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Research Article

Utilisation of fish protein isolate for maximizing fillet yield

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Abstract

The study examines the yield effectiveness of incorporating fish protein isolate (FPI) through injection into fillets of the same species *Pangasianodon hypophthalmus*, using a 1.5% salt solution as the injection medium. Subsequently, the yield of injected fillets was monitored under both chilled (4±1°C) and frozen (–18±1°C) storage conditions. The current study measured weight gain after injection, drip loss during storage, total yield after storage, and cooking yield post-storage. The strategic use of fish protein isolates by injection at 10% FPI level exhibited superior functional attributes, including higher weight gain, and reduced drip loss, projecting it ideal against quality deterioration during extended storage, thereby preserving the nutritional integrity and freshness of the product over time. Future research could focus on evaluating the nutritional consequences of FPI incorporation in fillets, and potential health benefits for consumers. Moreover, investigating a range of FPI concentrations, and combining them with natural preservatives or flavouring agents, may help identify optimal formulations that extend shelf life and maintain desirable sensory and nutritional qualities.

Keywords: Cooking yield, Drip loss, Functional protein isolate, Injection, Weight gain

Highlights

- Fish processing residues serve as efficient sources for producing functional protein isolates
- FPI exhibits strong water retention, minimizing drip loss and preserving fillet weight
- FPI injection enhances muscle integrity and reduces cooking and thawing losses in fillets

INTRODUCTION

Universally, fish is considered as a valuable muscle protein resource, mainly due to its higher nutrition value, digestibility and functional properties compared to other protein sources (Friedman, 1996; Simopoulos, 1997). Separation and alteration of fish muscle proteins increase the solubility, water holding capacity, emulsifying ability and gel forming properties, allowing them to be used as functional food additives. Several methods have been developed in order to isolate proteins from the fish muscle such as chemical extraction method uses solvents to remove fat and water from fish to get FPC (Fish Protein Concentrate); enzymatic hydrolysis method where proteolytic enzymes like alcalase, papain are used to breakdown the protein to get FPH (Fish Protein Hydrolysate); homogenisation method to get HFP (homogenised fish protein); and a relatively novel processing technique known as pH shift method, focuses on isolation of protein as fish protein isolate (FPI) involving acid or alkaline solubilisation. A more purified form of protein is FPI than other proteins extracted from fish muscle. FPI helps convert low value fish or byproducts into nutritious fillets and sausages. FPI also enhances the stability and texture of fish fillets. Three major benefits of pH-shifting method are as (a) manual separation of the bones from the flesh is not required, the bones and scales debris are removed easily as the sediment part after first centrifugation, (b) retention of more sarcoplasmic proteins and increasing of the proteinyield, and (c) efficient removal of lipid and hence minimizing the risk of lipid oxidation during frozen storage.

FPI can be used to develop superfoods/functional foods with acceptable sensory attributes and nutritional qualities. The fish processing industry has effectively implemented the practice of including functional proteins into fish fillets. Fish fillets are incredibly well-liked commodity that have been ruling the global fish market. As the characteristics of proteins grows, utilization of isolated fish protein (FPI) into the fish fillets could be converted into highly priced products, sometimes even surpassing the worth of the fish fillets or main flesh (Murmu et al., 2016). These isolated fish proteins used as additives shows promise for increasing the yield of fish products. One innovative approach to

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enhancing the quality of fish fillets is the injection of fish protein isolate (FPI) in order to ensure protein extracts back into the fillets to improve their structural integrity, juiciness, and overall sensory attributes. The advantages of this method include improved texture, enhanced protein content, increased yield, and better economic utilization of fish processing by-products.

Pangasius (*Pangasianodon hypophthalmus*) is a major cultured fish in India exported as skinless, boneless fillets popularly along with portions, steaks, fillets and its added value products (Thi et al., 2013). Its tender white flesh, mild flavour, firm texture and high nutritive value make it highly preferred by consumers (Rao et al., 2013). During the filleting, a considerable amount of meat retains with the fish frames is considered as waste. In view of addressing pollution problems associated with pangas processing waste, to minimize supply-demand gap and to meet increasing demand for nutritious food, these valuable compounds from the processing waste may be recovered in the form of FPI and be injected into Pangas fillets. The injection of FPI can help address challenges such as low water retention, texture degradation, and protein loss during processing and storage. The present study was aiming preparation of FPI solution from the fillet frames of *Pangasianodon hypophthalmus*, and injection into the same fish fillets to determine the fillet yield and during storage as well. Thus, the objective of the present study was to study the fillet yield following FPI injection and during storage.

MATERIALS AND METHODS

Fish: A total 12 kg of fresh *Pangasianodon hypophthalmus* (commonly known as Basa) were purchased from Garia Fish Market, Kolkata, by simple random sampling method. The samples were transferred to the laboratory within an hour, packed in ice in polystyrene boxes (0-2°C).

Fillet processing: The fishes were thoroughly cleaned with running potable water to remove filth, slime and other impurities followed by beheading, descaling, filleting and skinning manually. Filleting was done under good hygienic and sanitary conditions to prevent any cross contamination. The fillets (40-70 g) were used for injection and the fish frames were used for preparation of fish protein isolate (FPI).

Mince-preparation: Frames were ground using a laboratory mincer to form a fine and thick paste of minced meat which was used as raw material for preparation of fish protein isolate (FPI) as described by Surasani et al. (2018). The raw material processing and sample preparation steps were performed in a cold room at 4°C or on ice to maintain temperature below 5°C as described by (Surasani et al., 2018). The excess thick paste of minced meat, was transferred to zipped plastic bags for storage at –20°C temperature for further use.

Preparation of FPI by pH shift method

Preparation of homogenate: FPI was prepared by iso-electric solubilization/precipitation or pH shift method using both acid and alkali. Pangas fillets were grounded to mince and then were mixed with cold deionized water (4°C, 1:6, w/w) followed by homogenization for 60 s (2 × 30 s) as described by (Shafiq et al., 2021) with necessary modifications. The homogenate was transferred into a plastic beaker to adjust the desirable pH using 2M HCl and 2M NaOH respectively under continuous stirring condition (Surasani et al., 2017). The pH of the homogenate was kept constant throughout the process by adding required amount of diluted acid or alkali followed by a high-speed centrifugation (5000xg for 20 min) in a refrigerated centrifuge. The supernatant obtained after the centrifugation was quantified, filtered using a filter paper (Whatsman paper grade 1).

Precipitation: The supernatants obtained from the 1st centrifugation step were carefully separated and subjected to a pH change by using 2M NaOH and 2M HCl to bring back its pH to the isoelectric point (pH 5.5), as indicated by the thick white particles liquid (Shafiq et al., 2021). Post-precipitation step was followed by a second centrifugation (5000xg for 20 min) to separate the precipitated proteins from the liquid. The protein percentage was being calculated using Lowry method.

Determination of total protein content: The total protein content of FPI (prepared by both acid and alkali solubilisation process) was determined spectrophotometrically with Folin's reagent by using Folin-Lowry Reagent Kit. An aliquot of each of the protein standard solutions (200 µL) were mixed with 3 mL of Alkaline Copper Reagent and kept at room temperature for 10 minutes. After that, 0.5 mL of Folin's reagent is mixed and kept in boiling water bath for 10 minutes. The absorbance of the solutions was measured at 750 nm using double beam UV/VIS spectrophotometer. The reference cuvette contained all reagents except the extract sample. A standard curve was prepared by plotting the concentration of protein standard solutions (X axis) against the OD values

(Y axis) obtained from spectrophotometer. The total protein content of the FPI (prepared by both acid and alkali solubilisation process) sample was determined from the standard curve and expressed as "µg/µL".

Preparation of solutions for injection

Preparation of protein solution: FPI prepared by alkali solubilization pH shift method was used for injection into fish fillets by following the method as described by (Shafiq et al., 2021). The protein concentration of FPI solution was 3% for injection (Shaviklo & Etemadian, 2019), viscosity of the solutions for injection was a limiting factor with regard to protein concentration (Karlsdottir, 2009).

Salt: For the injection trials of FPI into fillets, 1.5% concentrations (w/w) of salt brines were prepared from food grade pure dried vacuum salt (>99.9% NaCl) with purity of 99.9% by following the method as described by (Qiancheng, 2008).

Experiment design and storage of samples: Three per cent FPI solution, prepared by alkali solubilization method, was injected immediately after filleting by adopting a brine injector manually as described by Qiancheng (2008) to obtain approximately 10% pick-up for injected fillets. The experiment was conducted by injecting FPI (3% protein+1.5% salt) at 4 levels of incorporation into pangas fillets. The treatments were: Control (1.5% salt injection) designated as C, 5% FPI injection to the weight of fillets (v/w) designated as T_1, 10% FPI injection to the weight of fillets (v/w) designated as T_2, 15% FPI injection to the weight of fillets (v/w) designated as T_3.

The temperature of the solutions injected and the processing workshop where the injection took place was 5°C and 16°C, respectively. After injection, fillets were placed carefully on a grid for 10 min to guarantee that the solutions injected effectively spread inside the muscle before packaging and to drain off excess solution liquid (Akse et al., 2008). Then half of the fillets (60 pieces of fillets) were packed in Styrofoam boxes with filter paper on the bottom to absorb excess water designated as first batch of fillets, stored under refrigerated (4±1°C) temperature. The other half of fillets (60 pieces of fillets) were packed in cartons lined with plastic bag regarded as second batch of fillets, are stored under frozen (-18±1°C) storage temperature.

The First batch of treated fillets were subjected in triplicate to yield study at 3 days interval during the refrigerated storage at 4±1°C for a period of 12 days. Samples stored were studied on 0th, 3th, 6th, 9th, 12th day. The Second batch of treated fillets were subjected in triplicate to yield study at 30 days interval during the frozen storage at -18±1°C for a period of 120 days. Samples stored in frozen storage were analysed on 0^{th}, 30^{th}, 60^{th}, 90^{th}, 120^{th} day respectively.

Yield study of fillets after injection

Determination of weight gain after injection: The weight gain of the injected fillets was measured by following the method as described by (Birkeland et al., 2007). The initial weight of the raw fillet was taken followed by weighing the fillets 15 minutes after injection. The weight gain of the fillets was calculated with respect to the weight of the raw fillets. Values less than 100% indicated that fillets had lost weight; while values over 100% indicate that fillets had gained weight. Fillet weight gain was determined based on fillet weight before injection as follows:

Weight gain (%) = 100 × (g injected fillet before storage - g fillet before injection)/(g fillet before injection). (1)

Determination of drip loss during storage: Drip loss was expressed as weight reduction during storage. Drip loss of refrigerated fillets was determined according to Larsen et al. (2008). Drip loss was determined based on injected fillet weight as follows:

Drip loss (%) = 100 × (g fillet before storage – g fillet after storage)/(g fillet before storage). (2)

Drip loss of frozen stored fillet was determined according to Bigelow and Lee (2007). Frozen fillets samples were put on plastic pellets with small size holes and thawed at 4°C overnight. Thawed fillets were removed from the pellet, left to drip on the plastic film on the top of pallet, and the drip was left on the plastic film.

Drip loss (%) = 100 × (g fillet before freezing – g thawed fillet after frozen storage)/(g fillet before freezing). (3)

Determination of fillet yield after storage: Fillet yield after storage was determined according to Thorarinsdottir et al. (2004b, 2002). The fillets were weighed as raw material and after storage; yield was determined by the observed changes in weight with respect to the weight of the raw fillets as follows:

Yield (%) = (g fillet after storage/g raw fillet) ×100 (4)

Determination of cooking yield: Cooking yield was determined according to Bigelow & Lee (2007). For

evaluation of cooking yield, the fillet needs to be trimmed so that it weighed about 50 g each piece. Cooking yield was determined by steam cooking the pieces at 95°C to 100°C for 8 min in a preheated conventional steam oven. After the cooking period, the pieces were cooled down to room temperature (25°C) for 15 min before weighing for cooking yield determination. The yield after cooking (%) was calculated as the weight of the cooked pieces in contrast with the weight before cooking.

Cooking yield (%) = g cooked fillet/g fillet before cooking × 100 (5)

Cooking yield of the whole fillet was the average of the three parts cut from the fillet.

RESULTS

The present study explores the effect of fish protein isolate (FPI) extracted by pH-shift method by using Pangas (*P. hypophthalmus*) as raw material and evaluates the benefits of FPI injecting into fillets of the same species using 1.5% brine. The yield of the injected fish fillet during refrigerated (4±1°C) and frozen (-18±1°C) storage was then evaluated. The results of these analyses are given in the following sections.

Determination of the total protein content of FPI (pH-shift method) using Folin-Lowry protein estimation kit: The recovery yields of Pangas fish protein isolate (FPI) at both pH levels such as pH 2.0 (acidic) and pH 12.0 (alkaline) were obtained from Standard curve for protein estimation by Folin-Lowry method at 750 nm absorbance. The acid and alkali-assisted extraction of FPI from pangas fillet frames resulted in total process yields of 75.64±0.24% and 87.33±0.11%, respectively.

The findings of this study indicate that the alkaline processing method achieved the highest protein yield, outperforming the other protein extraction technique evaluated. So FPI prepared by alkali solubilisation method was used for the injection into pangas fish fillets.

Yield study of fillets after injection: Weight gain of fillets after injection: In this experiment, fillets were injected with protein isolates obtained from alkaline pH shift method at the rate of 3% FPI+1.5% brine (Murmu et al., 2016) at 3 different levels of incorporation such as 5%, 10% and 15% of the weight of the fillets using an injector by following the method as described by Murmu et al. (2016) with necessary modifications. Weight gain (%) of fillets after injection is shown in (Fig. I). The injection of FPI resulted in 5% to 11% of weight gain. FPI injected fillets exhibited higher fillet weight gain (%) as compared to control fillets. Significantly highest ($p<0.05$) weight gain of fillets was observed in T2 sample (injected at 10% level).

Drip loss of fillets after storage: During storage, the leakage of myofibers leads to the loss of proteins, water,

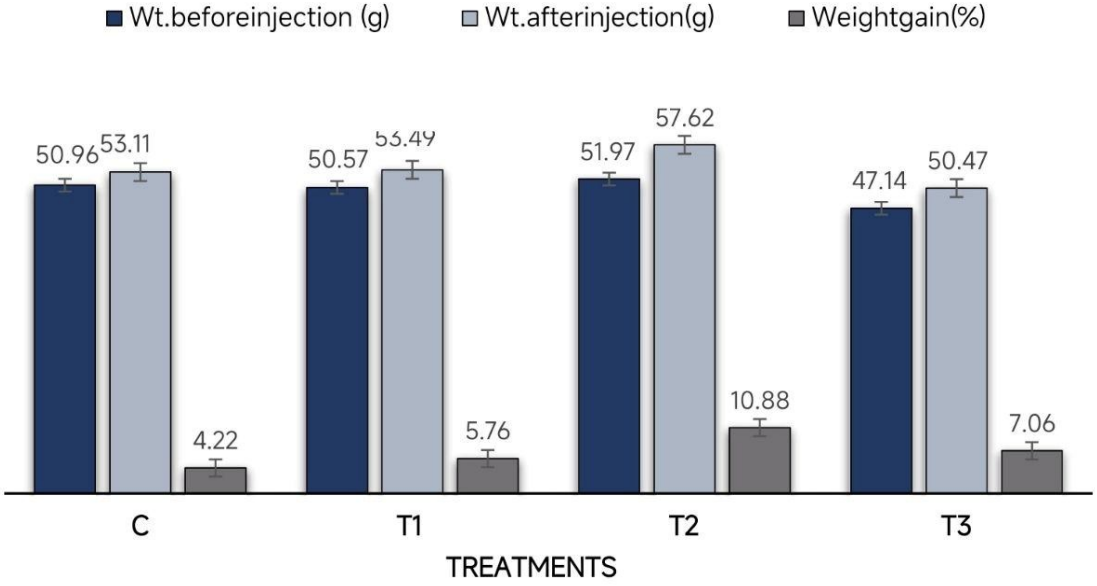

Fig. 1. Graphical view of weight gain of fillets after injection

and iron, a phenomenon known as drip loss (Ponsuksili et al., 2008). The drip loss of fish fillets after storage is presented in (Table 1). A significant increase ($p<0.05$) in drip loss was observed across all injected fillets over the refrigerated storage (4±1°C) period. Fillets injected with salt (Control) exhibited the highest drip loss, whereas the lowest drip loss was recorded in the T2 sample (10% FPI), indicating superior water retention in this treatment compared to others, However, no statistically significant differences ($p>0.05$) in drip loss were observed among treatments, except for the T2 sample, which demonstrated the lowest loss.

For fillets stored under frozen conditions, a significant increase in drip loss ($p<0.05$) was observed across all treatments over a 120-day period (Table 2). Fillets injected with fish protein isolate (FPI) exhibited a notable reduction in drip loss ($p<0.05$), with final values of 13.07±0.05% for T1, 14.11±0.09% for T2, and 15.32±0.03% for T3. In contrast, the control (C) fillets experienced the highest drip loss at 120 days, reaching 16.28±0.04%. Among all treatments, the lowest drip loss was recorded in the T1 sample (5% FPI) (Table 2). However, when compared to their refrigerated counterparts, frozen fillets exhibited substantially higher drip loss throughout the storage period.

Table 1. Changes in the drip loss (%) of different treatments of pangas fillets stored at (4±1°C) for 12 days of storage period

DAY	C	T1	T2	T3
0	0	0	0	0
3	4.45±0.11^{d}	4.11±0.17^{c}	3.27±0.15^{a}	3.7±0.11^{b}
6	6.21±0.08^{d}	4.6±0.13^{c}	3.7±0.09^{a}	4.34±0.10^{b}
9	8.8±0.18^{d}	5.4±0.21^{c}	3.9±0.13^{a}	5.1±0.14^{b}
12	9.37±0.20^{d}	5.67±0.11^{c}	4.55±0.13^{a}	5.38±0.13^{b}

*Results are mean of three determinations with standard deviation
#The mean value in the same column and row with different superscripts are significantly different ($p<0.05$)

Table 2. Changes in the drip loss (%) of different treatments of pangas fillets stored at (-18±1°C) for 120 days of storage period

DAY	C	T1	T2	T3
0	0	0	0	0
30	10.87±0.03^{a}	11.5±0.05^{b}	12.12±0.05^{c}	12.91±0.04^{c}
60	12.72±0.05^{b}	11.89±0.07^{a}	12.82±0.06^{b}	13.34±0.05^{c}
90	14.96±0.03^{c}	12.37±0.05^{a}	13.54±0.05^{b}	14.5±0.09^{c}
120	16.28±0.02^{d}	13.07±0.08^{a}	14.11±0.03^{b}	15.32±0.03^{c}

*Results are mean of three determinations with standard deviation
#The mean value in the same column and row with different superscripts are significantly different ($p<0.05$)

Table 3. Changes in the fillet yield (%) of different treatments of pangas fillets stored at (4±1°C) for 12 days of storage period

DAY	C	T1	T2	T3
0	101.82±0.03^{a}	105.55±0.02^{b}	112.66±0.07^{d}	108.55±0.06^{c}
3	99.11±0.14^{a}	102.24±0.10^{b}	109.4±0.13^{d}	104.14±0.17^{c}
6	96.6±0.18^{a}	100.72±0.19^{b}	105.98±0.15^{d}	102.88±0.12^{c}
9	93.37±0.21^{a}	99.43±0.20^{b}	108.28±0.17^{d}	99.53±0.27^{c}
12	92.84±0.40^{a}	96.46±0.17^{b}	102.33±0.12^{d}	98.9±0.16^{c}

*Results are mean of three determinations with standard deviation
#The mean value in the same column and row with different superscripts are significantly different ($p<0.05$)

Total yield of fillets after storage: In this present experiment, fillet yield (%) during refrigerated storage (4±1°C) was found to decrease in all treatments including control sample (Table 3). Fillet yield was significantly ($p<0.05$) different among the treatments. The highest initial fillet yield (112.66±0.06%) was recorded in T2 sample, which during refrigeratedstorage (4±1°C) reduced significantly ($p<0.05$) reaching a value of (102.33±0.12%). Control fillets showed at all times the lowest values of fillet yield as 92.84±0.4% after 12 day of refrigerated storage (4±1°C). After 12 days of storage, only the yield of T2 sample was still above 100% of original weight before injection.

The fillet yield differs among the treatments with different protein injection levels to the frozen fillets; in all treatments fillet yield decreased significantly ($p<0.05$) with storage time under frozen condition (-18±1°C) as represented in the (Table 4). FPI injected fillets (T2) exhibited highest fillet yield after 120 days of storage, the lowest was found in control (salt injection) (C). Compared with the count part of refrigerated fillets, frozen fillet yields during 120 days frozen storage (-18±1°C) were quite lower than refrigerated fillets during refrigerated storage (4±1°C) from day 0 to day 12.

Cooking yield of fillets after storage: Cooking yield is a crucial factor for consumers, as it ensures that they receive the full value of their purchase. A high cooking yield preserves the size and juiciness of the product,

preventing excessive shrinkage during cooking. Moreover, it helps to maintain the desired texture, ensuring a tender and satisfying eating experience without any undesirable changes (Karlsdottir, 2009).

In this present study, the cooking yield (%) (Table 5) of all fillets was found to decrease significantly ($p<0.05$) in all treatments including control under refrigerated storage (4±1°C). At the end of 12 days of storage under refrigerated temperature (4±1°C), the cooking yield of T1, T2 and T3 samples are significantly higher ($p<0.05$) than the cooking yield of control samples.

Likewise, the trend of refrigerated storage (4±1°C), the cooking yield of all the treatments under frozen storage (-18±1°C) significantly declined ($p<0.05$) over the storage period (Table 6). The control sample exhibited the most substantial reduction ($p<0.05$), with a 14.07% decrease, reaching a final cooking yield of 40.22±0.17% after 120 days of storage. Among all treatments, the highest cooking yield (46.25±0.21%) at the end of 120 days of frozen storage (-18±1°C) was recorded in the T1 sample (Table 6). These findings highlight the impact of 3% FPI with 1.5% salt injection solutions on the cooking yield of frozen fillets throughout storage.

Table 4. Changes in the fillet yield (%) of different treatments of pangas fillets stored at (-18±1°C) for 120 days of storage period

DAY	C	T1	T2	T3
0	101.62±0.18^{a}	105.13±0.36^{b}	112.77±0.18^{d}	108.25±0.09^{c}
30	92.88±0.18^{a}	93.61±0.21^{b}	99.45±0.18^{d}	95.93±0.19^{c}
60	90.02±0.30^{a}	92.79±0.56^{b}	95.93±0.08^{d}	93.45±0.13^{c}
90	87.06±0.13^{a}	92.07±0.59^{c}	97.33±0.12^{d}	91.12±0.15^{b}
120	85.65±0.19^{a}	88.78±0.11^{b}	92.33±0.34^{c}	88.82±0.29^{b}

*Results are mean of three determinations with standard deviation

#The mean value in the same column and row with different superscripts are significantly different ($p<0.05$)

Table 5. Changes in the cooking yield (%) of different treatments of pangas fillets stored at (4±1°C) for 12 days of storage period

DAY	C	T1	T2	T3
0	75.43±0.02^{b}	72.43±0.02^{a}	79.56±0.05^{d}	76.99±0.03^{c}
3	63.12±0.15^{b}	62.42±0.13^{a}	73.2±0.06^{d}	68.53±0.07^{c}
6	50.15±0.16^{a}	58.43±0.14^{b}	64.32±0.15^{d}	61.90±0.15^{c}
9	39.26±0.17^{a}	45.96±0.19^{b}	52.03±0.21^{d}	49.43±0.22^{c}
12	31.92±0.06^{a}	36.74±0.14^{b}	41.14±0.17^{d}	38.69±0.12^{c}

*Results are mean of three determinations with standard deviation

#The mean value in the same column and row with different superscripts are significantly different ($p<0.05$)

Table 6. Changes in the cooking yield (%) of different treatments of pangas fillets stored at (-18±1°C) for 120 days of storage period

DAY	C	T1	T2	T3
0	54.29±0.15^{c}	53.51±0.15^{b}	52.87±0.27^{a}	52.42±0.18^{a}
30	51.17±0.33^{b}	51.82±0.21^{b}	50.62±0.23^{a}	50.99±0.15^{a}
60	47.33±0.23^{a}	50.77±0.26^{d}	49.21±0.15^{c}	48.87±0.17^{b}
90	43.56±0.24^{a}	48.09±0.35^{d}	47.74±0.18^{c}	46.36±0.27^{b}
120	40.22±0.17^{a}	46.25±0.21^{d}	45.44±0.42^{c}	43.57±0.26^{b}

*Results are mean of three determinations with standard deviation

#The mean value in the same column and row with different superscripts are significantly different ($p<0.05$)

DISCUSSIONS

The weight gain in control fillets injected only with salt (1.5% salt) was 4.22%, was remarkably lower than in fillets injected with 10% FPI injection level (T2) (10.88%). Thorarinsdottir et al. (2004b) found the weight gain ranges from 4% to 7% in cod fillets injected with salt, protein, phosphate, or a combination of the three. In the present experiment, the weight gain from salt injections (4.22%) was comparable to the findings of Thorarinsdottir et al. (2004a) where the weight gain of salt injected cod fillets was 4%. Whereas, the weight gain from protein injections was significantly larger (10.88%) in the present study which may be due to the fact that different protein solutions contain identical protein components, particularly myofibrillar proteins effectively responsible for protein cross-linking and water holding capacity; thus, protein solutions are easier to maintain within fillets after injection (Karlsdottir, 2009).

Although, higher protein concentration is caused due to higher viscosity of protein making it difficult to inject into the fillet (Qiancheng, 2008). Once injected higher protein concentration helps to gain weight in the injected fillets. The protein concentration in Thorarinsdottir et al. (2004a, 2004b) trial was 10%, whereas, FPI concentration was 3% in this present trial. Experiments have shown that injecting cod and haddock fillets with brine containing FPI promotes weight gain by 5-20% , which also justifies present range of findings.

Despite having a protein injection level of 15%, the T3 sample gained less weight (7.06%) than T2 sample in the present study which may be due to inability of

the T3 fillets to retain the highly viscous protein at the higher concentration 15% injection level as stated by Qiancheng (2008). Therefore, increased protein concentrations have a negative impact on the weight gain of injected fillets.

Salt-injected fillets exhibited the highest drip loss at 9.37% during refrigerated storage (4±1°C), which was greater than that observed in FPI-injected samples. Supporting to the present study, Porcella et al. (2001) and Kristinsson & Rasco (2000) suggest that incorporating various protein materials can effectively reduce drip loss. Protein injection enhances water-holding capacity by providing binding sites for water molecules on protein structures, forming a gel matrix that entraps and retains water, thereby minimizing drip loss. In frozen storage (-18±1°C), thaw drip has been linked to partial denaturation of proteins during freezing, which leads to decreased water holding capacity (Shenouda, 1980; Mackie, 1993). Addition of salt to fish fillet before freezing has been demonstrated to improve water retention and reduce drip loss (Woyewoda, 1986; Ragnarsson, 1988). The lowest drip loss of 13.07±1.72% was observed in treatment T1 (5%) compared to T2 (10%) and T3 (15%) because high protein injection level can lead to increased drip loss due to the injected moisture being more susceptible to loss during frozen storage (Leygonie et al., 2012).

During refrigerated (4±1°C) and frozen storage (-18±1°C), protein injection (3% protein isolate + 1.5% salt) produced higher fillet yield than salt injection (1.5%). According to Paterson et al. (1988), the combination of salt, phosphate, and protein boosted the fillet yield. Qiancheng (2008) also observed that fillet yield varied across the treatments; fillets injected with fresh protein isolate (FePI) had the maximum fillet yield during the same storage time, followed by frozen protein isolate (FoPI) injection; control and salt injection had lower fillet yield. The current investigation found the same trend in fillet yield (%). The combination of salt and protein is effective in opening the myofibrillar structure, resulting in greater water retention and yield (Karlsdottir, 2009). Following frozen storage (-18±1°C) as in contrast to refrigerated storage (4±1°C), the treated pangas fillets generally displayed a significant drop in storage yield. As freezing alters the structure and physical characteristics of fish muscle, liquid escapes the muscle cells into the intercellular space as a result of their shrinkage (Bello et al., 1981; Hurling & McArthur, 1996). Slow freezing causes more harmful alterations to the fish's muscle resulted in significant loss of fillet yield after 120 days compared to refrigerated fillets. (Belitz et al., 2004).

The cooking yield of the refrigerated (4±1°C) and frozen (-18±1°C) fillets injected with (3% FPI+1.5% salt) protein injection was considerably higher than salt injected fillets. Shahidi et al. (1995) reported that the cooking yield of rainbow trout fillets increased with the rising concentration of capelin protein hydrolysate, from 0% to 3%. The combination of capelin hydrolysate and salt has been shown to improve the cooking yield of pork (Shahidi et al., 1995).The result of the present study also equivalents with that injection of 3% FPI solution with 1.5% brine had higher cooking yield than only salt injection. Cooking yield is influenced by both the concentration and functional properties of the injected protein. During refrigerated and frozen storage, FPI reduces protein denaturation and drip loss, helping the fillet retain more moisture and maintain higher cooking yield. The water-binding capacity of proteins also varies depending on the specific type of protein and its functional properties (Karmas & Turk, 1976), further contributing to differences in cooking yield outcomes.

The findings of this present research compelling evidence that the alkaline solubilisation method of protein isolate had higher protein recovery yield compared to acid solubilisation method. The injection of fish protein isolate had a transformative impact on the yield of pangas fillets stored under refrigerated and frozen conditions. The strategic use of fish protein isolates by injection at 10% FPI level exhibited superior functional attributes, including higher weight gain, and reduced drip loss, projecting it ideal against quality deterioration during extended storage, thereby preserving the nutritional integrity and freshness of the product over time. The ability of protein to retain water within their matrix against gravitational force, known as water-holding capacity (WHC), and proximate analysis is also a key factor influencing the overall quality of the fillet. Erikson et al. (2004) and Regenstein et al. (1984) have demonstrated that a salt concentration of 1.8% or higher can enhance the WHC of fish muscle, this effect can be attributed to the protein solution's ability to enhance water retention and improve water-holding capacity, ultimately resulting in increased moisture content and reduced drip loss. Ikasari and Suryaningrum (2015) observed a significant decline in protein content in pangasius fillets (*Pangasius hypophthalmus*) during frozen storage (-18±1°C). This protein loss can be attributed to the leaching of amino acids and water-soluble proteins as ice melts, where fish protein can be incorporated into fillets alongside

salt and phosphate to maintain relative protein content as described by Thorarinsdottir et al. (2004b).

Overall, this approach not only addresses consumer preferences but also contributes to an increased yield, providing economic benefits for producers aligning with the industry's ongoing quest for innovation and excellence.

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Data availability statement: This is an original research work, the data set generated during research work will be available from the corresponding author upon reasonable request.

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