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Profiling the Volatile Compound of Indonesian Rendang Using GC-MS/MS Analysis

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Abstract: Rendang is an indigenous food from west Sumatra with a distinct flavor and aroma. Different rendang production methods have an impact on its sensory qualities, including flavor and aroma. Finding the volatile substances that distinguish the aroma of each rendang was the aim of this investigation. In this experiment, five distinct Indonesian rendangs were used. Using the Head Space-Solid Phase Micro Extraction technique (HS-SPME), the volatile components of the rendang's meat and powder were extracted. The acquired data were examined utilizing a multivariate technique, which included clustering observation (Heat map) and Partial Least Squares Discriminant Analysis (PLS-DA). Fifteen volatile compounds from six volatile chemical classes, including pyrazines, furans, alcohols, aromatics, carboxylic, and aldehydes, were inferred from the GC-MS/MS analysis of the meat component. Based on VIP scores from significant features identified by PLS-DA, five volatile substances (2-methylpyrazine, 2,5-dimethylpyrazine, furfuryl alcohol, 2,6-dimethylpyrazine, and benzyl mercaptan) were discovered to be the discriminant of each process of rending manufacturing. Meanwhile, for the powder part, there were forty-four volatile compounds tentatively identified that couldn't be clustered and classified properly both in heatmap and PLSD-DA. The process of absorption of spices into the meat as a raw material for rendang turned out to provide more representative data in terms of profiling the compounds responsible for the flavor of rendang. Thus, the powder part can't be used as the representative for the discriminant of volatile compounds of Indonesian rendang.

Keywords: Aroma, Flavor, Fragrance, Hs-Spme, Gc-Ms/Ms, Volatolomics

Introduction

According to Azima *et al*. (2016; 2017; 2018); Nurmufida *et al*. (2017), Indonesia is a nation with a diverse ethnic and cultural population. There are many different ethnic foods available in Indonesian cuisine, but rendang stands out as an iconic one among people's tastes and has even come to symbolize the country's identity (Rahman, 2020). It is also linked to the Minangkabau culture of West Sumatra. Rendang was chosen as the world's most delectable cuisine in a reader survey carried out by Tim (2017) based on reader responses.

Rendang is one of the traditional foods of the Minangkabau society in West Sumatera, Indonesia which has a unique flavor due to the various kinds of spices used (Nazir *et al*., 2018). The Production of rendang utilizes many kinds of indigenous herbs and spices, beef, and coconut milk (Nurmufida *et al*., 2017). Each region in West Sumatra has its own characteristics in the process of making rendang, both in terms of the ingredients used and the method of cooking. Rendang from different regions goes through different production processes, resulting in rendang with a distinctive taste and unique aroma. Rendang is cooked for around 6-7 h at 80-90 °C, until the meat is soft and tender, turns dark or black and develops a distinct scent and flavour. Such cooking techniques are used to make food from raw ingredients into consumable foods and to extend the shelf life of rendang (Azima and Sayuti, 2016).

Particularly for wet or semi-wet foods, aroma is one of the indicators of qualities that impact how well a product is received by consumers. Rendang undergoes a variety of reactions during cooking, including protein rearrangement, enzyme activity degradation, and Maillard reaction. The Maillard reaction is the main reaction which is producing a distinctive brownish-black color and unique flavor and aroma (Azima and Sayuti, 2016; Syukri *et al*., 2018; Thammawong *et al*., 2019). It involves amino acids, reduced sugar from carbohydrates, or aldehyde components. The resulting Maillard Reaction Products (MRPs) may be used as a seasoning or scent for meat (Sun *et al*., 2022; Cao *et al*., 2017). The two primary steps in the processing of meat are the Maillard reaction and lipid degradation, which both produce large amounts of Volatile Organic Compounds (VOCs) that affect the scent of the meat (Liu *et al*., 2019). The characteristic aroma of meat is further enhanced by the formation of aromatic carbonyl compounds including aldehydes, ketones, alcohols, carboxylic acids, and esters as a result of lipid breakdown (Bassam *et al*., 2022). Therefore, the interplay between the Maillard process and lipid breakdown is essential for the emergence of meat aromas (Wang *et al*., 2020). According to Wu *et al.* (2022); Li *et al.* (2021), volatolomics was utilized to find VOC markers in different types of samples including meats such as chicken and beef. To examine and identify VOCs, Gas Chromatography coupled to a Mass Spectrometry detector (GC-MS) with a volatolomics approach was frequently utilized (Han *et al*., 2020; Pavlidis *et al*., 2019; Rini *et al*., 2020). Thus, utilization of GC-MS/MS was clearly expected to give a better result in the identification of the volatile compounds of each rendang part. Therefore, this study differentiated the quality of rendang based on the volatile species formed using GC-MS/MS detection and a volatolomics approach.

Materials and Methods

Materials and Chemicals

The main material used was rendang which was obtained from five different local restaurants (Asese; Lamun ombak; Rajo-Rajo; Silungkang and Andalas raya) in West Sumatera Province, Indonesia. The chemical reagents were purchased from Fujifilm Wako Tokyo Chemical Industry (TCI).

Samples Preparation

In this study, the production was described generally according to Azima and Sayuti (2016) as the reference. Despite practical use by the producers, some modifications might be applied. All of the spices were ground into a fine powder, mixed with coconut milk, and cooked for one and half hour at temperatures between 90 and 93°C to make a thick sauce for rendang. Put the beef in the sauce and continuously cook for another one and a half hour until the sauce becomes thicker and turns brown. At this stage, Kalio was formed. The Kalio was kept at the lower temperature ± 83°C for 2 h until the moisture became lost and the color changed to a dark brown or black. At this stage, rendang was formed. The product must be turned frequently when cooking to prevent burns. The obtained rendang was separated into meat and powder parts. The meat part was then minced and blended using a blender to get the powdered meat. The obtained samples were then kept in a very low-temperature condition for subsequent determination.

Solid-Phase Micro Extraction (SPME)

Approximately 200 mg sample was weighed on a screw tube and added 1.5 mL saturated NaCl. Then, crushed using shake master (BMS-M10N21) for 5 min and continued with centrifugation for 5 min at 4°C with 13.000 rpm. A 1 mL of supernatant was pipetted and transferred into a vial, then added 20 µL trans-2-hexenal 0.002 ppm (internal standard). Next, the vial was sealed with PTFE septa and a steel magnetic cap, and vortexed for 1 min. Volatile compounds were extracted by the specific apparatus (SPME equipment from Shimadzu-Japan). Extraction was done for 20 min at 80°C after heating for 10 min at 80°C using a water bath (TAITEC, Personal-11). Volatiles trapped in the fiber were desorbed in the injection port for 10 min. The volatiles were determined using a gas chromatography coupled to a mass spectrometry detector.

GC-MS/MS Analysis

The volatile flavor compounds were identified by a GCMS-TQ8040 NX (Shimadzu, Japan) and GC 2030 Nexis (Shimadzu, Japan) equipped with a polar fused column. The injection system was split. The temperature Program was set as step gradient increasing temperatures as 5 min (50°C), 10 min (250°C), Carrier gas: Helium, Flow rate: 1.43 mL/min. The injector temperature was set at 200°C and ionization energy for detection was set as 70 eV. The mass detection was set from 35-400 m/z. The internal standard was hexenal (Rini *et al*., 2021).

Statistical Analysis

The samples were classified using a multivariate technique called partial least squares discriminant analysis. Heat Map, which groups the constituents using correlation analysis and agglomerative clustering in a hierarchical manner, was used to enhance the understanding of the interactions between the samples and chemicals.

Meat Part

Heatmap-Samples Distribution and Its Volatiles' Temporal Correlations

Firstly, it was hypothesized that different production processes rendang (from different restaurants) produced distinctive and specific aromas. Figure 1 is a heatmap visualization of the interaction among samples and their volatile compounds from different production processes rendang. The heatmap was created based on the detected 15 volatile compounds. Moreover, Fig. 2 represents the chromatogram of volatile substances that are present in rendang namely Butyl acetate, Isobutanol, 2-Methyl-1-butanol, 2-Methylpyrazine, 2,5-Dimethylpyrazine, 2,6-Dimethylpyrazine, cis-3-Hexen-1-ol, Acetic acid, (E)-2-Octen-1-ol, Benzyl mercaptan, Furfuryl alcohol, Butylated hydroxytoluene, Butylated hydroxytoluene, Phenol, Hexadecanal and Eugenol. Heatmap colors indicated correlations between samples from 5 different production processes (different sources) and volatile compounds. A positive correlation between samples and volatile substances is indicated by the red color, while a negative correlation is indicated by the blue color. Rendang from different production processes (different restaurants) shows positive correlations on different volatile compounds. Silungkang shows strong positive correlations with 6 volatiles (acetic acid, benzyl mercaptan, furfuryl alcohol, 2, 6-Dimethylphyrazine, 2-Methylpyrazine, 2,5-Dimethylphyrazine) and these compounds have negative correlations with rendang from Andalas Raya. Meanwhile, Rajo-Rajo shows positive correlations with 8 volatile compounds (furfuryl alcohol, 2,6-Dimethylhydrazine, 2- Methylpyrazine, 2,5 - Dimethylhydrazine, 2 -methyl-1-butanol, isobutanol, butylated hydroxytoluene, butyl acetate). Both Silungkang and Rajo-Rajo indicate positive correlations on several of the same volatile species, especially for pyrazines. Pyrazine is a volatile compound class that is produced by the Maillard reaction and also interacts with other reactions (Sun *et al*., 2022; Diez-Simon *et al*., 2019). It can be suggested that Silungkang and Rajo-Rajo might have a few similarities in the process of rendang's production.

On the other hand, Lamun Ombak exhibits strong positive correlations with 3 volatile compounds (cis-3-hexen-1-ol, hexadecimal, eugenol) and Asese shows positive correlations with one feature only (2-methyl-1-butanol). Meanwhile, Andalas Raya shows strong positive correlations with 3 volatile compounds (butyl acetate, (E)-2-Octen-1-ol, phenol). In this case, it can be seen that the volatile components found in Asese, Lamun Ombak, and Andalas Raya are distinct. Rini *et al*. (2020) reported that rendang contains a variety of volatile chemicals that can be categorized as carbonyls, alkenes, alkynes, alcohols, and sulfides.

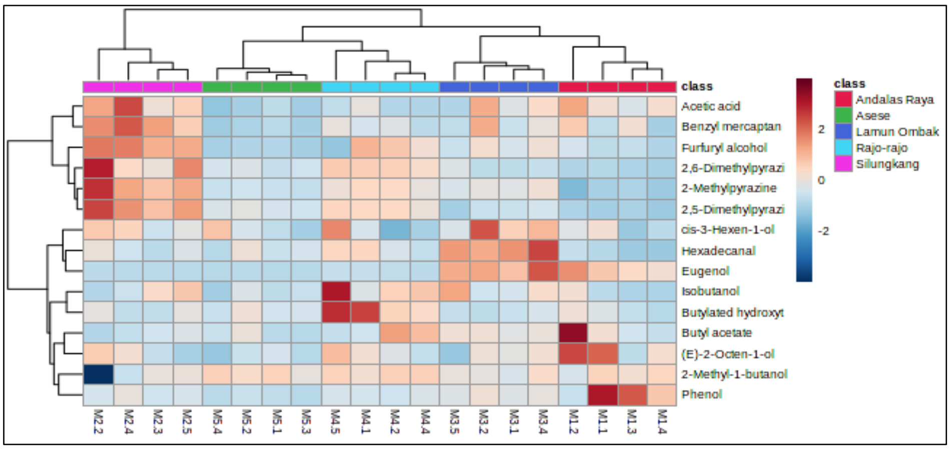


Fig. 1: Heat map diagram for clusterization of rendang (meat part) based on its volatiles

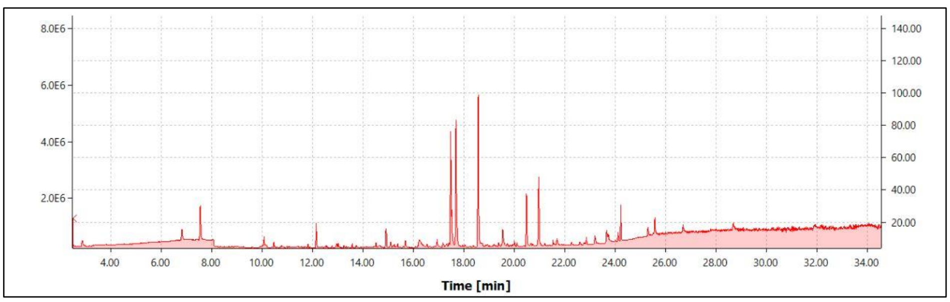
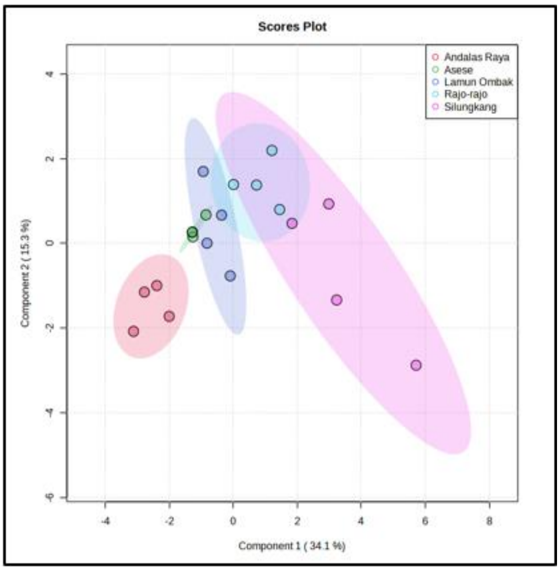
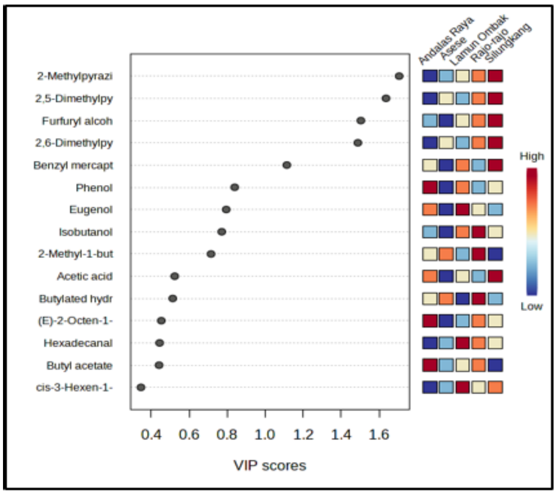


Fig. 2: GC-MS chromatogram of volatile compounds in Indonesian rendang



(a)



(b)

Fig. 3: The scores plot between the specified PCs; (3a) for the meat portion of Indonesian rending and the significant characteristics found by PLS-DA; (3b) for the same portion of meat. The colorful boxes on the right illustrate the relative concentrations of the relevant metabolite in each study group

Figure 1 shows that there are similarities between Andalas Raya, Asese, and Lamun Ombak. They all show positive correlations with 2-methyl-1-butanol which is identified as volatile alcohol. Alcohol has been proposed as one of the food's flavoring agents and is a byproduct of lipid oxidation (Mottram, 1998) and carbohydrate fermentation (Flores, 2018). Alcohol as the odor compound was also found in thermally processed beef (Sun *et al*., 2021; Rini *et al*., 2020; Zang *et al*., 2020). To sum up, each rendang exhibits positive correlations with the different volatile species, but some of them might have similarities in certain features.

Differentiating Groups Using Partial Least Squares Discriminant Analysis (Pls-Da)

A discriminative model was established in this research using the PLS-DA technique as a supervised analysis for categorizing the volatile components of rendang from distinct production processes (different restaurants). The training dataset of rendang samples from 5 different production processes (different restaurants) is shown in Fig. 3 as a PLS-DA sample plot. In Fig. 3, discrimination can be found either in Rajo-Rajo or Silungkang against the Andalas Raya, Asese, and Lamun Ombak. Differentiation of Andalas Raya, Asese, and Lamun Ombak can be seen from component 2 (15.3%) as shown in Fig. 3a.

Andalas Raya, Asese, and Lamun Ombak were rendang independently assembled their cluster in the negative side of Component 1 (34.1%). A relatively tiny eclipse separates Asese and Lamun Ombak. In this instance, these two rendangs might have a few volatile components in common. Lamun Ombak was a big and well-known restaurant from West Sumatra which have branches outside. It was very well known with the authentic flavor of Minangkabau society. Meanwhile, Asese was a well-known rendang brand that received a HACPP certificate and the quality of its products has met the Indonesian National Standards (SNI), also being known as the famous store to buy Minangnese foods. Both Lamun Ombak and Asese were famous and in high demand for Minangkabau foods.

The volatile components of Rajo-Rajo and Silungkang are separated with a very minor eclipse on the positive side of component 1 (34.1%) of the PLS-DA sample plot. This suggested that the volatile chemicals in these two rendangs might be comparable. Despite the fact that they were made in different ways, both originated in west Sumatra, Indonesia. Thus, they had their own uniqueness in terms of aroma and flavor. The PLS-DA sample plot revealed a separation of each rendang relating to its various volatile components.

The volatile species in the loading plot must show the critical contribution in each component indicated by a VIP score greater than 0.9, which was statistically significant, in order to identify the discriminant feature of each rendang in this study. Based on this comparison, five volatile species (benzyl mercaptan, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-methylpyrazine, and furfuryl alcohol) had been selected as the distinguishing characteristics of the five rendang from diverse production techniques, as shown in Fig. 3b. Various values may be utilized to satisfy the statistical model qualification for the significant volatile species used as discriminant characteristics from each rendang. Specifically, the rendang of the Silungkang was distinguished by eight volatile species, the Rajo-Rajo by nine volatile species, the Lamun Ombak by six, the Asese by two and the Andalas Raya by five volatile features.

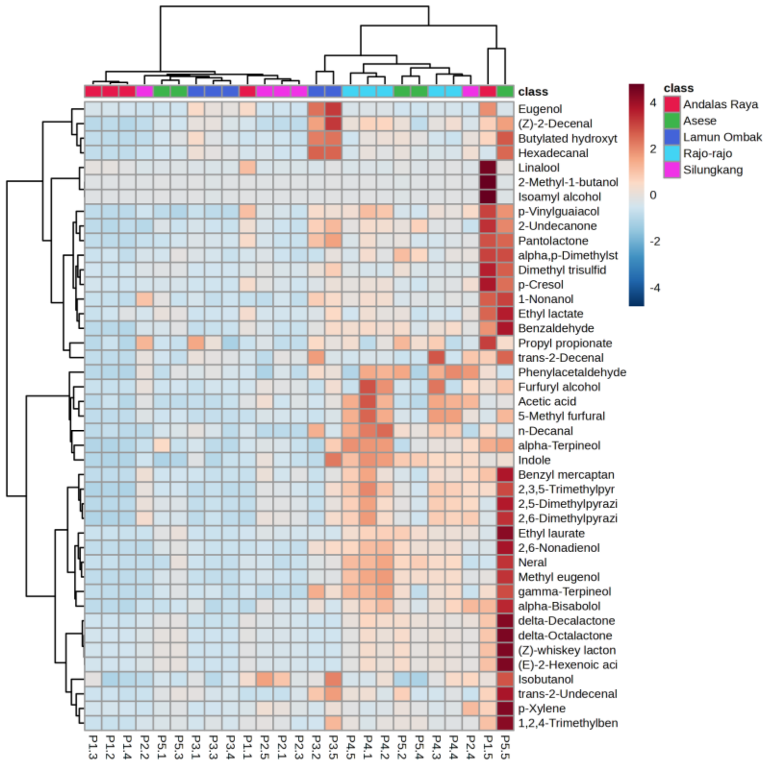


Fig. 4: Heat map diagram for clusterization of rendang (powder part) based on its volatiles

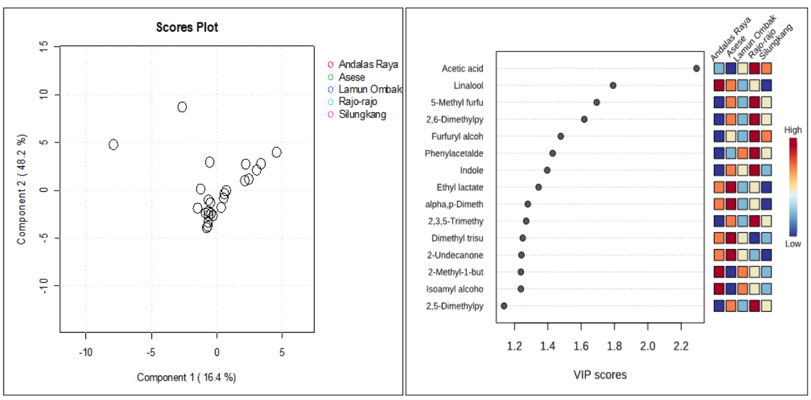


Fig. 5: The scores plot between the specified PCs; (3a) For the powder portion of Indonesian rendang and the significant characteristics found by PLS-DA; (3b) For the same portion of powder. The colorful boxes on the right illustrate the relative concentrations of the relevant metabolite in each study group

Powder Part

Heatmap-Samples Distribution and Its Volatiles' Temporal Correlations

In the powder part, about 44 volatile compounds were detected as shown in Fig. 4. The compounds detected are from the spices used in cooking rendang, as it is known that rendang is very rich in spices. Since the types of spices employed in the rendang cooking procedure in each region of Indonesia were generally similar, it can be noted that the volatile chemicals found in each rendang were not well grouped. This information contrasted with the findings for the volatile substances found in the rendang's meat component (Fig. 1), which were well grouped.

Partial Least Squares Discriminant Analysis (Pls-Da)-Distinguishing Different Groups

In this study, the results of PLS-DA rendang from 5 different cooking processes are shown in Fig. 5. Rendang from Rajo-Rajo was on the positive side of component 1 (16.4%) and component 2 (48.2%), while for other rendang such as Andalas Raya, Lamun Ombak, Asese and Silungkang were almost on the negative side of component 1 (16.4%) and component 2 (48.2%). This data shows that the volatile compounds found in the 5 types of rendang studied were in the same group.

Discussion

According to the information gathered during this investigation, it was observed that the volatile substances included in the rendang's meat part were well-classified and grouped and that their proportion to that of the powder part was inverse. This result was achieved as the raw materials (meat) used in rendang cooking underwent a process of spice absorption from the spices utilized. According to Azima and Sayuti (2016), the cooking process for rendang involves three stages: Gulai (where the sauce is cooked until it is thin and yellowish), Kalio (where the sauce is cooked until it is thick brown in color), and rendang (where the sauce was cooked until it was thick and dry and dark brown in color). These three stages involved a variety of reactions relating to protein decomposition and lipid oxidation producing the specific flavor and aroma.

The mechanism by which the spices were absorbed into the meat used as the primary raw material during the rendang cooking process revealed that this provided more accurate data for identifying the volatile substances that make up the aroma of Indonesian rendang. This was because the volatile compounds present in the spices used may be damaged due to exposure to various factors such as the use of high temperatures in the rendang cooking process, long cooking times, and exposure to light both in the cooking process and storage. The process of processing meat with the addition of spices and measuring process temperature settings has resulted in complex chemical reactions. The reaction process can be described from the various volatiles formed in rendang. From the food science perspective, this reaction also provides good health effects. From the perspective of the nutrients of the meat and the antioxidants from the spices, the method of creating rendang can also have a good impact on making meat protein more digestible (Azima and Sayuti, 2016). Food flavor is crucial in controlling hunger. The impact of flavor and scent, either separately or together, on feelings of hunger and subsequent food consumption. A favorable influence on health would result from the generation of food scents during processing, which would increase the desire to consume food.

Conclusion

The production process of rendang from five different restaurants that were originally from west Sumatera was successfully differentiated using GC-MS/MS volatilities profiling. The volatile species has the potential to distinguish the rendang production process with the use of a multivariate analysis, which results in the discovery of prospective markers. The selection of spices and the cooking method could be the reason why each tested rendang sample produced a different mixture of volatile chemicals. Although the qualities of the volatile compounds generated differ, consumers nevertheless have a high demand for each rendang despite these differences. There is still potential for advancement in the volatility’s studies of the sensory perspective.

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Author’s Contributions

Daimon Syukri: Conceptual and wrote the manuscript.
Abdi: Conducted the experiment.
Tuty Anggraini: Data analysis.
Alfi Asben: Responsible for GC MS measurement.
Rini: Responsible for laboratory chemicals.
Manasikan Thammawong: Reviewed the manuscript.
Kohei Nakano: Conceptual.

Ethics

This is an original data that hasn't been published previously. The corresponding author relates that no ethical concerns exist on the publication of this study.

Conflict of Interest

The authors have no conflict of interest.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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