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MicroRNA profiling as novel biomarkers for detecting gutter oil using machine learning

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Highlights:

- MiRNAs detected in edible oils reveal unique signatures for gutter oil.
- QRT-PCR & SVM classify gutter oil with 100% accuracy using miR-16 and let-7a.
- Novel miRNA biomarkers enable sensitive, low-cost gutter oil detection.

Abstract: Gutter oil, a major public health concern in East Asia, is often indistinguishable from pure edible oils using conventional physical and chemical methods. In this study, we present a novel approach for detecting gutter oil using microRNAs (miRNAs) as biomarkers. We proved that miRNAs exist in edible oils and can be used to differentiate between pure and recycled oils. A combination of qRT-PCR and machine learning techniques was employed to characterize miRNA profiles across commercial vegetable oils, animal oils, and gutter oil. Specifically, the relative abundances of miR-16 and let-7a were found to be significantly different among these oils, allowing for accurate differentiation via a support vector machine (SVM) model. The results indicate that miRNAs such as miR-16 and let-7a serve as reliable biomarkers, enabling classification of gutter oil even when it complies with national standards. This research provides a feasible and effective method for detecting gutter oil, with potential implications for improving food safety and public health.

Keywords: extracellular RNA; gutter oil; machine learning; miRNA; public health; SVM

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1. Introduction

Gutter oil, commonly known as used cooking oil or yellow grease, is commonly repurposed for livestock feed, soap manufacturing, textiles, cosmetics, rubber, detergents, or as a precursor for biodiesel production [1]. However, in East Asia, some illegal businesses recycle and repackage gutter oil as edible oil for sale[2]. During usage and reprocessing, these edible oils contain numerous harmful factors, including residues and contaminants formed from treating, storing, and processing waste oil, such as metal shavings, sawdust, solvents, halogens, or saline water [3]. Moreover, harmful substances are also generated during the recooking process of gutter oil. Chemical analyses indicate that gutter oil contains high levels of cholesterol, trans fatty acids (such as trans-4-hydroxy-2-nonenal (4-HNE)) [4], heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins, and occasionally aflatoxins [5]. Furthermore, consumption of gutter oil may result in acute symptoms such as diarrhea, abdominal pain and higher chronic health risks. Numerous experimental findings indicate that consuming gutter oil significantly raises the risk of cardiovascular incidents, leads to hypertension, atherosclerosis, anemia, gastrointestinal cancers, and so on [6–13].

The flood of gutter oil has now become a severe, persistent, and difficult-to-address food safety issue. Since the first recorded incident in Taiwan, China in 1985, gutter oil has grown into a multi-billion-dollar black-market industry. It is estimated that 2 to 3 million tons of gutter oil enter the Chinese market every year, potentially contaminating up to 1/10 of the food consumed in China. Therefore, the effective identification of gutter oil can protect the health of the general public from the harmful effects of recycled oil [14].

At present, methods for identifying gutter oil can be classified into three categories. Physical chemistry methods (utilize advanced sensors and spectroscopic techniques, e.g., metamaterial sensors [15], hybrid-waveguide couplers [16], Raman spectroscopy [17]), analytical chemistry methods (employ mass spectrometry and chromatography, e.g., MALDI-MS, UPLC-MS/MS [15,18–23]) and biochemical methods (encompass immunological techniques[24–26] and metabolomics [27,28]). However, these methods are limited by sensitivity and the cost of use, making them unsuitable for widespread use. (For a more detailed review, see Table S1)

Since Chen *et al.* [29] first discovered extracellular microRNAs (miRNAs) in serum, these molecules, once thought to be unstable, are now known to exist in highly stable, non-cellular forms in various body and non-body fluids, including serum, saliva, urine, milk, and traditional Chinese medicine extracts [30–34]. Chen *et al.* [35] demonstrated the use of several miRNAs as biomarkers for the quality assessment of milk and other dairy products [36]. These findings suggest that miRNAs may also exist in edible oils, and could be biomarkers for distinguishing between gutter oil and pure commercial oils. The miRNA profile of pure commercial animal or vegetable oils is expected to be relatively simple, whereas gutter oil, due to being mixed, processed, and consumed, would incorporate miRNAs from multiple biological origins, thereby displaying a blend of animal and vegetable oil characteristics.

Here, we introduce a novel approach to detect gutter oil using miRNAs. We hypothesize that: a) miRNAs exist in edible oils and are present at detectable concentrations; b) there are differences in miRNA profiles between pure commercial oils and gutter oil; c) a quantifiable method can effectively differentiate edible oils from different sources using miRNA analysis. We screened several highly abundant, stable, and specific miRNAs from both animal and plant sources [36–38], including MIR162a [39],

MIR168a [40,41], MIR166 [40,42], MIR156a [43], let-7a [44], miR-223 [45], and miR-16 [46]. Using qRT-PCR, we characterized the miRNA profiles of pure vegetable oils, animal oils, and gutter oil, and established decision boundaries for oil classification based on machine learning techniques.

2. Methods

2.1. Oil samples

Sunflower oil, olive oil, soybean oil, colza oil, camellia oil, and sesame oil—all pure commercial plant oils (COFCO Corporation)—as well as pure commercial lard (WH Group), were obtained from local stores in Nanjing, China. Additionally, we collected 37 samples of gutter oil from various sources, sequentially numbered (#1 to #37). These samples were obtained from restaurant kitchens, sewers, or directly during the cooking process. Notably, samples #1, #3, #17, #19, #22, #27, #29, and #37 were sourced from related testing agencies; these samples had undergone deep refining and were classified as hard-to-detect recycled oils that complied with the national standard for edible oils. When preparing samples of different concentrations, we diluted them with varying proportions of colza oil, in line with standard commercial practices.

2.2. RNA isolation

For qRT-PCR analysis, 100 μL of either diluted or undiluted oil sample was combined with 100 μL of RNase-free water. TRIzol Reagent or Trizol LS Reagent (Invitrogen) was then used for RNA extraction, following the manufacturer's instructions. Some samples underwent an additional chloroform extraction, resulting in the formation of four layers: a lipid droplet layer, an aqueous phase, an interphase, and an organic phase. The aqueous phase was collected, and a second TRIzol extraction was performed to remove any remaining lipid contaminants. More specifically, 100 μL of gutter oil sample was mixed with 1 mL of TRIzol Reagent. After adding TRIzol, the mixture was left at room temperature for 5 minutes and then centrifuged at 12,000 rpm for 5 minutes, after which the precipitate was discarded. For each 1 mL of TRIzol, 200 μL of chloroform was added, followed by vigorous shaking for 15 minutes and incubation at room temperature for another 15 minutes. The mixture was then centrifuged at 12,000 g and 4 °C for 15 minutes, and the upper aqueous phase was carefully transferred to a separate centrifuge tube. For samples that do not require secondary extraction, the aqueous phase was processed according to the manufacturer's standard protocol. For samples that require secondary extraction, chloroform was added at a 1:10 (chloroform: water) ratio, the mixture was vigorously shaken for 15 minutes, incubated at room temperature for 15 minutes, and centrifuged again at 12,000 g and 4 °C for 15 minutes. The upper aqueous phase was subsequently transferred to continue the experiment. All experiments were conducted using equal amounts of double-distilled water as controls.

2.3. qRT-PCR analysis

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was performed using TaqMan miRNA probes (see Table S2) according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). Briefly, total RNA was reverse transcribed to cDNA using AMV reverse transcriptase

(Takara) and a stem-loop RT primer (Applied Biosystems). Real-time PCR was performed using a TaqMan PCR kit and an Applied Biosystems 7300 Sequence Detection System (Applied Biosystems). All reactions, including no-template controls, were performed in triplicate.

After the reaction, the Ct values were determined using fixed threshold settings. The relative abundance of miRNA was calculated using the $2^{-\Delta Ct}$ method, where ΔCt is obtained by subtracting the Ct value of the no-template control from the Ct value of the sample. The relative miRNA abundance was then determined by calculating 2 to the power of −ΔCt.

2.4. Statistical analysis

All statistical analyses and visualizations were conducted using Origin 2023b. The Kruskal-Wallis non-parametric rank-sum test was used to determine statistical significance, followed by Dunn's post-hoc test for multiple comparisons. Hierarchical clustering was performed in Python 3.8.10 64-bit, using an algorithm provided by the Scipy library[47]. The dendrogram was visualized through iTOL (Interactive Tree Of Life) [48].

2.5. Machine learning using Support Vector Machine (SVM) algorithm

The miRNA relative abundance data from all samples were randomly divided into training and test sets in an 8:2 ratio.

The support vector machine (SVM) algorithm [49] was applied to predict the classification of edible oils. We utilized a linear kernel function (i.e., no special kernel transformation was applied, employing direct linear classification) and adopted a one-vs-rest (OvR) strategy to manage the multiclass classification of vegetable oil, gutter oil, and animal oil. After training, we tested the model on the test set and visualized the decision boundaries. All algorithms were implemented using Python 3.8.10 64-bit with Spyder IDE 5.5.1, and the SVM algorithm was sourced from the Scikit-learn package [50].

3. Results

3.1. Characterization of miRNAs in edible oils using different extraction methods

To screen for possible miRNAs in edible oils within a relatively pure environment and to explore appropriate extraction methods for miRNAs, we conducted two sets of RNA extraction trials on all commercial edible oils. In one set of experiments, RNA extraction was performed directly, while in the other, an additional chloroform extraction was conducted prior to using standard RNA extraction protocols (Figure 1A). A key characteristic of gutter oil is the blending of vegetable oil and animal oil caused by human cooking and consumption. To replicate these conditions, we mixed pure commercial vegetable oil and animal oil to create “simulate gutter oil” and carried out preliminary screening for miRNA molecules that might be used for gutter oil identification. Considering that soybean oil and pork are the most frequently used in Chinese cuisine, “simulate gutter oil” consisted of a mixture of commercial soybean oil and lard (Figure 1A). We measured the relative abundance of seven miRNAs—MIR162a, MIR168a, MIR166, MIR156a, let-7a, miR-223, and miR-16—in various commercial vegetable oils, animal oils, and gutter oil.

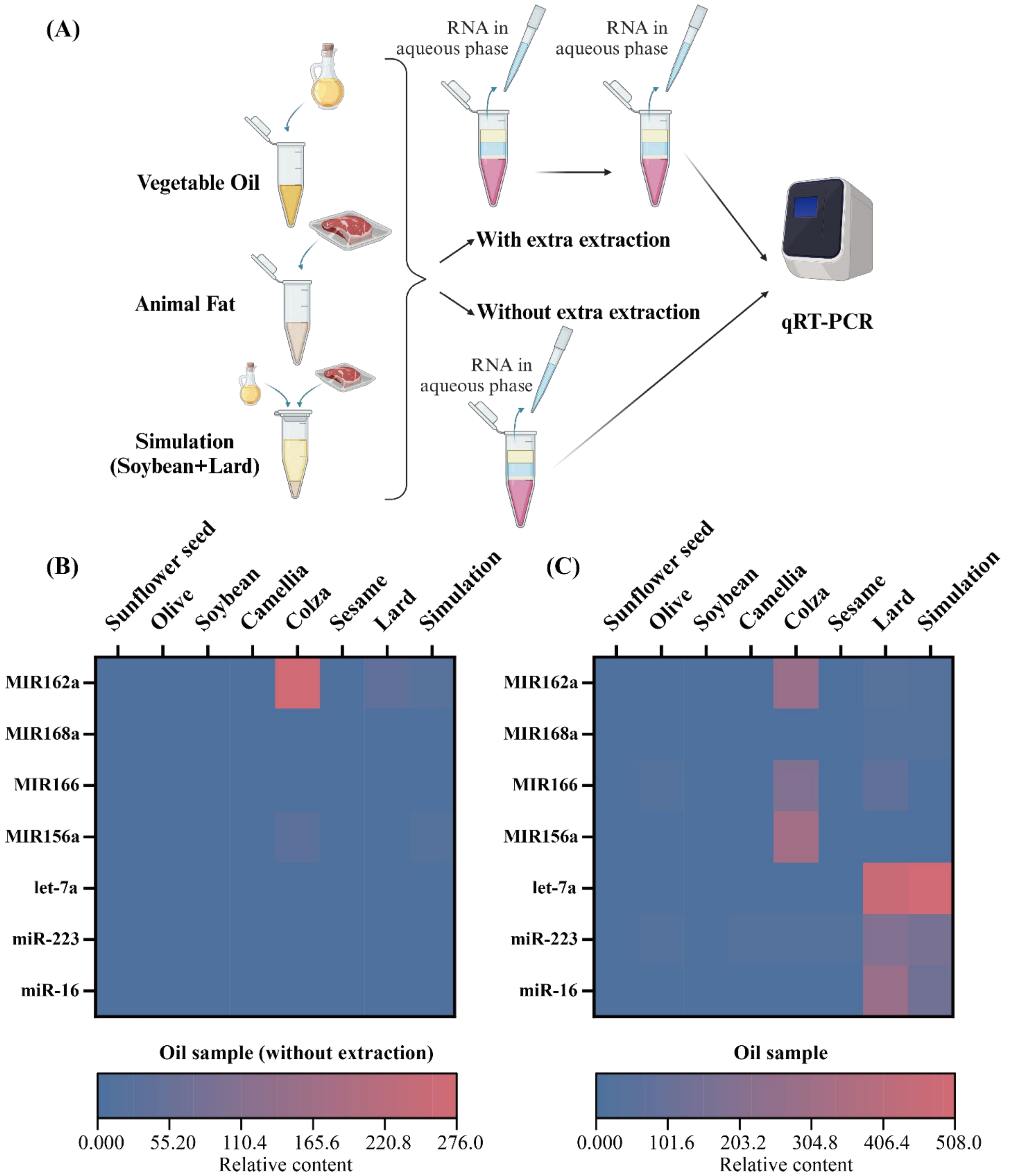

Figure 1. The relative abundance of various miRNAs in commercial oil and simulate gutter oil samples (Soybean oil+ Lard). (**A**) The experiment incorporated two RNA extraction methods: one with an additional extraction step and one without. Both methods were applied to RNA extraction from vegetable oil, animal oil, and simulated gutter oil. (**B**) Relative abundance of miRNAs in the oil samples without chloroform extraction. (**C**) Relative abundance of miRNAs in the oil samples after chloroform extraction. The horizontal axes of the two figures represent the biological sources of the samples, such as "Sunflower seed," indicating commercial sunflower seed oil. The vertical axis shows the measured miRNAs, while the color of each cell represents the abundance of the corresponding miRNA in the respective sample.

In Figure 1B, we observed that the differences in miRNA abundance between samples in the group without chloroform extraction were significantly smaller. Most samples exhibited miRNA levels similar to those of the no-template blank control, with the exception of the colza oil group, which showed a higher abundance of MIR162a. In contrast, the chloroform-extracted group exhibited clear distinctions in miRNA profiles among the samples (Figure 1C). MIR162a and MIR156a were found at higher levels in colza oil, while other vegetable oils showed no significant miRNA features. In animal oils and simulate gutter oil, let-7a, miR-223, and miR-16 were all present in high abundance. Importantly, these animal-derived miRNAs were not detected in vegetable oils, indicating their potential use as markers for the biological origin of edible oils. Therefore, in the following research, we used chloroform extraction method to extract samples of different types of edible oil, and miR-16 and let-7a were used as markers to distinguish edible oil from different sources.

3.2. Sensitivity of miRNA detection in edible oil samples

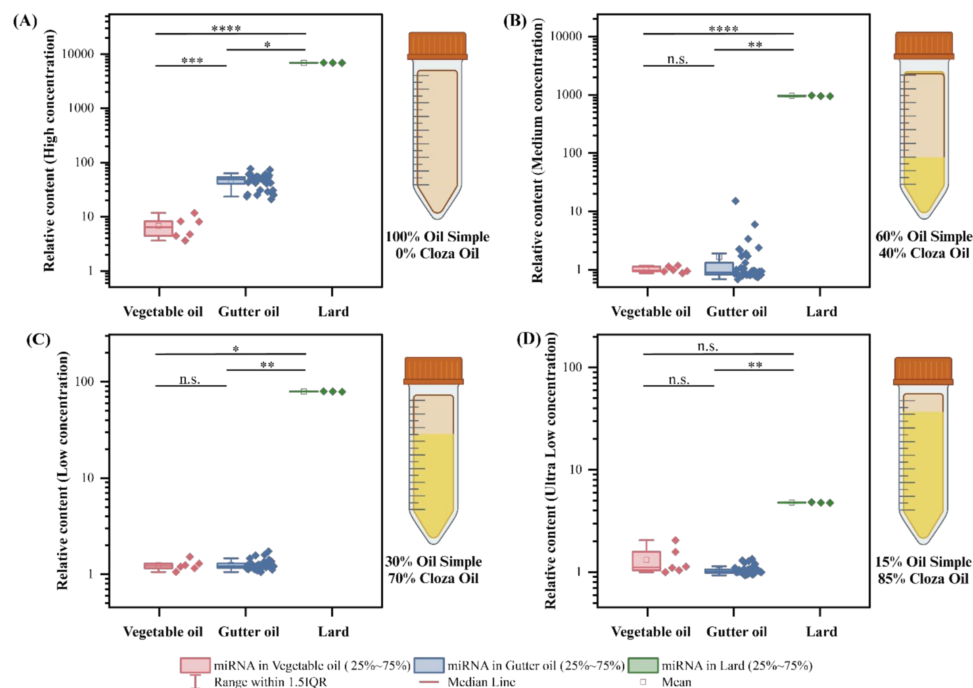

Figure 2. The variation in the relative abundance of miR-16 across four different concentrations of vegetable oil, lard, and gutter oil. The diagram on the right illustrates the dilution ratios used for different edible oil samples. (**A**) Differences in miR-16 relative abundance among vegetable oil, lard and gutter oil at high concentration condition (100%). (**B**) Differences in miR-16 relative abundance among vegetable oil, lard and gutter oil at medium concentration condition (60%). (**C**) Differences in miR-16 relative abundance among vegetable oil, lard and gutter oil at low concentration condition (~30%). (**D**) Differences in miR-16 relative abundance among vegetable oil, lard and gutter oil at ultra-low concentration condition (~15%). Different asterisks (*) on the boxplot represent significant differences among vegetable oil, gutter oil and lard by the Kruskal-Wallis test at α=0.05, 0.01, 0.001 or 0.0001 level.

To verify the presence of miRNAs in real-world gutter oil and to determine detection limits, we diluted commercial vegetable oil, commercial animal oil, and collected gutter oil samples to concentrations of

100%, 60%, 30%, and 15%. We selected miR-16, which exhibited significant variation across samples in the previous experiments, as the target miRNA to determine the sensitivity of using miRNAs for identifying the source of edible oils.

At 100% concentration, miR-16 abundance significantly differed between commercial vegetable oil, gutter oil, and commercial lard (Figure 2A). Lard exhibited a high level of miR-16, followed by gutter oil, while vegetable oil contained almost no miR-16. At 60% concentration, the detectable abundance of miR-16 in both lard and gutter oil dropped significantly, resulting in no notable difference between vegetable oil and gutter oil, though a few gutter oil samples still showed slightly elevated miR-16 levels (Figure 2B). At 30% concentration, miR-16 abundance differences between all gutter oil and vegetable oil samples were no longer significant; miR-16 was nearly undetectable in both vegetable oil and gutter oil samples, but it remained present in considerable amounts in lard (Figure 2C). At 15% concentration, statistically significant differences in miR-16 abundance were found only between gutter oil and lard, with no significant differences between other samples (Figure 2D). These findings indicate that the ability to distinguish edible oils based on miRNA abundance is concentration-dependent, and at original concentrations, miRNAs may effectively differentiate the biological origins of various edible oils.

3.3. Identification of candidate miRNA biomarkers for the classification of gutter oil

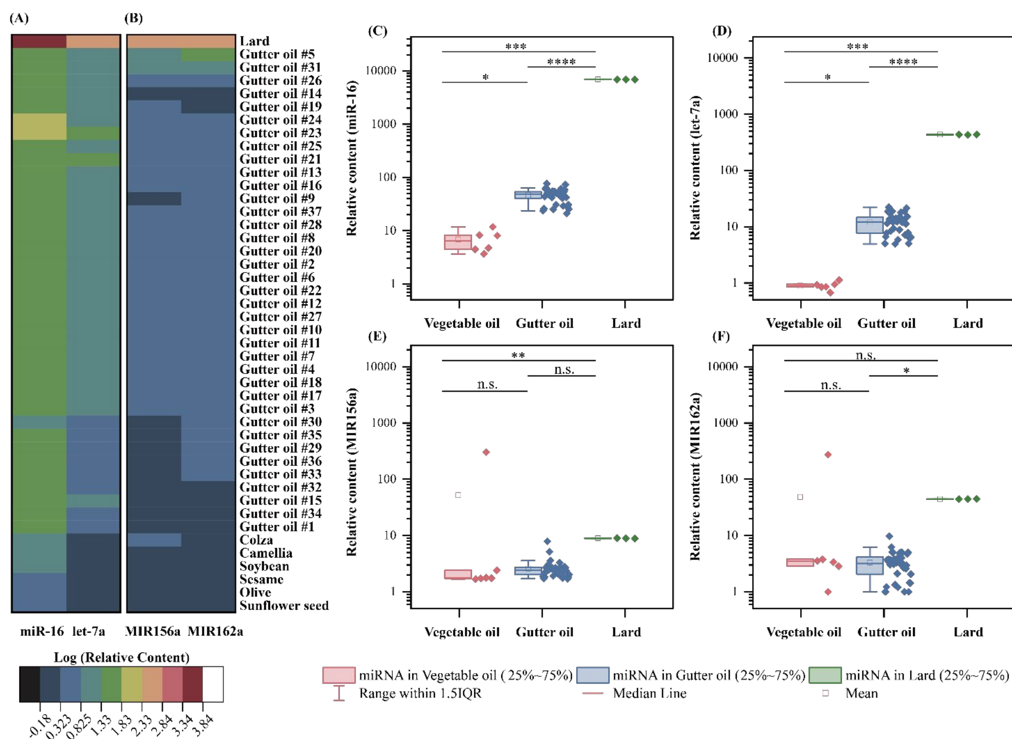

Figure 3. The relative abundance (log scale) of four different miRNAs in diverse categories of real-world samples. Different asterisks (*) on the boxplot represent significant differences among vegetable oil, gutter oil and lard by the Kruskal-Wallis test at α=0.05, 0.01, 0.001 or 0.0001 level. (**A**) The relative abundance of miR-16 and let-7a in the samples. (**B**) The relative abundance of MIR156a and MIR162a in the samples. (**C**) Differences in miR-16 relative abundance among vegetable oil, lard and gutter oil. (**D**) Differences in let-7a relative abundance among vegetable oil, lard and gutter oil. (**E**) Differences in MIR156a relative abundance among vegetable oil, lard and gutter oil. (**F**) Differences in MIR162a relative abundance among vegetable oil, lard and gutter oil.

In order to further investigate which miRNAs are more suitable for distinguishing gutter oil, we performed qRT-PCR analysis on commercial edible oils and gutter oil samples from different sources. For the two animal-derived miRNAs, miR-16 and let-7a, their abundance followed a similar pattern across different types of edible oils (Figure 3A). In vegetable oils, both miRNAs had low abundance; in gutter oil, they were moderately abundant; and in animal oils, they were highly abundant.

For the two plant-derived miRNAs, MIR156a and MIR162a, the distribution pattern across different types of edible oils differed significantly from that of animal-derived miRNAs (Figure 3B). Furthermore, we conducted statistical tests on the distribution of miR-16 and let-7a in edible oils. Figures 3C and 3D show that the abundance of miR-16 and let-7a differs significantly among vegetable oil, gutter oil, and animal oil. In contrast, Figures 4E and 4F indicate that the differences in abundance of the two plant-derived miRNAs cannot effectively distinguish between vegetable oil and gutter oil, or between vegetable oil and animal oil.

These findings suggest that miR-16 and let-7a are likely the most reliable biomarkers for classifying edible oils.

3.4. The classification of edible oils through hierarchical clustering and SVM

Then, we sought to classify various edible oil samples by miRNA profiling. We conducted clustering of all samples according to the similarity of their miRNA abundance. The analysis using miR-16 and let-7a revealed these distribution characteristics: animal oils, vegetable oils, and gutter oil clustered into distinct branches, with no overlap between them, demonstrating effective clustering (Figure 4A). The results also indicated that MIR156a and MIR162a could only separate animal oils from other types of edible oils, while gutter oil and vegetable oil were hardly to be distinguished, specifically, neither vegetable oil nor gutter oil forms a monophyletic group (Figure 4B).

To develop a quantifiable method to distinguish between vegetable oil, animal oil, and gutter oil, we plotted the relative abundance of miR-16 on the x-axis and that of let-7a on the y-axis, representing all sample data on a scatter plot (Figure 4C). This effectively mapped the distribution of miRNAs into a two-dimensional feature space, converting the classification problem of edible oils into finding a linear hyperplane that can separate different types of data points. There may be multiple lines capable of separating these points. To ensure robustness in our classification, we aimed to identify the hyperplane that maximizes the separation margin between different sample types, known as the maximum-margin hyperplane, reducing the risk of misclassification even in the presence of data noise. It should be noted that, based on our data analysis, this is a linearly separable problem; therefore, we employed the simplest linear SVM. By applying the SVM method, we determined this maximum-margin hyperplane, which not only incorporates the distribution information of miR-16 and let-7a in edible oils but also enables accurate classification of the edible oil samples.

In Figure 4C, the data points representing different edible oil samples were effectively classified into three categories using distinct color blocks, demonstrating that the SVM successfully leveraged miRNA abundance data for accurate classification. The one-vs-rest (OvR) strategy was employed, resulting in three separate decision boundary functions that distinguish vegetable oil from other edible oils, animal oil from other edible oils, and gutter oil from other edible oils. The three decision boundary functions obtained from training are presented below.

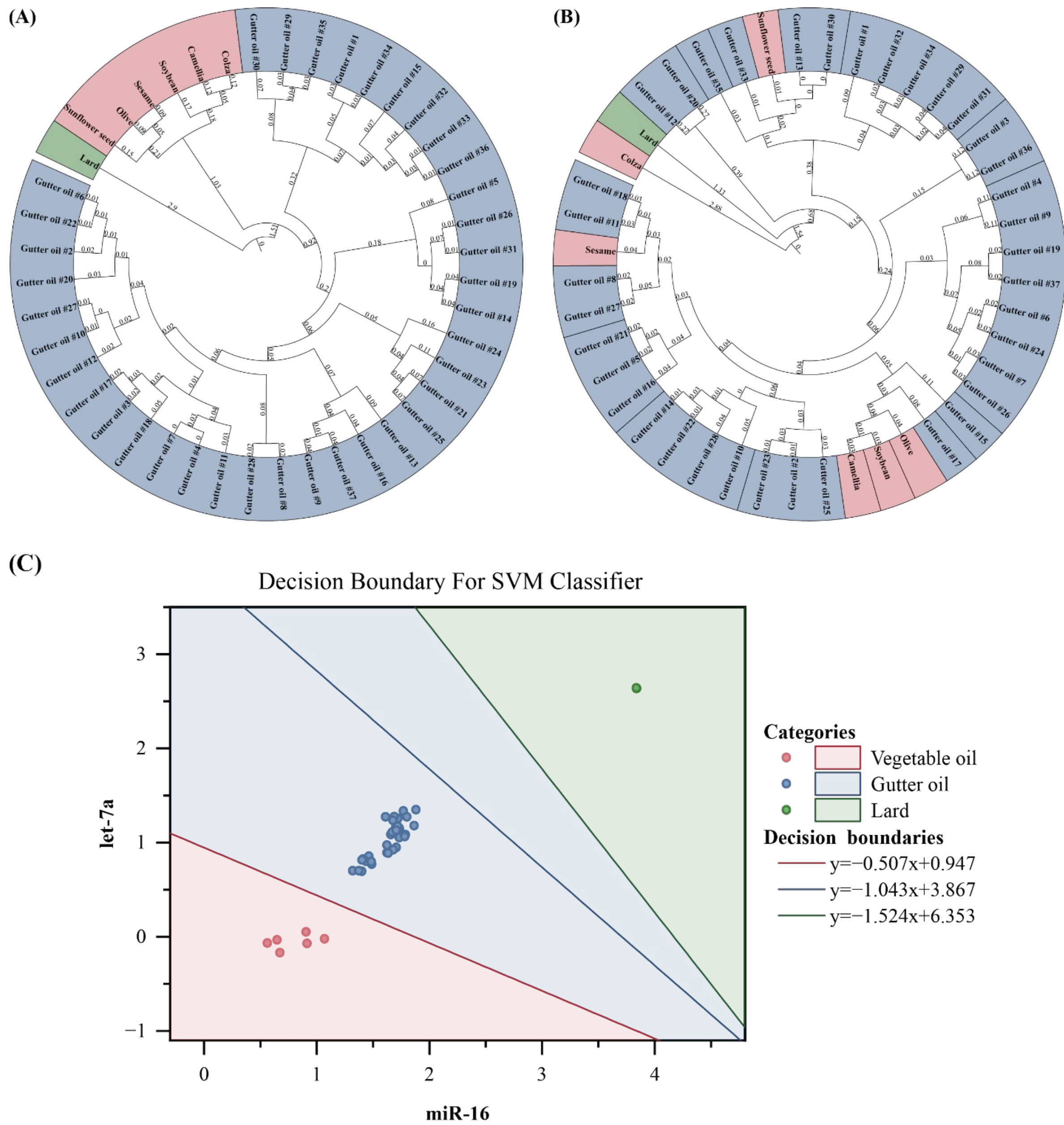

Figure 4. Using statistical methods and machine learning techniques to distinguish vegetable oil, gutter oil and lard samples based on the abundance of four different miRNAs. The dendrogram represents the clustering results, indicating that samples within the same branch contain miRNAs with greater miRNAs abundance similarity. (**A**) The hierarchical clustering analysis results using two animal-derived miRNAs are shown as a dendrogram. The red leaf nodes represent animal oil, the blue leaf nodes represent vegetable oil, and the green leaf nodes represent gutter oil. The numbers on each branch represent the branch length. (**B**) The hierarchical clustering analysis results using two plant-derived miRNAs are shown as a dendrogram. The red leaf nodes represent animal oil, the blue leaf nodes represent vegetable oil, and the green leaf nodes represent gutter oil. The numbers on each branch represent the branch length. (**C**) Each data point corresponds to an edible oil sample plotted within a two-dimensional space defined by the relative abundances of miR-16 and let-7a (log scale). Different colored lines represent the decision boundaries for vegetable oil, gutter oil, and lard. Consequently, a data point located within the red shaded region is categorized as vegetable oil, those within the green region as lard, and similarly for other classifications.

$$\text{Vegetable oil}_{\text{Vs Rest}} = -0.507*[\text{miR-16}] + 0.947 - [\text{let-7a}] \quad (1)$$

$$\text{Gutter oil}_{\text{Vs Rest}} = -1.403*[\text{miR-16}] + 3.867 - [\text{let-7a}] \quad (2)$$

$$\text{Lard}_{\text{Vs Rest}} = -1.524*[\text{miR-16}] + 6.353 - [\text{let-7a}] \quad (3)$$

In the functions above, [miR-16] and [let-7a] represent the relative abundances of the two miRNAs, respectively. The OvR strategy utilizes these three decision boundaries to make classifications. These boundaries do not directly assign an unknown sample to a specific category; instead, they calculate the likelihood that the sample belongs to a particular category compared to others. Specifically, equation (1) compares vegetable oil to other categories of edible oils. By inputting the relative abundances of miR-16 and let-7a for a sample into equation (1), a positive result indicates that the sample is more likely to be vegetable oil rather than other edible oils. Equation (2) compares gutter oil to other categories of edible oils, and equation (3) compares lard to other categories of edible oils. For classification purposes, the principles outlined below can be utilized to ascertain the category of a given sample: a) if any of the three function values is positive, the sample is assigned to the category corresponding to the highest positive value; b) if none of the function values is positive, the sample is assigned to the category with the smallest absolute negative value. Using this approach, all samples in this study were classified with 100% accuracy in our testing set (the model also achieved perfect accuracy (1.0) in 5-fold cross-validation), with F1-score equal to 1.

4. Discussion

Since gutter oil became a major threat to public health, extensive efforts have been made to develop an effective detection method. In this study, we provide a new method for distinguishing gutter oil from commercial edible oil. We found that: a) miRNAs are present in edible oils and can be detected at their original concentrations using qRT-PCR; while this was not unexpected, it further confirms that miRNAs are more resilient than previously thought, surviving under complex conditions such as edible oil and powdered milk processing [35]. b) The miRNA profiles of pure vegetable oils, animal oils, and gutter oil show significant differences, with miR-16 and let-7a being the most distinct. c) By mapping the relative abundances of miR-16 and let-7a into a two-dimensional feature space and applying an SVM algorithm, we successfully and accurately differentiated gutter oil samples.

In addition, we also found that plant-derived miRNAs, such as MIR156a and MIR162a, were detected in animal oils, whereas animal-derived miRNAs, such as miR-16 and let-7a, were entirely absent from vegetable oils. We hypothesize that this may be due to plant-derived miRNAs being ingested by animals through their diet, as previous research has demonstrated that miRNAs can be absorbed by animals via the digestive system [51]. In contrast, no substantial amounts of plant-derived miRNAs were detected in vegetable oils. Current research suggests that plant-derived miRNAs are abundant in leaves and flowers [52], although they are also found in seeds [37]. Since seeds—the main source of vegetable oil—are highly dormant organs, miRNA biosynthesis may not be very active. Additionally, vegetable oil is extracted through pressing rather than refining, as is the case for animal oil. These factors may collectively contribute to the lower miRNA content in vegetable oils. Consequently, the absence of animal-derived miRNA in vegetable oil provides a strong basis for identifying gutter oil. Furthermore,

beyond the miRNAs examined in this study, there may be other highly stable miRNAs that could serve as biomarkers for gutter oil detection, which warrants further exploration.

This study also has certain limitations. For example, when samples are excessively diluted, the experimental results may not be accurate. However, according to our experimental results, the accuracy of miRNA profiling is dependent on the extraction method. For example, our results demonstrated that samples processed with two extraction steps were more precise for miRNA detection. We believe that by further optimizing sample processing methods, such as introducing pre-amplification or utilizing higher-yield RNA extraction techniques, we can mitigate the inaccuracies caused by dilution effects. Moreover, in some extreme cases, using the miRNA approach may fail to detect gutter oil composed solely of vegetable oil, although such a scenario is highly unlikely.

Nevertheless, our method still exhibits significant advantages over traditional methods for detecting gutter oil: a) the miRNA profiles of different edible oils vary considerably, and miRNAs are not easily falsified, making them stable markers for detection. Notably, our experiment included the hard-to-detect gutter oils (i.e., sample #1, #3, #17, #19, #22, #27, #29, and #37). The results showed that using our method allows for the precise detection of these deeply processed gutter oils, firmly demonstrating that even gutter oil compliant with national standards can be recognized via miRNA analysis. b) During the COVID-19 pandemic, qRT-PCR technology was extensively used to detect viral infections [53], which led to widespread adoption of related equipment and techniques. Many institutions now have the necessary instruments and skilled technicians, enabling low-cost analysis of miRNAs in edible oils. Based on the latest data, conducting a single qRT-PCR test in different regions of China costs less than 20 yuan [54], granting this method a substantial cost benefit. Given these factors, it can be effectively incorporated into the current technological and regulatory infrastructures. c) Moreover, qRT-PCR is highly sensitive and facilitates the use of no-template controls, enabling rapid determination of sample characteristics through the application of decision boundary functions.

As in China, GB 2716-2018 (China's national standard for edible oils) only specifies detection items such as odor, acid value, peroxide value, polar components, solvent residue, and free gossypol, gutter oil often complies with the national standard after undergoing processes like heating, filtration, and alkali neutralization [55]. Thus, routine testing methods are not effective in distinguishing gutter oil. We propose that regional market supervision departments can employ our reported method to analyze miRNA profiles in edible oils. In tandem, they can build databases and machine learning models using gutter oil samples collected during law enforcement. This approach allows qPCR and SVM methods to be utilized in subsequent identification processes for oil quality. By detecting gutter oil with greater sensitivity, accuracy, and convenience, our method will aid the government in formulating policies, improving gutter oil detection capabilities, and protecting public health from the risks associated with gutter oil.

5. Conclusion

Our study reports the presence of miRNAs in edible oils and miR-16 and let-7a have the potential to serve as biomarkers for detecting gutter oil. The study also provided information on detection limitations and methodologies. This research offers a novel, feasible approach to solving the challenge of gutter oil detection. Because of the sensitivity and easy-to-adopt features of this technology, there is hope that it can be combined with legal and policy measures to help the government effectively regulate the misuse

of gutter oil and provide people with health protection. Furthermore, we believe that future research should focus on optimizing miRNA extraction methods and exploring additional types of miRNAs. Beyond miR-16 and let-7a, incorporating other miRNAs could expand the SVM classifier's two-dimensional space into a multidimensional one, thereby enhancing the sensitivity and robustness of detection.

Supplementary data

The authors confirm that the supplementary data are available within this article. Table S1. Reviews of technologies for detecting gutter oil [56]. Table S2. Sequences of primers and probes used for reverse transcription and TaqMan qPCR.

Acknowledgments

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Conflicts of interests

The authors declare no competing interests.

Ethical statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The authors state that no ethical approval is required for this study.

Authors' contribution

Conceptualization, Y.Z. and J.L.; methodology, Y.Z., L.L. and J.L.; software, J.L.; validation, J.L., L.C. and Y.L.; formal analysis, J.L.; investigation, L.L., J.L., L.C. and Y.L.; resources, L.L., Y.Z. and J.L.; data curation, L.L. and J.L.; writing—original draft preparation, J.L.; writing—review and editing, Y.J. and J.L.; visualization, J.L.; supervision, Y.Z., L.C., Y.L. and J.L.; project administration, Y.Z. and J.L.; funding acquisition, Y.Z. All authors have read and agreed to the published version of the manuscript.

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