Characterization of Chinese liquor aroma components during aging process and liquor age discrimination using gas chromatography combined with multivariable statistics

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Chinese liquor aroma components were characterized during the aging process using gas chromatography (GC). Principal component and cluster analysis (PCA, CA) were used to discriminate the Chinese liquor age which has a great economic value. Of a total of 21 major aroma components identified and quantified, 13 components which included several acids, alcohols, esters, aldehydes and furans decreased significantly in the first year of aging, maintained the same levels (p > 0.05) for next three years and decreased again (p < 0.05) in the fifth year. On the contrary, a significant increase was observed in propionic acid, furfural and phenylethanol. Ethyl lactate was found to be the most stable aroma component during aging process. Results of PCA and CA demonstrated that young liquor (fresh) and aged liquors were well separated from each other, which is in consistent with the evolution of aroma components along with the aging process. These findings provide a quantitative basis for discriminating the Chinese liquor age and a scientific basis for further research on elucidating the liquor aging process, and a possible tool to guard against counterfeit and defective products.

As one of the oldest alcoholic beverages, Chinese liquor possesses a long history of over 6000 years1. Due to the historical and cultural factors, this type of liquor plays a particular role in Chinese traditional culture, and it is now very popular in China and several other countries. Chinese liquor creates a sales income of over 60 billion dollars annually, with a consumption volume of over four million kiloliters2,3. Therefore, it takes up a significant position in the national economic development. Like some other alcoholic beverages such as whiskey and vodka, Chinese liquor aroma gets developed during the manufacturing process. Firstly, a mixture of several kinds of grains (sorghum is the main raw material) was milled and cooked, followed by fermentation in a specialized fermentor which coated with multiple layers of fermenting mud. Then the fermented product is distilled out with steam and saved as young liquor. The young liquor generally has unacceptable flavors which described as “pungent” or “coarse”, thus a prolonged aging process is needed to eliminate these negative attributes. Meanwhile, a well-balanced and mellow liquor aroma is formed adding economic value to the product. Therefore, aging plays an indispensable role in the process of producing high-quality Chinese liquor.

From quality perspective, aroma characteristic is the most important intrinsic factor making contributions to the overall quality of Chinese liquor, and is often used to discriminate liquors from different brands, regions and ages4. The overall aroma characteristic of Chinese liquor is generated by hundreds of chemical compounds balanced during the aging process; therefore, qualitative and quantitative analysis of liquor aroma components along with aging process is of great value for researchers and producers alike. During the past decade, aroma

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components of Chinese liquor have been studied extensively. Fan et al.\(^5\) used gas chromatography-olfactometry (GC-O) technique to investigate the aroma characteristic of Chinese liquor, and reported that 34 aroma components were important contributors for Chinese Chixiang-aroma-type liquor aroma, while 27 odorants were found to be important in light-aroma-type liquor by Gao et al.\(^6\) using the same method. The GC-O technique conduces to develop a better understanding of the aroma chemistry of Chinese liquor; however, it can be hard to provide concentration information since the odor threshold of aroma components is different for each component. To quantify aroma components in Chinese liquor, gas chromatography-mass spectrometry (GC-MS) has been widely used in a large number of researches\(^7\)–\(^12\). GC-MS technique equipped with commercial databases performs well in quantitative analysis, especially when aroma components of the sample are rather complicated as in Chinese liquor. Meanwhile, since the water present in Chinese liquor can cause damage to MS, as an alternative, headspace solid-phase microextraction (HS-SPME) or other extraction methods are normally chosen. However, the extraction process can also be easily affected by extraction time, extraction temperature, solvent burden ratio and fiber type. So the accuracy of quantitative analysis by GC-MS could be questionable. In addition, few researchers have focused on changes in aroma components of Chinese liquor during the manufacturing process. Ding et al.\(^13\) investigated changes in volatile compounds of Chinese Luzhou-flavor liquor during the fermentation and distillation process using HS-SPME-GC-MS. Ma et al.\(^14\) reported variations in physicochemical properties of Chinese Fenjiu during storage using HS-SPME-GC-MS with three internal standard substance; however, these results cannot represent the actual concentration changes of aroma components since the correction factors of aroma components are much different from each other. Above all, very limited research has been conducted on the characterization and quantification of Chinese liquor aroma components along with the aging process using gas chromatography with direct injection method.

Since the market price of Chinese liquor is closely related to the aging time, so the authentication of liquor age is of great importance to protect consumers from being cheated. Principal component analysis (PCA) and cluster analysis (CA) based on chromatographic profiles are two multivariate statistical techniques which have been proved in several studies to discriminate and classify groups effectively\(^15\)–\(^18\). The main objectives of this study were therefore: (1) to characterize Chinese liquor aroma components along with the aging process by accurate quantitative analysis, and to provide a theoretical basis for future researches which focus on the mechanism of aging process; (2) to explore the possibility of chromatographic profiles combined with multivariate statistic analysis to discriminate liquor age, and to fight against counterfeit and defective products.

**Results**

**Identification and quantification analysis.** Typical GC chromatograms with direct injection of a mixed standard solution and liquor samples are shown in Fig. 1a and b. It demonstrated that 53 compounds of the mixed standard cocktail can be efficiently separated within 80 min, and the retention time of the last component was 78 min. For liquor samples, aroma components were well separated from each other within 46 min, and no more peaks were detected after that. All major aroma components of the Chinese liquor could be found in the mixed standard cocktail by comparing the retention times. Considering the chemical groups of acids, alcohols, esters, aldehydes and fursans, 21 aroma components were identified.

![Figure 1. Typical Chromatogram map of standard solution (a) and liquor samples (b).](image-url)
to visualize the general variation of aroma components during the aging process, a radar map based on concen-
deviations obtained for most components confirmed the reliability and validity of the developed method. In order
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Table 2. One way ANOVA at the 5% significance level with Duncan method was used to compare statistical
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Acids.

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each aroma component in aged liquors was normalized by the one with young liquor. 17 components were
selected since other 4 components were not detected in young liquor, and the amounts of these 4 components
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was in accordance with the requirement of GC analysis (R² > 0.995). The slope of a regression curve stands for the
sensitivity of GC analysis for corresponding component19. As shown in Table 1, the lowest sensitive quantification
was 393.4 for acetal while the highest one was 4039.2 for phenylethyl alcohol. The detection limit was calculated
as three times the baseline noise19 and ranged from 0.340 mg/L for n-butanol to 7.959 mg/L for ethyl acetate. Mass
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As for the quantification analysis, detailed parameters of calibration curves and concentration ranges are sum-
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selected since other 4 components were not detected in young liquor, and the amounts of these 4 components
existing in aged liquors were also much lower as compared with other components.

Acids. Chinese liquor contains many acids, which are products of sugar oxidation or of ethanol fermentation
during the liquor making process. Acids can have impact on sensory characteristics, contribute to color stability
and increase antioxidant power20. Generally, acids are normally evaluated during the manufacturing of Chinese
liquor to monitor the whole process and to ensure product quality. In this research, acids were investigated as
possible markers of aging and also as an index for liquor age discrimination.

Five acids were detected: acetic acid, propionic acid, isobutyric acid, butanoic acid and isovaleric acid. As
shown in Table 2, acetic acid was the most abundant components of this group with concentration of 1194 mg/L
in young liquor, while the total amount of the other four acids was below 50 mg/L. As a matter of fact, total acid
content in Chinese liquor is usually expressed as the concentration of acetic acid, as more than 90% of the acidi-
ity is contributed by it. In the one-year-aged liquor, the acetic acid content decreased significantly (p < 0.05) to
701 mg/L; however, no additional change was observed in 2–5 year old liquors. As for isobutyric acid, butanoic
acid and isovaleric acid, a slight decline was continually observed during the aging process. As an exception,
the content of propionic acid in five-year-aged liquor was found to be higher than other stored liquor groups
(p < 0.05). The reduction in acids content may have been caused by some loss of volatile acids and esterification
reactions between acids and alcohols during the aging process. In the aroma composition of Chinese liquor, acids
are responsible for fruity, fatty as well as rancid notes. Short-chain acids which possess the aroma of sour and

| Number | Components        | Linearity range (mg/L) | Slope (a) | Intercept (b) | LOD (mg/L) | R² (n = 8) |
|--------|-------------------|------------------------|-----------|--------------|------------|------------|
| 1      | Acetaldehyde      | 6.06–606               | 505.4     | 15.9         | 2.097      | 0.9962     |
| 2      | Methanol          | 3.18–318               | 989.9     | 24.3         | 2.455      | 0.9959     |
| 3      | Ethyl acetate     | 23.25–2325             | 909.7     | 72.4         | 7.959      | 0.9958     |
| 4      | Acetol            | 6.46–646               | 393.4     | 34.1         | 4.334      | 0.9963     |
| 5      | 2-Butanone        | 3.58–358               | 1533.7    | 31.4         | 2.047      | 0.9988     |
| 6      | 1-Propanol        | 4.26–426               | 1904.4    | 45.0         | 2.363      | 0.9976     |
| 7      | Ethyl butyrate    | 1.27–127               | 1494.0    | 11.2         | 0.750      | 0.9981     |
| 8      | Isobutanol        | 10.92–1092             | 1530.8    | 27.5         | 1.796      | 0.9970     |
| 9      | n-Butanol         | 3.87–387               | 2174.2    | 7.4          | 0.340      | 0.9993     |
| 10     | Isomylolol        | 10.38–1038             | 2051.3    | 44.4         | 2.164      | 0.9979     |
| 11     | Ethyl hexanoate   | 2.98–298               | 1815.4    | 109.5        | 0.603      | 0.9972     |
| 12     | Ethyl lactate     | 9.17–917               | 2091.5    | 80.9         | 1.547      | 0.9981     |
| 13     | Ethyl oenanthate  | 3.64–364               | 2126.5    | 23.2         | 1.091      | 0.9983     |
| 14     | Acetic acid       | 15.48–1548             | 935.3     | 14.0         | 1.497      | 0.9952     |
| 15     | Furfural          | 5.41–541               | 1501.8    | 30.8         | 2.051      | 0.9978     |
| 16     | Propionic acid    | 3.88–388               | 1330.1    | 4.6          | 0.346      | 0.9972     |
| 17     | Isobutyric acid   | 1.40–140               | 1756.0    | 0.3          | 0.342      | 0.9995     |
| 18     | Butanoic acid     | 1.20–120               | 1631.3    | 7.3          | 0.179      | 0.9970     |
| 19     | Isovaleric acid   | 1.36–136               | 3802.7    | 15.0         | 0.394      | 0.9988     |
| 20     | Phenylethanol     | 2.10–210               | 4038.2    | 46.1         | 1.141      | 0.9979     |
| 21     | Ethyl palmitate   | 4.60–460               | 2517.0    | 49.0         | 1.947      | 0.9959     |

Table 1. Calibration curves for the quantification analysis of liquor aroma components. Regression equation: y = ax + b, where y is the peak area and x is the concentration mg/L. LOD: limit of detection, calculated by three times the ratio of signal/noise. R²: correlation coefficient.
Table 2. Quantitative analysis results of Chinese liquor aroma components with different age. All values are expressed as means (mg/L) ± standard deviation (SD). Different letters indicate significant differences (p < 0.05). ND: Not detected.

Figure 2. Radar map of aroma components. Acetaldehyde (1), Methanol (2), Ethyl acetate (3), Acetal (4), 2-Butanol (5), 1-Propanol (6), Isobutanol (7), n-Butanol (8), Isoamylol (9), Ethyl lactate (10), Acetic acid (11), Furfural (12), Propionic acid (13), Isobutyric acid (14), Butanoic acid (15), Isovaleric acid (16), Phenylethanol (17).

Acids are expressed as means (mg/L) ± standard deviation (SD). From this point of view, the observed decline in acids content in Chinese liquor during the aging process could be considered conducive to the formation of a well-balanced aroma.

Alcohols. Alcohols usually generate from deamination reactions of amino acids under anaerobic conditions and/or decarboxylation reactions of sugar under aerobic conditions during the fermentation process. Sorghum used for producing Chinese liquor contains a high content of amino acids, which offers abundant precursor to alcohols. Meanwhile, the reduction reaction of homologous aldehydes can also result in the increase of alcohols during the manufacturing process.
As can be seen from Table 2, 7 alcohols were detected by validated GC analysis method: methanol, 2-butanol, 1-propanol, isobutanol, n-butanol, isoamylol and phenylethanol. In this group, 1-propanol and isoamylol were the prominent alcohols since their concentrations found were 338, 637 and 800 mg/L, respectively, in the young liquor. After one year of storage, the content of 1-propanol decreased to 77% (p < 0.05), and maintained at this level during the following three years (p > 0.05). In the fifth year, however, the 1-propanol content increased rapidly and no significant difference was found between five-year-aged liquor and young liquor. Isobutanol and isoamylol seemed to be more stable during the first four years of aging (p > 0.05), however, reduction trends were observed in the fifth year, with the rate of 30% (isobutanol) and 21% (isoamylol) (p < 0.05). Though methanol exists in Chinese liquor with low content, the control of methanol level is still of great importance in consideration of its virulence. The content of methanol was 80 mg/L in young liquor, and then decreased to 46 mg/L after the first year, no significant change was observed after that. This level of methanol is within the commercial specification range for Chinese liquor (<400 mg/L). Small changes were also found in the content of 2-butanol, n-butanol and phenylethanol during aging process. It is recognized that there will be some decline in the alcohols content during the natural aging process of liquors, and this has been linked to the changes in the structure of water and alcohols molecules.

Esters. Esters are generally generated by esterification reactions between organic acids and alcohols, especially ethanol, during the fermentation and aging processes. The activities of hydrolase and esterase exist in the raw material of Chinese liquor Daqu are powerful, thus catalyse synthesis of esters could be possible during the active fermentation process. Since higher temperatures can lead to great losses in esters by ways of hydrolysis and volatilization, a relatively cooler temperature is needed during aging process. Ester group is the most affluent and crucial one exists in Chinese liquor, contributing to fruity, floral, and sweet aromas.

In Table 2, 6 esters were detected: ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl lactate, ethyl oenanthate and ethyl palmitate. Among those, ethyl acetate and ethyl lactate were the most prominent representatives, with concentrations of 1741 and 446 mg/L, respectively. Upon aging for one year, the concentration of ethyl acetate decreased to 694 mg/L (p < 0.05) and maintained at this level (p > 0.05) in the following three years, but then reduced by 29% in the fifth year (p < 0.05). Ethyl acetate is of great importance to the formation of distinctive aroma of distilled liquors, depending on its concentration. Although ethyl acetate is usually regarded as sending out the smell of nail polish at high concentrations, it displays fruity aroma at low concentrations on the contrary. It was observed that ethyl lactate was extremely stable during the aging process. Though the concentration fluctuated slightly in quantity, no significant difference was observed among six groups. Ethyl lactate is generally formed by lactic bacteria and plays an important role in stabilizing liquor flavor characteristics. Ethyl butyrate, ethyl hexanoate, ethyl oenanthate and ethyl palmitate had similar variation trends during aging process. As can be seen, long-chain esters were not found in young liquor, but formed in the first year of aging and decreased gradually in the following years. The reduction may have been caused by volatilization and permeation through the container with storage time. Overall, the decrease trend of total ester content found in this work (light-flavor Chinese liquor) was opposite with our previous research which focused on sauce-flavor Chinese liquor. This could be mainly resulted from different liquor types, since the raw materials and manufacturing process of these two liquors are much different from each other.

Aldehydes and furans. In this group, 3 kinds of aroma components were detected: acetaldehyde, acetal and furfural. The variation trends of acetaldehyde and acetal were similar as shown in Table 2. Acetaldehyde and acetal decreased significantly in the first year with the rates of 36% and 47%, respectively. Again no significant change was observed in the middle three years, but after the five year of aging, further reductions were observed from 153 mg/L to 119 mg/L for acetaldehyde (p < 0.05) and from 255 mg/L to 183 mg/L for acetal (p < 0.05). On the contrary, the level of furfural increased from 19 mg/L to 36 mg/L (p < 0.05) during the first year and maintained at the same level (p > 0.05) in the following aging period.

Acetaldehyde is one kind of poisonous compound generates from oxidation of alcohol in food products, which is often regarded as having sensorial properties of nut or overripe apples. Acetaldehyde is also one of the important metabolites of ethanol in human body. First, ethanol is oxidized into acetaldehyde by ethanol dehydrogenase, and then acetaldehyde dehydrogenase oxidizes acetaldehyde into acetic acid, finally acetic acid will be transformed into carbon dioxide and water and excreted out of the body through the respiratory process. For people who don’t have enough acetaldehyde dehydrogenase, acetaldehyde can accumulate in the body causing headache, nausea and vomiting, which is one of the reasons that some people do not tolerate consumption of heavy liquors. Acetal is a very common colorless aroma component exists in distilled alcoholic beverages, with a pleasant smell at low concentrations making positive contributions to the flavor characteristics. However, acetal at a higher concentration can be damaging to human health and can even cause death; the lethal dosage of acetal being about 300 mg/kg (body weight of consumer). At the level found which is about 250 mg/L, the acetal concentration levels are so far away from being lethal even if one were to consume the entire one liter volume liquor in one sitting. Furan can be formed by decomposition of carbohydrates under high temperature and dehydration of sugars through Maillard reaction, which occurs during fermentation and aging process and is usually considered as an aging marker.

Multivariate statistic analysis based on aroma components. Principal component analysis (PCA). PCA is one kind of multivariate statistic analysis method that can be used for the analysis of a database pertaining to several inter-corrected dependent variables, and it plays well in reducing the dimensionality of the database. PCA analysis involves the identification of different components: the first principal component contributes most to the total variance; similarly, each subsequent component has the largest contribution to the total variance with the orthorhombic restriction of previous components. Higher cumulative contribution rate of
the variance is indicative of better inclusion of the original information. Generally, the first and second principal component are selected and regarded as good representative of original information, when the accumulated variance exceed 80%.

The scores plot and loads plot are as presented in Figs 3 and 4. Variables in Fig. 4 were numbered according to the list from Table 1. In this research, scores plot was used to locate liquor groups with different ages, and the loads plot was used to further research the contribution of specific aroma component to each principal component. As can be seen from Fig. 3, the first principal component (PC1) and second principal component (PC2) were taken as coordinate axes for the PCA analysis on samples, and it was noted that the linear combination of PC1 and PC2 explained 98.27% of the total variance of liquor samples. PC1 led to the separation of young liquor and aged liquors, since young liquor samples were grouped on the positive side of PC1 while aged liquors were located on the negative side. On the other hand, PC2 contributed to the differentiation of aged liquors stored for different years. Five-year-aged samples were located on the negative side of PC2 whereas other samples were located on the positive side. It should be noted that one-year-aged and two-year-aged samples were clustered as one category, this may has been caused by the similarity of aroma composition in those two liquors. Similar result was also observed between three-year-aged and four-year-aged liquors. More information can be found from the loads plot in Fig. 3b. As can be seen, almost all aroma components were positively related to PC1 and PC2, indicating a general decrease in concentrations of these aroma components as the liquor age increased. In addition, acetaldehyde (1), acetal (4), ethyl butyrate (7), ethyl hexanoate (11), ethyl lactate (12), ethyl oenanthate (13), acetic acid (14), furfural (15), propionic acid (16), isobutyric acid (17), butanoic acid (18), isovaleric acid (19), phenylethanol (20), ethyl palmitate (21).

Cluster analysis (CA). In order to realize further separation among the six liquor samples, average values of 21 kinds of aroma components to six liquor samples were analyzed by CA using Euclidean distance. As shown in Fig. 5, all liquor samples were divided into two big groups. The first group consisted of aged liquors from one year
to five years, while only young liquor was found in the second group, which meant young liquor and aged liquors were well separated from each other. To be more specific, in the first sub-cluster, one-year-aged liquor was much similar with two-year-aged one, while three-year-aged and four-year-aged liquors were clustered as one category. These results were mainly caused by the similar composition of aroma components in liquors, which was also observed in PCA results. Furthermore, there must be much difference between five-year-aged liquor and other four aged liquors, since the former one was found to be existed as an independent category. These findings can be verified by the evolution of aroma components as presented before, since significant changes were observed in the fifth year during aging process. It can be concluded that cluster analysis based on aroma components performed well in the discrimination of Chinese liquor with different ages.

Conclusions
A gas chromatography analysis with direct injection technique was used for the identification and quantification of Chinese liquor aroma components along with the aging process. Based on the study, 21 aroma components were found to be important in the aroma profile of Chinese liquor. Of these, 13 aroma components decreased significantly with one year of aging, but maintained at the same level (p > 0.05) in the next three years, and decreased again (p < 0.05) after the five year aging. On the other hand, a significant increase was observed in propionic acid, furfural and phenylethanol. Ethyl lactate was found to be more stable than other aroma components during aging process. PCA and CA based on GC results were used to discriminate Chinese liquor with different ages based on the aroma components. Young liquor and aged liquors were well separated from each other, results of which were consistent with the evolution of aroma components during the aging process. The study contributes to a further understanding of the role of aroma components developed during the aging of Chinese liquor. A better comprehension of this knowledge will aid to distinguish the characteristic aroma of Chinese liquor with different liquor age and identify counterfeit and defective products.

Materials and Methods

Chinese liquor samples and chemicals. Chinese liquor “Junchang” (light-style) was provided by a local liquor factory in Sichuan province, and stored at room temperature before use. Junchang is a popular local brand of Chinese liquor produced by Junchang Liquor Factory, which was established more than ten years ago with an annual output of over 300 kiloliters in recent years. It is not as famous as Moutai and other major brands and is mostly sold locally. Young liquor without storage and liquors stored at the factory for 1, 2, 3, 4 and 5 years, were analyzed. One mL aliquot of liquor samples was injected into a 2 mL autosampler vials without any pre-treatment and then placed at room temperature before use. A solution containing mixed standards (Donglilong Information Technology Co. Ltd., Lanzhou, China), which contained 53 aroma components, was specially prepared for Chinese liquor gas chromatographic (GC) analysis (qualitative and quantitative analysis). The mixed standard solution was first diluted using ethanol with to give solution to ethanol ratio of 1:0, 1:1, 1:3, 1:7, 1:15, 1:31, 1:63 and 1:99, respectively, and then 1 mL of the diluted solution was injected into a 2 mL autosampler vials. This series of diluted solution was used for the protraction of standard curves.

Gas chromatography analysis. Gas chromatography analysis was performed using an Agilent 7890A gas chromatography (GC) unit equipped with a flame ionization detector (FID). The column carrier gas was nitrogen at constant flow rate of 1 mL/min. Liquor samples were analyzed on a LZP-950 column (50 m × 0.32 mm i.d., 1.0 μm film thickness), which was also special for Chinese liquor gas chromatographic analysis. A measured volume (1 μL) of test sample was injected into the GC, and the split ratio was 1:1. The oven temperature was held at 65 °C for 8 min, then raised to 200 °C at a rate of 5 °C/min, and held at 200 °C for 50 min; injector and detector temperature were 230 °C and 250 °C respectively.
The identification of aroma components in liquor samples was made by comparing the retention times with corresponding components in the mixed standard solution. The quantification of aroma components was made based on calibration curves which were obtained with the mixed standard solution under the same chromatographic conditions as those of the liquor samples. All liquor samples were analyzed by direct injection method, calculated in five replicates and the values were averaged.

**Statistical analysis.** Data of all groups obtained from GC analysis were initially processed by One Way ANOVA at the 5% significance level using IBM SPSS Statistics 21.0 to compare the differences statistically. Then multivariate statistical analysis was used to discriminate liquors with different ages, and to establish a correlation between aroma components and liquor age. In this research, PCA was applied to extract the first and second principal components from aroma components data and explore the possibility of discriminating differences between various samples. CA is an unsupervised clustering procedure based on the similarity or distances among observations. PCA and CA analysis were also conducted with IBM SPSS Statistics 21.0.

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Author Contributions
M.L.X. designed and performed the gas chromatography experiment under the supervision of S.M.Z. and Y.Y. M.L.X. analyzed the data and wrote the manuscript under the supervision of S.M.Z., H.S.R. edited the manuscript for both technical quality and use of English language. All of the authors contributed to the discussion of the research and manuscript.

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