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δ^{13}C-Ethanol as a Potential Exclusionary Criterium for the Authentication of Scotch Whiskies in Taiwan: Normal vs. 3-Parameter Lognormal Distributions of δ^{13}C-Ethanol Found in Single Malt and Blended Scotch Whiskies

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Abstract: With the difference in the photosynthesis process between C3- and C4-plants, the $^{13}C/^{12}C$ stable isotope ratio of ethanol, i.e., δ^{13}C-ethanol, can potentially be a basis for the discrimination of Scotch whiskies derived from different raw materials. This study analyzed 51 authentic single malt Scotch whiskies and 34 authentic blended Scotch whiskies by gas chromatography–combustion–isotope ratio mass spectrometry (GC-C-IRMS) and examined the resulting data by a series of fitting distribution processes. The evaluation result demonstrated that δ^{13}C-ethanol distribution of single malt Scotch whiskies fitted both normal and 3-parameter lognormal distribution. For blended Scotch whiskies, however, the data distribution of δ^{13}C-ethanol conformed with a 3-parameter lognormal distribution rather than a normal one. Moreover, 99.7% of the confidence intervals (CI) of δ^{13}C-ethanol for single malt Scotch whiskies would define between −23.21‰ to −30.07‰ for 3-parameter lognormal distribution, while from −11.19‰ to −28.93‰ for blended Scotch whiskies on the basis of the statistical properties. The simulative adulterated Scotch whiskies using more than 30% C4-derived edible distilled spirits can be effectively discriminated by means of CI of δ^{13}C-ethanol. Since the addition of rectified spirits produced from the C4 plant has been found in some cases of seized Scotch whiskies in Taiwan, establishing a CI of δ^{13}C-ethanol would be valuable for the purpose of Scotch whisky authentication.

Keywords: authenticity of Scotch whiskies; adulterated Scotch whisky; rectified spirits; δ^{13}C-ethanol; gas chromatography–combustion–isotope ratio mass spectrometry; GC-C-IRMS; 3-parameter lognormal distribution; fake whiskies

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1. Introduction

Over the past decades, Scotch whisky has become the most popular imported spirit in the alcohol market in Taiwan. According to the Scotch Whiskey Association report, Taiwan ranked third among the top 10 export markets for Scotch whisky by value in 2021 [1]. Meanwhile, the import market value of Taiwan is as high as 226.1 million GBP. The Taiwanese consumption habit includes two categories of Scotch whiskies, i.e., single malt Scotch whiskies and blended Scotch whiskies, which occupy nearly 92% of the whisky market in Taiwan. Unlike the consumption habits of other countries, the Taiwanese are particularly fond of single malt Scotch whisky. According to the marketing reports, the sales market for single malt Scotch whisky is almost as close as blended Scotch whisky in Taiwan [1,2]. Based on the great business profits, illicit single malt Scotch whiskies and illicit blended Scotch whiskies have been seized in Taiwan in some cases.

So far as the modus operandi is concerned, the fake whiskies in Taiwan can be classified into two types: adulterated Scotch whisky and counterfeit Scotch whisky. An adulterated

Scotch whisky commonly uses a bottle of a well-known brand of Scotch whisky blended with a small amount of genuine Scotch whisky and a large amount of rectified spirit. In contrast, counterfeit Scotch whisky does not even have an actual distillery set up in Scotland. Instead, counterfeiters create misleading Scotch whiskies using an obscuring Scotch whisky name by blending a large part of rectified spirit with some artificial food flavoring and/or a small amount of genuine Scotch whisky. To reduce manufacturing costs, large amounts of rectified spirit were found to be used in all the seized fake whiskies in Taiwan. According to case reports provided by the competent authorities in Taiwan, illegal whiskies are often sold in nightclubs, bars, KTVs, and other places where consumers are not easily aware of the authenticity of Scotch whisky. Sometimes, lawbreakers recycle empty bottles of high-priced Scotch whisky from these places, refill empty bottles with adulterated Scotch whisky, and sell them again. Therefore, the consumer and relative competent authorities eagerly require a more valuable and efficient method to support authenticity analyses.

The composition of Scotch whisky involves several aspects, including the ingredients used in production, the finished product's chemical composition, and the organoleptic characteristics of Scotch whisky. For the authentication purpose of whisky with qualitative techniques in earlier studies, gas chromatography with flame-ionization detection (GC-FID), gas chromatography–mass spectrometry (GC-MS), and liquid chromatography–mass spectrometry (LC-MS) have usually been employed to analyze the composition of whiskies [3–8]. Moreover, a number of spectroscopic methods combined with principal component analysis (PCA) or partial least squares (PLS) regression have been developed for rapid and reliable discrimination, including Fourier-transform infrared spectroscopy (FT-IR), near-infrared spectroscopy (NIR), mid-infrared spectroscopy, UV-Vis analysis, and Raman spectroscopy [9–14]. These investigations aimed to differentiate from the brand, age, or spectrum characteristics of whisky samples. Apart from qualitative research, mass spectrometry was also used based on the quantitative analysis of the contents of whiskies. Herein, GC-MS and LC-MS are regarded as powerful techniques to establish authenticity. Attributed to the high sensitivity of mass spectrometry, the concentration of compounds in whiskies can be determined and serve as the comparative identification targets for the authentication of whiskies [15–19].

In recent years, the isotope ratio mass spectrometer (IRMS) has been adopted to detect fake Scotch whiskies. The stable isotope analytical method is a highly efficient approach, normally used by archaeologists and biologists, to analyze and justify the origin or constituent information of an animal's bones or identify a plant's photosynthesis process. With its powerful analytical effectiveness, several studies have investigated the significant difference of stable isotopes to determine the origin of whiskies [20–24]. From the isotope analysis point of view, many factors affect the measured isotope ratio, such as the origins of raw materials, the species of raw materials, the reaction pathway, the procedure of treatment for samples, and the analysis process. Subtle fractionation involves both thermodynamics and kinetics effects. So far as thermodynamics is concerned, slightly different stable isotope ratios may give different equilibrium constants for a specific chemical reaction; that is, slightly different amounts of reaction products may result from reactants with slightly different isotope ratios. As to kinetics, the lighter isotopes will proceed faster through the photosynthetic process, resulting in products richer in the lighter isotopes. Therefore, these intrinsic properties are displayed in the variety of mass discrimination associated with various pathways of reactions [6]. Typically, the photosynthesis of plants can be sorted as C3, C4, and Crassulacean acid metabolism (CAM) photosynthesis. These different photosynthesis processes of plants lead to changes in observed stable isotope ratios [25–28]. Edible rectified spirit, also known as a neutral spirit, is a kind of spirit from a raw material such as grain, potato, beet, molasses, honey, or fruit. Because of the manufacturing process of the edible rectified spirit from the continuous distillation method, the alcohol content is usually greater than 95%, and it contains almost no other ingredients except water. The raw materials of edible rectified spirits vary on the basis of the customs and trade condition between different countries. In Taiwan, sugar cane is the primary raw material of edible rectified spirit. Sugarcane was once a heavily grown crop in Taiwan, especially in southern Taiwan. According to the photosynthesis of plants, sugarcane belongs to C4 plants; therefore, edible rectified spirits sold in Taiwan are mainly C4-derived spirits.

According to the definition given in Scotch Whisky Regulation 2009 [29], blended Scotch whisky can be made by blending various Scotch whiskies, including single malt Scotch whiskies and grain Scotch whiskies. Hence, it may be manufactured completely by C3-derived spirits or different proportions of C3- to C4-derived spirits, while single malt Scotch whiskies are only made from C3-derived spirits [30]. In view of the relationship between the photosynthesis processes of plants and stable isotope ratios, measurement of δ^{13}C-ethanol has been used to differentiate between the raw materials of spirits from C3 plants with δ^{13}C (‰) values of −21‰ to −34‰ and C4 plants with δ^{13}C values of −9‰ to −20‰, sequentially [20–25]. δ^{13}C is the ratio of two stable isotopes of carbon, ^{13}C and ^{12}C, used to represent the isotopic composition of carbon sources and fractionation during photosynthesis. For authentication purposes, however, the wide range of δ^{13}C for C3 and C4 plants may limit the practical forensic application if the seized Scotch whiskies were adulterated by adding rectified spirits in authentic Scotch whiskies. On the other hand, single malt Scotch whisky is only made from malted barley, which belongs to the C3 plant, according to the restriction from Scotch Whisky Regulation 2009 [29]. Although many researchers have studied the stable isotope ratios for C3 plants, the exact range of stable isotope values for δ^{13}C-ethanol produced through malted barley fermentation reaction is rarely discussed. Therefore, in practice, it sometimes affects the uncertainty of the authenticity of Scotch whisky, thus increasing the difficulty of discrimination.

In our previous work, we developed an exclusion method to successfully distinguish adulterated samples from authentic Scotch whiskies via the confidence interval (CI) of methanol (MeOH) concentration without any other reference samples of authentic whisky [19]. For discrimination purposes, many studies discuss various analytical methods for Scotch whiskies authentication, but only some evaluate the statistical implications for forensic meaning. In particular, statistical treatments, such as PCA and PLS, are mainly used to deal with data for the classified purpose. Much research has primarily focused on discussing characteristics of origins, including brand, geography, or botanical origin [31–35]. However, in modern statistical practice, a proper data distribution model could describe the data population of authentic Scotch whiskies to facilitate the discrimination procedures and consequently avoid bias due to analyzing a few scattered samples [19]. A model fitting method is a statistical tool usually used to find the most efficient and accurate statistical structure to describe the observed data distribution [36]. Otherwise, statistical parameters, such as means and standard deviation, should be explained under suitable distribution. Therefore, the distribution fitting method could summarize the data distribution by a proper statistical model, further predicting other data under the same observation, and avoiding statistical mistakes [36,37]. Especially in practice, collecting all reference samples to compare with each seized sample is difficult and challenging. Hence, it is helpful to establish reasonable and efficient discrimination methods for forensic purposes. Moreover, the case reports from competent authorities show that the illegal Scotch whisky seized in Taiwan are mainly adulterated Scotch whiskies, whose modus operandi is mainly through diluting the authentic Scotch whisky with edible rectified spirit. After dilution with edible rectified spirit, the stable isotope ratio of δ^{13}C-ethanol, MeOH concentration [19], and the intensity of fermentation congeners of Scotch whisky should all be affected. Therefore, it is crucial to define the exclusionary discriminating criterium established from real Scotch whisky samples and then compare it with the simulative adulterated Scotch whiskies diluting with different ratios of edible rectified spirit.

In this study, we attempted to fit and define the distribution range of δ^{13}C for ethanol in Scotch whiskies, including single malt Scotch whisky and blended Scotch whisky, in order to establish the CI of δ^{13}C-ethanol as a criterion for authentication purposes. In addition, this study expects to determine the stable isotope value of δ^{13}C-ethanol in Scotch whiskey through the analysis of a large number of reference samples and to define the stable isotope value range of δ^{13}C-ethanol produced by malted barley fermentation, thereby improving the reliability of discriminating power in practice. In addition, with a known modus operandi of illicit Scotch whiskies, a series of simulative experiments with adding rectified spirits to authentic Scotch whiskies may evaluate the parts of clues from seized Scotch whisky cases and further verify the importance of establishing a CI of δ^{13}C-ethanol for authentication of Scotch whiskies.

2. Materials and Methods

2.1. Preparation of Reference Whiskies and Simulative Samples

The reference materials prepared to establish the discrimination criteria of Scotch whisky were all imported from Scotland and purchased from legally operated tobacco and alcohol shops in Taiwan, including 51 single malt Scotch whiskies and 34 blended Scotch whiskies. Moreover, three edible rectified spirits from Taiwan Sugar Corp., Taiwan Tobacco & Liquor Corp., and Echo Chemical Co., Ltd. were also prepared to determine the δ^{13}C-ethanol value for reference.

The addition samples were prepared by adding a proper amount of diluted rectified spirit to a proper amount of authentic Scotch whiskies, where the diluted rectified spirit was prepared by diluting edible rectified spirit (purity of ethanol $\geq$ 96%, Taiwan Sugar Corp.) with distilled water to 40% alcohol by volume (ABV). As a result, serial addition solutions of authentic Scotch whisky and diluted rectified spirit were prepared with the addition ratio of 9:1, 8:2, 7:3, 6:4, and 5:5, respectively.

2.2. Stable Isotopic Measurement of Ethansol

The δ^{13}C measurement was performed by a gas chromatography–combustion–isotope ratio mass spectrometry (GC-C-IRMS) system. A GC 7890A with an Agilent J&W CP-Wax 57 CB fused capillary column (50 m $\times$ 0.25 mmID $\times$ 0.2 μm) were coupled with an IsoPrime IRMS (Elementar, Germany) via an open split. Before the δ^{13}C measurement of each sample batch, the GC-C-IRMS must conform to the criteria of stability of δ^{13}C $\leq$ 0.08‰ (Std. Dev. of fit) and linearity of δ^{13}C $\leq$ 0.03‰/nA. The CO_2 reference gas was calibrated against the acetanilide standard reference, which is certified by the Department of Geography, Indiana University (USA) with a δ^{13}C value of $-$29.53‰ ($\pm$0.01‰). A 1 mg acetanilide standard reference was dissolved in 1 mL of acetone (HPLC Grade, Burdick & Jackson). Samples of 0.2 μL each were injected with a split ratio of 1:20. The flow rate for carrier gas was set as 1.0 mL/min. Due to the high boiling point of acetanilide (b.p. = 304 °C), the injection port of temperature for the GC-FID system were at 325 °C. The oven temperature was set as follows: initially 100 °C, elevated to 200 °C at 20 °C min^{-1}, and then maintained for 35 min.

Samples of 12 μL each were diluted with 1.5 mL of acetone (HPLC Grade, Sigma Aldrich). Samples of 0.2 μL each were injected with a split ratio of 1:15. The oven temperature of GC-FID instrumental parameters were set as follows: initially 35 °C for 2 min, elevated to 60 °C at 3 °C min^{-1}, and to 200 °C at 30 °C min^{-1}, and then maintained for 10 min.

2.3. Statistical Analysis

The δ^{13}C-ethanol data analysis was executed by the statistical software SPSS (IPM), Minitab, and SPC (BPI Consulting, LLC, Katy, TX, United States). In subsequent, to appropriately deduce the distribution model, the data distribution fitting was exanimated by the software SPC. Furthermore, the Anderson–Darling and Kolmogorov–Smirnov tests were employed to verify the normality of the log-transformed δ^{13}C value of Scotch whiskies with a *p*-value of >0.05 to double confirm the fitting result.

3. Results and Discussion

3.1. Measurements and Statistical Analysis of δ^{13}C-ethanol Data in Scotch Whiskies

A typical chromatogram of GC-C-IRMS is displayed in Figure 1, where the ethanol peak appears at around 590 s. To avoid interference and clear the relevant data, all those signals from other constituents except ethanol were led to the FID detector. Three injections of all samples were performed for each measurement to evaluate the precision of the analytic method. All the relative standard deviations (RSD) of measurements were within 2%.

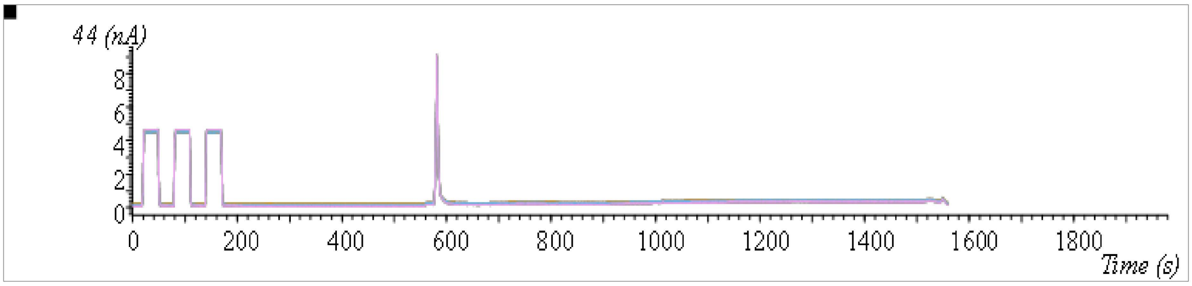

Figure 1. A GC-C-IRMS chromatogram with an ethanol peak at around 590 s. The colors in the figure present the isotopologues of CO_2, which are m/z 44,45,46.

A histogram plot of the δ^{13}C-ethanol data for 51 authentic single-malt Scotch whiskies is shown in Figure 2a and is slightly positively skewed. By the statistical definition of skewness, this positively skewed histogram may imply that the distribution of δ^{13}C-ethanol data fits another statistical model than a normal distribution. After checking by the software SPC, a fitting distribution demonstrated that the δ^{13}C-ethanol data of single malt Scotch whiskies not only conformed to the normal distribution but also to the 3-parameter lognormal distribution.

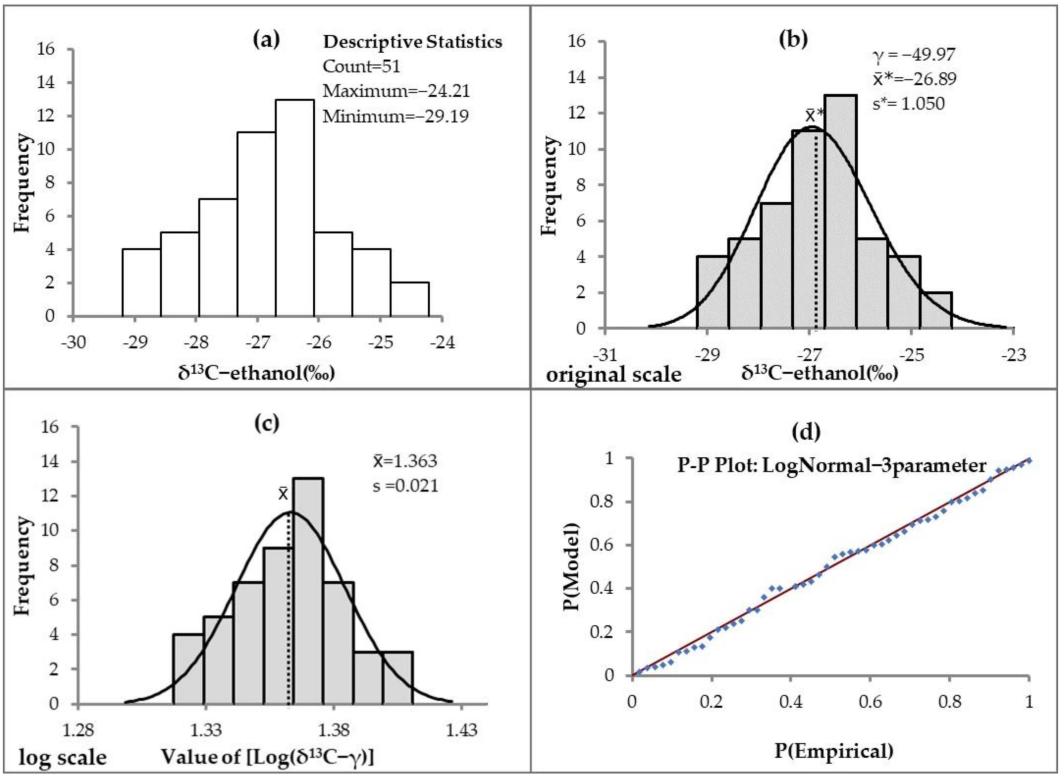

Figure 2. Statistical evaluating results of authentic single malt Scotch whiskies. (**a**) The histogram of δ^{13}C−ethanol. (**b**) The distribution of observed δ^{13}C−ethanol. (**c**) The distribution of transformed δ^{13}C−ethanol. (**d**) The P-P plot of δ^{13}C−ethanol.

Furthermore, a normality test was performed in terms of Anderson-Darling and Kolmogorov–Smirnov methods [38,39] by logarithm data transformed from the raw value of δ^{13}C-ethanol to certify the distribution attribute of the δ^{13}C-ethanol data of authentic single malt Scotch whiskies. On the basis of the characteristics of the 3-parameter lognormal distribution, the distribution shape and range can be described by three factors, the threshold(γ), the means ($\overline{x}$), and the standard deviation(s). In this study, the threshold (γ) of 3-parameter lognormal distribution was first calculated by both the SPC and Minitab software programs via the maximum likelihood estimation method and obtained the value of −49.97‰ for the δ^{13}C-ethanol data for authentic single malt Scotch whiskies. Subsequently, all data of δ^{13}C-ethanol as variable x were converted to log(x-γ) and tested for their normality on a log scale, as shown in Figure 2c. With their logarithmic data, both normality test results in Table 1 demonstrate the normality. As a result, the 3-parameter lognormal distribution corresponds with the distribution characters of δ^{13}C-ethanol in authentic single-malt Scotch whiskies.

Table 1. Normality test results of log-transformed δ^{13}C-ethanol data of authentic Scotch whiskies.

	Anderson–Darling Method			**Kolmogorov–Smirnov Method**		
	Statistic	**df**	**Sig.**	**Statistic**	**df**	**Sig.**
Single malt Scotch whisky	0.152	51	0.958	0.479	51	0.976
Blended Scotch whisky	0.526	34	0.167	0.762	34	0.607

According to the statistical characteristics, the δ^{13}C-ethanol distribution could be log-transformed to a normal one with a geometric mean $\overline{x}$; and a multiplicative standard deviation s, due to that a lognormal distribution is illustrative for the multiplicative central limit theorem [36]. Therefore, for authentic single malt Scotch whiskies, a distribution fitting procedure was performed on the δ^{13}C-ethanol observations in Figure 2a and redrawn as Figure 2b, fitting a 3-parameter lognormal distribution with $\gamma = -49.97$, $\overline{x}^* = -26.89$ and $s^* = 1.050$ on the original scale, where $\overline{x}^*$ and s* is back-transformed from $\overline{x}$ and s* on the log scale. The data descriptions of δ^{13}C-ethanol data for authentic Scotch whiskies are summarized in Table 2, including the min and max of the observed values. Moreover, after refitting distribution, the transformed data of δ^{13}C-ethanol data in single malt Scotch whiskies exhibited a normal distribution on the log scale, as shown in Figure 2c. Hence, it is concluded that the stable isotope analysis results of 51 authentic single malt Scotch whisky samples are representative, demonstrating that the δ^{13}C-ethanol data of authentic single malt Scotch whiskies not only conform to a normal distribution but also to a 3-parameter lognormal distribution. Moreover, a probability–probability plot (P-P plot) is used to evaluate the distribution's consistency and skewness via the visual comparison between the theoretical line and the actual spots out of the cumulative distribution functions. Figure 2d shows the P-P plot of the δ^{13}C-ethanol data of authentic Scotch whiskies compared to the theoretical line of a 3-parameter lognormal distribution. The straight diagonal line in the figure is essentially confirming that the δ^{13}C-ethanol data distribution in authentic single malt Scotch whiskies corresponds well to the 3-parameter lognormal distribution.

Table 2. Data descriptions of δ^{13}C-ethanol data for authentic Scotch whiskies.

	No. of Samples	Min	Max	Threshold	Mean	Standard Deviation *	Standard Deviation **
Single malt Scotch whisky	51	−29.19‰	−24.21‰	−49.97 ‰	−26.89‰	1.050	0.021
Blended Scotch whisky	34	−28.04‰	−17.14‰	−30.35 ‰	−25.13‰	1.542	0.188

* On the original scale; ** On the log scale.

On the other hand, statistical analysis of the δ^{13}C-ethanol data was also performed on authentic blended Scotch whisky, undergoing the same evaluation process as aforementioned. As a result, in Figure 3a, the histogram of the δ^{13}C-ethanol data from authentic blended Scotch whiskies presents an apparent skewed pattern with the right tail, compared to Figure 2a. Moreover, after performing the data fitting process, it is found that the δ^{13}C-ethanol distribution of the authentic blended Scotch whiskies could be described as a 3-parameter lognormal one, as shown in Figure 3b,c. After logarithmic transformation, as can be observed in Figure 3c, the data of δ^{13}C-ethanol of authentic blended Scotch whiskies complies with a normal distribution. Meanwhile, the normality test of the transformed data, shown in Table 1, also certifies the same result.

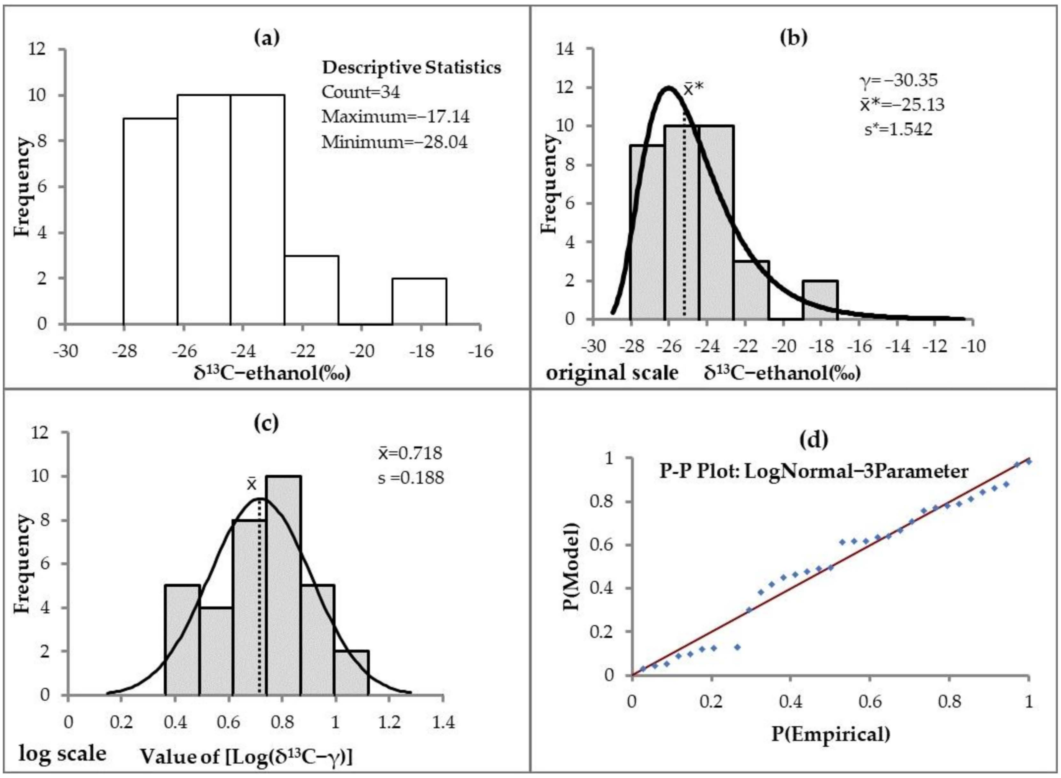

Figure 3. Statistical evaluating results of authentic blended Scotch whisky. (**a**) The histogram of δ^{13}C-ethanol. (**b**) The distribution of observed δ^{13}C−ethanol. (**c**) The distribution of transformed δ^{13}C−ethanol. (**d**) The P-P plots of δ^{13}C−ethanol.

Similarly, to obtain the threshold γ, the means $\overline{x}^*$, and the standard deviation s* of the δ^{13}C-ethanol data, the mentioned evaluation, and back-transformed procedure was also carried out for the authentic blended Scotch whisky samples, resulting in the values in Table 2. With all investigated results, it is certified that the distribution of δ^{13}C-ethanol data for authentic blended Scotch whiskies corresponds to a 3-parameter lognormal distribution.

Likewise, Figure 3d demonstrates an excellent fit to the distribution of δ^{13}C-ethanol data in authentic blended Scotch whiskies.

3.2. Confidence Interval of δ^{13}C-Ethanol Data and Its Implication for Scotch Whiskies

Ideally, many experiments assume that the observed data could be described as the normal distribution, which is usually used to study the random variation of data in many investigations. However, several researchers have reported that their experimental data could not be entirely consistent with normal distribution but with the lognormal distribution [36,40–43]. Therefore, statistical evaluation should be processed under accurate distribution and result in reasonable interpretation. In the whisky production process, various factors such as raw material characteristics, microbial mechanism, and environmental conditions may lead to a multiplicative effect, resulting in a non-normal distribution of the observed δ^{13}C-ethanol data. Ethanol is the major product of yeast fermentation, which may react with other ingredients in various ways. From the viewpoint of such variations, it is reasonable that the distribution of δ^{13}C-ethanol data for single malt Scotch whisky and blended Scotch whisky follows a 3-parameter lognormal distribution.

With characteristics of the empirical rule, calculating the confidence intervals for δ^{13}C-ethanol in Scotch whiskies can also be performed by their logarithm values. For the 3-parameter lognormal distribution, the confidence intervals (CI) on the log scale can be defined with the probability of 68.3% as ($\bar{x} \pm s$), of 95.5% as ($\bar{x} \pm 2s$), and of 99.7% as ($\bar{x} \pm 3s$) after transforming the raw observed data to their logarithmic value. Once turned into the original scale, the corresponding CI can be performed with a probability of 68.3% as [$\gamma + (\bar{x}^* - \gamma) \times/s^*$], of 95.5% as [$\gamma + (\bar{x}^* - \gamma) \times/(s^*)^2$], and of 99.7% as [$\gamma + (\bar{x}^* - \gamma) \times/(s^*)^3$]. Table 3 shows the back-transformed data of δ^{13}C-ethanol in Scotch whiskies. For a 95.5% confidence level, the boundaries of the δ^{13}C-ethanol data are −24.50‰ to −29.06‰ for single malt Scotch whiskies, and −17.93‰ to −28.15‰ for the blended Scotch whiskies, respectively.

Table 3. Confidence intervals of δ^{13}C-ethanol data for authentic Scotch whiskies.

	68.3%		**95.5%**		**99.7%**	
	Upper Bound	**Lower Bound**	**Upper Bound**	**Lower Bound**	**Upper Bound**	**Lower Bound**
	Unit: (‰)					
Single malt Scotch whisky	−25.73	−28.01	−24.50	−29.06	−23.21	−30.07
Blended Scotch whisky	−22.29	−26.96	−17.93	−28.15	−11.19	−28.93

3.3. Exclusionary Criterium for Scotch Whiskies Authentication on the Basis of the Confidence Interval of δ^{13}C-Ethanol Data

To make adulterated or counterfeit Scotch whiskies, three commonly-used edible rectified spirits by forgers, which comply with regulations of "The Tobacco and Alcohol Administration Act" in Taiwan [44], were selected to examine the δ^{13}C-ethanol for reference. Two samples belonged to C4-derived spirits, and one was a C3-derived spirit, as shown in Table 4. Since the single malt Scotch whisky belongs to C3-derived spirits, C3-derived edible rectified spirit is not suitable by using the exclusory criterium for seized Scotch whiskies by stable isotope analysis in this study. On the basis of the types of rectified spirits found in some seized cases of Scotch whiskies in Taiwan, the C4-derived rectified spirit was selected to prepare the simulative Scotch whiskies in this study. The simulative results are shown in Figure 4.

Table 4. δ^{13}C-ethanol data of commonly-used edible rectified spirit in Taiwan.

Supplier	Taiwan Sugar Corp.	Taiwan Tobacco & Liquor Corp.	Echo Chemical Co., Ltd.
δ^{13}C-ethanol	−13.50 (‰)	−14.63 (‰)	−24.76 (‰)
Type	C4-derived spirit	C4-derived spirit	C3-derived spirit

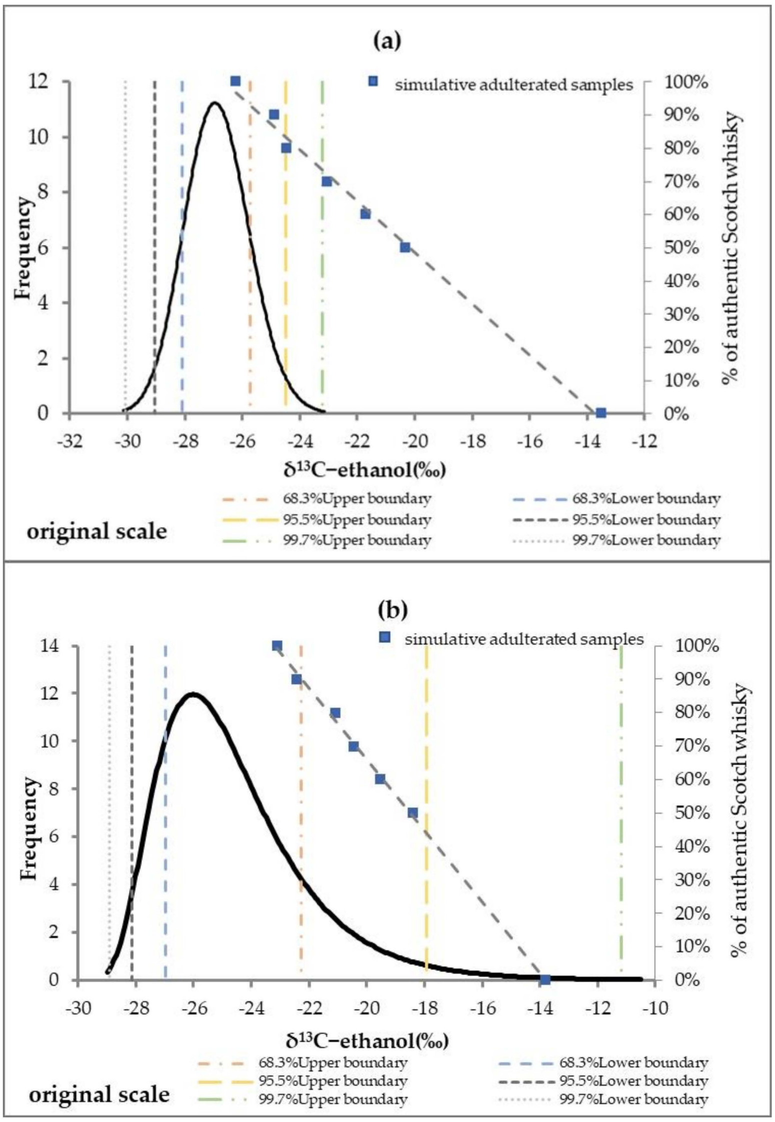

Figure 4. The relation between the percentage of authentic Scotch whisky and the observed δ^{13}C-ethanol after adding various amounts of rectified spirit with a δ^{13}C−ethanol of −13.50‰ (avg.) to (**a**) an authentic single malt Scotch whisky with a δ^{13}C−ethanol of −26.21‰ (avg.) and (**b**) an authentic blended Scotch whisky with a δ^{13}C−ethanol of −23.12‰ (avg.).

In Figure 4a, a series of the simulative adulterated single malt Scotch whiskies were prepared by adding 40% ABV rectified spirit with a δ^{13}C-ethanol of −13.50 to an authentic single malt Scotch whisky with a δ^{13}C-ethanol of −26.21 with various ratios. The selection of the rectified spirit for use in these spikes was due to its easy acquirement and extensive use in Taiwan. In this case, the δ^{13}C-ethanol data of authentic single malt Scotch whiskies would become more positive when a more diluted rectified spirit of 40% ABV was added. Once the adding volume ratio of diluted rectified spirit becomes more than 30% of the total solution, i.e., the volume ratio of authentic single malt Scotch whisky less than 70% of the total solution, the δ^{13}C-ethanol for simulated adulterated single malt Scotch whisky would be above the 99.7% CI boundary. Therefore, it turns out to be fake. Based on the reports from seized cases in Taiwan, most forgers adulterated or counterfeit Scotch whiskies with more than 30% dilution in order to gain high profits. Consequently, the simulation experiment of adding C4-derived spirits to authentic single-malt Scotch whisky proves the importance of establishing δ^{13}C-ethanol CI and provides a reference for seized Scotch whisky cases in Taiwan.

Similarly, Figure 4b is the relation between the observed δ^{13}C-ethanol data of simulative adulterated blended Scotch whiskies and their corresponding adding volume ratio of an authentic blended Scotch whisky and diluted rectified spirit. As the δ^{13}C-ethanol of the rectified spirit is −13.50, which is still within the domains of 95.5% and 99.7% CIs of authentic blended Scotch whiskies, the δ^{13}C-ethanol of simulative adulterated blended Scotch whiskies explains less exclusive power for authentication. In other words, the elimination of authentic single malt Scotch whiskies is more evidential than authentic blended Scotch whiskies in this study. Taking into consideration the CIs and the experimental results from simulative adulterated samples, these data could provide significant clues for the authentication of Scotch whiskies. As a result, the CI established in this study could be considered as a role as an eliminative tool for the discrimination of seized Scotch whiskies.

For the δ^{13}C-ethanol investigation, some research for wine authentication was also studied with a statistical approach. In addition, Spitzke and Fauhl-Hassek established confidence intervals for regression lines of ethanol and other components in wine [45]. Based on their results, the correlation of δ^{13}C values between ethanol and other components may provide more evidence for authentication after the first exclusory evaluation.

4. Conclusions

In this study, we first demonstrate that a suitable distribution model, a 3-parameter lognormal distribution, can describe the δ^{13}C-ethanol values measured by GC-C-IRMS through a series of rigorous statistical interpretations. In addition, we further evaluated and narrowed the distribution of δ^{13}C-ethanol in single malt Scotch whisky and blended Scotch whisky by the 3-parameter lognormal distribution principle. Moreover, we evaluated the exact range of stable isotope values for δ^{13}C-ethanol produced by malted barley fermentation reaction as between −23.21‰ to −30.07‰ on the basis of δ^{13}C-ethanol data in single malt Scotch whisky through a series of reliable statistical processes. With these CI boundaries, the simulative adulterated Scotch whiskies could also be exclusively screened while the seized Scotch whisky containing more than 30% C4-derived edible distilled spirits without comparing specific authentic reference samples. This result has been proven to effectively discriminate most adulterated and counterfeit Scotch whiskies in Taiwan. Therefore, based on the above results, the importance of establishing the CI with δ^{13}C-ethanol is demonstrated to provide an effective method for primary discriminating seized Scotch whiskies.

It should be emphasized that the CI boundaries established in this work for exclusive authentication are based on the δ^{13}C-ethanol value, which is highly associated with the characteristics of raw materials used in the process of Scotch whiskies. For those adulterated Scotch whiskies whose δ^{13}C-ethanol values fell into the CI boundaries, those results can not be directly recognized as authentic Scotch whiskies. To obtain other evidence, some of the analytical methods, such as pH measurement, the gas chromatography–mass spectrometry analysis of methanol or other Scotch whisky congeners, or other spectra measurements, should be considered to establish more authentication criteria.

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