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Evaluation of microbiological and biochemical quality characteristics of croaker (*Johnius dussumieri*) fish balls processed at different sous vide temperature during refrigerated storage

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Abstract

The present study evaluated the microbiological and biochemical quality characteristics of croaker (*Johnius dussumieri*) fish balls processed at different sous vide temperatures during refrigerated storage. Fish balls prepared from croaker were vacuum packaged and subjected to different treatments: T₀ (control: vacuum packaging without heat treatment), T₁ (60 °C for 20 min), T₂ (70 °C for 20 min), and T₃ (80 °C for 20 min). Samples were stored under refrigerated conditions (4±1 °C) for a period of 30 days. Quality changes were assessed at regular intervals using biochemical parameters including pH, trimethylamine nitrogen (TMA-N) and total volatile basic nitrogen (TVB-N), along with microbiological parameters such as total plate count and psychotrophs count. Significant changes were observed in all quality parameters throughout storage, with control samples showing faster deterioration compared to sous vide-treated samples. Sous vide processing effectively reduced microbial growth and delayed biochemical spoilage during refrigerated storage. Among all treatments, T₃ showed better preservation efficiency by maintaining lower microbial counts and reduced biochemical changes throughout storage. The findings suggest that sous vide processing combined with vacuum packaging can be an effective preservation technique for improving quality and extending the shelf life of croaker fish balls during refrigerated storage.

Keywords: Croaker (*Johnius dussumieri*), fish balls, sous vide processing, refrigerated storage, microbiological quality, biochemical quality, shelf life, vacuum packaging

Introduction

Fish and fishery products (FFP) are very nutritious and play an important role in human diet. They contain rich sources of essential amino acids, contributing around 20% to the per capita intake of animal protein. They are also rich in omega-3 polyunsaturated fatty acids, which help to lower the risk of heart disease, along with other vital macronutrients that support overall health (Bjorndal *et al.*, 2024) ^[9].

In developed countries, fish processing has expanded, particularly focusing on high value-added products such as ready-to-eat seafood meals. Fish consumption in India has grown significantly, contributing to the nation's food and nutrition security. India registered a per capita yearly consumption of over 13 kg in 2022-23 from 7kg in 2011-12 (NCAER, 2023) ^[32]. During the financial year 2024-25, India achieved a record seafood export volume of 16,98,170 metric tonnes, generating revenue of ₹ 62,408.45crore (approximately US\$ 7.45 billion) (MPEDA, 2025) ^[30].

Fish is highly perishable and can spoil quickly if not handled properly. It is prone to quality deterioration and microbial contamination due to its near-neutral pH, high moisture content, rich nutrient composition and the presence of enzymatic and oxidative reactions in fish tissues (Cortes-Sanchez *et al.*, 2021) ^[14]. Therefore, if fish is not consumed immediately after harvest, proper preservation and processing methods are necessary to maintain its safety and quality. Fish processing mainly aims to extend freshness and reduce spoilage while also creating value-added products (Datta, 2015) ^[17]. Mince-based fish products are especially useful because they can improve consumer acceptance by masking the original appearance of fish, making them more appealing to people who do not prefer whole fish (Siddiqui *et al.*, 2024) ^[39].

Over the last decade, people's eating habits have changed, leading to the growth of the ready-made food industry (Monfort, 2010) [29]. Nowadays, busy lifestyles, population growth and changing work patterns have increased the demand for foods that are nutritious, tasty and quick to prepare. As a result, ready-made foods have become more common in daily life (Oguzhan & Yangilar, 2014) [35]. Especially for young people and school-age children, products prepared with fish (meatball, croquette, nugget, finger and sausage) are healthy and convenient choices. At the same time, there is growing interest in modern food processing methods that help improve food safety, extend shelf life and maintain nutritional quality. In this context, sous vide cooking has been getting growing interest from consumers as well as the catering and food service industries worldwide. Sous vide processing is now seen as a reliable and efficient alternative to traditional cooking for meat products. Thanks to better technology and packaging materials, methods like this have become more common (Bongiorno *et al.*, 2018) [10].

Sous vide French for "under vacuum" is a professional cooking technique has commonly applied in homes, restaurants, catering and the food industry. The sous vide technique is a three-step process and is a good example of the hurdle principle. It includes (i) vacuum packaging that removes oxygen and creates anaerobic conditions (ii) mild heat treatment (55-90 °C) with carefully temperature control and (iii) rapid cooling followed by refrigerated storage after heating (Kenesei *et al.*, 2024) [26]. These foods can be cold stored for 3-5 weeks before reheating and consumption (Creed & Reeve, 1998; Nyati, 2000) [15, 34].

Sous vide processing helps maintain the natural appearance and quality of fish while extending shelf life. However, high heat treatments can reduce sensory quality; therefore, moderate temperatures of 60-80 °C for 20-40 minutes are preferred. As sous vide uses lower cooking temperatures, controlling surviving microorganisms remains an important safety concern (NACMCF, 1990) [31].

Sous vide technology offers several major advantages like reducing the need for additives and preservatives, helping control microbial contamination and extending the shelf life of food (Biyikli *et al.*, 2020; Thathsarani *et al.*, 2022) [8, 45]. Besides this, it enhances sensory characteristics such as taste and flavour while helping to reduce moisture and nutrient loss during cooking and preserve the nutritional quality of the meat (Biyikli *et al.*, 2020; Silva *et al.*, 2016) [8, 41]. Furthermore, sous vide cooking can reduce lipid oxidation, helping to maintain the tenderness and texture of fish. By allowing steady change protein denaturation, it ensures controlled texture development, improving overall meat quality (Choi *et al.*, 2024) [12], while also, reduced flavour deterioration and the risk of cross-contamination during storage (Douglas *et al.*, 2023) [18].

In 2025, India's marine fish landings along the mainland were estimated at 3.57 million tonnes, reflecting a 3% increase from the previous year. Gujarat recorded the 2nd in fish landings among all states, with a total of 6.43 lakh tonnes, accounting for 18% of the country's overall marine fish production (CMFRI, 2025) [13]. In the Indian Ocean, about 48 species from 27 genera are found. Among them, the Sin croaker (*Johnius dussumieri*), described by Cuvier in 1830, holds significant commercial value in India (Abinaya & Sajeevan, 2022) [1]. It is often caught using bottom trawl nets along the Gujarat coast, where it is locally referred to as

dhoma. Even though *Johnius dussumieri* is one of the most abundant croaker species in Indian waters, its small size limits its utilization. It is mainly eaten in dried and salted form within local markets. A small fraction will also go towards fish powder and fish mince production by fish meal and surimi-based plants, respectively (Rahangdale *et al.*, 2022) [38]. It is also used to make mince-based value-added products. Because it is low in cost, most of the catch is not properly preserved on board. (Annamalai *et al* 2018) [2].

Materials and Methods

Raw Materials

Croaker (*Johnius dussumieri*) was obtained from the Veraval fish landing center for experimental purposes. The fish measured an average total length of 21.83±1.26 cm and an average weight of 122.33±5.72 g. A total of 20 kg of fish was used for the experiment. The fish were transported to the Fish Processing Laboratory, College of Fisheries Science, K.U., Veraval under iced conditions (0-2 °C) for further processing and analysis. After arrival, they were manually dressed and converted into skinless and boneless fillets. The fillets were washed with chilled potable water to remove blood and impurities after that drained well and then minced using a hand-operated mincer. The fish mince obtained was used to prepare fish balls.

Methods

The fish balls were prepared by thoroughly mixing minced fish (83.75%) with breadcrumbs (10%), salt (2%), sweet red pepper (0.5%), ground black pepper (0.5%), cumin (0.5%), allspice (0.5%), sunflower oil (1%) and garlic powder (1.25%) to obtain a uniform dough. The mixture was kneaded thoroughly to ensure even distribution of ingredients and to achieve a consistent texture. Once the desired consistency was attained, the dough was shaped into 20 g fish balls. The prepared fish balls were divided into four lots, namely T₀, T₁, T₂ and T₃ then vacuum-packaged for further processing.

Procedure for Experiment

In the experiment, for treatment T₀ (control), the fish balls were vacuum-packaged without applying any heat treatment. In treatment T₁, the vacuum-packaged fish balls were subjected to sous-vide cooking at 60 °C for 20 min. In treatment T₂, the vacuum-packaged fish balls were subjected to sous-vide cooking at 70 °C for 20 min. In treatment T₃, the vacuum-packaged fish balls were subjected to sous-vide cooking at 80 °C for 20 min. After heat treatment, the cooked fish balls were rapidly cooled in ice water for 30 minutes and subsequently stored under refrigerated conditions. The effect of different sous-vide temperatures on the biochemical, microbiological and sensory quality of fish balls was evaluated during refrigerated storage.

Biochemical analysis

Determination of pH (pH meter method)

The pH of the sample was measured following the method described by AOAC (2006) [3]. For this purpose, 10 g of the fish ball sample was homogenised with 20 mL of distilled water and the mixture was filtered. The pH of the filtrate was recorded at room temperature using a digital pH meter (U TECH INSTRUMENTS-733060).

Determination of Trimethylamine Nitrogen (TMA-N)

Preparation of the Trichloroacetic acid (TCA) extract: A trichloroacetic acid (TCA) extract was prepared by homogenising 10 g of the fish ball sample with 30 mL of 20% TCA using a mortar and pestle. The homogenate was filtered through Whatman No. 1 filter paper and the filtrate volume was adjusted to 50 mL with distilled water. The extract was stored under refrigeration and used for the estimation of trimethylamine nitrogen (TMA-N) and total volatile base nitrogen.

TMA-N content was determined by the micro-diffusion method described by Beatty and Gibbons (1937) [6]. For analysis, 1 mL of N/100 sulphuric acid was placed in the inner chamber of the micro-diffusion apparatus. The outer chamber received 1 mL of the TCA extract, followed by the addition of 0.5 mL of neutralised formaldehyde and 1 mL of saturated potassium carbonate. The unit was sealed with a glass cover and allowed to stand undisturbed overnight. The acid in the inner chamber was then titrated with N/100 sodium hydroxide using Tashiro's indicator. A reagent blank was run simultaneously, and TMA-N values were calculated and expressed as mg per 100 g of sample using the standard formula.

$$\text{TMA-N (mg/100 g)} = \frac{(V_s - V_b) \times 0.14 \times \text{Volume of extract}}{\text{Vol. of sample taken} \times \text{Weight of sample}} \times 100$$

Where,

V_s = Titre value of 0.01 N NaOH for the sample (ml)

V_b = Titre value of 0.01 N NaOH for the blank (ml)

Determination of Total Volatile Base Nitrogen (TVB-N)

The TCA extract used for the analysis of total volatile base nitrogen (TVB-N) was prepared following the same procedure described for TMA-N analysis. TVB-N content was determined using the micro-diffusion method of Beatty and Gibbons (1937) [6]. In this procedure, 1 mL of N/100 sulphuric acid was placed in the inner chamber of the diffusion apparatus, while 1 mL of the TCA extract was added to the outer chamber, followed by 1 mL of saturated potassium carbonate solution. The unit was sealed with a glass lid and kept undisturbed overnight to allow diffusion. The residual acid in the inner chamber was then titrated against N/100 sodium hydroxide using Tashiro's indicator. A blank determination was carried out using the same method without the sample, and the TVB-N content was calculated and expressed as mg per 100 g of the sample.

$$\text{TVB-N (mg/100 g)} = \frac{(V_s - V_b) \times 0.14 \times \text{Volume of extract}}{\text{Vol. of sample taken} \times \text{Weight of sample}} \times 100$$

Where,

V_s = Titre value of 0.01 N NaOH for the sample (ml)

V_b = Titre value of 0.01 N NaOH for the blank (ml)

Microbiological analysis

Preparation of Sample

Ten grams of the fish ball sample were aseptically weighed, placed on a sterile plate and transferred into a sterile polyethylene bag. The sample was homogenised with 90 mL of sterile phosphate buffer or normal saline solution. The homogenate was processed using a stomacher blender to obtain the initial tenfold dilution (10^{-1}). Subsequent serial dilutions were prepared by transferring 1 mL of the previous dilution into 9 mL of sterile diluent, producing dilutions of 10^{-2} , 10^{-3} and 10^{-4} for microbiological analysis.

Media used and methodology adopted for microbiological evolution

SR NO	Target bacteria	Media	Method	Incubation	Reference
I	Total Viable Count (TVC)	Plate count agar	Spread plate	37 °C for 48 h	Mailoa <i>et al.</i> , (2017) [27]
II	Total Psychotropic Count	Plate Count Agar	Spread plate	7±1 °C for five days	Hassan <i>et al.</i> , (2014) [22]

Statistical analysis

Statistical analysis was performed in triplicate for each sample at every storage interval. Significant differences among treatments were determined using Analysis of Variance (ANOVA), following the standard procedures outlined by Snedecor and Cochran (2014) [42]. The ANOVA was conducted with IBM BASIC Windows Release 1.13 to compare the variances among different treatment combinations and identify the most effective treatment.

Results and Discussion

Biochemical analysis

pH

The changes in pH values during storage are presented in Table 1. The initial pH values were recorded as 6.46 ± 0.05 for T_0 (control) and 6.62 ± 0.15 , 6.65 ± 0.06 and 6.67 ± 0.08 for T_1 , T_2 and T_3 , respectively. A significant interaction effect was observed between treatments and storage periods ($P < 0.05$). The pH of fish meat generally increases after heating, particularly at higher temperatures. In the present study, pH increased significantly after heat treatment, which may be attributed to protein denaturation. Similar findings were reported by Ayub and Ahmad (2019) [4], who observed increased pH in sous vide-cooked trout, while Becker *et al.* (2016) [7] suggested that increasing temperature causes pH elevation due to protein denaturation. Maskan *et al.* (2024) [28] also reported increased pH in sous vide-cooked rainbow trout fish balls.

During storage, a significant increase ($P < 0.05$) in pH was observed in all treatments. The control (T_0) recorded the highest pH value of 7.93 ± 0.08 , followed by T_1 , T_2 and T_3 with values of 7.47 ± 0.10 , 7.27 ± 0.15 and 7.12 ± 0.06 , respectively. Fish and fishery products are generally considered acceptable up to a pH of 6.8, while values above 7.0 indicate spoilage (Azaman *et al.*, 2015) [5]. Control samples exceeded this limit earlier than sous vide-treated samples, whereas T_3 maintained greater stability throughout storage. The increase in pH may be associated with the accumulation of volatile basic compounds formed through microbial and enzymatic degradation. In contrast, lower pH values in sous vide-treated samples suggest that vacuum packaging combined with heat treatment effectively slowed spoilage. Similar findings were reported by Cropotova *et al.* (2019) [16], who observed increasing pH trends in sous vide-treated tilapia fillets during chilled storage.

TMA-N

The Trimethylamine Nitrogen (TMA-N) content of fish balls increased progressively during refrigerated storage (Table 2), with a significant interaction ($P < 0.05$) between treatment and storage time. Trimethylamine (TMA), formed through microbial and enzymatic breakdown of trimethylamine oxide (TMAO), is an important spoilage

indicator responsible for the characteristic fishy odour in seafood products (Howgate, 2010). Initially, TMA-N values were 3.25 ± 0.21 , 4.15 ± 0.12 , 4.35 ± 0.28 and 4.70 ± 0.37 mg/100 g for T₀, T₁, T₂ and T₃, respectively. Similar increases in TMA-N after sous vide processing were reported by Nieva-Echevarria *et al.* (2017) [33].

A significant increase ($P < 0.05$) in TMA-N values was observed in all treatments during storage. The control recorded the highest value (20.78 ± 0.34 mg N/100 g), followed by T₁, T₂, and T₃ with 18.62 ± 0.33 , 17.70 ± 0.23 , and 16.32 ± 0.28 mg N/100 g, respectively. The acceptable limit for TMA-N in fish products is 10-15 mg N/100 g (Tavares *et al.*, 2021) [44]. The increase in TMA-N may be associated with the formation of volatile amines during protein degradation. Among all treatments, T₃ showed the lowest TMA-N values and a slower increase throughout storage, indicating its greater effectiveness in delaying spoilage and maintaining product quality. Similar findings were reported by Gokoglu *et al.* (2024) [21], who observed lower TMA-N values in sous vide-treated meagre fillets compared to control samples during chilled storage.

TVB-N

TVB-N refers to the total amount of volatile basic nitrogenous compounds, including trimethylamine, dimethylamine, and ammonia, formed due to microbial and enzymatic activity during storage and is considered an important freshness indicator for seafood products (Ozogul & Ozogul, 2000) [36]. The changes in TVB-N values of fish balls are presented in Table 3. A significant interaction ($P < 0.05$) between treatment and storage duration was observed. Initially, TVB-N values were 13.25 ± 0.17 , 14.36 ± 0.26 , 15.03 ± 0.09 and 15.50 ± 0.22 mg/100 g for T₀, T₁, T₂ and T₃, respectively. These values increased during storage and reached 47.48 ± 0.55 , 37.65 ± 0.24 , 35.30 ± 0.23 and 33.37 ± 0.45 mg/100 g, respectively, on day 30. A similar increasing trend was reported by Pongsetkul *et al.* (2022) [37] in sous vide-treated tilapia during chilled storage.

The acceptable limit for TVB-N in fish products is 30 mg/100 g, beyond which spoilage is indicated (Sikorski *et*

al., 1990) [40]. In the present study, control samples exceeded this limit earlier than sous vide-treated samples, while T₃ maintained lower TVB-N values throughout storage. The increase in TVB-N may be attributed to microbial activity and protein degradation during storage. However, sous vide processing effectively delayed the increase by reducing microbial growth and slowing enzymatic activity. Among all treatments, T₃ showed the greatest effectiveness in controlling TVB-N levels, indicating better preservation efficiency at higher processing temperatures. Similar findings were reported by Gokoglu *et al.* (2024) [21], who observed lower TVB-N values in sous vide-treated fish than in control samples. Likewise, Caglak *et al.* (2017) [11] reported an increasing trend in TVB-N during chilled storage of sous vide-processed fish and noted that higher processing temperatures were more effective in controlling TVB-N levels.

Table 1: Changes in pH of Croaker (*Johnius dussumieri*) fish balls during refrigerated storage

Storage Day	T ₀	T ₁	T ₂	T ₃
0	6.46 ± 0.05	6.52 ± 0.15	6.55 ± 0.06	6.57 ± 0.08
5	6.70 ± 0.05	6.61 ± 0.07	6.59 ± 0.08	6.58 ± 0.02
10	6.90 ± 0.03	6.70 ± 0.06	6.65 ± 0.08	6.63 ± 0.20
15	7.25 ± 0.08	6.87 ± 0.08	6.74 ± 0.03	6.68 ± 0.04
20	7.35 ± 0.03	6.95 ± 0.06	6.88 ± 0.07	6.71 ± 0.11
25	7.69 ± 0.05	7.21 ± 0.16	7.10 ± 0.11	6.75 ± 0.05
30	7.93 ± 0.08	7.47 ± 0.10	7.27 ± 0.15	7.12 ± 0.06

Table 2: Changes in TMA-N of Croaker (*Johnius dussumieri*) fish balls during refrigerated storage

Storage Day	T ₀	T ₁	T ₂	T ₃
0	3.25 ± 0.21	4.15 ± 0.12	4.35 ± 0.28	4.7 ± 0.37
5	5.79 ± 0.29	5.65 ± 0.11	5.38 ± 0.44	5.08 ± 0.09
10	10.06 ± 0.33	6.82 ± 0.39	6.29 ± 0.29	5.90 ± 0.28
15	15.44 ± 0.12	9.71 ± 0.32	8.93 ± 0.17	8.09 ± 0.23
20	17.87 ± 0.29	13.24 ± 0.32	12.58 ± 0.35	11.91 ± 0.23
25	19.61 ± 0.24	16.85 ± 0.29	15.8 ± 0.33	13.71 ± 0.23
30	20.78 ± 0.34	18.62 ± 0.33	17.7 ± 0.23	16.32 ± 0.28

Table 3: Changes in TVB-N of Croaker (*Johnius dussumieri*) fish balls during refrigerated storage

Storage Day	T ₀	T ₁	T ₂	T ₃
0	13.25 ± 0.17	14.36 ± 0.26	15.03 ± 0.09	15.50 ± 0.22
5	20.48 ± 0.21	18.55 ± 0.51	18.16 ± 0.24	17.47 ± 0.46
10	26.86 ± 0.16	23.42 ± 0.37	22.20 ± 0.42	21.51 ± 0.41
15	33.92 ± 0.16	26.46 ± 0.37	25.12 ± 0.49	23.90 ± 0.24
20	40.70 ± 0.34	29.27 ± 0.54	27.44 ± 0.41	26.46 ± 0.31
25	45.34 ± 0.61	34.34 ± 0.62	31.26 ± 0.61	28.45 ± 0.43
30	47.48 ± 0.55	37.65 ± 0.24	35.30 ± 0.23	33.37 ± 0.45

Microbiological analysis

Total Plate Counts (TPC)

Total plate count (TPC) is widely used as an indicator of spoilage in fish and seafood products, reflecting the microbial load in a sample. The changes in TPC of fish balls are presented in Table 4. Initial TPC values for T₀, T₁, T₂ and T₃ were 3.61 ± 0.23 , 2.75 ± 0.15 , 2.21 ± 0.13 and 1.45 ± 0.11 log CFU/g, respectively. A significant interaction effect between treatment and storage period was observed. Fish balls processed at higher sous vide temperatures showed a greater ($P < 0.05$) reduction in microbial load, with T₃ exhibiting the lowest bacterial count, followed by T₂ and T₁. Similar findings were reported by Tang *et al.* (2017) [43],

who observed increased microbial inactivation at higher temperatures. The reduction in bacterial count after sous vide processing may be attributed to protein denaturation, membrane damage and altered cell wall permeability of microorganisms, along with restricted growth of aerobic bacteria under vacuum conditions, as reported by Erdem *et al.* (2022) [19].

A significant increase ($P < 0.05$) in TPC was observed in all samples during refrigerated storage. Similar results were reported by Shakila *et al.*, who observed lower microbial growth in sous vide-treated fish cakes than in conventionally vacuum-packed samples. According to FSSAI (2017) [20], the maximum permissible microbial limit for fish products

is 6 log CFU/g. In the present study, control samples exceeded this limit after 15 days, reaching 8.15 ± 0.07 log CFU/g. In contrast, T₁, T₂ and T₃ recorded TPC values of 7.24 ± 0.11 , 6.88 ± 0.10 and 6.45 ± 0.11 log CFU/g, respectively, on day 30. T₁ and T₂ exceeded the acceptable limit on the 25th day, whereas T₃ exceeded to the limit at the end of storage, indicating the effectiveness of higher sous vide temperatures in controlling microbial growth. Similar observations were reported by Hosseini *et al.* (2023) [23] in sous vide silver carp fillets.

Total Psychotropic Counts

The changes in total psychotropic count of fish balls during refrigerated storage are presented in Table 5. A significant interaction effect between treatments and storage duration was observed. Initially, the control (T₀) showed a psychotropic count of 3.15 ± 0.11 log CFU/g, whereas sous vide-treated samples T₁, T₂ and T₃ recorded lower values of 2.51 ± 0.09 , 2.03 ± 0.10 and 1.45 ± 0.19 log CFU/g, respectively. Psychotropic counts increased significantly in all treatments during storage. On day 30, the control reached 9.97 ± 0.12 log CFU/g, exceeding the acceptable limit of 7 log CFU/g (ICMSF, 1986; FSSAI, 2017) [25, 20], while T₁, T₂ and T₃ recorded comparatively lower values of 8.33 ± 0.12 , 7.89 ± 0.06 and 7.08 ± 0.10 log CFU/g, respectively. The control remained acceptable up to 15 days, T₁ and T₂ up to 25 days, while T₃ remained within acceptable limits throughout the 30-day storage period. These findings indicate that sous vide processing reduced psychotropic bacterial growth and delayed spoilage during refrigerated storage. Similar findings were reported by Hosseini *et al.* (2023) [23] and Maskan *et al.* (2024) [28], who observed slower psychotropic bacterial growth in sous vide-treated samples compared to controls.

Table 4: Changes in TPC (log CFU/g) of Croaker (*Johnius dussumieri*) fish balls during refrigerated storage

Storage Day	T ₀	T ₁	T ₂	T ₃
0	3.61 ± 0.23	2.75 ± 0.15	2.21 ± 0.13	1.72 ± 0.21
5	4.51 ± 0.16	3.02 ± 0.15	2.73 ± 0.13	2.16 ± 0.18
10	5.33 ± 0.10	3.80 ± 0.10	3.45 ± 0.12	2.72 ± 0.17
15	6.25 ± 0.10	4.23 ± 0.09	3.96 ± 0.09	3.16 ± 0.15
20	7.13 ± 0.10	5.57 ± 0.10	5.01 ± 0.17	4.43 ± 0.11
25	7.74 ± 0.13	6.58 ± 0.16	6.14 ± 0.12	5.37 ± 0.07
30	8.15 ± 0.07	7.24 ± 0.11	6.88 ± 0.10	6.45 ± 0.11

Table 5: Changes in Total Psychotropic Count (log CFU/g) of Croaker (*Johnius dussumieri*) fish balls during refrigerated storage

s	T ₀	T ₁	T ₂	T ₃
0	3.15 ± 0.11	2.51 ± 0.09	2.03 ± 0.10	1.45 ± 0.19
5	4.97 ± 0.10	3.25 ± 0.14	2.81 ± 0.10	1.93 ± 0.11
10	6.07 ± 0.20	4.53 ± 0.12	3.87 ± 0.10	2.92 ± 0.16
15	7.23 ± 0.13	5.77 ± 0.14	4.75 ± 0.12	3.72 ± 0.17
20	8.56 ± 0.21	6.49 ± 0.14	5.93 ± 0.09	4.93 ± 0.13
25	9.15 ± 0.29	7.40 ± 0.06	7.12 ± 0.11	6.03 ± 0.08
30	9.97 ± 0.12	8.33 ± 0.12	7.89 ± 0.06	7.08 ± 0.10

Conclusion

This study demonstrated the effectiveness of sous-vide processing in preserving the biochemical and microbiological quality of croaker (*Johnius dussumieri*) fish balls during refrigerated storage. Sous-vide treated samples exhibited slower increases in pH, total volatile base nitrogen (TVB-N), and trimethylamine nitrogen (TMA-N), along with lower total plate count (TPC) and total psychotropic

count (TPCy) values compared to the vacuum-packaged control, indicating improved product stability and delayed spoilage. Among the treatments evaluated, T₃ (80 °C for 20 min) showed the most favourable biochemical and microbiological characteristics throughout storage and maintained acceptable quality for up to 25 days under refrigerated conditions. The results further indicated that higher sous-vide processing temperatures were more effective in preserving product quality and suppressing microbial growth. Therefore, sous-vide processing can be considered an effective preservation technique for croaker fish balls, extending shelf life while maintaining product quality. In addition, this technology offers potential for the development of value-added, ready-to-eat fish products, thereby enhancing consumer convenience and promoting the efficient utilization of fish resources.

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