

ORIGINAL ARTICLE

Assessment of ghee adulterated with oils and fats in Bangladesh

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ABSTRACT

Objective: This study aimed at determining the existence of oils and fats in ghee manufactured in Bangladesh and to validate the nature of the impurity.

Materials and Methods: In this study, a ghee sample was prepared in the laboratory by following standard methods and was used as a control sample. On the other hand, 19 ghee samples, including five branded samples (B1–B5), and 14 local samples (L1–L14) were collected from different manufacturers. The ghee samples were assessed for fat composition, Reichert Meissl (RM), saponification, Polenske, acid, Kirschner, and butyro refractometer (BR) values. To validate the ghee samples, vegetable oils and body fats were mixed in different ratios and then analyzed.

Results: All the branded samples contained more than 99.5% fat, but only three local samples showed more than 97% fat. Admixing of soybean oil and coconut oil in different ratios showed the RM value from 1.57 ± 0.09 to 4.14 ± 0.21 , whereas incorporation of hydrogenated vegetable oils and tallow showed 6.36 ± 0.03 to 14.10 ± 0.14 . Nine local samples revealed RM values similar to external fat admixed samples. B2, B4, B5, L2–L8, and L10–L14 samples' saponification values differed from the standard limits. Polenske, acid, Kirschner values and BR reading for L4, L6, L7, L8, L10, L12, L13, and L14 showed the worst results. All values varied significantly ($p < 0.01$).

Conclusion: Local samples, L4, L6, L7, L8, L10, L12, L13, and L14, were assumed to be adulterated with external oils and fats. The quality of local ghee is questionable, as the samples contained more than 8% moisture, whereas pure ghee had less than 0.5% moisture.

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Introduction

Ghee is the top-rated fat-rich milk product in the Indian subcontinent. It is prepared on both small and large scales. One gram of ghee generates 9-Kcal of energy [1,2]. It has a long shelf life (around 1 year) under tropical storage conditions [3,4]. In Bangladesh, ghee is also a top-rated dairy product and rich in milk fat sources. Many people consume it regularly. Ghee can be formulated from butter or cream [5,6]. Both ripened or unripened cream can be used for the preparation of ghee. Butter may be either indigenous (deshi) or creamery [7,8]. Butter is prepared by fermenting whole milk to curd and then churning [9]. Finally, the cream or butter is boiled to prepare ghee [10].

The high price and limited supply of ghee lead to several malpractices. Admixing low-price fats or oils in ghee is done mainly for gaining more profit. Ghee is primarily

adulterated by vegetable/plant oils and animal body fats [11]. Adulterants whose physical texture resembles that of ghee were preferred, as they are difficult to detect visually. Hydrogenated vegetable oils (dalda) are considered as the most suitable material for adulteration [12]. It is not uncommon to encounter ghee samples containing over 50% hydrogenated vegetable oils. Other vegetable oils and fats which may be conveniently admixed with ghee are palm oil, coconut oil, and partially hydrogenated refined groundnut oil [13].

Similarly, animal body fats are also admixed as an adulterant in ghee preparation. Their detection is more critical as the physical and chemical test values are within the standard range of clarified butter. Moreover, characteristics of ghee prepared from cottonseed-fed buffaloes akin to animal fats admixed adulterated ghee samples [14]. Animal body

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fats are widely used in textiles, soaps, and other industries. These are byproducts of the meat industry, and a large volume is also imported, some of which have high possibilities to be misused for adulterating ghee [15]. In Bangladesh, the current retail average price of branded ghee is Bangladeshi Taka (BDT) 1,100–1,200/kg. On the other hand, many local/non-branded types of ghee are sold only by BDT 400–500/kg, indicating a considerable price difference.

There are several methods to detect the presence of fats/oils in ghee. Reichert Meissl (RM), saponification, Polenske, acid, Kirschner values, and butyro refractometer (BR) readings for milk fat are constant [16,17]. These methods precisely determine the existence of oils and body fats in ghee [18]. Therefore, we can detect whether oils and fats are present or absent by determining these values. Milk fat constants for RM, saponification, Polenske, acid, Kirschner and BR value are 17–35, 225–230, 1.2–2.4, 0.0–1.0, 20–26, and 37–44, respectively [19–21]. In this study, five branded and 14 local/non-branded samples were purchased from different markets and super shops of Gazipur, Bangladesh, and analyzed for chemical composition and adulteration detection. One control sample was prepared in the Dairy Science Laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) for comparison. Attempts were also made to identify the added agent of adulteration.

Materials and Methods

Ethical statement

Ethical consent was obtained from shopkeepers, but brand and trade names were kept hidden.

Collection and storage of samples

Five branded and 14 local ghee samples (prepared by non-branded industries/agents/manufacturers) were purchased from Jaydebpur Bazar, Tongi Bazar, and BSMRAU Employees Cooperative Society super shop, Gazipur, Bangladesh. Branded samples were designated as B1–B5, and local samples were defined as L1–L14. Then, samples were stored at room temperature [22]. One sample was taken from each manufacturing company, and three replications were done for each sample.

Preparation of ghee in the laboratory

Raw milk was collected from BSMRAU dairy farm. The cream was separated from the collected raw milk by a centrifugal cream separator (SURYODAY/EMCS 300 LPH; Suryoday Engineering Company, Gujarat, India). Then, the cream was taken in an iron pan for heating. It started melting at 30°C, and at 60°C all the cream was melted. As the temperature increased, contents became thicker and bubbles were formed. The content appeared as a clean

yellowish liquid and then filtered with a twofold muslin cloth. Finally, ghee was prepared. Ghee was prepared in the laboratory (positive control) to compare the quality of commercial ghee. This operation was carried out in a cool and convenient place, aside from direct sunlight, and completed within the least possible time [23]. This control sample was designated as A.

Validation of the adulterants

We collected some common adulterants to validate ghee, such as hydrogenated vegetable oil, tallow, coconut oil, and soybean oil. The adulterants were mixed with the pure ghee at different ratios to validate the presence of specific adulterants [15].

Determination of chemical composition

Moisture and fat percentages were determined by the Gerber method using the procedure described by the Food Safety and Standards Authority of India [24].

Determination of fat constants

RM, Polenske value, and Kirschner value

RM and Polenske values were analyzed using the standard methods described by the Food Safety and Standards Authority of India [24]. RM value is the unit of 1/10 ml normal alkali solution required to neutralize the water-soluble volatile fatty acids distilled from 5 gm of milk fat. Polenske value signifies volatile and water-insoluble fatty acids. Five grams of ghee is saponified by applying glycerol soda diluted with water and acid (H_2SO_4), and afterwards, steam distillation in a glass distillation apparatus (condenser) is done at a controlled rate. The cooled and condensed distillate is filtered. The water-soluble acids that pass through are calculated using titration with sodium hydroxide to quantify the RM value. On the contrary, the water-insoluble acids are collected on the filter paper and dissolved in ethyl alcohol, and titrated to measure the Polenske value.

Kirschner value is determined by adding 0.5 gm powder of silver sulfate to the neutralized solution obtained from RM procedure [25]. A flask containing neutralized solution is made to stand in the darkroom for 1 h and then the contents are filtered in the dark. 100 ml of the filtrate is transferred to a dry flask, after which 35 ml chilled distilled water, 10 ml diluted H_2SO_4 , and a loose aluminum wire (5 mm loop of 30 cm) are added. The flask is connected with a condenser, and 100 ml of distillate is collected and then titrated with NaOH solution.

Saponification value

Two grams of ghee is taken into a conical flask and dissolved in 5 ml distilled ethyl alcohol. 25 ml of 0.5N potassium hydroxide (alcoholic) is mixed together with it. The

flask is attached with a condenser and refluxed till the liquid becomes clear. At the same time, without taking ghee a blank experiment is done. Then, both the flasks are cooled down and phenolphthalein is added and titrated with 0.5N HCl until the color disappears [24]. Saponification value of ghee = (blank-ghee) × 0.5 × 56/weight of the sample; here, 56 is the equivalent mass of potassium hydroxide.

Acid value

Thirty grams of ghee is taken into a round bottom flask, and 50 ml of 95% ethyl alcohol is added. The flask is heated until the content becomes clear and transparent. Then, it is gently shaken to dissolve all free fatty acids. Finally, the sample is titrated with 0.1N NaOH solution. Here, 1 ml of N-sodium hydroxide = 1 ml of N-oleic acid = 0.282 gm of oleic acid [24]. Acid value = no. of ml of 0.1N NaOH × 2.82/weight of the sample.

BR reading

The ghee sample was filtered through Whatman filter paper and placed in a glass bottle. Before taking the BR reading, it was confirmed that the sample's temperature was around 40°C. Finally, BR reading was determined by BR (Digital Butyro refractometer; PR-BUTYRO, Atago Co. Ltd., Japan) [24].

Statistical analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences (IBM@version25) statistical package. All results are represented as mean ± S.E. For comparison, a one-way analysis of variance was carried out. Differences were considered to be statistically significant when *p*-value was less than 0.01.

Results and Discussion

Chemical composition

Pure ghee contains 99.5% milk fat and the rest are considered as moisture percentage [19,20]. The mean fat value for the laboratory-prepared control ghee was 99.87 ± 0.03, which was in the range of the ideal ghee. The percentage of fat of branded ghee varies from 98.70% to 99.83%. The mean fat value for B5 was 98.70 ± 0.40, and for B4 it was 98.70 ± 0.10. The value of these samples were slightly lower than the standard value. The remaining three samples mean fat values were within the limit of pure ghee (Table 1). The local samples' fat and moisture percentages varied remarkably. For local samples, the highest fat percentage was found in sample L1, 98.13%, and the lowest fat percentage for sample L11 was 91.33%. The mean fat value for sample L1 was 98.13 ± 0.03 and for L11 it was 91.33 ± 0.12. Subsequently, the mean moisture value for sample L1 was 1.87 ± 0.03

Table 1. Chemical composition of analyzed samples.

Samples	Parameters measured	
	Fat (%)	Moisture (%)
A	99.87 ^a ± 0.03	0.13 ⁱ ± 0.03
B1	99.60 ^a ± 0.10	0.40 ^j ± 0.10
B2	99.57 ^a ± 0.07	0.43 ⁱ ± 0.07
B3	99.83 ^a ± 0.03	0.17 ⁱ ± 0.03
B4	98.70 ^b ± 0.10	1.30 ^h ± 0.10
B5	98.70 ^b ± 0.40	1.30 ^h ± 0.40
L1	98.13 ^c ± 0.03	1.87 ^e ± 0.03
L2	97.27 ^d ± 0.12	2.73 ^f ± 0.12
L3	97.47 ^d ± 0.12	2.53 ^f ± 0.12
L4	94.13 ^f ± 0.09	5.87 ^d ± 0.09
L5	93.13 ^g ± 0.03	6.87 ^c ± 0.03
L6	92.53 ^h ± 0.15	7.47 ^b ± 0.15
L7	93.42 ^g ± 0.04	6.58 ^c ± 0.04
L8	93.07 ^g ± 0.38	6.93 ^c ± 0.38
L9	94.53 ^{ef} ± 0.09	5.47 ^{de} ± 0.09
L10	93.43 ^g ± 0.12	6.57 ^c ± 0.12
L11	91.33 ⁱ ± 0.12	8.67 ^a ± 0.12
L12	91.47 ⁱ ± 0.20	8.53 ^a ± 0.20
L13	94.73 ^e ± 0.12	5.27 ^e ± 0.12
L14	92.37 ^h ± 0.06	7.63 ^b ± 0.03
LSD	0.421	0.421
LS	**	**

LS = Level of significance; LSD = Least significant difference; A = Control sample (prepared in lab); B1–B5 = Samples manufactured by five different branded industries in Bangladesh; L1–L14 = Samples prepared by 14 different local manufacturers in Bangladesh, In a column, means of the similar letter (s) do not significantly differ.

** = Significance at 1% level.

and for L11 it was 8.67 ± 0.12. Although local samples (L1, L2, and L3) contain a better amount of fat percentage, the remaining 11 samples' fat percentage was not good (Table 1). Significant differences were found among all the samples (*p* < 0.01). The inclusion of soybean oil and buffalo body fat plunged the fat percentage in pure ghee up to 5% [26].

Fat constants

RM value

Butyric acid makes up around 75% and caproic acid makes up 25% of the RM values. The Food Safety and Standards Authority of India rules determine that the RM value is the prime quality parameter of ghee. In milk fat, the RM value ranges from 17.00 to 35.00, which is much higher than all other fats and vegetable oils [19]. The mean RM value for the control ghee was 22.46 ± 0.69, which was in the standard

RM value range. The mean RM value of branded ghee varied from 21.10 ± 0.38 to 26.60 ± 0.28 . These values were within the range of the standard RM value for pure ghee (Table 2). RM value indicates whether the ghee is prepared from milk fat or not [15]. It can be said that the branded industries tried to make ghee from milk fat. Local samples' RM value varied significantly. The highest RM value was found in sample L9 (34.29 ± 0.06) and the lowest was in L7 (3.24 ± 0.05). We mixed different adulterants to validate the samples; for example, hydrogenated vegetable oils, tallow, coconut oil, and soybean oil in different ratios (i.e., 5%, 10%, and 20%). Mean RM values for L4, L5, L8, L12, and L14 were found within 6.40 ± 0.21 – 14.01 ± 0.21 . The addition of hydrogenated vegetable oils and tallow in ghee in different ratios showed the mean RM value to be from 6.36 ± 0.03 to 14.10 ± 0.14 . Mean RM values for L6, L7, L10, and L13 varied from 3.24 ± 0.05 to 4.72 ± 0.08 . The addition of coconut oil and soybean oil in different ratios showed the mean RM value to be from 1.57 ± 0.09 to 4.14 ± 0.21 (Table 3). Statistical

analysis showed that there were significant differences among all the samples. This experiment is in agreement with the work of Jirankalgikar et al. [12] and Ayari et al. [27]. Using more than 10% of vegetable oil adulteration significantly reduced the RM value [28]. Hazra et al. [29] described that cow ghee was admixed with animal body fat at 10% and buffalo ghee at 20%, which can be detected by the RM technique. They found a reduced RM number in their experiments [29]. Pure cow ghee adulterated with soybean oil at 5% or above plunged the RM value [27].

Table 3. Validation of the RM value with admixing different adulterants.

Ratios	Hydrogenated vegetable oils	Tallow	Coconut oil	Soybean oil
5%	14.10 ± 0.14	12.29 ± 0.36	3.27 ± 0.10	4.14 ± 0.21
10%	9.39 ± 0.05	9.30 ± 0.13	2.01 ± 0.09	2.23 ± 0.11
20%	7.25 ± 0.38	6.36 ± 0.03	1.29 ± 0.13	1.57 ± 0.09

Table 2. Milk fat constants of analyzed samples.

Samples	Parameters measured					
	RM value	Saponification value	Polenske value	Acid value	Kirschner value	BR Reading
A	$22.46^a \pm 0.69$	$228.07^d \pm 0.91$	$2.27^b \pm 0.03$	$0.75^{gh} \pm 0.01$	$24.43^c \pm 0.95$	$40.33^i \pm 0.33$
B1	$22.77^a \pm 0.48$	$228.94^d \pm 0.22$	$1.92^c \pm 0.08$	$0.69^{hi} \pm 0.04$	$22.89^f \pm 0.34$	$43.00^j \pm 0.00$
B2	$22.84^a \pm 0.35$	$234.47^b \pm 0.13$	$2.22^b \pm 0.01$	$0.60^{hi} \pm 0.02$	$21.23^e \pm 0.26$	$40.33^i \pm 0.33$
B3	$23.93^d \pm 0.31$	$224.61^e \pm 0.14$	$2.23^b \pm 0.05$	$0.61^{hi} \pm 0.04$	$27.26^c \pm 0.06$	$47.00^g \pm 0.00$
B4	$26.60^c \pm 0.28$	$232.29^c \pm 0.28$	$1.55^d \pm 0.14$	$1.21^f \pm 0.07$	$24.96^{de} \pm 0.32$	$44.00^h \pm 0.00$
B5	$21.10^f \pm 0.38$	$231.58^c \pm 0.14$	$2.71^a \pm 0.05$	$0.55^j \pm 0.05$	$18.15^h \pm 0.13$	$41.00^j \pm 0.58$
L1	$22.81^a \pm 0.11$	$227.79^d \pm 0.25$	$1.19^e \pm 0.03$	$0.71^h \pm 0.03$	$22.10^f \pm 0.16$	$47.00^g \pm 0.00$
L2	$18.36^e \pm 0.04$	$211.05^f \pm 0.26$	$0.69^{gh} \pm 0.04$	$0.54^i \pm 0.03$	$25.50^d \pm 0.27$	$35.67^k \pm 0.33$
L3	$15.22^h \pm 0.07$	$223.38^c \pm 0.15$	$0.54^h \pm 0.03$	$0.85^{gh} \pm 0.05$	$20.56^e \pm 0.13$	$34.00^l \pm 0.00$
L4	$11.32^l \pm 0.05$	$210.48^f \pm 1.08$	$0.74^g \pm 0.03$	$1.15^f \pm 0.02$	$11.51^j \pm 0.09$	$52.00^e \pm 0.58$
L5	$14.01^i \pm 0.21$	$208.56^e \pm 0.73$	$1.26^e \pm 0.03$	$0.58^{hi} \pm 0.03$	$14.54^i \pm 0.17$	$49.00^f \pm 0.00$
L6	$3.31^n \pm 0.12$	$159.68^k \pm 0.21$	$0.93^{fg} \pm 0.02$	$0.87^g \pm 0.04$	$32.26^b \pm 0.10$	$52.33^e \pm 0.67$
L7	$3.24^n \pm 0.05$	$156.19^m \pm 0.06$	$2.64^a \pm 0.10$	$1.69^{cd} \pm 0.01$	$34.40^a \pm 0.24$	$55.00^c \pm 0.58$
L8	$7.66^k \pm 0.22$	$245.53^a \pm 0.19$	$2.62^a \pm 0.06$	$1.64^d \pm 0.05$	$11.45^j \pm 0.17$	$56.00^b \pm 0.00$
L9	$34.29^b \pm 0.06$	$228.95^d \pm 0.15$	$0.84^{fg} \pm 0.04$	$2.25^b \pm 0.06$	$9.18^l \pm 0.31$	$43.67^{hi} \pm 0.33$
L10	$4.72^m \pm 0.08$	$158.30^l \pm 0.55$	$0.82^{fg} \pm 0.02$	$2.39^a \pm 0.03$	$14.63^i \pm 0.35$	$59.33^a \pm 0.33$
L11	$39.62^a \pm 0.35$	$205.37^h \pm 0.12$	$0.87^{fg} \pm 0.05$	$1.11^f \pm 0.06$	$26.62^c \pm 0.16$	$43.33^{hi} \pm 0.33$
L12	$6.40^j \pm 0.21$	$193.86^i \pm 1.16$	$0.81^{fg} \pm 0.05$	$1.81^c \pm 0.14$	$10.26^k \pm 0.09$	$53.33^d \pm 0.33$
L13	$4.63^m \pm 0.09$	$210.91^f \pm 0.39$	$0.36^i \pm 0.02$	$1.49^e \pm 0.04$	$11.84^j \pm 0.24$	$56.00^b \pm 0.58$
L14	$11.33^j \pm 0.28$	$190.30^j \pm 0.09$	$0.97^f \pm 0.11$	$1.88^c \pm 0.04$	$11.67^j \pm 0.11$	$56.33^b \pm 0.33$
LSD	0.754	1.325	0.160	0.137	0.805	0.988
LS	**	**	**	**	**	**

LS = Level of significance; LSD = Least significant difference; A = Control sample (prepared in the lab); B1–B5 = Samples manufactured by five different branded industries in Bangladesh; L1–L14 = Samples prepared by fourteen different local manufacturers in Bangladesh. In a column, means of the similar letter (s) do not significantly differ.

** = Significance at 1% level.

Saponification value

When milk fat is adulterated with either animal or vegetable fat, the saponification value changes. The saponification value for milk fat varies from 225.00 to 230.00 [30]. The mean saponification value for the control ghee was 228.07 ± 0.91 . The mean saponification value of branded ghee varied from 224.61 ± 0.14 to 234.47 ± 0.13 . Samples B2, B4, and B5 slightly exceeded the range. The saponification value for local samples also showed variation. The mean saponification value for local ghee ranged from 156.19 ± 0.06 to 245.53 ± 0.19 . L2, L4–L7, and L10–L14 samples' mean saponification values were below 225 (Table 2). Enriching ghee with animal body fat and vegetable oil somewhat changed the saponification value [31]. For animal fats, plant oils, and hydrogenated fats, the value ranged from 192.00 to 203.00, 170.00 to 197.00, and 197.00 to 199.00, respectively. Coconut oil and palm oil show higher saponification values ranging from 243.00 to 262.00 [32,26].

Polenske value

The mean Polenske value for the control ghee was 2.27 ± 0.03 . The mean Polenske value of branded ghee samples varied from 1.55 ± 0.14 to 2.71 ± 0.05 . Polenske value for milk fat varies from 1.2 to 2.4 [27]. Sample B5 slightly exceeds the range. Polenske value for local samples also showed dissimilarities. The mean Polenske value for local ghee ranged from 0.36 ± 0.02 to 2.64 ± 0.10 . The highest value was found in sample L7 and the lowest was in L13. Except for L1 and L5 samples, no sample was found within the standard range. L2–L4, L6, L9, and L10–L14 samples differed from the ideal range (Table 2). These indicated additions of other fats or oils rather than milk fat. Statistical analysis showed that there were significant differences among all the samples. Polenske value for vegetable oils and body fats are less than 1.0. Butter oil mixed with vegetable oil and body fat considerably reduced the Polenske value [33]. Patel [28] reported that the overall range of Polenske value was 1.30–1.80 for both unadulterated cow and buffalo ghee. When 15% of animal fat is added, the range deviated to 1.0 compared to the standard range [28].

Acid value

The mean acid value for the control ghee was 0.75 ± 0.01 . The mean acid value of the branded ghee varied from 0.55 ± 0.05 to 1.21 ± 0.07 . For ghee, the acid value ranged from 0.0 to 1.0 [34]. Only sample B4 exceeded the range. The acid value measures the ghee's hydrolytic rancidity, indicating the edibility of milk fats [12]. The acid value for local samples showed variations. The highest value was found in sample L10, which was 2.39 ± 0.03 , and the lowest was

in L2, which was 0.54 ± 0.03 . L4, L7, and L8–L14 samples' mean saponification values exceeds 1.0 (Table 2). The acid value measured the breakdown of triglycerides to free fatty acids. This value gets overrated if another vegetable or animal body fat is admixed [12].

Kirschner value

Kirschner value ranges from 20.00 to 26.00 in milk fat [35]. Sample B3 slightly exceeded the range, but B5 was under the range (Table 2). Kirschner value for local samples also showed differentiation. The mean Kirschner value for local ghee ranged from 10.26 ± 0.09 to 34.40 ± 0.24 . The highest value was found in sample L7 and the lowest was in L12. However, L4–L8, L10, and L12–L14 samples' mean values exceeded the range. Incorporation of vegetable and animal fat in clarified butter fat plunged the standard Kirschner value [36].

BR reading

The mean BR reading for sample A was 40.33 ± 0.33 , which is in the normal range. The mean BR reading of the branded ghee was from 40.33 ± 0.33 to 47.00 ± 0.00 . The B3 sample exceeded the range. The standard BR value for milk fat ranges from 37.00 to 44.00, whereas for vegetable oils and fat, it is more than 50.00. Moreover, the incorporation of cottonseed oil sometimes increases the BR reading [29]. The BR reading for local ghee samples differed; of which, L4, L6, L7, L8, L10, and L12–L14 varied significantly (Table 2). These samples' mean BR readings were higher than 50.00. Statistical analysis showed that there were significant differences among the samples. These results are in agreement with Gosewade et al. [16]. BR reading or refractive index increases with unsaturation and number of carbons as well as fatty acid length. It included animal fats and hydrogenated oils at 5–25% in butter oil, triggering BR reading [37,38]. Patel [28] reported that the addition of vegetable oil to ghee up to a 10% level could easily be detected by BR [28].

Conclusion

The chemical composition of branded ghee samples was almost excellent. No impurity was found in the five branded samples. Most of the local ghee samples did not contain the standard amount of fat percentage. Among the 14 local samples, eight were adulterated with oils and fats.

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Conflict of interest

The authors declare there is no conflict of interests related to any matter of this research article.

Authors' contribution

SA, MMR, and MAH designed the research plan. SA and MAH collected the samples. SA analyzed the samples. SA, MMR, and MAH prepared and edited the manuscript. All authors critically revised and approved the research paper before the final submission.

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