



# Heat Penetration Characteristics and Quality Evaluation of Pangasius (*Pangasius hypophthalmus*) Chunks in Masala Using Retort Pouches

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

A South Indian dish was developed using Pangasius fish (*Pangasius hypophthalmus*) fillet chunks in masalas as a medium. The aim of the current study was to optimize the various  $F_0$  values for Pangasius fillet chunks packed in masala, using flexible, retortable pouches. After being processed

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at different lethality levels, the product showed a similar heating and cooling lag factor. For  $F_0$  values of 7.37 and 8.12 minutes, the total process time were 26.94 and 31.37 minutes, respectively. The  $fh$  values for products processed at  $F_0$  values of 7.37 and 8.12 minutes were 13 and 16.5 minutes, respectively. The cook value obtained for the thermally processed product was 64.70 minutes for  $F_0$  7.37 minutes and 65.87 minutes for  $F_0$  8.12 minutes. Over a storage duration of up to 90 days, all instrumental texture indicators exhibited a declining pattern. The product processed at  $F_0$  value of 7.37 and 8.12 minutes were achieved commercial sterility. The product processed at  $F_0$  7.37 minutes was considered more favourable based on organoleptic assessment.

**Keywords:** *Pangasius hypophthalmus*;  $F_0$  value; total process time; cook value;  $fh$  value; organoleptic evaluation.

## 1. INTRODUCTION

Conventional thermal methods of processing (e.g., boiling, frying, roasting, grilling, and smoking) produce organoleptically acceptable and tasty goods, but they have a number of limitations, including limited heat penetration, long processing times due to poor heat transfer, and nonhomogeneous heat distribution, which can result in overheating or underheating issues (Dominguez-Hernandez et al., 2018, Troy et al., 2016, Sobral et al., 2018). If food products like fish are not handled and prepared properly, they will deteriorate quickly due to microbial development, oxidative reactions, and enzymatic autolysis. This is one of the several preservation techniques that have evolved (Rahman, 2020), and thermal processing is one of them. The idea behind thermal processing is to heat foods for a given amount of time at a specified temperature.

Heat treatment used for canning of fish is designed to kill pathogenic bacteria as well as other spoilage-causing bacteria during storage. *Clostridium botulinum* is an anaerobic bacterium that produces toxin in low-acid products with pH values greater than 4.5, such as canned fish (Méndez and Abuín 2012). Thermal treatment of fish in sealed metallic cans prevents both bacterial and autolytic spoilage, resulting in products with shelf lives of 1 to 2 years at room temperature. Bigelow et al. (Bigelow et al., 1920) mathematically expressed the death kinetics of bacterial spores. According to Esty and Meyer (Esty and Meyer, 1922), the heat resistance of a population of *Clostridium botulinum* was determined by the number of microorganisms present at the beginning, and it was concluded from the data that the decline was exponential with time. Microorganism inactivation is still measured in decimal reduction time, or the time it takes to lower the number of microbial cells by a factor of ten, or D value. The z value is the increase in temperature that occurs when the D value is reduced by a factor of 10. Esty and

Meyer, (1922) reported that the highest heat resistance was recorded at 121.1°C for the proteolytic *C. botulinum* in canned food. The heat penetration parameters ( $f_h$ ,  $f_c$ ,  $j_h$ ,  $j_c$ ) given by Ball (1923) are the traditional method of measuring the lethal effect of thermal processing.

Ready-to-eat (RTE) fish products are highly profitable and flavourful, packaged in sealed containers that require no additional cooking before consumption. These products are efficient in terms of both time and storage, making them an excellent option for quick meal solutions at home. The RTE sector has played a significant role in revitalizing India's seafood canning industry, which had previously declined (Gopinath et al., 2007). Recognized as "Value-added products" by India's Marine Product Export Development Authority (MPEDA), these RTE items contribute to the export market; in one recent year, India shipped 0.14 million metric tons of such value-added seafood, generating \$378.3 million in revenue (MPEDA 2022). Thermal processing is essential for RTE products to ensure food safety, prolong shelf life, and enhance both taste and texture.

Retort pouches are becoming more and more popular because of their many advantages over metal containers. These include being less cheap, thin compared to jars and cans, requiring less time to process, having a simpler opening, the processed food can be consumed right out of the pouch, taking up less storage space, and having easier disposal (Trinh and Glasgow, 2012). When compared to empty cans, retort pouches take up 85 % less space and weigh much less (Yang et al., 2007).

*Pangasius*, a catfish with characteristics like rapid development, air breathing, low dissolved oxygen tolerance, and polyculture compatibility, has gained interest in several Asian countries. Due to the influence of international markets, a

large number of farmers in India have become interested in cultivating *P. hypophthalmus* (Mermelstein 1978). Because of the absence of a fishy odor, as well as little bones and skin, Pangasius fillets characterize Their flavor is delicate, and their texture is firm when cooked, allowing for a wide variety of food treatments (Coles and Kirwan, 2011). Various types of value-added products have been developed from Pangasius fish (Singh and Lakra, 2012), but the data regarding thermally processed Pangasius fish fillet chunks in masala packed in retort pouches is very scanty. In light of this, the present study has been done to evaluate the textural, biochemical and sensory changes that occur in the processed Pangasius fish fillet chunks.

## 2. MATERIALS AND METHODS

### 2.1 Materials

The freshly farm reared Pangasius fish (*Pangasius hypophthalmus*) was used as the raw material. It weighed 1.5 kg each and was procured from private fish farms located at Nellore, Andhra Pradesh, India, through the Karnataka Fisheries Development Corporation (KFDC), Mangalore, India and transported to the laboratory in an aseptic and chilled condition. A four-ply laminated flexible retort pouch with a capacity of 250± 10 g was used for packaging of chunks with masala. It was made of 12 µm polyester (outer layer) with 9 µm aluminum foil, 15 µm nylon (middle layer), and 70 µm

polypropylene (inner layer). The pouches were purchased from Floeter India Retort Pouches Pvt. Ltd., Haryana, India. The pilot-scale horizontal overpressure retort (steam/water spray) used in the study was procured from Lakshmi Engineering Works, Chennai, India, having a loading capacity of 25-30 pouches per process.

### 2.2 Preparation of Pangasius Fillets Chunks in Masala

The Pangasius was dressed aseptically by removing the entrails and washed with the chilled potable water. Fish fillets were made into small chunks having a thickness of 3-5 cm. Cleaned fillet chunks were subjected to marination with salt, turmeric and a slight amount of oil and kept in the refrigerator for 30 minutes to enhance the flavour. Pangasius fish masala was prepared by following methods as follows. Mustard seed, cumin seed, fenugreek seed, coriander seed, big onion, tomato, garlic, ginger and red chili were fried until the colour changed to brown. After frying, fine paste was made with it. On a pan, vegetable oils and green chili, curry leaves, turmeric powder, shallots (small onions), coconut water and turmeric were added along with the other ingredients and fried in a low flame for a few minutes and curry paste was added and heating was gradually carried out over a modest flame. Salt was added to enhance the taste. Water was added to increase the consistency of fish masala as per requirements. Pangasius fillet chunks in masala prepared using the recipe given in Table 1.

**Table 1. Recipe for preparation of Pangasius fillet chunks in masala**

Ingredients	Weight
Big onion	200g
Tomato	300g
Garlic	50g
Turmeric powder	10g
Shallots (Small onion)	100g
Chilli powder	10g
Coriander leaves	25g
Coriander seeds	300g
Cumin seed	10g
Fenugreek	10g
Red chilli small	20g
Coconut	1 piece
Curry leaves	10g
Ginger	50g
Mustard seed	15g
Button red chilli	20g
Vegetable oil	150 ml
Salt	As per requirement
Water	As per requirement

### 2.3 Filling and Sealing of the Pouches

About 250 ± 10 g pre-prepared Pangasius fish fillet chunks with masala were added in retort pouches. Few pouches were punched from the bottom (after determining the slowest heating point) using a punching tool and the packing gland was tightly screwed for heat penetration. The punched hole was positioned so that the thermocouple tip would eventually be at the cold spot. Steam exhaustion was done in an autoclave at 100 °C to remove the air entrapped in the pouches. The sealing region of the pouches was carefully monitored to prevent contamination and then pouches were sealed using a continuous vertical band sealing machine (Gempack, Chennai, India).

### 2.4 Thermal Processing of Pangasius Fillets Chunks in Masala

Heat penetration data of the processed product was recorded using the Ellab eval Flex Four Channel Thermal Validation and Sterilization Monitoring System, Cat. 21401004 (Ellab A/S, Trollesmindealle 25, DK-3400 Hilleroed, Denmark), with an Ellab CTF 9004 Precision Thermometer and  $F_0$  value integrator was used. Copper/cupronickel thermocouples (Ellab SSA 12050-G700-TS) of stainless-steel electrode probe was used with a length of 40 mm and diameter of 1.2 mm. A total of four thermocouple probes were employed, with two being used to record pouch temperature and the other two being used to monitor retort ambient temperature. The data was recorded at a 1 second interval. Process time was determined using the Ball formula method (Ball, 1923) and total process time was calculated by adding 58% of the retort's come-up time to the ball's process time given by Stumbo, (2013). Cook value (CV) was computed using the reference temperature of 100 °C and the z value of 33 °C, which is required for thiamine denaturation.

### 2.5 Quality Evaluation

#### 2.5.1 Proximate, biochemical, and microbiological analysis

Moisture, ash and crude protein were analysed using AOAC (2010) and crude lipids were analysed using Bligh and Dyer (1959). Raw and processed Pangasius fillet chunks were analyzed for biochemical characteristics such as TVB-N, FFA and TBA-RS. TVB-N were analysed using Conway's micro diffusion method (Conway et al., 1962). TBA value was analysed using the

Raghavan and Hultin, (2005) method and FFA was analysed using Dyer and Morten, (1956). Total coliforms, total plate count, faecal Coliforms, *Escherichia coli*, *Vibrios*, *Salmonella*, *Listeria spp.* and *Staphylococcus spp.* were estimated for raw Pangasius fish using APHA (2000).

#### 2.5.2 Commercial sterility test

To verify the growth of any survivors of mesophiles and thermophiles in the product, the pangasius fillet pieces in masala were processed at various  $F_0$  values (7.37 and 8.12 minutes) and then incubated at 37 °C for 15 days and 55 °C for at least 5 days, respectively. A sterile forceps was used to remove about 1-2 g of the samples from the incubated pouches, which were then aseptically opened in a laminar air flow chamber and inoculated into test tubes having pre-sterilized fluid thioglycolate broth. The tubes of fluid thioglycolate broth were overlaid with sterilized liquid paraffin to create an anaerobic condition and incubated at 37 °C for 48 hours and at 55 °C for 5 days, respectively. After the incubation period, the tubes were checked for turbidity as per the IS 2168 (1971).

#### 2.5.3 Texture profile analysis

The texture profile analysis (TPA) of fresh fish and processed product was carried out. using texture analyser (TA-XT Plus, Stable Micro Systems, UK) having a load cell of 75 mm diameter cylindrical probe equipped with a sensor of 50 N. Raw Pangasius fillet chunks as well as cooked Pangasius fillet chunks from the pouch were used for studying the TPA (n-3). The texture analysis consisted of two simultaneous 40 % compressions at a crosshead speed of 12 mm/min, a trigger force of 0.5 kg, and a time and distance of 5 seconds and 6 mm. Mean values of the TPA parameters were calculated with force by time data as described by Bourne (1978).

#### 2.5.4 Sensory evaluation

Sensory evaluation was based on characterization and differentiation of the various sensory characteristics such as appearance colour, flavour, chewiness, succulence, toughness, and overall acceptability. The score was given based on the 9-point hedonic scale (5 = limit of acceptability) by a 25-member panel as per the guidelines given by Peryam and Pilgrim (1957). To assign the score, thermally processed

pouches were heated in boiling water for 5 minutes and served warm to panelists. Sensory analysis was carried out for the product before thermal processing as a control and the thermally processed product after an interval of 15 days for a period of 90 days of storage at ambient temperature.

### 2.5.5 Statistical analysis

The biochemical, sensory and texture profiles of Pangasius fillet chunks were statistically analysed using the SPSS 10.00 statistical package. The mean  $\pm$  standard deviation was used to express the results. A one-way ANOVA was used to determine the mean difference, and Duncan's multiple range tests were employed to compare the means at the 5% significant level.

## 3. RESULTS AND DISCUSSION

### 3.1 Thermal Process Evaluation

In this study, ready to eat Pangasius fillet chunks in masala products were developed based on the different literature and culinary styles followed in the southern parts of India. The product was standardised based on the organoleptic evaluation and texture profile attributes. For fishery products, Frott & Lewis, (1994) recommended a  $F_0$  value of 5–20 minutes. The literature reports that the  $F_0$  value for ready to eat fish ranges from 5 to 10 minutes. (Bindu et al., 2010, Gopal et al., 1998, Majumdar 2015). Initially, a number of trials have been conducted at 121.1 °C before the actual experiment, which helps to decide the lethality requirements of the product. The cumulative lethality ( $F_0$  value) obtained after the two-process trails were  $F_0$  7.37 and 8.12 min. In the initial trials the product was processed at  $F_0$  values of 7,8,9 & 10 min at a temperature of 121.1°C and based on organoleptic evaluation, the fish masala product was processed at  $F_0$  values of 7.37 and 8.12 min was selected for optimization.

### 3.2 Heat Penetration Characteristics

Heating and cooling factors of Pangasius fillet chunks in masala in retort pouches at  $F_0$  7.37 min and  $F_0$  8.12 min, respectively, are given in Fig. 1 and Fig. 2, and Table 2. The come up time was obtained 9 and 8 min for  $F_0$  7.37 and  $F_0$  8.12, respectively. Lag factor for heating ( $J_h$ ) and cooling ( $J_c$ ) during the thermal processing were determined and  $J_h$  values were found to be 1.15 min and 1.09 min for two different  $F_0$  7.37 min and  $F_0$  8.12 min, respectively and  $J_c$  values were 1.12 min and 1.01 min, respectively. The total

processing time was 26.94 min and 31.37 min for  $F_0$  7.37 min and  $F_0$  8.12 min, respectively. The cook values achieved for the thermally processed Pangasius fillets chunks in masala were 64.70 min and 65.87 min for  $F_0$  7.37 min and  $F_0$  8.12 min, respectively. Some of the researchers also reported that cook value of 89.79 min in seer fish moilee in retort pouch (Manju et al., 2004), 57.19 to 92.12 min in sardine (Ali et al., 2005), 86.14 min in Shrimp kuruma (Mohan et al., 2006), 69.73 to 125.65 min for mackerel (2006), 83.19 to 86.14 min for prawn kuruma and fish peera (Bindu et al., 2010, Mohan et al., 2008), cook value of fish peera at 66.02 min. Bindu et al., (2007) reported that in thermally processed ready to eat black clam total process time recorded was 44 min at  $F_0$  value of 9.29. For the thermal processed Mackerel (*Rastrelliger kanagurta*) in retort pouches total process time obtained was 53.087, 31.790, and 22.303 for three different temperature 115 °C, 121 °C, and 126 °C, respectively by Xavier et al., (2013). Total process time at  $F_0$  8.15 min was recorded 48.3 min for seer fish moilee packed in retortable pouches (Manju et al., 2004). Puthanangadi et al., (2021) reported that total process time of shrimp in masala at  $F_0$  8.86 min and  $F_0$  9.96 was recorded 37.11 min and 39.88 min, respectively.

### 3.3 Proximate Analysis

The proximate composition of fresh Pangasius fish constitutes moisture, fat, crude protein and ash contents are shown in Table 3. The proximate composition of fresh Pangasius fish constitutes moisture, crude protein, crude fat and ash contents were found to be  $70.29 \pm 0.19$  %,  $16.70 \pm 1.37$  %,  $5.28 \pm 0.33$  % and  $1.89 \pm 0.05$  %, respectively. The results of this study were in agreement with the findings of Kumar et al. (2017) and Karl et al., (2010). Karl et al., (2010) observed that the moisture content of  $79.32 \pm 0.87$  %, protein  $16.96 \pm 0.31$  %, fat  $2.02 \pm 0.06$  % and ash  $1.44 \pm 0.18$  % in Pangasius fish. More or less similar results were obtained by Rao et al., (2013) were moisture 78.2%, protein 17.24%, fat 2.84% and ash 1.3%. Addition of oil during masala preparation that would have contributed to the increases fat content in processed product, (2012). The results obtained by the study conducted by Orban et al., (2008), Sokamte et al., (2020) were moisture  $83.57 \pm 2.30$ , protein  $13.60 \pm 1.34$ , fat  $1.84 \pm 0.92$ , ash  $1.25 \pm 0.19$  and moisture  $77.32 \pm 2.15$ , protein  $19.66 \pm 0.46$ , lipid  $1.00 \pm 0.02$ , ash  $1.24 \pm 0.14$ , respectively. There is a decreasing trend in

moisture content from 70.29% value in the fresh material to about 64.67 % in the processed product. This may be attributed to loss of water holding capacity of the cells due to protein denaturation upon heat treatment. The crude protein, fat and ash percentage of processed Pangasius fish fillets chunks were found to be 17.10%, 7.89% and 2.38%, respectively. This

result was in agreements of Mai et al., (1978), Gall et al., (1983), Gallardo et al., (1990), and Biji et al., (2015). The increasing protein, Fat and ash contains in the study may be due to the loss water from muscle during sterilization. Also adding an oil during masala preparation that would have contributes to the increase of fat content (Kumar et al., 2017).

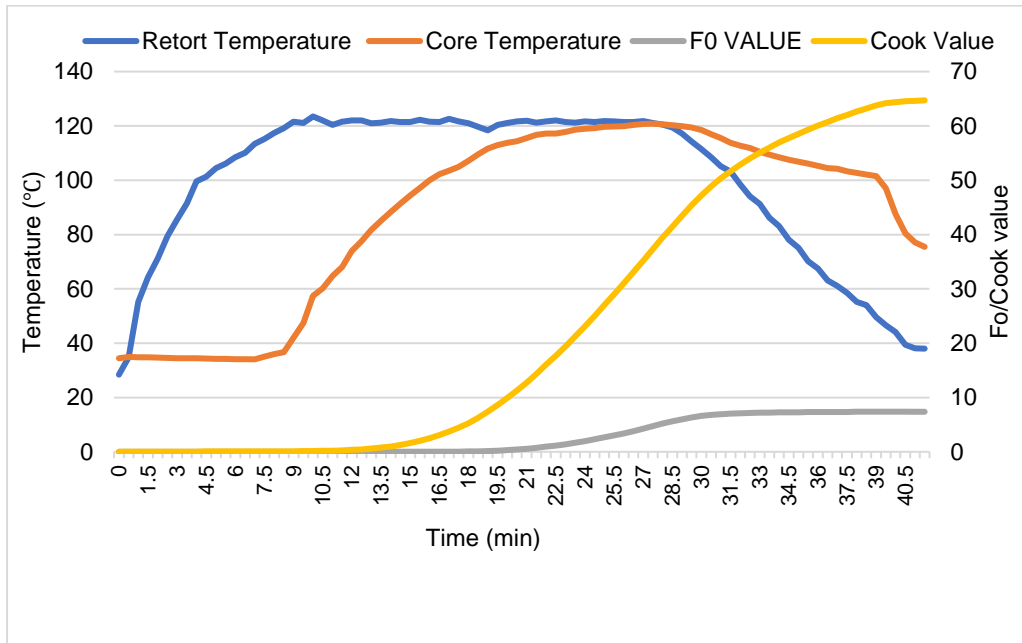


Fig. 1. Heat penetration rate, Fo value and cook value of Pangasius fish fillet chunks in masala processed at F0 7.37 min

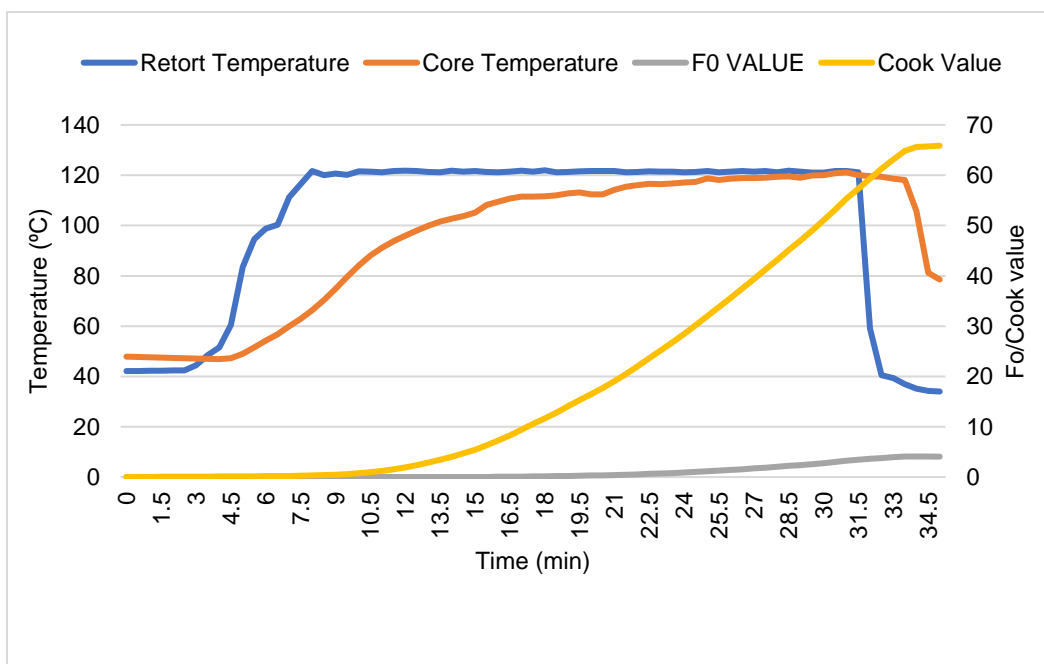


Fig. 2. Heat penetration rate, Fo value and cook value of Pangasius fish fillet chunks in masala processed at F0 8.12 min

**Table 2. Heat penetration characteristics of Pangasius fillet chunks in masala processed at  $F_0$  7.37 and 8.12 min**

Parameters	$F_0$ 7.37	$F_0$ 8.12
Come-up-time (min)	9	8
Heating lag factor ( $J_h$ )	1.15	1.09
Cooling lag factor ( $J_c$ )	1.12	1.01
$f_h$ slope of heating curve (min)	13	16.5
$g$ ( $^{\circ}\text{C}$ )	1.56	1.928
Cook value (min)	64.70	65.87
Ball's process time (min)	23.45	26.73
Total process time (min)	26.94	31.37

**Table 3. Proximate composition of fresh Pangasius fish and processed Pangasius chunks**

Sl. No.	Parameter	Fresh Pangasius fish (%)	Processed Pangasius fillet chunks (%)
1.	Moisture	70.29 $\pm$ 0.19	64.67 $\pm$ 0.40
2.	Crude protein	16.70 $\pm$ 1.37	17.10 $\pm$ 0.67
3.	Total lipid	5.28 $\pm$ 0.33	7.89 $\pm$ 1.05
4.	Ash	1.89 $\pm$ 0.05	2.38 $\pm$ 0.42

Values are expressed as mean  $\pm$  standard deviation ( $n=3$ )

### 3.4 Biochemical Evaluation of Pangasius Fish Fillets Chunks

TVBN, FFA and TBARS were determined and compared with the fresh material used in the study (Table 4). The biochemical indices showed an increasing trend with respect to storage days and it was found that all biochemical parameters were within the limit. Proteins, amino acids, and other nitrogenous substances, such as TMAO, may have broken down during thermal processing, which might account for the higher level of biochemical compounds in processed products [Gallardo et al., 1990, Biji et al., 2015 Chia et al., 1983]. The research done by Bindu et al., (2007) and Kamalakanth et al., (2018) shows a similar trend of FFA values.

TVBN, FFA and TBARS were determined and compared with the fresh material used in the study and the same are given in the Table 4. For fresh material the values of TVBN, FFA and TBARS content 2.8  $\pm$  0.06 mg N/100g, 0.27 $\pm$  0.02 mg% of Oleic acid and 0.54 $\pm$ 0.02 malonaldehyde/kg, respectively. For the processed sample, the TVBN content was within the range of 8-17 mg N/100g, FFA content was 0.2-0.9 and TBARS value was 0.55-1.5 malonaldehyde/kg. All the biochemical indices showed on increasing trend with respect to storage days. The increased content of TVBN in the processed sample may be due to the breakdown of proteins, amino acids, and other

nitrogenous compounds including TMAO during sterilization (32,46,48,50). TBARS value is an indication of secondary oxidation. TBARS values increased during storage of processed Pangasius fillets chunks in masala packed in flexible retortable pouches and it was minimal increasing trend. However, the increasing trend of TBARS in processed samples were within the acceptable limit. The results are in agreement with finding of various authors by Bindu et al., 2010, Kamalakanth et al., 2018, Gill et al., 1987, Ahn and Min 2005, Devadasan, 2001, Ammu et al., 2017).

Medina et al., (1995) reported that precooking of fisheries products prior to thermal processing significantly increases the level of free fatty acid content in the muscle. According to Aubourg et al. (1990) FFA values were seen to be increasing on storage. This is because due to increased temperature, lipid hydrolysis takes place which enhances the FFA fraction in the lipids of fish flesh. Hence FFA levels progressively increase in storage. The previous research done by Bindu et al., (2010, 2007, 2004), Kamalakanth et al., (2018), Maheswara et al., (2011) shown the similar increasing trend for FFA value in thermally processed fishery products. For the Pangasius fillets chunks in masala all biochemical parameters were within the limit during the storage period of 90 days at ambient temperature.

**Table 4. Changes in biochemical parameters of fresh and thermally processed Pangasius chunks in masala processed at  $F_0$  7.37 and  $F_0$  8.12 minutes**

Attributes	Fresh fish	Processed fish													
		5 days		15 days		30 days		45 days		60 days		75 days		90 days	
		$F_0$ 7.37	$F_0$ 8.12	$F_0$ 7.37	$F_0$ 8.12	$F_0$ 7.37	$F_0$ 8.12	$F_0$ 7.37	$F_0$ 8.12	$F_0$ 7.37	$F_0$ 8.12	$F_0$ 7.37	$F_0$ 8.12	$F_0$ 7.37	$F_0$ 8.12
TVB-N (mgN/100g)	2.83± 0.06 <sup>i</sup>	8.06± 0.23 <sup>h</sup>	8.69± 0.01 <sup>g</sup>	11.25± 0.01 <sup>f</sup>	11.20± 0.76 <sup>f</sup>	12.65± 0.57 <sup>e</sup>	13.01± 0.02 <sup>e</sup>	13.53± 0.56 <sup>d</sup>	15.14± 0.01 <sup>c</sup>	15.25± 0.05 <sup>c</sup>	16.75± 0.03 <sup>b</sup>	17.17± 0.30 <sup>ab</sup>	17.43± 0.02 <sup>a</sup>	17.22 ± 0.08 <sup>ab</sup>	17.53 ± 0.09 <sup>a</sup>
TBA-RS (mg MAD /kg)	0.54± 0.02 <sup>h</sup>	0.55± 0.01 <sup>h</sup>	0.85± 0.02 <sup>g</sup>	0.9± 0.01 <sup>g</sup>	0.97± 0.02 <sup>f</sup>	1.10± 0.01 <sup>e</sup>	1.11± 0.01 <sup>e</sup>	1.10± 0.02 <sup>e</sup>	1.16± 0.01 <sup>d</sup>	1.2± 0.02 <sup>cd</sup>	1.22± 0.04 <sup>c</sup>	1.32± 0.01 <sup>b</sup>	1.49± 0.04 <sup>a</sup>	1.34 ± 0.01 <sup>b</sup>	1.5 ± 0.05 <sup>a</sup>
FFA (% of oleic acid)	0.27± 0.02 <sup>i</sup>	0.28± 0.28 <sup>i</sup>	0.51± 0.05 <sup>g</sup>	0.46± 0.01 <sup>h</sup>	0.57± 0.02 <sup>f</sup>	0.52± 0.02 <sup>g</sup>	0.61± 0.01 <sup>ef</sup>	0.59± 0.01 <sup>f</sup>	0.70± 0.01 <sup>d</sup>	0.64± 0.02 <sup>e</sup>	0.89± 0.02 <sup>a</sup>	0.73± 0.02 <sup>cd</sup>	0.80± 0.05 <sup>b</sup>	0.77 ± 0.02 <sup>bc</sup>	0.9 ± 0.02 <sup>a</sup>

Values are expressed as mean ± standard deviation. Different superscript in small letters (a,b,c,...) same rows indicate significant differences ( $p < 0.05$ ) ( $n=3$ )

**Table 5. Instrumental texture profile analysis for fresh and thermally processed Pangasius fillet chunks in masala processed at  $F_0$  7.37 and  $F_0$  8.12 minutes**

Product Parameter	Fresh fish	Processed fish													
		5 days		15 days		30 days		45 days		60 days		75 days		90 days	
		$F_0$ 7.37	$F_0$ 8.12	$F_0$ 7.37	$F_0$ 8.12	$F_0$ 7.37	$F_0$ 8.12	$F_0$ 7.37	$F_0$ 8.12	$F_0$ 7.37	$F_0$ 8.12	$F_0$ 7.37	$F_0$ 8.12	$F_0$ 7.37	$F_0$ 8.12
Hardness (kg.f.)	1.88± 0.53 <sup>a</sup>	0.80± 0.33 <sup>b</sup>	0.71± 0.11 <sup>b</sup>	0.72± 0.42 <sup>b</sup>	0.72± 0.07 <sup>b</sup>	0.68± 0.08 <sup>b</sup>	0.53± 0.13 <sup>b</sup>	0.61± 0.21 <sup>b</sup>	0.52± 0.49 <sup>b</sup>	0.53± 0.12 <sup>b</sup>	0.42± 0.56 <sup>b</sup>	0.31± 0.16 <sup>b</sup>	0.30± 0.05 <sup>b</sup>	0.32 ± 0.02 <sup>b</sup>	0.32 ± 0.01 <sup>b</sup>
Springiness (mm)	1.00± 0.42 <sup>ab</sup>	0.87± 0.23 <sup>b<sup>c</sup></sup>	0.82± 0.11 <sup>bcd</sup>	0.86± 0.23 <sup>a</sup>	0.73± 0.02 <sup>cdef</sup>	0.75± 0.11 <sup>cde</sup>	0.65± 0.04 <sup>defg</sup>	0.63± 0.12 <sup>efgh</sup>	0.60± 0.01 <sup>efgh</sup>	0.49± 0.04 <sup>ghi</sup>	0.55± 0.18 <sup>fgh</sup>	0.44± 0.06 <sup>hi</sup>	0.33± 0.09 <sup>i</sup>	0.42 ± 0.06 <sup>hi</sup>	0.33 ± 0.05 <sup>i</sup>
Cohesiveness	0.96± 0.21 <sup>a</sup>	0.59± 0.21 <sup>bcd</sup>	0.56± 0.04 <sup>bc</sup>	0.53± 0.12 <sup>b</sup>	0.52± 0.02 <sup>bcd<sup>e</sup></sup>	0.51± 0.02 <sup>bcd<sup>e</sup></sup>	0.49± 0.0 <sup>bcd<sup>e</sup></sup>	0.47± 0.0 <sup>bcd<sup>e</sup></sup>	0.38± 0.14 <sup>defgh</sup>	0.43± 0.02 <sup>defgh</sup>	0.36± 0.06 <sup>gh</sup>	0.37± 0.06 <sup>gh</sup>	0.37± 0.01 <sup>efgh</sup>	0.33 ± 0.10 <sup>h</sup>	0.33 ± 0.04 <sup>h</sup>
Chewiness (kgf.mm)	1.81± 0.16 <sup>a</sup>	0.55± 0.40 <sup>bc</sup>	0.34± 0.11 <sup>bc</sup>	0.42± 0.22 <sup>bc</sup>	0.27± 0.04 <sup>bc</sup>	0.27± 0.06 <sup>bc</sup>	0.17± 0.05 <sup>bc</sup>	0.19± 0.11 <sup>bc</sup>	0.13± 0.23 <sup>bc</sup>	0.11± 0.03 <sup>bc</sup>	0.16± 0.13 <sup>bc</sup>	0.05± 0.03 <sup>c</sup>	0.04± 0.01 <sup>c</sup>	0.04 ± 0.01 <sup>c</sup>	0.03 ± 0.01 <sup>c</sup>

Values are expressed as mean ± standard deviation. Different superscript in small letters (a,b,c,...) indicate significant differences ( $p < 0.05$ ) ( $n=3$ )



### 3.5 Microbial Evaluation of Raw Pangasius Fish Fillets Chunks

The total plate count of fresh Pangasius fish fillet chunks was  $4.96 \times 10^4$  CFU/g. The sample was also subjected to check the presence of important bacterial pathogens such as *Salmonella*, *Vibrio*, *Escherichia coli*, *Staphylococcus aureus*, and *Listeria* spp. However, none of the pathogens were present in the sample. This results in agreement with the study conducted on *Litopenaeus vannamei* by Puthanangadi et al. (2021).

### 3.6 Commercial Sterility Test

The commercial sterility of thermally processed chunks in masala was examined, and no growth was observed in the medium. This suggests that the total lethality given to the product was sufficient to achieve commercial sterility. Thermal processed pangasius fillet chunks masala pouches processed at  $F_0$  7.37 and  $F_0$  8.12 minutes were subjected to a commercial sterility test. The sample was incubated in thioglycolate broth tubes for 48 hours at 37 °C and for five days at 55 °C. The tubes showed no signs of turbidity. The study was carried out throughout the study period of 90 days and there was no growth of any thermal resistance bacteria. This indicates that both  $F_0$  values were good enough to reach the commercial sterility of the product.

### 3.7 Texture Profile Analysis

The texture profile analysis was done for the fresh Pangasius fillet chunks as well as thermally processed Pangasius fish fillet chunks in masala processed to two different  $F_0$  values. Hardness (kgf), Springiness (mm), Cohesiveness, Chewiness (kgf.mm) values for fresh Pangasius fillet chunks were found to be  $1.88 \pm 0.53$ ,  $1.00 \pm 0.42$ ,  $0.96 \pm 0.21$ ,  $1.81 \pm 0.16$ , respectively. The texture of the fish is influenced by a number of things. Vácha et al., (2014) studied the instrumental texture profile analysis parameter for female Tench (*Tinca tinca*) and found that the hardness (N), springiness (mm), cohesiveness and Chewiness (kgf.mm) values were  $9.49 \pm 0.6$ ,  $0.67 \pm 0.01$ ,  $0.47 \pm 0.02$  and  $2.85 \pm 0.26$ , respectively. The decrease in hardness values upon thermal processing is due to the effect of temperature on the collagen and the resultant softening of the muscle. Instrumental texture profile analysis for fresh and thermally

processed Pangasius fillets chunks in masala processed at  $F_0$  7.37 and  $F_0$  8.12 min. are given in Table 5.

The hardness for the  $F_0$  7.37 and  $F_0$  8.12 min of Pangasius fillet chunks in masala were  $0.80 \pm 0.33$  and  $0.71 \pm 0.11$  kgf, respectively. It was observed that the hardness decreased as  $F_0$  values increases. The hardness value of the processed fish product upto 90 days of storage decreased after thermal processing than the fresh Pangasius. These results were in agreement with the findings of Mallick et al. (2010), Tanaka et al., (1985). They reported that the thermal processing at higher temperature produces firmer products. Other parameters like springiness, cohesiveness and chewiness followed the same trend as like that of hardness. The springiness values ranged from  $0.40 \pm 0.06$  mm to  $0.33 \pm 0.05$  mm for the  $F_0$  value of 7.37 and 8.12 min, respectively. Cohesiveness values ranged from  $0.96 \pm 0.10$  to  $0.33 \pm 0.04$  for the  $F_0$  values of 7.37 and 8.12 min, respectively. As the  $F_0$  value increases the textural parameters like hardness springiness, cohesiveness and chewiness decrease as reported by Ali et al. (2005), Gopinath et al., (2007), Mallick et al. (2010), Majumdar et al. (2015), Putanangadi et al., (2021). After thermal processing there was a change in textural attributes of the product, as compared with the fresh Pangasius. Low values of hardness, springiness, cohesiveness and chewiness of the thermally processed Pangasius fillet chunks in masala than fresh material may be due to the slight alteration in protein denaturation. Heat treatments altered the native protein conformation which leads to denaturation of muscle protein resulting in change in texture of the products (Ma et al., 1983).

### 3.8 Sensory Analysis

Score obtained for sensory analysis at  $F_0$  7.37 min and  $F_0$  8.12 minutes were in the range of 8.9 to 6.2. Sensory parameters showed a decreasing trend with the storage period and are mentioned in Fig. 3. A similar trend is seen in the previous study done by Vijayan et al., (1998), Puthanangadi et al., (2021), Shankar et al., (2002). According to Ma et al. (1983), the muscle firmness and texture of the product are affected by thermal processing. The longer the product heated, the more the denaturation, coagulation, and oxidation in the native protein, and the combined effect influence of all of these factors that control the organoleptic quality of the product (2015).



**Fig. 3. Sensory Evaluation of fresh and thermally processed Pangasius fillet chunks in masala processed at  $F_0$  7.37 and  $F_0$  8.12 minutes**

#### 4. CONCLUSION

The present study optimized the total lethality ( $F_0$ ) for developing RTE pangasius fish fillets chunks in masala using retort pouches by selecting  $F_0$  values of 7.37 min and 8.12 min based on the evaluation of sensory parameters. Assessment of the heat penetration characteristics and commercial sterility test revealed that both  $F_0$  values satisfied the requirements for commercial sterility. Microbiological, biochemical, and proximate analysis showed that the processed product

parameter levels were within the acceptable limits for the duration of 90 days storage. The texture profile analysis showed decreasing trends of textural parameters during the storage period, which reveals that heat processing had a significant effects on the product texture. The product processed at  $F_0$  7.37 min received higher sensory scores and rated better for its overall quality. The results summarize that pangasius fish fillet chunks could be an excellent raw material for developing various RTE ethnic dishes and meeting the global demand for fish products.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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