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Chemical and Microbiological Characteristics of Kombucha Beverage Produced from Robusta and Arabica Coffee with Varied Roasting Profiles

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Abstract. *Kombucha is a fermented beverage product often produced by adding kombucha culture into sweetened tea, juices, coffee, and herb extracts. Coffee is a refreshing ingredient commonly served as a beverage prepared from roasted coffee beans. Therefore, this study aimed to investigate the chemical and microbiological characteristics of kombucha beverage produced from robusta and arabica coffee through natural methods with light, medium, and dark roasting profiles. Ground-roasted robusta and arabica coffee were dissolved into a sucrose solution, then the kombucha culture was added to these treatments and fermented for 14 days at ambient temperature. Subsequently, each kombucha beverage was analyzed for Total Soluble Solid (TSS), pH, Total Titrated Acid (TTA), Soluble Vitamin C, Caffeine Content, and Total Lactic Acid Bacteria (LAB). The results showed that TSS decreased on the 14th day due to sucrose degradation by microbes. TTA increased at the end of the processing, followed by a lower pH (2.87–3.49), signifying a safe value for human consumption. The soluble Vitamin C increased on the seventh day and decreased on the 14th day, which was related to the oxidation process during fermentation. The monoanion of ascorbic acid became oxidized by oxygen molecules and formed other unstable compounds. The total LAB and caffeine content elevated along with fermentation time due to the caffeine solubility which increased in low pH solution.*
Keywords: *arabica; fermentation; kombucha; roasting; robusta.*

Type of the Paper: Regular Article

1. Introduction

Fermented products are known for the beneficial effects on improving flavor, prolonging the shelf life of products, preserving products, enhancing nutritional value, removing harmful components, reducing cooking duration, increasing antioxidant activity, and contributing to human health [1–3]. In fermentation, microorganisms help break down large organic molecules into simpler forms. This process is influential in determining the biochemical, physical, and sensory characteristics of final products [3]. Food, beverage, animal-based, plant-based, and traditional, as well as novel fermented products are variations often obtained [4–6].

Kombucha is a fermented beverage product commonly produced by adding kombucha culture into sweet black or green tea brewing [7,8]. This contains various probiotics, mainly including acetic acid bacteria, yeast, and lactic acid bacteria (LAB), which are beneficial for humans. The acetic acid bacteria and yeast are symbiotic and play an essential role in the early

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stages of yeast reproductive fermentation [9]. During fermentation, a floating whitish cellulose layer appears due to the metabolism results of bacteria and yeast [10,11]. However, kombucha beverages can be prepared using other brews including butterfly pea [12], cascara [13], dragon fruit peel [14], herbal tea from roselle, mangosteen peel, soursop leaf, moringa leaf, and yellow leaf [15], as well as coffee [16].

Coffee is a refreshing ingredient commonly served as a beverage produced from roasted coffee beans [17]. Robusta and arabica are the two types often consumed, with each possessing distinctive characteristics. Arabica is believed to be more advantageous in commercial value and sensory profiles [18], while robusta has unique profiles that consumers prefer [19,20]. Consuming coffee at a moderate level was reported to be beneficial in reducing the risk of many diseases, although some studies detected the side effects of overconsumption [21,22].

Light, medium, and dark roasting are often applied during the processing of coffee, with physical, chemical, and sensory characteristics changing as the roasting temperature increases. The true density and water content decrease, but the true density and weight loss remain constant by applying high-temperature short-time roasting [23]. Studies showed that antioxidant capacity decreased in darker roasted coffee [24], with sensory profiles presenting a more bitter taste than the type subjected to a lower roasting degree [25].

The application of coffee for kombucha production has been explored for gastrointestinal simulation [26], characterization and biological activity during fermentation, in vivo toxicity [27], caffeine and chlorogenic acid content when using robusta green beans [7], as well as sensory characterization [28]. However, the use of various roasting profiles for the production process is not yet investigated. Each type of roasting profile produces distinguishing properties, which can be relatable during application for kombucha fermentation. Therefore, this study aimed to investigate the chemical and microbiological characteristics of kombucha beverages produced from robusta and arabica coffee through natural methods with different roasting profiles.

2. Materials and methods

2.1. Materials and Equipment

Materials used in this study included Robusta and Arabica coffee roasted into light, medium, and dark profiles. Others were kombucha starter, sugar, mineral water, MgO, H_2SO_4, KOH, chloroform, 0.01 N iodine, 1% alum, 1% phenolphthalein indicator, 0.1 N NaOH, and distilled water. The equipment applied were sterilized glass bottles for coffee kombucha fermentation, spatula, Erlenmeyer, beaker glass, pH meter, brix meter, burette, burette stand, measuring cup, pipette, and water bath.

2.2. Coffee Roasting and Grinding

Robusta and arabica coffee beans obtained from farmers of Bondowoso, Indonesia were used and processed through a natural method with a water content of 12%. These were transformed into light, medium, and dark profiles using a NOR roasting machine with final temperatures of 208°, 215°, and 223°C, respectively, then the samples were ground into fine particle size.

2.3. Kombucha Beverage Production

Kombucha beverage was produced by boiling two liters of mineral water at 100°C, then 110 g of ground coffee and 210 g of sugar were added and stirred until completely dissolved. The optimum coffee-to-water ratio for a Golden cup comprises 8.25 g of coffee per 150 ml of water, which is referred to as the Cupping Specialty Coffee recommended by the Specialty Coffee Association of America [29]. After all the ingredients dissolved, the sweet brewed coffee was allowed to rest until it reached room temperature before pouring into a 3.5L glass bottle previously sterilized by steaming for 30 minutes. This was followed by adding 200 ml of liquid kombucha culture as well as a symbiotic culture of bacteria and yeast (SCOBY) with a 10 cm diameter and 1 cm thickness. The bottle top was covered using kitchen paper and tied with rubber bands, then the content was fermented for 14 days at room temperature without exposure to direct sunlight [28,30]. This procedure was repeated for all treatments, including robusta and arabica coffee with light, medium, and dark roast profiles.

2.4. pH Measurement

Approximately 100 ml of the coffee solution was added into a glass beaker, then the pH of this kombucha sample was measured with a pH meter. Standardization was performed before using the pH meter by rinsing the electrode with distilled water and drying. The calibrated pH meter was dipped into the culture and a reading was obtained after a few moments when the value appeared stable. This pH test was specifically carried out on days 0, 7, and 14 of kombucha fermentation [31,32].

2.5. Determination of Total Soluble Solid (TSS)

Testing for total dissolved solids was conducted using a hand-refractometer, from which the prism component was initially rinsed in distilled water and wiped with a soft cloth. The kombucha sample was dropped onto the refractometer prism and the degree of Brix was measured [33].

2.6. Determination of Total Titrated Acid (TTA)

A total of 10 ml of kombucha was measured in a 250 ml measuring flask and shaken until homogeneous. Subsequently, 10 ml of the resulting sample solution was obtained, and 3 drops of 1% phenolphthalein indicator were added. The sample was titrated with 0.1 N NaOH until the color changed to pink and the content of TTA was calculated using equation (1) [34,35].

$$\text{Total Titrated Acid (\%)} = \frac{\text{Volume NaOH (ml)} \times \text{N NaOH} \times \text{Molecular Weight of Acid} \times \text{Dilution Factor}}{\text{Volume of Sample (ml)} \times 1000} \times 100\% \quad (1)$$

Note: TTA was determined by total lactic acid (Molecular Weight = 90)

2.7. Determination of Soluble Vitamin C

A total of 10 ml of kombucha was measured in a 250 ml measuring flask and shaken until homogeneous. Subsequently, 10 ml of this sample and 2 ml of 1% starch indicator were collected for titration with 0.01 N iodine until the color changed to a stable blue. In addition to estimating vitamin C in the sample, a blank solution was prepared using 10 ml of distilled water. The blank solution had the same treatment as the analyte but did not contain the analyte component. Analyte refers to the solution analyzed and the purpose of preparing the blank solution is to determine the amount of absorption by non-analyte substances [36].

$$\text{Soluble Vitamin C (\% b/v)} = \frac{(\text{N.V})\, I_2 \times (\text{Molecular Weight of Vit C/Valence})}{\text{Volume of Sample (ml)} \times 1000} \times 100\% \text{ b/v} \quad (2)$$

Note: Molecular Weight of Vit C/Valence = 176.13/2

2.8. Determination of Caffeine Content

The absorption of the sample solution was measured at a maximum wavelength of 285 nm and then recorded. The caffeine concentration was determined based on the regression equation of the standard calibration curve and could be calculated using equation (3) [37].

$$\text{Caffeine content (mg/g)} = (\text{M . V . Df}) \,/\, (\text{m}) \quad (3)$$

M is the concentration (ppm) or (mg/L), V denotes volume (L), Df represents the dilution factor, and m is the sample weight (g).

2.9. Determination of Total Lactic Acid Bacteria (LAB)

Total LAB testing was conducted using the plate counting method with de Man Ragosa and Sharpe (MRS) agar sterile medium, 660 µl of galactose, and 15 ml cysteine HCl. The materials were cooled to 50 °C and the results of this microbiological analysis were obtained using a Standard Plate Count [38].

3. Result and Discussion

Kombucha beverage was produced by dissolving ground roasted coffee in a sucrose solution and adding kombucha culture. The sucrose solution provided a primary carbon source for the growth of bacteria and yeast during fermentation [39]. Fig. 1 shows TSS data at 14 days of fermentation, representing the content of materials dissolved in the kombucha solution [40]. The highest TSS measuring 11.5 - 14.63 °Brix was obtained on the first day, but the value decreased ranging from 10.8 – 12.3 °Brix in each sample after 14 days. The fermentation process was characterized by a reduction in the substrate in the form of sucrose and the improvement of products such as acids and bacterial cellulose [40]. Therefore, TSS reduction during fermentation correlated to the consumption of sugar by microbes. Based on Fig. 1, TSS was slightly lower in the kombucha sample of arabica coffee compared to robusta. These results correlated with another study in which arabica and robusta coffee TSS were 33.32 ± 0.07 g/100 g and 33.96 ± 0.52 g/100 g, respectively [41].

Fig. 2 shows that the pH value of kombucha at the onset of fermentation ranged from 3.43-4.02, and it decreased to 2.87–3.49 on day 14. The pH decreased with fermentation time, signifying the metabolic activity of bacteria and yeast. During fermentation, sucrose is converted into alcohol, and bacteria form other organic acids [42], which release protons to lower the pH value [43]. For food safety issues, the recommended pH of kombucha must be between 2.5 and 3.5, while values of 2.5 to 3.3 are needed to prevent the growth of pathogenic bacteria [44].

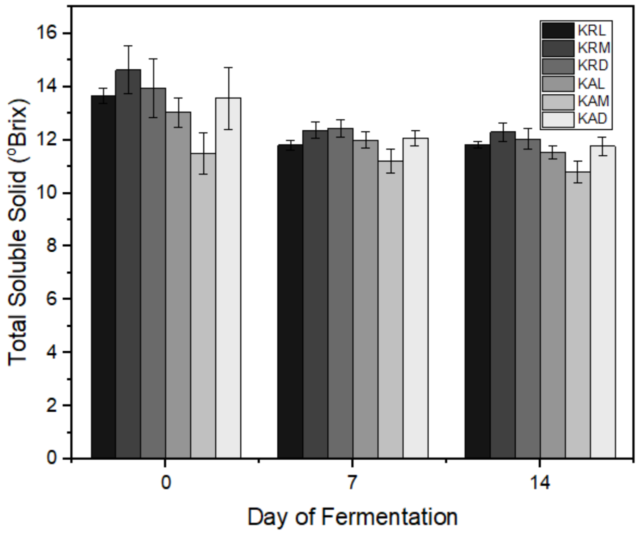

Fig. 1. TSS in (a) Kombucha-Robusta Light Roast (KRL), (b) Kombucha-Robusta Medium Roast (KRM), (c) Kombucha-Robusta Dark Roast (KRD), (d) Kombucha-Arabica Light Roast (KAL), (e) Kombucha-Arabica Medium Roast (KAM), (f) Kombucha-Arabica Dark Roast (KAD)

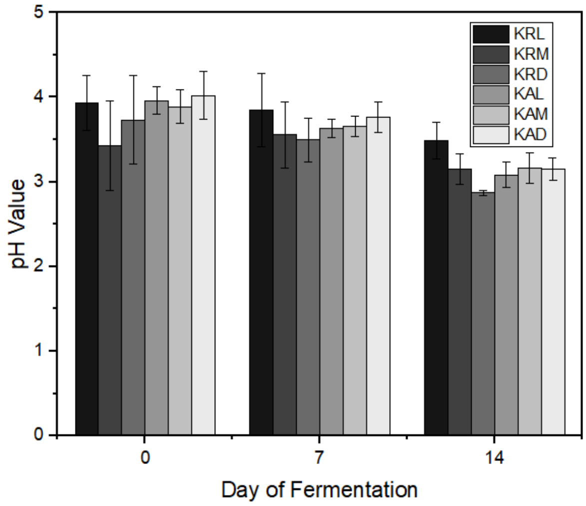

Fig. 2. pH Value in (a) KRL, (b) KRM, (c) KRD, (d) KAL, (e) KAM, (f) KAD

Kombucha TTA (Fig. 3) at the onset of fermentation was 0.75–1.5%, and it increased from 1.18-2.63 % on day 14. Furthermore, the sugar content broke down due to microbial activity, leading to the formation of organic acids and alcohol [45]. TTA is related to the growth of microbes, specifically LAB known to produce organic acids such as acetic, gluconic, and glucuronic acid, which increase with longer fermentation [46].

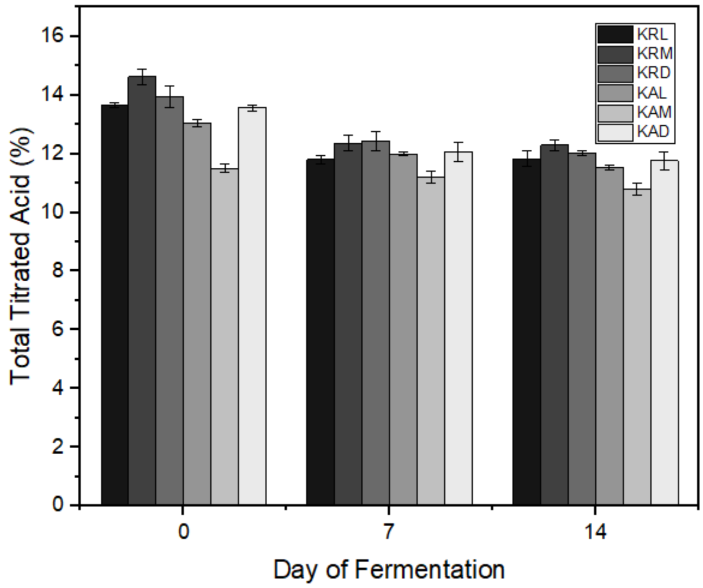

Fig. 3. TTA in (a) KRL, (b) KRM, (c) KRD, (d) KAL, (e) KAM, (f) KAD

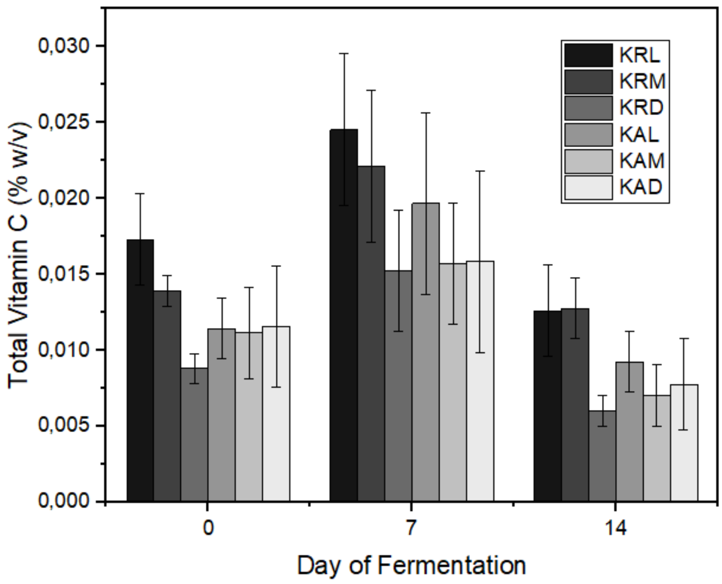

Fig. 4. Total Vitamin C in (a) KRL, (b) KRM, (c) KRD, (d) KAL, (e) KAM, (f) KAD

Fig. 4 shows that fermented kombucha contains four water-soluble vitamins, including B1, B6, B12, and C [45]. Another investigation reported vitamin C as highest at 1.51 mg/ml, followed by 0.84 mg/ml B12, 0.74 mg/ml B1, and 0.52 mg/ml B6 [47]. In this study, the total vitamin C at the onset of fermentation was 0.009–0.017 % w/v, which increased from 0.015–0.025 %w/v on the seventh day, while after 14 days the value decreased to 0.006-0.013 %w/v. At the onset of fermentation, vitamin C increased due to glucose breakdown and the reduction of D-glucose compound to D-sorbitol, which was oxidized to L-sorbose by the bacteria *Acetobacter xylinum*. *L-sorbose* generated from the fermentation process was subsequently converted into ascorbic acid. The glucose as an energy source for bacteria and yeast activities in kombucha solution diminished with time [48], leading to the inhibition of the ascorbic acid formation. After 14 days, the vitamin C content decreased, corresponding with the study of Winandari [30] which reported vitamin C reduction to 0.0325 mg/mL on the nineth day of fermentation [30]. The main factors influencing vitamin C degradation include oxygen and other oxidizing agents, high pH, elevated temperature, metal ions [49], and water activity [50]. During oxidation, the monoanion of ascorbic acid reacted with oxygen molecules and formed dehydro-L-ascorbic acid (DHA). The compound DHA can be further hydrolyzed to 2,3-diketo-L-gulonate (DKG) or oxidized to a range of products such as L-threonic acid (ThrO), oxalic acid (OxA), and esters [51], while ascorbic acid often maintains maximum stability near pH 3.0 and 6.0 [52]. KRD showed the lowest pH on the 14th day (2.87) compared to other treatments, which impacted the lowest vitamin C content (0.006 % w/v). To maintain the ascorbic acid content of the product, storage temperature is recommended to be less than 25 °C, and heat treatment should be conducted at a relatively mild temperature of 75 °C [53]. In this study, the decrease in vitamin C was probably caused by oxygen presence that led to the oxidation process.

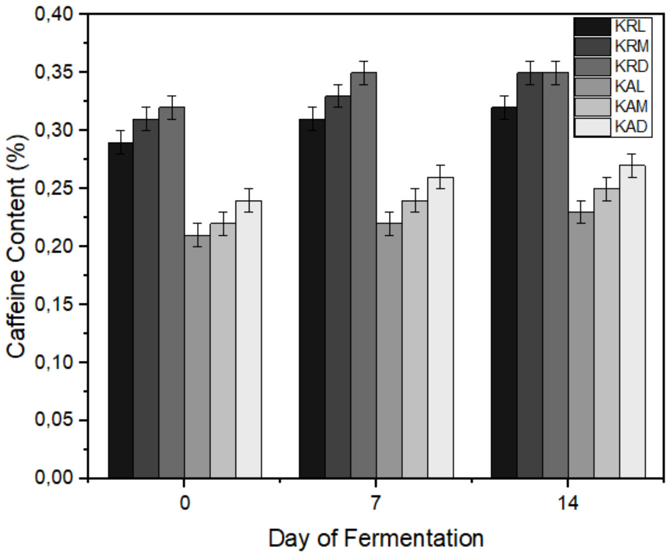

Fig. 5. Caffeine Content in (a) KRL, (b) KRM, (c) KRD, (d) KAL, (e) KAM, (f) KAD

Kombucha was produced using light, medium, and dark roast profiles of arabica and robusta coffee. Based on Fig. 5, the beverage prepared from robusta showed a higher caffeine content compared to arabica. This result was consistent with another study that reported the caffeine content of 2.382 g/100 g and 1.454 g/100g in green bean robusta and arabica, respectively [41]. The caffeine levels increased along with elevation in roasting degree, correlating to other reports, which stated that higher temperature treatments would increase caffeine [54,55]. In addition, the caffeine content of each kombucha increased with time, where the type produced from robusta dark roast coffee presented the highest content at the onset of fermentation (0.29-0.32%), seventh day (0.31-0.35%), and 14th day (0.32-0.35%).

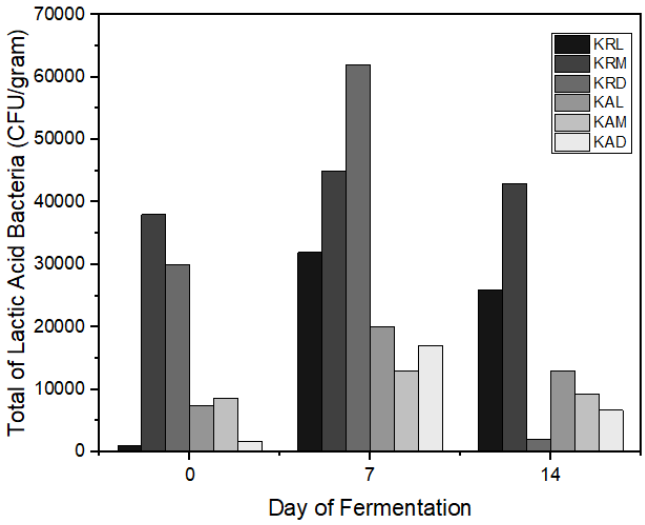

Fig. 6. Total LAB in (a) KRL, (b) KRM, (c) KRD, (d) KAL, (e) KAM, (f) KAD

Arabica coffee kombucha presented slightly lower caffeine content at the onset of fermentation (0.21-0.24%), seventh day (0.22-0.26%), and 14th day (0.23-0.27%). Caffeine, as an alkaloid, is known to taste bitter, therefore a longer fermentation process leads to higher caffeine content due to increased solubility associated with acid solution [56,57], generating more bitter products [58]. The caffeine content of kombucha fulfills the maximum amount allowed for daily human consumption based on the Indonesian National Standard (0.9-2% w/w) [59].

Total LAB in kombucha increased from the onset of fermentation to the seventh day and decreased until the 14th day (Fig. 6). This beverage is produced by fermenting sweetened solution from plant material such as tea, juices, and herb extracts using a mixed culture of acetic acid bacteria, yeast, and LAB [9]. Kombucha is commonly subjected to alcoholic and acetic acid fermentation processes. At first, the yeast in the symbiotic culture breaks down the sugar into alcohol, then the resulting alcohol is oxidized to acetic acid by lactic acid and acetic acid bacteria [60]. Fermentation by LAB helps to prevent toxin formation [61] and microbial spoilage [62], extends shelf life, changes the taste of the original ingredients, improves the digestibility of foods and nutritional value, as well as offers health benefits [63,64].

4. Conclusion

In conclusion, this study found kombucha to be a prevalent beverage mainly due to the claim of improving human health positively. Many types of materials were used in kombucha fermentation, including coffee, with a unique taste as a refreshing beverage, whose profiles could be enhanced through symbiotic culture. Robusta and arabica coffee were roasted to light, medium, and dark profiles, then ground, dissolved in sucrose solution, mixed with kombucha culture, and fermented for 14 days. During the fermentation process, the pH of kombucha decreased, while the TTA value increased, and TSS reduced due to microbial growth. Total vitamin C was found to be highest on the seventh day before declining on the 14th day, while the caffeine content and total number of LAB were highest on the 14th day. All formulations showed promising prospects to be developed as kombucha beverages based on investigation into chemical and microbiological properties, and a pH range of 2.5 to 3.5 was recommended for safe consumption. A sensory evaluation should be conducted in future studies to obtain the perspective of consumers regarding the acceptable kombucha type for daily consumption.

Abbreviations

LAB	Lactic Acid Bacteria
KRL	Kombucha-Robusta Light Roast
KRM	Kombucha-Robusta Medium Roast
KRD	Kombucha-Robusta Dark Roast
KAL	Kombucha-Arabica Light Roast
KAM	Kombucha-Arabica Medium Roast
KAD	Kombucha-Arabica Dark Roast
TTA	Total Titrated Acid
TSS	Total Soluble Solid

Data availability statement

Data will be shared upon request by the readers.

CRediT authorship contribution statement

D.A.S.: conceptualization, methodology, research work, data curation, preparation, investigation, original draft writing, review and editing; S.: supervision, conceptualization; S.B.P.S.M: resources, investigation; A.P.A.: resources, investigation; N.N.: supervision, methodology; C.P.H.: preparation, resources.

Declaration of Competing Interest

The authors declare no competing interest.

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