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Thermal Processing and Shelf-Life Evaluation of Red-Bellied Pacu (*Piaractus brachypomus*) Fish Masala in Retort Pouches

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Abstract

A convenient and shelf-stable ready-to-eat fish masala dish was developed using red-bellied pacu (*Piaractus brachypomus*) in the present study. The work focused on optimizing thermal processing conditions and evaluating their impact on the product's safety, sensory quality, and storage stability at ambient temperature. Flexible retortable pouches were used for packaging, and heat penetration was monitored using ELLAB thermal data. The *fh* values recorded were 14.5 and 13.5 min for F_0 values of 6.0 and 7.0, with corresponding process times of 24.83 and 25.51 min and cook values of 59.67 and 60.90 min, respectively. Sensory, biochemical, and microbiological quality tests were carried out during storage. Heat processing caused only minimal changes in the fatty acid and amino acid profiles. No microbial growth was observed for up to 150 days. The product processed at an F_0 value of 6.0 retained better overall quality during storage. The results highlight the potential of pacu as a promising raw material for the development of ethnic ready-to-eat fish dishes.

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Introduction

India's inland fisheries sector is growing significantly due to rising fish demand and sustainable aquaculture practices. In recent years, the increasing consumer demand for fish and the ornamental fish trade have contributed to a significant increase in the cultivation of non-native fish species in India. Species such as Pacu, Tilapia, Carp, Catfish, and Trout, originally from Africa, Europe, and Southeast Asia, are among the commonly cultured varieties. The red-bellied pacu fish (*P. brachypomus*) is a species native to the Amazon River basins in South America and is widely known in India as Roopchand or Red-bellied Pacu. This species was introduced to India in 2004 via Bangladesh and has since spread to Kerala, Andhra Pradesh, and the North Eastern region (Singh & Lakra, 2011). It has a shape similar to a pomfret and is often marketed as a freshwater pomfret. This fish offers nutritional and culinary benefits, providing high-quality protein, vitamins, minerals, and essential omega-3 fatty acids. Its firmer texture and mild flavour make it versatile in various culinary preparations, making it suitable for flavourful dishes like ready-to-eat (RTE) masala or curries.

RTE fish products are very lucrative and delicious, packed in air-tight containers and ready for consumption without further cooking. In today's busy lifestyle, RTE products save both time and storage space, making them a convenient and better alternative for home-cooked meals. The once-declining Indian seafood canning industry has revived because of the RTE sector (Gopinath, Anthony, Nagarajarao, Jaganath, & Krishnaswamy, 2007). India's Marine Product Export Development Authority (MPEDA) categorizes these RTE products as 'Value-added products'. India exported 0.14 MMT of other item categories, including value-added seafood, worth 378.3 million US dollars (MPEDA, 2022). Thermal processing is crucial in RTE products in ensuring safety, extending shelf life, and improving flavour and texture.

Thermal processing is a food preservation technique that involves heating the food in hermetically sealed containers to kill microorganisms and pathogens at a specific time and temperature. This technique allows flexible retort pouches with three or four plies as an alternative to the traditional metal containers for packing, and these retort pouches are considered as a value-added packaging material (Juliano, Koutchma, Sui, Barbosa-Cánovas, & Sadler, 2010; Dasan et al., 2021). Compared to conventional metal or glass containers, retort pouch enables quicker heat transmission due to its high surface area to volume ratio and thinner profile (Rodriguez et al., 2003; Awuah, Ramaswamy, & Economides, 2007). Thermal processing in retortable pouches can reduce processing time by around 35% compared to cans, with equivalent lethality (Mohan, Ravishankar, Bindu, Geethalakshmi, & Gopal, 2006). Retorted products are also more cost effective and can be kept at room temperature for over a year without refrigeration (Bindu, Ravishankar, Gopal, & Mallick, 2010).

Red-bellied Pacu fish is one of the commonly cultured freshwater fish species. 'Pacu fish in masala' is a traditional ethnic dish and popular among Asian communities. The present study aims to optimize the thermal process conditions to develop an RTE Red-bellied pacu fish masala that can be stored at ambient temperature for a extended periods, ensuring year-round availability. Apart from this, during periods of glut production, this highly efficient technology can be utilized to preserve pacu fish in ready-to-eat (RTE) form.

Materials and Methods

The freshly harvested red-bellied pacu fish was procured from commercial fish farms located in the southern part of Karnataka, India, in an aseptic and chilled condition. Each fish was weighed approximately 800-900 g. A unique, distinct, and characteristic masala was prepared using commercially available spices in India and different culinary recipes practiced in the southern part of Karnataka. Four-ply laminated flexible retortable pouches (M/s. Floeter India Retort Pouches Pvt. Ltd., Haryana, India) having dimensions of 15×20 cm and a capacity of 300 g were used for packaging the pacu fish steaks with masala. The pilot-scale horizontal overpressure retort with programmable logistic control (PLC) (M/s. Lakshmi Engineering Works, Chennai, India) with a loading capacity of 25-30 pouches per process was used for steam heat sterilization. AR-grade chemicals and glassware were used throughout the study.

The red-bellied pacu fish were washed and hygienically dressed, followed by cleaning with chilled potable water. The fish was cut into small pieces of steaks of approximately 1.5-2 cm thickness. The cleaned steaks were kept in a saturated brine solution for 30 min to allow the brining process to harden the texture of the meat. Then, the fish steaks were pre-cooked at 100 ± 2ºC for 10 to 15 min. After frying all the ingredients (Table 1) together, a delicate paste was prepared. This paste was added to the pre-cooked pacu fish steaks and the mixture was gently heated over a low flame until a distinctive and characteristic aroma emerged. Salt was added to improve the flavour, and the consistency of the fish masala was adjusted by adding water.

Approximately 250 ± 10 g of pacu fish steaks with masala were packed in each retort pouch. The 6 pouches (2 F_0 values × 3 replicas) were fixed with thermocouple packaging glands (50 mm) (GKJ 13009C052) at the slowest heating point. These fish steaks were fixed on packaging glands to record the core temperature. Exhausting and sealing were performed using a vacuum packaging machine (DZ-400) to exhaust the air entrapped in pouches. Copper/cupronickel thermocouples (Ellab SSA 12050-G700-TS) with stainless-steel electrode probes were used, measuring 4 cm in length and 1.2 mm in diameter. The probes were fixed into the packaging glands, and all pouches were kept in trays and loaded into the overpressure retort (Water immersion type). Further, these pouches were thermally processed at the required F_0 values (6.0 & 7.0 min).

Table 1. Ingredients used for the preparation of Red Bellied Pacu fish in masala

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

Heat processing was carried out at 121.1°C for the desired F_0 values. Heat penetration data was recorded every second using the Ellab Eval Flex Four Channel Thermal Validation and Sterilization Monitoring System, Cat. 21401004 (Ellab A/S, Trollesmindealle 25, DK-3400 Hilleroed, Denmark) as per the methodology used by Dasan et al. (2021). Process time was determined using Ball's mathematical formula, and total process time was calculated using the formula approach suggested by Stumbo (2013). Cook value was estimated to measure the deterioration of nutrients during the process (Ranganna, 2000). Pouches were cooled, dried, labelled, and stored at room temperature for shelf-life analysis.

The proximate composition of the fresh and processed fish products was analyzed using AOAC (2010) methods. Biochemical parameters like Total Volatile Basic Nitrogen (TVBN), Peroxide value (PV), Thiobarbituric Acid Reactive Substances (TBARS), Free Fatty Acid (FFA), and pH were assessed. TVBN analysis was performed using Convey's micro diffusion technique (Conway, 1962). The TBARS values were calculated using the Raghavan and Hultin (2005) approach. PV was estimated using the method suggested by Jacobs (1951). Free fatty acid (FFA) was analyzed using the procedure prescribed by Pearson (1976). The spread plate technique (APHA, 2017) was used to estimate the total plate count (TPC) for fresh pacu fish.

Amino acid content was determined by HPLC (M/s. Shimadzu HPLC-LC 10 AS, Shimadzu Scientific Instruments, Columbia, MD, USA) using the method described by Ishida, Fujita, and Asai (1981). Tryptophan was determined using the technique outlined by Sastry and Tummuru (1985). Fatty acids were analyzed using the Metcalfe, Schmitz, and Pelka (1966) method, after total lipid extraction by the Folch, Lees, and Stanley (1957) method.

A commercial sterility test was performed using IS 2168 (ISI, 1971). Processed pouches were incubated at 55°C for 5 days and at 37°C for 15 days to assess the survival of thermophilic and mesophilic bacteria, respectively. After incubation, pouches were aseptically opened in a biosafety cabinet after specific time intervals, and about 1-2 g of the sample was collected using sterilized forceps and inoculated into test tubes containing pre-sterilized fluid thioglycolate broth (Hi-Media, Laboratories, Mumbai). The broth tubes were over-layered with sterilized liquid paraffin on top to create an anaerobic environment to determine the product's commercial sterility. Inoculated samples were incubated at 37°C for 48 h and 55°C for 5 days, respectively.

The texture was assessed for fresh and processed products using the Texture Profile Analyzer (TA-XT Plus, Stable Micro Systems, UK). A cylindrical probe with a 75 mm diameter and a 50 N sensor was used as the load cell. The texture measurement consisted of two consecutive 40% compressions, with a trigger force of 0.5 kg and a time interval of 5 sec and distance of 6 mm, at a crosshead speed of 12 mm/min (Mohan et al., 2006; Dasan et al., 2021). The TPA parameters were calculated using the force-by-time data from each test, which following Bourne's (1978) methodology.

The colour of both the processed product and fresh fish was measured using a ColorFlex-EZ colourimeter (HunterLab, Virginia, USA), following calibration with black and white standard tiles. About 5 g of fish meat was placed in an optically transparent glass cup, which was then placed over the port and sealed with an opaque cup cover to block off any outside light interference. L* (lightness), a* (+a is redness, -a is greenness), b* (+b is yellowness, -b is blueness), and the Overall Whiteness of the fresh fish as well as the processed product was estimated as per Lubana, Randhawa, Surasani, and Singh (2016).

Sensory evaluation was conducted for various characteristics, including colour, appearance, flavour, succulence, chewiness, toughness, and overall acceptability. The score was determined based on a 9-point hedonic scale assessed by 25 panellists, as described by Peryam and Pilgrim (1957). Thermally processed pouches were randomly selected and heated in boiling water for 5 min. The sample contents were placed in coded white enamel plates and served hot to panellists. The panellists were asked to assign a score of 1-9 (1 = extremely dislike, 2 = dislike very much, 3 = moderately dislike, 4 = slightly dislike, 5 = neutral (neither like nor dislike), 6 = slightly like, 7 = moderately like, 8 = like very much, and 9 = extremely like). Sensory evaluation was carried out for the products before thermal processing as a control and for the thermally processed product after an interval of every 30 days for 150 days of storage at ambient temperature.

Statistical analysis was conducted using the IBM SPSS Statistics 27.0.1.0 statistical package. One-way ANOVA test was used to determine significant differences in the means ($p < 0.05$), and the Duncan multiple range test was used to compare means. The results are tabulated as mean ± standard deviation (SD).

Results and Discussion

In the present investigation, the RTE ethnic and highly lucrative red-bellied pacu fish masala dish was developed based on the different culinary styles followed in Southern Karnataka, India. The recipe for pacu fish in masala was standardized based on the organoleptic evaluation (Table 1). Frott and Lewis (1994) studied and recommended an F_0 value in the range of 5–20 min for fish products. F_0 value in the range of 5-10 min has been reported in several studies for RTE fish products (Majumdar, Dhar, Roy, & Saha, 2015). Therefore, in the present study, the product was processed at F_0 values of 6, 7, 8, and 9 min at a temperature of 121.1°C, and based on organoleptic evaluation (Fig. 1), fish masala product processed at F_0 values of 6 and 7 min was selected for further study.

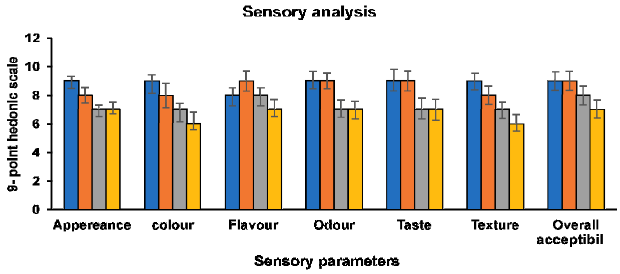

Fig. 1. Sensory analysis for optimization of Total Lethality (F_0) of Red Bellied Pacu fish in masala processed at F_0 6,7,8 and 9 min

The data obtained from the thermal validation and temperature monitoring system (Ellab) was used to evaluate the heat penetration characteristics of pacu fish in masala (Table 2). Fig. 2 represents the heating and cooling behaviour of pacu fish masala in retortable pouches at F_0 6 and F_0 7 min. Heat penetration characteristics showed a shorter come-up time of 6 and 7 min to reach the retort at reference temperature (121.1° C). The total process time of 24.83 and 25.51 min was taken by F_0 6.0 and F_0 7.0, respectively. To complete the operation, F_0 7.0 had taken 2.7 % more time than F_0 6.0. Cook value

Table 2. Heat penetration characteristics of thermally processed Red Bellied Pacu fish in masala at F_0 6.0 and F_0 7.0 min

Parameters	F_0 6.0 min	F_0 7.0 min
Come-up-time (min)	6	7
Heating lag factor (J_h)	0.95	0.99
Cooling lag factor (J_c)	1.01	1.02
f_h slope of the heating curve (min)	14.5	13.5
U	6.15	7.19
f_h/U	2.35	1.87
g (°C)	2.69	2.31
Cook value (min.)	59.67	60.90
Ball's process time (min.)	21.35	21.45
58 % CUT	3.48	4.06
Total process time (min.)	24.83	25.51

and total process time values were found to be on an increasing trend with an increase in F_0 value. The result of a rise in cook value with respect to increasing F_0 value was consistent with the conclusions reported by Ali, Sudhir, and Gopal (2005). The freshwater prawn took 57 and 63 min to process in curry medium at F_0 8 and 9 min, respectively (Majumdar, Roy, & Saha, 2017).

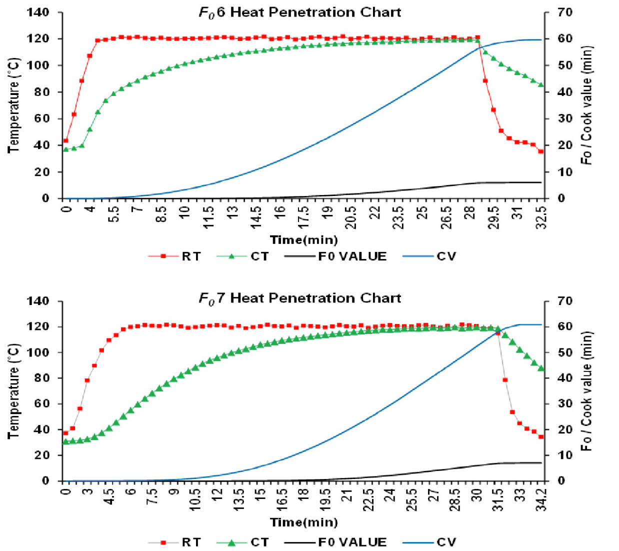

Fig. 2. Heat penetration rate of red-bellied pacu fish in masala processed in flexible retort pouches at F_0 values of 6 and 7 min (121.1°C). RT = Retort Temperature; CT = Core Temperature; CV = Cook Value

The proximate composition of fresh red-bellied pacu fish showed moisture, crude protein, total lipid, and ash contents of 76.39%, 18.03%, 3.38%, and 1.77%, respectively. It indicated that the fish used in this study was of good quality. As compared to fresh fish, the moisture content was significantly decreased ($p < 0.05$) in processed product by 7.81% and 9.32% for the F_0 values of 6 and 7 min, respectively. Total lipid, crude protein, and ash contents were found to be increased in processed products compared to fresh fish, but intense heat treatments did not affect these parameters. Water loss from muscle may result in increased levels of lipid, protein, and ash (Juárez et al., 2010). The main factor causing the reduction in moisture content is the thermal denaturation of the muscle protein (Bell, Farkas, Hale, & Lanier, 2002).

The results of changes in biochemical parameters of fresh and thermally processed pacu fish in masala are shown in Table 4. The Total Volatile Base Nitrogen (TVB-N) values showed a significant increase ($p < 0.05$) throughout the storage period in all retorted samples, with F_0 7.0 reaching the highest value of 15.03 mg N/100 g by day 150, followed by F_0 6.0 (13.70 mg N/100 g). This trend is in accordance with the gradual breakdown of proteins and non-protein nitrogenous compounds during storage, which is commonly observed in thermally processed seafood (Nguyen, Hilmarsdóttir, Tómasson, Arason, & Gudjónsdóttir, 2022). Although TVB-N increased, the values remained well below the commonly accepted spoilage threshold of 25–30 mg N/100 g (Connell, 1990; Castro et al., 2012), indicating that the retort processing was effective in maintaining the product quality over time. Similarly, Free Fatty Acid (FFA) content, which serves as a marker of lipid hydrolysis, significantly increased in all treatments ($p < 0.05$), with F_0 7.0 showing the highest level (1.09 mEq/g). The rise in FFA levels may be due to residual lipolytic enzymes or microbial lipases that may have survived or been reactivated during storage (Biji et al., 2015; Rather et al., 2021).

The Peroxide Value (PV), indicative of primary lipid oxidation, also increased over time, with F_0 7.0 samples showing higher values than F_0 6.0. This suggests that more intense thermal processing (higher F_0) may lead to the degradation of natural antioxidants and stimulation of prooxidants, increasing peroxide value (Medina, Sacchi, & Aubourg, 1995; Gao et al., 2022). By day 150, the PV in F_0 7.0 reached 5.59 mEq O_2/kg, which remained within the acceptable limit for processed fish products. The TBARS (thiobarbituric acid reactive substances) values, which indicate the presence of secondary oxidation products, particularly malondialdehyde (MDA), showed an increasing trend. Among the treatments, F_0 7.0 recorded the highest value of 1.62/ mg MDA/kg. Although the values increased over time, they remained below the 2.0/ mg MDA/kg threshold typically associated with the onset of noticeable rancidity (Sangsari, 2013). The pH also showed a gradual decline during the storage period, from an initial value of 5.53–5.54 to 5.33–5.34 by day 150, which could be attributed to the accumulation of acidic compounds resulting from protein and lipid degradation. Applying thermal treatment at 121°C can effectively slow down the rate of pH decline in meatballs during storage (Li, Wang, Zhou, Li, & Ye, 2021). In a study on salted duck stored at 4°C, Song, Liu, Li, Li, and Xu (2019) reported that thermal processing at 121°C for 30 min slowed the

Table 3. Proximate composition of fresh and thermally processed Red Bellied Pacu fish in masala

Parameter	Fresh Pacu Fish	Processed Pacu fish	
		F_0 6.0 min	F_0 7.0 min
Moisture (%)	76.39 ± 0.48^a	70.42 v± 0.63^b	69.27 ± 0.40^c
Crude protein (%)	18.03 ± 0.55^a	19.23 ± 0.54^b	19.40 ± 0.57^b
Total lipid (%)	3.38 ± 0.33^a	6.49 ± 0.57^b	6.34 ± 0.42^b
Ash (%)	1.77 ± 0.12^a	3.08 ± 0.33^b	3.35 ± 0.38^b

decline in pH, which is consistent with the present findings.

Microbial quality analysis of fresh pacu fish showed a total plate count of 3.30×10^4 cfu/g. The potential bacterial pathogens, like *Salmonella*, *Escherichia coli*, *Vibrio* spp., *Listeria* spp., and *Staphylococcus aureus,* were not detected in both fresh and processed products. These findings are consistent with the study by Murthy, Rao, Asha, and Prasad (2015), which reported that fresh pacu had an aerobic plate count (APC) of 1.8×10^5 cfu/g, and that *E. coli*, total coliforms, and faecal coliforms were not detected (<3 MPN/g).

Thermally processed red-bellied pacu fish masala pouches processed at F_0 6.0 and F_0 7.0 min were subjected to a commercial sterility test. The samples were incubated in thioglycolate broth tubes for 48 h at 37°C and for five days at 55°C. The tubes showed no signs of turbidity. The test was carried out throughout the 150-day study period at regular 30-day intervals, and no growth of any thermally resistant bacteria was observed. This indicates that both F_0 values were sufficient to achieve commercial sterility of the product.

The amounts of aspartic acid and glutamic acid, the most abundant non-essential amino acids in pacu fish, showed a slight reduction as the thermal processing level increased from F_0 6.0 to F_0 7.0 min. Aspartic acid decreased from 3.11 g/100 g to 2.94 g/100 g, and glutamic acid decreased from 3.78 g/100 g to 3.50 g/100 g (Table 5). These alterations may be related to the Maillard reaction or the thermal denaturation of amino acids, which is known to happen at higher temperatures (Tamanna & Mahmood, 2015). Glycine and serine levels showed a decrease with increased heat processing, following the typical pattern observed for non-essential amino acids under thermal treatment.

Valine and leucine showed a slight decrease in concentration with increasing heat processing. The increase in tyrosine content from 0.16 g/100 g to 0.24 g/100 g may be attributed to the degradation of phenylalanine, as tyrosine is a metabolic product of phenylalanine. These findings are in agreement with those reported by Murthy et al. (2015).

As the thermal processing intensity increased from F_0 6.0 to F_0 7.0 min, the overall saturated fatty acid (SAFA) content decreased from 18.2% to 16.46% (Table 6). In particular, the observed decrease in C18:0 from 15.36% at F_0 6.0 to 13.65% at F_0 7.0 suggests that elevated heat processing may lead to the degradation of specific long-chain saturated fatty acids. The polyunsaturated fatty acid (PUFA) content decreased from 43.09 % at F_0 6.0 to 40.75 % at F_0 7.0 due to its higher sensitivity to heat treatments. The fatty acid composition of fish can be influenced by various heating techniques, with higher temperatures resulting in a lower PUFA content (Koubaa, Mihoubi, Abdelmouleh, & Bouain, 2012). The concentration of omega-3 fatty acids and MUFAs remained relatively stable under both processing conditions. This suggests that they are more resistant to heat-induced oxidation compared to omega-6 polyunsaturated fatty acids. The present investigation supports the conclusions of Peter, Sukumar, Shakila, and Sundaramoorthy (2015), who reported no decline in MUFA concentrations in rohu fish fillets. These findings are also consistent with the results reported by Murthy et al. (2015).

Fresh pacu fish showed values of hardness, springiness, chewiness and cohesiveness as 2.79 ± 0.15, 1.52 ± 0.05, 1.20 ± 0.06, and 0.86 ± 0.06, respectively. These values indicate the excellent quality of the fresh fish used for product development (Table 7**)**. A highly significant ($p < 0.05$) decrease was observed in the texture parameters of both the products throughout the 150-day storage period. It was revealed that thermal processing has significantly affected the

Table 4. Changes in biochemical quality parameters of fresh and thermally processed red bellied pacu fish in masala

Parameters	DAYS	Fresh Pacu Fish	F_0 6.0	F_0 7.0
TVB-N (mg N/100 g)	0	3.64 ± 0.35	7.51 ± 0.26^{a}	8.39 ± 0.26^{a}
	30		8.12 ± 0.17^{b}	9.55 ± 0.10^{b}
	60		9.15 ± 0.31^{c}	10.83 ± 0.46^{c}
	90		10.88 ± 0.29^{d}	12.12 ± 0.17^{d}
	120		11.86 ± 0.09^{e}	13.92 ± 0.18^{e}
	150		13.70 ± 0.13^{f}	15.03 ± 0.11^{f}
	p value		<0.001	<0.001
FFA (mEq/g)	0	0.29 ± 0.02	0.37 ± 0.09^{a}	0.43 ± 0.12^{a}
	30		0.44 ± 0.04^{ab}	0.56 ± 0.08^{ab}
	60		0.51 ± 0.03^{b}	0.62 ± 0.04^{b}
	90		0.63 ± 0.07^{c}	0.77 ± 0.04^{c}
	120		0.72 ± 0.04^{c}	0.90 ± 0.03^{c}
	150		0.91 ± 0.07^{d}	1.09 ± 0.10^{d}
	p value		<0.001	<0.001
PV (mEq of O_2/kg)	0	0.92 ± 0.05	1.02 ± 0.18^{a}	1.08 ± 0.13a
	30		1.77 ± 0.15^{b}	2.04 ± 0.09^{b}
	60		2.53 ± 0.06^{c}	3.15 ± 0.29^{c}
	90		3.23 ± 0.23^{d}	3.97 ± 0.14^{d}
	120		4.12 ± 0.14^{e}	4.82 ± 0.09^{e}
	150		4.88 ± 0.17^{f}	5.59 ± 0.28^{f}
	p-value		<0.001	<0.001
TBARS				
(mg malonaldehyde/kg)	0	0.31 ± 0.04	0.42 ± 0.04^{a}	0.53 ± 0.04^{a}
	30		0.54 ± 0.04^{b}	0.61 ± 0.06^{a}
	60		0.71 ± 0.04^{c}	0.83 ± 0.04^{b}
	90		0.84 ± 0.06^{d}	1.08 ± 0.12^{c}
	120		1.24 ± 0.03^{e}	1.36 ± 0.06^{d}
	150		1.42 ± 0.04^{f}	1.62 ± 0.04^{e}
	p value		<0.001	<0.001
pH	0	5.68 ± 0.04	5.53 ± 0.03^{a}	5.54 ± 0.04^{a}
	30		5.47 ± 0.02^{ab}	5.49 ± 0.04^{ab}
	60		5.40 ± 0.06^{bc}	5.42 ± 0.05^{bc}
	90		5.38 ± 0.05^{c}	5.40 ± 0.03^{c}
	120		5.35 ± 0.04^{c}	5.38 ± 0.04^{c}
	150		5.33 ± 0.04^{c}	5.34 ± 0.03^{c}
	p value		<0.001	0.002

Superscripts in lowercase letters (a, b, c, d, e, and f) indicate significant differences ($p < 0.05$) between days of study and processed products. Values are expressed as mean ± SD (n = 3).

Table 5. Amino Acid profile (%) of Thermally processed Pacu fish in masala

Amino acid (g. 100 g^{-1})	F_0 6.0	F_0 7.0
Aspartate	3.11	2.94
Glutamate	3.78	3.5
Serine	1.83	1.69
Histidine	23.07	22.44
Glycine	7.86	7.16
Proline	2.15	2.11
Arginine	0.78	0.61
Alanine	2.44	2.51
Tyrosine	0.16	0.24
Cystine	ND	ND
Valine*	2.09	1.92
Methionine*	1.34	1.33
Phenylalanine*	6.88	5.72
Tryptophan*	ND	ND
Isoleucine*	2	1.89
Leucine*	3.78	3.5
Lysine*	ND	ND
Threonine*	2.01	2.12

*Essential Amino Acids; ND- Not Detected.

product's texture. The high heat applied during retort processing can denature proteins in the meat, leading to the breakdown of muscle fibres and resulting in a softer, more tender texture. Similar trends have been observed in other thermally

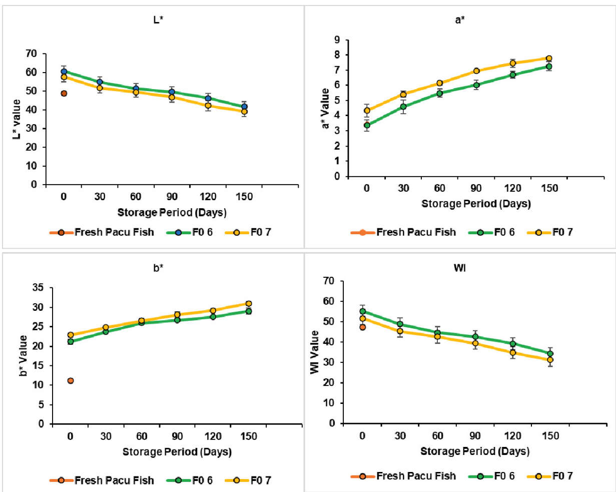

Fig. 3. Changes in colour attributes of fresh and thermally processed Red-bellied Pacu fish in masala

Table 6. Fatty Acid profile (%) of Thermally processed Pacu fish in masala

Fatty Acid	F_0 6.0	F_0 7.0
C16:0	0.11	0.13
C18:0	15.36	13.65
C20:0	1.49	1.29
C22:0	1.24	1.39
ΣSAFA	**18.2**	**16.46**
C16:1	37.86	38.93
C18:1	4.98	4.71
C22:1	ND	ND
ΣMUFA	**40.84**	**41.64**
C18:2 n-6	12.11	11.54
C18:3 n-3	11.70	11.47
C20:2 n-6	0.95	0.81
C20:3 n-6	10.42	9.73
C20:4 n-6	1.02	0.97
C20:5 n- 3	1.47	1.47
C22: 6 n-3	1.45	1.43
C24: 1	3.97	3.33
ΣPUFA	**43.09**	**40.75**

processed seafood products (Ali et al., 2005; Majumdar et al., 2017).

The colour results (Fig. 3**)** revealed that the fresh fish had colour attributes such as L*, a* and b* values of 48.7 ± 0.78, 3.41 ± 0.37 and 11.23 ± 0.42, respectively. The processed products showed higher L values, with F_0 6.0 and F_0 7.0 recording 60.63 ± 0.72 and 57.61 ± 0.43, respectively. The a and b values showed an increasing trend in both the thermally processed products, likely due to the severity of heat treatment. The overall whiteness value of the processed product significantly decreased during storage for both the products. The L* value of both the products decreased significantly ($p < 0.05$) with increasing exposure time to heat processing. The Maillard reaction is responsible for this decrease in L* with increased heating time. Prolonged storage stimulates the activity of endogenous enzymes and microorganisms in fish fillets, leading to lipid oxidation and a decrease in L value and overall whiteness. Exposure to high temperatures causes colour changes in meat, primarily due to the oxidation of myoglobin, which increases redness and can reduce lightness (Boles, 2010). An

Table 7. Texture profile analysis for fresh and thermally processed Red Bellied Pacu fish in masala

Parameters	DAYS	Fresh Pacu Fish	F_0 6.0	F_0 7.0
Hardness (Kg.f.)	0	2.79 ± 0.15	2.09 ± 0.07^{a}	2.04 ± 0.05^{a}
	30		1.78 ± 0.12^{b}	1.62 ± 0.02^{b}
	60		1.57 ± 0.10^{c}	1.40 ± 0.06^{c}
	90		1.42 ± 0.06^{d}	1.24 ± 0.05^{d}
	120		1.29 ± 0.04^{d}	1.05 ± 0.04^{e}
	150		0.99 ± 0.05^{e}	0.84 ± 0.11^{f}
	p value		<0.001	<0.001
Springiness (mm)	0	1.52 ± 0.05	1.29 ± 0.06^{a}	1.11 ± 0.03^{a}
	30		0.87 ± 0.09^{b}	0.86 ± 0.03^{b}
	60		0.74 ± 0.04^{c}	0.68 ± 0.04^{c}
	90		0.64 ± 0.07^{cd}	0.56 ± 0.07^{d}
	120		0.58 ± 0.01^{d}	0.47 ± 0.05^{de}
	150		0.44 ± 0.03^{e}	0.39 ± 0.05^{e}
	p value		<0.001	<0.001
Cohesiveness	0	0.86 ± 0.06	0.79 ± 0.05^{a}	0.76 ± 0.09^{a}
	30		0.68 ± 0.02^{b}	0.64 ± 0.02^{b}
	60		0.55 ± 0.05^{c}	0.52 ± 0.03^{c}
	90		0.52 ± 0.03^{c}	0.48 ± 0.02^{c}
	120		0.37 ± 0.05^{d}	0.35 ± 0.02^{d}
	150		0.32 ± 0.07^{d}	0.30 ± 0.03^{d}
	p value		<0.001	<0.001
Chewiness (Kgf.mm)	0	1.20 ± 0.06	0.79 ± 0.03^{a}	0.74 ± 0.03^{a}
	30		0.65 ± 0.04^{b}	0.61 ± 0.01^{b}
	60		0.59 ± 0.01^{c}	0.56 ± 0.03^{b}
	90		0.46 ± 0.02^{d}	0.41 ± 0.01^{c}
	120		0.39 ± 0.01^{f}	0.36 ± 0.03^{cd}
	150		0.36 ± 0.01^{e}	0.32 ± 0.05^{d}
	p value		<0.001	<0.001

Note: Different lowercase superscripts (a, b, c, d, e, and f) indicate significant differences ($p < 0.05$) between days of study and processed products. Values are expressed as mean ± SD (n = 3).

increasing trend in colour parameters with rising F_0 values was also observed in thermally processed tilapia in masala (Vaghabhai et al., 2023).

Products processed at F 6.0 min received higher sensory scores in all attributes compared to those processed at F_0 7.0 min (Fig. 4). At the same time, significant differences were found during 0 to 150 days of storage in all the parameters of the processed product ($p < 0.05$). The scores for sensory parameters such as appearance, colour, flavour, firmness, succulence, toughness, and overall acceptability decreased with extended storage periods. Extended storage period can stimulate the off-flavours and spoilage indicators in the product due to enzyme activity (Biji et al., 2015). Earlier research on shrimp kuruma in retortable pouches (Mohan et al., 2006), shrimp in masala (Dasan et al., 2021), and seer fish curry (Ravishankar, Gopal, & Vijayan, 2002) revealed that thermally processed products lose their sensory quality over long storage time.

The study successfully optimized the total lethality (F_0) for developing RTE red-bellied pacu fish in masala using flexible retortable pouches. Based on sensory evaluation, F_0 values of 6.0 and 7.0 min were selected for product development. Assessment of the heat penetration characteristics and commercial

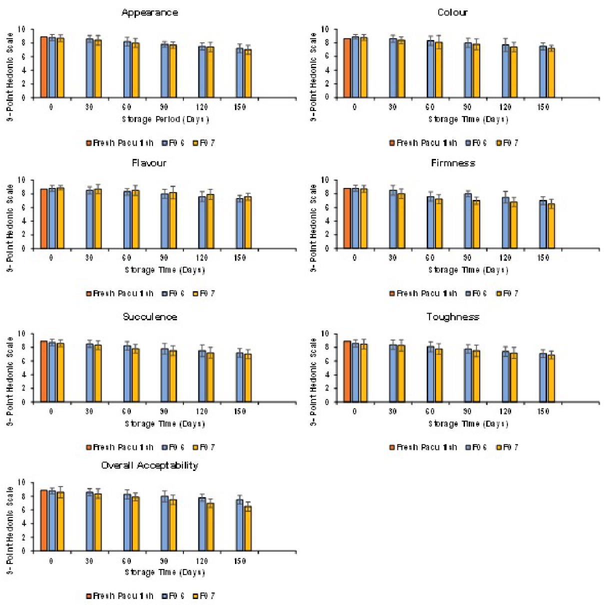

Fig. 4. Sensory evaluation of fresh and thermally processed Red Bellied Pacu fish in masala

sterility test revealed that both F_0 values satisfied the requirements for commercial sterility. Microbiological and biochemical quality assessments confirmed that the processed product remained within acceptable safety and quality limits throughout the 150-day storage period. Thermal processing affected the product's amino acid and fatty acid profile at F_0 7.0 min compared to F_0 6.0 min. Texture profile analysis revealed a declining trend in texture parameters during storage, indicating that heat processing had a significant impact on the product's texture. Colour analysis showed expected changes due to heat processing. The product processed at F_0 6.0 min received higher sensory scores and was rated better for its overall quality. The findings of the study suggest that red bellied pacu fish holds strong potential as a raw material for developing a variety of RTE ethnic dishes, catering to the growing global demand for convenient and value-added fish products.

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