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VALUE ADDITION OF TILAPIA (*Oreochromis niloticus*): PREPARATION OF FILLET AND SHELF-LIFE ASSESSMENT

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A B S T R A C T

Although tilapia is one of the most cultivated fish species in Bangladesh owing to its high growth rate, comparatively easy culture practices, higher survival rate and short culture period but low market preference marked it as a low-priced fish. Various approaches have been adopted to enhance consumer acceptability and ensure better use of tilapia, including the production of value-added products such as tilapia-prepared fillets, which attract increasing interest. Therefore, the current study was designed and carried out in order to prepare tilapia fillets and to estimate their shelf life at frozen (-18 ± 2^0C) and refrigerated (4 ± 1^0C) temperatures. Fillets were produced from tilapia collected from local fish markets of Sylhet city, Bangladesh with according to Good Manufacturing Practices (GMP) established by International Standards Organization (ISO) and packed in polyethylene bags. Proximate composition, total volatile base nitrogen (TVBN), peroxide value (PV), pH, total plate count (TPC) and sensory properties of fillets were analyzed to determine shelf-life. A portion of the fresh fillet sample was immediately analyzed and the remaining portions were preserved for up to 12 and 90 days at refrigerated and frozen storage temperatures, respectively. Results revealed that, at a definite interval of time, changes in the chemical, microbial and sensory attributes of tilapia fillets were found to be more pronounced in refrigerated storage conditions than fillets stored in frozen conditions. The shelf life of refrigerated and frozen tilapia fillet samples was 9 and at least 90 days, respectively, per the results of sensory, microbial and chemical evaluation.

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INTRODUCTION

Bangladesh is one of the most suitable countries for freshwater aquaculture in the world because of its favorable resources and environmental conditions, thus become one of the world's leading fish producing countries (Debnath et al., 2020; FAO, 2020). The country produced a total of 4.384 million MT fish in FY 2018-19, where nearly 57% of the total fish production contributed by aquaculture (FRSS, 2019). Tilapia is one of the most widely cultivated fish species in the world including Bangladesh due to better growth performance, relatively easy raring practices, better survival rate in adverse condition and short culture period (da Silva Santos et al., 2017; Yasin et al., 2020). Bangladesh produced an amount of 390,559 MT Tilapia in FY 2018-19 which is accounted for 8.91 percent of total fish production (FRSS, 2019). However, tilapia is often considered as low-priced fish species owing to its higher availability and low consumer preference (Mohan Dey et al., 2005; Venugopal et al., 1995). Value addition through production of fish fillet can be an effective strategy to increase consumer acceptability and commercial value, and ensure better utilization of tilapia. With the rapid modernization of the society, demand for ready to eat and/or ready to cook products are gradually growing owing to their convenience (Arason et al., 2010; Yerlikaya et al., 2005). In this viewpoint, value adding and variation of fish products will help to fulfil the ever shifting and diverse demands of the consumers. Value addition is one of the most promising sectors in food processing industry (Vanitha et al., 2013). The value that is added to any product or service through a particular process that may change the nature of raw material is called value addition and the products thus produced are called value-added products (Hu et al., 2012). With the changes in consumer consumption expectations as well as the development of reliable cold-chains, fresh preprocessed fishery products such as fillets are more popular with consumers and producers because of their convenience for processing and cooking (Yu et al., 2020).

Fish is a highly perishable food product due to its chemical composition, neutral pH, and weak connective tissues (Ghaly, 2010; Hassoun and Emir Çoban, 2017; Sumon et al., 2020). Though fish muscle of live fish is usually considered as sterile, fish spoilage commences very swiftly after catch or harvest and during the different stages of the production chain, processing, and subsequent storage conditions (Ghaly, 2010; Mei et al., 2019). Mechanism of fish spoilage is well reported, where it is categorized into three types such as microbial, chemical and enzymatic spoilage (Olatunde and Benjakul, 2018; Hussain et al., 2021). Components of fish body decomposed and new compounds formed during spoilage, which lead to protein degradation and lipid oxidation, as well as changes in fish odor, flavor, and texture (Mei et al., 2019). Therefore, it is of importance to develop effective treatment methods to preserve the quality and extend the shelf life of fish. To date, information regarding tilapia fillet and its shelf life are very scarce but is of importance to identify the time after the tilapia is primarily safe for consumption and its sensory properties and nutritional value remain unchanged and acceptable to the consumer as well as to ensure quality avoid spoilage. Considering the aforementioned facts, the present research is designed and conducted to increase consumer acceptability and better utilization of tilapia through production of tilapia fillet and assess its shelf life preserved at refrigerator (4 ± 1^0C) and freezing (-18 ± 2^0C) temperature.

MATERIALS AND METHODS

Collection and preparation of the sample

Fresh Tilapia fish were collected from local fish markets of Sylhet district. Immediately after collection, the fish were iced properly with crushed ice in an insulated ice box with proper care to maintain premium quality and transported to the laboratory of Fisheries Technology and Quality Control, Sylhet Agricultural University where they were weighed and then washed with clean water, beheaded, eviscerated, skinned and washed with chilled water. The skinned fishes were then filleted according to Good Manufacturing Practices (GMP) established by ISO – International Standards Organization (ISO, 2005) and packed in polyethylene bags. A portion of the fresh sample was analyzed immediately and the remaining portions were stored in refrigerated (4 ± 1^0C) and frozen storage (-18 ± 2^0C) temperature for up to 12 and 90 days, respectively to determine its shelf life.

Shelf-life assessment

Shelf-life of Tilapia fillet preserved at refrigerated temperature was assessed up to 12 days at an interval of 3 days. While shelf-life of frozen fillet was evaluated at an interval of 10 days up to 90 days. The samples were obtained from freezer and then thawed properly prior to conduct different experiments. During the whole experiments handling and hygiene were maintained strictly following the EC guidelines. To determine the shelf-life of the product sensory, chemical and microbial quality were analyzed.

Sensory analysis

Sensory evaluation was performed by selected panel members of Fisheries Faculty, Sylhet Agricultural University who have experience in evaluation of similar products using the acceptance test with a 9-point hedonic scale, scored from 9 (strongly like) to 1 (strongly dislike) (Stone and Sidel, 1998). The attributes evaluated were color, odor, taste, texture and overall acceptability. A score of 5 (neither liked nor disliked) was considered as the indifference region of the affective relationship of the judge to the product. Scores from 6 to 9 were considered as the acceptance region and scores from 1 to 4 as the rejection region.

Chemical analysis

pH

A 1 g sample of the fish flesh was homogenized in 10 mL of distilled water and the mixture was filtered. The pH of homogenized filtrate sample was measured using a pH meter (YSI, Model pH10A, USA) at ambient temperature (Erkan et al., 2011).

TVB−N

Total volatile basic nitrogen (TVB-N) was estimated according to the method delineated by Antonacopoulos and Vyncke (1989). Fish fillet (10 g) was homogenized with 6 % perchloric acid (90 mL) for 1 min in a food blender. The homogenates were filtered through a filter paper (Whatman no. 1) and filtrates alkalized by NaOH (20 %) before distillation duplicate filtrates were distilled in the apparatus (Velp Scientifica, Model UDK 129, Italy). The distillate was titrated with 0.01 N HCl.

Peroxide value (PV)

Samples of 0.5 g were mixed with 25 mL of a solution of glacial acetic acid and chloroform (ratio 3:2) in a conical flask, and then 1 mL of saturated potassium iodide (14 g KI/10 mL) was added. The mixture was kept in the dark for about 10 min, and then 30 mL of distilled water and 1 mL of freshly prepared 1 % starch were added. After shaking, the samples were titrated again with 0.01 N sodium thiosulfate until the blue color disappeared. The peroxide values were expressed in units of meq/kg of sample (Erkan et al., 2011). The PV was calculated using the following formula: Peroxide value (meq O2/kg muscle) = $((V–B) \times Nf/W) \times 1{,}000$ where V is the volume of sodium thiosulfate consumed, B is the volume of normal sodium thiosulfate consumed during a blank titration, W is the weight of the sample (grams) and Nf is the normality of sodium thiosulfate multiplied by a factor.

Microbial analysis

Total plate count (TPC) was determined according to the method of Hussain et al. (2018). About 20 g fish sample was homogenized with 180 mL sterile saline solution (0.85% NaCl) to prepare 1:10 sample suspension. Additional 10-fold dilutions were prepared with sterile saline solution. An aliquot (0.1 mL) of each sample dilution was spread on triplicate plate count agar (Difco, Haryana, India) and incubated at 37^0C for 48 h. Microbial counts were expressed as the logarithm of colony forming units per g (log10 CFU/g).

Data analysis

All experimental data were subjected to statistical analysis. One-way ANOVA and post hoc Tukey's test were used to analyze values of parameters and the differences within variables employing SPSS (Version 23.0, IBM, USA). ANOVA of TPC was performed on log-transformed values to assure data normality (Hussain et al., 2018; Khalafalla et al., 2015). Values are expressed as mean $\pm$ SD; p values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

pH

The pH value of muscle indicates the freshness of a fish and also one of the most important factors for preventing food spoilage and microbial growth (Anvari et al., 2013; Ucar et al., 2020). The usual pH of live fish muscle ≈ 7.0 but after death, this value falls noticeably because of rigor mortis and the glycogen is changed to lactic acid (Abbas et al., 2008). In the early period of the study both refrigerated and frozen temperature storage, the low muscle pH reflected the good nutritional condition of the fillets. The initial pH of the study fish sample was 6.45, a similar value has been reported by Simeonidou et al. (1997). In the present study, a gradual increase was seen in pH values of the samples during 12 days storage at 4^{0}C which are presented in Table 1. At day zero, pH value was 6.45±0.04 which is very close to day three and 6.69±0.02 and 6.88±0.05 were respectively at day sixth and ninth. However, significantly highest pH (7.16±0.06) recorded at the end of experimental refrigerator period. From the study of Khalafalla et al. (2015), a gradual increase in pH values with storage time from 6.2±0.02, 6.2±0.02 and 6.2±0.01 at day zero has been observed to reach 7.16±0.11, 7.23±0.04 and 7.27±0.01 at 9th, 9th and 18th day of refrigerated storage for C, R and T groups, respectively. Similar results of pH values at day zero of chilled storage were recorded by Sallam (2007) and Zambuchini et al. (2008), while Hernández et al. (2009) reported higher pH values at day zero. Abouel-Yazeed (2013) reported gradual increase in day 3 and then found unacceptable level of pH values in tilapia fish stored at 4˚C in control condition on day 6 till the end of the storage period (day 15). Differences among the initial pH values than those reported in other studies may be due to the species, diet, and season, level of stress during the catch as well as type of muscle.

Table 1. Changes in the pH, TVBN, PV and TPC of tilapia fish fillets stored at 4^{0}C for 12 days

Storage period (Days)	Parameter			
	pH	TVB-N (mg N/100 g)	PV (meq/kg)	TPC (log cfu/g)
0	$6.45±0.04^{d}$	$7.24±0.35^{e}$	$0.02±0.01^{d}$	$4.68±0.26^{e}$
3	$6.52±0.06^{d}$	$17.52±0.46^{d}$	$1.64±0.23^{c}$	$5.39±0.34^{d}$
6	$6.69±0.02^{c}$	$26.09±0.67^{c}$	$3.48±0.35^{a}$	$5.87±0.13^{c}$
9	$6.88±0.05^{b}$	$30.80±0.62^{b}$	$2.44±0.14^{b}$	$6.65±0.24^{b}$
12	$7.16±0.06^{a}$	$33.23±0.48^{a}$	$1.93±0.14^{c}$	$7.54±0.28^{a}$

Means with the same superscript letters within a column are not significantly different at P < 0.05. Values are means of triplicate determinations ± standard deviation

Frozen temperature observation is presented on Table 2. Initial pH turned between 6.45±0.04 and 6.69±0.01 after 1 month. The value of 30 days was not significantly differed with 40 days. The highest pH value was 6.95±0.07 on the 90th day of the storage which was significantly similar to 70th and 80th days. The pH changes of the present study are in agreement with the findings of Manthey et al. (1988), Ryder et al. (1993) and Arannilewa et al. (2005).
However, it was observed that pH value increased significantly for both the storage temperature. Abbas et al. (2008) reported the increasing value of pH reflect the formation of alkaline bacterial metabolites in spoiling fish. The increasing pH values could be associated to the production of basic components induced by the growth of bacteria Simeonidou et al. (1997). According to Özyurt et al. (2009) pH values above 7.1 are indicative of decomposition in fish. It was evident from the present study that except pH of refrigerated stored fillets at day 12 none of the value exceeded the acceptable limit.

TVB-N

Total volatile bases-nitrogen (TVB-N) is a product of bacterial spoilage and it is often used as an index to assess the keeping quality and shelf life of seafood products (Goulas and Kontominas, 2007; Kostaki et al., 2009). Total volatile basic nitrogen (TVB-N) consists mainly of trimethylamine, dimethylamine, ammonia and other volatile basic nitrogenous compounds (Sallam, 2007a, 2007b). The increase in the TVB-N is due to the activation of spoilage bacteria and endogenous enzymes (Huss, 1995). For several fish species, TVB-N values were reported to increase curvilinearly or linearly with time, and a level of 30 mg muscle TVB-N/100 g has been considered the upper limit above which some fishing products are considered spoiled and unfit for human consumption (Gökodlu et al., 1998). Gimenez et al. (2002) has been proposed a level of more than 25 mg N/100 g flesh is considered as an unacceptable value in fish and fishery products. Jeya et al. (2005) reported that the TVB-N values of good quality fish are generally less than 25 mg /100 g muscle and above 25–30 mg /100 g indicate that fish is decomposed and inedible.

Table 2. Changes in pH, TVBN, PV and TPC of tilapia fish fillets stored at -18±2^{0}C for 90 days

Storage period (Days)	Parameters			
	pH	TVB-N (mg N/100 g)	PV (meq/kg)	TPC (log cfu/g)
0	6.45±0.04^{f}	7.82±0.36^{i}	0.02±0.01^{g}	4.68±0.26^{a}
10	6.56±0.03^{e}	10.76±0.89^{h}	0.06±0.03^{g}	4.52±0.16^{a}
20	6.61±0.03^{e}	11.68±0.43^{g}	0.11±0.04^{f}	3.87±0.10^{bc}
30	6.69±0.01^{d}	13.47±0.23^{f}	0.16±0.01^{ef}	3.66±0.15^{bcd}
40	6.72±0.02^{cd}	14.89±0.16^{e}	0.19±0.01^{e}	3.55±0.15^{cde}
50	6.78±0.07^{c}	15.36±0.20^{de}	0.21±0.04^{ef}	3.24±0.21^{ef}
60	6.86±0.04^{b}	15.92±0.22^{cd}	0.26±0.03^{d}	2.91±0.17^{f}
70	6.89±0.02^{ab}	16.59±0.27^{bc}	0.32±0.06^{c}	3.32±0.33^{de}
80	6.92±0.02^{ab}	16.86±0.17^{b}	0.46±0.02^{b}	3.68±0.27^{bcd}
90	6.95±0.07^{a}	17.76±0.43^{a}	0.57±0.04^{a}	3.98± 0.23^{b}

Means with the same superscript letters within a column are not significantly different at P < 0.05. Values are means of triplicate determinations ± standard deviation.

Total volatile bases-nitrogen (TVB-N) of refrigerated and frozen stored samples is depicted in Table 1 and 2. The initial TVB-N value of fresh fish fillet was 7.24±0.35mg N/100 g muscle. The TVB-N value of the fillet stored at 4^{0}C increased significantly on 3rd day and it rose up to 25 mg at 6th day which reached to 33.23±0.48 at the last day of refrigeration which can be considered acceptable. Similar findings on refrigerated Nile tilapia fillets were reported from the study of Khalafalla et al. (2015). Abouel-Yazeed (2013) found unacceptable level of TVB-N values in tilapia fish stored at 4˚C in control condition on day 6 till the end of the storage period (day 15).

In the current study, the concentration of TVB-N increased during frozen storage from 7.24±0.35 to 17.76±0.43 mg N/100 g (Table 2). The TVB-N content of fish fillets increased progressively during storage in the frozen temperature but did not attain the unacceptable limit (35 mg N/100 g) for human consumption on the 90th day of storage. Based on the standard unacceptable limit for the TVB-N values, the fish samples were acceptable for human consumption up to 90 days of storage. As compared with the initial value, a significant (P < 0.05) increase was observed in this parameter on day 10, followed by an increasing trend up to the end of the storage period (Table 4).

As for many fish species, the formation of TVB-N for tilapia fish fillets in the present study increased with the time of frozen storage and by the end of the storage period (day 90), a significantly (P < 0.05) higher value of 17.76±0.43 mg N/100 g was detected for TVB-N in the fish sample (Table 2). The findings of the present study are in agreement with the findings of Emire and Gebremariam (2010). Similar results were reported by Ola and Oladipo (2004) for croaker and by Xue (2000) for yellowtail. A similar increase in TVB-N has been also reported in iced cuttlefish by Subramanian (2007). Slight differences may be due to the differences in the types of species, catching season and region, age and sex of the fish (Sadok et al., 1996).

PV

The peroxide value (PV) is a very important characteristic of lipid quality which provides a quantitative measure of hydroperoxide (ROOH) levels. The measurement of hydroperoxides provides an estimate of the overall oxidation status for lipids and lipid-containing foods especially in the primary phase of oxidation, generally known as the induction period

(Emir Çoban and Patir, 2013). The hydroperoxide subsequently decompose and turn into secondary products such as hydrocarbons, alcohols, aldehydes, and ketones (Gardner, 1989). For estimating the secondary oxidation, the most common method is titrimetric and colorimetric, used for monitoring the oxidation state of lipids include the peroxide value (PV), anisidine value (AV), 2-thiobarbituric acid reactive substances (TBARS), carbonyl value, and total polar compounds (Fritsch, 1981).

The mean PV value of refrigerated and frozen stored tilapia fillets is depicted in Table 1 and 2, respectively. The initial PV value of fresh tilapia fish fillet was 0.02±0.01 meq/kg. Alparslan et al. (2016) reported the primary PV in shrimps was 1.02 meq O_2/kg at the Control condition which is higher than tilapia. At 4^0C temperature storage, significantly the highest (3.48±0.35 meq/kg) PV recorded on day 6 and then it fell gradually and at day 12 it was 1.93 meq/kg. There was no significant difference in PV of refrigerated stored fillets between 3rd and 12th day. There was a slow growth of PV concentration with increasing storage time, after 90 days at frozen storage. At the end of the frozen storage period the value of PV reached 0.02±0.01 to 0.57±0.04 meq/kg. Boran et al. (2006) estimated the limit of peroxide value for quality and acceptability of oils for human consumption is 8 meq/kg. From the findings of the present study, it is evident that the PV value of tilapia fillets stored in refrigerated and frozen storage is in acceptable limits throughout the storage periods.

TPC

Total plate count (TPC) is used as an acceptability index for fish products because of the effect of bacteria in spoilage. Yearly, almost thirty percent of world's food production is lost due to microbial spoilage. The changes in TPC with 4^0C and -18^0C storage time in all groups were presented in Table 1 and 2, respectively. At refrigerator temperature the initial quality of the fish used in the study was good, as indicated by a low initial bacterial count (4.68±0.26 log cfu /g), which further significantly increased at the next two observation. The significantly highest (7.54±0.28 log cfu /g) TPC recorded at 12th The initial bacterial count of the untreated double filleted sardine was higher than the values reported by Ababouch et al. (1996) for fresh sardines, which could be due to the handling in preparation of double filleted sardine. With the storage period, the counts showed an increasing trend. Ozogul et al. (2017) reported that the initial TMAB count for the control group of cold-stored sea bass fillets was 2.97 log cfu/g and increased with the storage period, reaching to maximum value of 11.13 log cfu/g on the last day of the storage (day 12). In this respect, ICMSF (1986) stated that the upper acceptability limit of total viable bacterial count in fresh fish is 7 log CFU/g flesh, and 6 log CFU/g is the maximum permissible limit of APC recommended by EOS (2005) in chilled fish. At the beginning of the frozen storage, TBC was determined as 4.68±0.26 log cfu/g and after 10 days it was not significantly differed. The lowest value estimated 60th days.

Table 3. Sensory scores of tilapia fillet stored at 4^0C for 12 days

Storage period (Days)	Parameter				
	Colour	Odour	Taste	Texture	Overall acceptability
0	$9.00±0.00^a$	$8.77±0.22^a$	$8.83±0.10^a$	$8.73±0.15^a$	$8.82±0.06^a$
3	$8.09±0.30^b$	$7.78±0.15^b$	$7.55±0.34^b$	$6.86±0.28^b$	$7.59±0.31^b$
6	$7.36±0.22^c$	$6.41±0.39^c$	$6.39±0.23^c$	$5.76±0.10^c$	$6.40±0.17^c$
9	$5.79±0.10^d$	$5.45±0.27^d$	$5.36±0.53^d$	$5.03±0.18^d$	$5.63±0.13^d$
12	$4.97±0.18^e$	$4.68±0.24^e$	$4.17±0.17^e$	$3.97±0.29^e$	$4.36±0.25^e$

Means with the same superscript letters within a column are not significantly different at $P < 0.05$. Values are means of triplicate determinations ± standard deviation.

Sensory evaluation

Sensory evaluation is the most popular way of assessing the freshness of fish. It is fast, simple, and provides immediate quality information (Reineccius, 1991). The sensory evaluation of the product is an important quality criterion by which the consumer can judge the acceptability of product appearance. Sensory score for the attributes like appearance, color, odor and overall acceptability of the of Tilapia fillets during 16 days of cold storage (4^0C and -18^0C) are shown in Table 3 and 4, respectively. All the sensory attributes scores showed a declining trend. It is well known that fish spoilage causes the subsequent development of strongly fishy, sour and putrid odours, and fish are then distinctly rejected for consumption by any taste panel.

Table 4. Sensory scores of tilapia fillet stored at -18^{0}C for 90 days

Storage period (Days)	Parameter				
	Colour	Odour	Taste	Texture	Overall Acceptability
0	9.00±0.00^a	8.77±0.22^a	8.83±0.10^a	8.73±0.15^a	8.82±0.06^a
10	8.72±0.17^b	8.57±0.09^a	8.69±0.07^a	8.27±0.25^b	8.63±0.09^a
20	8.49±0.15^c	8.29±0.14^b	8.47±0.11^b	8.10±0.20^{bc}	8.29±0.13^b
30	8.18±0.26^d	8.04±0.10^c	8.25±0.17^c	7.85±0.33^c	8.02±0.28^c
40	8.01±0.16^d	7.61±0.16^d	8.02±0.22d	7.04±0.28^d	7.78±0.09^c
50	7.73±0.19^e	7.53±0.18^d	7.91±0.10^d	6.84±0.21^{de}	7.47±0.17^d
60	7.33±0.23^f	7.28±0.19^e	7.61±0.14^e	6.57±0.23^e	7.23±0.18^d
70	7.11±0.12^{fg}	6.79±0.26^f	7.00±0.27^f	6.21±0.24^f	6.68±0.37^e
80	6.89±0.11^{gh}	6.51±0.17^g	6.80±0.21^f	6.09±0.19^f	6.49±0.23^e
90	6.67±0.22^h	6.17±0.31^h	6.36±0.16^g	5.73±0.31^g	6.16± 0.18^f

Means with the same superscript letters within a column are not significantly different at P < 0.05. Values are means of triplicate determinations ± standard deviation.

In this evaluation test, frozen temperature showed high acceptability (P <0.05) in most of the studied sensory characteristics throughout the storage period than the refrigerator temperature. Sample was considered to be acceptable for human consumption until the sensory score reached 4 (Ojagh et al., 2010). At the present study, Acceptability reached at 4.36±0.25 on the 12th days in 4^{0}C storage. However, without the highlighted day never the acceptability as low near 4 estimated throughout the refrigerator and frozen experimented period.

CONCLUSION

The production of tilapia fillets could be a promising option for fish farmers in Bangladesh looking to improve profits by increasing market acceptability and optimizing tilapia utilization. Proper fillet preparation and storage are important for a higher-quality product. The shelf-life of tilapia fillet stored in refrigerated conditions was found to be 9 days in this research. Frozen fillet, on the other hand, had a shelf life of at least 90 days. As a result, future research should concentrate on the shelf-life of fillets that have been frozen for a longer period of time. Furthermore, government and non-government initiatives are critical for providing fillet preparation training as well as the requisite monetary and technical support to fish farmers and entrepreneurs, which will aid in ensuring better tilapia utilization and increasing market acceptability.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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