CHAPTER V

CONCLUSIONS

Thailand is one of the most important surimi production countries in Southeast Asia. In 1995, total production of surimi production was approximately 60,000 metric tons per year (Morrissey and Tan, 2000). In general 65-75% of fish mucle protein is myofibrillar proteins and 20-35% of fish muscle is water soluble, sarcoplasmic proteins (Mackie, 1996). The amount of sarcoplasmic proteins discarded in surimi wash-water was about 16,000-32,000 metric tons per year. Some of this surimi protein byproduct is recovered for use as low-cost feed. Using surimi wash-water protein, as high value, food ingredient could add to the profitability of Thailand fish process industry. Research on the functional properties of surimi wash-water proteins from threadfin bream, is essential to encourage the use of this byproducts as a functional food ingredient.

This research presents one of the first extensive studies of sarcoplasmic proteins from threadfin bream species. Threadfin bream is a major species of fish used in surimi production. Little or no work has been published on the functional properties of threadfin bream sarcoplasmic proteins relevant to the use as a food ingredient (Chapter 2). The research was mainly carried out on four parts as, physicochemical properties of TBSP (section 4.1), biochemical characteristics of proteases from TBSP, (section 4.2.) functional properties of TBSP (section 4.3) and modification of TBSP (section 4.4).

5.1 Physicochemical properties of TBSP

Determination of proximate analysis is basic information on the composition of food ingredients. Food manufactures require such information on the chemical and nutritional properties of their ingredients. In addition to proximate analysis, the amino acid profile, molecular weight (by SDS-PAGE and gel filtration) and differential scanning calorimetry profiles were determined for TBSP.

Proximate analysis showed TBSP contained 44.20% of crude protein, 1.98% fat, 0.20% carbohydrate, 5.97% moisture, 47.65% ash and the total calories content was 195 Calories/100 g sample. The high ash in the TBSP sample may be attributed to the use of 0.1 M Sodium phosphate buffer for extraction of sarcoplasmic proteins. It was decided to use buffer for the extraction of whole sarcoplasmic proteins and for better control of pH. Salt free extractant may be used to reduce the salt content of TBSP samples. Distilled water extraction will change the composition of TBSP (Morioka et al., 1997). Alternatively, the content of salt can be reduced by including a dialysis or filtration step in the production process for TBSP.

The amino acid data for TBSP shows that this ingredient is an excellent source most of the essential amino acids (phenylalanine, methionine, threonine, leucine, isoleucine, valine and lysine).

Molecular weight analysis of TBSP by SDS-PAGE showed the presence of equal amounts of 42 kDa, 40 kDa and 35 kDa components as expected for demersal or bottom fish. The patterns for 3-types of threadfin bream and 4 others species studied also had this pattern. In contrast, Nile tilapia and Ruby tilapia, freshwater fish, had rich in 42 kDa component as expected for fresh water fish. The main major component is 42 kDa, actin. However, sarcoplasmic proteins also have myosin component (205 kDa). Therefore, the method for extraction of fish sarcoplasmic proteins can extract myofibrillar proteins.

Gel filtration analysis of freeze-dried powder sample which redissolved in phosphate buffer showed only one peak that was outside the molecular size range of Sephacryl S-200 HR (i.e. 5 – 250 kDa).

The DSC profile for soluble TBSP showed three endothermic transitions with Tm values at 56°C, 67°C and 76°C. Because of the small amount of connective tissue in fish muscle, these transitions can be assigned to denaturation of sarcoplasmic proteins and myofibrillar proteins. There are no literature measurements with which to compare the TBSP result. However, the DSC profile compares with other fish species as discussed previously (section 4.1).

5.2 Biochemical characteristics of proteases from TBSP

The characteristic of crude proteases in TBSP included the effect of substrates, pH-activity profile, temperature-activity profile, inhibitors, activators, frozen storage and freeze-drying of crude protease of TBSP and also the activity of enzyme transglutaminase in TBSP were investigated. The proteases from fish muscle can affect fish quality and gelation of myofibrillar proteins. So, the presence of protease activity is important information and also the effect of the protease on the functional properties of TBSP.

TBSP had high proteolytic activity toward natural fish protein substrate (endogenous substrate activity assay) and casein, but no activity was detected with azocasein. The activity of crude protease from TBSP was maximum at pH 7 and optimum temperature at 55°C with endogenous protein substrate at pH 7.

The enzyme activity inhibition pattern indicated the presence of a mixture of serine, cysteine and metallo-proteases though the major enzyme in TBSP was a serine protease. This study identified metal ion activators which is Ca^{2+} ions that enhanced the TBSP proteolytic activity by 83% while Mg^{2+} had the most inhibition of the TBSP protease.

Activity of protease from TBSP was not changed after storing fish in the freezer for up to 1.5 years. However, freeze-drying fish proteins led to 44% loss of protease activity. To check validity of this result, a brief study using cod showed that muscle protease from this source was also inactivated by freeze-drying. The loss of protease activity due to freeze-drying was the same (43-44%) in both samples prepared from fresh frozen fish and from the frozen storage fish that stored for 1.5 years.

The cold temperature denaturation is highly important. First, these results show that fish waste intended to be used for TBSP manufacture could be stored frozen for a long period before use. Therefore, more fish waste could be collected before processing to give TBSP. This means that the protein powders manufacturer has more flexibility. Furthermore, freeze-drying is a common method for drying food protein ingredients. Perhaps the method of drying (freeze-drying, spray-drying) may contribute to the effect on fish protein ingredients. This area could be researched further in the future.

There was no transglutaminase activity detected in TBSP for 1% (w/v) or concentrated 5% (w/w) solution. Many researches suggest that fish muscle contain transglutaminases (section 2.2). But in this study of fish protein ingredient showed there was no transglutaminase.

5.3 Functional properties of TBSP

TBSP powder had solubility of 42-46% at pH 2, 3, 4, 7, 8 and 9. This level of solubility was better than other fish protein concentrates and isolates (section 4.3.1). The reasons for such differences could be because other investigators used different fish, types of storage, different drying methods, etc. TBSP showed lowest solubility (16.09%) at pH 5.

The water holding capacity of TBSP was 0.73±0.03 g water/g protein. The oil holding capacity of TBSP is 5.26±0.04 g oil/g protein. This is new results, and there is no previous work published on threadfin bream sarcoplasmic protein to compare. The foaming capacity for 5% TBSP (44.20% protein) was 150%, while the foaming capacity of beta-lactoglobulin and casein were 60%.

The foaming capaicty for TBSP was not different in presence of the cations tested, except that Ca^{2+} reduced the foam capacity of casein. Anions (SCN^-, Cl^-, SO_4^{2-}) also had no effect on protein foam capacity compared to the control (water). Such results suggest that TBSP could be useful ingredient as a foaming agent in food.

TBSP (44.20% protein) had an EAI value of 46 m^2/g sample and ESI value of 60.45 min. Both values are low when compared with beta-lactoglobulin (~100% protein). However, as described below modification of TBSP could improve the emulsification characteristics.

The storage modulus (G') of TBSP significantly increased with temperature $\geq$ 50°C which showed TBSP was heat induced gelation. TBSP also undergoes gelation transitions in two stages, i.e. at the temperature of 50-80°C and also at 80-90°C. The gelation of TBSP increased in strength in the presence of calcium chloride but only moderately in the presence of sodium chloride. For both TBSP and beta-lactoglobulin, gelation increased in the presence both of NaCl and $CaCl_2$. TBSP gelation was also higher pH 7 > pH 8.5 > pH 5, but the gelation of beta-lactoglobulin

decreased in the order, pH 5 > pH 8.5 ≈ pH 7. The gelation of TBSP was not affected by protease inhibitors (iodoacetate, PMSF, pepstatin A, 1,10-phenanthroline).

5.4 Modification of TBSP

The chemical modification, such as acylation (succinylation, acetylation) and enzymatic modification (trypsin) are performed to expand the range of functional properties available. No previous work has been published on TBSP chemical modification. In this study, the effect of acetylation, succinylation, trypsinolytic treatment on TBSP functional properties were determined.

Original TBSP had free amino group (mainly lysine ε-NH_2) levels of 3.18 (± 0.23) x 10^{-4} moles/g protein. Acylation of TBSP with succinic acid and acetic acid anhydride reduced free amino group by 98.83 and 100%, respectively. So, most of free amino groups were modified. Incubation of TBSP at 55°C, under conditions used to assay endogenous protease, led to a net loss of 55.72% free NH_2 groups. Trypsin hydrolysis increased the free amino group content by 86.54% because peptides were hydrolyzed and gave free amino group (-NH_2). This treatment can improve functional properties of proteins, such as solubility and foam capacity.

Chemical modification of TBSP affected many physical properties the protein. Surface hydrophobicity increased with acylation, with large effects obtained with succinylation compared with acetylation. The solubility of TBSP was also increased at pH range of 5-9 following acylation, but the solubility is greatly reduced below pH 4. Trypsin hydrolysis also increased solubility due mainly to the increasing DH. This connection between solubility and DH is believed to be primarily due to the decrease in peptide size, because smaller and more soluble peptides are produced at higher DH.

Modification also improved many functional properties of TBSP. Succinylation, acetylation, and trypsin modification all increased % overrun compared with normal TBSP. EAI of succinylated TBSP was the highest. This may be because of the effect of exposed hydrophobic group which can result in higher interaction at the air/water interface. The good emulsification properties of TBSP suggest this can be used as binding ingredient in sausage. Modification can reduced

gelation of TBSP. Highest effect was obtained with acylation probably because chemical reagents caused proteins to denature so the hydrophobic groups was exposed which affect the functional properties of TBSP. Such results suggest that chemically modification of TBSP may be useful for extending some functional properties of TBSP such as solubility and foaming properties.

5.5 Overal conclusion

In this research, ornate threadfin bream fish was used as sample. TBSP was prepared by extraction fish muscle with 0.1 M phosphate buffer pH 7, freeze-dried to powder and kept in plastic bag at 4°C until use. The physicochemical properties of TBSP, biochemical characteristics of proteases from TBSP and functional properties of TBSP with effect of modification by chemical modification (succinylation and acetylation) and enzymatic modification (trypsinolytic) were studied.

TBSP composed of 44.20% protein with high ash content (47.65%) due to the use of sodium phosphate buffer as extractant of sarcoplamic proteins. High ash may be the reason why there was no different in foaming properties at different pH. TBSP had most of essential amino acid with high lysine content. This protein could be used for nutritional purposes, such as protein supplement in some food for malnutrition children in rural province of Thailand. The determination of molecular weight by SDS-PAGE and DSC analysis revealed many small peptides, enzymes and also myofibrillar proteins. The presence of myofibrillar proteins TBSP extraction was the reason for better functional properties of TBSP such as solubility, foaming properties and gelation. Myofibrillar proteins are the main proteins that cross-link to form complex of gel, because molecular structure of myosin is more suitable for the reaction than sarcoplasmic proteins

The proteases in TBSP had been characterized and data showed they are mixture of serine, cysteine and metallo-protease. They have optimum activity at pH 7 and 55°C. At this point, the presence of protease should be taken into consideration during the study of TBSP functionality because these proteases can be digest proteins to smaller proteins. Proteins have short chains they are not strong enough for gelation.

Functional properties: solubility, water holding capacity and oil holding capacity, foaming properties, emulsifying properties and gelation of TBSP were studied. The data show that TBSP had lowest solubility at pH 5 and more soluble at extreme acid pH and basic pH. This is important for the application of TBSP in acid food such as fruit juice. The present of myofibrillar proteins could affect the solubility. Water holding capacity and oil holding capacity relate to the acceptability of a given food product such as texture, juiciness or mouthfeel. These functional properties of TBSP seem to be lower than fish hydrolysates. Foaming capacity of TBSP was higher than beta-lactoglobulin and casein. However, TBSP had low EAI but good ESI value.

The effects of modifications on TBSP were studied to expose the advantages of TBSP. Highest effect was obtained with succinylation and acetylation, probably because chemical reagents caused proteins to denature so exposing hydrophobic groups, which affect almost all of the main functional properties of TBSP. The results, suggest that chemically modification of TBSP may be useful for extending some functional properties of TBSP such as solubility and foaming properties. However, typsinolytic modification can improve solubility and foaming of TBSP. In conclusion, TBSP which is normally discarded with surimi wash-water could be use as a functional food ingredient. However, suitable modification should be selected for any particular purpose.

5.6 Recommentdations and Suggestion for future work

This research has shown, using a laboratory prepared sarcoplasmic protein extract from threadfin bream, that proteins from surimi wash-water (currently estimate as 16,000-32,000 metric tons per year for Thailand) could be useful as:

- Dietary supplement: TBSP has excellent profile of EAA.
- Foaming agent: TBSP has good foaming properties approaching values for milk proteins. Chemical modification also improved foaming properties.
- Emulsification agent: TBSP could be used for food emulsions or binding ingredient in sausage.

- Gelling agent: TBSP had low gelling properties. However, this could be positive feature for many liquid foods, where gelation is not important, e.g. For fruit drink, soups.

As recommendation for future work there is need for

1. Studies for the method of drying and their effect on TBSP ingredient.
2. Studies on the extraction of sarcoplasmic protein using water as extractant which may give ingredients with different functional properties.
3. Appropiate method to recover proteins from wash-water in surimi industry and study on the physicochemical, biochemical and functional properties of the recovered proteins in order to use as food proteins supplies and help to improve the environment.
4. More research on the enzyme activity from TBSP including protease, transglutaminase and their effect on TBSP. It is not clear why TGase activity was not detected in the TBSP and more research is needed in this area.
5. Investigations on using TBSP as ingredient in food model systems, such as binding properties in meat emulsions, sausage or in bakery products.