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Article

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Development of a monitoring system for Huangjiu storage based on electrical conductivity

Jian Hu[1,2,4], Shuangping Liu[1,4], Mujia Nan[3], Caixia Liu[4], Xiao Han[1], and Jian Mao[1,4,*]

[1]National Engineering Research Center for Cereal Fermentation and Food Biomanufacturing, School of Food Science and Technology, Jiangnan University, Wuxi, China
[2]Shanghai Jinfeng Wine Co., Ltd., Shanghai, China
[3]Basic Department, University of Tibetan Medicine, Lhasa, China
[4]Jiangnan University (Shaoxing) Industrial Technology Research Institute, Shaoxing, China

***Correspondence to*: Jian Mao, National Engineering Research Center for Cereal Fermentation and Food Biomanufacturing, School of Food Science and Technology, Jiangnan University, Wuxi 214122, China. E-mail: maojian@jiangnan.edu.cn

Abstract

In order to quickly detect the rancidification of Huangjiu in pottery jars, this study developed a fast detection method based on the principle of electrical conductivity changes caused by microbial contamination. The change in total acid in Huangjiu was positively correlated with the increase of electrical conductivity. This method was applied to an online monitoring system for Huangjiu storage in stainless steel tanks. When the electrical conductivity exceeds the normal fluctuation range (mean+3 standard deviations) of previous data, the monitoring system recognizes microbial contamination. By optimizing the conductivity-temperature compensation coefficient and conductivity statistical method, the standard deviation of the method was reduced and the sensitivity of microbial pollution monitoring was improved. The ranges of conductivity and compensation coefficient of common types of Huangjiu were estimated. Interference in conductivity measurements due to environmental factors was minimised through the synchronous comparison of conductivity data for multiple tanks of Huangjiu. The standard deviation, which indicates the fluctuation range of the system, decreased from 143 to 2 μS/cm. The monitoring system was then applied in Huangjiu storage tanks with capacities of 60 t and 300 t. Through the comparison of conductivity data change, the abnormal signals caused by microbial contamination during the storage of Huangjiu were found over time. Meanwhile, through offline detection of total acid in Huangjiu, the effectiveness of microbial contamination online detection was verified.

Keywords: Huangjiu; online monitoring; electrical conductivity; rancidification; microbial contamination.

Introduction

Huangjiu (Chinese rice wine) is the traditional fermented wine of China and one of three ancient wine products in the world. It has the title of China's 'national wine' due to its rich nutritional content and healthcare value (Lu *et al.*, 2007; Yu *et al.*, 2018). The brewing process of Huangjiu involves soaking, stewing, fermentation, squeezing, mixing, aged wine storage, blending, filtering, bottling, and sterilising (Mo *et al.*, 2010; Yu *et al.*, 2019). In particular, aged wine storage can significantly influence the quality of Huangjiu. The pleasant flavour of aged wine is one of the special flavours of Huangjiu and storage time is conducive to the production of aromatic compounds in Huangjiu (Shen *et al.*, 2011; Feng *et al.*, 2020). Traditional Huangjiu is usually stored in jars for at least 3 a before entering the market (Chen *et al.*, 2013; Yu *et al.*, 2020). This incurs a high cost, is labour-intensive, and can involve storage-related losses (Jiao *et al.*, 2017; Shen *et al.*, 2021). Additionally, the quality of Huangjiu can only be determined when the jar is opened, and it is impossible to monitor quality changes during storage. Huangjiu may become rancid upon microbial pollution, thus resulting in great losses. To address the technological defects related to the storage of Huangjiu in pottery jars, Huangjiu storage in large tanks was developed, and traditional jar storage was partly replaced by mechanical and pipeline storage, aiming to improve quality control.

Huangjiu in large storage tanks has been studied for nearly 40 a, during which some key problems such as container material, sanitation, and disinfection as well as flavour changes have been successively overcome. The qualification rate of Huangjiu after storage is higher than 95%, but lower than 99%. However, because tanks have large capacities, relatively great losses may occur even when only 1%–3% of the Huangjiu suffers abnormal changes such as increased acidity. Major microorganisms that cause rancidification of Huangjiu include lactic acid bacteria, *Saccharopolyspora*, acetic bacteria, *Pseudomonas*, etc. (Hong *et al.*, 2016; Xie *et al.*, 2021). Most of these bacteria can only grow in special culture media. Hence, it is necessary to collect samples continuously for offline detection in the storage process. The storage process should also be monitored by analysing physicochemical indicators or high-throughput sequencing, aiming to assure a higher quality level (>99%). However, sampling detection involves some food safety risks and high sampling frequency or professional detection technology can increase the burden

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of laboratory detection. Online monitoring is a fast detection method that can decrease safety risks. Currently, researchers around the world are investigating the possibility of dynamic monitoring in the fermentation process and determining the termination of the fermentation process using instruments and equipment (Jiang *et al.*, 2018). In some alcoholic beverage products, microfluidic membrane devices, proton transfer reaction mass spectrometry and time of flight mass spectrometer (PTR-ToF-MS), and electrical conductivity detection technology have been applied for online monitoring of the fermentation process (Paquet *et al.*, 2000; Zou *et al.*, 2016; Li *et al.*, 2019; Berbegal *et al.*, 2020). These online monitoring techniques are used to study the indicators in the fermentation process to monitor whether fermentation can proceed normally to completion. The storage stage of Huangjiu is an important and special stage to form flavor after fermentation, and there are still few studies and application cases of online monitoring in the Huangjiu storage process. Among existing monitoring technologies, conductivity detection technology is characterised by fast detection, high sensitivity (Poghossian *et al.*, 2019), and relatively good application feasibility. Some studies have indicated that online monitoring of the conductivity of meats and plants is conducive to production and can improve the quality and safety level of products (Jeon *et al.*, 2017; Bohuslávek, 2018). Moreover, conductivity can be used to directly detect or predict the rancidification of milk, because the charged ion metabolites produced by bacterial growth in milk can increase conductivity. Therefore, conductivity can be used as a quality indicator of milk rancidification (Lien *et al.*, 2016; Yanthi *et al.*, 2018). In the study of wine, electrical conductivity is applied to monitor the impact of ultrasonic radiation on wine quality (Yan *et al.*, 2017). Huangjiu is rich in nutrients, and when microbial contamination occurs, the electrical conductivity increases with the degree of rancidity (Jian *et al.*, 2022). Therefore, changes in conductivity are influenced by microbial contamination. In this study, the rancidification (abnormal increase in total acid) and conductivity changes of Huangjiu were investigated, and a fast detection method for microbial contamination was developed. The method is applied to the online monitoring system of Huangjiu in large storage tanks, and the effectiveness of the monitoring system is verified.

Materials and Methods

Experimental samples

Huangjiu samples were provided by Shanghai Jinfeng Wine Co., Ltd. (Shanghai, China). Huangjiu is fermented using rice and wheat as the raw materials, with a fermentation time of 2 months.

Main reagents and instruments

Reagents including NaOH were analytically pure and purchased from Shanghai Anpu Experimental Technological Co., Ltd. (Shanghai, China). The 1413 µS/cm conductivity calibration buffer solution was obtained from Hanna Water Environment Engineering Co., Ltd. (Shanghai, China).

The instruments used included a Metrohm 785 potentiometric titrator (Herisau, Switzerland), Anton Paar Alcolyzer Wine Analyzing System (Graz, Austria), Hanna HI 99300 conductivity meter (electrode constant=1.0 cm^{-1}; Rhodes, Italy), Hamilton online conductivity sensor Conducell 4USF Arc 120 (electrode constant=0.36 cm^{-1}; Bonaduz, Switzerland), 60 t stainless steel tanks, and 300 t stainless steel tanks (Shaoxing, China).

Detection method of Huangjiu

Alcoholic strength detection method

After filtration by qualitative filter paper, 50 mL of Huangjiu samples were injected into the sample pool of Anton Paar Alcolyzer Wine. The numerical value of alcoholic strength was gained after the stabilization of numerical values (Jian *et al.*, 2022).

Total acid and amino acid nitrogen detection method

The total acid and amino acid nitrogen were tested by an automatic acid-base titration method according to the national standards for Huangjiu (GB/T 13662-2018) (Liu *et al.*, 2021). The total acid of Huangjiu was calculated based on lactic acid equivalent, and the unit of calculation is g/L. Amino acid nitrogen is the content of nitrogen in free amino acids.

To measure the conductivity of Huangjiu in storage tanks, 4–20 mA analog signals were output directly by the intelligent online conductivity sensor Conducell 4USF Arc 120 to the PLC and then transferred to a computer. The real-time electrical conductivity value (EC_t) and the real-time temperature value (T) were collected by an Inscan HIS 6.41 and stored. The online monitoring system collected conductivity data every 5 min. To measure the conductivity of Huangjiu in jars, an HI 99300 conductivity meter was used. The sensor was immersed in alcoholic liquor and the real-time values of EC_t and T were read directly after stabilisation. All conductivity sensors and meters were calibrated using a 1413 µS/cm buffer solution before the experiment.

The conductivity value under a standard temperature of 25 °C (EC_{25}) was calculated from the real-time values of EC_t, T, and conductivity-temperature compensation coefficient (a):

$$\mathrm{EC}_{25} = \mathrm{EC}_t + (25 - T) \times a$$

Storage of Huangjiu in stainless steel tanks

The storage tanks were cleaned and the outlet valve was closed. The 85–90 °C-sterilised Huangjiu samples were pumped into the tank until the storage volume was reached, and then the inlet valve was closed. Huangjiu was naturally cooled to room temperature in the tank and stored for at least 1 a. Physicochemical indicators of Huangjiu were analysed through sampling before and during storage if necessary.

Results and Discussion

Changes in the conductivity of Huangjiu after rancidification

In this study, 100 pottery jars of the same batch of 3-year-old Huangjiu were collected randomly to detect real-time conductivity, real-time temperature, and total acid value. The detection results were arranged in ascending order according to the real-time conductivity. The results are shown in Table 1. The temperatures of all samples were basically constant at 12.3±0.3 °C. The conductivity of samples 98#–100# increased significantly, accompanied by a large increase in total acid. These samples had all developed

Table 1. Real-time conductivity, real-time temperature, and total acid of Huangjiu in jars

No.	Real-time conductivity (μS/cm)	Real-time temperature (°C)	Total acid (g/L)
1–92	1023±19	12.3±0.3	4.75±0.17
93–97	1108±26	12.2±0.3	5.32±0.34
98	1177±3	12.3±0.1	9.61±0.04
99	1289±2	12.4±0.1	14.5±0.03
100	1298±3	12.4±0.1	12.4±0.03

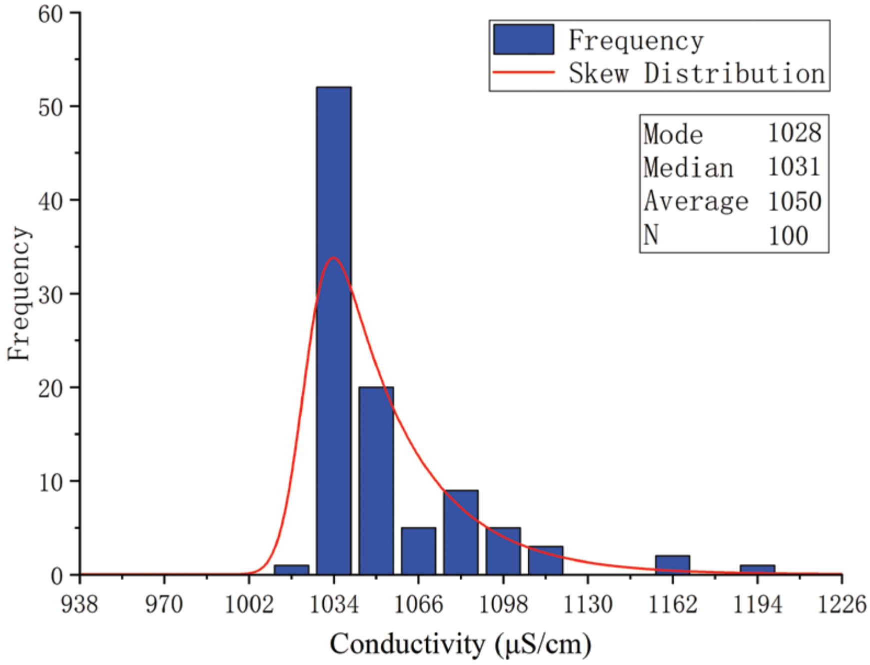

Figure 1. Distribution of the conductivity of Huangjiu in 100 pottery jars.

rancidification (total acid>7 g/L) due to microbial pollution, which exceeded the limit of the national standard for Huangjiu. The conductivity distribution pattern of Huangjiu in pottery jars in this batch is shown in Figure 1. The average value was higher than the median and mode values with the curve showing a rightward skewed distribution. The standard deviation (SD) of conductivity was 48 μS/cm. Before storage, there were minimal differences among the Huangjiu in jars of the same batch and the conductivities were normally distributed with SD<5 μS/cm. After storage, the SD increased and the conductivity changed from a normal distribution to a rightward skewed distribution. Hence, some jars had become rancid due to microbial contamination in the storage process, causing a positive growth in conductivity. According to the correlation between conductivity and total acid, the rancidification of Huangjiu in jars can be quickly identified through conductivity detection technology.

Composition of the online monitoring system for Huangjiu in tanks

The conductivity of Huangjiu changes in response to microbial contamination. Hence, a conductivity sensor was applied to the online monitoring system of Huangjiu in stainless steel tanks. The whole online monitoring system for Huangjiu in large tanks was composed of stainless steel tanks of different scales (60 t and 300 t) and conductivity sensors, cables, PLC, and PC. The intelligent sensor and PLC were connected through the RS485 port and then connected to the PC through the RS232 serial port. The conductivity sensor outputs a 4–20 mA analog signal to the PLC, which then outputs the digital signal to the PC.

Each tank for the storage of Huangjiu has a conductivity sensor, an inlet valve, an outlet valve, a sampling valve, a pressure balance system, and an air purification system (Figure 2). Both 60 t and 300 t tanks were placed in outdoor areas. Specifically, a stainless steel canopy at the top of the 60 t tank area was used to protect the Huangjiu from sunlight and rainwater. The 300 t tanks were directly exposed to sunlight and rainwater.

Effects of the conductivity-temperature compensation coefficient of Huangjiu on the online monitoring method

During the fast conductivity detection of Huangjiu in jars, the temperatures of the same batch of wine fluctuated within ±0.5 °C, and no temperature compensation was needed for the real-time conductivity measurements. However, the temperature of the Huangjiu in large tanks fluctuated across a range of 0–35 °C and real-time conductivity had to be calibrated to the conductivity under standard temperature (25 °C). Therefore, the conductivity-temperature compensation coefficient of the wine was measured in advance. After the Huangjiu samples in tanks were collected, the conductivity values under different temperatures were measured using a table-type conductivity meter. The conductivity-temperature compensation coefficient of this batch was 44.95 μS/(cm·°C) after fitting and the calculation formula for the linear fitting is shown in Figure 3A.

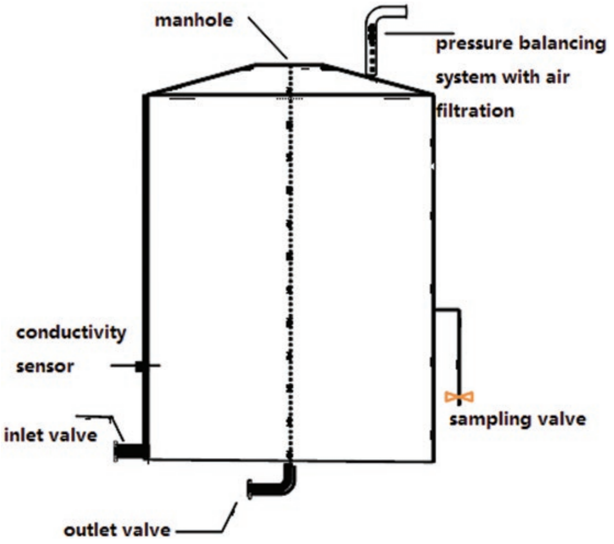

Figure 2. Structure of stainless steel tanks for Huangjiu storage.

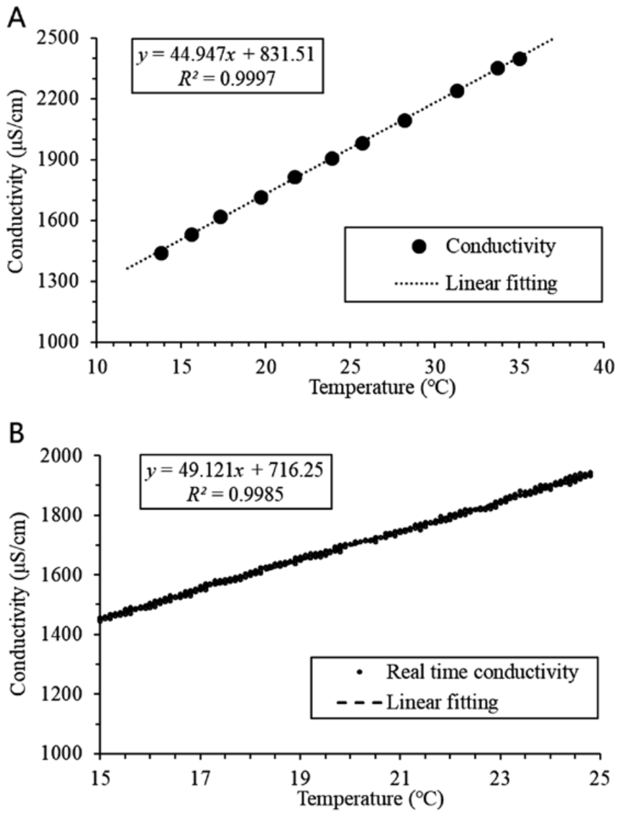

Figure 3. (A) Calculation of offline conductivity-temperature compensation coefficients. (B) Calculation of online conductivity-temperature compensation coefficients.

To decrease sampling error and sampling-induced microbial contamination risks, the online real-time conductivity and real-time temperature of the Huangjiu in tanks can be applied for correction during times when the temperature rises or decreases. The conductivity-temperature compensation coefficient of this batch was 49.12 μS/(cm·°C) after fitting. The calculation formula for the linear fitting is shown in Figure 3B. Both calculation methods for the conductivity-temperature compensation coefficient had some errors, which are mainly attributed to sampling error and inconsistencies in inner versus outer temperatures during temperature fluctuation (the sensor measured the tank surface temperature near the alcohol rather than the overall temperature).

Conductivity is a physical property of Huangjiu and may not change significantly during storage in stainless steel tanks. Instead, it displayed a normal distribution in a narrow range. The changes in the real-time conductivity of the Huangjiu in large tanks over time after temperature compensation are shown in Figure 4. The real-time temperature of Huangjiu decreased from 24.8 to 14.9 °C in 3 d. Influenced by the temperature, the real-time conductivity dropped from 1939 to 1440 μS/cm, with an average of 1675±143 μS/cm. After temperature correction, the fluctuation amplitude of conductivity (the conductivity hereinafter refers to the value after temperature compensation) of Huangjiu decreased, and the average value was 1894±27 μS/cm. The corresponding SD decreased from 143 to 27 μS/cm.

The conductivity and conductivity-temperature compensation coefficient ranges of different types of Huangjiu are listed in Table 2. Different batches of Huangjiu have different

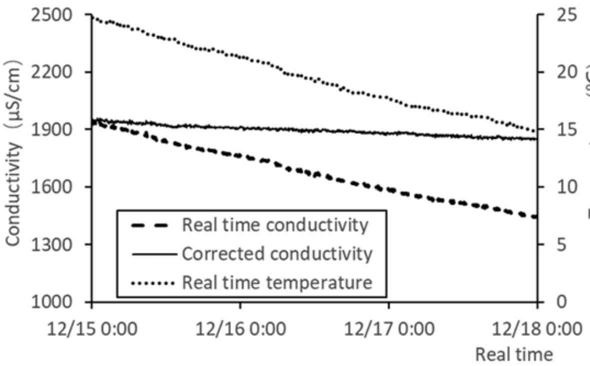

Figure 4. Effects of the conductivity-temperature compensation coefficient on the conductivity calibration of Huangjiu.

Table 2. Conductivity and compensation coefficient ranges of different types of Huangjiu

Type	Conductivity (μS/cm)	Compensation coefficient (μS/(cm·°C))
Dry type	1400–1900	40–55
Semi-dry type	1500–2000	40–60
Semi-sweet type	1200–1700	35–50
Sweet type	900–1400	30–45

qualities due to differences in raw materials, technologies, and bacterial species, thus resulting in different ranges of conductivity-compensation coefficient and conductivity. Hence, the conductivity-temperature compensation coefficient must be established for each batch of Huangjiu.

Application of the online monitoring system in the 60 t tank area

Conductivity detection technology was applied to the online monitoring system of the 60 t tanks of Huangjiu. Conductivity data were sensitive to the detection error of the sensor, the metabolic activities of microbial pollutants in Huangjiu, and environmental factors. Hence, the accuracy of the conductivity detection technology is assured when there is some sensitivity provided. During normal storage of Huangjiu, the conductivity data were normally distributed and the real-time conductivity was within 3-sigma. The proportion of numerical values within the mean value+3 times SD accounted for 99.7% of all numerical values (Poghossian *et al.*, 2019). The real-time conductivity of Huangjiu was positively offset in the presence of microbial contamination. When the conductivity exceeded the fluctuation range observed previously in normal storage (mean value+3 times SD), the state of balance of the Huangjiu was destroyed by microorganisms and microbial contamination occurred. Therefore, during the storage of Huangjiu, the system had a higher sensitivity when the SD of the conductivity was smaller, allowing more timely recognition of signals of abnormal growth in conductivity.

The normal distributions of conductivity (5 min/event, 25642 times) and daily conductivity (91 d) for the 60 t tanks of Huangjiu are shown in Figures 5A and 5B, respectively. The mean values of the results from the two calculation methods were consistent and the data were normally distributed within a narrow range. However, the SD of the daily conductivity decreased from 15 to 11 μS/cm. This demonstrates that monitoring based on daily conductivity can decrease the SD and improve the sensitivity of the method, which is conducive to online monitoring and generating alerts for storage in tanks.

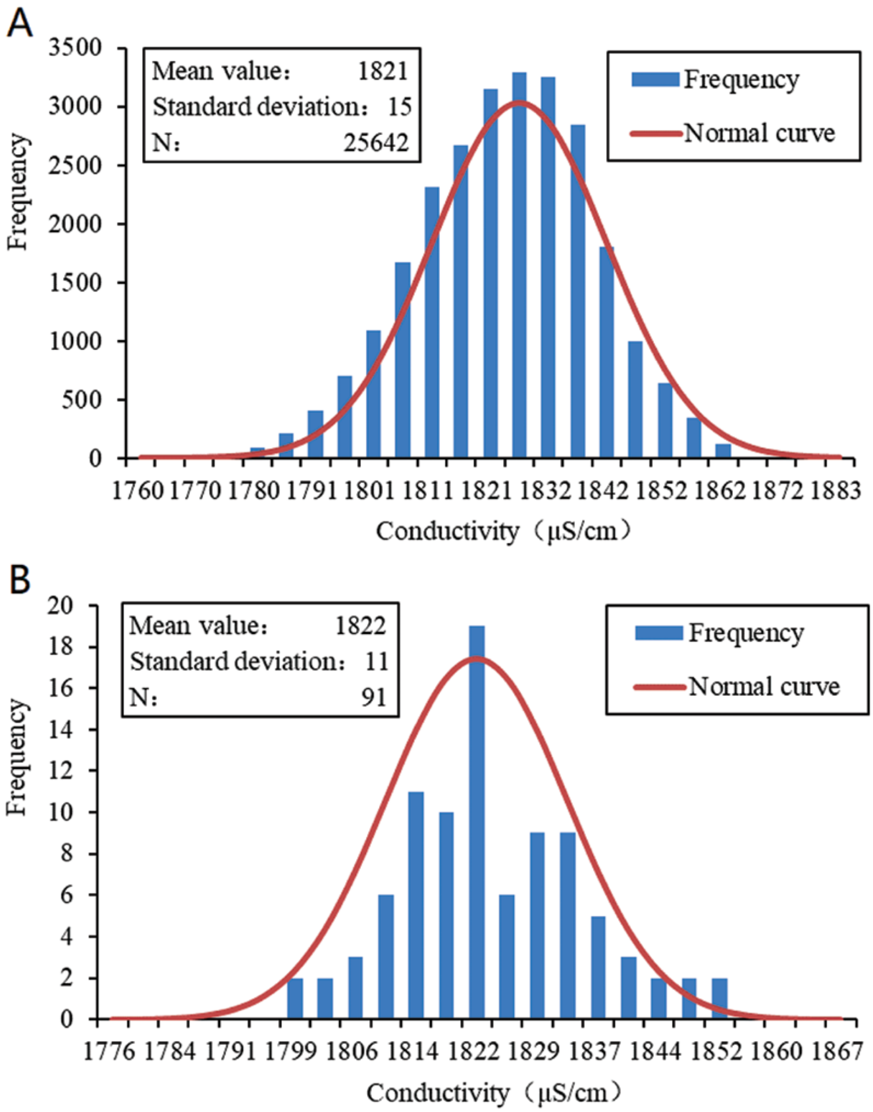

Figure 5. (A) Normal distribution of real-time temperature-corrected conductivity of Huangjiu. (B) Normal distribution of daily temperature-corrected conductivity of Huangjiu.

The changes in conductivity and total acid of Huangjiu in 60 t tanks are shown in Figure 6. The storage period could be divided into three stages according to the changes in temperature: the liquid cooling stage, the normal storage stage, and the abnormal storage stage.

The liquid cooling stage occurred from 15 December to 22 December, when the Huangjiu temperature was far higher than the ambient temperature. The daily conductivity of Huangjiu decreased by approximately 5% in this stage. The conductivity sensor is usually installed approximately 2–4 cm behind the stainless steel of the tank (the diameter of the tank is approximately 5 m) and is connected to the external base, sheath, and other metal pieces. As a result, the measured real-time temperature was close to the surface temperature of the tank and lower than the central temperature of the tank. The calculated conductivity after temperature compensation was higher than the real value of the liquid, thus resulting in data distortion. Therefore, data from the liquid cooling stage cannot be listed in the statistical range of the monitoring system data.

The normal stage occurred from 23 December to 24 March (3 months), during which the Huangjiu temperature was close to the ambient temperature. In this stage, the daily conductivity was 1822±11 µS/cm and the total acid was 4.24±0.04 g/L. Over nearly 3 weeks from 25 March to 17 April, daily conductivity increased and exceeded the upper limit (1855 µS/cm) for normal storage before 29 March (mean value of daily conductivity+3 times SD). At this point, it entered the abnormal storage stage. Samples were collected on 14 April and 17 April for detection, and the total acid values of Huangjiu were increased by 0.5 g/L and 1.1 g/L, respectively. The variation in conductivity and total acid content of Huangjiu in the 60 t tanks verified that the proposed conductivity detection technology was suitable for the online monitoring of abnormal changes such as rancidification.

Application of the online monitoring method in the 300 t tank area

The proposed online monitoring method was then applied to the 300 t tank area after its accuracy was verified in the 60 t tank area. Variations in the total acid and daily conductivity of four batches of Huangjiu were measured across two weeks from 16 September to 29 September, with results shown in Figures 7 and 8, respectively. In the 3-month normal storage stage (1 June to 31 August) of Huangjiu stored in tank #329, the average total acid content was 4.86±0.04 g/L and the daily conductivity was 1682±14 µS/cm. The fluctuation range of daily conductivity was higher for the 300 t tanks than for the 60 t tanks (11 µS/cm), which compromised the sensitivity of the system. The total acid of Huangjiu in tank #329 increased significantly on 28 September, while the daily conductivity was within the fluctuation range in the normal storage stage (<1724 µS/cm). The 300 t tanks were placed in an open area. The conductivity and temperature of Huangjiu tended to fluctuate suddenly due to environmental changes (sunshine, rain, and snow). Moreover, the 300 t tanks' volume was larger and the unevenness of the wine was more prominent, thus decreasing the sensitivity of the monitoring method. By comparing real-time conductivity data with historical data during the normal storage stage, it was found that the variation amplitude of abnormal signals was smaller than three times the SD when there were many interfering factors, and the system failed to recognise signals of abnormal change.

Therefore, in the case of storing Huangjiu in multiple tanks (*N*≥3), the conductivity data of multiple tanks are compared synchronously to reduce the interference of the environment on the online detection. The daily conductivity of Huangjiu on 16 September was used as the benchmark (set to zero). The relative deviation of daily conductivity in the 300 t tanks with the change in date was calculated (Figure 9). Combining this with the total acid data and conductivity curve of Huangjiu, the variation amplitudes of the relative deviation were basically consistent among the four batches from 16 September to 22 September. Meanwhile, the total acid was in the range of the normal storage period, without any abnormal changes. Data for Tank #329 began to deviate from the conductivity curves of the other tanks (#328, #330, and #331) on 23 September, showing abnormal changes. The positive deviation amplitude became more significant following 26 September. This revealed an acceleration of the abnormal changes in Huangjiu caused by microbial contamination. The SD was calculated by the deviation of daily conductivity values of four batches in the normal storage stage, and the maximum was 2 µS/cm. Thus, the sensitivity of the

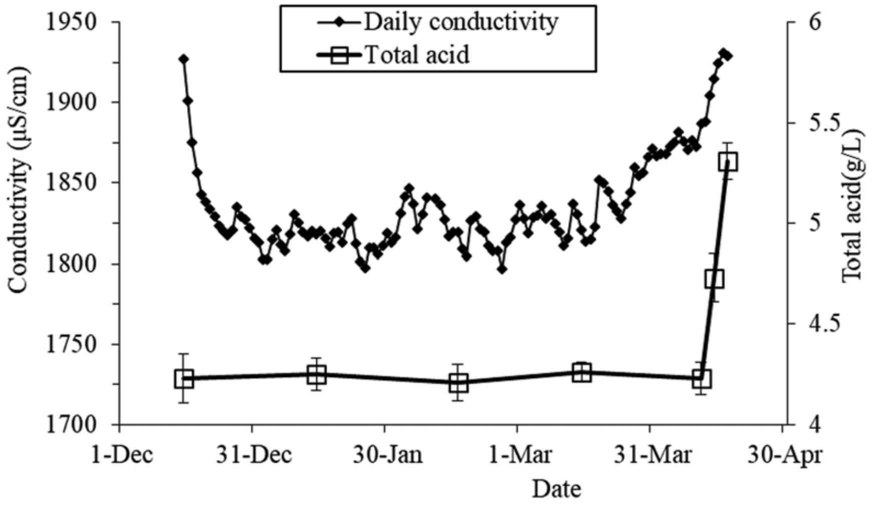

Figure 6. Variations in daily temperature-corrected conductivity and total acid of Huangjiu in 60 t tank during storage.

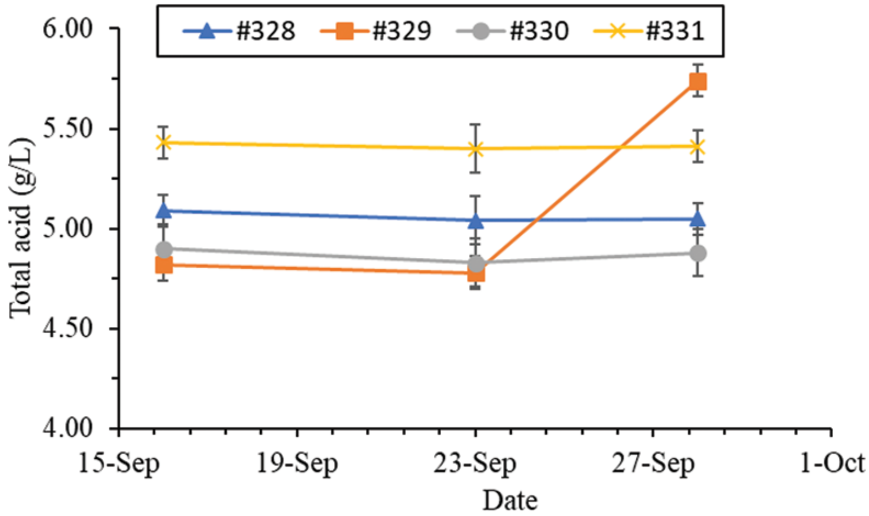

Figure 7. Variations in the total acid of Huangjiu in 300 t tanks.

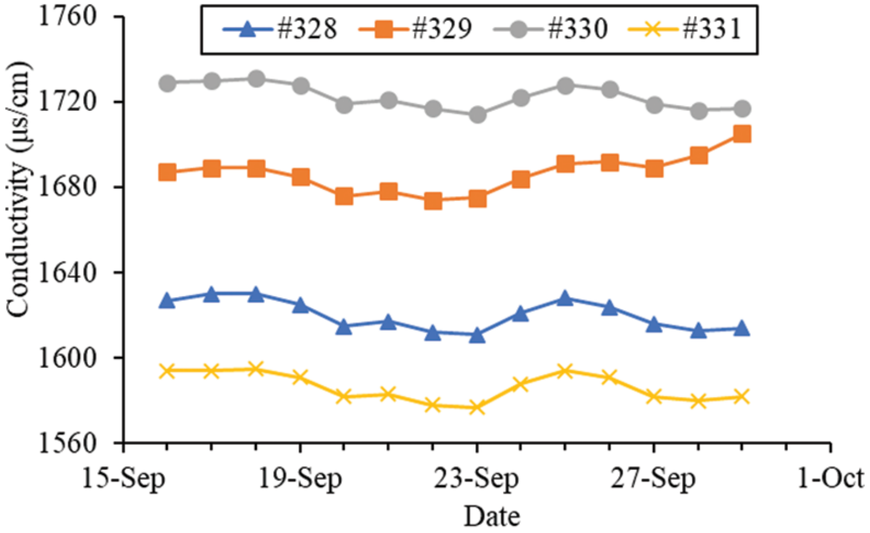

Figure 8. Variations in the daily temperature-corrected conductivity of Huangjiu in 300 t tanks.

method was improved significantly. On 27 September, the positive deviation of daily conductivity of Tank #329 from other batches reached three times the SD (6 μS/cm). On 28 September, the total acid content in Huangjiu was analysed through sampling and was nearly 1 g/L higher than that 5 d before. It was therefore confirmed that microbial contamination occurred in wine storage tank #329, which verified the accuracy of the system in recognising abnormal storage signals.

Conclusions

Huangjiu is usually stored in pottery jars. With the greater mechanical development and automation of the Huangjiu industry, an increasing number of enterprises have begun to use large tanks instead of pottery jars to store Huangjiu. Both storage methods have risks of rancidification caused by microbial contamination. By studying the conductivity and total acid in Huangjiu in jars, it was found that the conductivity of Huangjiu increased upon rancidification caused by microbial pollution during storage. Therefore, an online monitoring system for Huangjiu in large stainless steel tanks was designed based on the principle of rancidification-induced changes in conductivity. Two calculation methods for the conductivity-temperature compensation coefficient were established according to two daily conditions of sampling and non-sampling, to achieve temperature correction of conductivity. After real-time conductivity and real-time temperature were transformed to conductivity at 25 °C, they were further transformed into daily average conductivity. The fluctuation range (SD) of the system decreased from 143 to 11 μS/cm. Finally, interference by environmental factors on conductivity and temperature detection was minimised through the synchronous comparison of data change of multiple tanks. This decreased the SD of the online monitoring system to 2 μS/cm. In the study process, the monitoring system was applied to 60 t and 300 t tank areas, respectively. The method was verified as valid and accurate according to changes in the conductivity and total acid content of Huangjiu.

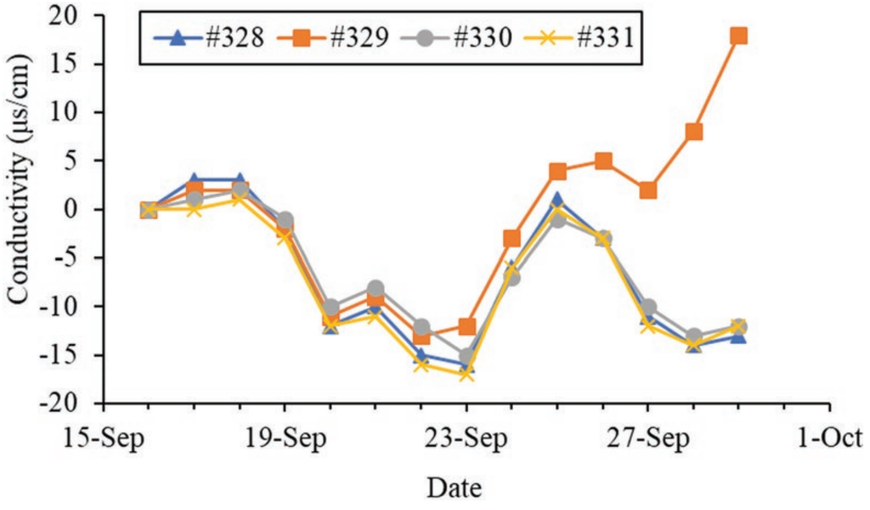

Figure 9. Variations in the relative deviation of daily temperature-corrected conductivity in 300 t tanks.

Author Contributions

Jian Hu: investigation, software, methodology, writing original draft. Shuangping Liu: investigation, methodology. Caixia Liu: software, methodology. Xiao Han: software, methodology. Mujia Nan: resources. Jian Mao: conceptualization, methodology, review and editing, supervision. All authors reviewed the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability

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

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