Physical Education, Health and Social Sciences

https://journal-of-social-education.org

E-ISSN: 2958-5996
P-ISSN: 2958-5988

# Production and Evaluation of Functional Probiotic Ice Cream

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*DOI:https://doi.org/10.63163/jpehss.v3i1.140*

## Abstract

In the present study an approach was made to produce functional probiotic ice cream by using the four probiotic organisms namely type-1 (Sterptococcus thermophilus), type-2 (Lactobacillus bulgaricus), type-3 (Bifidobacteria), type-4 (Saccharomyces), type-5 (Mixture of Streptococcus thermophilus, Lactobacillus bulgaricus, Bifidobacteria and Saccharomyces and type-6 (Plain ice cream; without probiotic organisms) using conventional ice cream-making method. All types were assessed for proximate and rheological analysis. Total solids and carbohydrate contents were found slightly high ($P<0.05$) in type-III and low in type-IV; whereas in other types non-significant variation was noted. Fat content (%) was observed high in type-VI followed by IV, II, III, V and Type-I respectively. Remarkably high protein content (%) marked for the type I followed by type-II, III, IV, V and VI, respectively. Similarly, high ash content (%) was noted in Type-I and V while low in the type-II probiotic ice cream. Acidity (%) and pH value vaired among all the probiotic isecComparatively more expansion in volume (overrun %) appeared in the type III and little in type- II and VI ice cream. Type-V and I ice cream melted quickly while slowly in the type VI ice cream. On the basis of composition, rheological features, physical attributes Sacchromyces and Bifidobacterial probiotic containing types of ice cream were found better.

**Keywords:** Probiotic, Production, Overrun, Melt down rate, Ice-cream

## Introduction

Probiotics are called living microorganisms conferring beneficial effects on the consumer by harmonizing microbial flora of GIT system (Mahmoudi et al., 2015; FAO/WHO, 2001). Probiotic dairy foods comprise one of the most important and advanced segments of functional food industry (Mahmoudi et al., 2015) because the dairy foods are the cheif probiotic carrier (Champagne et al., 2018) enriched numerous therapeutic benefits to end users (Sarfraz et al., 2019; Shafi et al., 2019; Vasconcelos et al., 2019) and technological parameters (Guimaraes et al., 2020) among those, functional ice cream/frozen desserts and bio-yoghurt are well known probiotic dairy foods (Mohammadi et al., 2011). Among the dairy foods ice cream is typically widely consumed by all age groups throughout the world due to its pleasant mouth feel, cooling properties as well as its rich nutritional worth (Durmaz et al., 2020). Its constituents, such as milk, sweeteners, emulsifiers and stabilizers, may have a great impact on the final quality (Goktas et al., 2022). It possesses a huge potential and could be utilized as beneficial dairy product due to the consumer's more preference; hence ice cream can be utilized

with modifications in the form of fruits rich in phenolics, prebiotics compounds and/or probiotics (Ozturk et al., 2018). Additional aspect rising the choice of ice cream as a probiotic carrier dairy commodity is the protecting features of milk macro-nutrients and other constituents such as minerals and vitamins etc. for the viability of probiotics as highlighted earlier (Cruz et al., 2009). Probitotic microorganisms must possess some unique characteristics like combating to alimentary canal environment, auto-aggregation and acting as beneficial microbiota along with being non-pathogenic in nature to be ranked as probiotics (Gut et al., 2018). Among the beneficial bacteria, Bifidobacteria and Lactobacillus species are frequently consumed as probiotics in the food commodity (Holzapfel, et al., 2006, Lacerda et al., 2022). Conversely, among the yeast species Saccharomyces boulardii is the only choice probiotic marketed enriched with probiotic qualities (Tomicic et al., 2016, Lacerda et al., 2022). The needs for therapeutic food commodities have been increased recently. Probiotic foods are the food products having enough amounts of live probiotic microorganisms which support useful effects on the consumers. Keeping in view, the demand of ice cream, therapeutic effect of probiotic organisms, an approach has been made to explore the impact of probiotic bacteria on the physicochemical and rheological quality of ice cream.

## Materials and Methods

### Experimental design

Buffalo milk was distributed into six equal portions and used for the production of probioitc ice cream (three trials) i.e. type-1 (Sterptococcus thermophilus), type-2 (Lactobacillus bulgaricus), type-3 (Bifidobacteria), type-4 (Saccharomyces), type-5 (Mixture of Streptococcus thermophilus, Lactobacillus bulgaricus, Bifidobacteria and Saccharomyces and type-6 (Plain ice cream; without probiotic organisms) by using conventional ice cream-making method.

### Probiotic organisms

Probiotic organisms for the production of probiotic ice creams used in this study i.e. Sterptococcus thermophilus, Lactobacillus bulgaricus, Bifidobacteria and Saccharomyces were purchased commercially from China.

### Preparation of probiotic ice cream

Graphical experimental layout is shown in Figure 1. Initially the procured buffalo milk was filtered/clarified then it was pasteurized at 90°C for ten minutes and the ice cream constituents (i.e. Full cream powder, sugar, emulsifier starch /sahlep and stabilizer etc.) were added according to protocol. The ice cream mix was cooled to 45±2°C. The ice cream mixture was inoculated with respective probiotic cultures (for fermentation) except type VI (plain ice cream), stirred at room temperature (for 45 min).

### Aging of functional ice cream

Each type ice cream mix was kept overnight at 4°C for aging purpose.

### Freezing of functional ice-cream mix

Mixture of ice cream was added in the ice-cream machine and allowed to freeze for~30 min. The freezed probiotic ice-cream in plastic cups (~100ml quantity) was kept in the freezer (Dawlance Refrigerator, Model No. 9188WBM) at -20±1°C for hardening and stored for analysis.

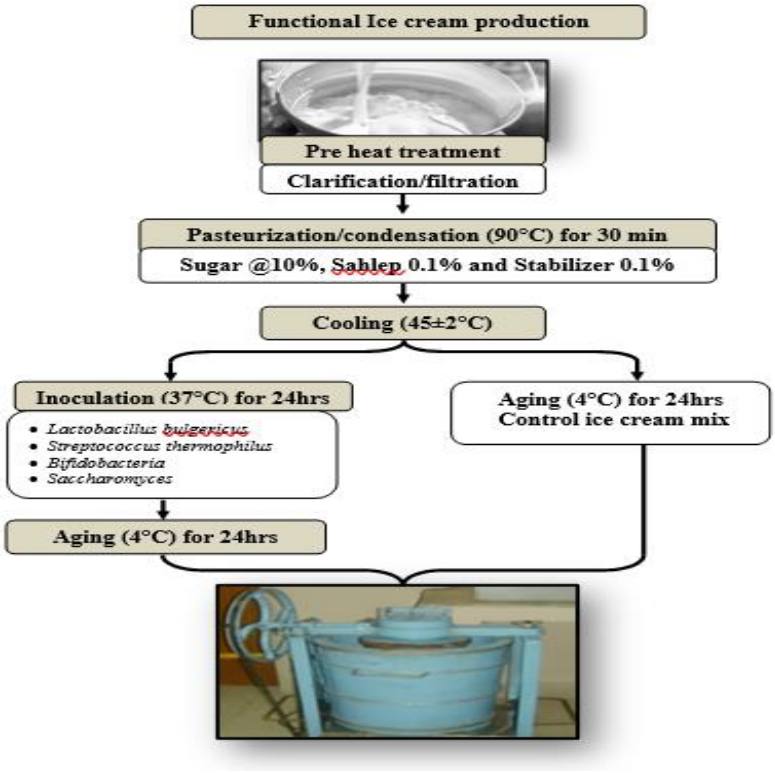
### Physicochemical Analysis

Total solid (TS), protein, fat, ash and carbohydrates content, acidity (%) and pH value, of probiotic ice cream was analyzed as per method quoted in AOAC (2000).

**Rheological (Melt-down rate and Overrun %) properties**

Expansion (overrun) into the product during the freezing process of the ice cream samples was observed according to Rinaldi et al. (2014); whereas the melt-down rate was noted according to standard protocol (Guner et al., 2007).

**Figure 1: Production of probiotic ice cream**




## Results and Discussion

### Chemical composition of probiotic ice cream

In our study an approach was made to produce the functional probiotic ice cream from buffalo milk admixed with probiotic organisms. Similarly, Caisip and Resubal (2001) and Jaswinder et al. (2006) also made yoghurt ice cream by incorporating probiotic into ice cream mixture at the time of allowing freezing. Overall composition of different types of probiotic ice cream samples is tabulated in Table-1. Results exposed that dry matter content in type-III probiotic ice cream was found high ($P<0.05$) 35.14±0.59 % whereas, low in type-IV 33.76±.53 % while the total solid content (%) of type-V 34.93±.61, type-II 34.90±0.82, type-VI 34.75±.52, and type-I 34.011±0.34%, respectively varied non significantly ($P>0.05$) with both type-III and type-IV probiotic ice cream. The results are in line with the observations of Owni et al. (2009), authors revealed that the dry matter content of ice cream in between 31.82 to 33.41%. Whereas, the current observations are not in agreement with those of Barnard et al. (1984) who found that the TS content of samples were in between 37.0 to 40.0%. This higher level of total solids content could be due to formulation/figuring of ice mixture, milk base which could cause increase in total solids contents in their study. However, Jaswinder et al. (2006) noted a remarkable decline in total solid content of ice cream supplemented with yoghurt base. Ozdemir (2019) reported total solids content >34%. Similarly, findings of Hassan and Barakat study (2018) also supported current findings they reported 35.51% total solids. Present results are also in line with the findings of Mangsi et al. (2011) and Kanta et al. (2018) they also made

and analyzed yogurt ice cream and mentioned a dry matter contents ~33%. The fat (%) in probiotic ice cream was observed with mean values of type-1, type-2, type-3, type-4, type-5 and type-6 as 10.71±0.25, 11.16±0.35, 10.97±0.26, 11.14±.33, 10.92±.36 and 11.81±.36%, respectively (Table 1). These observations are matching with that of Ayar et al. (2018), who noted in between 8.32 to 11.07%. Whereas, the findings of current research are not concurrent to the result of Mangsi et al. (2011) they observed 4.84% fat in the yoghurt ice cream. This inconsistency in the results could be due to variation in figuring of ice cream mix preparation because authors mentioned 30:70 yoghurt and ice cream mix; hence the yoghurt they used might be low in fat content. Similarly, Ozdemir (2019) who reported 8.10% fat in yoghurt and fruits formulated ice cream that might cause a slight decrease in fat content with the current observations. However, the mean value of fat examined in the present study may categorized it in regular class (10%) as mentioned by Aimee et al. (2001) who ranked the vanilla ice cream on the basis fat content as 10%(regular), 5%(light), 2.5%( low) and 0.4%( fat free).

Average protein content of different probiotic ice cream type-1, type-2, type-3, type-4, type-5 and type-6 was observed as 5.13±0.19, 5.06±0.28, 4.92±0.23, 4.73±.19, 4.79±0.21 and 4.28±0.27%, respectively (Table 1). Our results are divergent than that of Emata et al. (2001) they noted 3.72g/100g protein. This discrepancy in the current findings with the reported work could be credited with figuring of mixture i.e. in the present research naturally rich in protein content milk (buffalo's milk) was utilized as a basic ingredient in the ice cream mix preparation supposed to be the reason for higher percentage of protein. Furthermore, Bajad et al. 2016, higher acidity in the mix results in proteins destabilization that may cause significant decline in the protein content which supports present results where a slight decline in the protein content was noted in the probiotic yoghurt ice cream contrast to that of plain/simple frozen dessert. Moreover, our research results matching to that of observations of Nikhitha et al. (2022) mentioned 4.4 to 5.6% protein. It is of interesting to highlight that protein content in our study was as per specified standard reported by Bajad et al. (2016) they mentioned that ice cream inevitably possess at least 2.5% protein (milk origin), similarly Kenya standard (DKS 1517:2021) stated 3.5% and Punjab foods standards also specified protein <3.5% in frozen desserts.

**Ash content**

The ash contents (%) of probiotic ice cream samples differed significantly in all type of probiotic ice cream and average content of ash in probiotic ice cream type-1, type-2, type-3, type-4, type-5 and type-6 was observed as 0.97±0.02, 0.90±0.02, 0.94±0.02, 0.91±0.01, 0.97±0.01 and 0.95±0.02%, respectively (Table 1). Current findings of ash contents are matching with observations of Durak, (2006) reported that the ash values of ice creams made with the incorporation of yoghurt who found ash in between 0.59-0.92%. However, the highest ash value (1.27%) in the grape seed ice cream sample was observed by Kavaz et al. (2016).

**Carbohydrate contents**

Average content of carbohydrates in probiotic ice cream type-1, type-2, type-3, type-4, type-5 and type-6 was observed as 17.21±0.42, 17.79±1.07, 18.30±0.92, 16.71±0.72, 18.27±0.95 and 17.72±0.6 %, respectively (Table 1). The analysis of carbohydrates revealed that the carbohydrate percentage of buffalo milk was high. Nikhitha et al., (2022) reported slightly higher carbohydrate content (20.7 to 25.25%) in probiotic ice cream. However, the present results fall within the specified ISI standards i.e. not less than 15% sugars.

**Acidity (%)**

Average acidity values of probiotic ice cream type-1, type-2, type-3, type-4, type-5 and type-6 was observed as 0.22±0.004, 0.24±0.004, 0.37±0.003, 0.25±0.003, 0.43±0.008 and 0.16% ± 0.002, respectively (Table 1). The titratable acidity of the fermented ice creams (0.24–0.29%) was found in probiotic and synbiotic ice creams (Sarvestani et al., 2021**)** this vast discrepancy

in the current findings (0.16 to 0.37%) could be due to the ability of probiotic organisms to convert lactose into lactic acid (Sarvestani et al., 2021**)**. **Furthermore**, increased level of lactic acid is not enviable for the patrons since it might bring objectionable flavor/and taste. Another drawback of alleviated lactic acid contents during its production is as "higher developed acidity higher would be the degradation of casein content" (Goff & Hartel, 2013). **Authors are agreed with the reported work of** Turgut & Cakmakci (2009), who also reported that incorporation of cultured milk in the ice cream mix during its production influenced the lactic acid content which could be due to varying probiotic strains.

**pH value**

Average pH of probiotic ice cream type-1, type-2, type-3, type-4, type-5 and type-6 was observed as 6.11±0.04, 6.37±0.02, 6.2±0.04, 6.52±0.02, 5.92±0.03 and 6.45±0.06, respectively (Table 1). Even though pH value of supplemented cultured milk varied from 4.36 (L. reuteri) to 5.66 (L. acidophilus); whereas it was more in overall ice cream mixture, that could be because of the elevated buffering capability of the mixture. To such an extent, this could restrain the probiotic induced flavor. Saleem et al. (2005) observed the desired level of pH in probiotic ice cream mixture admixed with varying amount of fermented and also prepared by mixed at the rate of 25-50% of commercially fermented milk (L. acidophilus and B. bifidum); where it was ranged from 6.67 and 5.51 pH for plain and probiotic ice cream samples, respectively. The present findings are identical with previous reported work (Alamprese et al., 2005); Abghari et al (2011); Niamah, Al-Manhel, & Al-Sahlany, 2018; Sarwar et al., 2021) highlighted decline pH in probiotic containing ice cream.

**Table 1: Physicochemical characteristics of probiotic ice cream**

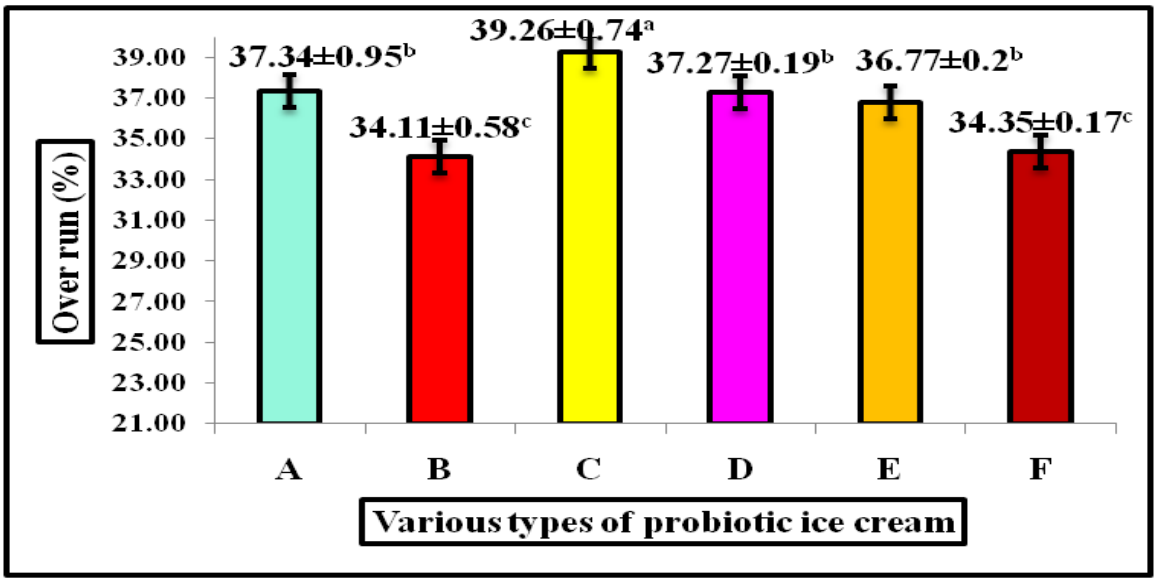
Superscripts with different letters within columns/same row shows significant difference

| **Parameters** | **Physicochemical characters of probiotic ice cream** | | | | | | **LSD (0.05)** |
|---|---|---|---|---|---|---|---|
| | **Type- I** | **Type- II** | **Type- III** | **Type- IV** | **Type -V** | **Type-VI** | |
| TS (%) | 34.011±0.34$^{ab}$ | 34.90±0.82$^{ab}$ | 35.14±0.59$^{a}$ | 33.76±.53$^{b}$ | 34.93±.61$^{ab}$ | 34.75±.52$^{ab}$ | 0.649 |
| Fat (%) | 10.71±0.25$^{e}$ | 11.16±0.35$^{c}$ | 10.97±0.26$^{d}$ | 11.14±.33$^{b}$ | 10.92±.36$^{d}$ | 11.81±.36$^{a}$ | 0.086 |
| Protein (%) | 5.13±0.19$^{a}$ | 5.06±0.28$^{b}$ | 4.92±0.23$^{c}$ | 4.73±.19$^{d}$ | 4.79±.21$^{e}$ | 4.28±.27$^{f}$ | 0.008 |
| Ash (%) | 0.97±0.02$^{a}$ | 0.90±0.02$^{d}$ | 0.94±0.02$^{b}$ | 0.91±0.01$^{c}$ | 0.97±0.0 $^{a}$ | 0.95±0.02$^{b}$ | 0.004 |
| Carbohydrates (%) | 17.21±0.42$^{ab}$ | 17.79±1.07$^{ab}$ | 18.30±0.92$^{a}$ | 16.71±0,72$^{b}$ | 18.27±0.95$^{a}$ | 17.72±0.64$^{ab}$ | 0.660 |
| Acidity (%) | 0.22±0.004$^{d}$ | 0.24±0.004$^{c}$ | 0.37±0.003$^{b}$ | 0.25±0.003$^{c}$ | 0.43±0.008$^{a}$ | 0.16±0.002$^{e}$ | 0.006 |
| pH Value | 6.11±0.04$^{c}$ | 6.37±0.02$^{b}$ | 6.2±0.04$^{b}$ | 6.52±0.02$^{a}$ | 5.92±0.03$^{d}$ | 6.45±0.06$^{ab}$ | 0.048 |

**Over run (%)**

No doubt that the structure and general quality attributes of frozen desserts are linked with over run rate. According to different probiotic ice cream types results, overrun values type 1, type-2, type-3, type-4, type-5 and type-6 are 37.34±0.95, 34.12±0.58, 39.62±0.74, 37.27±0.19, 36.77±0.2 and 34.35±0.17 %, respectively (Figure 2). Furthermore, a minor decline in the ice crystal mass expanded the ice cream volume (Flores and Goff, 1999) this could be due heat exchange rate upon high aeration. Moreover, increase in expansion of ice cream volume caused the reduction in the hardness of frozen dessert. Whereas Abd El-Rahman et al. (1997) and Prindiville et al. (1999) disagreed and declared that air bubbles/air cell and iced crystals had prominent impact on the hardening properties compared to that of overall expansion in the volume of ice cream. These observations are alike to our observations.

**Figure: 2 Showing Overrun (%) of probiotic ice cream**




| | | |
|---|---|---|
| **A:** Str. thermophilus | **B.** L.bulgaricus | C. Bifidobacteria |
| **D.** Saccharomyces | **E.** Mixed probiotic | **E.** System |

**LSD (0.05): 0.771**

**SE±:0.3854**

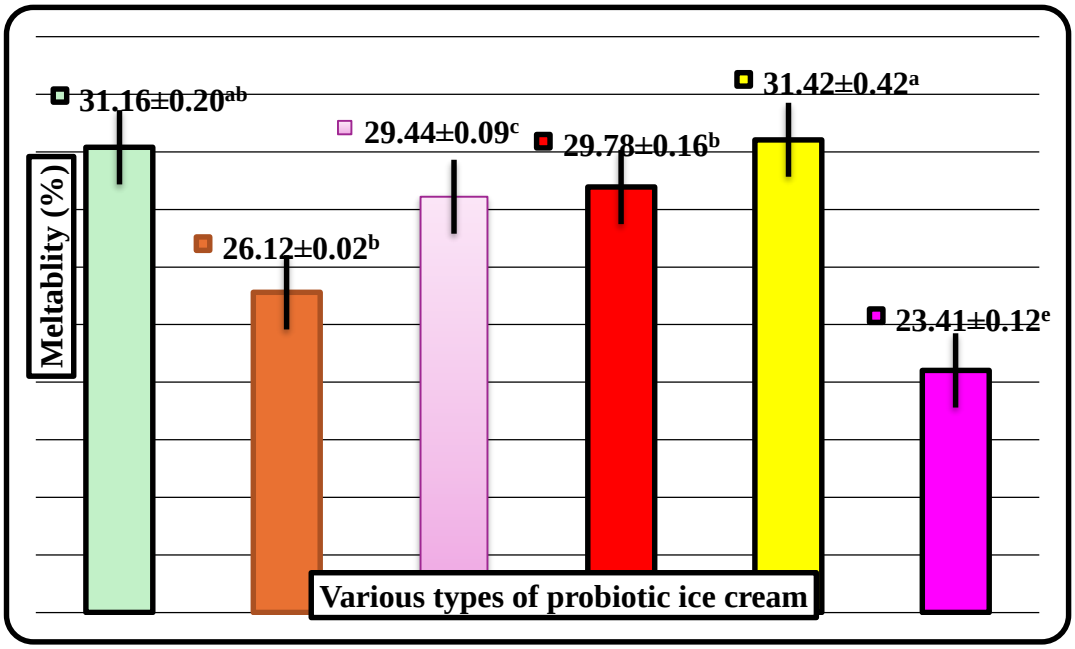
**Data are the average of 6 batches and each in triplicate.**

**Meltdown Rate (%)**

The melting points attributes are said to be prime quality characteristics of frozen dairy food commodities (Erkaya et al., 2012). Corresponding to this in the present study, addition of varying probiotic strains in ice cream mixture revealed significantly different melting behavior (Figure 3). Melting rate at 45 minutes of probiotic ice cream i.e. type 1, type-2, type-3,type-4,type-5 and type-6 melt down was observed as 31.16, 26.12, 29.44, 29.78, 31.42 and 23.41, respectively. Parallel to plain ice cream all the probiotic ice cream melted slightly faster. Whereas, type-2 ice cream had slow melting ability whilst type-3 and type-4 probiotic ice

creams showed similar kind of melting behavior upon 45 minutes @ 4°C. This could be due to variation in the acid production, freezing point, viscosity of mixtures, moisture/ total solids contents and fat content (Granger et al 2005), overrun (Sofjan & Hartel, 2004) and sources of milk (Pandya &Ghodke, 2007) etc.

**Figure: 2 Showing Meltablity (%) of probiotic ice cream**




| | | |
|---|---|---|
| **A:** Str. thermophilus | **B.** L.bulgaricus | **C**. Bifidobacteria |
| **D.** Saccharomyces | **E.** Mixed probiotic | **E.** System |

**LSD (0.05): 0.267**

**SE±: 0.1336**

**Data are the average of 6 batches and each in triplicate.**

## Conclusion

Keeping in view proximate composition, rheological features, physical attributes Sacchromyces and Bifidobacterial probiotic containing types of ice cream were found better.

## Acknowledgement

All the authors of this manuscript earnestly acknowledge the support of respective Universities and Institutes.

## Author contribution

QRA and GBK; Conceived and designed methodology, performed experiments. AHS, and MTS analyzed the data and wrote the manuscript. All authors edited and contributed to the article.

## Conflicts of interest

There are no potential conflicts of interest, as stated by the authors.

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