individual and preserved in 95% ethanol and stored at -20°C until further use.

3.2.2 DNA extraction

Approximately 50 mg of fin tissue was utilized for DNA extraction. Tissue samples were cut into small pieces, approximately 1 mm cube sample size. DNA was extracted from each sample using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocols. The quality of the extracted DNA was evaluated by the measurement of the concentration and purity based on the UV absorption ratio at 260/280 nm using Nanovue (Version 4282 V2. 0.4) spectrophotometer. DNA samples were diluted to a final concentration of 10 ng/µl and stored at -20°C for next use.

3.2.3 Polymerase chain reaction amplification and sequencing analysis

Polymerase chain reaction (PCR) of the COI gene was performed in a 25 µl reaction mixture containing 2.5 µl of 10x buffer [magnesium (Mg^{2+}) plus, 2.0 µl of 2.5 mM of deoxynucleoside triphosphate (dNTP), 1.25 µl of 10 µM of each primer, 0.125 µl of *Taq* polymerase (TakaraBio Inc., Shiga, Japan) and 2.0 µl of DNA template using the thermal cycler LifeECO (Bioer). The primers used were universal primers CoxI Fish F2 and CoxI Fish R2 (Ward et al., 2005), and designed primers 5346F and 6446R (Table 3.1). The thermal profile used was 35 cycles of a three-step cycle consisting of denaturation at 95°C for 4 min, annealing at 95°C for 40 sec, 51°C for 40 sec, 72°C for 90 sec and final extension step at 72°C for 7 min (Table 3.2) as reported by Lo et al. (2017).

On the other hand, the mitochondrial 16S rRNA gene was amplified using universal primers 16SAR and 16SBR (Palumbi et al., 2002) in a 25 µl reaction volume with 2.5 µl of 10x buffer with $MgCl_2$, 2.0 µl of 2.5 mM of dNTP, 1.25 µl of 10 µM of each primer, 0.125 µl of *rTaq* polymerase (TakaraBio, Shiga, Japan) and 2.0 µl of DNA template. The thermal cycle profile included initial denaturation step of 94°C for 4 min, followed by 36 cycles of a three-step cycle consisting of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, 72°C for 90 sec and final extension step at 72°C for 7 min (Table 3.3) as outlined by Lakra et al. (2009).

The PCR products were separated and visualized on a 1.5% agarose gel (Nacalai Tesque, Japan). Two microliters of PCR product mixed with 1 µl loading dye containing GelRed (1:100 dilution) nucleic acid gel stain (Biotium, CA, USA) was loaded into the agarose gel and electrophoresed (1x TAE) at 100 V for 20 min. The size of the amplicons was determined using Broad Range DNA Ladder One

(TakaraBio, Shiga, Japan) as a marker added with diluted GelRed (Biotium, CA, USA) in distilled water (1:10 dilution) and viewed under UV transilluminator.

The PCR product was purified by Agencourt AMPure XP (Beckman Coulter, CA, USA), and then it was bidirectionally sequenced using BigDye® Terminator v.3.1 cycle sequencing kit (Applied Biosystems, Inc., CA, USA) following the manufacturer's instructions on an ABI PRISM® 3130 Genetic Analyzer at the Research Instrument and Radio Isotope Research, Division of Biological Research, Science Research Center, Kochi University, Japan.

The obtained sequence electropherograms were edited manually. The sequences of amplified COI and 16S rRNA fragments were subjected to nucleotide BLASTn (Basic Local Alignment Search Tool) search in the NCBI GenBank to identify any closely related species. The COI and 16S rRNA sequences of abo (DDBJ accession numbers: LC594682 – LC594687) were aligned with reference sequences of some species belonging to the Sciaenidae family, *Otolithes* (accession numbers: EF534126, EF528214, EF534114, EF528197) and *Otolithoides* (EF534127) by Lakra et al. (2009) using the ClustalW program and then shortened to obtain the consensus length. Nucleotide differences of each sequence were counted, and neighbor-joining (NJ) tree was constructed with the Kimura 2-Parameter model using the Molecular Evolutionary Genetics Analysis (MEGA7) (Kumar et al., 2015). *Thunnus orientalis* (accession no. AB185022) was set as the outgroup species (Lakra et al., 2009). The bootstrap method with 2,000 replications was applied to test the robustness of topology nodes.

3.3 Results

3.3.1 Species identification of abo

Alignment of the COI and 16S rRNA sequences with their sequences from *Otolithes* and *Otolithoides* estimated a consensus length of 651 bp and 571 bp, respectively (Fig. 3.1 and Fig. 3.2). The COI sequences of all the three abo individuals have a nucleotide identity of 100%. On the other hand, the nucleotide differences between abo and *Otolithes ruber* sequences (EF534126) was approximately 13.1% (85/651) (Fig. 3.1). Among abo and *Otolithes cuvieri* (EF534114) sequences, nucleotide differences were 12.0% (77/651). On the other hand, 16.0% (104/651) variable sites were found among abo and *Otolithoides biauritus* (EF528214). The NJ tree-based on COI sequences produced two main groups (Fig. 3.3a). Abo formed a separate subclade with the clustered *Otolithes ruber* and *Otolithes cuvieri* with a 98% bootstrap support, whereas the *Otolithoides* species formed a separate group.

All three 16S rRNA sequences of abo were completely the same (100%). Only 0.9% nucleotide differences (5/571) were found among abo and *Otolithes ruber* (EF528214) sequences whereas 8.0% nucleotide differences (46/571) among abo and *Otolithes cuvieri* (Fig. 3.2). On the other hand, NJ tree clearly showed that abo is clustered with *Otolithes ruber* with 100% bootstrap support (Fig. 3.3b).

3.4 Discussion

A higher percentage of nucleotide differences (13.1%) was observed in the alignment of COI, however, the NJ tree (Fig. 3.3a) indicated that abo formed a close phylogeny in the *Otolithes* clade with 98% bootstrap support. The nucleotide

differences and the NJ tree inferred from COI sequences demonstrated that abo might belong to the *Otolithes* genus. On the other hand, the NJ tree inferred from 16S rRNA sequences have shown that abo sequences formed the same cluster with *Otolithes ruber* suggesting that abo inhabiting SMB is likely to be *Otolithes ruber*. Overall, molecular identification of the three collected abo specimens bought from the local market at Calabanga, Camarines Sur in SMB, have obtained identical sequences that matched with *Otolithes ruber.*

Molecular DNA identification techniques have been proven to be analytically powerful (Jefri et al., 2015). The knowledge on the species identification of the collected abo could serve as a baseline information that may be useful during its processing and food composition analyses of the dried products. Reliance on morphology can sometimes present difficulty in recognition and differentiation among croaker species in cases where similar morphological characteristics could be misleading. Abo is a higher value fish species, being more expensive than pagotpot and arakaak. Hence, molecular identification of the other croaker species, pagotpot and arakaak, should therefore be also clarified. The correct identification of fishery resources in San Miguel Bay may be essential for the efficient assessment and management of the stocks, to assure their availability for domestic consumption, processing of products and marketing.

Table 3.1 Primers for amplification of COI and 16S rRNA genes used in the study

Target Fragment Gene	Sequence (5'-3')	Oligo name	Reference
COI	*TCGACTAATCATAAAGATATCGGCAC*	CoxI Fish F2	Ward et al. 2005
COI	*ACTTCAGGGTGACCGAAGAATCAGAA*	CoxI Fish R2	Ward et al. 2005
COI	*CTTAGTTAACAGCTAARCGC*	5346F	Designed primers
COI	*GTRTCTACGTCTATTCCGAC*	6446R	Designed primers
16S rRNA	*CGCCTGTTTATCAAAAACAT*	16SAR	Palumbi et al. 1991
16S rRNA	*CCGGTCTGAACTCAGATCACGT*	16SBR	Palumbi et al. 1991

Table 3.2 PCR thermal conditions for the amplification of COI gene

Step	Temperature	Time	Cycle
Initial denaturation	95°C	4 min	1 cycle
Denaturation	95°C	40 sec	
Annealing	51°C	40 sec	
Elongation	72°C	90 sec	35 cycles
Final extension	72°C	7 min	1 cycle

Table 3.3 PCR thermal conditions for the amplification of 16S rRNA gene

Step	Temperature	Time	Cycle
Initial denaturation	94°C	4 min	1 cycle
Denaturation	94°C	1 min	
Annealing	55°C	1 min	
Elongation	72°C	90 sec	36 cycles
Final extension	72°C	7 min	1 cycle

Figure 3.1 Partial alignment of DNA sequences for the PCR amplicons of the mitochondrial COI gene

Multiple alignment of partial DNA sequences of 16S rRNA resulted to a consensus length of 651 sites. Dots indicate nucleotide identity.

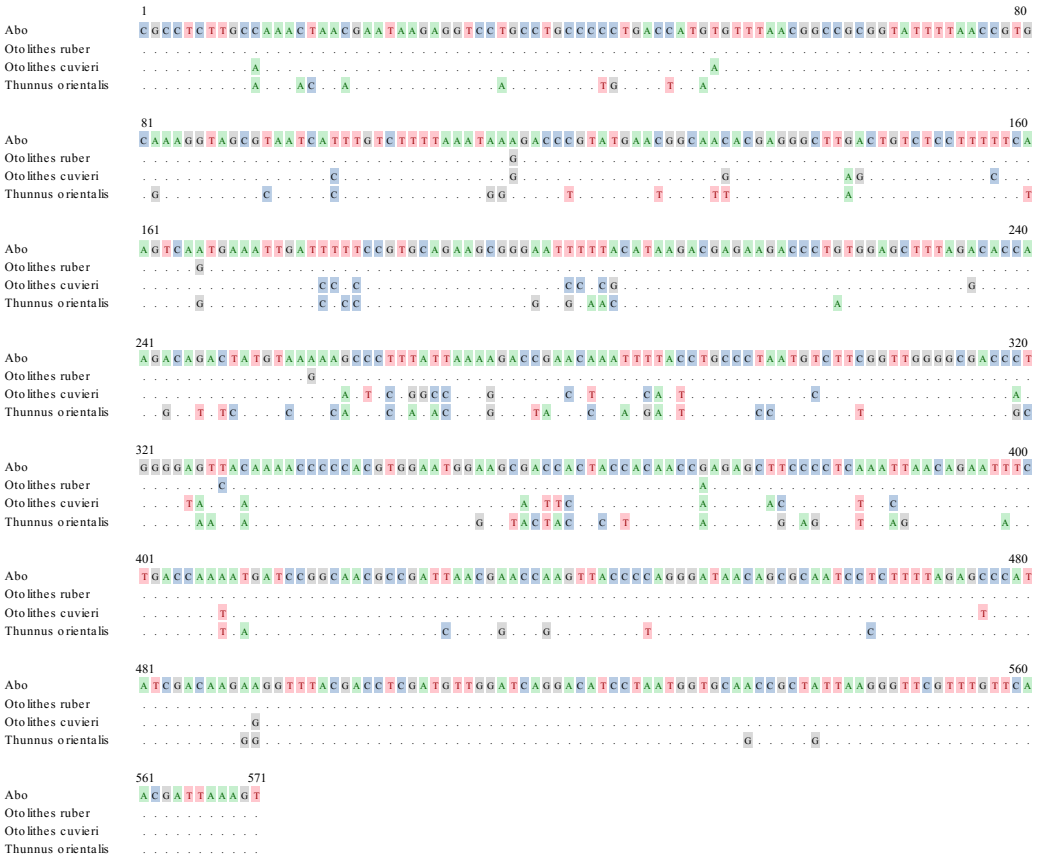
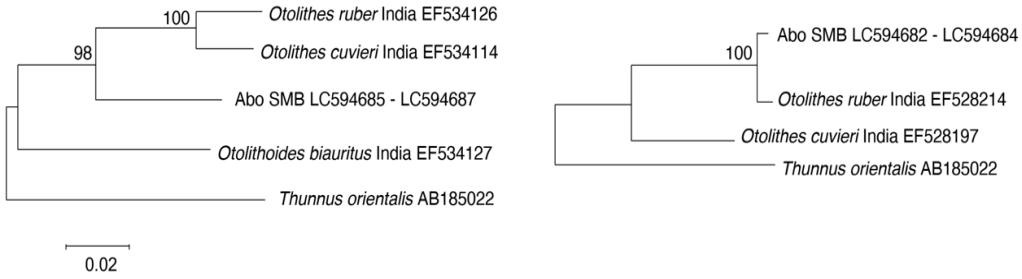


Figure 3.2 Partial alignment of DNA sequences for the PCR amplicons of the mitochondrial 16S rRNA gene

Multiple alignment of partial DNA sequences of 16S rRNA resulted to a consensus length of 571 sites. Dots indicate nucleotide identity.



(**a**) (**b**)

Figure 3.3 Neighbor-joining trees

(**a**) NJ tree inferred from COI sequences (**b**) NJ tree inferred from 16S rRNA sequences

Chapter 4: Proximate composition and salt content of dried salted abo

Abstract

Salting, in combination with the traditional sun-drying method, remains to be widely employed by dried fish processors at San Miguel Bay, Philippines. The present quality of dried abo products are highly salted and dried. To maximize the potential of this foodstuff as a high market value product with high consumer acceptability, standardization of processing and characterization of dried abo products is important. Therefore, this study was conducted to produce dried salted abo with different salt concentrations and drying times and evaluate its proximate composition and salt content. In this study, the dried abo processing conditions gathered from the interviews were optimized to produce six groups of dried salted abo with different salt concentrations (4%, 8%, and 12%) and drying times (6 h and 12 h). Proximate composition analysis of moisture, ash, crude protein, and crude lipid were analyzed according to the methods of AOAC. Salt content was determined using a digital salt meter. Values of moisture, crude protein, ash, crude lipid content of commercially available dried abo product were almost similar to that of dried abo samples dried at 12 h, indicating that the commercial dried abo was processed with long drying time. In the laboratory-produced dried abo subjected to 6 h and 12 h drying time, moisture was the major constituent of the dried fish samples. Moisture significantly reduced in 8% and 12% salt concentration samples at 6 h drying. Ash content increased with increasing salt concentration in both 6 h and 12 h drying times. The high values of ash corresponded to the increasing salt concentration suggested that ash was mainly derived from the added salt in the brine solution. This highlighted the need for

modification of the salt content of the product. The 12% salt concentration samples dried for 6 h have significantly higher crude protein than 4% and 8% salt concentration samples could indicate an alteration in the conformation of muscle proteins as crude powder-like and paste-like textures were observed, respectively. Hence, further investigation of proteins would be essential. On the other hand, no significant difference was found in crude lipid content among samples dried with different salt concentrations for both drying times. Overall, different salt concentrations and drying times have influenced the proximate composition of the dried salted products. Results of this study implied that when processing dried salted abo, salt concentration can be controlled between 8 – 12% at 6 h drying. Thus, this could be an opportunity to develop dried fish products with improved salt content and texture that could be further evaluated for nutritional content and consumer acceptability.

4.1 Introduction

Salting and sun-drying are some of the oldest means of preserving fish. These processes remain popular in the Philippines due to their low capital investment, simplicity of processing, and other advantages. At Calabanga, Camarines Sur, Philippines, abo *(Otolithes ruber)* is typically salted by brine salting. The fish is butterfly-split and then immersed in brine solution in high salt concentration followed by the traditional sun-drying (Chapter 2). At present, dried abo products are highly salted and dried which has a high market demand due to its desirable taste and flavor. Dried salted abo could also represent as a low-cost source of protein.

Proximate composition is an important aspect of fish quality that influences the fish's keeping quality and technological characteristics (Islam et al., 2013). In traditionally sun-dried fishes (*Rhamphochromis* fish species), the reported values for moisture was 16.7%, crude protein of 34.1%, crude lipid of 25.8%, and ash of 16.4% (Msusa et al., 2017). Studies on dried salted fish investigating the effects of different salting methods on proximate composition have reported that by brine salting and dry salting, there was a considerable loss in moisture due to heavy uptake of salt and an increase in ash and protein contents (Martínez-Alvarez & Gómez-Guillén, 2006). Moreover, salting affects the conformation of muscle proteins and alters the water holding capacity and subsequent protein denaturation (Offer and Trinick, 1983).

There has been no attempt to examine the proximate composition and chemical quality of dried abo products in San Miguel Bay. Few studies have investigated the proximate composition of fresh *Otolithes ruber* as reported by Jalili et al. (2014), Sharifian et al. (2011) and Edirisighe et al. (2000). Little information on

the chemical composition is known regarding dried-salted products utilizing species under the Sciaenidae family.

Moreover, it is hypothesized that characterization of the chemical properties of dried abo and the standardization of the drying processes, particularly salt concentration and drying time, could help develop the strategies to improve it as a foodstuff with low-salt content and high consumer acceptability. Therefore, this study aimed to produce dried salted abo with different salt concentrations and drying times. Specifically, it aimed to evaluate the dried salted abo in terms of its proximate composition and salt content.

4.2 Methods

4.2.1 Materials

Fresh abo was purchased in the local market at Calabanga, Camarines Sur, Philippines. Commercially available dried abo product was also obtained from a local retail market to investigate its proximate composition. Chemicals (analytical grade) used in all experiments were obtained from Nacalai Tesque, Inc. (Japan) or Thermo Fisher Scientific (USA).

4.2.2 Dried abo processing

Preparation of dried abo was optimized based on the processing conditions described from the interview conducted to dried fish processors at Calabanga, Camarines Sur (Chapter 2). The purchased fresh abo was immediately packed in a cooler box and preserved with clean ice blocks before being transported to the Fish Processing laboratory of Partido State University, Sagñay, Camarines Sur,

Philippines. The temperature was kept low (5 ± 2°C) and monitored using a THA-02L digital temperature logger (As One, Japan).

Approximately seven (7) kg abo were divided into six groups with a combination of salt concentration for brining and drying time: i) 4% salt concentration, 6 h drying, ii) 8% salt concentration, 6 h drying, iii) 12% salt concentration, 6 h drying, iv) 4% salt concentration, 12 h drying, v) 8% salt concentration, 12 h drying, vi) 12% salt concentration, 12 h drying. The three salt concentrations were decided based on the estimated standard 8% salt concentration as indicated from the results of the survey (Chapter 2). A higher (12%) and lower (4%) salt concentration were selected and added to the standard salt concentration. The two drying times were also decided based on the 12 h drying time practiced by majority of the respondents.

The weight and length (standard length and total length) of each fish were measured using a digital weighing scale and ruler, respectively. The average weight, standard length, and total length of abo were 70.0 ± 11.1 g, 17.1 ± 0.94 cm and 20.0 ± 1.06 cm, respectively.

The collected abo samples (Fig. 4.1a) were washed with running tap water. Fish were then degutted and split into a butterfly fillet, starting from the head to the tail (Fig. 4.1b). The remains of viscera and blood were quickly washed off with tap water. Fish were then soaked in a prepared brine solution with a controlled salt concentration of 4%, 8%, and 12% (w/w) for 30 min (Fig. 4.1c). Brined fish were then rinsed with tap water for two times and then drained (Fig. 4.1d). After draining, brined fish were placed and arranged in racks with flesh side facing upwards for sun-drying (Fig. 4.1e). Sun-drying was done for 6 h and 12 h. While drying, the racks

were covered with a net to protect it from infestation of insects and stray animals. Fish were turned over from time to time to ensure homogenous drying. Dried abo samples were collected and cooled at room temperature. Sharp teeth of dried fish were cut before samples were vacuum-packed, weighed and labeled (Fig. 4.1f).

4.2.3 Sample preparation for chemical analyses

The dorsal part of dried fish muscle was used for all the chemical analyses. Sample preparation was done in an iced box to prevent the evaporation of moisture from samples. First, the head was cut, followed by the bones, skin, and tail, and then discard. Collected flesh was further cut into smaller pieces. Samples were then crushed until completely homogenized with EG-45 electronic coffee mill (Kalita, Japan), and further pounded by a mortar and pestle. Powdered samples were divided and collected utilizing the conical quadrant method. Samples were then stored in storage bags at -20°C until analysis.

4.2.4 Proximate analysis

The commercial and laboratory-produced dried abo were subjected to proximate analyses. Proximate analyses for moisture, ash, crude protein, and crude lipid were carried out following the official methods described by the Association of Official Analytical Collaboration (AOAC) International (AOAC, 2000).

4.2.4.1 Moisture

One (1) gram of dried fish sample was taken in triplicate in a pre-weighed moisture bottle and dried in a drying oven maintained at 105°C, with the lid kept

partially opened. Samples were first dried for three hours, followed by one hour for the succeeding drying time. Drying and cooling was done repeatedly until constant weight was obtained. Moisture content (%) was estimated by this formula:

$$\text{Moisture content (\%)} = \frac{W1 - W2}{W1 - W0} \, x \, 100$$

where:

W0: weight of weighing container at constant weight (g)

W1: weight of weighing container containing sample before drying (g)

W2: weight of weighing container when the sample reaches constant weight after drying (g)

4.2.4.2 Ash

About 0.7 g of sample was taken in a pre-weighed crucible and then heated on a hot plate controlled at 150°C until no smoke was emitted for preliminary ashing. Samples were incinerated in a muffle furnace at 550°C for 18 hours until ash was grayish-white. Crucibles were then cooled in a desiccator and weighed. Ash content was calculated from the weight difference of the crucibles. It is expressed as % ash content using the following formula:

$$\text{Ash content (\%)} = \frac{W2 - W0}{W1 - W0} \, x \, 100$$

where:

W0: weight of crucible (g)

W1: weight of sample + crucible (g)

W2: weight of ash + crucible (g)

4.2.4.3 Crude protein

About 0.5 g of sample was digested with 20 ml of concentrated sulfuric acid and about 5 g of cracking catalyst (mixture of K_2SO_4 and $CuSO_{4.5}H_2O$) in a digestion flask. The contents of the digestion flask were heated in a digestion chamber. Digestion was continued until a blue color was attained and then heated for another hour. After cooling down, the volume was made by adding distilled water in a 100 ml volumetric flask. A 10 ml aliquot was taken and distilled in the Kjeldahl distillation unit with 10 ml of 30% sodium hydroxide and washed with little distilled water. The liberated ammonia was absorbed in 10 ml of 0.025 M H_2SO_4 with two (2) drops of mixed indicator (methyl red and methylene blue) and was titrated against the standard 0.05 M NaOH until a faint gray color was developed. Crude protein was calculated by multiplying the total nitrogen content by 6.25.

$$\text{Nitrogen (\%)} = (0.0007\ x\ F\ x(V2 - V1)/S)\ x \frac{100}{10}\ x\ 100$$

$$\text{Crude protein content (\%)} = Nitrogen\ (\%)\ x\ (nitrogen - protein\ conversion\ factor, 6.25\ )$$

where:

V1: 0.05 M NaOH titration value (ml) of this test

V2: 0.05 M NaOH titration value (ml) for blank test

S: Sample amount (g)

F: titer of 0.05 M NaOH

4.2.4.4 Crude lipid

About 6 g of sample kept inside a thimble was dried for 2 h in a drying oven controlled at 105°C. The dried thimble filter was placed in the Soxhlet extraction unit. About 100 ml of diethyl ether was used as the solvent. The water bath was heated such that the dripping of diethyl ether from the condenser was 80 drops per minute. Extraction was continued for 16 h. After the extraction, the pre-weighed receiver flask containing the extracted lipid was dried for one hour in a drying oven at 105°C. The flask was cooled in a desiccator, and drying and cooling were repeated until a constant weight was obtained. The crude lipid of the samples was calculated using the following formula:

$$\text{Crude lipid content (\%)} = \frac{(W1 - W0)}{W}\ x\ 100$$

where:

W0: weight of flask (g)

W1: weight of flask after lipid extraction (g)

W: amount of sample (g)

4.2.5 Salt content determination

The salt (NaCl) content was determined by the conductivity of the solution using a PAL-SALT Probe digital salt meter (ATAGO CO. LTD., Japan).

Approximately 1 g of sample was mixed with 4 ml distilled water and then homogenized in a 15 ml centrifuge tube. Samples were then centrifuged at 10°C, 2,147 *x g* for 40 min until the suspended matter was completely precipitated. About 2 ml of collected supernatant was transferred to the probe and analyzed.

4.2.6 Statistical analysis

The proximate analysis results and salt content are expressed as mean ± standard deviation (SD) of three determinations. One-way analysis of variance (ANOVA) followed by Tukey's honest significance test (Tukey's HSD) was performed to determine the effect of salt concentration on proximate composition. All statistical analyses were tested at $p \leq 0.05$ significance level using R version 4.0.1.

4.3 Results

4.3.1 Proximate composition

The main chemical components such as water, crude protein, lipid, and ash have the largest impact on the nutritive value, functional properties, sensory quality and the storage ability of the flesh (Islam et al., 2013). Table 4.1 shows the proximate composition of abo samples at different salt concentrations and drying times. Moisture content ranged from 38.34 – 66.28%. The moisture content of all samples significantly decreased with an increasing salt concentration in samples dried for 6 h. The highest reduction in moisture was observed at 12 h drying in 12% salt concentration. The ash content ranged from 3.94 – 12.79% and was found to be increased with an increasing salt concentration in both 6 h and 12 h drying times.

Highest ash content was estimated in samples dried for 12 h with 12% salt. The crude protein content varied from 26.4 – 47.9%. The salt concentrations did not reveal a significant effect in crude protein content except between 8% and 12% salt concentration samples at 6 h drying time. The crude lipid content ranged from 0.41 – 1.04%. Similar effects were also observed for crude lipid content with no significant differences between the samples dried with different salt concentrations for both drying regimes. On the other hand, the values of moisture, crude protein, ash, and crude lipid content of commercially available dried abo were 37.72% ± 0.61, 50.7% ± 0.47, 9.56% ± 0.09 and 0.55% ± 0.03, respectively.

In addition, during sample preparation for chemical analyses, different textures of the samples were observed. In 12 h of drying, fine powder-like texture was obtained from all samples. On the other hand, in the 6 h of the drying regime, drying with 12% salt concentration revealed a crude powder-like texture and drying with 4% and 8% salt concentrations produced paste-like texture.

4.3.2 Salt content

The salt contents of dried abo in 6 h and 12 h drying ranged from 4.20 – 7.87% and 3.31 – 12.5%, respectively (Table 4.2). It was found that the increasing salt concentration of the brine solution increased the salt concentration for both drying times.

4.4 Discussion

The moisture, crude protein, crude lipid, and ash content of the commercially available dried abo was almost similar to the values of proximate composition of

samples dried at 12 h. This indicated that the obtained commercial dried abo is indeed processed with a longer drying time.

The main constituent of fish flesh is moisture. In 6 h drying, moisture significantly reduced in 8% and 12% salt concentration samples. Although no significant difference was found among samples in 12 h drying, a reduction in moisture content was also observed. Hwang et al. (2012) also found a significant decrease in moisture between 5 – 10% salt concentration samples of sun-dried milkfish. Lower moisture content in the 12% salt concentration samples can be attributed to the decrease in the water-holding capacity of proteins. Hamm (1960) suggested that at higher salt concentrations (approximately above 2 M), protein is denatured, resulting in cross-linking between proteins, increasing shrinkage, and consequently losing water from the muscle and, therefore, decreased the water-holding capacity. This could be observed in the development of crude powder-like texture in 12% salt concentration samples. Thus, in the processing of dried salted abo, a salt concentration between 8 – 12% can be considered at 6 h drying to improve the product's texture.

Ash content represents the total mineral content in foods. Both ash and salt content increased as the salt concentration of the brine was increased, suggesting that ash was mainly derived from the added salt in the brine solution during processing. These results conform to the previous report by Hwang et al. (2012), in which the salt content of sun-dried milkfish increased with increased salt concentrations and was found significant between 5 – 10% salt concentrations. In this study, the 12% salt concentration samples have a salt content of 7.87% and 12.5% for 6 h and 12 h drying, respectively. In contrast, the sodium chloride content of dried split fish

products reported in the Philippine Food Composition Table ranged from 11.64 – 22.39% (DOST-FNRI, 2020). This result indicates that the laboratory-produced dried abo samples contain lower salt content than these reported dried fish. Nevertheless, the salt content of 12% salt concentration samples is still on the high side. Long-term consumption of high-salt products is not conducive to human health as it increases the burden of diseases such as kidney disease, heart disease and, hypertension (Tian et al., 2020). To reduce the global burden of cardiovascular diseases, reduction of the salt intake has been one of the public health initiatives being promoted by the WHO. The food industry is being encouraged to lower the level of salt content in processed foods known as product reformulation. Hence, increasing demand for low-salt food is expected. Processing of dried abo can therefore be modified to lower the level of salt content.

Crude protein content among samples of 12 h drying time was not significantly different. However, in 6 h drying, the 12% salt concentration samples have significantly higher crude protein content than 4% and 8% salt concentration samples. Soaking in the high brine solution decreases the solubility of proteins due to the competition between the salt ions and protein for the water molecules, which may have caused salting-out of proteins that led to precipitation and dehydration of proteins (Jittinandana et al., 2002). Consequently, the precipitation could have contributed to the accumulation of the protein molecules, thus increasing the crude protein content. This increase in crude protein could result in the alteration of physical and chemical properties of protein (Abraha et al., 2018). Hence, further analysis might be needed to investigate these conformational changes in the muscle proteins.

Moreover, lipids are the primary energy storage material in fish (Edirisinghe et al., 2014). Crude lipid values of dried abo were low. This is similar to the lipid content of tiger tooth croaker (*Otolithes ruber)* in iced storage, which has been reported to be low, a characteristic of tropical fish species (Sharifian et al., 2011). Low lipid content makes the muscle less susceptible to lipid oxidation; therefore, it could be advantageous for dried fish products to control the product's quality deterioration.

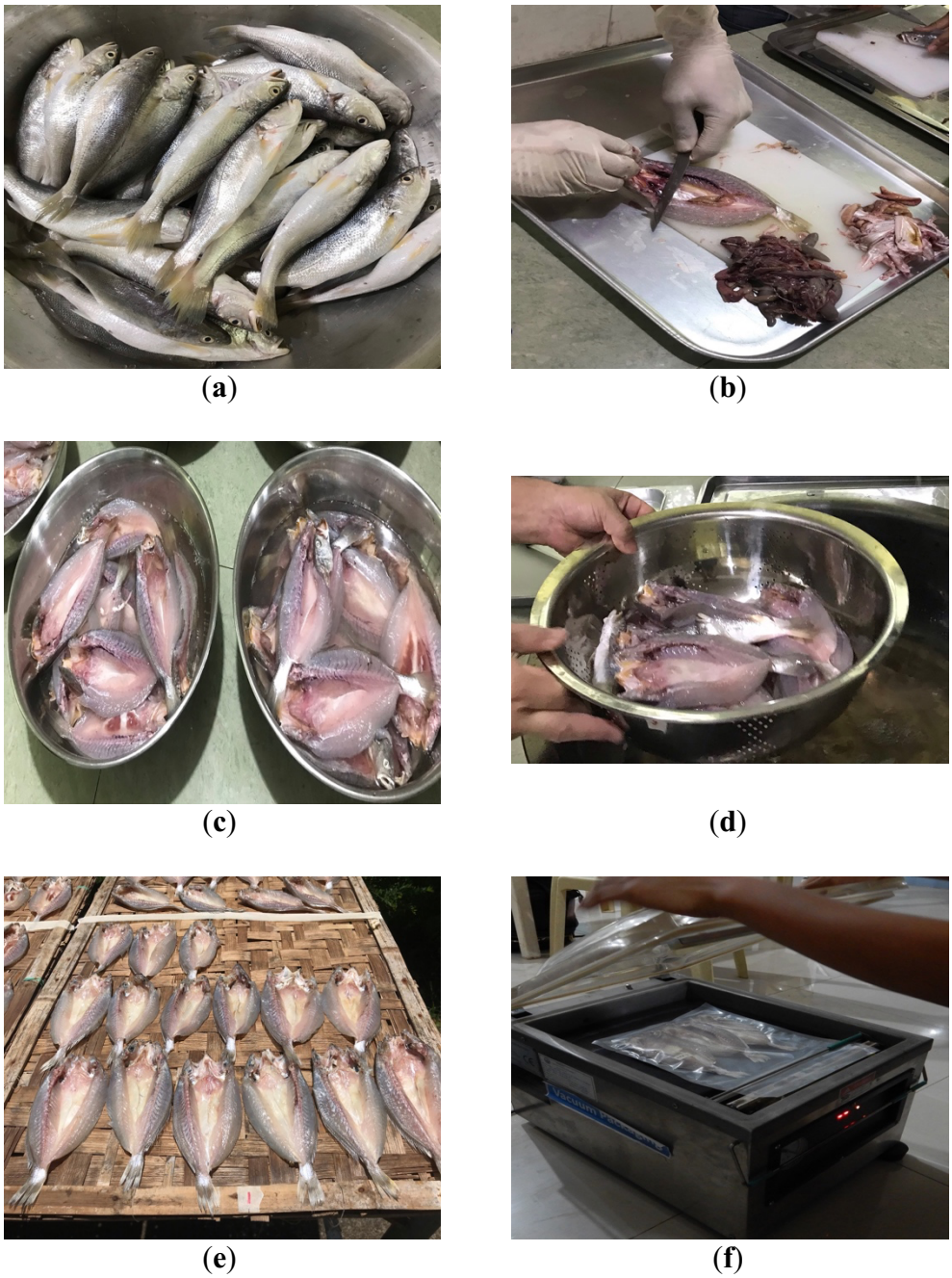


Figure 4.1 Processing steps in the laboratory production of dried salted abo with three salt concentrations and two drying times

(a) washed fresh abo samples **(b)** degutting and butterfly-splitting **(c)** soaking in brine solution (4%, 8%, 12%) for 30 min **(d)** washing and draining **(e)** sun drying (6 h, 12 h) **(f)** vacuum packing

Table 4.1 Proximate composition of dried Abo samples prepared with various salt concentration and drying time

Drying Time (hr)	Salt Concentration (%)	Moisture (%)	Ash (%)	Crude Protein (%)	Crude Lipid (%)
6	4	66.28 ± 0.89 ^{A}	3.94 ± 0.09 ^{A}	27.7 ± 1.22 ^{AB}	0.52 ± 0.03 ^{A}
	8	65.60 ± 0.71 ^{A}	5.68 ± 0.10 ^{B}	26.4± 0.66 ^{A}	0.41 ± 0.05 ^{A}
	12	58.61 ± 1.91 ^{B}	8.67 ± 0.37 ^{C}	29.2 ± 0.68 ^{B}	0.71 ± 0.23 ^{A}
12	4	41.35 ± 2.40 ^{a}	6.55 ± 0.46 ^{a}	47.9 ± 2.02 ^{a}	1.00 ± 0.11 ^{a}
	8	42.98 ± 1.63 ^{a}	9.59 ± 0.27 ^{b}	45.5 ± 2.81 ^{a}	0.94 ± 0.33 ^{a}
	12	38.34 ± 0.96 ^{a}	12.79 ± 0.79 ^{c}	46.0 ± 0.50 ^{a}	1.04 ± 0.16 ^{a}

Values represent the mean of three determinations ± SD. Uppercase and lowercase letters denote samples dried for 6 hours and 12 hours, respectively. Values in the same column followed by a common letter are not significantly different by the Tukey's HSD ($p > 0.05$).

Table 4.2 Ash content and salt content of dried abo samples prepared with various salt concentration and drying time

Drying time (hr)	Salt concentration (%)	Ash content (%)	NaCl content by salt meter (%)
6	4	3.94 ± 0.09	4.20 ± 0.27
	8	5.68 ± 0.10	5.39 ± 0.46
	12	8.67 ± 0.37	7.87 ± 0.80
12	4	6.55 ± 0.46	3.31 ± 1.63
	8	9.59 ± 0.27	9.33 ± 0.43
	12	12.8 ± 0.79	12.5 ± 0.89

Values represent the mean of three determinations ± SD.

Chapter 5: Protein profile of dried salted abo

Abstract

Biochemical changes occur in the fish tissues during the salting and drying processes. The significant reduction in the moisture content and increase in crude protein content observed among the dried salted abo samples might have resulted in conformational changes of proteins. Hence, this study was undertaken to examine the changes in the protein profile of dried salted abo samples. Protein profile was analyzed by Tricine SDS-PAGE and LC-MS/MS. Sequence analysis was also conducted to clarify the characteristics of the peptides of myosin heavy chain and actin. A total of 23 bands were detected in 10% and 20% gel Tricine SDS-PAGE and nine proteins were identified by LC-MS/MS. Among the identified proteins, myofibrillar proteins, myosin heavy chain (MHC) and actin, together with keratin, dissociated into fractions that were different from their theoretical molecular weights. On the other hand, tropomyosin, beta-enolase, L-lactate dehydrogenase, triosephosphate isomerase, nucleoside diphosphate kinase, and parvalbumin were found to be stable. Changes in the band intensities as salt concentration increased at 6 h and 12 h drying time, have indicated degradation of MHC and polymerization of actin that could have occurred by the influence of salt curing and drying processes. Characterization of the individual peptide fragments obtained from mass spectrometry were observed to elucidate the formation of these fragments. Six fragments identified as MHC seemed to be derived from the light meromyosin region. On the other hand, all three peptides identified as actin seemed to be found in the four subdomains of actin with larger predicted sizes than the molecular weight

estimated by SDS-PAGE. Furthermore, since both MHC and actin fragments contained lysine and arginine as the amino acid residues at the C-terminal end and before the N-terminus peptides as estimated by mass spectrometry, it was unlikely to estimate the proteolytic cleavage site and proteases that may have caused the degradation of myofibrillar proteins that occurred during the salting and drying processes. Overall, findings revealed that the applied salting and drying conditions led to the degradation of myosin heavy chain and polymerization of actin, in addition to an overall alteration of protein profile. The protein fragments observed could be useful indicators to monitor the changes in adjusting the salt concentration and textural properties of the product. Assessment of the small peptides and amino acids generated from the degradation of proteins will be further studied.

5.1 Introduction

Salting and ripening are the two main stages in the salting process. The ripening of salted fishes is a series of complex biochemical processes, including proteolysis, lipolysis, and lipid oxidation, which takes place wherein changes occur in the tissue of fish induced by enzymes that break down proteins and fats. The physical and chemical changes that occur during ripening determine the overall sensory qualities of salted fish-products (Voskresensky, 1965). The salting process affects the conformation of the muscle proteins, causing changes in the water-holding capacity and subsequent denaturation (Martínez-Alvarez & Gómez-Guillén, 2006).

The muscle proteins generally comprise water-soluble sarcoplasmic proteins, salt-soluble myofibrillar proteins, and insoluble stroma proteins, which occupy about 20-50%, 50-70%, and ~3% (in the case of fish) of the total muscle protein, respectively (Ochiai & Ozawa, 2020). Myofibrillar proteins contribute to the textural quality of flesh food. Biochemical changes in fish myofibrillar proteins had been studied to introduce scientific quality control into the production of dried fish products. Structural changes in proteins as observed in the production of cross-linked myosin heavy chains in some cured and dried meats suggested its effects on the textural formation of dried meat (Ito et al., 1990).

The glycine-sodium dodecyl sulfate polyacrylamide gel electrophoresis (or Laemmli SDS-PAGE) and Tricine SDS-PAGE are widely used SDS electrophoretic techniques for separating proteins. Glycine-SDS-PAGE is based on glycine-Tris buffer system, is used for separation of high-molecular mass proteins. On the other hand, Tricine–SDS-PAGE is based on Tricine-Tris buffer system, commonly used to

separate proteins in the mass range 1–100 kDa, specifically for the resolution of proteins smaller than 30 kDa (Schagger, 2006). SDS-PAGE analyses used to identify different muscle proteins have also revealed the changes in the protein pattern of salted fish (Martínez-Alvarez & Gómez-Guillén, 2006; Sannaveerappa et al., 2004; Thorarinsdottir et al., 2002). However, the myofibrillar proteins involved in these changes during different processing conditions were only tentatively identified by molecular weight. None of the studies have reported the precise identification of the degraded proteins through advanced techniques, such as mass spectrometry.

Mass spectrometry is the most common method of choice for the high–throughput identification, characterization and quantification of proteins. The analysis of the mass spectra can provide information about the peptide sequence and abundance by applying different bioinformatic tools (Ortea et al., 2016). In addition, the use of mass spectrometry-based proteomic techniques also allows the identification of peptides generated during ripening (Mora et al., 2015) and the cleavage sites of proteolytic enzymes to understand the proteolytic mechanisms during processing. Hence, this study aimed to assess the muscle protein profile of dried salted abo with different salt concentrations and drying time. It also aimed to clarify the profile of myosin heavy chain and actin fragments.

5.2 Methods

5.2.1 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

For fish myofibrillar protein extraction, about 50 mg sample was homogenized with 1 ml buffer (5% NaCl, 0.02 M Tris-HCl, pH 7.2) and 0.1% protease inhibitor cocktail (Nacalai Tesque, Inc., Japan). The supernatant was

obtained by centrifugation at 0°C, 13,416 *x g* for 20 min. The protein concentration was determined by the Coomassie protein assay reagent kit (Thermo Fisher Scientific, USA) following the manufacturer's instructions.

Five micrograms of sample were treated with the sample buffer solution with and without 2x 2-Mercaptoethanol (2-ME; Nacalai Tesque, Inc., Japan) for SDS-PAGE and electrophoresed in 10% polyacrylamide gel by Tricine SDS-PAGE system (Schägger, 2006). Electrophoresis was carried out at a constant voltage of 100 volts and current of 100 mA for 3 hours. The protein bands were stained with 0.04% Coomassie Brilliant Blue R-250 in 40% methanol and 10% acetic acid. After staining, gels were destained with 10% acetic acid.

In order to visualize the low molecular weight proteins, Tricine SDS-PAGE was performed using a 20% polyacrylamide gel. The protein bands were stained with the 0.04% Coomassie Brilliant Blue R-250 followed by silver staining with Sil-Best stain (Nacalai Tesque, Inc., Japan). The molecular weights were estimated by reference to the relative mobilities of standard proteins.

5.2.2 Protein identification by liquid chromatography-tandem mass spectrometry (LC-MS/MS)

The proteins were electrophoresed in 10% and 20% SDS-PAGE gels, and were stained with SimplyBlue SafeStain (Invitrogen, USA) for 1 h. After the excision of the targeted protein band, the gel was incubated in a destaining solution (50 mM NH_4HCO_3, 50% acetonitrile) for 10 min at room temperature, and the destaining solution was replaced. This procedure was repeated until the gel was clear. The gel was then sequentially incubated with a reduction solution (10 mM

dithiothreitol, 100 mM NH_4HCO_3) for 60 min at 56°C and an alkylating solution (50 mM iodoacetamide, 100 mM NH_4HCO_3) for 45 min at room temperature in the dark. After reduction and alkylation of the protein, the gel was rinsed by 100 mM NH_4HCO_3 for 10 min at room temperature. The gel was vortexed in 100 mM NH_4HCO_3 for 5 min, and then again in acetonitrile for 5 min before being dried by vacuum centrifugation. The dried gel was soaked in a digestion solution (80 μL of 10 ng μL−1 trypsin gold, mass spectrometry grade, Promega, Madison, WI, USA: 50 mM NH_4HCO_3) on ice for 60 min. The gel was then incubated overnight in 100 mM NH_4HCO_3 at 37°C. The digested peptides were extracted by sonication in 50% acetonitrile and 70% acetonitrile in 0.1% trifluoroacetic acid. The sample was concentrated by vacuum centrifugation and suspended in 5% acetonitrile–0.1% trifluoroacetic acid, then desalted using GL-Tips SDB (GL Sciences Inc.) and dried by vacuum centrifugation, and then dissolved in 5% acetonitrile–0.1% trifluoroacetic acid. The sample was analyzed using a Finnigan LTQ XL mass spectrometer (Thermo Fisher Scientific, Inc.), coupled with liquid chromatography (Michrom BioResources, Inc. AUBURN CA, USA) and equipped with a nanoelectrospray ion source (Thermo Fisher Scientific, Inc.). The MS/MS data were analyzed with an in-house database by Mascot server, using Proteome Discoverer software version 1.2 (Thermo Fisher Scientific, Inc.).

5.2.3 Peptide sequence analysis

Six myosin heavy chain and three actin fragments were subjected to peptide sequence analysis. The reference sequence was selected based on the sequence with the highest frequency (100%) observed in the sequence data (Appendix 2). These

sequences were downloaded from the NCBI protein sequence database. The reference sequence for MHC was the common carp (*Cyprinus carpio*) with accession number Q90339. On the other hand, the reference sequence for actin was African clawed frog (*Xenopus laevis*) with accession number P04751. In the LC-MS/MS data, peptide sequences with high, medium (data not shown) and low confidence scores were aligned with the reference sequence in the MEGA X. The sequences with high confidence and low confidence scores were referred to as the high-quality peptides and low-quality peptides, respectively. The assigned score for spectral correlation by MASCOT was calculated by the probability that the matching spectrum originates from the matched candidate sequence. High confidence, medium confidence and low confidence hits are interpreted as 99%, 95% and 90% accuracy to the matched peptides in the database. Positions of the N-terminus peptides and C-terminus peptides among the peptide sequences were located based on the alignment with the reference sequence. To calculate the predicted molecular weight of the high-quality peptides and low-quality peptides, the number of amino acids was estimated by subtracting the position of the N-terminus amino acid of N-terminus peptide from the position of the C-terminus amino acid of the C-terminus peptide and then multiplied by 110 Da, which is the average molecular mass of an amino acid. The N-terminus amino acid residue of N-term peptide and C-terminus amino acid residue of C-term peptide were recorded. In addition, the amino acid residue before the N-terminus amino acid of N-terminus peptide and the amino acid following the C-terminus amino acid of the C-terminus peptide were noted from the reference sequence.

5.3 Results

5.3.1 SDS-PAGE pattern

A total of 23 bands of different molecular weights were detected. In the 10% Tricine SDS-PAGE gel, seventeen bands were detected (Figure 5.1). High intensity of protein bands with MW of 46 kDa (band # 7), 37 kDa (band # 10), 10 kDa (band # 18) and at the top of the gel (276 kDa) were observed.

In 12 h drying (lanes 5 – 7), higher staining intensity was observed in MW of 276 kDa (band # 1) while the bands at sizes of 136 kDa (band # 2), 58 kDa (band # 6), 43 kDa (band # 8), 33 kDa (band # 11), 25 kDa (band # 13) and 19 kDa (band # 14) revealed a lower intensity. However, at 6 h drying (lanes 2 – 4), staining intensity at MW of 276 kDa (band # 1) and 136 kDa (band # 2) were higher as compared to the bands at 73 kDa (band # 4), 58 kDa (band # 6), 43 kDa (band # 8), 33 kDa (band # 11), 27 kDa (band # 12) and 19 kDa (band # 14). The protein profile of 12% salt concentration samples was different from the 4% and 8% salt concentration samples (Figure 5.1).

On the other hand, Tricine SDS-PAGE using the 20% gel (Fig, 5.2 and Fig. 5.3) revealed additional six low molecular weight protein bands below 20 kDa (bands # 16, # 17, # 20, # 21, # 22, and # 23). At 12 h drying (lanes 5 – 7), higher staining intensity bands was observed at MW of 5.0 kDa (band # 21) and 4 kDa (band # 22) compared to bands at 15 kDa (band # 16), 12 kDa (band # 17) and 2 kDa (band # 23). On the other hand, at 6 h drying (lanes 2 – 4), bands at 15 kDa (band # 16), 12 kDa (band # 17), 5.0 kDa (band # 21) and 4 kDa (band # 22) revealed higher staining intensity than at MW of 2 kDa (band # 23).

5.3.2 Identification of proteins by mass spectrometry

The identified protein in each band corresponds to the most abundant protein with the highest score for each band as analyzed by the Proteome Discoverer 1.2 software (Table 5.1, Fig. 5.1 and Fig. 5.2). Among the 23 bands, 14 identified proteins were the same protein with the second-highest score (data not shown). The identified proteins included the fragments of myofibrillar proteins (bands 1 – 6, 8 – 10, 12, 14, 16 – 17, 20 – 21) and sarcoplasmic proteins (bands 7, 11, 13, 15, 18 – 19, 22 – 23).

5.3.3 Sequence analysis

Figures 5.4 present the schematic representation of the six MHC fragments. The N-terminus amino acid of the N-terminus peptide of band 4 (Fig. 5.4a) was found at position 1421 (high quality) and 1143 (low quality) while in band 5 (Fig. 5.4b), it was at position 1398 (high quality) and 1453 (low quality) of the reference sequence. In both band 8 (Fig. 5.4c) and band 14 (Fig. 5.4e), these were located at position 1398 (high quality) and 354 (low quality). In band 12 (Fig. 5.4d), it was positioned at 1398 (high quality) and 1384 (low quality) whereas in band 16 (Fig. 5.4f) it was found at position 1701 (high quality) and 953 (low quality). On the other hand, the positions of the C-terminus amino acid of the C-terminus peptides of bands 4, 8 and 12 were located at 1910 (high and low quality). In band 5, it was found at position 1713 (high quality) and 1910 (low quality). In band 14, it was positioned at 1713 (high quality) and 1910 (low quality), and in band 16, also found at position 1713 (high quality) and 1698 (low quality).

The predicted sizes of high-quality peptides were in the range of 56 kDa (Fig. 5.4c, 5.4d, 5.4e), 54 kDa (Fig. 5.4a), 35 kDa (Fig. 5.4b), and 1 kDa (Fig. 5.4f). Among the MHC fragments, bands 4, 5, and 16 have predicted sizes of 54 kDa, 35 kDa, 1 kDa, respectively. These sizes are smaller than the apparent MW in SDS-PAGE of 73 kDa (Fig. 5.4a), 61 kDa (Fig. 5.4b), and 16 kDa (Fig. 5.4f), respectively. On the other hand, the other three MHC peptides, bands 8, 12, and 14 have larger predicted sizes of 56 kDa (Fig. 5.4c, 5.4d, 5.4e). In addition, in the case of low-quality peptides, bands 4, 8, 12, 14, and 16 have larger predicted sizes than the apparent MW of SDS-PAGE whereas only band 5 has smaller predicted size.

Bands 4, 5, 8, 12, and 16 were observed to contain arginine (R) as the C-terminus amino acid of the C-terminus peptide (both high and low-quality) whereas in band 14, it contained arginine (high quality) or lysine (low quality). Moreover, by the reference sequence, it was confirmed that arginine was the amino acid residue before the N-terminus amino acid of the N-terminus peptide for bands 4 and 12 (both high and low quality). On the other hand, arginine or lysine (K) were the amino acids found in bands 5, 8, 14 and 16.

Figure 5.5 shows the schematic representation of three actin fragments. The N-terminus amino acid of the N-terminus peptide of band 17 (Fig. 5.5a) was at position 53 (high quality) and 65 (low quality), in band 20 (Fig. 5.5b), it was found at position 53 (high quality) and 87 (low quality) and in band 21 (Fig. 5.5c), it was positioned at 53 (high quality) and 180 (low quality). Moreover, the positions of the C-terminus amino acid of the C-terminus peptides of bands 17 and 20 were found at 374 (high quality) and 337 (low quality). In band 21, it was positioned at 374 (high and low quality).

Predicted sizes of the three actin (bands 17, 20 and 21) were larger than the observed sizes in SDS-PAGE. The three actin fragments (band 17, 20, 21) also contained arginine (both high and low quality) as the C-terminus amino acid residue in the C-terminus peptide. In the reference sequence, lysine (high quality) and arginine (low quality) were found as the amino acid residues before the N-terminus amino acid of the N-terminus peptides of bands 17 and 21 whereas lysine (both high and low quality) for band 20.

5.4 Discussion

5.4.1 Protein profile

SDS-PAGE electrophoretic patterns of 12 h and 6 h dried samples have shown clear and defined bands. This could be attributed to the addition of protease inhibitors during the protein extraction to minimize proteolysis. By LC-MS/MS, nine proteins were identified. The high-intensity protein bands corresponded to tropomyosin (37 kDa), actin (276 kDa), beta-enolase (46 kDa), and parvalbumin (10 kDa). Among these identified proteins, tropomyosin, beta-enolase, L-lactate dehydrogenase, triosephosphate isomerase, nucleoside diphosphate kinase, and parvalbumin were found stable in their native MW. On the contrary, myosin heavy chain, actin, and keratin had dissociated into fractions that were different from their theoretical MW. SDS-PAGE of raw muscles of herring and pompano have shown myosin and actin at approximately 200 kDa and 42 kDa, respectively (Christensen et al., 2011; Zhang et al., 2020). In both reducing (data not shown) and non-reducing SDS-PAGE conditions, the original MW of the myosin heavy chain (220 kDa), actin (42 kDa), and keratin (62 kDa) were not observed. This could be due to the loss of

moisture imposed by soaking in the brine solution and exposure to sun-drying resulting in the denaturation of proteins. During processing, these proteins could have been degraded by proteolytic enzymes. An earlier study has shown similar results that the endogenous enzymes such as cathepsins B, H, K, and D during the processing of dried salted scad have contributed to the degradation of the fish protein (Wu & Cao, 2018).

In the present study, changes in the protein profile were observed for both samples in 6 h and 12 h drying time. Among different salt concentrations in 12 h drying, similar band patterns were observed except for bands 1, 2, 6, 8, 11, 13 and 14. By mass spectrometry, these bands corresponded to actin (276 kDa and 136 kDa), MHC (43 kDa and 19 kDa), L-lactate dehydrogenase (33 kDa), and triosephosphate isomerase (25 kDa). Between 8% and 12% salt concentration samples, the band intensity of actin (276 kDa) had increased. On the contrary, decreased band intensities were observed in actin (136 kDa), MHC (43 kDa and 19 kDa), and triosephosphate isomerase (25 kDa). This indicates the occurrence of fragmentation of myosin heavy chain and polymerization of actin.

Moreover, at 6 h drying, similar band patterns were observed except for proteins identified as actin, MHC, and L-lactate dehydrogenase. In the 12% salt concentration samples, the band intensity of high-molecular-weight actin (276 kDa and 136 kDa) increased, whereas the intensity of actin with low-molecular-weight decreased (58 kDa), suggesting that actin might have polymerized. In the case of MHC fragments (73 kDa, 43 kDa, 27 kDa, and 19 kDa), band intensities markedly decreased. These observed changes were in contrast to the SDS-PAGE pattern of processed anchovies, which detected heavy band patterns tentatively identified as

myosin and actin. The boiling process before drying the anchovies could have inactivated the proteases; thus, myosin and actin were still found intact and retained in their original MW (Dewi, 2002).

On the other hand, the electrophoretic profile of the low molecular weight proteins (less than 20 kDa) was clearly visualized in the 20% Tricine gel (Fig. 5.2 and Fig. 5.3). Silver staining is a highly sensitive method for detecting proteins and is about 10 – 20 times more sensitive than Coomassie Blue staining (Scopes, 2010, p. 303). At 12 hours drying, there was decreased staining intensities of MHC (19 kDa), actin (12 kDa and 5 kDa), and keratin (4 kDa and 2 kDa) in the 12% salt concentration samples. However, MHC (15 kDa) was not detected among all samples at 12 h drying. In the case of 6 h drying, keratin (4 kDa and 2 kDa) was not detected in 12% salt concentration samples. Decreased intensities of actin (12.0 kDa and 5.0 kDa) were observed in 8% and 12% salt concentration samples. Moreover, MHC (15 kDa) had disappeared in the 8% and 12% salt concentration samples.

The present study was similar to the SDS-PAGE patterns of dried salted fish that have shown degradation of myofibrillar proteins influenced by different processing conditions. Thorarinsdottir et al. (2002) stated that myosin and actin were degraded to some extent during the salting of cod. Actin molecule appeared to be more resistant while myosin appeared to be more vulnerable to denaturation by heavy salting. In another previous study, intense aggregation of myofibrillar proteins were observed, particularly myosin, as a consequence of the overall salting process of wet-salting followed by dry-salting (Martínez-Alvarez & Gómez-Guillén, 2006). Sannaveerappa et al. (2004) also found immediate loss of both high- and low-MW protein bands in dried milkfish due to the combined effects of salting and sun-drying.

5.4.2 Peptide sequence profile

Characterization of the peptide fragments from the excised bands from SDS-PAGE and identified as myosin heavy chain and actin through LC-MS/MS was presented in this study. Knowing the position of the of N-terminus peptides and C-terminus peptides of each myosin heavy chain fragment (Fig. 5.4) by alignment with the reference sequence (*Cyprinus carpio*) may have revealed the estimated location of the generated N-terminus and C-terminus peptides of the six protein bands in the myosin molecule. Structurally, myosin heavy chain is a highly asymmetric protein with a globular head and a long rod or tail domain. The heavy meromyosin subfragment-1 is the N-terminal head domain of the myosin heavy chain (MHC) (about 800 amino acid residues) (Asghar & Pearson, 1980; Offer, 1987). On the other hand, the myosin rod domain can be proteolytically cleaved into heavy meromyosin subfragment-2 (HMM-S2), the N-terminal-third, and light meromyosin (LMM) corresponding to the C-terminal two-thirds of the rod (Wick, 1999). Since the N-terminus amino acids of the N-terminus peptides were found at positions 1398 (bands 5, 8, 12, 14), at 1421 (band 4), and at 1701 (band 16) of the reference sequence, this suggest that the six MHC fragments were derived from the LMM region, located on the C-terminal side of the myosin heavy chain.

During the heating process, aggregation of two heads of myosin molecules takes place followed by denaturation of tail portions. Further aggregation of the resulting oligomers occurs forming a clump through the tails which contributes to the formation of gel network (Lanier et al., 2014). Similar changes in the myosin heavy chain could also be observed during the salting and drying processes. This could be

evident in all of the six MHC fragments which seemed to be found in the LMM region.

On the other hand, the cleavage sites of the MHC fragments were not clarified in this study. Among the 23 bands detected in SDS-PAGE, six fragments identified as MHC could have been degraded by endogenous proteases. During curing and drying, the presence of different cleavage sites induced by the enzymes involved during these processes can be speculated. However, there could be a low possibility to find arginine and lysine in the cleavage sites of the amino acid sequence. After mass spectrometry, there could be a possibility of finding at least one peptide which may contain other amino acid in the C-terminal side. In contrast, the peptide sequence data have shown that the C-terminus amino acids of the C-terminus peptides appeared to be arginine and lysine in the six MHC fragments (bands 4, 5, 8, 12, 14 and 16), suggesting that the native C-terminus cleavage site cannot be speculated. In addition, the N-terminus cleavage site cannot be estimated by mass spectrometry analysis. Hence, the N-terminus amino acid among all the peptides was identified and compared to the reference sequence to estimate its position. The amino acid before the N-terminus peptides can therefore be estimated. Because the preceding amino acid of the N-terminus peptides of the six MHC fragments was found to be arginine or lysine, it can be speculated that these fragments detected by mass spectrometry were derived from trypsin digestion, hence, it was unlikely to identify the native N-terminus cleavage site.

Furthermore, predicted sizes from mass spectrometry and the apparent MW in SDS-PAGE of the six MHC fragments were compared. Smaller and larger predicted sizes of the six high-quality MHC peptides were observed. Since the native

N-terminus and C-terminus cleavage sites were unlikely to be identified, this could have contributed to the smaller predicted sizes of three MHC fragments (bands 4, 5, and 16), thus, sequence coverage of the identified peptides was limited.

On the other hand, three MHC fragments (bands 8, 12, and 14) exhibited similar pattern of larger predicted sizes compared to the apparent molecular weight in SDS-PAGE. This could be attributed to the distribution of the identified peptides sequences that were distant from each other, thereby, larger coverage of sequences was observed. Furthermore, the larger predicted sizes may indicate that it covered a larger portion of the repetitive sequence of the myosin tail domain. The rod-like tail sequence has a 28-residue repeat pattern composed of four heptads (Strehler et al., 1986; Wick, 1999). The amino acid sequence data of abo was observed to be arranged into repetitive sequence pattern with hydrophobic amino acid residues, which might have contributed to a larger range of sequences, suggesting the larger predicted sizes.

In the case of actin, the position of the N-terminus amino acid of the N-terminus peptides of the three actin fragments (bands 17, 20, and 21) when compared to the reference sequence (*Xenopus laevis*) could have indicated its location at the four subdomains (subdomains 1, 2, 3, and 4) of the actin molecule (Fig. 5.5). Similar to MHC fragments, arginine and lysine were found to be the generated C-terminus amino acid residues of the C-terminus peptides and the residue before the N-terminus amino acids of the N-terminus peptides of the three actin fragments. This suggests the possibility that the native N-terminus and C-terminus cleavage sites of proteases could not be identified. Moreover, the predicted sizes of the actin fragments (bands 17, 20, 21) were larger compared to the apparent MW in SDS-PAGE. This could

have been influenced by the distribution of the identified peptides that were distant from each other in the actin sequence. This could also indicate that the actin molecule appeared to be less susceptible to proteolytic actions. Taken together, all the three actin fragments seemed to be generated from the four subdomains of the actin sequence as observed from the coverage of predicted sizes, however, the exact point of cleavage of proteases was unlikely to be identified.

Table 5.1 Protein identification by LC-MS/MS in dried abo

Band No.	Accession No.	Identified Protein	Mascot score	Sequence coverage	No. of peptides	Theoretical molecular weight	Estimated molecular weight
1	P68140	Actin, alpha skeletal muscle	4607.48	83.29	26	41.9	276
2	P04751	Actin, alpha cardiac muscle	850.43	42.71	17	42.0	136
3	P84335	Tropomyosin alpha-1 chain	935.96	67.61	30	32.7	86
4	Q90339	Myosin heavy chain, fast skeletal muscle	1224.96	12.45	29	221.5	73
5	Q90339	Myosin heavy chain, fast skeletal muscle	556.46	5.12	9	221.5	61
6	P04751	Actin, alpha cardiac muscle	35.54	497.14	15	42.0	58
7	B5DGQ7	Beta-enolase	2795.37	46.31	19	47.3	46
8	Q90339	Myosin heavy chain, fast skeletal muscle	1182.88	14.16	32	221.5	43
9	P84335	Tropomyosin alpha-1 chain	774.90	72.18	31	32.7	39
10	P84335	Tropomyosin alpha-1 chain	4269.82	85.92	48	32.7	37
11	O13276	L-lactate dehydrogenase A	926.30	36.4	13	36.4	33

Table 5.1 continued

12	Q90339	Myosin heavy chain, fast skeletal muscle	610.36	7.03	19	221.5	27
13	Q90XG0	Triosephosphate isomerase B	2985.31	43.55	12	26.8	25
14	Q90339	Myosin heavy chain, fast skeletal muscle	459.04	6.98	17	221.5	19
15	P85292	Nucleoside diphosphate kinase B	512.91	23.81	3	14.1	16
16	Q90339	Myosin heavy chain, fast skeletal muscle	235.08	2.22	5	221.5	15
17	P04751	Actin, alpha cardiac muscle	397.56	33.16	14	42.0	12
18	P09227	Parvalbumin alpha	355.42	28.44	3	11.4	9
19	P35527	Keratin, type I cytoskeletal 9	379.03	9.79	4	62.0	8
20	P04751	Actin, alpha cardiac muscle	297.07	28.38	10	42.0	7
21	P04751	Actin, alpha cardiac muscle	309.51	19.10	10	42.0	5
22	A5A6M6	Keratin, type II cytoskeletal 1	120.51	7.85	15	65.4	4
23	A5A6M6	Keratin, type II cytoskeletal 1	186.46	9.42	6	65.4	2

*Estimated MW from SDS-PAGE analysis

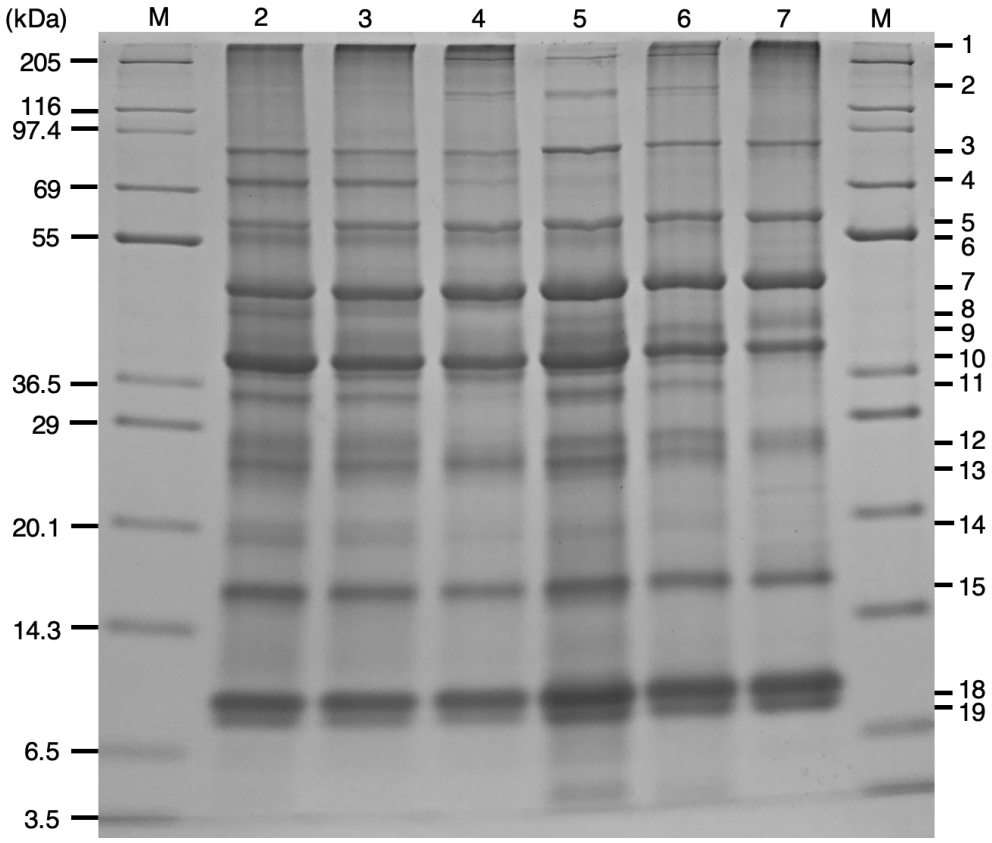


Figure 5.1 SDS-PAGE pattern of dried abo with different salt concentration and drying time in 10% Tris-Tricine gel

Protein marker (lanes M); 4% salt concentration, 6 hours (lane 2); 8% salt concentration, 6 hours (lane 3); 12% salt concentration, 6 hours (lane 4); 4% salt concentration, 12 hours (lane 5); 8% salt concentration, 12 hours (lane 6); 12% salt concentration, 12 hours (lane 7)

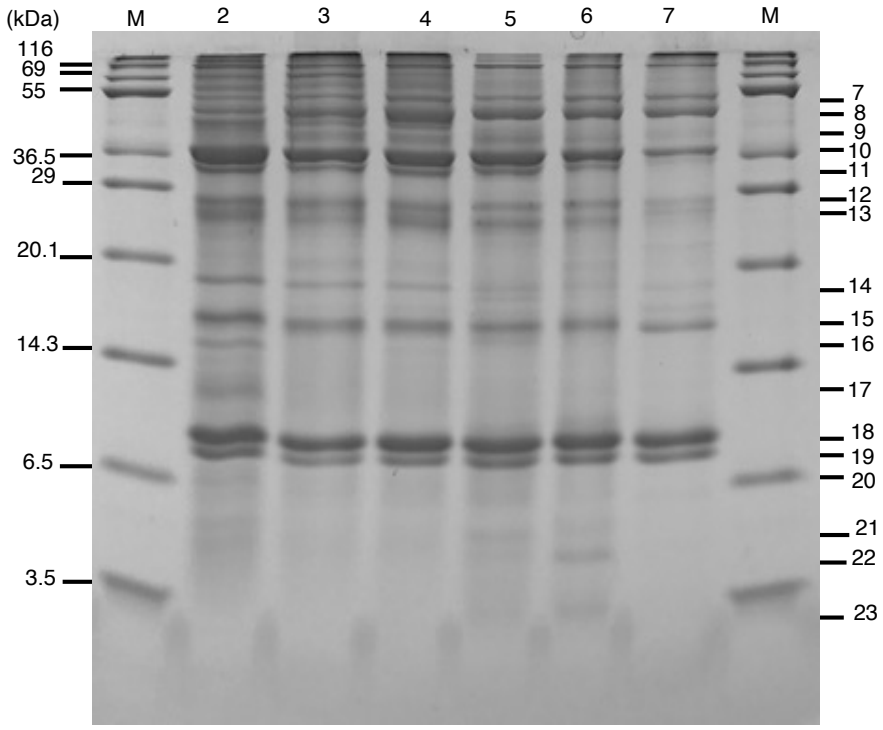


Figure 5.2 SDS-PAGE pattern of dried abo with different salt concentration and drying time in 20% Tris-Tricine gel

Protein marker (lanes M); 4% salt concentration, 6 hours (lane 2); 8% salt concentration, 6 hours (lane 3); 12% salt concentration, 6 hours (lane 4); 4% salt concentration, 12 hours (lane 5); 8% salt concentration, 12 hours (lane 6); 12% salt concentration, 12 hours (lane 7)

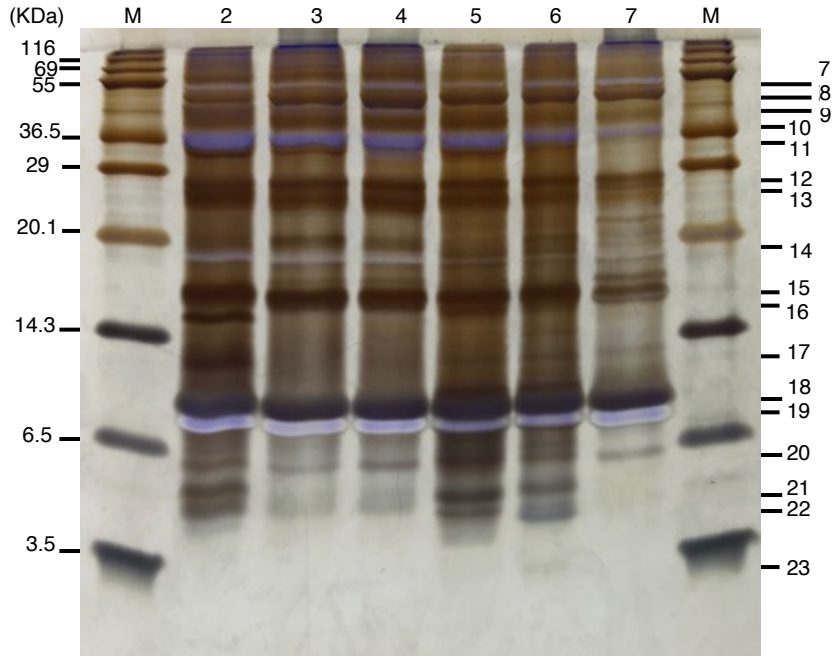


Figure 5.3 SDS-PAGE pattern of dried abo with different salt concentration and drying time in 20% Tris-Tricine gel with silver staining

Protein marker (lanes M); 4% salt concentration, 6 hours (lane 2); 8% salt concentration, 6 hours (lane 3); 12% salt concentration, 6 hours (lane 4); 4% salt concentration, 12 hours (lane 5); 8% salt concentration, 12 hours (lane 6); 12% salt concentration, 12 hours (lane 7)

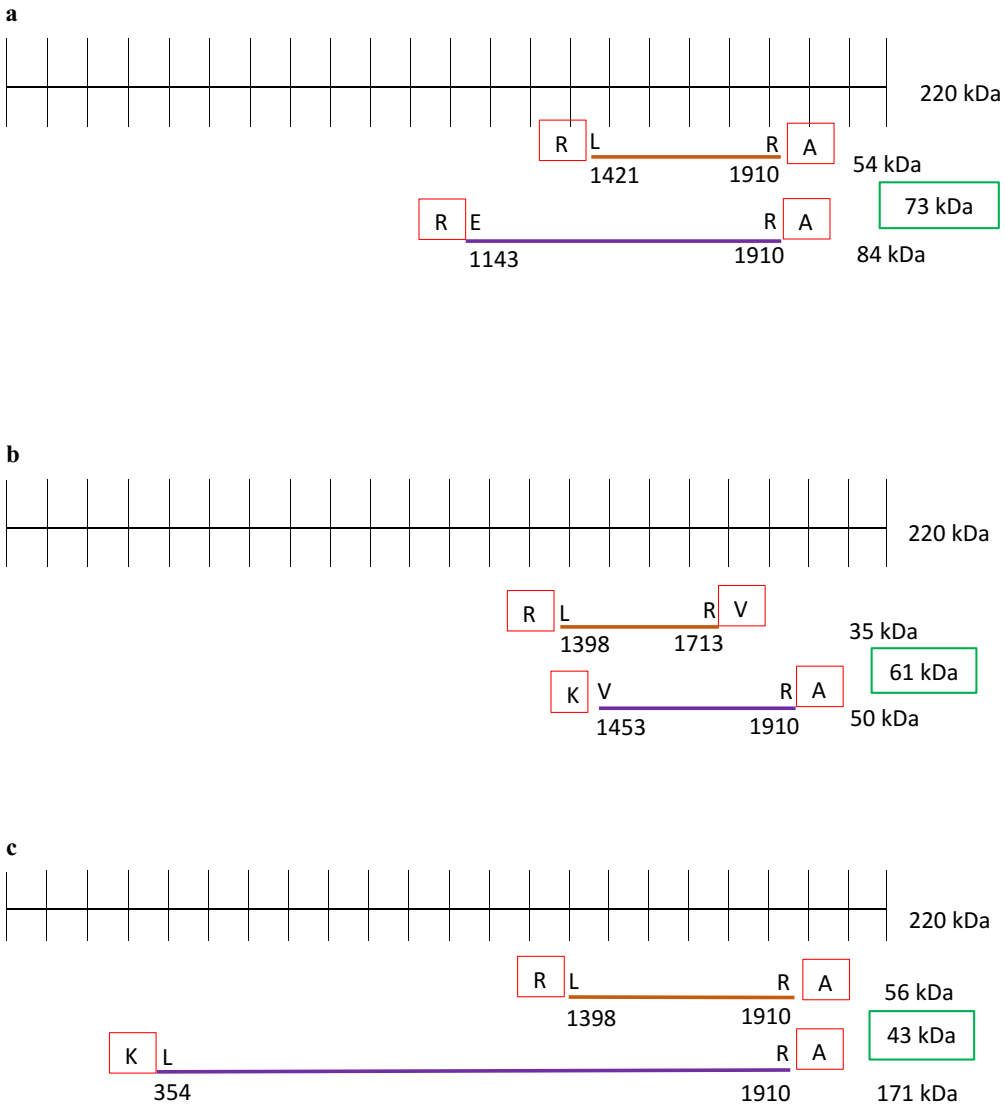


Figure 5.4 Map of peptide sequences from myosin heavy chain fragments

(a) MHC band 4 **(b)** MHC band 5 **(c)** MHC band 8

high-quality sequences with predicted MW
low-quality sequences with predicted MW
MW (kDa) in SDS-PAGE

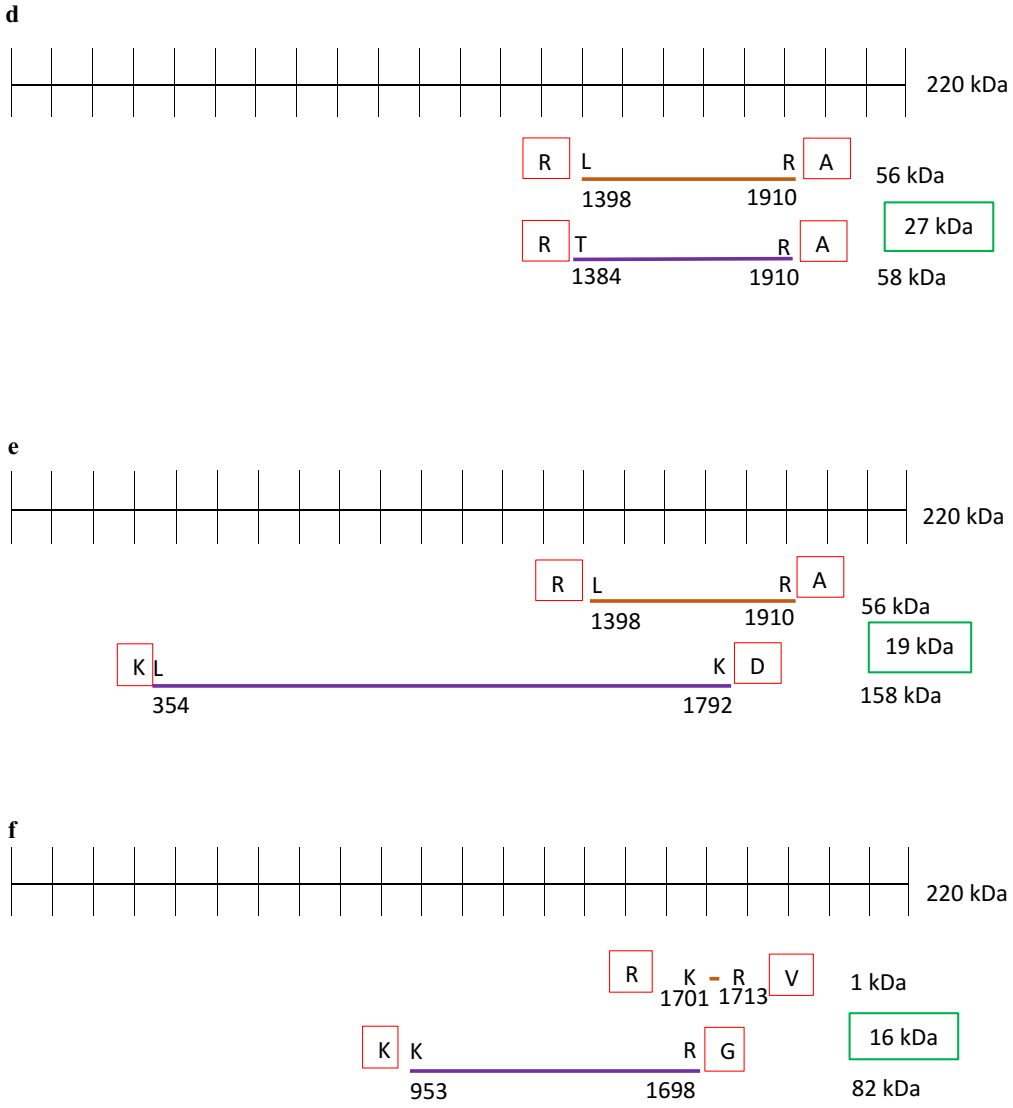


Figure 5.4 Map of peptide sequences from myosin heavy chain fragments

(**d**) MHC band 12 (**e**) MHC band 14 (**f**) MHC band 16

high-quality sequences with predicted MW
low-quality sequences with predicted MW
MW (kDa) in SDS-PAGE

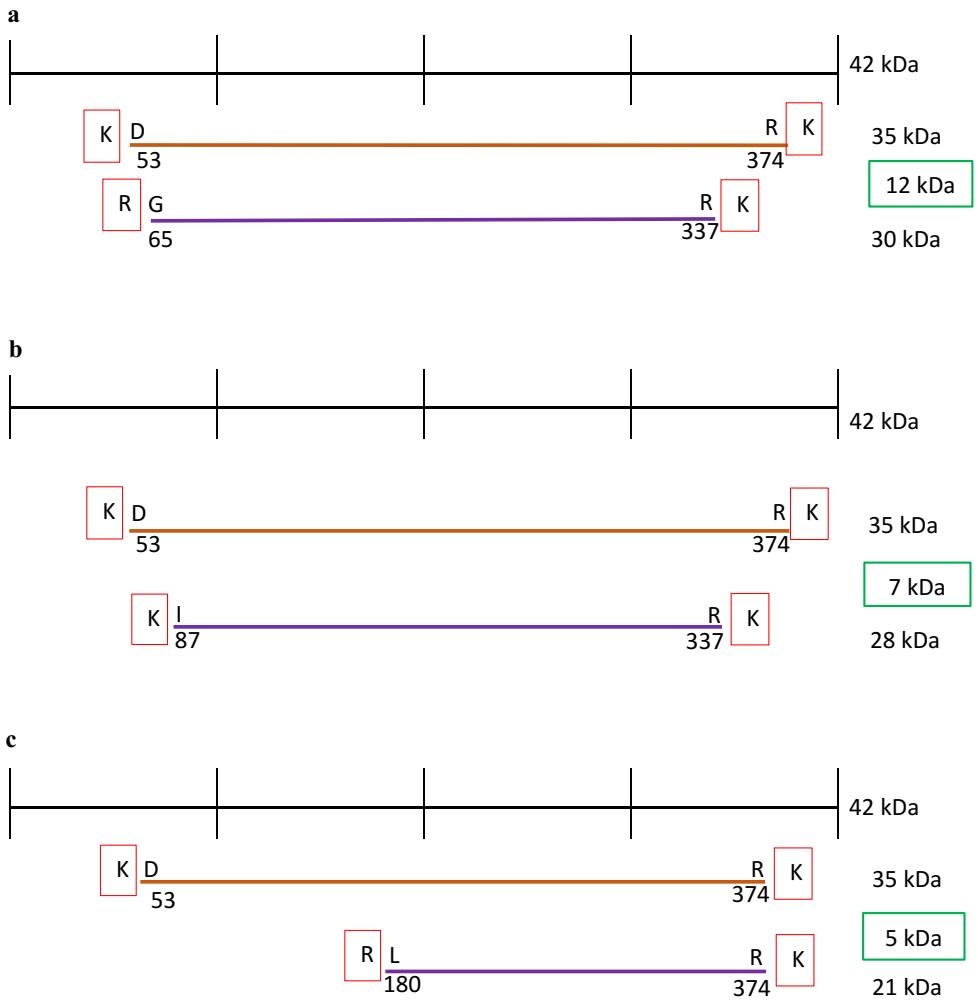


Figure 5.5 Map of peptide sequences from actin fragments

(a) Actin band 17 **(b)** Actin band 20 **(c)** Actin band 21

high-quality sequences with predicted MW
low-quality sequences with predicted MW
MW (kDa) in SDS-PAGE

Chapter 6: General Discussion

6.1 Status of abo fishing, dried abo processing, and species identification

The Bicol region is a top fishery region in the Philippines, one of the leading contributors to the municipal fisheries in the country. San Miguel Bay is one of the most productive fishing grounds in the region, with abo fisheries as the leading industry in the bay. Since abo is a commercially important fish species and popularly marketed as dried fish products, there is a need to assess the status of this fishery resource to determine the strategies to sustain the fishery resource production that would eventually sustain the livelihood of the local fisherfolk and processors. The status of fisheries in the Bicol region was characterized by declining fish catch and higher fishing efforts (Silvestre and Hilomen, 2004; Nieves et al., 2009; Olaño et al., 2018). This is in agreement with the respondents' claim that declining fish stock was one of their challenges. Although Lanzuela et al. (2020) reported that abo is still in good condition of stock based on the spawning potential ratio data and the NSAP-BFAR data (unpublished) showed increasing landed catch trend of abo, it is still important that management measures be directed towards the sustainability of the *O. ruber* stock.

For sustainable resource management, the production of abo should be kept stable to prevent overexploitation of resources. As reported by the respondents, small-scale fishers using gillnet could not compete against the commercial trawlers, hence, they have lower income. The utilization of more efficient fishing gear could increase the catch rate and increase their income. However, the regulated use of fishing gears is important to prevent unsustainable fishing that could lead to

depletion of fishery resources (Olaño et al., 2018). In addition, strong enforcement of fishery rules and regulations, specifically on the closed season, is recommended.

Moreover, improving the market value by producing acceptable and highly graded dried abo products may contribute to the increase of income of dried fish processors. Observation of the drying facility revealed that sanitation requirements and availability of cold storage should be complied to minimize microbiological contamination of the products. This is in agreement with the reported problems faced by the fish processing industry in the country (Guevara et. al., 1984), wherein lack of adequate facilities necessary for handling, processing, and distribution could lead to inferior quality of processed fish products. It should also be emphasized to the processors to take advantage of the opportunity to use vacuum packaging technology to maintain the quality and extend the shelf life of the dried fish. Through vacuum packaging, product presentation will be improved, thus attracting new consumers, especially among the middle-income class of society.

In Chapter 3, it was presented that there has been no molecular data yet available in SMB. The present study was able to clarify the species identification of abo, which could provide the first molecular report of abo species in SMB. In addition, despite the respondents' claim that they could distinguish abo from other species having similar features, there could be a possibility that misidentification of fish species could occur on the field and during marketing by fish traders. Abo is considered a higher value fish species than pagotpot and arakaak. Thus, enhancing the fishers' and stakeholders' awareness with the correct identification of fish species would be important to prevent the substitution of a less valuable species for a valuable fish species, which might result in an economic loss on the part of the fish

trader or processor. Correct identification would also be helpful for efficient assessment and management of the *Otolithes ruber* stocks.

6.2 Chemical composition of dried abo products

The knowledge on the chemical composition of the dried abo products with different salting and drying time conditions could contribute to understanding the influence of these processes to the nutritional value and sensory characteristics of the products. For fish processors, the knowledge on the nature of the raw material is essential for them before they can apply the proper processing techniques. For the consumers, they are mainly interested to the sensory quality and its contribution to health and safety. The food composition information would also guarantee product quality. However, there have been no studies conducted yet on the chemical composition of dried fish products in SMB.

In Chapter 2, the practice of heavy salting or brining at high salt concentration and long drying time by the processors in SMB highlighted the need for development of the product, particularly, the reformulation of the salt content. The standard salt concentration and drying time was estimated at 8% salt concentration and 12 h drying time. Analysis of the salt content (Chapter 4) of the laboratory-produced dried abo indicated that the salt (NaCl) content of the samples corresponded to the added salt during processing. The proximate composition data of dried abo samples also revealed a significant decrease in moisture at 12% salt concentration at 6 h drying, which was consistent with the observed crude powder texture of the sample, which could be another control point for modification of the product. Therefore, salt concentration between 8% and 12% can be controlled at 6 h

drying. Precise control of quality parameters such as salt concentration, brining time, size of fish for processing, drying time, and cold storage, are the key components that will be monitored in the product. Further investigation should include preference testing to determine the most-liked product by consumers.

Physical and chemical changes that occur during ripening, accompanied by drying, determine the overall sensory qualities of salted fish products (Sikorski, 1995). In Chapter 5, the protein profile of dried abo samples with 12% salt concentration revealed degradation and polymerization of myosin heavy chain and actin, respectively. The changes in the conformation of myofibrillar proteins associated with protein denaturation and aggregation may have contributed to the textural changes from paste-like to crude powder-like texture of samples with high salt concentration at 6 h drying. By protein sequencing analysis approach, the characteristics of the individual peptide fragments of myosin heavy chain and actin obtained by mass spectrometry has elucidated the changes that occurred during salting and drying. Processors should note that increasing the salt concentration and drying time would exhibit changes in the muscle protein profile influencing the texture of the products. Hence, these protein fragments are considered essential indicators to monitor the changes in adjusting the physical properties of the product. Analysis of the degradation of proteins into small peptides and free amino acids content should be further studied.

The present situation of the dried abo processing industry at Calabanga, Camarines Sur, in San Miguel Bay, necessitates the different local government agencies and the academe to assist and involve the processors and fishermen in training courses and dissemination of results of research studies. This study could

represent the first report regarding the biochemical composition of dried salted abo produced from different salt concentrations and drying times that could be beneficial to processors and consumers. The development of an acceptable dried salted abo product can pave the way for possibilities in improving a traditional Filipino product to a wider domestic market and contribute to the improvement of the dried fish industry and boost the economy of San Miguel Bay.

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Appendix 1a

QUESTIONNAIRE

A. **RESPONDENT INFORMATION**

Name:_____

Age: _____

Address:_____

Abo producer classification: [ ] small [ ] medium [ ] large

B. METHOD OF DRYING ABO

1. How long have you been drying "Abo"?
   [ ] less than one year [ ] 2 - 3 years [ ] 4 - 5 years
   [ ] 1 - 2 years [ ] 3 - 4 years [ ] more than 5 years
2. Do you have suppliers of the fresh abo? [ ] Yes [ ] No
   If yes, please state your supplier/s_____
   If no, where do you get or buy your raw materials?
   _____
3. What vehicle do you use in transporting the fish?
   [ ] refrigerated truck van
   [ ] jeepney
   [ ] tricycle
   [ ] Others, (please specify) _____
4. How long does it take to transport the fresh abo to your processing plant?
   [ ] less than 1 hour
   [ ] 1 hour
   [ ] 2 hours
   [ ] more than 3 hours
5. What do you use in transporting fresh abo?
   [ ] bañera
   [ ] styrofore
   [ ] pail
   [ ] Others, (please specify) _____
6. Do you chill the fish before drying? [ ] Yes [ ] No
   If yes, what is the chilling temperature? _____
7. What do you use for chilling?
   [ ] ice cooler
   [ ] styrobox

[ ] chiller equipment
[ ] none

8. Do you sort the “abo” before processing? [ ] Yes [ ] No
9. What are the qualities of fish do you consider for it to be included or discarded?
   [ ] freshness of the abo
   [ ] size of the abo
   [ ] freshness of the abo and size of the abo
   [ ] Others, (please specify) _____
10. What is the acceptable size or weight of abo suited for drying?
   [ ] less than 3 inches
   [ ] 4 inches
   [ ] 5 inches
   [ ] more than 5 inches
   [ ] Others, (please specify) _____
11. What type of dried abo do you produce?
   [ ] whole
   [ ] splitted
   [ ] butterfly fillet
   [ ] Others, (please specify) _____
12. If splitted or butterfly fillet, what do you use in splitting?
   [ ] bolo
   [ ] splitting knife
   [ ] cutting knife
   [ ] butchering knife
13. Where do you process the dried fish?
   [ ] at home [ ] processing facility
14. Do you salt the “abo” before drying? [ ] Yes [ ] No
   If yes, what method of salting do you use?
   [ ] dry salting
   [ ] kench salting
   [ ] brine salting
15. If dry salting, what is the ratio of salt and fish?
   [ ] 1:1
   [ ] 1:2
   [ ] 1:3
   [ ] Others, (please specify) _____
16. If brine salting, what is the ratio of salt and water?
   [ ] 1:1
   [ ] 1:2
   [ ] 1:3
   [ ] Others, (please specify) _____

17. What do you use in salting or brining?
    [ ] brining tank
    [ ] plastic basin
    [ ] stainless basin
    [ ] Others, (please specify) _____
18. What is the salting time for dry & kench salting?
    [ ] 1 hour
    [ ] 3 hours
    [ ] 8 Hours
    [ ] overnight
    [ ] Others, (please specify) _____
19. What is the brining time?
    [ ] 1 hour
    [ ] 3 hours
    [ ] 8 hours
    [ ] overnight
    [ ] Others, (please specify) _____
20. How many times do you wash the fish after salting or brining?
    [ ] once
    [ ] 2 times
    [ ] 3 times
    [ ] Others, (please specify) _____
21. What method of drying do you use?
    [ ] sun-drying
    [ ] artificial drying
    [ ] cabinet drying
22. If sun-drying, how many hours do you dry the abo?
    [ ] 6 hours
    [ ] 12 hours
    [ ] 24 hours
    [ ] Others, (please specify) _____
23. If artificial drying or cabinet drying, how many hours do you dry the abo?
    [ ] 6 hours
    [ ] 12 hours
    [ ] 24 hours
    [ ] Others, (please specify) _____
24. If the weather is rainy, how do you dry abo?

    _____
25. What material do you use in drying abo?
    [ ] bamboo rack
    [ ] aluminum rack
    [ ] stainless rack

[ ] Others, (please specify) _____

26. What equipment or instruments do you use for drying “abo”?
    [ ] solar dryer
    [ ] cabinet dryer
    [ ] thermometer
    [ ] Others, (please specify) _____
27. After drying, what packaging method do you use?
    [ ] vacuum packaging
    [ ] polyethylene bag packaging
    [ ] paper packaging
    [ ] carton packaging
    [ ] Others, (please specify) _____
28. Before packing, how do you sort the dried abo in terms of:
    [ ] quality _____
    [ ] size _____
    [ ] Others, (please specify) _____
29. What should be the condition in packaging the dried fish?

    _____
30. To generalize, what are the step-by-step procedure in drying “abo”?
    1._____
    2. _____
    3. _____
    4. _____
    5. _____

Appendix 1b

Questionnaire

No. _____

INTERVIEW GUIDE

A. RESPONDENT INFORMATION

Name: _____

Age: _____

Address: _____

Occupation: _____ Yrs of fishing/processing: _____

B. STATUS OF "ABO"

1 [ ] When is the peak season of "Abo"? (in months)

2 [ ] When is the lean season of "Abo"? (in months)

3 [ ] When is the spawning period of "Abo"?

4 [ ] During what period can you observe a drop in the catch of "Abo"?

5 [ ] What is the impact of the peak of Amihan and Habagat on the SMB fisheries catch?

6 [ ] Is there a fishing ban period here in San Miguel Bay?

7 [ ] Average catch per fisherman of "Abo"? What is the maximum and minimum catch? What is the total volume of fish catch?

8 [ ] Average weight of fish bought by dried fish processors?

9 [ ] What fishing gear/s used for catching "Abo"?

10 [ ] How do you classify the size of "Abo"? What is the length and weight?

[ ] small

[ ] medium

[ ] large

[ ] Others, (please specify) _____

11 [ ] Do you practice measuring the weight of "Abo" before drying? Is there a standard size and length?

12 [ ] What is the matured size of "Abo"?

13 [ ] How do you know if the fish is "Abo"? Do you know other related species?

14 [ ] Is there an available association/organization between fishermen/local officials?

C. FISH PROCESSING

1 ☐ Where do you supply your dried fish?

2 ☐ Fish drying starts and ends in what months?

3 ☐ How many kilos of Abo is being dried per day?

4 ☐ How long do you dry "Abo"? How many hours or days?
How long is the processing duration?

5 ☐ What is the endpoint of sundrying?

6 ☐ What is the salt concentration of dried Abo? How do you control the solution? Do you change the solution or not? Do you add/decrease seawater or freshwater or change it?

7 ☐ Maximum & minimum selling price of raw and dried "Abo" based on body size and season? Does price varies or not?

8 ☐ Number of pieces of "Abo" per 1 kg?

9 ☐ Type of packaging used for dried Abo? How do you store dried "Abo"?

10 ☐ What is your recommendation in cooking and eating dried Abo?

D.

1 ☐ How do you select the fishing area for the day? For the next day?

2 ☐ Which area or part is the fishing ground for Abo? What direction or landmark?
How long is the travel time?

3 ☐ Size of Abo during peak season?

4 ☐ Size of Abo during spawning season?

5 ☐ How do you recognize the sex of Abo as Male or Female?

6 ☐ How is "bulongan" or whispering method being practiced?

7 ☐ Who decide for the price of fresh Abo? How to decide the price?

8 ☐ Best size of Abo to be processed dried?

9 ☐ What is the preferred saltiness of dried fish by most of the consumers?

10 ☐ What is the preferred saltiness of dried fish by you and your family?

Appendix 2

Peptides of myosin heavy chain identified by LC-MS/MS (band 4)

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on Q90339	Position of AA residue of the peptide sequence (N-terminus) based on Q90339	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
1	High	Q90339	R	1593	1594	VIDSMQSTLDSEVR	S	1608
2	High	Q90339	R	1700	1701	KVAEQELVDASER	V	1714
3	High	Q90339	R	1593	1594	VIDSMQSTLDSEVR	S	1608
4	High	Q90339;Q8JIP5	R	1421	1422	LQGEVEDLMIDVER	A	1436
5	High	Q90339	K	1504	1505	NLQQEISDLTEQLGETGK	S	1523
6	High	Q90339;Q8JIP5	R	1421	1422	LQGEVEDLMIDVER	A	1436
7	High	P13535;P11055;A7E2Y1;Q076A3;P02565;P12847;Q28641;Q29RW1;Q02566;P02563;Q076A4;P13542;P13538;Q90339;Q8JIP5	K	1758	1759	AITDAAMMAEELKK	E	1773
8	High	Q90339	R	1700	1701	KVAEQELVDASER	V	1714
9	High	Q90339	R	1593	1594	VIDSmQSTLDSEVR	S	1608
10	High	Q90339	K	1504	1505	NLQQEISDLTEQLGETGK	S	1523
11	High	Q90339	R	1593	1594	VIDSmQSTLDSEVR	S	1608
12	High	P13535;P11055;A7E2Y1;Q076A3;P02565;P12847;Q28641;Q29RW1;Q02566;P02563;Q076A4;P13542;P13538;Q90339;Q8JIP5	K	1758	1759	AITDAAmMAEELKK	E	1773
13	High	P13535;P11055;A7E2Y1;Q076A3;P02565;P12847;Q28641;Q29RW1;Q02566;P02563;Q076A4;P13542;P13538;Q90339	K	1772	1773	EQDTSAHLER	M	1783
14	High	Q90339;Q8JIP5	R	1421	1422	LQGEVEDLmIDVER	A	1436
15	High	Q90339	K	1701	1702	VAEQELVDASER	V	1714
16	High	Q90339	K	1701	1702	VAEQELVDASER	V	1714
17	High	Q90339	R	1898	1899	KVQHELEEAQER	A	1911
18	High	P13535;P11055;A7E2Y1;Q076A3;P02565;P12847;Q28641;Q29RW1;Q02566;P02563;Q076A4;P13542;P13538;Q90339;Q8JIP5	K	1758	1759	AITDAAMmAEELKK	E	1773
19	High	P13535;P11055;A7E2Y1;Q076A3;P025	K	1758	1759	AITDAAmmAEELKK	E	1773

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on Q90339	Position of AA residue of the peptide sequence (N-terminus) based on Q90339	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
		65;P12847;Q28641;Q29RW1;Q02566;P02563;Q076A4;P13542;P13538;Q90339;Q8JIP5						
20	High	Q90339	R	1898	1899	KVQHELEEAQER	A	1911
21	High	Q076A3;P02565;Q90339	R	1690	1691	AALEQTER	G	1699
22	High	P13535;P11055;A7E2Y1;Q076A3;P02565;P12847;Q28641;Q29RW1;Q02566;P02563;Q076A4;P13542;P13538;Q90339;Q8JIP5	K	1758	1759	AITDAAMmAEELKK	E	1773
23	High	Q076A3;P02565;Q90339	R	1690	1691	AALEQTER	G	1699
24	High	P13535;P11055;A7E2Y1;Q076A3;P02565;P12847;Q28641;Q29RW1;Q02566;P02563;Q076A4;P13542;P13538;Q90339	K	1772	1773	EQDTSAHLER	M	1783
25	High	P13535;P11055;A7E2Y1;Q076A3;P02565;P12847;Q28641;Q29RW1;Q02566;P02563;Q076A4;P13542;P13538;Q90339;Q8JIP5	K	1758	1759	AITDAAmmAEELKK	E	1773
26	High	P13535;P11055;A7E2Y1;Q076A3;P02565;P12847;Q28641;Q29RW1;Q02566;P02563;Q076A4;P13542;P13538;Q90339;Q8JIP5	K	1758	1759	AITDAAmMAEELKK	E	1773
27	High	Q90339	K	1899	1900	VQHELEEAQER	A	1911
28	High	Q90339	K	1899	1900	VQHELEEAQER	A	1911
29	High	Q90339	K	1504	1505	NLQQEISDLTEQLGETGK	S	1523
30	High	Q02566;P02563;Q90339;Q8JIP5	K	1849	1850	ELTYQTEEDKK	N	1861
31	Medium	Q02566;P02563;Q90339;Q8JIP5	K	1849	1850	ELTYQTEEDKK	N	1861
32	Medium	Q90339	R	1898	1899	KVQHELEEAQER	A	1911
33	Medium	Q90339	R	1397	1398	LQDAEESIEAVNSK	C	1412
34	Medium	P02565;Q90339	R	1561	1562	VQLELNQVK	S	1571
35	Medium	P13535;P11055;A7E2Y1;Q076A3;P02565;P12847;Q28641;Q29RW1;Q02566;P02563;Q076A4;P13542;P13538;Q90339;Q8JIP5	K	1758	1759	AITDAAmmAEELKK	E	1773
36	Low	Q90339	K	1576	1577	LAEKDEEmEQIKR	N	1590
37	Low	P13535;Q076A3;Q29RW1;Q076A4;P13542;Q90339;Q8JIP5	R	1607	1608	SRNDALR	V	1615
38	Low	P13535;P11055;A7E2Y1;Q076A3;P025	R	1383	1384	TEELEEAKK	K	1393

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on Q90339	Position of AA residue of the peptide sequence (N-terminus) based on Q90339	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
		65;P12847;Q28641;Q29RW1;Q02566;P02563;Q076A4;P13542;P13538;Q90339;Q8JIP5						
39	Low	P13535;P11055;P02565;P12847;Q29RW1;Q02566;P02563;P13538;Q90339	R	1476	1477	SLSTELFK	M	1485
40	Low	Q90339	K	1701	1702	VAEQELVDASER	V	1714
41	Low	P02565;P13538;Q90339	K	1669	1670	EQVAMVER	R	1678
42	Low	Q90339	K	1899	1900	VQHELEEAQER	A	1911
43	Low	Q90339	K	1899	1900	VQHELEEAQER	A	1911
44	Low	P02565;Q90339	R	1561	1562	VQLELNQVK	S	1571
45	Low	P13535;P11055;P02565;P12847;Q29RW1;Q02566;P02563;P13538;Q90339	R	1476	1477	SLSTELFK	M	1485
46	Low	Q90339;Q8JIP5	K	1580	1581	DEEMEQIKR	N	1590
47	Low	P12847;Q90339;Q8JIP5	K	1522	1523	SIHELEK	A	1530
48	Low	P02565;P13538;Q90339	K	1669	1670	EQVAMVER	R	1678
49	Low	P13535;Q076A3;Q29RW1;Q076A4;P13542;Q90339;Q8JIP5	R	1607	1608	SRNDALR	V	1615
50	Low	P13535;P11055;A7E2Y1;Q076A3;P02565;P12847;Q28641;Q29RW1;Q02566;P02563;Q076A4;P13542;P13538;Q90339;Q8JIP5	R	1383	1384	TEELEEAK	K	1392
51	Low	P13535;P11055;A7E2Y1;Q076A3;P02565;P12847;Q28641;Q29RW1;Q02566;P02563;Q076A4;P13542;P13538;Q90339;Q8JIP5	R	1383	1384	TEELEEAK	K	1392
52	Low	Q90339	R	1397	1398	LQDAEESIEAVNSK	C	1412
53	Low	P13535;Q076A3;P02565;Q28641;Q29RW1;Q02566;P04460;P02563;Q076A4;P13542;P13538;Q90339;Q8JIP5	R	1142	1143	ELEEISER	L	1151
54	Low	P11055;Q076A3;P12847;Q29RW1;Q076A4;Q90339;Q8JIP5	K	1452	1453	VLAEWK	Q	1459
55	Low	Q90339;Q8JIP5	K	1785	1786	NLEVTVK	D	1793
56	Low	Q90339	K	1701	1702	VAEQELVDASER	V	1714
57	Low	Q90339;Q8JIP5	K	1785	1786	NLEVTVK	D	1793
58	Low	Q90339;Q8JIP5	K	1785	1786	NLEVTVK	D	1793
59	Low	Q90339;Q8JIP5	K	1785	1786	NLEVTVK	D	1793
60	Low	P13535;P11055;P02565;P12847;Q29RW1;Q02566;P02563;P13538;Q90339	R	1476	1477	SLSTELFK	M	1485

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on Q90339	Position of AA residue of the peptide sequence (N-terminus) based on Q90339	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
61	Low	Q90339;Q8JIP5	R	1645	1646	NVQGQLK	D	1653
62	Low	Q90339;Q8JIP5	R	1645	1646	NVQGQLK	D	1653
63	Low	P13535;Q076A3;P02565;Q28641;Q29RW1;Q02566;P04460;P02563;Q076A4;P13542;P13538;Q90339;Q8JIP5	R	1142	1143	ELEEISER	L	1151
64	Low	P13535;P11055;A7E2Y1;Q076A3;P02565;P12847;Q28641;Q29RW1;Q02566;P02563;Q076A4;P13542;P13538;Q90339	K	1772	1773	EQDTSAHLER	M	1783
65	Low	P13535;P11055;A7E2Y1;Q076A3;P02565;P12847;Q28641;Q29RW1;Q02566;P02563;Q076A4;P13542;P13538;Q90339	K	1772	1773	EQDTSAHLER	M	1783
66	Low	P11055;Q076A3;P12847;Q29RW1;Q076A4;Q90339;Q8JIP5	K	1452	1453	VLAEWK	Q	1459
67	Low	P11055;Q076A3;P12847;Q29RW1;Q076A4;Q90339;Q8JIP5	K	1452	1453	VLAEWK	Q	1459
68	Low	Q90339;Q8JIP5	K	1785	1786	NLEVTVK	D	1793
69	Low	Q90339	K	1701	1702	VAEQELVDASER	V	1714
70	Low	Q90339;Q8JIP5	R	1782	1783	mKKNLEVTVK	D	1793
71	Low	Q90339;Q8JIP5	R	1782	1783	mKKNLEVTVK	D	1793
72	Low	Q90339	K	977	978	NLTEEmASQDESIAK	L	993
73	Low	P12847;Q90339;Q8JIP5	K	1522	1523	SIHELEK	A	1530
74	Low	Q90339	R	1663	1664	GQEDmKEQVAMVER	R	1678
75	Low	Q076A3;P02565;Q90339	R	1690	1691	AALEQTER	G	1699
76	Low	P13535;P11055;A7E2Y1;Q076A3;P02565;P12847;Q28641;Q29RW1;Q02566;P02563;Q076A4;P13542;P13538;Q90339;Q8JIP5	K	1758	1759	AITDAAMMAEELK	K	1772
77	Low	Q076A3;P02565;Q90339	R	1690	1691	AALEQTER	G	1699
78	Low	P13535;P11055;P02565;P12847;Q29RW1;Q02566;P02563;P13538;Q90339	R	1476	1477	SLSTELFK	M	1485

Appendix 3

Peptides of myosin heavy chain identified by LC-MS/MS (band 5)

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on Q90339	Position of AA residue of the peptide sequence (N-terminus) based on Q90339	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
1	High	Q90339	K	1504	1505	NLQQEISDLTEQLGETGK	S	1523
2	High	Q90339	R	1700	1701	KVAEQELVDASER	V	1714
3	High	Q90339;Q8JIP5	R	1421	1422	LQGEVEDLMIDVER	A	1436
4	High	Q90339	K	1504	1505	NLQQEISDLTEQLGETGK	S	1523
5	High	Q90339	R	1593	1594	VIDSMQSTLDSEVR	S	1608
6	High	Q90339	R	1397	1398	LQDAEESIEAVNSK	C	1412
7	High	Q90339	R	1700	1701	KVAEQELVDASER	V	1714
8	High	Q90339	R	1397	1398	LQDAEESIEAVNSK	C	1412
9	High	Q90339	K	1701	1702	VAEQELVDASER	V	1714
10	Medium	P13533;Q9Y623;P13535;P11055;Q9TV62;Q29RW1;P49824;P13538;Q90339	R	1476	1477	SLSTELFK	M	1485
11	Medium	P13533;Q9Y623;P13535;P11055;Q9TV62;Q29RW1;P49824;P13538;Q90339	R	1476	1477	SLSTELFK	M	1485
12	Low	Q9Y623;P11055;Q076A3;Q29RW1;Q076A4;Q90339;Q8JIP5	K	1452	1453	VLAEWK	Q	1459
13	Low	Q9Y623;P11055;Q076A3;Q29RW1;Q076A4;Q90339;Q8JIP5	K	1452	1453	VLAEWK	Q	1459
14	Low	P13533;Q9Y623;P13535;P11055;Q9TV62;Q29RW1;P49824;P13538;Q90339	R	1476	1477	SLSTELFK	M	1485
15	Low	Q90339	R	1898	1899	KVQHELEEAQER	A	1911
16	Low	Q90339	K	1504	1505	NLQQEISDLTEQLGETGK	S	1523

Appendix 4

Peptides of myosin heavy chain identified by LC-MS/MS (band 8)

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on Q90339	Position of AA residue of the peptide sequence (N-terminus) based on Q90339	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
1	High	Q90339	R	1700	1701	KVAEQELVDASER	V	1714
2	High	Q90339	R	1593	1594	VIDSMQSTLDSEVR	S	1608
3	High	Q90339	R	1397	1398	LQDAEESIEAVNSK	C	1412
4	High	Q90339	R	1700	1701	KVAEQELVDASER	V	1714
5	High	Q90339;Q8JIP5	R	1421	1422	LQGEVEDLMIDVER	A	1436
6	High	Q90339	R	1593	1594	VIDSMQSTLDSEVR	S	1608
7	High	Q9UKX3;P13533;Q9Y623;A7E2Y1;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339;Q8JIP5	K	1758	1759	AITDAAMMAEELKK	E	1773
8	High	Q90339	R	1593	1594	VIDSmQSTLDSEVR	S	1608
9	High	Q9UKX3;P13533;Q9Y623;A7E2Y1;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339;Q8JIP5	K	1758	1759	AITDAAmMAEELKK	E	1773
10	High	Q90339	K	1504	1505	NLQQEISDLTEQLGETGK	S	1523
11	High	Q90339	R	1397	1398	LQDAEESIEAVNSK	C	1412
12	High	Q9UKX3;P13533;Q9Y623;A7E2Y1;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339;Q8JIP5	K	1758	1759	AITDAAmMAEELKK	E	1773
13	High	Q90339	R	1593	1594	VIDSmQSTLDSEVR	S	1608
14	High	Q9UKX3;P13533;Q9Y623;A7E2Y1;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339	K	1772	1773	EQDTSAHLER	M	1783
15	High	Q9UKX3;P13533;Q9Y623;A7E2Y1;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339;Q8JIP5	K	1758	1759	AITDAAMmAEELKK	E	1773
16	High	Q90339	K	1701	1702	VAEQELVDASER	V	1714
17	High	Q90339	K	1701	1702	VAEQELVDASER	V	1714

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on Q90339	Position of AA residue of the peptide sequence (N-terminus) based on Q90339	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
18	High	Q90339	K	1504	1505	NLQQEISDLTEQLGETGK	S	1523
19	High	Q9UKX3;P13533;Q9Y623;A7E2Y1;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339;Q8JIP5	K	1758	1759	AITDAAMmAEELKK	E	1773
20	High	Q9UKX3;P13533;Q9Y623;A7E2Y1;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339	K	1772	1773	EQDTSAHLER	M	1783
21	High	Q90339	R	1898	1899	KVQHELEEAQER	A	1911
22	High	Q90339	K	1504	1505	NLQQEISDLTEQLGETGK	S	1523
23	High	Q90339	K	1701	1702	VAEQELVDASER	V	1714
24	High	Q076A3;P02565;Q90339	R	1690	1691	AALEQTER	G	1699
25	High	Q076A3;P02565;Q90339	R	1690	1691	AALEQTER	G	1699
26	High	Q90339	K	1899	1900	VQHELEEAQER	A	1911
27	High	Q9UKX3;P13533;Q9Y623;A7E2Y1;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339;Q8JIP5	K	1758	1759	AITDAAMMAEELKK	E	1773
28	High	P13533;P02563;Q90339;Q8JIP5	K	1849	1850	ELTYQTEEDKK	N	1861
29	High	P13533;P02563;Q90339;Q8JIP5	K	1849	1850	ELTYQTEEDKK	N	1861
30	High	Q90339	K	1899	1900	VQHELEEAQER	A	1911
31	Medium	Q90339	R	1898	1899	KVQHELEEAQER	A	1911
32	Medium	P02565;P13538;Q90339	K	1669	1670	EQVAmVER	R	1678
33	Medium	Q9UKX3;P13533;Q9Y623;Q9H6N6;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339;F1PT61;Q8JIP5	K	1375	1376	YETDAIQR	T	1384
34	Medium	Q90339	K	1576	1577	LAEKDEEmEQIKR	N	1590
35	Medium	Q90339	R	1663	1664	GQEDmKEQVAMVER	R	1678
36	Medium	Q9UKX3;P13533;Q9Y623;Q076A6;P02565;P12847;Q29RW1;P02563;P13540;P13538;Q90339	R	1476	1477	SLSTELFK	M	1485
37	Low	P02565;Q90339	R	1561	1562	VQLELNQVK	S	1571
38	Low	Q9UKX3;P13533;Q9Y623;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339;Q8JIP5	R	1864	1865	LQDLVDK	L	1872

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on Q90339	Position of AA residue of the peptide sequence (N-terminus) based on Q90339	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
39	Low	Q90339	K	1899	1900	VQHELEEAQER	A	1911
40	Low	Q90339	K	1899	1900	VQHELEEAQER	A	1911
41	Low	P12847;P13540;Q90339;Q8JIP5	K	1522	1523	SIHELEK	A	1530
42	Low	P12847;P13540;Q90339;Q8JIP5	K	1522	1523	SIHELEK	A	1530
43	Low	Q9UKX3;Q9Y623;Q076A3;Q29RW1;Q90339;Q8JIP5	R	1607	1608	SRNDALR	V	1615
44	Low	Q90339;Q8JIP5	K	1580	1581	DEEMEQIKR	N	1590
45	Low	Q90339;Q8JIP5	K	1785	1786	NLEVTVK	D	1793
46	Low	Q90339;Q8JIP5	K	1785	1786	NLEVTVK	D	1793
47	Low	P13533;Q9Y623;Q076A6;P02565;P12847;Q28641;Q29RW1;P04460;P02563;P13538;Q90339;Q8JIP5	R	942	943	KLEDEcSELKK	D	954
48	Low	Q9UKX3;P13533;Q9Y623;Q076A6;P02565;P12847;Q29RW1;P02563;P13540;P13538;Q90339	R	1476	1477	SLSTELFK	M	1485
49	Low	Q9UKX3;P13533;Q9Y623;A7E2Y1;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339;Q8JIP5	R	1383	1384	TEELEEAK	K	1392
50	Low	P02565;P13538;Q90339	K	1669	1670	EQVAmVER	R	1678
51	Low	Q9UKX3;P13533;Q9Y623;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339;Q8JIP5	R	1864	1865	LQDLVDK	L	1872
52	Low	Q90339	R	1663	1664	GQEDmKEQVAMVER	R	1678
53	Low	Q90339	R	1898	1899	KVQHELEEAQER	A	1911
54	Low	P13533;P13540;P13538;Q90339	K	1617	1618	KMEGDLNEmEIQLSHANR	Q	1636
55	Low	Q90339	R	1476	1477	VQHELEEAQER	A	1911
56	Low	Q90339;Q8JIP5	R	1645	1646	NVQGQLK	D	1653
57	Low	Q9UKX3;P13533;Q9Y623;Q076A6;P02565;P12847;Q29RW1;P02563;P13540;P13538;Q90339	R	1476	1477	SLSTELFK	M	1485
58	Low	Q90339	R	1700	1701	KVAEQELVDASER	V	1714
59	Low	Q9UKX3;P13533;Q9Y623;A7E2Y1;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339;Q8JIP5	K	1758	1759	AITDAAmMAEELK	E	1773
60	Low	Q9UKX3;P13533;Q9Y623;Q076A6;P0256	R	1476	1477	SLSTELFK	M	1485

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on Q90339	Position of AA residue of the peptide sequence (N-terminus) based on Q90339	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
		5;P12847;Q29RW1;P02563;P13540;P13538;Q90339						
61	Low	Q90339;Q8JIP5	R	1645	1646	NVQGQLK	D	1653
62	Low	Q9UKX3;P13533;Q9Y623;A7E2Y1;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339	K	1772	1773	EQDTSAHLER	M	1783
63	Low	Q9UKX3;P13533;Q9Y623;A7E2Y1;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339	K	1772	1773	EQDTSAHLER	M	1783
64	Low	Q076A3;P02565;Q90339	R	1690	1691	AALEQTER	G	1699
65	Low	Q9UKX3;Q9Y623;Q076A3;P12847;Q29RW1;Q90339;Q8JIP5	K		1453	VLAEWK	Q	1459
66	Low	Q9UKX3;P13533;Q9Y623;A7E2Y1;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339;Q8JIP5	R	1383	1384	TEELEEAK	K	1392
67	Low	P13533;P13540;P13538;Q90339	K	1617	1618	KKMEGDLNEmEIQLSHANR	Q	1636
68	Low	Q9UKX3;P13533;Q9Y623;A7E2Y1;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339;Q8JIP5	K	1757	1758	KAITDAAmMAEELKK	E	1773
69	Low	Q9UKX3;P13533;Q9Y623;A7E2Y1;Q076A3;Q076A6;P02565;P12847;Q28641;Q29RW1;P02563;P13540;P13538;Q90339;Q8JIP5	K	1758	1759	AITDAAMmAEELK	E	1772
70	Low	Q076A3;P02565;Q90339	R	1690	1691	AALEQTER	G	1699
71	Low	Q90339	R	1700	1701	KVAEQELVDASER	V	1714
72	Low	Q076A3;P02565;Q90339	R	1690	1691	AALEQTER	G	1699
73	Low	Q90339;Q8JIP5	K	1785	1786	NLEVTVK	D	1793
74	Low	Q90339	K	353	354	LTGAVmHHGNmKFK	Q	368
75	Low	Q90339;Q8JIP5	R	1421	1422	LQGEVEDLmIDVER	A	1436

Appendix 5

Peptides of myosin heavy chain identified by LC-MS/MS (band 12)

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on Q90339	Position of AA residue of the peptide sequence (N-terminus) based on Q90339	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
1	High	Q90339;Q8JIP5	R	1421	1422	LQGEVEDLMIDVER	A	1436
2	High	Q9Y623;P13535;Q076A3;Q8MJV1;P02565;P12847;Q28641;P02563;A2AQP0;P02564;Q076A4;P13542;P29616;Q90339;P02562;Q8JIP5	K	1758	1759	AITDAAMMAEELKK	E	1773
3	High	Q90339	R	1397	1398	LQDAEESIEAVNSK	C	1412
4	High	Q90339;Q8JIP5	R	1421	1422	LQGEVEDLmIDVER	A	1436
5	High	Q9Y623;P13535;Q076A3;Q8MJV1;P02565;P12847;Q28641;P02563;A2AQP0;P02564;Q076A4;P13542;P29616;Q90339;P02562;Q8JIP5	K	1758	1759	AITDAAMmAEELKK	E	1773
6	High	Q90339	R	1898	1899	KVQHELEEAQER	A	1911
7	High	Q9Y623;P13535;Q076A3;Q8MJV1;P02565;P12847;Q28641;P02563;A2AQP0;P02564;Q076A4;P13542;P29616;Q90339;P02562;Q8JIP5	K	1758	1759	AITDAAmMAEELKK	E	1773
8	High	Q90339;Q8JIP5	R	1847	1848	VKELTYQTEEDKK	N	1861
9	High	Q9Y623;P13535;Q076A3;Q8MJV1;P02565;P12847;Q28641;P02563;A2AQP0;P02564;Q076A4;P13542;P29616;Q90339;P02562	K	1772	1773	EQDTSAHLER	M	1783
10	High	Q9Y623;P13535;Q076A3;Q8MJV1;P02565;P12847;Q28641;P02563;A2AQP0;P02564;Q076A4;P13542;P29616;Q90339;P02562	K	1772	1773	EQDTSAHLER	M	1783
11	High	Q90339	R	1898	1899	KVQHELEEAQER	A	1911
12	High	Q9Y623;P13535;Q076A3;Q8MJV1;P02565;P12847;Q28641;P02563;A2AQP0;P02564;Q076A4;P13542;P29616;Q90339;P02562;Q8JIP5	K	1757	1758	KAITDAAMmAEELKK	E	1773
13	High	Q9Y623;P13535;Q076A3;Q8MJV1;P02565;P12847;Q28641;P02563;A2AQP0;P	K	1758	1798	AITDAAmmAEELKK	E	1773

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on Q90339	Position of AA residue of the peptide sequence (N-terminus) based on Q90339	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
		02564;Q076A4;P13542;P29616;Q90339;P02562;Q8JIP5						
14	High	Q9Y623;P13535;Q076A3;Q8MJV1;P02565;P12847;Q28641;P02563;A2AQP0;P02564;Q076A4;P13542;P29616;Q90339;P02562;Q8JIP5	K	1758	1759	AITDAAmmAEELKK	E	1773
15	High	P02563;Q90339;Q8JIP5	K	1849	1850	ELTYQTEEDKK	N	1861
16	High	P02565;Q90339	R	1561	1562	VQLELNQVK	S	1571
17	High	P02563;Q90339;Q8JIP5	K	1849	1850	ELTYQTEEDKK	N	1861
18	High	P02563;Q90339;Q8JIP5	K	1849	1850	ELTYQTEEDK	N	1860
19	Medium	Q9Y623;P13535;Q076A3;Q8MJV1;P02565;P12847;Q28641;P02563;A2AQP0;P02564;Q076A4;P13542;P29616;Q90339;P02562;Q8JIP5	K	1757	1758	KAITDAAmMAEELKK	E	1773
20	Medium	Q90339	K	1899	1900	VQHELEEAQER	A	1911
21	Medium	Q90339	K	1899	1900	VQHELEEAQER	A	1911
22	Low	Q9Y623;Q076A3;P12847;Q076A4;Q90339;P02562;Q8JIP5	K	1452	1453	VLAEWK	Q	1459
23	Low	Q9Y623;Q076A3;P12847;Q076A4;Q90339;P02562;Q8JIP5	K	1452	1453	VLAEWK	Q	1459
24	Low	Q9Y623;P13535;Q076A3;Q8MJV1;P02565;P12847;Q28641;P02563;A2AQP0;P02564;Q076A4;P13542;Q90339;P02562;Q8JIP5	R	1383	1384	TEELEEAKK	K	1393
25	Low	Q90339;Q8JIP5	R	1421	1422	LQGEVEDLmIDVER	A	1436
26	Low	Q90339	R	1397	1398	LQDAEESIEAVNSK	C	1412
27	Low	Q90339;Q8JIP5	K	1785	1786	NLEVTVK	D	1793
28	Low	P12847;P02564;Q90339;Q8JIP5	K	1522	1523	SIHELEK	A	1530
29	Low	P12847;P02564;Q90339;Q8JIP5	K	1522	1523	SIHELEK	A	1530
30	Low	Q90339;Q8JIP5	K	1580	1581	DEEMEQIKR	N	1590
31	Low	Q9Y623;P13535;Q076A3;Q8MJV1;P02565;P12847;Q28641;P02563;A2AQP0;P02564;Q076A4;P13542;Q90339;P02562;Q8JIP5	R	1383	1384	TEELEEAK	K	1392
32	Low	Q9Y623;P13535;Q076A3;Q8MJV1;P02565;P12847;Q28641;P02563;A2AQP0;P02564;Q076A4;P13542;Q90339;P02562;Q8JIP5	R	1383	1384	TEELEEAK	K	1392

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on Q90339	Position of AA residue of the peptide sequence (N-terminus) based on Q90339	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
33	Low	Q90339;Q8JIP5	K	1785	1786	NLEVTVK	D	1793
34	Low	Q90339;Q8JIP5	R	1782	1783	mKKNLEVTVK	D	1793
35	Low	Q9Y623;P13535;P02565;P12847;P02563;P02564;P29616;Q90339	R	1476	1477	SLSTELFK	M	1485
36	Low	Q90339;Q8JIP5	K	1785	1786	NLEVTVK	D	1793
37	Low	Q9Y623;P13535;Q076A3;Q8MJV1;P02565;P12847;Q28641;P02563;A2AQP0;P02564;Q076A4;P13542;P29616;Q90339;P02562	K	1772	1773	EQDTSAHLER	M	1783
38	Low	Q90339	K	1899	1900	VQHELEEAQER	A	1911
39	Low	Q90339	K	1899	1900	VQHELEEAQER	A	1911

Appendix 6

Peptides of myosin heavy chain identified by LC-MS/MS (band 14)

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on Q90339	Position of AA residue of the peptide sequence (N-terminus) based on Q90339	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
1	High	Q90339	R	1700	1701	KVAEQELVDASER	V	1714
2	High	Q90339;Q8JIP5	R	1421	1422	LQGEVEDLMIDVER	A	1436
3	High	Q90339	R	1397	1398	LQDAEESIEAVNSK	C	1412
4	High	Q90339	R	1397	1398	LQDAEESIEAVNSK	C	1412
5	High	Q90339	K	1701	1702	VAEQELVDASER	V	1714
6	High	Q90339	K	1701	1702	VAEQELVDASER	V	1714
7	High	Q90339	K	1899	1900	VQHELEEAQER	A	1911
8	High	Q076A3;P02565;Q90339	R	1690	1691	AALEQTER	G	1699
9	High	Q076A3;P02565;Q90339	R	1690	1691	AALEQTER	G	1699
10	High	Q02566;Q90339;Q8JIP5	K	1849	1850	ELTYQTEEDKK	N	1861
11	High	Q02566;Q90339;Q8JIP5	K	1849	1850	ELTYQTEEDKK	N	1861
12	High	Q90339	R	1898	1899	KVQHELEEAQER	A	1911
13	Medium	Q90339	K	1899	1900	VQHELEEAQER	A	1911
14	Medium	Q90339	R	1898	1899	KVQHELEEAQER	A	1911
15	Low	Q9Y623;P13535;P02565;P12847;Q29RW1;Q02566;P13540;Q90339	R	1476	1477	SLSTELFK	M	1485
16	Low	Q9Y623;Q076A3;P12847;Q29RW1;Q076A4;Q90339;Q8JIP5	K	1452	1453	VLAEWK	Q	1459
17	Low	Q9Y623;P13535;P02565;P12847;Q29RW1;Q02566;P13540;Q90339	R	1476	1477	SLSTELFK	M	1485
18	Low	P12847;P13540;Q90339;Q8JIP5	K	1522	1523	SIHELEK	A	1530
19	Low	P12847;P13540;Q90339;Q8JIP5	K	1522	1523	SIHELEK	A	1530
20	Low	Q90339;Q8JIP5	K	1785	1786	NLEVTVK	D	1793
21	Low	Q9Y623;P13535;Q076A3;Q8MJV1;P02565;P12847;Q29RW1;Q02566;P13540;Q076A4;Q90339;Q8JIP5	R	1383	1384	TEELEEAK	K	1392
22	Low	Q90339;Q8JIP5	R	1645	1646	NVQGQLK	D	1653
23	Low	Q90339;Q8JIP5	R	1645	1646	NVQGQLK	D	1653
24	Low	Q90339	K	1701	1702	KVAEQELVDASER	V	1715
25	Low	Q90339;Q8JIP5	R	1782	1783	mKKNLEVTVK	D	1793
26	Low	Q90339	K	353	354	LTGAVMHHGNMKFKQK	Q	370

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on Q90339	Position of AA residue of the peptide sequence (N-terminus) based on Q90339	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
27	Low	Q9Y623;P13535;Q076A3;Q8MJV1;P02565;P12847;Q29RW1;Q02566;P13540;Q076A4;Q90339;Q8JIP5	R	1383	1384	TEELEEAK	K	1392

Appendix 7

Peptides of myosin heavy chain identified by LC-MS/MS (band 16)

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on Q90339	Position of AA residue of the peptide sequence (N-terminus) based on Q90339	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
1	High	Q90339	R	1700	1701	KVAEQELVDASER	V	1714
2	High	Q90339	K	1701	1702	VAEQELVDASER	V	1714
3	Low	Q90339	R	1690	1691	AALEQTER	G	1699
4	Low	Q90339	R	1645	1646	NVQGQLK	D	1653
5	Low	Q90339	K	952	953	kDIDDLELTLAKVEK	E	968

Appendix 8

Peptides of actin identified by LC-MS/MS (band 17)

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on P04751	Position of AA residue of the peptide sequence (N-terminus) based on P04751	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
1	High	P04751	K	240	241	SYELPDGQVITIGNER	F	257
2	High	P04751	R	97	98	VAPEEHPTLLTEAPLN PK	A	116
3	High	P04751	K	52	53	DSYVGDEAQSK	R	64
4	High	P04751	K	52	53	DSYVGDEAQSKR	G	65
5	High	P04751	K	317	318	EITALAPSTMK	I	329
6	High	P04751	R	198	199	GYSFVTTAER	E	209
7	High	P04751	K	361	362	QEYDEAGPSIVHR	K	375
8	High	P04751	R	185	186	DLTDYLMK	I	194
9	High	P04751	K	86	87	IWHHTFYNELR	V	98
10	Low	P04751	R	185	186	DLTDYLmK	I	194
11	Low	P04751	R	179	180	LDLAGR	D	186
12	Low	P04751	R	64	65	GILTLK	Y	71
13	Low	P04751	K	330	331	IIAPPER	K	338
14	Low	P04751	K	193	194	ILTER	G	199
15	Low	P04751	K	317	318	EITALAPSTmKIK	I	331

Appendix 9

Peptides of actin identified by LC-MS/MS (band 20)

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on P04751	Position of AA residue of the peptide sequence (N-terminus) based on P04751	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
1	High	P04751;P53467	K	240	241	SYELPDGQVITIGNER	F	257
2	High	P04751	R	97	98	VAPEEHPTLLTEAPLN PK	A	116
3	High	P04751;P53467;P07828	K	317	318	EITALAPSTmK	I	329
4	High	P04751	K	361	362	QEYDEAGPSIVHR	K	375
5	High	P04751;P53467	K	52	53	DSYVGDEAQSKR	G	65
6	High	P04751	R	185	186	DLTDYLmK	I	194
7	Low	P04751	R	185	186	DLTDYLMK	I	194
8	Low	P04751;P53467;P07828	K	86	87	IWHHTFYNELR	V	98
9	Low	P04751;P53467;P07828	R	179	180	LDLAGR	D	186
10	Low	P04751;P53467;P07828	K	330	331	IIAPPER	K	338
11	Low	P04751;P53467;P07828	K	193	194	ILTER	G	199

Appendix 10

Peptides of actin identified by LC-MS/MS (band 21)

No.	Confidence Icon	Protein Group Accession #	AA residue preceding the peptide sequence (N-terminus)	Position of AA residue preceding the peptide sequence (N-terminus) based on P04751	Position of AA residue of the peptide sequence (N-terminus) based on P04751	Peptide Sequence	AA residue following the peptide sequence (C-terminus)	Position of AA residue following the peptide sequence (C-terminus)
1	High	P04751	R	97	98	VAPEEHPTLLTEAPLNPK	A	116
2	High	P04751	K	317	318	EITALAPSTMK	I	329
3	High	P04751	K	52	53	DSYVGDEAQSK	R	64
4	High	P04751	K	317	318	EITALAPSTmK	I	329
5	High	P04751	K	52	53	DSYVGDEAQSKR	G	65
6	High	P04751	K	361	362	QEYDEAGPSIVHRK	C	376
7	High	P04751	K	361	362	QEYDEAGPSIVHR	K	375
8	Low	P04751	R	314	315	MQKEITALAPSTMK	I	329
9	Low	P04751	K	330	331	IIAPPER	K	338
10	Low	P04751	R	179	180	LDLAGR	D	186
11	Low	P04751	K	330	331	IIAPPERK	K	339
12	Low	P04751	K	361	362	qEYDEAGPSIVHR	K	375