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THE DEVELOPMENT, OPTIMIZATION, AND SHELF-LIFE STUDY OF 'FISH JELLY' FROM HILSA (*Tenualosa ilisha*): NUTRITIONAL, SENSORY, AND MICROBIOLOGICAL EVALUATION

Md. Masud Rana[1*], Md. Abu Talha[2], Mst. Tasmim Sultana[3], Md. Kamal Hossain[4] and Md. Alamgir Kabir[5]

[1]Department of Fishing and Post-Harvest Technology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh
[2]Department of Aquaculture, Habiganj Agricultural University, Habiganj-3300, Bangladesh
[3]Department of Dairy Science, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh
[4]Laboratory of Soil, Water and Environment, Bangladesh Council of Scientific & Industrial Research, Dhaka, Bangladesh
[5]Institute of Food Science and Technology, Bangladesh Council of Scientific & Industrial Research, Dhaka, Bangladesh

[*]rana.fpht@sau.edu.bd.

ABSTRACT

Hilsa (*Tenualosa ilisha*) is nutritionally rich and highly demanded fish species in South Asians regions due to its unique taste and flavor. Value added fish products becoming the popular items in human diets, value added products can be stored for longer time. "Fish Jelly" is a novel product which was developed from Hilsa fish that caters to the growing demand for convenient and nutritious seafood items. Hilsa meat is carefully processed to extract the purest form of fish protein to develop fish juice, subsequently some innovative techniques were employed to prepare jelly structure from the Hilsa fish juice by keeping the nutrients and flavor intact. Three treatments were examined viz. Fish Jelly-1 (FJ1) which contain 60% fish juice, Fish Jelly-2 (FJ2) which contain 55% fish juice, Fish Jelly-3 (FJ3) which contain 50% fish juice. The prepared fish jelly stored at different storage conditions to assess the shelf life of the developed products. In case of sensory attributes, all three jellies showed higher scores when stored under refrigerated conditions compared to those kept at room temperature. FJ3 showed significantly higher protein content at 4.0%, FJ1 had the highest lipid content at 26.9%, and FJ3 also recorded the highest ash content at 2.79%. All samples to have diversified fatty acid profile and palmitoleic acid was the predominant fatty acid in all jelly samples. Since the acceptable limit for total bacterial count is ≤105 CFU/gm, all three jellies remained safe for consumption for up to 45 days when stored at ambient temperature and when stored in refrigerated temperature, FJ2 jellies were found satisfactory for up to 90 days, while FJ1 and FJ3 jellies remained satisfactory for up to 135 days. The findings of this study have the potential to introduce a unique and value-added seafood product to the market, promoting the sustainable utilization of Hilsa fish resources and diversifying the product range for the seafood industry

Keywords: *Fish Jelly, Quality, Shelf-life, Seafood, Health Benefits*

1. Introduction

Food has become increasingly diverse as the world has modernized. Jelly is a popular leisure food owing to its good taste and swallowability (Wu et al., 2023) and is also an exceptionally diversified, adaptable, and nutritious food item. Jelly is a fruit-flavored condiment that is clear, translucent, sparkling, quivers, tasty, shelf-stable, and prepared from fruit or fruit juice, sugar, and pectin (Eshita, 2022). The history of fruit jelly dates back centuries and has evolved. The practice of preserving fruits in sugar or honey in the form of jelly was known to ancient civilizations. Fruits are processed into shelf-stable goods like jams and jellies to make them available for consumption throughout the off-season (Shinwari & Rao, 2018). Fruit jelly is a flavorful and versatile product that serves as a source of natural sugars and is rich in bioactive compounds—such as carotenoids, flavonoids, phenolics, and vitamins—which possess antioxidant properties (De et al., 2019). The use of sugar and pectin in fruit jelly may have certain adverse effects on human health. Blood sugar control and low-glycemic foods will be prominent developments in the health food market, considering sugar consumption is directly associated with diabetes and other diseases like obesity (Acosta et al., 2008). Compared to fruit jelly, fish jelly is a more obscure culinary invention with roots in several different cultures. Fish is a highly nutritious food that is high in protein and comprises a variety of vitamins and minerals, including selenium, phosphorus, magnesium, iodine, and vitamins D and A.

Nevertheless, hilsa fish jelly can be an excellent source of nutrition. Hilsa (*Tenualosa ilisha*) is renowned for being especially rich in omega-3 fatty acids, such as docosahexaenoic acid (DHA) and eicosatetraenoic acid (EPA), and it is an excellent source of high-quality protein. The hilsa fish is rich in omega-3 fatty acids, which are beneficial for heart health, reducing inflammation, and supporting brain function. DHA (docosahexaenoic acid) is essential for development of children's brain and nervous system. According to particular research, having enough omega-3 fatty acids during pregnancy may lower the chance of premature delivery. Hilsa contains zinc, which is essential for immunological function, wound healing, and DNA synthesis. The moderate cholesterol content in hilsa contributes to the body's structural and functional needs, particularly in cell membranes and hormone synthesis. The selenium content in hilsa enhances its nutritional value by providing antioxidant support, promoting immune function, and contributing to thyroid health. In Bangladesh, hilsa shad (*Tenualosa ilisha*) is a fish of biological, nutritional, economic, and socioeconomic significance (Bandara & Wijewardene, 2023). The iconic flagship species, also known as the national fish of Bangladesh, is a geographical indication (GI) product that accounts for 12% of the country's annual fish production, or 517,198 MT, with a value of approximately US$ 4 billion. Of this, 232,698 MT (or 6% of the inland catch) are harvested from inland waters, and the remaining 284,500 MT (or 44% of the marine catch) are harvested from marine waters. A total of 4,450 MT of hilsa were sold at an average price of Tk 800 per kg, with 2,200 MT of other fish kinds sold at an average price of Tk 400 (The Daily Star). Since hilsa fishing is prohibited at certain periods of the year, fish jelly can be precious in providing nutritional demands all year round, even though its price is slightly higher during peak season.

2. Materials and methods

2.1 Experimental site

The study was conducted in the Fishing and Post Harvest Technology laboratory of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

2.2 Collection and storage of samples

Tenualosa ilisha samples were collected from Chandpur the district of Bangladesh, weighing between 600 and 800g. This was done to obtain individuals with better proximate composition and to reduce production costs. The collected samples were then carried to the laboratory for product development and quality analysis and kept at refrigeration temperature until the end of the study.

2.3 Treatments of the experiment

The experiment consisted of three Treatments. They are product-01 (FJ1), product-02 (FJ2) and product-03 (FJ3). Their proximate composition is given in Table 1.

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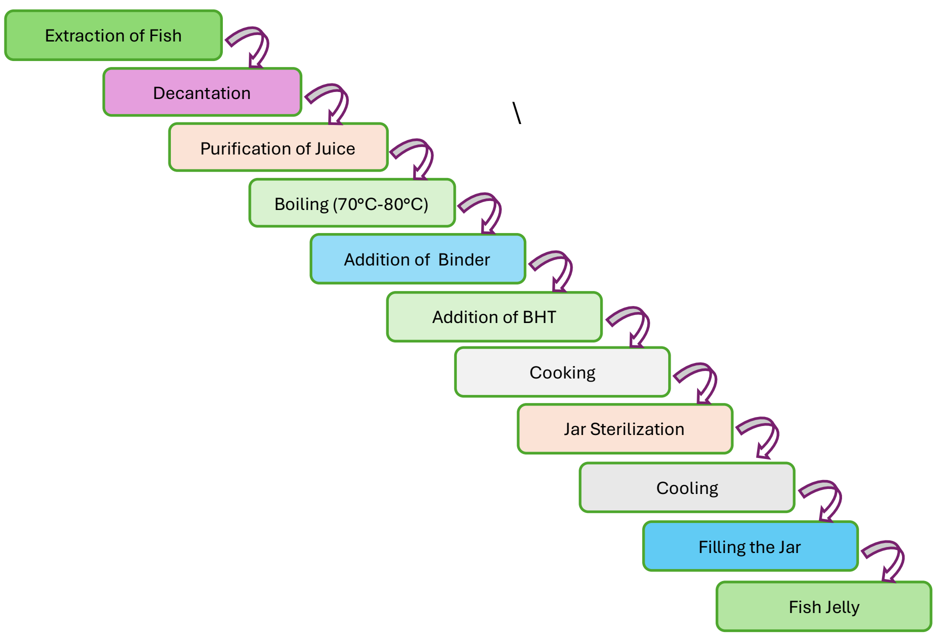
Table 1. Ingredient's ratio used to prepare product-01 (FJ_1), product-02 (FJ_2) and product-03 (FJ_3)

Ingredients	Product-01 (FJ_1)	Product-02 (FJ_2)	Product-03 (FJ_3)
Clear Fish Juice	60%	55%	50%
Sugar	30%	33%	37%
Citric Acid	0.7%	0.7%	0.7%
Pectin	1%	1%	1%
Agar Powder	0.5%	1.5%	2.5%
Chili Powder	1%	1.5 %	2%
Cumin Powder	0.3%	0.3%	0.3%
Fruit Pulp	6.0%	6.5%	8%
BHT	0.5%	0.5%	0.5%

2.4 Procedure of premium fish jelly preparation

The experiment was conducted in a completely hygienic condition in the Fishing and Post Harvest Technology laboratory. The following procedure (Fig 1) was followed for the production of premium quality fish jelly from hilsa fish.

Fig 1. Procedure of premium Fish Jelly preparation.



2.5 Product development

All ingredients were measured carefully. The juice was extracted from fish flesh, and other edible portions were filtered correctly. Extracted fish flesh juice, food grade color, spices, and sugar were mixed, taken in a kettle, and started boiling with continuous stirring. The solution was boiled until total soluble solids reached 55°C. Then, separate pectin and sugar were added to it and stirred well. Citric acid was added when the Brix reached 58°C. The suspension was cooked 70-80°C until Brix reached 65°C. After that, KMS (mixed with a small amount of water) was added and then filled into a clean, sterilized jar. Then, the jar is properly cooled, labeled, and stored in a cool, dark place.

2.6 Sensory assessment

A sensory test requires a person, an assessor, to examine a sample using one or more of the human senses of vision, smell, taste, and kinesthesis corresponding to the properties in the product of appearance, odor, taste, and texture, A five-person team was involved during the assessment. This method is based on the response or tendency of sense organs to accept food products. Sensory evaluation was carried out at a 10-day interval for the fish jelly stored at ambient (26-28℃) and refrigeration (4℃) temperatures. During sensory evaluation, the changes in color, flavor/odor, texture, taste, and general appearance were evaluated based on a 9-point hedonic scale, considering 9.0 as best and 1.0 as worst.

2.7 Analytical methods

Determination of proximate composition *viz.* moisture, crude protein, crude lipid, ash, amino acid profile, fatty acid profile, and crude fiber were carried out in the Fishing and Post-Harvest Technology laboratory at Sher-e-Bangla Agricultural University, Dhaka, and Food Safety laboratory of Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka. The proximate composition of the samples was analyzed in triplicate according to the standard procedure given by the Association of Official Analytical Chemists (Association of Official Analytical Chemists, 2000).

2.8 Microbial quality analysis

Total Bacterial Count (TBC) of fresh fish samples was done by standard plate count (SPC) method. This method consists of growing the bacteria in a nutrient culture petridish and counting the colonies in the petridish which developed at defined incubation period. This method was used to determine the general quality of the processed fish products “Fish Jelly”. The process of standard plate count (SPC) is shown in Fig 2.

2.9 Statistical analysis

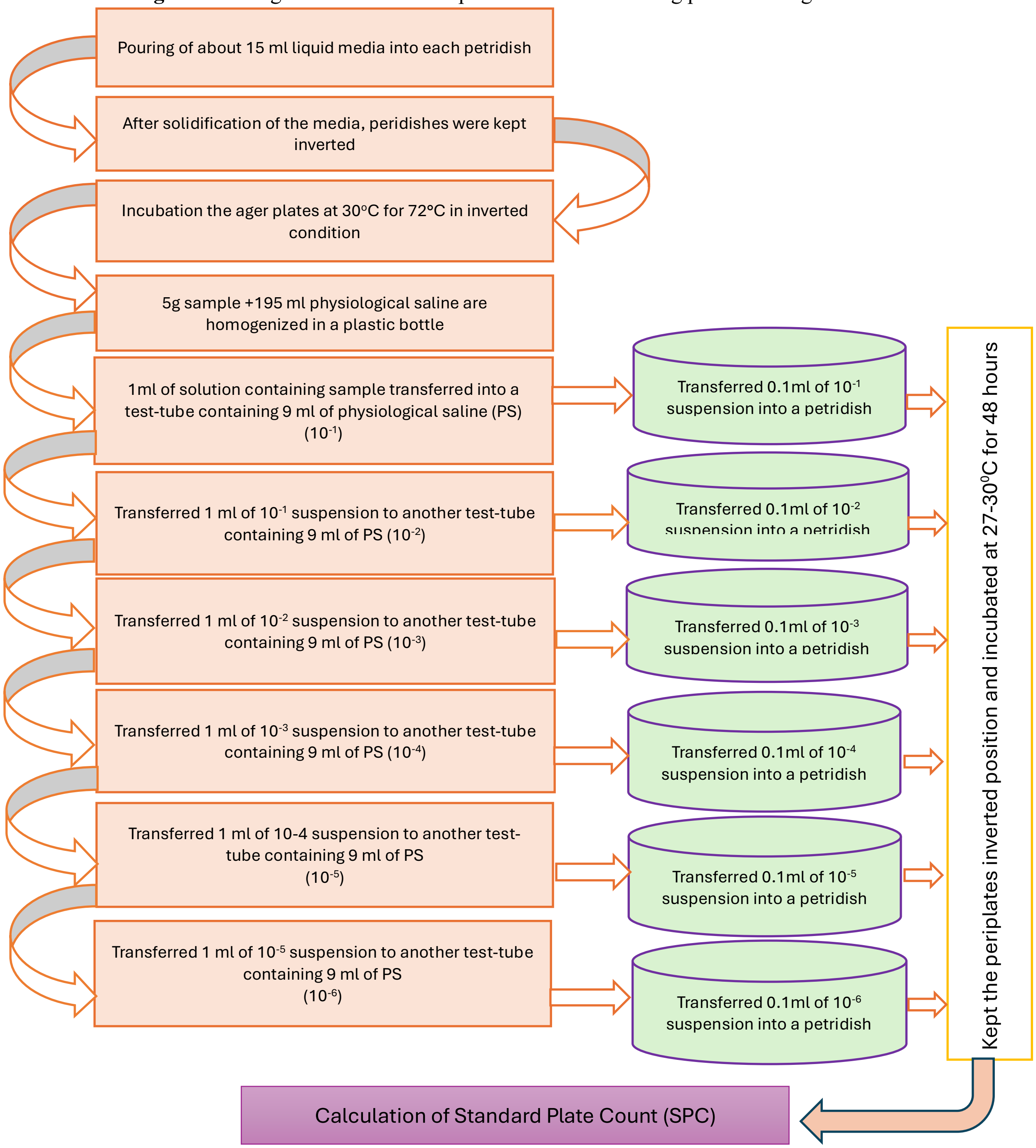
Collected data were analyzed and graphically presented with the help of Microsoft Office Excel 2010 software and SPSS version 20.0.

3. Results and Discussion

3.1 Sensory Characteristics

The mean value of sensory characteristics of the developed products is shown in the graphs 3 to 5 below. The results of the organoleptic properties in terms of mean acceptability score of the products obtained from the study of one hundred thirty-five (135) and two hundred fifty (250) days storage period in ambient temperature and refrigerated conditions, respectively.

Fig 2. Flow diagram of the standard plate count method using plate count agar medium.



These sensory qualities changed at different levels with the time of storage. The sensory evaluation was done on different days in all three products: FJ1, FJ2, and FJ3. The hedonic score for color, flavor, and taste of FJ1 when stored at ambient temperature was noted as 6 or above, except the score of texture, which was found just below 6 till 90 days, which is usually indicative of a ‘like slightly’ to ‘like extremely’ product. All sensory scores were recorded below 5 on the 135th day, which denotes the deterioration of product quality. Whereas, in refrigerated conditions, hedonic scores for FJ1 were found above 6 for 205 days and then drastically reduced to 4 or just above when recorded on 250th day, indicating consumers dislike. Their it showed a better storage life of FJ1in refrigerated conditions when compared to ambient temperature. For FJ2, the scores were reported as 6 or above for all sensory attributes till 90 days in case of products stored in ambient temperature, and after the 120th day, scores regarding color, flavor, and taste were noted below 6 and near or below 5 on the 135th day, indicating quality degradation at this point. When FJ2 stored in refrigerated condition scores were reported around six till 195th day, showing better acceptance of the product and when the values were measured on 250th day total deterioration of the product was observed. In case of FJ3, sensory scores were recorded 6 or above till 100 days when stored in ambient temperature and after 120th day scores were noted below 6 and near or below 5 on 135th day indicating quality degradation of the product. On the other hand, when FJ3 was stored in refrigerated conditions, scores were noted as 6 or above to 165 days, and when measured on the 250th day, the scores were drastically reduced to 4, indicating total quality deterioration of the product. In this study, higher sensory values were recorded for all three products when stored in refrigerated conditions during all the panel tests compared to those stored in ambient temperature which is quite similar to those reported by Sagona et al. (2022) where the author reported frozen conditions are desirable way to ensure a longer preservation period of royal jelly. In case of products stored at ambient temperature, FJ1 was reported to be in a better state than FJ2 and FJ3 in terms of consumer acceptance on the basis of sensory attributes (Fig 3, 4 & 5). Hence, it is indicated that premium quality fish jelly can be accepted by ready-to-eat seafood consumers, health-conscious individuals, and culinary enthusiasts.

3.2 Proximate Composition

Nowadays, appropriate knowledge about the proximate composition of fish is increasingly finding application to food scientists in establishing high-protein foods with high nutritive value (Mohamed et al., 2010). The nutritional value especially protein, lipid, and ash were found in significant amounts in all three processed fish jellies. Their contribution in product-01 (FJ1), product-02 (FJ2) and product-03 (FJ3) are shown in following graphs.

Fig 3 (a & b): Fish jelly 1 when stored in ambient temperature & refrigeration condition.

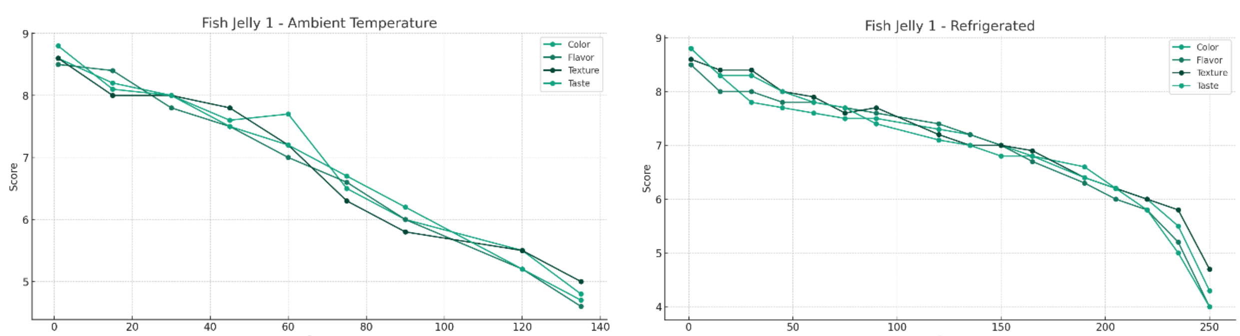


Fig 4 (a & b): Fish jelly 2 when stored in ambient temperature & refrigeration condition.

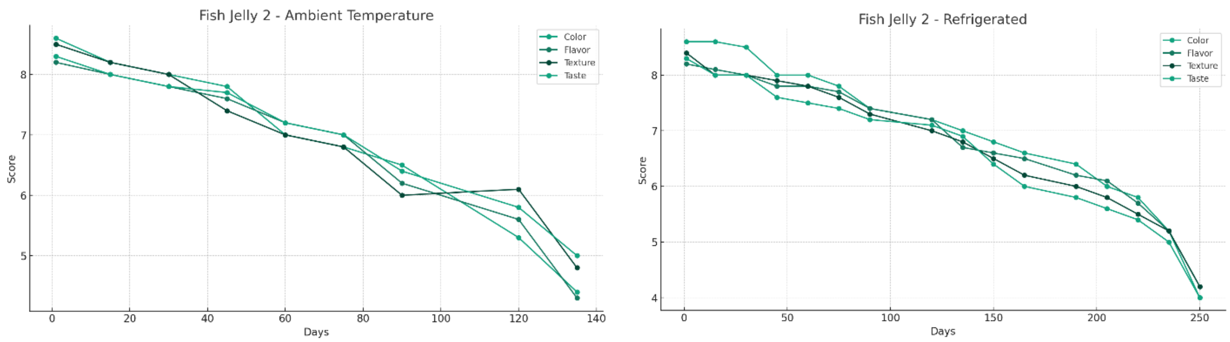
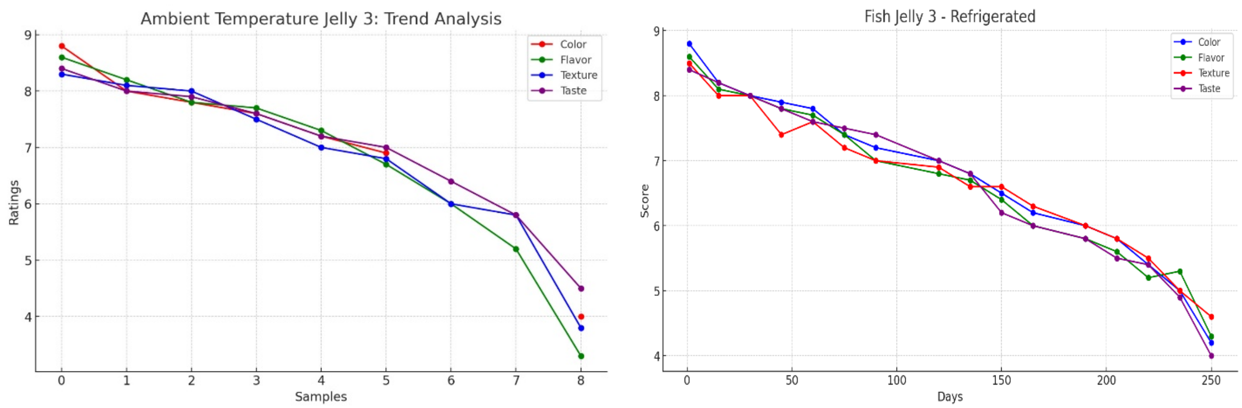
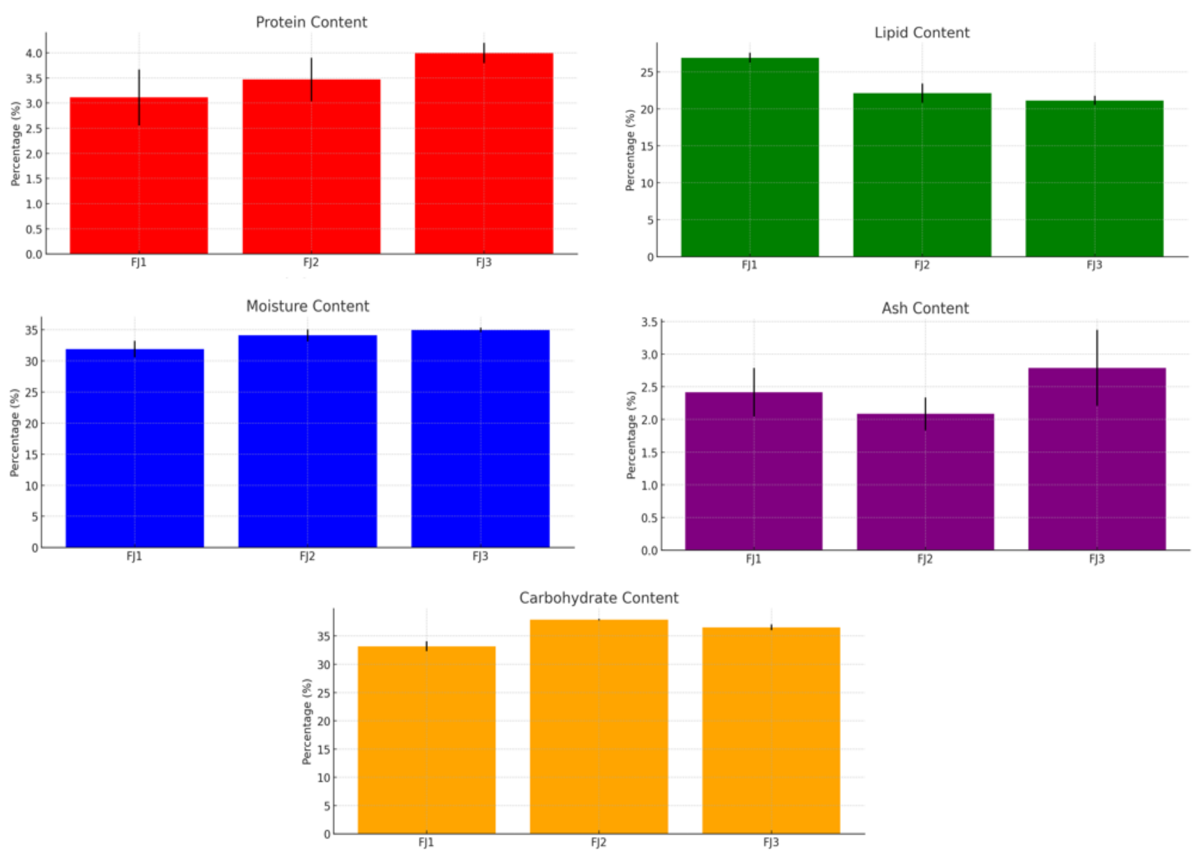


Fig 5 (a & b): Fish jelly 3 when stored in ambient temperature & refrigeration condition.



Fish protein is of high quality and contains sufficient amounts of all the essential amino acids required by the body for growth, maintenance of lean muscle tissue and active metabolism (Nath & Majumdar, 2013). The highest protein content was seen for product-03 (FJ3) (Fig 6) which is 4.0% and the lowest was noted for product-01 (FJ1) which is 3.11%. Clear fish juice contains more fat, so it contains less protein. Protein content in the present study was found to be almost similar to that reported by Lee et. al. (2010) where the authors recorded 5.50% protein in banana peel jelly. Whereas, protein content reported (0.59%) by Anuar & Salleh (2019) in bilimbi jam was far lower than the current finding. Lipid is an important constituent, which that determines both functional and sensory properties. Lipids including their fatty acids provide several quality attributes on the basis of their composition, content and properties (Faridullah et al., 2022). Maximum lipid content was noted in product-01 (FJ1) which is 26.9% whereas minimum was recorded in product-03 (FJ3) as 21.2% (Fig 6). Hilsa jelly is a novel product. Hilsa is a fatty fish, so the fat content is higher. Also, we used the belly region of the fish, so the fat is higher here. Lipid content in present study was found far higher than those reported by Lee et al. (2010) and Anuar & Salleh (2019) where the authors recorded 2.24% and 0.46% lipid in banana peel jelly and bilimbi jam. This might be due to the presence of a higher number of fatty acids in the Hilsa fish which is used to prepare fish jellies. The moisture level in each food product varied based on its ingredients and processing methods (Zubair et al., 2014). The moisture content of the samples was found to vary from 31.9% to 35.0%, where the highest percentage reported in FJ3. FJ3 contains lower fat, so the moisture is higher here (Fig 6). Findings of this study is found in accordance with previous studies of Carvalho et al. (2012), Lee et al. (2010) and Zubair et al. (2014), where the authors reported moisture content from sapota, banana peel and strawberry jellies ranging within 30 to 42%. However, Teixeira-Lemos et al. (2021) recorded water content of 8–18% in the fruit gummy jellies which was slightly higher than the current finding. According to Rana et. al. (2021), ash content represents the quantity of minerals like calcium, phosphorus, and iron present in the sample. The highest ash content was seen in product-03 (FJ3) which is 2.79% and the lowest in product-02 (FJ2) which is 2.09% (Fig 6). Ash content in present study was found lower than those reported by Lee et al. (2010) where the author recorded 13.22% ash in banana peel jelly. We prepared it from fish muscle, so the ash content is higher. Whereas, ash content reported (0.37%) by Anuar & Salleh (2019) in bilimbi jam was slightly lower than the current finding. Carbohydrate content was recorded highest in product-02 (FJ2) as 37.9% whereas the lowest one found in product-01 (FJ1) as 33.2% (Fig 6). Present study showed comparatively lower amount of carbohydrate than those reported by Amini Khoozani et al. (2019), where the author recorded a higher carbohydrate content (75.0%) in banana fruit.

Fig 6 (a, b, c, d & e): Protein, lipid, moisture, ash and carbohydrate content



3.3 Nutritional Quality

Fatty Acid Profile

Fatty acid composition was analyzed for determining the nutritional value of product-01 (FJ1), product-02 (FJ2) and product-03 (FJ3) and the obtained results are shown in Table 2 & Fig 7.

Twenty-five fatty acids were identified in the samples and they are of three different types: saturated fatty acids (SFAO), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Primary fatty acids recorded in product-01 (FJ1) were palmitoleic, oleic, myristic, stearic, EPA and vaccenic acid. In product-02 (FJ2) and product-03 (FJ3), similar primary fatty acids were noted which are palmitoleic, oleic, myristic and stearic acid. The most predominant fatty acid in all products was palmitoleic acid which contributed 28.64%, 43.23% and 39.74% in FJ1, FJ2 and FJ3, respectively. Palmitoleic acid is an essential fatty acid. This is beneficial for human health; three samples contain it, which is best for the human body. Oleic, myristic and stearic acids were recorded as 2nd, 3rd and 4th highest contributors in all three samples and were present between 14.02% to 19.75%, 7.67% to 12.34% and 7.4% to 12.25%, respectively. Among all three products, highest amount (19.75%) of oleic acid was observed in FJ1 whereas myristic (12.34%) and stearic acids (12.25%) were found highest in FJ2. EPA also contributed a considerable proportion in FJ1 but its contribution to FJ2 and FJ3 is comparatively low. Pentadecanoic, eicosadienoic and arachidoneic acid were absent in all the products. Considering the amount of total unsaturated fatty acids, all samples had a rich composition with a sum of unsaturated fatty acids (monounsaturated and polyunsaturated) in general higher than half of total fatty acids content. Present finding is quite similar to those reported by Ferrão et al. (2013), where the authors observed highly enriched and diversified fatty acid profile with sixteen fatty acids identified in which palmitic, linoleic and alpha-linolenic acids were found in higher amounts.

Fig 7: GC chromatogram of the sample FJ_1, FJ_2 and FJ_3

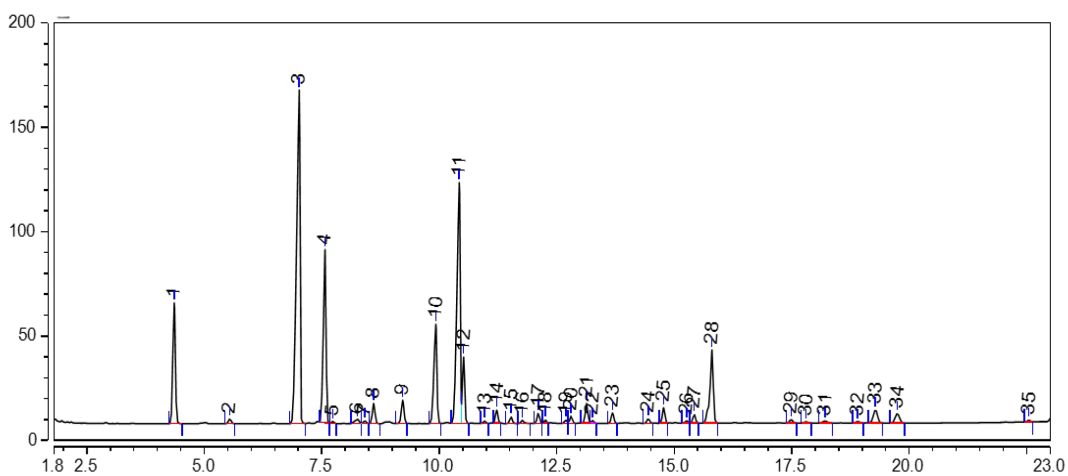


Table 2: Fatty acids composition of samples stored at ambient temperature

Fatty Acids	Products-01 (FJ_1) g/100g extracted fat	Products-02 (FJ_2) g/100g extracted fat	Products-03 (FJ_3) g/100g extracted fat
6 C 14:0 Myristic acid	7.67	12.34	12.23
8 C 15:0 Pentadecanoic acid	0.34	0.42	0.49
9 C 15:1 Pentadecanoic acid	0	0	0
10 C 16:0 Palmitoleic acid	28.64	43.23	39.74
11 C 16:1 Palmitoleic acid	11.88	9.46	12.34
12 C 17: 0 Heptadecanoic acid	1.34	0.22	0.17
13 C 17: 1 Heptadecanoic acid	1.53	0	0.07
14 C 18:0 Stearic acid	7.4	12.25	10.99
16 C 18:1 c9 Oleic acid	19.75	14.02	15.44
17 C 18:1 c11 Vaccenic acid	3.67	2.06	2.56
18 C 18:2c Linoleic acid	0.81	0.12	0.12
20 C 18:3 Alpha Linoleic acid	0.19	0	0
21 C 20:0 Arachidic acid	0.22	0.29	0.33
22 C 20:1 Eicosenoic acid	1.3	1.04	1.26
23 C 20:2 Eicosadienoic acid	0	0	0
24 C 20:3 Eicosatrienoic acid	0	0.53	0.41
25 C 21:0 Henicosanoic acid	0.31	0	0
26 C 20:3 Eicosatrienoic acid	0.99	0	0
27 C20:4 Arachidoneic acid	0	0	0
28 C 22:0 Behnic acid	0.59	0.38	0.3
29 C 20:5 EPA	6.19	0.55	1.04
31 C 24:0 Ligonoceric acid	0.25	0.15	0.07
32 C 24:1 Nervoneic acid	0.15	0.12	0.15
33 C 22:5 DPA	1.39	0	0.11
34 C 22:6 DHA	1.08	0	0.38
Total	95.69	97.18	98.2
Unknown	4.31	2.82	1.8
Total Saturated fatty acids	46.76	69.28	64.32
Total Monounsaturated fatty acids	38.28	26.7	31.82
Total Polyunsaturated fatty acids	10.65	1.2	2.06

3.4 Microbial Quality

Total bacterial count of the samples stored in ambient temperature and refrigerated condition are shown in table 3 and table 4, respectively. After production microbial quality of fish jelly samples (FJ1, FJ2 and FJ3) was assessed in ambient temperature and refrigeration condition by analyzing total bacterial count. The bacterial counts in the food samples were observed to increase with increase in storage time. Total bacterial count was measured in CFU/gm unit. In case of products stored at ambient temperature, when the samples were examined within one day of processing, very low microbial contamination was found while highest being recorded in FJ3 (2.11×103 CFU/gm). Samples were then assessed on 45th and 90th day and highest bacterial count was noted in FJ1 and FJ2 with values being 3.44×104 CFU/gm and 3.18×106 CFU/gm, respectively. At the end of 135 day, the bacterial counts from the samples ranged from 1.34×108 to 1.06×109 CFU/gm where the highest being reported in FJ1 (1.06×109 CFU/gm). When it comes to determining microbial load of products stored at refrigeration condition, samples were assessed for 270 days period. On day 1 of sampling total bacterial count was ranged between 1.03×103 to 2.06×103 CFU/gm. Then the microbial load of samples was further analyzed on day 45, 90, 135, 180 and

225 where the highest bacterial count was recorded in FJ3 (2.53×103 CFU/gm), FJ3 (2.14×103 CFU/gm), FJ2 (2.84×105 CFU/gm), FJ1 (1.84×106 CFU/gm) and FJ3 (3.14×106 CFU/gm), respectively. During last assessment which took place on 270th day, total bacterial count was noted to range between 3.04×107 CFU/gm to 3.54×108 CFU/gm where the highest being reported in FJ1 which is 3.54×108 CFU/gm. This finding is quite similar to those reported by Chaudhry et al. (2023), where the author reported continuous increase in total bacterial count during storage which ended as 2.9×105 CFU/gm on 120th day. As the acceptable limit for total bacterial count is ≤105 CFU/gm, so all three jellies had remained satisfactory for consumption up till 45 days for products stored in ambient temperature and till 90 days for FJ2 and till 135 days for FJ1 & FJ3 products to be stored in refrigeration temperature.

Table 3. Total bacterial count of samples stored at ambient temperature

Products Name	Total Bacterial Count (APC) CFU/gm			
	Day 01	**Day 45**	**Day 90**	**Day 135**
FJ_1	1.04×10^3	3.44×10^4	2.13×10^6	1.06×10^9
FJ_2	1.24×10^3	2.06×10^4	3.18×10^6	1.34×10^8
FJ_3	2.11×10^3	1.64×10^4	2.74×10^6	4.04×10^8

Table 4. Total bacterial count of samples stored at refrigeration temperature

Products Name	Total Bacterial Count (APC) CFU/gm						
	Day 01	**Day 45**	**Day 90**	**Day 135**	**Day 180**	**Day 225**	**Day 270**
FJ_1	1.24×10^3	2.11×10^3	2.07×10^3	3.24×10^3	1.84×10^6	2.1×10^6	3.54×10^8
FJ_2	1.03×10^3	1.29×10^3	1.22×10^3	2.84×10^5	2.34×10^5	1.44×10^6	3.04×10^7
FJ_3	2.06×10^3	2.53×10^3	2.14×10^3	3.19×10^4	2.22×10^5	3.14×10^6	3.17×10^7

4. Conclusions

The study was conducted to develop a quality jelly from hilsa (*Tenualosa ilisha*). On the basis of sensory preferences Fish Jelly 2 (FJ2) got better consumer acceptance. Besides this, a significantly higher nutritional composition (protein, lipid, and ash) was observed in FJ2. In terms of fatty acid profile all three jellies (FJ1, FJ2 and FJ3) were found containing highly beneficial unsaturated fatty acids. Additionally, during the storage period, all samples were found to be least contaminated in terms of total bacterial count because the boiling and cooking temperatures are high, whether measured in ambient temperature or refrigeration conditions. These findings suggest the possibility of using FJ2 as premium quality fish jelly which will be highly nutritious and have healthy functional attributes.

5. Acknowledgement

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