

Influence of Gac aril, yeast, and sugar in high quality sandwich bread making

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ABSTRACT

Research was conducted to develop a sandwich bread with added Gac aril. Effects of Gac aril (5, 7, and 9%), added sugar content (8.6, 10.3, and 12%), and instant dry yeast used (0.3, 0.4, and 0.5%) to bread quality and sensory value of the products were performed. The relationship between the dependent variables such as β -carotene, lycopene, and volume expansion and three independent variables including Gac aril, sugar and dry yeast content was analyzed using multiple regression analysis. It was observed that the optimum responses of β -carotene, lycopene, and volume expansion were determined as 14.78 µg/g, 59.14 µg/g, and 5.3 times, respectively. To achieve those optimal values, the optimization conditions of various input variables, the percentage of Gac aril, sugar, and dried yeast were found to be 8.55%, 9.76%, and 0.5%, respectively, which, on confirmation, showed a high quality sandwich bread. The optimal parameters were also verified. Sensory evaluation showed that the sandwich bread prepared with 8.55% and 9% Gac aril gave a higher acceptance score than the lesser content and control sample. Preference mapping also showed more than 80% of the participants loved these products.

1. INTRODUCTION

In Vietnam, bread is one of the most commonly consumed foods on a daily basis. However, bread mainly provides energy, the micronutrient content in breads is often low [1]. Gac fruit (*Momordica cochinchinensis* spreng) is widely grown in Southeast Asian countries [2], they have been used as food and medicine for a long time due to the very good properties that Gac fruit possesses [3].

In Vietnam, Gac aril have also been used for the purpose of improving eyesight and reducing dry eyes, because they have been shown to contain particularly high levels of lycopene and β -carotene [4,5]. Studies have published that Gac fruit contains 10 times more β -carotene than carrots or sweet potatoes [6], the highest β -carotene content of all other fruits, about 84–720 µg/g of Gac aril, lycopene content was about 380–2300 µg/g, which has been shown to be 70 times higher than that of tomatoes [7]. Other studies also showed that some chemical components of Gac have broad pharmacological activities, such as anti-tumor, anti-oxidant, and anti-inflammatory [8].

Many studies have investigated the possibility of replacing wheat flour with other flours to increase the good nutritional components of

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Department of Food Technology, Institute of Food and Biotechnology, Can Tho University, Can Tho city, Vietnam. E-mail: nmthuy @ ctu.edu.vn this product [9,10]. Bread was produced from wheat flour, corn and orange flesh sweet potato [11], unbreaded wheat and rice bran [12], and bread produced from wheat flour in combination with plantain and soybeans [13]. Bread fortification with whole green banana flour was studied by Khoozani *et al.* [14]. Guardado-Félix *et al.* [15] investigated the effect of partial replacement of wheat flour with sprouted chickpea flours in bread making. Bread fortified with moringa seed powder was also studied to increase both the micro and macronutrient of conventional bread [1].

The alarming nutritional status of children in rural Vietnam is chronic vitamin A deficiency. Nutrition intervention programs have been implemented such as vitamin A capsules. However, this is not a solution. In the long term, food-based interventions are considered part of an effective strategy to reduce vitamin A deficiency. Research on the addition of Gac to sandwiches is still unknown, both in Vietnam and around the world. Therefore, the addition of Gac aril and also fiber from Gac can significantly improve the nutritional quality of sandwiches made from a mixture of wheat flour and Gac aril.

Besides, yeast and the necessary amount of added sugar are two important ingredients that affect the quality of sandwiches. During fermentation in sandwich technology, yeast uses sugar to produce carbon dioxide and ethyl alcohol.

In the manufacture of bread, sugar is added to feed the yeast. The yeast *Saccharomyces cerevisiae* makes efficient use of maltose in the

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dough, resisting the osmotic stress caused by the semi-solid state of the dough. Moreover, it is the high sugar content in some doughs that, when subjected to different processing conditions, give the desired aroma and taste to the finished product [16]. In baked goods, the sugar component interacts significantly with all other ingredients, for example, sugar also contributes to the browning reaction. During the preparation phase, sugar actively aids in the aeration of the dough and results in a characteristic soft cake [17].

Mutiple regression analysis was successful to optimize production process of various food as jam [18] and bread [19,20]. The study aims to determine the effect of simultaneous combination of Gac aril, sugar, and instant dry yeast on the quality (β -carotene, lycopene, and volume expansion) of sandwich bread. The optimal parameters were determined and verified to produce the high quality Gac–sandwich bread.

2. MATERIALS AND METHODS

2.1. Effect of Gac Aril, Instant Dry yeast, and Sugar on Sandwich Bread Quality

Gac fruit was harvested when the peel was completely red [5]. The Gac aril was collected, pureed, and frozen at -18° C for further research. Other ingredients used: wheat flour (Bakers' Choice), salt, sugar, eggs, fresh milk, instant dry yeast (Mauripan), and margarine.

Prepare the ingredients according to the fixed weight, including flour 290 g, egg 60 g, butter 26 g, salt 3 g. Gac (5, 7 and 9%), sugar (8.6, 10.3 and 12%), and instant dry yeast (0.3, 0.4 and 0.5%), were added according to the experimental setup, in which percentage of Gac aril and sugar is calculated according to the weight of wheat flour and the percentage of yeast is calculated according to the total weight of all ingredients. Put all ingredients into automatic bread machine (PETRUS, China). Total time to make bread (including 4 times of stuffing, 3 times of fermentation and baking) is 3 h and 25 min. Baking temperature is 165°C. At the end of the process, the sandwich bread loaf was removed from the machine and analyzed for physical (expansion volume) and chemical parameters (β -carotene and lycopene). The sensory evaluation and analysis were followed by procedure of Thuy *et al.* [21,22].

2.2. Quality Analysis

The moisture content of the product was analyzed according to the AOAC standard method [23]. The β -carotene content was analyzed using the method of Fikselová *et al.* [24]. Lycopene was quantified according to the published method of Kakubari *et al.* [25]. Bread expansion is calculated through the volume ratio of the sandwich bread before baking and after finishing the product.

2.3. Statistical Analysis

Multiple regression was used to analyze the relationship between a dependent variable (β -carotene content, lycopene content, and volume expansion) and several independent variables (Gac aril, sugar, and yeast) (equations 1).

$$Y = b_o + \sum_{n=1}^{3} b_n X_n + \sum_{n=1}^{3} b_{nn} X_n^2 + \sum_{n \neq m=1}^{3} b_{nm} X_n X_m \quad (1)$$

Where b_0 : Y intercept (constant), b_n : regression coefficient for the linear effect of X_n on Y, b_{nn} and b_{nm} : regression coefficient for the quadratic effect on Y and X_n , X_m : independent values.

2.4. Data Analysis

Collected data were analyzed using STATGRAPHICS Centurion XV software (U.S.A.). Values are presented as mean \pm SD.

3. RESULTS AND DISCUSSION

3.1. Effect of Gac, Sugar, and Yeast Supplements on β-Carotene, Lycopene Content, and the Volume Expansion of Sandwich Bread

In this study, Gac aril created beautiful natural color and provided highvalue bioactive compounds for sandwich bread. Sugar adds sweetness and contributes to the browning of the bread. The main role of sugar in bread yeast is to provide food for the yeast. As yeast grows and multiplies, it uses sugars, forming by-products of carbon dioxide and alcohol, which gives bread its distinctive flavor. Sugar softens bread by preventing gluten from forming. It also keeps moisture in the finished product. The obtained data showed that the content of β -carotene, lycopene, and expansion volume of bread increased when increasing the amount of Gac aril from 5% to 7%. However, when continuing to increase the amount of Gac fruit to 9%, the expansion volume of the bread almost did not increase or decrease slightly; however, both β -carotene and lycopene content still increased slightly [Table 1].

Bread usually does not contain β-carotene and lycopene compounds, so it is completely dependent on the percentage of Gac supplement. Similarly, Wanjuu et al. [26] similarly reported that adding orangefleshed sweet potatoes to bread also increased β -carotene content. The sucrose and yeast added to bread recipes have shown a significant effect. The right concentration of sugar will facilitate fermentation and increase the volume (cause swelling) of the dough. It was observed that the amount of sugar used from 8.6% to 10.3% caused the bread volume increased. However, at 12% of sugar content, the expansion tended to equalized or decreased [Table 1]. Excessive sugar content used in bread production can also adversely affect quality [27]. At high concentrations, sugar has the effect of weakening the gluten network by competing with gluten for the water content available in the dough. The higher sucrose concentration in the dough (about 30%) can cause severe osmotic stress on yeast cells, which damages the cell components and reduces their fermentability [28,29]. Sugar in bread production improves crust color through browning [30]. It also helps keep bread moist and is considered an emollient and flavor enhancer.

The percentage of yeast added significantly affected the volumetric expansion of bread, the volume of bread after baking increased from 5.23 to 5.34 times when the yeast content used increased from 0.3% to 0.5%. As mentioned, *S. cerevisiae* is responsible for fermenting and making the dough rise to form a loaf. After using the sugar, the yeast converts the sugar into ethanol and carbon dioxide [31], which inflates the air bubbles in the dough, causing the dough to rise.

3.2. Establish the Relationship between a Dependent Variables and Independent Variables Using Multivariable Regression Models

3.2.1.The content of β *-carotene*

Optimizing the parameters of Gac aril (%), sugar (%), and instant dry yeast (%) were performed by multivariable regression method. The statistical significance of the model after acquisition was tested through analysis of variance (ANOVA) [Table 2].

In this case, the four effects [Gac aril (X_1) , sugar (X_2) and yeast (X_3) , and interact (X_1^2)] show P value less than 0.05, indicating high confidence 95.0%. The analysis results also showed that there was no contribution

Table 1: The content of β -carotene, lycopene, and volume expansion of sandwich bread varies with the content of Gac aril, sugar, and instant dry yeast used.

Gac aril (%)	Sugar (%)	Instant dry yeast (%)	β-carotene content (μg/g)	Lycopene (µg/g)	Expansion volume (times)
5	8.6	0.3	$10.84{\pm}0.06$	43.37±0.30	5.38±0.03
		0.4	$10.77 {\pm} 0.07$	43.07±0.35	5.48 ± 0.01
		0.5	10.62 ± 0.04	41.47 ± 0.80	5.51±0.02
	10.3	0.3	$10.84{\pm}0.09$	43.36±0.47	5.44±0.03
		0.4	$10.72{\pm}0.02$	42.88±0.67	5.48 ± 0.04
		0.5	10.61 ± 0.04	41.43±1.16	$5.54{\pm}0.07$
	12.0	0.3	10.99 ± 0.03	43.96±0.15	5.24±0.03
		0.4	$10.87 {\pm} 0.07$	43.46±0.77	5.32 ± 0.05
		0.5	$10.83{\pm}0.09$	56.27±0.78	5.38±0.04
7	8.6	0.3	13.35±0.02	53.41±0.08	5.34±0.03
		0.4	13.16±0.05	52.63±0.26	5.38±0.02
		0.5	$13.00{\pm}0.07$	52.00±0.33	5.45 ± 0.03
	10.3	0.3	$13.54{\pm}0.10$	$54.14{\pm}0.49$	5.35 ± 0.01
		0.4	$13.48{\pm}0.11$	$53.94{\pm}0.86$	5.44 ± 0.03
		0.5	$13.39{\pm}0.06$	$53.54{\pm}0.67$	5.51 ± 0.01
	12	0.3	$13.50{\pm}0.09$	54.01 ± 0.47	5.22 ± 0.02
		0.4	$13.42{\pm}0.03$	53.68±0.15	5.24 ± 0.01
		0.5	$13.35 {\pm} 0.07$	52.06 ± 0.84	5.27±0.04
9	8.6	0.3	$15.13{\pm}0.11$	$60.53{\pm}0.55$	5.07 ± 0.02
		0.4	$15.36{\pm}0.09$	$61.44{\pm}0.45$	5.14 ± 0.03
		0.5	$15.16{\pm}0.03$	60.65±1.13	5.17 ± 0.02
	10.3	0.3	15.21±0.11	$60.82{\pm}0.59$	5.12 ± 0.02
		0.4	$15.14{\pm}0.08$	$60.55{\pm}0.58$	5.15 ± 0.02
		0.5	$15.12{\pm}0.10$	$60.49{\pm}1.08$	5.22 ± 0.01
	12.0	0.3	$15.41{\pm}0.09$	61.63±0.45	4.91±0.02
		0.4	15.35 ± 0.10	$61.39{\pm}0.50$	4.95±0.01
		0.5	$15.31{\pm}0.02$	61.25±0.11	5.00 ± 0.02

Mean±standard deviation.

of X_1X_2 , X_1X_3 , X_2^2 , X_2X_3 , and X_3^2 interactions to β -carotene content (P > 0.05). Therefore, unimportant terms can be removed from the model to improve the regression model and optimization results. Equation 2 of the fitted model is:

$$\beta - carotene(\mu g / g) = 1.159 + 2.304X_1$$

+0.053X₂ - 0.781X₃ - 0.085X₁²
R² = 99.39%, R²_{adi} = 99.36%, SEE = 0.138 (2)

where: X_1 , X_2 , and X_3 are the percentage of Gac aril, sugar, and instant dry yeast, respectively.

It was observed that the lack of fit P > 0.05 (Lack-of-fit is 0.1245data not shown) is not significant, indicating that the model is fitted to all data [32]. The R-Squared statistic indicates that the model as fitted explains 99.39% of the variability in β -carotene. The adjusted R-squared statistic is 99.36%, which is more suitable for comparing models with different numbers of independent variables. Guan and Yao [33] suggested that the correlation model is good when the

Source	Sum of	DI	Mean	F-ratio	<i>P</i> -value		
	squares		square				
X ₁ :Gac aril	268.135	1	268.135	14043.04	0.0000		
X_2 :Sugar	0.439	1	0.439	23.00	0.0000		
X ₃ :Dried yeast	0.330	1	0.330	17.27	0.0001		
$\mathbf{X}_{1}\mathbf{X}_{1}$	2.079	1	2.079	108.86	0.0000		
X_1X_2	0.0007	1	0.0007	0.04	0.848		
X_1X_3	0.056	1	0.056	2.93	0.093		
X_2X_2	0.007	1	0.007	0.38	0.538		
X_2X_3	0.004	1	0.004	0.21	0.649		
X ₃ X ₃	0.006	1	0.006	0.31	0.580		
Lack-of-fit	0.545	17	0.032	1.68	0.076		
Pure error	1.031	54	0.019				
Total (corr.)	272.633	80					
R ² =99.42%	R^2 =99.42% R^2 (adjusted for d.f.) = 99.35%						

Table 2. Analysis of Variance for B-carotene

coefficient of determination of correlation R^2 is >0.8. The combination of factor levels maximizes the β -carotene content on the specified region and the optimization is performed. It shows that the optimal value of β -carotene (15.41 µg/g) at the optimal values of Gac aril, sugar, and yeast is 9%, 12% and 0.3%, corresponding.

3.2.2. The content of lycopene

Humans cannot synthesize lycopene, so a dietary intake of lycopene is necessary to take advantage of its beneficial health properties. Many significant health benefits in lycopene, including its protective effects on cardiovascular diseases [34]. Lycopene plays an important role in chronic disease prevention according to published epidemiological, tissue culture, and animal studies [35]. The analysis of variance for lycopene content is presented in Table 3. It was observed that six effects had P < 0.05 (X₁, X₂, X₃, X₁X₃, X₁², and X₃²), indicating that they were significantly different (95% confidence level).

The unimportant terms were omitted and the equation (equation 3) of the fitted model is:

$$Lycopene\left(\frac{\mu g}{g}\right) = 5.995 + 8.617X_1 + 0.126X_2$$

$$+6.59X_3 - 0.363X_1^2 + 2.585X_1X_3 - 38.722X_3^2$$

$$R^2 = 99.26\%, \qquad R^2_{-x} = 99.20\%, \qquad \text{SEE} = 0.631$$
(3)

Where: X_1, X_2 , and X_3 are the percentage of Gac aril, sugar, and instant dry yeast, respectively.

P-value of Lack-of-Fit >0.05 demonstrating that a good model was established with high R-Squared and adjusted R-squared values (99.26 and 99.20%, respectively). Similar to β -carotene, a combination of factor levels that maximized lycopene content in the indicated region was identified. The optimal lycopene content (μ g/g) was obtained at the optimal concentrations of Gac aril (9%), sugar (12%), and yeast (0.385%).

3.2.3. Volume expansion

Similarly, the results of ANOVA statistical analysis are shown in Table 4. Five effects $(X_1, X_2, X_3, X_1^2, \text{ and } X_2^2)$ can be observed with P < 0.05. *P*-value for the lack of fit in the ANOVA table is >0.05, so the model showed a good fit with the observed data at the 95% confidence

Source	Sum of squares	Df	Mean square	F-ratio	<i>P</i> -value
X ₁ :Gac aril	4522.85	1	4522.85	11350.94	0.0000
X ₂ :Sugar	2.470	1	2.470	6.20	0.0159
X3:Dried yeast	21.395	1	21.395	53.69	0.0000
X_{1}^{2}	37.845	1	37.845	94.98	0.0000
X_1X_2	0.319	1	0.3192	0.80	0.3747
X_1X_3	9.620	1	9.620	24.14	0.0000
X_{2}^{2}	0.098	1	0.098	0.25	0.6215
$X_{2}X_{3}$	1.103	1	1.103	2.77	0.1020
X_{3}^{2}	2.699	1	2.699	6.77	0.0119
Lack-of-fit	11.356	17	0.668	1.68	0.0769
Pure error	21.517	54	0.398		
Total (corr.)	4631.27	80			
R-squared=99.29%		R-squared (adjusted for d.f.)=99.20%		Standard Error of Est.=0.63	

Table 4: Analysis of variance for volume expansion.

Table 3: Analysis of variance for lycopene content.

Source	Sum of squares	Df	Mean square	F-ratio	<i>P</i> -value
X ₁ :Gac aril	1.540	1	1.540	1143.55	0.0000
X ₂ :Sugar	0.319	1	0.319	236.79	0.0000
X ₃ :Dried yeast	0.163	1	0.163	121.28	0.0000
X_{1}^{2}	0.203	1	0.203	151.00	0.0000
X_1X_2	0.0017	1	0.0017	1.29	0.2613
X_1X_3	0.0020	1	0.0020	1.50	0.2255
X_{2}^{2}	0.236	1	0.236	175.60	0.0000
$X_{2}X_{3}$	0.0009	1	0.0009	0.67	0.4173
X_{3}^{2}	0.0001	1	0.0001	0.08	0.7818
Lack-of-fit	0.017	17	0.00098	0.73	0.7611
Pure error	0.0727	54	0.00134		
Total (corr.)	2.557	80			
R ² =96.50%	R^2 (adjusted for d.f.) = 96.06%			Standard Error of Est. = 0.037	

level. The unimportant terms (P > 0.05) are also removed; the equation is improved and shown in Equation 4.

Volume expansion (times) =
$$0.759 + 0.288X_1 + 0.772X_2$$

+ $0.55X_3 - 0.027X_1^2 - 0.040X_2^2$
R² = 96.32%, R²_{adj} = 96.07%, SEE = 0.037 (4)

It was observed that the model as fitted with high R^2 and R^2 adjusted values (96.32% and 96.07%, respectively) of the variability in volume expansion. With this model, the optimal (highest) volume expansion value obtained (5.57 times) was determined when processing under the condition of combining 3 optimal parameters of gac, sugar, and yeast, respectively, which are 5.41%, 9.73%, and 0.5%.

3.2.4. Simultaneous optimization of multiple responses

Multi-response graphic optimization is performed by overlaying the surfaces of each response by looking for intersection points between the optimal regions of each response. Contour plots facilitate this considerably; they exhibit responsive surface design, allowing for easier processing and verification of information [36].

In this study, the dependent variables were identified, including β -carotene (μ g/g), lycopene (μ g/g), and the volume expansion (times) were optimized separately using response surface methodology. Therefore, the independent variables have different optimal values. Desirability optimization should be performed to produce a combination of response surfaces to maximize beta-carotene, lycopene, and volume expansion with the same optimal values of Gac aril, sugar and dry yeast. Contour plot showing the effects of gac aril, sugar, and yeast on β -carotene, lycopene, and volume gain optimized with an asterisk (*), as shown in Figure 1. For each figure, one variable is fixed; the fixed contents of dry yeast, sugar, and Gac aril are shown in Figure 1a-c, respectively.

To get the highest values of β -carotene (14.78 µg/g), lycopene (59.14 µg/g), and expansion volume (5.3 times), the optimal values of Gac aril, sugar, and instant dry yeast should be used 8.55%, 9.76%, and 0.5%.

The optimal value of sugar content obtained from this study is quite similar to the study results of Campbell *et al.* [37], they reported that the concentration of added sugars in bread flour varied from zero to about 8% or slightly higher. However, the optimal concentration of dry yeast optimized in this study was much lower than in the previous study. Birch *et al.* [38] suggested a high yeast concentration of 6% for low-temperature fermentation (5°C) to produce bread in a short production time.

This difference is probably due to the type of yeast used and the different processing conditions. To test the optimal values found from the predictive models, sandwich bread was processed according to the optimal parameters, including Gac aril 8.55%, sugar 9.76%, and dry yeast 0.5%. The experimental results showed that β -carotene, lycopene, and volume expansion values of bread are close to the results predicted from the model, only 2.4 to 3.1% difference [Table 5], where β -carotene and lycopene were 2.4% and 3.1% higher, respectively, while the expansion volume was 2.6% lower than predicted. These differences are within the allowable limit (<5%).

Overall organoleptic evaluation showed that the M2 (8.55% Gac aril) and M3 (9% Gac aril) samples [Figure 2] scored the highest of all recipes, showing 8.55–9% of the Gac aril supplementation as the preferred levels in sandwich bread making. The preference mapping also showed similar results [Figure 3]. Bread samples were evaluated according to preference scores. The results from the preference mapping again confirmed that formula M2 and M3 are the most preferred by consumers (80–100%). Sample M1 received a relatively moderate preference (40%) and the control sample received the lowest acceptance from consumer.

Bread is a popular food all over the world, these research results have added to the processing technology of high quality bread, with higher β -carotene and lycopene content than traditional product (as mentioned above). This new type of bread not only serves the daily needs of people but also support good health. The effects of other ingredients (yeast and sugar) on quality support (volume expansion) besides Gac composition were also identified.



Figure 1: The overlay plot of β-carotene, lycopene, and volume expansion of bread and optimum value*: (a) instant dry yeast 0.4%; (b) sugar 10.6%; and (c) Gac aril 7%.



Figure 2: Sandwich breads have been supplemented with 8.55% (M2 – left side) and 9% Gac aril (M3 – right side).



Figure 3: Preference mapping of sandwich bread with different formulas. M0: Control sample (Without Gac aril addition); M1: 7% Gac aril; M2: 8.55% Gac aril, and M3: 9% Gac aril.

4. CONCLUSION

The obtained results confirmed that the addition of 8.55-9% Gac aril could be an effective way to enhance the nutrition (β -carotene and lycopene) content of sandwich bread. The special feature in this study is that the carotenoids and the volume expansion of the sandwich bread increased when the appropriate combination of Gac

Table 5: Predicted and actual value of responses from optimal conditions.

Parameters	Predicted values	Actual values
β -carotene (μ g/g)	14.78	15.13±0.09
Lycopene (µg/g)	59.14	$60.97 {\pm} 0.48$
Volume expansion (times)	5.30	5.16 ± 0.05
Mean+SD		

aril, sugar, and instant dry yeast. High sensory scores were obtained with bread containing 8.55% and 9% Gac aril compared with the control sample (without Gac addition). Based on these findings, Gac aril has a natural colorant, a potential functional food ingredient, and could become a high-quality source for the production of healthful foods. This also showed a new use and the ability to develop a combination of Gac aril in the sandwich manufacturing industry, effectively taking advantage of the rapidly growing Gac fruit in some localities in Vietnam and supporting the improvement of economic efficiency of the region.

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

11. PUBLISHER'S NOTE

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