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Fermentation characteristics and bread baking attributes of bread dough fermented with glutinous rice

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Abstract: In this study, 5 kinds of Chinese traditional starters (Qu) were selected and made into fermented glutinous rice, then the fermentation products were used in bread-making, a starter with special flavor was screened, and its fermentation characteristics were determined. Fermented glutinous rice was made by the selected starter, and its effects on dough and bread-baking properties were studied. The results showed that the number of molds was stable after 24 hours of fermentation and decreased later. Yeasts grew into the stationary phase after 40 h. Then, after 48 h of fermentation, the content of main metabolites: organic acids, reducing sugar, and ethanol, was all stabilized.
Keywords: fermented glutinous rice; functional ingredients of bread; flavor; baking; enzymes

1. Introduction

Glutinous rice, also known as Jiangmi, is processed from glutinous rice. It is one of the important food resources in my country. Its main nutritional components are carbohydrates, protein, fat, and some vitamins, among which starch accounts for about 75% to 77%. Since the starch in glutinous rice is almost all amylopectin (98%~99%), after cooking, amylopectin can be entangled with each other like branches, so glutinous rice is more viscous after cooking, and has the characteristics of softness and fragrance. In addition to being eaten directly, glutinous rice has high processing and utilization value, and is the raw material for making many traditional Chinese specialties, such as sweet wine, rice dumplings, glutinous rice balls, and eight-treasure rice, etc. (Lv et al., 2013).

Tianjiuniang is one of the representative traditional Chinese folk foods and has a long history of eating. Its brewing technique can be traced back to the Han Dynasty and flourished in the Ming and Qing Dynasties. It is made by steaming glutinous rice, mixing it with sweet wine koji, and fermenting (Gui-you et al., 2004). Tianjiu Niang has different names in various parts of China, such as glutinous rice, rice wine, distilled juice wine, glutinous rice wine, sweet rice wine, distiller's grains, etc. "Compendium Supplements" records that the wine is "sweet and pungent in taste, warm in nature. Adjuvant medicine produces acne pulp, promotes blood circulation and nourishes marrow veins, and produces body fluid." Its sweet and sour taste, mellow aroma, is attractive, and is deeply loved by the common people. The alcohol content of Tianjiu Niang is low. During the fermentation process, the macromolecular nutrients in the glutinous rice are degraded into small molecular nutrients, which is more conducive to human body absorption. It is rich in carbohydrates, amino acids, vitamins, minerals, and organic acids. Moderate consumption can improve immunity, promote metabolism, nourish blood and beautify skin, strengthen body and mind (Kim et al., 2015).

In addition to delicious nutrition, sweet fermented glutinous rice has certain medicinal value and can be used as an auxiliary treatment for some chronic diseases. Drinking fermented glutinous rice regularly can promote the secretion of gastric juice in patients with chronic atrophic gastritis, increase appetite, and help digestion. Fermented glutinous rice can also speed up blood circulation, which is beneficial for patients with chronic arthritis to promote blood circulation and collaterals, increase high-density lipoprotein content, reduce lipid deposition in blood vessels, and help lower blood lipids and prevent arteriosclerosis. In addition, fermented glutinous rice has the effects of refreshing, relieving fatigue, and promoting milk secretion in parturients, which

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is beneficial to the physical recovery of patients after serious illness. From the perspective of traditional Chinese medicine, tianjiu niang can also warm the meridians, activate blood circulation, nourish yin, and moisten the lungs, which is good for women's beauty, treatment of dysmenorrhea, and postpartum recovery (Chai et al., 2015). Studies have shown that fermented glutinous rice has the inhibitory effect of α-glucosidase, which is beneficial to control the blood sugar level of the human body and prevent diseases such as diabetes and obesity. In the mouse experiment, an appropriate amount of rice wine can help improve the learning and memory ability of mice and has the effect of delaying brain aging and preventing Alzheimer's disease (Li et al., 2015). However, since alcohol has a certain stimulating effect on the liver, it is not suitable for patients with liver diseases (hepatitis, cirrhosis, etc.) to drink. At the same time, there are certain differences in the nutritional components of sweet wine made from different sweet wine kojis, and the consumption should also be adjusted.

The thermomechanical properties, dynamic rheological properties, fermentation rheological properties, free sulfhydryl content, and protease activity of dough with different fermentation times were compared and analyzed, and the corresponding bread baking quality and sensory scores were compared. To explore the reasons why Tianjiu niang affects the rheological properties and biochemical indicators of dough and the baking quality of bread. (4) Satisfaction function-response surface optimization to optimize the addition of compound enzyme preparations to improve the baking quality of Tianjiuniang bread. The amount of compound enzyme preparation was optimized by the satisfaction function-response surface optimization method, so as to improve the baking quality of Tianjiuniang bread. (5) Effects of enzyme preparations and fermented glutinous rice on dough characteristics, bread baking characteristics, and storage quality.

Comparing the free sulfhydryl content, protease activity, molecular weight distribution of gluten protein and dough microstructure of wheat bread, sweet wine glutinous rice bread and the optimal enzyme compound group sweet rice wine bread dough, and comparing the baking characteristics and storage moisture and hardness of the three kinds of bread, aging enthalpy and the total number of bacterial colonies on the surface of the skin to comprehensively evaluate the bread quality.

2. Materials and methods

2.1. Experimental materials and equipment

Sweet wine koji: Angel koji (AQ), Angel Yeast Co., Ltd.; Suzhou koji (SZ), Suzhou grain, oil and food Co., Ltd.; Anhui distiller's yeast (AH), Guangxi distiller's yeast (GX), Hubei's distiller's yeast (HB), commercially available farm sweet wine. Instant active dry yeast: Le Sifu (Mingguang) Co., Ltd.; Butter: COFCO Donghai Cereals and Oils Industry (Zhangjiagang) Co., Ltd.; Wheat flour: COFCO Noodle Industry Pengtai Co., Ltd.; Glucose oxidase (GOX, 17200 U/g): Netherlands Royal DSM Group; transglutaminase (TG, 100 U/g): Jiangsu Yiming Biological Co., Ltd.; xylanase (XYL, 678400 U/g): Dongguan Pan Asia Pacific Biotechnology Co., Ltd.; glutinous rice, salt, white sugar: commercially available.

2.2. Experimental method

2.2.1. Comparison and screening of sweet wine koji used in making sweet wine bread

(1) Production of Tianjiu Niang Wash 250 g of glutinous rice three times, add enough water to soak for 3-5 hours, until the glutinous rice can be crushed by hand. Drain the water, steam on high heat for 30 minutes (the glutinous rice is cooked but not mushy), pour cold boiled water over the rice and let it cool, when the temperature drops to about 36°C, add sweet wine koji (0.5% inoculum) and stir evenly and set aside The dimples were then fermented in a 30°C incubator for 48 hours.

(2) Production of sweet wine glutinous rice bread. The recipes of wine, glutinous rice bread, and common wheat bread made with different sweet rice wine koji fermented are shown in Table 1. Jiuniang Bread AQFRB, Suzhou Jiuniang Bread SZFRB, Guangxi Jiuniang Bread GXFRB, Anhui Jiuniang Bread AHFRB, Hubei Jiuniang Bread HBFRB. Mix the ingredients except butter for 2 minutes at low speed and 4 minutes at high speed to form a dough. After adding the butter, stir slowly for 1 min, then stir rapidly for 3 min until the gluten network is formed. After the film was relaxed for 10 minutes at room temperature, it was cut and rounded, and the film was relaxed for 10 minutes again, and shaped (90 g/piece). Then put it into a proofing box (temperature 38°C, relative humidity 85%) to proof for 65 minutes, put it in an oven (upper heat 170°C, lower heat 210°C) and bake for 21 minutes, demold and cool.

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2.3. Determination of pH and total titratable acidity (TTA) of sweet fermented glutinous rice

Determination of pH and TTA According to AACC method (2000) 02-52, take 10 g of sweet wine fermented with different sweet wine kojis into Erlenmeyer flasks, add 90 mL of distilled water, stir magnetically for 30 minutes, let stand for 10 minutes, and measure pH. Titrate with 0.1 mol/L NaOH and stir to pH 8.6. The volume of NaOH consumed is the total titratable acidity. Repeat the operation at least three times for each sample.

2.4. Determination of reducing sugar content in sweet fermented glutinous rice

The DNS colorimetric method was used for the determination of reducing sugar, referring to and improving the experimental method of Breuil and colleagues (1985). Take 5 g of sweet fermented glutinous rice, add 10 mL of 0.1 mol/L NaOH solution to inactivate the enzyme, then add 35 mL of water, shake in a water bath at 45 °C for 1 h, cool down, dilute to 100 mL, mix well, let stand, precipitation.

Draw an appropriate amount of supernatant into a 250 mL volumetric flask, add 5 mL of 10% zinc acetate solution and 5 mL of 10% potassium ferrocyanide solution, add water to the mark, mix well, let stand for 30 min, and filter the supernatant (Discard the initial filtrate). Take 1 mL of the filtrate in a 10 mL colorimetric tube, add 2 mL of DNS reagent, add water to 5 mL, shake well, take out the boiling water bath for 5 min, cool under running water, and finally add Water to 10 mL, shake well, and measure the absorbance at 520 nm. The reducing sugar content was calculated according to the absorbance value and the glucose standard curve.

2.5. Quality Evaluation of Tianjiu Niang Bread

(1) Determination of the specific volume of bread. After baking, the bread was cooled at room temperature for 2 h, its mass and volume were measured, and the specific volume was calculated (specific volume (mL/g) = volume (mL)/mass (g)). Volume was determined by the rapeseed displacement method.

(2) Determination of the overall texture of bread. After the bread was cooled at room temperature for 2 hours, it was immediately cut into 10 mm uniform slices with a slicer, and two complete and uniform slices of bread in the center were taken for overall texture determination. The parameters are set as follows: probe model P/36, induction force 5 g, compression degree 40%, speed before test 3.0 mm/s, speed during test 1.0 mm/s, speed after test 3.0 mm/s, test interval 1 s. The experiment was repeated three times for each group of bread to obtain the average value.

(3) Sensory evaluation of bread. The flavor, taste, appearance, texture, and overall acceptability of bread were evaluated by the nine-point preference method. Scores from 1 to 9 represent liking for the sample; the higher the score, the more you like the sample, 1 represents extremely dislike, and 9 represents extremely like. The evaluation panel consisted of 20 (10 female, 10 male) persons trained in sensory evaluation.

2.6. Quality assessment of sweet fermented bread

(1) Determination of the specific volume of bread. After baking, the bread was cooled at room temperature for 2 h, its mass and volume were measured, and the specific volume was calculated (specific volume (mL/g) = volume (mL)/mass (g)). Volume was determined by the rapeseed displacement method.

(2) Determination of the overall texture of bread (Birch et al., 2013). After the bread was cooled at room temperature for 2 hours, it was immediately cut into 10 mm uniform slices with a slicer, and two complete and uniform slices of bread in the center were taken for overall texture determination. The parameters are set as follows: probe model P/36, induction force 5 g, compression degree 40%, speed before test 3.0 mm/s, speed during test 1.0 mm/s, speed after test 3.0 mm/s, test interval 1 s. The experiment was repeated three times for each group of bread to obtain the average value.

(3) Sensory evaluation of bread. The flavor, taste, appearance, color, and overall acceptability of bread were evaluated by the nine-point preference method (Butt et al., 2008). Scores from 1 to 9 represent liking for the sample; the higher the score, the more you like the sample. 1 represents extremely dislike, and 9 represents extremely like. The evaluation panel consisted of 20 (10 female, 10 male) persons trained in sensory evaluation.

2.7. Data analysis and processing

Using Excel 2013, Origin9.1, Design-Expert.V8.0.6, SPASS19.0, and other software to analyze the experimental results, using ANOVA (variance analysis method) for significant analysis, the level of significance difference is set to $P < 0.05$.

3. Results and Discussion

Comparison and screening of sweet wine fermented bread with different sweet wine koji

3.1. Comparison of pH, TTA, and reducing sugar content of sweet wine fermented with different sweet wine koji

Table 1. pH, TTA, and reducing sugar content of different fermented glutinous rice

Sample	pH	TTA/mL	Reducing Sugar Content
AQ	3.98±0.05^{a}	5.94±0.40^{c}	30.16±0.55^{a}
SZ	3.61±0.04^{d}	9.05±0.15^{a}	27.70±0.45^{c}
GX	3.92±0.03^{a}	6.85±0.37^{b}	30.59±0.92
AH	3.74±0.03^{c}	8.65±0.54^{a}	24.65±1.01^{d}
HB	3.81±0.02^{b}	7.15±0.69^{b}	29.29±0.50^{b}

Sweet wine koji generally contains abundant fungi such as rhizopus and yeast, which metabolize and hydrolyze nutrients such as carbohydrates and proteins, and generate various small molecular compounds such as reducing sugars, organic acids, amino acids, etc., and endow fermented foods with specific flavors and tastes (Driss et al., 2013). As shown in Table 3-1, there are some differences in the basic physical and chemical indicators between the different sweet wine kojis fermented with different sweet wine koji (Beck et al., 2012). According to the description of Ahn et al. (2005), a high-quality starter should have a soft sour taste and a pH range of around 3.7-4.1. It can be found that the pH value of most fermented rice is in this range, which has the potential to become a high-quality starter. The results of TTA and pH basically corresponded, and the fermented glutinous rice with higher TTA (SZ, AH, etc.) had lower pH, indicating that the increase of titratable acid content would decrease the pH. The reducing sugar content in different sweet wines samples exceeded 20 g/100 g sweet wines, and the reducing sugar content in AQ, GX and HB three kinds of sweet wines even reached 30 g/100 g sweet wines, indicating that the Rhizopus saccharification in the sweet wine koji Higher reducing sugar content can bring a sweet taste to bread, which is conducive to better taste of bread.

3.2. Comparison of Baking Quality of Tianjiu Niang Bread Fermented with Different Liquor Koji

The sweet wine fermented with different sweet wine koji was used as bread ingredients to make sweet wine bread, and the specific volume and texture parameters of the bread were measured to characterize the baking quality of the bread. The results are shown in Table 2. It can be found that although there are differences in the basic physical and chemical indicators of sweet wine fermented by different sweet wine kojis, the difference is not obvious in terms of bread baking quality.

Bread-specific volume is one of the most important bread-baking characteristics, which can directly affect consumer choice and is therefore an important parameter to measure bread quality (Lopes-da-Silva et al., 2007). In general, the introduction of a certain amount of gluten-free grains into bread formulations will dilute the gluten, affect the CO2 retention capacity, and generally decrease the specific volume of the bread (Irakli et al., 2015).

However, the results of this study show that the specific volume of bread containing Tianjiu Niang is similar to that of common wheat bread (Table 2), and the difference is not very significant. The gas production and gas holding capacity of the dough during fermentation and baking are the main factors affecting the specific volume of bread. Appropriate acid activates the α-amylase in the flour, the yeast in the rum, and baker's yeast produce gas together, reducing sugar, etc (Singh & Singh, 2013). Supplementation of fermentation substrates and other reasons may enhance the gas production capacity of sweet fermented rice dough, thereby increasing the specific

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volume. The dilution of gluten by sweet fermented gluten, the hydrolysis of gluten by sweet fermented gluten and wheat flour endogenous protease, and the destruction of gluten by a reducing environment may all lead to a decrease in the gas-holding capacity of the dough, thereby reducing the specific volume. Therefore, the specific volume of bread containing sweet fermented glutinous rice is the result of the joint action of many factors, among which GXFRB presents the largest specific volume of 4.87 mL/g. The impact on the dough reaches a more coordinated state (Rai et al., 2012).

Table 2. Specific volume and texture analysis of bread with different fermented glutinous

Samples	Hardness /g	Cohesion	Elasticity	Adhesiveness/g	Chewiness /g
WB	288.0±14.0^{ab}	0.813±0.012^{a}	0.897±0.006^{a}	242.0±11.0^{a}	217.0±11.5^{a}
AQFRB	319.7±6.0a	0.743±0.006^{d}	0.880±0.010^{b}	251.0±5.6^{a}	221.0±7.5^{a}
SZFRB	294.0±18.0^{ab}	0.773±0.015^{c}	0.900±0.010^{a}	238.7±11.0^{a}	215.3±7.6^{a}
GXFRB	282.3±10.6^{b}	0.780±0.010^{bc}	0.897±0.006^{a}	232.3±9.3^{a}	207.7±7.6^{a}
AHFRB	282.3±8.4b	0.793±0.006^{b}	0.906±0.011^{a}	234.3±8.4^{a}	216.3±8.6^{a}
HBFRB	309.7±32.2^{ab}	0.783±0.006^{bc}	0.897±0.006^{a}	254.3±27.1^{a}	228.0±24.9^{a}

3.3. Sensory analysis of sweet wine fermented bread with different sweet wine koji

The sensory analysis results of sweet wine fermented with different sweet wine kojis are shown in Figure 1. In terms of appearance, color, flavor, and other indicators, bread containing sweet wine has certain advantages over ordinary wheat bread. Sweet wine contains a large amount of reducing sugar. During the baking process of bread, it can intensify the Maillard reaction, making the bread easier to color and more attractive in color, while GXFRB and AHFRB have larger specific volumes, so the appearance score is higher. In terms of flavor, GXFRB scored 7.6 points, significantly higher than other breads, indicating that it has a unique flavor and is the most popular. However, in terms of taste, the bread made with sweet fermented glutinous rice is obviously not as good as wheat bread (Cai et al., 2016). Combined with the results of the overall texture analysis (Table 2), it may be that sweet fermented glutinous rice reduces the cohesiveness of the bread, resulting in sticky, unpleasant bread and a lower sensory score. Although sweet wine has a negative impact on bread in terms of taste, in terms of overall acceptance, sweet wine bread is still higher than wheat bread, indicating that, compared with ordinary wheat bread, bread containing sweet wine has more advantages than disadvantages, and is generally more acceptable. GX Amazake koji fermented bread is the most popular (Giannone et al., 2016).

3.4. Optimizing the changes of pH and TTA in the process of fermenting sweet wine koji

In the early stage of sweet fermented rice fermentation, due to the metabolism of mold to produce organic acids such as acetic acid, malic acid, and succinic acid (De Marchi et al., 2015), The pH of fermented glutinous rice dropped rapidly, and stabilized at about 4.0 after 18 hours, reaching a soft acidity suitable for dough. The continuous accumulation of organic acids also made the titratable acidity (TTA) gradually increase, and the increasing trend gradually slowed down after 20 hours. Combined with the change of the number of molds, it can be found that the number of molds just reached the stable period at this time, and the acid production

ability was not as good as that of the rapid growth of molds. After 48 hours of fermentation, the TTA of Tianjiu Niang basically tended to be stable, which is consistent with (Wu et al., 2012).

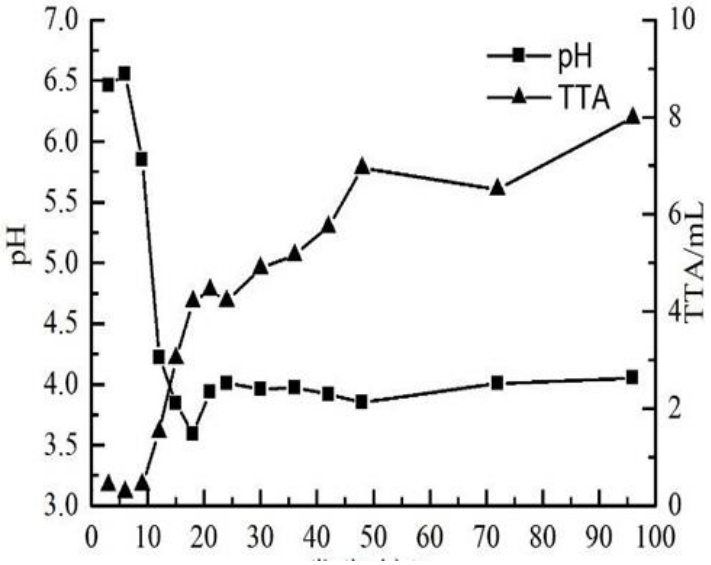

Fig.1 Changes of pH and TTA value during fermentation of fermented glutinous rice

3.5. Changes in reducing sugar and ethanol content in the process of optimizing sweet wine koji fermentation

Figure 2 shows the changes of reducing sugar content and ethanol content during the fermentation process of Tianjiu Niang. It can be clearly found that after 12 hours of fermentation, the content of reducing sugar in sweet fermented glutinous rice starts to increase significantly, while the starting point of rapid increase of ethanol content is around 24 hours of fermentation. This time difference is due to the fact that Rhizopus proliferates in large numbers and saccharifies the gelatinized glutinous rice to produce a large amount of glucose in the early stage of fermented glutinous rice, and then the yeast decomposes the glucose and ferments to produce ethanol (Harinder & Bains, 1988). After 40 h of fermentation, reducing sugar and ethanol in Tianjiu Niang accumulated to a certain extent (reducing sugar 30.2 g/100 g Tian Jiuniang, ethanol 2.2%), and the increasing trend slowed down significantly. Interestingly, although the growth rate of yeast slowed down after 48 h, the increasing trend of ethanol content slowed down after about 36 h of fermentation, which may be due to the

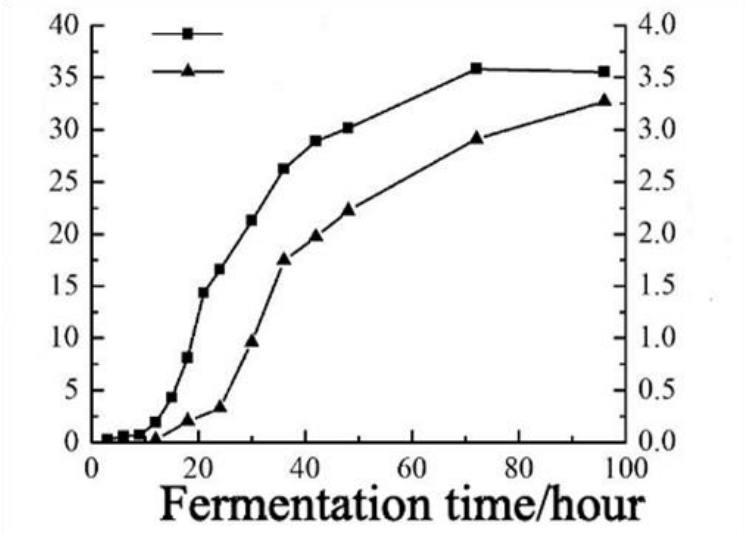

Fig. 2. Changes of ethanol and reducing sugar during fermentation of glutinous rice

catalysis of esterase, alcohol acyltransferase, etc. Fatty acids react to produce esters (Schermers et al., 1976), which is conducive to the unique flavor of sweet wine. The presence of reducing sugar and ethanol makes tianjiu niang taste sweet, delicious, and mellow, but also makes tianjiu niang have a strong reducing property, which may be unfavorable for the cross-linking of disulfide bonds of gluten protein.

4. Conclusion

There are some differences in the basic physical and chemical indicators of the five kinds of sweet wine koji fermented with sweet wine. The pH and TTA are similar to those of a high-quality acidic starter, and the reducing sugar content exceeds 20 g/100 g of sweet wine. Different sweet fermented rice had little effect on the specific volume of bread, but it could significantly reduce the cohesion of bread, resulting in a significant decrease in the sensory score of bread mouthfeel. However, the type and content of flavor substances in bread containing sweet fermented glutinous rice were improved, and a variety of ester substances were added. According to the results of principal component analysis and sensory analysis, GXFRB had the most unique flavor and the highest overall acceptability score. The results of microscopic and macroscopic analysis showed that the enzyme preparation re-crosslinked the gluten protein in the dough containing liqueur fermented gluten, improved the gluten network structure, improved the baking quality of bread, and greatly increased the bread mouthfeel score. However, during the storage process, although the enzyme preparation slowed down the water loss of bread and significantly reduced the hardness of sweet fermented bread, the effect on the aging enthalpy of amylopectin was not obvious. In addition, the bread containing fermented glutinous rice has weak antibacterial ability, which helps to prolong the shelf life of bread. Enzyme preparations and sweet fermented glutinous rice as bread ingredients can improve the flavor and texture of bread, and effectively prolong the storage period of bread.

CRediT authorship contribution statement

Xiaorong Wei, Experiment and analyze data, Tao Wu, and Zhao Jie, Data analysis, writing-original draft, editing, proofreading.

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Declaration of competing interest

The authors declare no conflict of interest.

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