



DEVELOPMENT OF READY-TO-COOK (RTC) HILSA (*TENUALOSA ILISHA*) CURRY UNDER VACUUM AND MODIFIED ATMOSPHERE PACKAGING DURING REFRIGERATED STORAGE

MT Islam^{1*}, R Sultana¹, MA Rahim¹, MM Hasan², FA Flowra¹, MA Bashar² and Y Mahmud³

¹Department of Fisheries, University of Rajshahi, Rajshahi-6205, Bangladesh

²Bangladesh Fisheries Research Institute, Riverine Station, Chandpur-3602, Bangladesh

³Bangladesh Fisheries Research Institute, Headquarter, Mymensingh-2201, Bangladesh

Abstract

The aim of the study was to evaluate quality of ready-to-cook (RTC) hilsa curry under not sealed pack as control, vacuum as T₁, MAP-1 (50% CO₂ & 50% N₂) as T₂, and MAP-2 (40% CO₂, 30 N₂ & 30% O₂) pack as T₃ during storage at 4±1°C. For this purpose, pH, total volatile base nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARS) and aerobic plate count (APC) of three samples from each treatment were analyzed at four days interval during 28 days of storage. The pH and TVB-N values of RTC hilsa curry were within the standard limit in all samples during the storage period. However, significantly ($p < 0.05$) lower values were observed on and after the 12th day for pH and 16th day for TVB-N in all samples compared to the control. TBARS gradually increased from the 4th day for all samples except vacuum packaged sample. However, significantly ($p < 0.05$) lower TBARS were observed in the vacuum and MAP-1 samples on and after the 8th day of storage compared to the control and MAP-2 samples. APCs gradually increased from the initial value of 5.25 log CFU/g with time in all samples. However, significantly ($p < 0.05$) lower APCs were observed on and after the 16th day of storage in all samples compared to the control sample. The APCs exceeded the 7 log CFU/g, which is considered as the upper acceptable limit on approximately 16th day for the control, 24th day for vacuum, 22nd day for MAP-1, and 20th day for MAP-2 sample. Therefore, the vacuum packaging demonstrated the better results, which the superstores can utilize conveniently to display RTC hilsa curry with prolonged shelf life.

Key words: Fish curry, Hilsa, MAP, Quality, Ready-To-Cook, Shelf life, Vacuum

Introduction

Hilsa shad (*Tenualosa ilisha*) is Bangladesh's national fish and is known as the fish king for its delicious taste and unique flavors (Rahman et al. 2020). Hilsa is rich in amino acids, minerals, and lipids, especially with several essential and polyunsaturated fatty acids (PUFA) such as eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) (Mohanty et al. 2012). In general, hilsa fishes are traded in domestic markets in fresh, un-gutted, whole, and under icing conditions (Alam et al. 2012). In order for this common high-priced fish to enter the market fresh, careful handling is therefore important to control and slow down spoilage. In terms of preservation, hilsa cannot be sun-dried due to its high lipid content. Icing is the most common short-term method of preservation, and salting or salt-fermentation is a long-term one (Majumdar et al. 2005, Nowsad 2007, Ay 2011). Some of the hilsa has been frozen (Toufique 2015). Smoking was also practiced

*Author for correspondence: tariqrubd@gmail.com

previously, but they are now not on the market (Saha 2015). Therefore, combining two or more methods could effectively retain the quality and flavor, which eventually increases the shelf life of hilsa.

On the other hand, people prefer a fast, simple way of cooking rather than spend too much time cooking because of the busy lifestyle strain. For this purpose, ready-to-cook foods are considered to fit the busy lifestyle of people as well as do not compromise on the part of their health (Malik et al. 2018). Food preference has been changing with the social and economic development of Bangladesh. Now, city-dwellers seek ready-to-cook or prepared foods instead of raw ingredients. There are no such value-added products from fish, including hilsa fish in the market. Hilsa curry, prepared with various spices, is a very popular dish for its distinctive flavor and texture in South Asian countries (Singha et al. 2020). Therefore, hilsa curry could be prepared as an RTC product, which can be introduced in the market for those clusters of consumers.

The combination of vacuum or modified atmosphere packaging (MAP) and chilled storage is a widely used method for displaying and storing highly perishable fish, meat, and their products in many countries. The shelf life of raw fish fillets can thus be extended by up to 25-400% (De Witt and Oliveira 2016). This kind of packaging has not been practiced yet in Bangladesh. Therefore, the development of proper vacuum and modified atmosphere (MA) packaging along with low-temperature storage is required for fresh hilsa fish and its value-added products, including RTC products, to ensure the supply of quality fishery products with longer shelf life in the market. The aim of the study was to assess the quality and shelf life of RTC hilsa curry under vacuum and MA packaging at refrigerated (4°C) storage conditions.

Materials and Methods

Sample collection

Hilsa fishes (*Tenualosa ilisha*) were collected in February 2020 from the hilsa landing center in Chandpur, Bangladesh. The fishes were then brought to the Quality Control Laboratory of the Department of Fisheries in the University of Rajshahi under icing conditions.

Preparation of RTC hilsa curry

Upon arrival at the laboratory, fishes were washed with tap water to clean the dirt of the skin. The fishes were then dressed and subsequently cut into small pieces of about 50 g. Slices were washed twice using tap water, and the last wash was performed using distilled water. To make RTC hilsa curry from one kg of fish, various spices and ingredients were used. Those were ginger paste (12 g), garlic paste (20 g), red chili powder (6 g), coriander powder (3 g), turmeric powder (3 g), lemon juice (2 tablespoons), and salt (12 g). All the spices and ingredients were put into a stainless steel bowl, and a small quantity of water was added to make a paste. The hilsa slices were then rubbed adequately with the paste and kept in the refrigerator for 20 min after covering them with wrapping plastic.

Packaging of RTC hilsa curry

Around 100 g of RTC hilsa curry was packaged in each plastic pouch with low gas and moisture permeability. The multi-layered transparent pouch (PE/PA/PE) with 100 µm densities was used for this purpose. According to Nayma et al. (2020), four packaging conditions were applied under vacuum and MA packaging with various gas ratios. The packaging was carried out using a packaging unit combined with a packaging machine (C100 Multivac, Germany) and a gas mixer (KM100-3 MEM, WITT, Germany) by following the machine's manual instructions. After packaging, the gas levels in the samples' headspace were determined with a gas analyser (Oxybaby M+, WITT, Germany). Those four packaging conditions were regarded as treatments i.e., (1) not sealed packaged as control, (2) vacuum packaged as treatment 1 (T₁),

(3) MAP-1 (50% CO₂ & 50% N₂) as treatment-2 (T₂) and (4) MAP-2 (75% CO₂ & 25% O₂) as treatment-3 (T₃). All packaged samples were preserved at 4±2°C in a refrigerator. Three samples from each treatment were analysed every four days during 28 days of the storage in the laboratory.

Biochemical and microbiological analyses

In order to assess the shelf life of RTC hilsa curry at refrigerated storage, various microbiological and biochemical parameters were investigated in the Quality Control Laboratory of the Department of Fisheries in the University of Rajshahi. After adding 50 ml of distilled water, ten grams of cut flesh was homogenized with a grinder (REX 500, Bajaj, India), and then the pH of the slurry was measured using a pH meter (HI2002 Edge, Hanna Inst, USA) (Islam et al. 2020). According to the Jinadasa (2014) method, total volatile base nitrogen (TVB-N) was measured. Thiobarbituric acid reactive substance (TBARS) was estimated by a colorimetric method, according to Park et al. (2007). According to the APHA (1992) method, aerobic plate count (APC) was performed on plate count agar (Sigma-Aldrich, USA) by the decimal dilution technique. After incubating for 48 h at 35°C in an incubator (Poleko, Poland), the plates were counted for colonies and expressed as log colony forming units (CFU/g).

Statistical analysis

The differences among mean values of various treatments were performed by one-way ANOVA with post hoc Tukey's test using SPSS-20 (IBM, Chicago, USA), and a significant difference was defined as $p < 0.05$.

Results and Discussion

pH value

In the current study, the preliminary pH of RTC hilsa curry was 6.10 and then fluctuated between 5.82 and 6.49 during the storage time. The pH values did not exceed the acceptable limit (6.8–7.0) in all packaging conditions (Metin et al. 2001). During the storage period, no significant ($p > 0.05$) differences were observed in pH values among treatments (Table 1). However, significantly ($p < 0.05$) lower pH values were found on the 12th, 16th, and 20th day of storage in all treated samples compared to the control. In the case of vacuum packaging and MAP, lower pH value perhaps caused by lactacidogenic bacteria, linked to the inhibition of gram (-) aerobic bacteria (mainly pseudomonads). The gram-negative aerobic bacteria become predominant during the storage period as their number increased (Françoise 2010). In MAPs, the dropped in pH value possibly occurred as a result of the dissolution of CO₂ in muscle tissues (Ježek and Buchtová 2012). Moreover, the fish muscle surface absorbs CO₂, thus acidifying it with carbonic acid formation (Banks et al. 1980).

Table 1. pH values of RTC hilsa curry under vacuum and MA packaging during refrigerated (4±1°C) storage condition (n = 3)

Treatments	Storage days							
	0 d	4 d	8 d	12 d	16 d	20 d	24 d	28 d
Not sealed packaged (Control)	6.10±0.11 ^a	6.45±0.01 ^a	6.18±0.14 ^a	6.26±0.08 ^b	6.45±0.04 ^b	6.49±0.08 ^b	-	-
Vacuum packaged	6.10±0.11 ^a	6.14±0.08 ^a	6.00±0.11 ^a	5.97±0.04 ^a	6.08±0.06 ^{ab}	5.99±0.04 ^a	5.95±0.21	6.14±0.03
MAP-1 (50% CO ₂ & 50% N ₂)	6.10±0.11 ^a	6.20±0.23 ^a	5.97±0.37 ^a	5.98±0.01 ^a	5.85±0.11 ^a	6.16±0.12 ^{ab}	6.04±0.14	6.08±0.18
MAP-2 (40% CO ₂ , 30% N ₂ & 30% O ₂)	6.10±0.11 ^a	6.04±0.08 ^a	5.99±0.09 ^a	5.82±0.10 ^a	5.90±0.25 ^{ab}	5.91±0.19 ^a	5.92±0.04	5.96±0.13

Values are expressed as mean ± SD. Values with different superscript letters in a column are significantly different ($p < 0.05$).

Total volatile base nitrogen (TVB-N) value

In order to predict bacterial spoilage of fish, TVB-N is a widely used parameter as an indicator. The TVB-N value of 30-35 mg N/100 g is generally considered a standard limit for chilled stored fish (Connell 1995). The preliminary TVB-N value was 2.66 mg/100g and then remained constant until the 8th day and steadily increased with storage time over the rest of the storage period (Table 2). The gradual increase in TVB-N values during the storage time possibly occurred due to increased bacterial growth and presence of endogenous enzymes (Ruiz-Capillas and Moral 2001, Islami et al. 2015). This finding is similar to the earlier studies, where a correlation was detected between quality deterioration and grown TVB-N values of MA packaged seafood (Özogul et al. 2004, Nayma et al. 2020). There was no significant difference ($p > 0.05$) observed in TVB-N values among treatments until the 12th day of storage. However, on the 16th and 20th day of storage, significantly ($p < 0.05$) lower TVB-N were found in all treated samples compared to the control. In the current study, MAP showed a slower increase in TVB-N values supported by a previous study where Silver Carp fillets were preserved at 4°C (Rahmatipoor et al. 2017). The better performance of MAP in the current study may be due to the effect of bacteriostatic properties of CO₂. A past study claimed that CO₂ gas is responsible for partial prevention and delay of the growth of spoilage bacteria (Farber 1991).

The maximum TVB-N value reached 7.14 mg/100 g on the 20th day of storage for not sealed packaged and 28th day for MAP-2 packaged sample. Nevertheless, the TVB-N values were within the standard limit in all packaged samples (Table 2). A similar result was also observed in the case of RTC pangas curry where TVB-N values did not surpass the standard limit in any treatments (not sealed packaged, MAP-1 with 50% CO₂ & 50% N₂, and MAP-2 with 75% CO₂ & 25%N₂) at similar storage condition (Nayma et al. 2020).

Table 2. Total volatile base nitrogen (TVB-N) values (mg/100 g) of RTC hilsa curry under vacuum and MA packaging during refrigerated (4±1°C) storage condition (n = 3)

Treatments	Storage days							
	0 d	4 d	8 d	12 d	16 d	20 d	24 d	28 d
Not sealed packaged (Control)	2.66±0.20 ^a	3.14±0.31 ^a	2.53±0.41 ^a	4.76±0.40 ^a	7.00±0.40 ^b	7.14±0.20 ^b	-	-
Vacuum packaged	2.66±0.20 ^a	2.41±0.16 ^a	2.24±0.40 ^a	3.08±0.79 ^a	3.64±0.40 ^a	5.18±0.20 ^a	5.18±0.99	6.38±0.20
MAP-1 (50% CO ₂ & 50% N ₂)	2.66±0.20 ^a	2.94±0.20 ^a	2.80±0.40 ^a	3.16±0.11 ^a	5.04±0.40 ^a	5.32±0.40 ^a	6.10±0.48	6.30±0.20
MAP-2 (40% CO ₂ , 30% N ₂ & 30% O ₂)	2.66±0.20 ^a	3.11±0.04 ^a	2.66±0.20 ^a	3.80±0.62 ^a	4.34±0.59 ^a	6.10±0.68 ^{ab}	6.44±0.40	7.14±0.59

Values are expressed as mean ± SD. Values with different superscript letters in a column are significantly different ($p < 0.05$)

Thiobarbituric acid reactive substance (TBARS) value

TBARS is a popular method to evaluate lipid oxidation and subsequently the quality of foods. It measures the amount of malonaldehyde (MDA) as a secondary product of the oxidation of PUFAs (Bremner 2003) in which modification of peroxide occurs that results in the production of materials such as aldehydes and ketones (Feliciano et al. 2010). Oxidative rancidity may be the primary concern for MA packaged fish's shelf life, especially for fatty fishes (Sivertsvik et al. 2002). The standard limit for TBARS was set as 2 mg MDA/kg fish, and beyond this limit, an unpleasant odor and taste can be formed (Connell 1995).

The preliminary value of TBARS was 0.26 mg MDA/kg hilsa curry. The TBARS were steady up to the 4th day and then gradually increased for all treated samples except the vacuum packaged sample. In the case of the vacuum sample, the TBARS gradually increased very slowly and reached 1.26 mg MDA/kg on the last day of storage (Table 3). However, significantly ($p < 0.05$) lower TBARS were observed in the vacuum and MAP-1 samples on the 8th to 20th day of storage compared to the control and MAP-2 samples. The absence of O₂ may have caused the oxidation of PUFAs to be delayed. This finding has been agreed by Masniyom et al. (2013) and Alice et al. (2020), who found that in the absence of O₂ vacuum and MA packaged tilapia and goonch fish had the lower MDA compared to the control sample.

In the case of the MAP-2 sample, significantly ($p < 0.05$) higher TBARS were found on the 8th to 20th day of storage compared to other samples. This increase might be due to the presence of oxygen in the MAP-2 sample. This oxygen interacts with the fatty acids and produces hydroperoxide without modifying the odorous compounds (Church 1998). The TBARS exceeded the acceptable limit (2 mg MDA/kg) before the 8th day of storage for control and MAP-2 sample. On the other hand, the TBARS were within the standard limit in the MAP-1 sample until almost the 24th day and in the vacuum sample until the end of storage (Table 3). Since MDA can interact with other fish compounds such as amines, nucleic acid, and nucleosides, amino acids, aldehydes, and proteins, the levels of TBARS do not represent the actual levels of fat oxidation (Domínguez et al. 2019).

Table 3. Thiobarbituric acid reactive substance (TBARS) values (mg MDA/kg) of RTC hilsa curry under vacuum and MA packaging during refrigerated ($4 \pm 1^\circ\text{C}$) storage condition (n = 3)

Treatments	Storage days							
	0 d	4 d	8 d	12 d	16 d	20 d	24 d	28 d
Not sealed packaged (Control)	0.26±0.08 ^a	0.36±0.1 ^a	2.36±0.04 ^e	2.19±0.07 ^c	3.04±0.06 ^c	3.78±0.09 ^c	-	-
Vacuum packaged	0.26±0.08 ^a	0.35±0.05 ^a	0.46±0.06 ^a	0.57±0.07 ^a	0.61±0.07 ^a	0.59±0.03 ^a	0.93±0.03	1.26±0.08
MAP-1 (50% CO ₂ & 50% N ₂)	0.26±0.08 ^a	0.36±0.01 ^a	1.19±0.06 ^b	1.84±0.08 ^b	1.70±0.16 ^b	2.37±0.03 ^b	1.78±0.06	3.28±0.06
MAP-2 (40% CO ₂ , 30% N ₂ & 30% O ₂)	0.26±0.08 ^a	0.34±0.04 ^a	3.66±0.24 ^d	4.07±0.08 ^d	4.18±0.10 ^d	5.13±0.10 ^d	5.11±0.05	5.41±0.04

Values are expressed as mean ± SD. Values with different superscript letters in a column are significantly different ($p < 0.05$).

Aerobic plate count (APC)

For the evaluation of the microbiological quality of a product as well as for shelf life calculation, APC is an important indicator. The APC of RTC hilsa curry was 5.25 log CFU/g indicated an acceptable initial quality of fish curry. The bacterial counts of 2 - 6 log CFU/g are considered acceptable of newly caught freshwater fishes (rainbow trout, tilapia, sea bass, and silver perch) (Gelman et al. 2001). The APC of hilsa curry gradually decreased up to the 8th day of storage and then progressively increased in all packaged samples (Table 4). The primary decrease in APC was aligned with the primary decrease in pH and TVB-N value (Table 1 & 2). There is no significant difference ($p > 0.05$) was observed in APCs among all treated samples until the 12th day of storage. Nevertheless, significantly ($p < 0.05$) lower APCs were evident on the 16th and 20th day of storage in all treated samples compared to the control.

Table 4. Aerobic plate count (APC) (Log CFU/g) of RTC hilsa curry under vacuum and MA packaging during refrigerated ($4\pm 1^\circ\text{C}$) storage condition ($n = 3$)

Treatments	Storage days							
	0 d	4 d	8 d	12 d	16 d	20 d	24 d	28 d
Not sealed packaged (Control)	5.25 \pm 0.29 ^a	5.53 \pm 0.46 ^a	4.91 \pm 0.32 ^b	6.18 \pm 0.15 ^a	7.04 \pm 0.12 ^c	7.82 \pm 0.09 ^c	-	-
Vacuum packaged	5.25 \pm 0.29 ^a	4.74 \pm 0.02 ^a	4.65 \pm 0.18 ^a	5.24 \pm 0.48 ^a	5.69 \pm 0.10 ^a	6.63 \pm 0.15 ^{ab}	6.99 \pm 0.33 ^a	7.36 \pm 0.13 ^a
MAP-1 (50% CO ₂ & 50% N ₂)	5.25 \pm 0.29 ^a	4.95 \pm 0.63 ^a	4.78 \pm 0.11 ^a	5.06 \pm 0.81 ^a	5.70 \pm 0.05 ^a	6.40 \pm 0.08 ^a	7.57 \pm 0.14 ^a	8.40 \pm 0.08 ^b
MAP-2 (40% CO ₂ , 30% N ₂ & 30% O ₂)	5.25 \pm 0.29 ^a	5.01 \pm 0.15 ^a	4.72 \pm 0.13 ^a	5.90 \pm 0.39 ^a	6.19 \pm 0.07 ^b	7.00 \pm 0.19 ^b	7.35 \pm 0.16 ^a	8.28 \pm 0.22 ^b

Values are expressed as mean \pm SD. Values with different superscript letters in a column are significantly different ($p < 0.05$).

ICMSF (1986) set 7 log CFU/g as the upper acceptable limit of APC for frozen and fresh fish. In this study, the APC exceeded the 7 log CFU/g on approximately 16th day for the not sealed packaged (control), 24th day for vacuum packaged, 22nd day for MAP-1 (50% CO₂ & 50% N₂) sample, and 20th day for MAP-2 (40% CO₂, 30% N₂ & 30% O₂) sample (Fig. 1).

Based on the 7 log CFU/g as the maximum acceptable limit for ready-to-cook fish and fishery products, the shelf life of not sealed packaged, vacuum packaged, MAP-1 (50% CO₂ & 50% N₂), and MAP-2 (40% CO₂, 30% N₂ & 30% O₂) packaged sample during refrigerated storage is determined at approximately 16 days, 24 days, 22 days and 20 days, respectively (Fig. 1). Nayma et al. (2020) reported lower shelf life of MAP (50% CO₂ & 50% N₂) packaged RTC pangas fish curry stored at 4°C that is of 13 days based on the microbial evaluation, which is lower than the finding from this study.

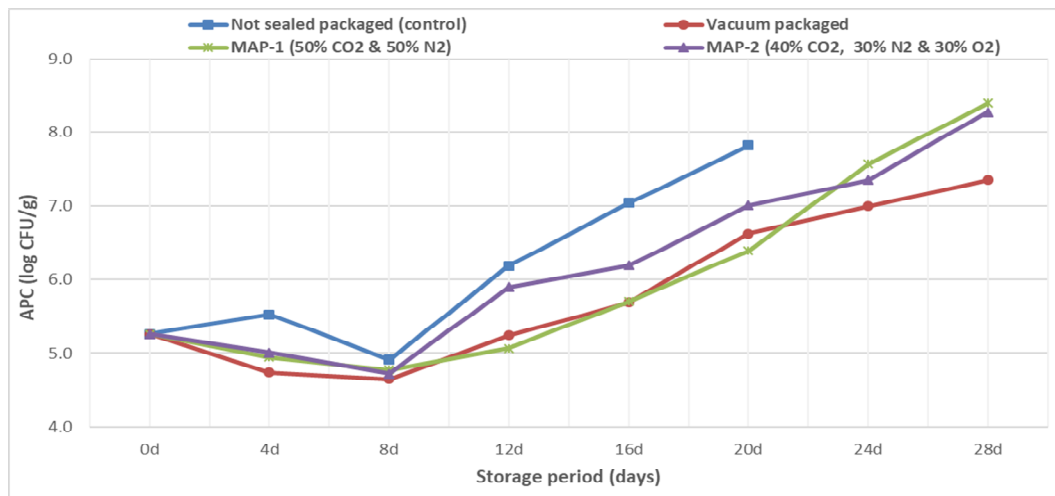


Fig. 1: Changes in aerobic plate count (APC) (log CFU/g) of RTC hilsa curry under vacuum and MA packaging during refrigerated ($4\pm 1^\circ\text{C}$) storage condition ($n = 3$).

During low-temperature storage, the high CO₂ levels prevented psychotropic bacteria's growth in fish, suggesting the high CO₂ sensitivity characteristics exhibited by those bacteria (Masniyom et al. 2011). In contrast, lower APCs of samples held in vacuum packaged compared to the air packaged showed that the absence of oxygen could hamper the growth of aerobic spoilage bacteria, mainly *Pseudomonas* and

Aeromonas (Masniyom et al. 2011). In addition, the vacuum packaged sample presented lower APCs than the MAP-2 (50% CO₂ / 50% O₂) packaged sample in the entire storage period. There is an agreement with the previous study of Alice et al. (2020) where vacuum-packaged goonch fish stored at 4°C indicated a longer shelf life (10 days) than MAP-2 (50% CO₂ & 50 O₂) pack (9 days).

The current study finding was in agreement with Masniyom et al. (2013), who indicated that APC persisted low in tilapia fish for MAP with 60% CO₂. It was also stated that the bacterial growth in chub mackerel (*Scomber colias*) was inhibited by 50% CO₂ (Stamatis and Arkoudelos 2007), in swordfish (*Xiphias gladius*) by 40% CO₂/30% N₂/30% O₂ (Pantazi et al. 2008) and in *Pangasius hypophthalmus* fillets by 50% CO₂/50% N₂ (Noseda et al. 2012) during refrigerated storage. In another study, it was found that the shelf life of RTC pangas curry at 4°C increased from 12 to 15 days by increasing the amount of CO₂ along with N₂ from 50% to 75% (Nayma et al. 2020).

Conclusion

It can be concluded that all packaging treatments had satisfactory results during the storage time, with the exception of the control, which exhibited APC above the standard limit on the 16th day of the storage. On the other hand, vacuum packaging retarded the bacterial growth and decreased the TBARS amounts significantly during the storage period, extending the shelf life by 24 days. Similarly, MAP-1 (50% CO₂ & 50% N₂) reduced the APCs and TBARS amount effectively, extending shelf life by 22 days. In contrast, MAP-2 (40% CO₂, 30% N₂ & 30% O₂) effectively reduced the APCs during the storage period and extended the shelf life by 20 days. Therefore, vacuum packaging demonstrated the highest shelf life, which is recommended to utilize by the processors or superstores to display the RTC hilsa curry under refrigeration condition, which will increase the shelf life and convenience of the product. This RTC hilsa curry can be cooked quickly by adding only the required amount of onions and vegetable oil.

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