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Development of Dietetic Misti Dahi (Indian Yoghurt) Using Natural and Artificial Sweeteners

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Authors' contributions

This work was carried out in collaboration among all authors. Authors PB, TB, RNB and RRS conceptualized and designed the work. Author PB executed the experimental work and carried out the laboratory analysis. Authors TB and RNB provided the necessary guidelines and contributed critically to revise the manuscript. Author RRS contributed critically to revise the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Dairy based sweetmeats usually contain high sugar with a high calorific value which in turn is linked to various non-communicable diseases. To reduce this high calorie content, sugar can be replaced in part or wholly by non-nutritive artificial sweeteners, as well as natural sweetener like honey. In the present study natural and artificial sweetener sources (honey and sucralose, respectively) were used to replace sucrose in part or completely to develop low calorie fat reduced *misti dahi,* an Indian yoghurt. Effect of sweeteners on setting time, proximate composition, microbiological quality and calorific value of *mistidahi,* and sensory quality were analysed as per the standard methods. Protein and total solids (TS) content decreased from 4.22 to 3.96% or 4.39 to 4.23% in honey or sucralose and from 24.20 to 21.99% in honey or 21.57 to 16.70% in sucralose, respectively with increase in honey or sucralose used to replace sucrose in *mistidahi*, while fat content was variable. Microbiological analysis revealed that with an increase in the content of honey, the total aerobic plate count (TAPC) of *mistidahi* tended to show an increase, while for sucralose containing samples a decreasing trend in TAPC in *mistidahi* was noted.

Keywords: Bovine milk; artificial sweetener; lactic acid bacteria; low fat; low calorie.

1. INDRODUCTION

Sweetener is a substance that occurs naturally or is synthetically produced and provides sweet taste to food or beverages; it maybe nutritive (caloric) or non-nutritive (non-caloric). The most common nutritive sweetener used in the food industry is sucrose, chemically consist of fructose and glucose. Consumption of the sucrose or sugar has been found to link to various non-communicable diseases like diabetes [1]. So, nutritionists and health professionals around the world are increasingly focusing on the development of healthier foods by reducing the calorie and fat content and thus helping in the management of various health conditions. Indians have a strong preference for dairy based sweetmeats. Nevertheless, these dairy products are not free from sucrose whose energy value is quite high. One of the most popular traditional fermented milk products of India which finds its place even in ancient scriptures is *Dahi* or Indian Yoghurt due to its health beneficial properties [2]. A sweetened variety of *dahi*, popularly known as *mistidahi*, is popular in Eastern India. However, *mistidahi* contains a high level of sugar (6-25%) and its fat content ranges between 1-12% [3]. In order to reduce the calorie content, sugar is usually replaced in part or wholly by non-nutritive artificial sweeteners, as well as natural sweetener like honey [4].

Honey, a natural sweetener from the nectar of plants, may be used as an alternative to sucrose as it contains high level of fructose and small amount of glucose. Since fructose is sweeter than glucose, so less amount of honey is required to obtain the same sweetness of sugar [5]. On average honey is 1.0-1.5 times sweeter than sugar on dry matter basis, while liquid honey is dense and approximately as sweet as sugar. Honey contains only 82.4g carbohydrates/100g while sugar has 100g carbohydrates and provides energy of 304 Kcal/100g and 400 Kcal/100g, respectively [6]. Nevertheless, honey possesses several health beneficial properties [7].

Artificial sweeteners, like sucralose, may also be used as a substitute of sucrose in preparing low calorie sweetmeats. Sucralose is a chemically synthesised non-nutritive potent sweetener and is cost effective. It is 450-650 times sweeter than sucrose and provides desirable sweetness without getting metabolized in the body and hence, it adds no calorie [8]. U.S. Food and Drug Administration approved sucralose as general-purpose sweetener with an acceptable daily intake of 5mg/kg body weight/day. Considering the above facts, an attempt has been made in the present study to develop a low calorie, reduced fat fermented *mistidahi* with replacement of sugar with honey or sucralose, in part or completely.

2. METHODOLOGY

The study was undertaken in the laboratories of the Department of Livestock Products Technology, College of Veterinary Science, Assam Agricultural University, Khanapara Campus, Guwahati.

2.1 Procurement of Raw Materials

Fresh raw cow's milk was procured from the institution's cattle farm. Sugar, Honey (Dabur,

India) and food grade sucralose tablets ('Zero' from M/s. Alembic Ltd, Ahmedabad, India) were purchased locally. Yoghurt culture NCDC 263 (National Collection of Dairy Cultures) comprising of mixed culture of *Lactobacillus delbrueckeii* ssp*. bulgaricus* and *Streptococcus thermophilus* was obtained from the National Dairy Research Institute, Karnal, India.

2.2 Starter Culture

Freeze dried yoghurt culture was inoculated in reconstituted and sterilized skim milk (12%). Working cultures were maintained in skim milk, sub-cultured at weekly interval and stock cultures were maintained in nutrient agar slants (-) 20°C and sub-cultured at 3 month's interval. Sixteen to eighteen hour active culture was prepared in sterilized skim milk (12% w/v) by inoculating the stock culture at 2% level. Repeated sub-culturing, at least three times, was done before using it as starter culture for preparation of *mistidahi*.

2.3 Quality Judging and Pre-treatment of Raw Milk

Rapid judging of milk was done by subjecting it to various physico-chemical and bacteriological tests like pH, acidity, Rapid Platform Test (RPT) and Methylene Blue Reduction Test (MBRT). The pH was determined using a digital pH meter Model 780 (Metrohm, Switzerland). Titratable acidity, RPT and MBRT were done as per method of Artherton and Newlander [9]. Composition of the raw milk analysed in Ultrasonic Milk Analyser (Master Classic, Bengaluru, India) was found to contain 4.46% milk fat, which was pre-treated to reduce the fat content to 4.04% by removing the scum formed after boiling and subsequently cooling down the milk to room temperature several times. Solids-not-fat content calculated using Richmond's formula was standardized to 12% using skim milk powder (HiMedia, India). Milk was sterilized by autoclaving at 115^{0}C for 20min.

2.4 Product Preparation

Misti dahi was prepared by using fat reduced standardized milk. Only sucrose in control, and sucrose with honey and sucralose in different combinations were added to the treatment groups of standardized milk. Followed by inoculating the milk with active culture of NCDC 263 (2%) and incubated at 42±1°C for 4–5h or till formation of curd. After proper setting, the products were stored at 4±1°C for 3–4h. Immediately after setting, the products were analyzed for their microbiological quality and proximate composition. Allotted treatment are shown in Table 1.

Table 1. Sweeteners used for part or full substitution of sucrose in *Misti dahi*

Treatments	Sucrose %	Honey %	Sucralose %
Control	100	-	-
T_1	75	25	-
T_2	50	50	-
T_3	25	75	
T_4	-	100	-
T_5	75	-	25
T_6	50	-	50
T_7	25	-	75
T_8	-	-	100

2.5 Setting Time of *Misti Dahi*

Setting time (min) was noted from the time of starter culture inoculation in milk till formation of set curd. This was recorded for all the treatment groups. A total of five replications were done for each treatment group.

2.6 Proximate Analysis

Fat content of the set products was determined by the Gerber's method [10]. Protein content of the set milk product was determined by following Kjeldahl method. Moisture, Total solids and ash content of the set products were estimated by the method as per AOAC [11].

2.7 Microbiological Analysis

Total viable count and yeast and molds counts of the products were analyzed by following pour plate technique [12]. Colonies were then counted in a bacteriological colony counter. For Colititre, a set of nine tubes containing 9 ml of Brilliant Green Lactose Bile broth, with inverted Durham's tube were inoculated with 1ml of the inoculum from the required dilutions of the set products of all treated groups. Tubes were incubated at 37°C for 24h. After which, tubes were checked for production of gas, change of colour and development of turbidity and the Most Probable Number (MPN) was calculated as per AOAC [13] guidelines.

2.8 Calorific Value of the Product

The calorific value of the set products was calculated using Atwater system. The total

calorie value was calculated by adding up the calories provided by the energy-containing nutrients *i.e.,* protein, carbohydrate and fat which were taken as 4, 4 and 9kcal/g, respectively to get the calorie value [14]. The total carbohydrate content in *mistidahi* was determined by difference (fibre is included) [15].

2.9 Sensory Evaluation

After proper setting, the products were brought to 4±1^{o}C before serving to the 9-membered semi trained panel for sensory evaluation. The products were rated for appearance, colour, body and texture, flavour and taste through a 9-point hedonic scale. Overall acceptability of the products was calculated out by taking the mean score of the different sensory parameters stated above.

2.10 Statistical Analysis

Experimental data obtained were expressed as the average of mean values ± standard error. To highlight significant differences among the samples Analysis of variance (ANOVA) with Honest Significant Difference (HSD) test for mean comparison were used. Statistical tests were performed with a 5% or 1% significance level using the SPSS program version 20 [16]. A total of five batches were studied for proper statistical analysis.

3. RESULTS AND DISCUSSION

3.1 Composition of Raw Milk

The average chemical composition of the raw cow's milk is given in Table 2. Variations in the composition might be due to the variation in lactation period, feeds, etc. The pH and acidity of the freshly drawn cow's milk were 6.6-6.8 and 0.16-0.17% lactic acid, respectively. These findings are in accordance with the findings of Aneja et al. [17]. RPT including organoleptic, Clot on Boiling (COB) and alcohol test results indicate suitability of raw milk for heat processing. Resazurin and MBRT test results were also indicative of good quality milk and, hence, was found to be suitable for preparation of *mistidahi*.

3.2 Setting Time

In treatment where sugar was replaced by varying levels of honey (i.e., T_1 to T_4), the setting time decreased with an increase in the replacement level of sucrose with honey. This might be due to thick consistency of honey which might have thickened the consistency of the milk resulting in reduced setting time of the product. Contrary to the results of honey added products, the setting time of sucralose added *mistidahi* products increased as the level of sucralose was increased to replace sucrose.

3.3 Effect of Honey and Sucralose on Proximate Composition of *Misti Dahi*

3.3.1 Fat

Effect of sweeteners on fat content of the *mistidahi* showed highly significant difference among the groups. Fat content of honey added products were found to gradually decrease from a mean of 2.96 to 2.52% however, at 75% sugar replacement (T_3), a higherfat level was noted (Table 3). The findings of lesser mean fat content of honey treated samples than that of control, is in disagreement with the findings of Bakr et al. [18] who reported an increase in the mean fat content in honey containing bioyoghurt. Sucralose added sample exhibited an increasing trend in the fat content which was also in harmony with the findings of Chethana et al. [19] on gulab jamun made with sugar substitutes.

3.3.2 Protein

The mean protein content of the *Misti dahi* samples showed highly significant (P<0.01) difference. As the proportion of honey to replace sucrose in the different treatment groups increased, there was a gradual decrease in the mean protein content from 4.22% (T_1) to 3.96 % (T_4). The lowest mean protein value of 3.87 %was found inT_2 group (why group?) of *mistidahi* (Table 3). This might be generally due to absent of protein content of honey which used in the experiments was 0.1-3.3% [20] and particularly for *Dabur* honey the protein was absent [21]. These findings were also supported by the findings of Bakr et al. [18].

Mean protein content of sucralose containing *Misti dahi* samples decreased from 4.39±0.15 to 4.23±0.16% as the sugar replacement was increased from 25 to 100%. This might be due to very less protein content of sucralose. The findings were supported by that of Chethana et al. [19] in *dahi* containing sucralose.

Table 2. Physico-chemical properties of raw cow's milk

Batch No.	pH	Acidity (%LA)	Fat (%)		SNF (%)	RPT				MBRT
			Raw	Defatted		Organoleptic	COB	Alcohol	Resazurin	
1	6.6	0.16	4.6	4.1	8.40	Pale yellow colour, normal sweet taste	-ve	-ve	Good	Good
2.	6.7	0.17	4.3	3.8	9.64	-do-	-do-	-do-	-do-	-do-
3.	6.6	0.16	4.7	5.2	8.73	-do-	-do-	-do-	-do-	-do-
4.	6.6	0.16	4.5	3.7	9.65	-do-	-do-	-do-	-do-	-do-
5.	6.7	0.18	4.2	3.4	8.74	-do-	-do-	-do-	-do-	-do-

-do- represent 'same result'
-ve represent 'negative'.

Table 3. Effect of honey and sucralose on proximate composition of *Misti Dahi*

Treatment Groups	Proximate Composition (%) (Mean±SE)			
	Fat	Protein	Ash	Total solids
CONTROL	3.40±0.23e	3.83±0.08a	4.08±0.06a	24.55±0.32e
T_1	2.96±0.22c	4.22±0.14ab	3.90±0.23a	24.20±0.81de
T_2	2.52±0.23b	3.87±0.05ab	4.02±0.18a	23.98±0.73de
T_3	3.16±0.27cde	3.86±0.21ab	4.38±0.28a	22.80±0.63cd
T_4	2.52±0.23b	3.96±0.23ab	4.20±0.23a	21.99±0.53c
T_5	1.98±0.17a	4.39±0.15b	4.72±0.20a	21.57±0.20c
T_6	3.04±0.21cd	4.29±0.12ab	4.42±0.18a	21.70±0.48c
T_7	2.92±0.21c	4.22±0.13ab	5.80±0.15b	19.00±0.27b
T_8	3.26±0.25de	4.23±0.16ab	6.74±0.19c	16.70±0.28a

Figures with differing alphabets are significantly different

3.3.3 Ash

Ash content of the sugar replaced *mistidahi* showed an increase in the mean value as the level of sugar replacers was raised. Ash content differed (P<0.01) significantly among the groups. The highest ash value (i.e. 6.74%) was recorded for 100% sucralose added group while the least value (i.e. 3.90%) was associated with *Misti dahi* utilizing 25% honey. Similar findings have been reported by Bakr et al. [18] with a maximum ash content of 8.43g/kg of *dahi* in both sac-sweet and sucrol containing products.

The honey also contained higher amount of minerals (0.04-0.2% [20]) (Dabur honey contain 0.1-0.2% minerals) and this might be the main factor for the increasing ash content of the honey added products. Metry and Owayss [22] and Bakr et al. [18] reported a decrease in the ash content of *dahi* prepared when fennel honey was used at incremental higher levels which they attributed to the lesser content of ash in fennel honey.

3.3.4 Total solids

A highly significant (P<0.01) difference was found for TS content of *mistidahi* samples. When either of honey or sucralose was used at incremental higher levels to replace sucrose, a decrease in the TS content was noted. This might be due to the gradual reduction in the sugar content of *mistidahi* from 25 to 100%. Highest TS content was recorded in the control sample and the least value was associated with product containing 100% sucralose. Islam et al. [23] also reported lower TS content of *dahi* prepared utilizing sac-sweet (149.73 g/kg) and sucrol (149.83 g/kg) versus use of 8% sucrose in *dahi*. Metry and Owayss [22], Rashid and Thakur [24], Bakr et al. [18] have reported an increasing trend in the TS content of bioyoghurt or *dahi* when sugar content was added at incremental levels.

3.4 Effect of Honey and Sucralose on Microbiological Quality of *Misti Dahi*

3.4.1 Total Viable Count (TVC)

Highly significant (P<0.01) difference was found for TVC among the *mistidahi* samples. TVC of the treatment groups, containing honey increased from 4.34 to 4.52 $\log_{10}$cfu/ml as the concentration of honey increased from 25 to 100% (Fig. 1). TVC of the honey added groups were found to be than the control group (4.70 $\log_{10}$cfu/ml). The antibacterial effect of honey has been reported by several researchers [25,26].

Islam et al. [23] reported higher count of TVC (i.e. 7.63$\log_{10}$cfu/g in *dahi* samples containing sucrolas against the one containing sac-sweet (5.77 – $\log_{10}$cfu/g) as sugar replacer and Chethana et al. [19] enumerated a lower count of 1×10^7cfu/ml in 1.5% sucralose containing low calorie *mistidahi* compared to samples containing 0.5 and 1.5% sucralose.

In the present study, TVC was found to be lower significantly in 100% sucralose added sample, which had the least TVC (i.e. 4.02 logcfu/ml). The antibacterial property of sucralose [27] may be a major factor towards this decreasing trend in TVC, besides the 'no carbohydrate factor'.

3.4.2 Coliform count

None of the samples of the present study revealed presence of coliform organisms per ml or g of product. This might be due to using good quality raw milk and hygienic procedures followed in the preparation of *mistidahi* as well as due to the probable production of bacteriocins by starter cultures as reported by Rashid and Thakur [24] and Bakr et al. [18].

3.4.3 Yeast and mould count

In the present study, yeast and mould count was found to be less than the minimum countable number of 25 per plate. Bogdanov et al. [25] opined that lesser yeast and mould count in control and treatment groups might be due to hygienic practices followed during manufacturing of the product as well as to the antifungal property of honey.

3.4.4 Calorific value of *Misti Dahi*

Reduction in the calorific value from 97.33 in control group to 81.25 in T_4 group is evident since the calorie value of honey (304 Kcal/100g) is less than that of sugar (400 Kcal/100g) (Table 4) [6], so honey added groups showed lower calorific value than control product. The energy value of the honey brand (Dabur, India) used in the study was printed to be320 Kcal/100g [21]. The drastic reduction in the calorific value in T_8 group (56.26k.cal./100 g) was attributed to the type of artificial sweetener used. Sucralose has been grouped as 'no calorie' sweetener [8]. The decrease in the calorific value of sugar replaced *mistidahi* was supported by the findings of Hussein et al. [28] and Mittal and Bajwa [29].

Table 4. Effect of honey and sucralose on microbiological quality of *Misti Dahi

Treatment Groups	Total Viable Count (log_{10}cfu/ml)	Coliform count	Yeast and Moulds count
CONTROL	4.70 ± 0.25c	ND	<25
T_1	4.34 ± 0.31abc	-do-	-do-
T_2	4.37 ± 0.31abc	-do-	-do-
T_3	4.56 ± 0.20bc	-do-	-do-
T_4	4.52 ± 0.20bc	-do-	-do-
T_5	4.23 ± 0.21ab	-do-	-do-
T_6	3.99 ± 0.17a	-do-	-do-
T_7	4.22 ± 0.24ab	-do-	-do-
T_8	4.02 ± 0.18a	-do-	-do-

n=5
**Mean ± SE;*
Figures with letters non similar are significantly different
ND- Not detected, -do- 'same result'

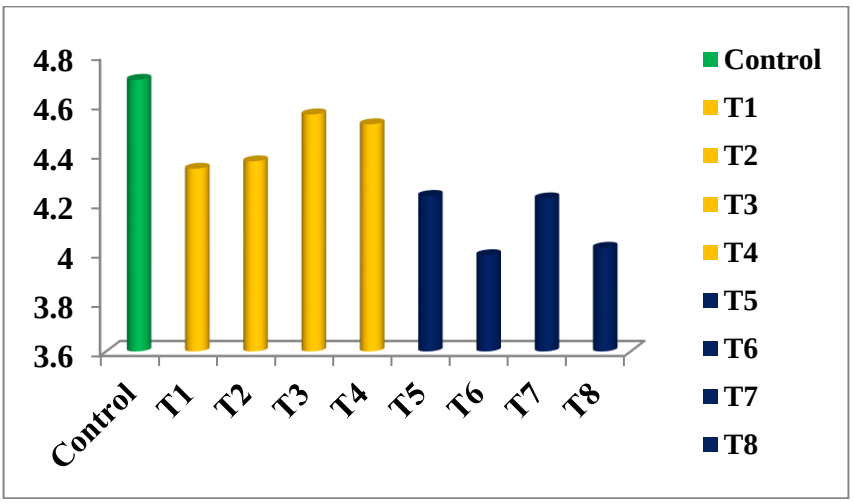

Fig. 1. Effect of honey and sucralose on total viable count of *Misti Dahi*

Table 5. Effect of replacing sucrose with honey or sucralose on the calorific value of *Misti Dahi*

Treatment	Calorific Value (Kcal/100g) (Mean ± SE)
CONTROL	97.33±1.33a
T_1	94.80±3.63a
T_2	91.18±2.85ab
T_3	82.39±0.82c
T_4	81.25±1.51c
T_5	83.54±2.37c
T_6	85.12±2.23bc
T_7	67.13±1.93d
T_8	56.26±2.07e

Dissimilar supercripted alphabets in column wise are significantly (P<0.05) different from each other

3.5 Sensory Evaluation

Appearance: From the findings of the present experiment, it could be seen that the amount of sugar in *mistidahi* replaced by honey or sucralose greatly influenced the eating quality characteristics of the *mistidahi*. There was no significant difference among the samples for appearance. The highest mean value for the parameter was found in T7 group with 7.44 ± 0.12 score and the lowest was found in T5 with 6.39 ± 0.21 score.

Colour: The scores for colour of *mistidahi* do not differ significantly between the samples. The highest and lowest mean values were found in T6 (7.44 ± 0.15) and T8 (6.96 ± 0.19).

Body and Texture: The score for body and texture of the product differ highly significantly between (*P*<0.01) the groups. The control sample enjoyed superior ratings for body and texture (7.38±0.19) followed by T_6 (7.24±0.16) which had highest rating for colour attribute too.

Table 6. Effect of addition of honey and sucralose on the organoleptic properties of *Misti dahi**

Treatment	Appearance	Colour	Body & Texture	Flavour	Taste	Overall acceptability
CONTROL	7.29 ± 0.20	7.42 ± 0.19	7.38 ± 0.19ab	7.18 ± 0.17b	7.18 ± 0.18b	7.11 ± 0.19b
T_1	7.24 ± 0.17	7.42 ± 0.18	7.09 ± 0.21b	7.29 ± 0.19b	7.33 ± 0.20b	7.42 ± 0.15b
T_2	7.11 ± 0.20	7.22 ± 0.16	7.22 ± 0.16ab	7.22 ± 0.15b	7.24 ± 0.18ab	7.24 ± 0.14ab
T_3	7.13 ± 0.17	7.36 ± 0.18	7.09 ± 0.18b	7.24 ± 0.17b	7.20 ± 0.18b	7.27 ± 0.15b
T_4	7.36 ± 0.13	7.42 ± 0.14	7.11 ± 0.18b	7.20 ± 0.15ab	7.33 ± 0.17b	7.40 ± 0.12b
T_5	6.39 ± 0.21	7.42 ± 0.20	7.24 ± 0.19ab	7.22 ± 0.19ab	7.16 ± 0.20ab	7.16 ± 0.19ab
T_6	7.24 ± 0.18	7.44 ± 0.15	7.24 ± 0.16ab	7.13 ± 0.15ab	7.04 ± 0.15ab	7.20 ± 0.13ab
T_7	7.44 ± 0.12	7.29 ± 0.10	7.09 ± 0.16b	7.11 ± 0.14ab	7.18 ± 0.15b	7.11 ± 0.12b
T_8	7.02 ± 0.19	6.96 ± 0.19	6.91 ± 0.18a	7.02 ± 0.19a	7.13 ± 0.19a	6.96 ± 0.17a

**Mean ± SE;*
Letters not similar are significantly different

Flavour: Flavour score differ significantly between the samples (P<0.01) with the highest score in T1 (7.29 ± 0.19) and lowest in T8 (7.02 ± 0.19**).** Starter culture was also reported to contribute to the flavour of dahi.

Taste: Study revealed a highly significant differenve for taste score among the samples of *mistidahi*. Scores ranges between 7.04 ± 0.15 to 7.33 ± 0.20.

Overall Aceptibility: Overall aceptibility was found to be differ significantly (P<0.01) among the groups. Panel members rated T_1 *mistidahi* samples the best while T_8 was rated the poorest (6.96±0.17) for overall acceptability. T_1 enjoyed superior ratings not only for overall acceptability but also for other sensory attributes like flavour and taste followed by T_4 which was rated superior for appearance and taste.

4. CONCLUSION

Study on the proximate composition of *mistidahi* showed variable results for fat content. Protein and TS content of product tended to decrease, while a gradual increase in the ash and moisture content was noted as the level of honey or sucralose was raised in the formulation. Microbiological analysis of the products revealed that with an increase in the usage level of honey in, the total aerobic plate count (TAPC) of *mistidahi* increased. However, in samples T_5 to T_8 groups, a decreasing trend in TAPC was noted with an increase in the level of sucralose. Based on sensory evaluation, low fat content, higher protein and TS content, treatment group T_1 is recommended for producing low-calorie fat reduced *mistidahi*. Honey was preferred over sucralose as sweetener; the former being natural ingredient too.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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