TẠP CHÍ KHOA HỌC
TRƯỜNG ĐẠI HỌC SƯ PHẠM TP HỒ CHÍ MINH
Tập 22, Số 9 (2025): 1554-1563
Website: https://journal.hcmue.edu.vn
ISSN: 2734-9918

HO CHI MINH CITY UNIVERSITY OF EDUCATION
JOURNAL OF SCIENCE
Vol. 22, No. 9 (2025): 1554-1563
https://doi.org/10.54607/hcmue.js.22.9.4449(2025)

Research Article

STABLE ISOTOPE COMPOSITION $\delta^{15}N$ IN ORGANIC AND CONVENTIONAL STRAWBERRY FRUIT SAMPLES

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Received: September 12, 2024; Revised: October 28, 2024; Accepted: November 01, 2024

ABSTRACT

Strawberry fruit samples collected in Japan and Dalat (Lam Dong) were analyzed for stable isotope composition δ15N using the Isotope Ratio Mass Spectrometry system (EA-IRMS). The results of the ANOVA test showed a statistically significant difference at $= 0.05$ *among the stable isotope composition δ15N in the organic strawberries from Japan compared to conventionally grown and greenhouse grown strawberries in Dalat and Japan with* $p - values < 0.001$. *Specifically, the mean δ15N value in organic strawberries grown in Japan (4.720‰) was more enriched than that obtained from samples conventionally grown (0.020‰ and 0.864‰) and greenhouse grown (0.103‰) in Dalat and Japan. Additionally, the average δ15N value in hydroponically grown strawberries in Dalat (5.283‰) did not differ significantly from the organic strawberries in Japan at the α=0.05 level of significance.*

Keywords: conventional; Dalat; Japan; organic; stable isotope composition; strawberry

1. Introduction

Strawberries are among the most delicious and soft types of berries, playing a significant role in the berry category. There are numerous wild varieties of strawberries found worldwide, but cultivated strawberries are primarily derived from two American species, Fragaria chiloensis and Fragaria virginiana. The hybridization of these two species serves as the ancestor to all modern strawberry cultivars. The cultivated strawberry (Fragaria

Cite this article as: Nguyen, H. N., Tran, Q. T., Tran, T. A., Nguyen, M. D., Le, X. T, Nguyen, T. H. L., Vo, T. M. T., Tran, T. T. P., Le, V. T., Tuong, T. T. H., Pham, D. H., Truong, T. S., & Phonesavanh Lathdavong (2025). Stable isotope composition $\delta^{15}N$ in organic and conventional strawberry fruit samples. *Ho Chi Minh City University of Education Journal of Science, 22*(9), 1554-1563. https://doi.org/10.54607/hcmue.js.22.9.4449(2025)

x ananassa Duch) is renowned as a legendary fruit in temperate countries. However, it can also be grown in tropical and subtropical climates (Kumar et al., 2018).

In the world, the high demand for organic berry fruit plants has increased in recent decades, which makes strawberry cultivation a very demanding enterprise. Its fruit is a good source of Vitamin C (40-120 mg/100g fruit), proteins, and minerals such as potassium, phosphorus, calcium, and iron, as well as folate and phytochemical compounds like ellagic acid. Compared to other berry varieties, strawberries contain higher levels of Vitamin C, phenolics, and flavonoids (Kumar et al., 2018).

The studies have demonstrated that strawberries are beneficial for health, such as in reducing cholesterol, blood pressure, and inflammation, and promoting cardiovascular health. Furthermore, strawberries may help prevent large fluctuations in blood sugar levels and insulin. The health-protective effects of strawberries are attributed to ellagic acid and ellagitannins, which have been shown to inhibit the growth of cancer cells. Therefore, strawberries are an excellent addition to a healthy diet (Afrin et al., 2016; Basu et al., 2014; Bebek et al., 2022; Giampieri et al., 2012).

In Vietnam, strawberries are known as a specialty fruit of Dalat, in Lam Dong province. French settlers introduced strawberry cultivation to Dalat in the 1940s. This highland city boasts the most suitable soil conditions and climate for growing strawberries. Dalat strawberries are renowned among the varieties grown in Vietnam. With its cool climate year-round, strawberries from Dalat yield large quantities of high-quality fruit, known for their deliciously sweet and fragrant taste. Strawberry farming in Dalat not only improves the lives of farmers but also supplies the market with premium agricultural products, meeting the demand for clean fruit among Vietnamese consumers. In recent years, there has been a growing consumer trend towards healthier eating habits. Particularly, consumers are increasingly interested in understanding and tracking food labels. Research on consumer preferences indicates that in some cases, consumers are willing to pay for labels that guarantee origin protection (Protected Designation of Origin - PDO) and geographical indication (Protected Geographical Indications - PGI). Consumers want to know where their food comes from and what it includes (Aprile et al., 2012).

In the world, there have been numerous published studies on the quality, health benefits, as well as authentication of organic and conventional strawberries using stable isotope techniques (Covaciu et al., 2016; Perini et al., 2018; Strojnik et al., 2022). Bebek's study (Bebek et al., 2022) utilized the stable isotope component $\delta^{15}N$ to differentiate organic strawberries from various farms. Results showed that the average $\delta^{15}N$ value of 40 Albion strawberry samples grown in greenhouse conditions in Zagreb, Croatia, was $5.09 \pm 0.82‰$, whereas for strawberry juice and pulp samples at 75% and 100% ripeness stages, the values were $4.37 \pm 0.74‰$ and $3.94 \pm 0.15‰$, respectively. Strawberries are an agricultural product where fertilizers and soil play a crucial role in determining their yield. For soilless cultivation methods, organo-mineral fertilizers exhibit lower δ15N values (approximately 0‰) compared to conventional organic fertilizers (2-20‰) (Bateman et al., 2007).

According to Perini's study (Perini et al., 2018), Group A, comprising strawberry, blackberry, gooseberry, and strawberry samples collected from Trentino PV, Romania, and Poland, grown in soil with organic fertilizer, exhibited δ15N values greater than +2‰ compared to Group B samples collected from Trentino VV, grown without soil and using mineral fertilizers. The cultivation method also significantly influences the δ15N composition of strawberries. Inácio's study (Inácio et al., 2020) on Fragaria strawberries collected under two cultivation methods in Rio Grande do Sul, Brazil, organic and hydroponic, showed average δ15N values ranging from 1.2‰ to 9.8‰ for organic cultivation and from +2.9‰ to +4.0‰ for hydroponic cultivation. These studies underscore the importance of stable isotopes, specifically δ15N, in distinguishing between organic and conventional strawberries and understanding the impact of cultivation practices on their isotopic composition.

However, in Vietnam, the number of studies on strawberries is still limited, with publications primarily focused on breeding and geographical origin tracing (Ha et al., 2022). Recently, with increased consumer demand for strawberries, there have been incidents of organic origin falsification to inflate product prices. The stable isotope composition of δ15N has been scientifically validated as a reliable basis for distinguishing organic strawberries from those grown using other cultivation methods. This could potentially serve as a promising scientific tool for differentiating organic produce and ensuring food traceability.

In this study, we initially analyzed the stable isotope composition of δ15N in strawberries from five groups: organically grown strawberries in Japan, hydroponically grown strawberries in Dalat, and conventionally grown and greenhouse grown strawberries in both Japan and Dalat. Assessing the differences in δ15N values among these strawberry samples will provide a basis for verifying the origin and cultivation methods of the strawberries. This study aims to develop a reliable scientific tool to protect consumer rights while enhancing the credibility of strawberry product labeling.

2. Material and methods

2.1. Sampling site and datasets

A total of 30 strawberry samples were collected for δ15N isotope analysis. These included 10 organic strawberry samples collected from farms in southern and central Japan (Kumamoto, Kyoto, Miyazaki, Togochi province) (Figure 1); 4 hydroponically grown strawberry samples; 5 greenhouse-grown strawberry samples from Da Lat; and 11 outdoor field-grown strawberry samples using conventional agriculture methods in Japan (Togochi, Fukuoka, Tamana province) and Da Lat. These samples were collected at two time points in February 2024 and March 2024. Details of the samples are provided in Table 1.

2.2. Analysis of the stable isotope composition $\delta^{15}N$ using the EA-IRMS system

The sample is rinsed with distilled water to remove dirt, and then approximately 20 grams are cut into small pieces and placed into a 200 ml flask for vacuum-freeze drying (Figure 2). After completely drying for 72 hours at -55^{0}C, the sample is finely ground using a mortar and pestle. The freeze-drying of the sample will minimize the isotopic fractionation process of Nitrogen within the sample. A weighed amount of 0.4 ± 0.1 mg or 1 ± 0.1 mg is then packaged into Tin capsules. The EA-IRMS system, after being started up and leak-tested, is set up to run the EA and IRMS methods for analyzing $\delta^{15}N$ isotopes in plant samples shown in Table 2.

***Figure 1.** Organic strawberries from Japan*

Nitrogen has two stable isotopes in nature, ^{14}N and ^{15}N, mostly found as the lightest isotopic form, ^{14}N (99.634 %), whereas the heaviest form, ^{15}N, represents 0.366 % of the total. Variations in nitrogen isotope compositions are measured as the relative deviation of the sample heavy-to-light isotope ratio $^{15}N/^{14}N$ from the international reference, that is, atmospheric N_2 gas, i.e., the nitrogen isotope ratio ($\delta^{15}N$), calculated as detailed in Eq. 1, and expressed either as its per mille (‰) value or as mUr (1 mUr = 1‰)(Santesteban et al., 2024)

$$\delta^{15}N[‰] = \frac{R_{sample} - R_{reference}}{R_{reference}}.1000 = \left(\frac{R_{sample}}{R_{reference}} - 1\right) \times 1000. \quad (1)$$

Where: $R_{sample} = {}^{15}N_{sample}/{}^{14}N_{sample}$ and $R_{reference} = {}^{15}N_{reference}/{}^{14}N_{reference}$ are the ratios of the sample heavy-to-light isotope ratio in the sample and international reference, respectively.

Figure 2. *The process of freeze-drying strawberry samples*

In the ISOdat 3.0 program, $\delta^{15}N$ values exported are typically raw values. These raw values may not be the correct values due to factors such as the accuracy of standard gases and variations in analysis sequences. To correct these raw values to true values, standard values are used to establish a regression line between the raw and certified values. This regression line is then applied to recompute all samples, including isotope standard samples (Certified Reference Materials), quality control samples (QC), and test samples. The multi-point normalization method and the regression method are given by (Dunn et al., 2018):

$$\delta_{true(s)} = \frac{\delta_{true(R_1)} - \delta_{true(R_2)}}{\delta_{raw(R_1)} - \delta_{raw(R_2)}} \times \delta_{raw(s)} + \delta_{true(R_1)} - \left(\delta_{raw(R_1)} \times \frac{\delta_{true(R_1)} - \delta_{true(R_2)}}{\delta_{raw(R_1)} - \delta_{raw(R_2)}}\right). \quad (2)$$

Where : $\delta_{true(s)}$ is the $\delta^{15}N$ value in the analyzed sample; $\delta_{raw(s)}$ is the $\delta^{15}N$ value in the analyzed sample measured with the EA-IRMS system; $\delta_{true(R_1)}$ is the $\delta^{15}N$ value in the certified standard USGS61; $\delta_{raw(R_1)}$ is the $\delta^{15}N$ value of USGS61 measured with the EA-IRMS; $\delta_{true(R_2)}$ is the $\delta^{15}N$ value in the certified standard USGS63; and $\delta_{raw(R_2)}$ is the $\delta^{15}N$ value of USGS63 measured with the EA-IRMS.

The measurement uncertainty $u_{true(s)}$ is calculated using the formula:

$$u_{true(s)} = \sqrt{u^2_{true(R_1)} + u^2_{raw(R_1)} + u^2_{true(R_2)} + u^2_{raw(R_2)} + u^2_{raw(s)}}\,. \quad (3)$$

The expanded measurement uncertainty U is calculated as follows:

$$U = k \times u_{true(s)}. \quad (4)$$

Where: k is the test statistic factor (k = 2 for a confidence level of approximately 95%);

Table1. *The information on the analyzed strawberry samples*

Origin	Type	Sample	Amount	$\delta^{15}N$ (‰)	SD
Japan	Organic	A1	0.446	4.406	0.117
Japan	Organic	A2	0.409	6.383	0.116
Japan	Organic	A3	0.437	5.868	0.116
Japan	Organic	A4	0.451	10.155	0.116
Japan	Organic	A5	0.432	-1.742	0.119
Japan	Organic	A9	0.429	5.024	0.117
Japan	Organic	A10	0.411	6.651	0.116
Japan	Organic	A11	0.46	4.405	0.117
Japan	Organic	A12	0.388	5.731	0.116
Japan	Organic	A13	0.47	0.316	0.118
Dalat	Hydroponic	A7	0.429	4.983	0.117
Dalat	Hydroponic	A8	0.38	4.519	0.117
Dalat	Hydroponic	A14	0.455	5.010	0.117
Dalat	Hydroponic	A16	0.466	6.620	0.116
Dalat	Greenhouse	A20	0.451	1.241	0.118
Dalat	Greenhouse	A21	0.434	-0.510	0.118
Dalat	Greenhouse	A22	0.419	0.164	0.118
Dalat	Greenhouse	A29	0.472	0.561	0.118
Dalat	Greenhouse	A34	1.011	-0.942	0.118
Japan	Conventional	A27	0.403	2.981	0.117
Japan	Conventional	A28	0.408	-1.267	0.119
Japan	Conventional	A28	0.409	0.877	0.121
Dalat	Conventional	A18	0.441	2.626	0.117
Dalat	Conventional	A23	0.409	0.472	0.118
Dalat	Conventional	A24	0.438	0.463	0.118
Dalat	Conventional	A30	1.005	-0.924	0.118
Dalat	Conventional	A31	1.001	-0.087	0.118
Dalat	Conventional	A32	1.135	-0.748	0.118
Dalat	Conventional	A33	1.042	-0.520	0.118
Dalat	Conventional	A35	1.025	-1.119	0.118

The standard samples are measured three times each, with a standard deviation (SD) achieved for $\delta^{15}N$ values of 0.019. Standard samples are used to calculate the adjustment of analysis results, including the isotopic standard samples from Reston Stable Isotope Laboratory: USGS61, USGS62, USGS63, and the Sulfanilamide standard from Thermo. The specific details regarding the mean value and the measurement uncertainty (U) of these standard samples are provided in Table 3.

Table 2. *Measuring conditions in EA and IRMS*

Parameter	EA Method
Matrix	Plant
Type oven	NCS
Isotopes	$\delta^{15}N$
Furnace temperature	950 °C
Carrier flow (He)	140 ml/min
Oxygen flow O2	180 ml/min
Reference flow (CO2)	70 ml/min
Measuring time (Cycle run)	300 sec

Table 3. *The information on standard samples*

ID	CRM	matrix	$\delta^{15}N$ (‰)	%N
1	USGS61	caffeine	-2.87 (-3.04 ± 0.03)	*
2	USGS62	caffeine	20.17 (20.26 ± 0.08)	*
3	USGS63	caffeine	37.83 (37.81 ± 0.05)	*
4	Sulfanilamide	Crystalline Powder	*	16.27 (16.34 ± 0.44)

3. Results and discussion

The results of the $\delta^{15}N$ stable isotope analysis of 30 strawberry samples collected from Japan and Dalat were statistically evaluated using ANOVA and are presented in Table 4 and Figure 3.

Table 4. *Statistical results of the $\delta^{15}N$ isotope composition in the strawberry samples*

$\delta^{15}N$ (‰)	Organic (Japan)	Hydroponic (Dalat)	Indoor agriculture (Dalat)	Conventional (Japan)	Conventional (Dalat)
N	10	4	5	3	8
Mean	4.720	5.283	0.103	0.864	0.020
Minimum	-1.742	4.519	-0.942	-1.267	-1.119
Maximum	10.155	6.620	0.561	2.981	2.626
SD	3.330	0.920	0.863	2.124	1.211
ANOVA test p-value (= 0,05)		1.79E-04			

The statistical comparison (ANOVA test) results in Table 4 show a significant difference in the average $\delta^{15}N$ values among different strawberry samples at a significance level of α=5% ($p=0.000179<0.05$). Specifically, organic strawberries from Japan and hydroponic strawberries from Dalat had average $\delta^{15}N$ values (4.720‰ and 5.283‰, respectively) more enriched than conventional strawberries grown in Japan (0.864‰) and Dalat (0.020‰), as well as those grown in a greenhouse (0.103‰). Table 1 shows that the average $\delta^{15}N$ values of both greenhouse and conventional cultivation strawberries are less than 3.00‰. In the organic strawberry group, there were two samples with average $\delta^{15}N$ values lower than 4.00‰ (-1.742‰ and 0.316‰, respectively), whereas the majority of other samples exhibited higher values. The two samples with low $\delta^{15}N$ values both originate from frozen Mousso strawberries, which were processed into products intended for juice or fresh cream. In contrast, the remaining eight samples were fresh strawberries harvested from an organic strawberry farm. The processing, storage, and freezing of these strawberries may cause isotopic fractionation, resulting in lower $\delta^{15}N$ values for these two samples compared to the others. Additionally, it is possible that the Mousso strawberry variety inherently has lower $\delta^{15}N$ values than other varieties. The number of organic strawberry samples examined in this study set size is not large enough, and future research will focus on expanding the range of $\delta^{15}N$ values in organic strawberries. The low $\delta^{15}N$ values of these two samples result in a lower average $\delta^{15}N$ value for the Japanese organic strawberry group and an increased standard deviation (SD). Therefore, when using t-test, no significant difference in the mean $\delta^{15}N$ values between organic strawberries and hydroponic ones was found at the significance level α=5% (p-value = 0.063). This implies that there is no difference between organic strawberries and hydroponic ones based on the data in this study.

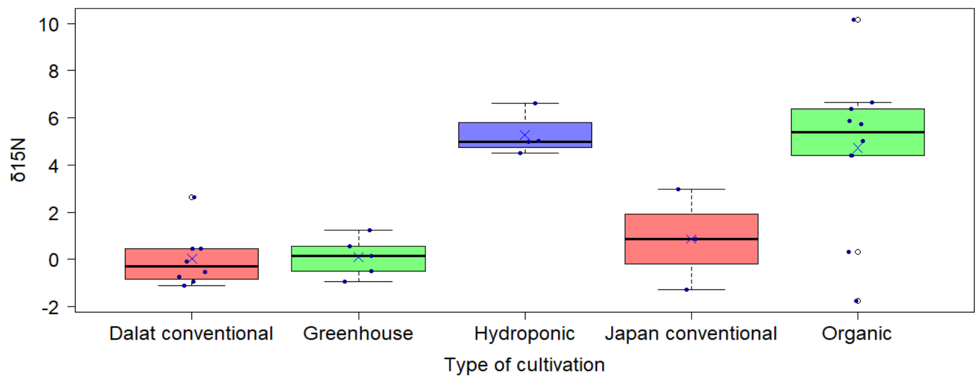

Figure 3. *A box plot shows the $\delta^{15}N$ values in the strawberry samples*

These differences may be influenced by factors such as cultivation conditions (soil, irrigation water, fertilizers, and care practices) and geographical conditions such as climate, rainfall, altitude relative to sea level, and specific characteristics of strawberry plants in each region. Depending on the organic farming practices for a particular crop, organic agriculture typically utilizes natural fertilizers such as manure, poultry litter, fish emulsion, and other organic materials to enhance plant growth. In contrast, conventional agriculture often employs chemical fertilizers like urea, potassium nitrate, and various compound fertilizers directly applied to crops to promote growth. These are key factors influencing the productivity of strawberries, thus choosing conventional cultivation methods to increase yields often involves the use of specific fertilizers or growth enhancers, resulting in differences in $\delta^{15}N$ values among groups. In summary, the analytical results present significant differences in $\delta^{15}N$ values between organic strawberries and those grown under different cultivation practices.

4. Conclusion

In this study, the analysis of stable isotope $\delta^{15}N$ composition in strawberry samples collected from Japan and Dalat (Lam Dong, Vietnam) was conducted. The analytical results and statistical evaluations clearly indicated significant differences in $\delta^{15}N$ values among the strawberry sample groups. These differences are influenced by factors such as soil type, irrigation water, fertilizers, cultivation practices, climate, rainfall, altitude, and strawberry plant characteristics specific to each region where the samples were collected. The initial exploratory research results using $\delta^{15}N$ values to differentiate strawberries cultivated by various farming methods have shown promising signals. This paves the way for developing a reliable scientific basis for distinguishing between organic and conventional strawberries, with potential applications for other agricultural products.

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

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THÀNH PHẦN ĐỒNG VỊ BỀN $\delta^{15}N$ TRONG QUẢ DÂU TÂY HỮU CƠ VÀ DÂU TÂY THÔNG THƯỜNG

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Ngày nhận bài: 09-12-2024; ngày nhận bài sửa: 28-10-2024; ngày duyệt đăng: 01-11-2024

TÓM TẮT

Các mẫu quả dâu tây thu thập tại Nhật Bản và Đà Lạt (Lâm Đồng) được phân tích thành phần đồng vị bền $\delta^{15}N$ bằng hệ thống phổ khối lượng tỉ lệ đồng vị (EA-IRMS). Kết quả kiểm định ANOVA cho thấy ở mức ý nghĩa thống kê $= 0{,}05$ có sự khác biệt rõ ràng giữa thành phần đồng vị bền $\delta^{15}N$ trong các mẫu dâu tây hữu cơ tại Nhật Bản với các mẫu dâu tây trồng theo hình thức truyền thống và trong nhà kính tại Đà Lạt và Nhật Bản với các giá trị p<0,001. Cụ thể, giá trị $\delta^{15}N$ trung bình trong quả dâu tây hữu cơ trồng ở Nhật Bản (4,720‰) cao hơn so với giá trị thu được trong mẫu trồng truyền thống ở Đà Lạt và Nhật Bản (lần lượt là 0,020‰ và 0,864‰) và trong nhà kính (0,103‰) ở Đà Lạt. Ngoài ra, giá trị $\delta^{15}N$ trung bình trong quả dâu tây trồng thủy canh tại Đà lạt (5,283‰) không có sự khác biệt với nhóm dâu tây hữu cơ Nhật Bản ở mức ý nghĩa thống kê $= 0{,}05$.

Từ khóa: thông thường; Đà Lạt; Nhật Bản; hữu cơ; thành phần đồng vị bền; dâu tây