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## Research Article

### Sorghum flour in breadmaking: opportunities for sorghum sourdough, gluten-free bread quality and shelf life

Rosen Chochkov<sup>1✉</sup>, Angel Angelov<sup>2</sup>, Aleksandra Krasteva<sup>1</sup>, Ivan Rangelov<sup>3</sup>

<sup>1</sup> Department: Technology of cereals, fodder, bread and confectionary products, University of food technologies, Plovdiv, Bulgaria

<sup>2</sup> Center of Competence AgriFoodSystems and Bioeconomy, Plovdiv, Bulgaria

<sup>3</sup> Index 11 JSC, Plovdiv, Bulgaria

#### Abstract

Our study aimed to obtain gluten-free bread from sorghum (*Sorghum bicolor* (L.) Moench) with applying single cultures of *Pediococcus pentosaceus* SAP 4.4 (PP), *Levilactobacillus brevis* SAP 1.4 (LB) and *Weissella cibata* SAP 8.7 (WC) as sourdoughs. The tested strains reached 108 cfu.g<sup>-1</sup> at the end of fermentation process and produced sourdoughs with a pleasant lactic aroma. The most significant influence on the volume, respectively the specific volume of the bread with sorghum flour and the sourdough has its enrichment with sample PP. When conducting an organoleptic analysis, it was found that the appearance, the color of the crust and the medium, the hollowness, the aroma and the chewiness of the product tend to improve. Also, the addition of sourdough improves both the taste and aroma. As the duration of storage increases, the compressibility of the medium decreases in all investigated samples, but with different degrees of intensity.

#### Keywords

sorghum flour, sourdough, bread quality, shelf life, lactic acid bacteria

#### Abbreviations

LB – *Levilactobacillus brevis* SAP 1.4; PP – *Pediococcus pentosaceus* SAP 4.4; P.U. – penetrometric units;

WC – *Weissella cibata* SAP 8.7

✉Corresponding author: Assoc. Prof. Rosen M. Chochkov, PhD, Department Technology of cereals, fodder, bread and confectionary products, Technological Faculty, University of Food Technologies, 26 Maritza blvd., 4002 Plovdiv, Bulgaria, tel.: +359 32 603 635; E-mail: [rosen4o4kov@abv.bg](mailto:rosen4o4kov@abv.bg)

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## Introduction

Sorghum plays a decisive role in food security in developing countries. It is used in many types of food recipes such as breads, porridges, pastes, and griddlecake (Coulibaly et al. 2020).

Sorghum (*Sorghum bicolor* L. Moench) is the fifth largest crop in the world. Sorghum grain has a protein content varying from 4.4 to 21.1 % with an average value of 11.4% (Olojede et al. 2020; Ogunsakin et al. 2015). Sorghum grain is gluten free, high in resistant starch, a rich source of nutrients, and most importantly, it contains a diverse range of bioactive phenolic compounds (Awika et al. 2004; Dykes et al. 2007). It has been proven safe for people with celiac disease, but literature on its use in gluten-free foods is relatively scarce compared to that for corn and rice.

In recent years, several studies on composite breads have confirmed the technological usefulness of hydrocolloids to compensate for quality losses (Ozkoc et al. 2009; Shittu et al. 2009). Using stabilizers, gums and pre-gelatinized starches could mimic the wheat gluten function in gluten free or composite doughs (Onyango & Lindhauer 2011; Ozkoc et al. 2009; Shittu et al. 2009). Among food hydrocolloids, xanthan gum plays a special role for improving the composite breads quality through enhancing the dough viscoelastic properties even at low concentrations (Ahmed & Thomas 2017; Crockett Ie & Vodovotz 2011).

The use of sorghum in wheat products could be a possible future solution to counteract the high climate-induced protein content of wheat. In addition, end products consisting of sorghum can gain valuable nutrients (Salim-ur-Rehman et al. 2006; Rumler et al. 2021).

Gluten-free bread (GFB) can be classified as a source of fiber since the fiber content is higher than 3 g per 100 g, while GFB made with 100 % WS/BS (bronze sorghum) can be classified as high in fiber, as the content of this component is higher than 6 g per 100 g (Centeno et al. 2021).

The use of sourdough is the oldest biotechnological process to leaven bakery goods and to improve bread texture, aroma, and shelf life (Cappa et al. 2016; Chochkov et al. 2022; Sun et al. 2020; Teleky et al. 2020). A number of studies have shown that sourdough can be used successfully to improve the

processing characteristics of gluten-free doughs (Moore et al. 2006; Moore et al. 2007; Ngemakwe et al. 2015; Matos et al. 2015). In some cases, this effect may be attributed to the potential of lactic acid bacteria to secrete extracellular polysaccharides, which could be a beneficial alternative to conventional thickeners used to modify the viscosity, structure and stability of a wide range of gluten-free foods (Moroni et al. 2009).

From the seven industrial strains *Saccharomyces cerevisiae* was selected as a perspective on the grounds of its biosynthetic and baker's properties. *S. cerevisiae* strain 3 had over 14% improvement of the fermentation activity correlating with the characteristics of the maltase activity. A detailed analysis of both strains established that Strain 10 had higher zymase activity while strain 3 had higher maltase activity (Angelov et al. 1996).

The quality of sorghum flour, dough, and bread can be improved by the addition of different ingredients and using novel and traditional methods (Ari Akin et al. 2022).

While the formulation of sorghum-wheat composite flour is crucial for value-added nutritionally improved products, the products produced should be characterized by acceptable consumer sensory attributes (Khating et al. 2014).

For gluten-free bread, increasing white sorghum flour levels in the blends primarily affected specific volume, color, flavor and texture characteristics, leading to decreases in specific volume, cohesiveness, springiness, chewiness and moistness, but increases in color intensity, brown and nutty flavors, graininess and roughness (Adzqia et al. 2023).

Therefore, the aim of the present study was to explore the application of three different LAB strains isolated from sorghum grains to produce three sorghum sourdoughs for the development of gluten-free bread with improved sensory and quality characteristics.

## Materials and Methods

**Materials.** The flour used in this study was obtained from sorghum hybrid KALATUR (Lidea, 31700 Mondonville, France) provided by Agricultural University, Plovdiv. The grains were ground by laboratory grain mill at the University of Food

Technologies, Plovdiv. Compressed yeast was supplied by Lesaffre Ltd. (Sofia, Bulgaria). Guar gum (E412), xanthan gum (E415), Gum arabic (E414) and Tapioca flour were supplied by Cargill (Minneapolis, MN, USA).

Three lactic acid bacteria (LAB) strains were used in this study: *Pediococcus pentosaceus* SAP 4.4 (PP), *Levilactobacillus brevis* SAP 1.4 (LB) and *Weissella cibaria* SAP 8.7 (WC). The strains were isolated from KALATUR sorghum grains, identified and stored in culture collection of the Dept. of Biotechnology, University of Food Technologies, Plovdiv. Starter cultures for the sourdoughs were prepared from stock cultures of each strain stored in Microbank™ (Pro Lab Diagnostics Inc., Richmond Hill, ON, CA) by cultivation in MRS broth (de Man–Rogosa–Sharpe, Merck KGaA, Darmstadt, Germany) at 37°C for 48 h.

## Methods

**Sourdough preparation.** Sorghum flour was used to prepare a separate sourdough with each LAB strain. Equal weights of flour and sterile water were mixed to obtain a dough yield (DY) of 280. Each starter culture was added to a batch of sorghum dough at an inoculum amount of 5 log colony forming units (CFU).g<sup>-1</sup> of dough. The sourdough variants were then fermented in an incubator at 37°C for 24 h.

**Analyses of sourdoughs.** Active acidity (pH) was determined by a pH meter (FiveEasy FE20, Mettler-Toledo GmbH, Greifensee, Switzerland). Total titratable acidity (TTA) was determined by titration with 0.1 N NaOH to pH 8.4 and expressed as mL of NaOH.10 g<sup>-1</sup> of sourdough, as described in AACC 02-31 (AACC 2010).

**Bread production.** Test sorghum breads with sourdoughs and control bread were prepared. The control sample bread (CSB) was composed of sorghum flour (100%), yeast (3% of flour basis), salt (1.5% of flour basis), and water (70% of flour basis). The same ingredients (except the water, which was increased by 5%) were used to prepare the test breads with the addition of sourdoughs PP, LB and WC, named after the respective LAB strains and also guar gum (0.5%), xanthan gum (0.5%), gum arabic (0.5%) and tapioca flour (10%). Kneading was done by a single-phase method of

dough preparation to obtain a dough with a homogeneous mass and an initial temperature of 26 - 28°C. The dough was left to rest for 60 min (T - 35°C; W (relative humidity) - 75% and was then divided into pieces of 440 g - for pan bread. After shaping, the dough was imposed to cold fermentation for 420 min (T - 4°C), and final fermentation at 35°C for 60 min in a fermenting chamber (Tecnopast CRN 45-12, Novacel Rovimpex Novaledo, Italy). The dough was then baked in an electric floor oven Salva E-25 (Salva Industrial S.L.U., Spain), preheated to a temperature of 200 °C, for 19 – 20 min. After baking, the breads were allowed to cool for 3 h at room temperature (Di Cagno et al. 2002).

**Bread quality.** Bread volume was determined after baking and cooling the breads for 3 h at room temperature by a rapeseed displacement method (Ngemakwe et al. 2015). The specific volume was calculated by the ratio between volume (cm<sup>3</sup>) and weight (g) of each sample. Bread loss (%) was determined following weighing each loaf before and after baking. The bread loaves were wrapped in plastic bags and stored at room temperature (27 ± 2°C) to determine the storage time (in hours) until mold growth became visible (Agagündüz et al. 2021).

**Crumb elasticity.** Total, elastic and plastic deformation were measured by an automatic penetrometer (Chochkov et al. 2022). A 4 mm thick crumb sample was cut from the bread and placed on the flat surface of the lifting table, which was raised until the upper surface of the sample lightly touched the lower end of the immersion body.

**Sensory analysis.** Sensory analysis of the obtained breads was performed by a descriptive panel consisting of 25 panelists (52% women and 48% men) aged 22 - 60 years, who were familiar with sensory analysis of foods, but not specifically trained in the evaluation of sourdough breads. The analysis was carried out according to ISO 6658:2017 (Olojede et al. 2020). The panelists were asked to score eight parameters, namely shape, crust color, crumb color, porosity, aroma, chewability, taste, and aftertaste. They expressed the intensity of each attribute on a 9 – point hedonic scale (9 - extremely good; 1 - extremely bad).

**Statistical analyses.** All analyses were conducted in triplicate. The obtained data were subjected to

one-way ANOVA using XLSTAT version 2019.1.2 (AddinSoft Inc., New York, NY, USA). Comparison among LS means were performed by Tukey's test; differences were considered significant at  $P < 0.05$ .

## Results and Discussion

**Sorghum sourdough fermentation.** Sorghum sourdoughs were prepared by using three single-strain LAB cultures: *Pediococcus pentosaceus* SAP 4.4 (PP), *Levilactobacillus brevis* SAP 1.4 (LB) and *Weissella cibata* SAP 8.7 (WC). Fermentation was carried out for 24 h at 37°C, and the obtained sourdoughs were characterised according to final pH, TTA and LAB viable counts (Table 1).

These strains were selected to compare their potential in developing sourdough and later gluten-free bread made from sorghum. The strain *Pediococcus pentosaceus* has a homofermentative metabolism, where the pyruvate molecules formed from glucose metabolism are primarily reduced to lactic acid. This results in a pleasant aromatic profile of the obtained sourdoughs. On the other hand, heterofermentative strains of *Levilactobacillus brevis* and *Weissella cibaria* produce acetates, which complement the aromatic profile of the sourdoughs. Lactic acid bacteria in the sourdough contribute to the aroma of the bread and

slow down its spoilage which is may be due to the acids they produce.

There was an increase in the microbial population of lactic acid bacteria. Data analysis showed no significant differences in biomass concentration at the end of the fermentations among the three types of sourdough. The growth of lactic acid bacteria increased from 3.00 log cfu.g at the beginning to 8.46–9.84 log cfu.g up to 24 h. The highest value was recorded for the homofermentative strain *Pediococcus pentosaceus*.

Sorghum proteins are unable to form a gas-retaining, viscoelastic dough, which is a basic requirement for making leavened bread and many other baked goods (Taylor and Dewar 2001). The other major issue with sorghum proteins is that their digestibility can be reduced if subjected to heat treatment during the technological process (Arbab and El Tinay 1997).

Sorghum fermentation can have both probiotic and prebiotic activity. For example, fermented sorghum sourdough inoculated with the probiotic gram-positive bacterium *Weissella cibaria* produces significant levels of prebiotics, which can stimulate beneficial gut microbiota in the colon (Schwab et al. 2008). The quality indicators of bread with sorghum flour and sourdough are presented in Table 2.

**Table 1.** Fermentation kinetics of sorghum sourdoughs

| Strain/<br>Time | <i>Pediococcus pentosaceus</i> SAP 4.4<br>(PP) |           |           | <i>Levilactobacillus brevis</i> SAP 1.4<br>(LB) |           |           | <i>Weissella cibata</i> SAP 8.7 (WC) |           |           |
|-----------------|------------------------------------------------|-----------|-----------|-------------------------------------------------|-----------|-----------|--------------------------------------|-----------|-----------|
|                 | pH                                             | TTA       | Log cfu.g | pH                                              | TTA       | Log cfu.g | pH                                   | TTA       | Log cfu.g |
| 0 h             | 6.36±0.04                                      | 3.8±0.12  | 3.00±0.25 | 6.32±0.06                                       | 4.1±0.16  | 3.00±0.36 | 6.40±0.08                            | 4.1±0.18  | 3.00±0.06 |
| 4 h             | 6.01±0.06                                      | 4.2±0.14  | 3.28±0.41 | 5.98±0.14                                       | 4.8±0.24  | 3.40±0.38 | 5.84±0.10                            | 4.6±0.26  | 3.18±0.28 |
| 8 h             | 5.38±0.10                                      | 6.8±0.21  | 6.43±0.32 | 5.36±0.21                                       | 5.4±0.26  | 4.94±0.48 | 5.12±0.10                            | 5.4±0.32  | 4.00±0.36 |
| 12 h            | 4.86±0.11                                      | 8.6±0.18  | 6.98±0.46 | 4.62±0.16                                       | 8.9±0.18  | 6.82±0.22 | 4.73±0.08                            | 7.6±0.24  | 5.64±0.44 |
| 16 h            | 4.02±0.09                                      | 10.0±0.25 | 7.84±0.28 | 4.01±0.11                                       | 10.4±0.30 | 7.64±0.26 | 4.10±0.11                            | 9.4±0.30  | 6.88±0.38 |
| 20 h            | 3.86±0.15                                      | 11.8±0.16 | 8.45±0.36 | 3.84±0.16                                       | 11.5±0.26 | 8.44±0.28 | 4.08±0.06                            | 10.9±0.14 | 7.74±0.24 |
| 24 h            | 3.72±0.10                                      | 12.6±0.21 | 9.84±0.44 | 3.70±0.24                                       | 12.1±0.24 | 9.12±0.36 | 4.02±0.06                            | 11.5±0.26 | 8.46±0.42 |
|                 | a                                              | A         | a         | a                                               | a         | a         | a                                    | b         | a         |

\*Mean values of the same row differ significantly ( $p < 0.05$ ).

The specific volume of sourdough breads increases compared to the control, which has the lowest - 1.24 cm<sup>3</sup>.g<sup>-1</sup>. The highest specific volume was recorded for culture PP - 1.48 cm<sup>3</sup>.g<sup>-1</sup>. Similar results were reported by (Adepehin et al. 2022; Velázquez et al. 2012) where the specific loaf volume varied from 1.46 to 1.80 cm<sup>3</sup>.g<sup>-1</sup>. For gluten-free bread, increasing white sorghum flour levels in the blends primarily affected specific volume (Adzqia et al.

2023). The obtained results form Table 2 clearly indicate that the highest values for technological loss are reported for the control sample (20.20%), while all test samples have lower losses. The obtained results thus show that the most significant influence on the volume of bread has the addition of sourdough with test culture PP. In comparison with the other culture the difference is lower. This increase is probably due to the increased amount of

water for kneading of bread samples, since the flour has a high-water absorption, as well as the hydrocolloids and the tapioca flour that were added.

**Table 2.** Baking characteristics of sorghum bread formulations

| Bread samples  | Weight, g | Specific volume, cm <sup>3</sup> /g | Bread loss, % |
|----------------|-----------|-------------------------------------|---------------|
| Control sample | 402.3     | 1.24                                | 20.20         |
| Sample PP      | 413.4     | 1.48                                | 18.33         |
| Sample LB      | 415.9     | 1.45                                | 17.83         |
| Sample WC      | 414.5     | 1.44                                | 18.11         |

In another study, the nutritional properties of the sorghum flour were improved and the physical parameters met the expectation for sourdough breads (Ogunsakin et al. 2015). Furthermore, breads made with *L. acidophilus* (at 20 and 30% addition levels) showed better quality attributes such as softer texture, higher total phenolic content and antioxidant capacity (Turksoy et al. 2023). Other authors reported a significant decrease in bread volume with sorghum replacement (higher than 20%) of wheat flour (Sibanda et al. 2015).

The results from analyzing the sensory profile in sorghum bread with sourdough are presented in Fig. 1.

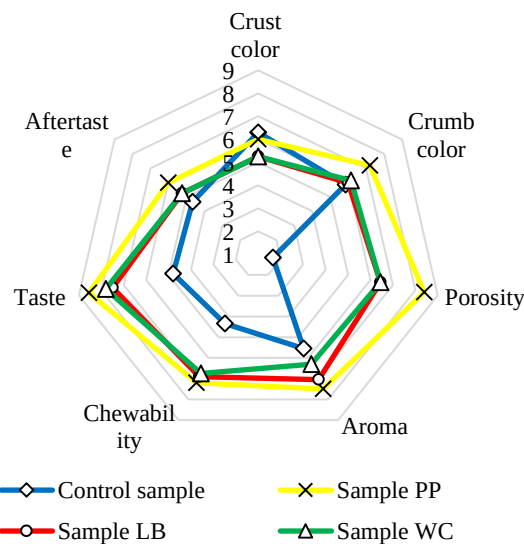


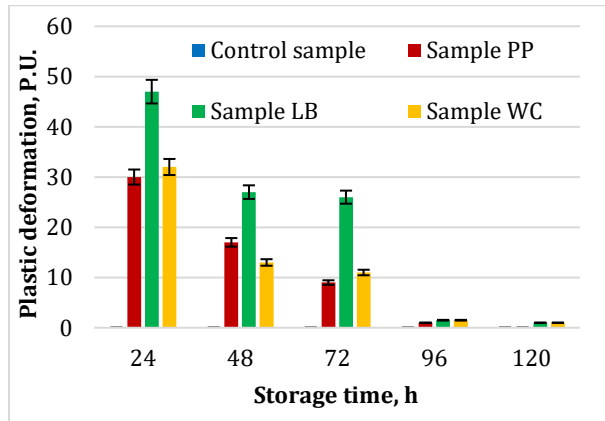
**Figure 1.** Sensory profile of sorghum bread with sourdoughs

In the control sample, the results for the color of the crust and crumb, porosity, aroma and chewiness are low. The bread is very hard. The control sample is characterized by an unpleasant taste (according to the evaluators), and a not so pleasant aftertaste, with a feeling of bitterness and an intrusive aftertaste. The crumb and crust color are satisfactory, with slightly higher values in culture PP. The same results regarding the sourdough were reported by Turksoy et al. (2023). This research showed that the bread crust color and titratable acidity were comparable; although, the crumb color of sourdough bread made with *L. acidophilus* was measured darker. Their aroma is significantly better. It can be feel the typical sourdough aroma. Also, the lactic acid bacteria added improve the taste and aroma. The test breads have relatively good results for aftertaste. For gluten-free bread, increasing white sorghum flour levels in the blends primarily affected color, flavor and texture characteristics but increases in color intensity, brown and nutty flavors, graininess and roughness (Adzqia et al. 2023).

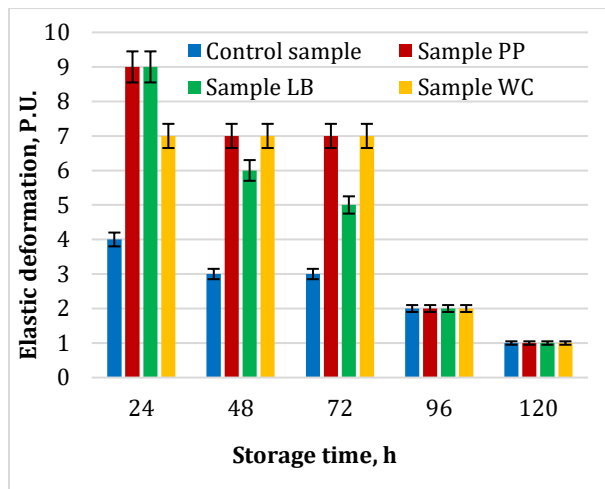
**Shelf life estimation of sorghum bread with sourdough.** The shelf life of bread with sorghum flour and sourdough was determined by determining the deformation characteristics of the breadcrumb – plastic (Hp) and elastic deformation (He) (Chochkov 2016). Fig. 2 clearly shows that for all samples, with increasing of storage time, the values characterizing the deformations of the crumb decrease, which indicates that the breadcrumb becomes harder. 24 h after bread baking, the results in the test samples with sourdough are much higher than the control sample. The values for culture PP and culture WC are the same, 39 P.U. Culture LB has the highest results – 56 P.U. The control sample is distinguished by 4 P.U. With increasing storage time, the compressibility of the crumb decreases in all test cultures, but with varying degrees of intensity. In the control sample, the breadcrumb is characterized by the lowest compressibility. Secil Turksoy et al. (2023) reported that both starter cultures, sourdough bread had a softer texture than the control, with significant ( $p < .05$ ) differences in hardness.

The first 24 h of the analysis the control sample has plastic deformation – 0 P.U., culture PP and WC similarly to the total deformation have almost similar results. The highest results are for sample LB – 47 P.U. According to study, it was found that

the greatest change is noted in culture LB. Culture PP and WC also decrease significantly by 30 and 31 P.U. respectively. By the end of the study, the control sample does not change the results – the plasticity remains at a constant, unchanged level – 0 P.U. From the obtained results and based on the visual assessment of the products, the test samples with sourdough retain their plasticity until the end of storage. The changes in the deformation characteristics are shown in Fig. 2.



(a)



(b)

**Figure 2.** Influence of sourdoughs on deformation characteristics of sorghum bread (a) – plastic; (b) – elastic. Control sample (CS); *Pediococcus pentosaceus* SAP 4.4 (PP), *Levilactobacillus brevis* SAP 1.4 (LB) and *Weissella cibaria* SAP 8.7 (WC).

Elastic deformation measurement showed the same average low values for all tested cultures. This can be explained by the fact that the experimental

samples were made with gluten-free flour, and hence the elasticity of the samples was negligible, typical for this type of products. Analogously to the indicators of the total and plastic deformations, in the first 24 h of the analysis, the control had the lowest values – 4 P.U., while in all three test samples, the results were higher.

During the study, a decrease was found in all samples. At the end (at 96 and 120 h) the results for all samples were the same, respectively 2 and 1 P.U. In summary, it can be concluded that during the study, the samples with sourdough had better results for elasticity, until the end of the study, where the results became uniform. Organic acids in sourdough breads induced hardening of the bread crumb. Sandra Galle et al. (2012) indicated that the exopolysaccharides formed during sourdough fermentation masked the effect of the organic acids and led to a softer crumb in the fresh and stored sorghum bread. Among EPS, dextran showed the best shelf life improvements. Sibanda et al. (2015) reported that sorghum flour addition at 30% in white bread, there was a complete loss of dough elasticity (Sibanda et al. 2015).

## Conclusions

In this study, the addition of LAB strains as a sourdough in sorghum flour dough has a different effect on the individual characteristics of the dough and the final product. The application of sourdough (culture PP) resulted in a significant pronounced increase on bread specific volume. The obtained sorghum gluten-free breads had positive sensory acceptance in appearance, crumb and crust color, porosity and chewability. The study demonstrated that with increasing the storage time, the compressibility of the crumb decreases in all test cultures, but with varying degrees of intensity. In the control sample, the bread crumb was characterized by the lowest compressibility.

## Author Contributions

Conceptualization, methodology, and analysis – original draft preparation. All authors have read and agreed to the published version of the manuscript.

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## Institutional Review Board Statement

Not applicable.

## Informed Consent Statement

Not applicable.

## Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

## Conflicts of Interest

The authors declare no conflicts of interest.

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