Article


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Abstract: The demand for organic food products has increased in recent years due to them being perceived healthier, safer, and eco-friendlier by consumers, boosting the development of this industry. The higher retailing price of organic products increases the risk of fraudulent practices, making it necessary to establish control mechanisms to authenticate these products. However, the authentication of organic foodstuffs is a great analytical challenge that still requires further research. In the case of organic agriculture, regulations mainly determine the nutrient inputs that can be used by farmers, and generally prohibit the use of pesticides and/or synthetic fertilisers, aiming at maintaining soil fertility using green manures, composts, animal manures, etc. These inputs affect the final food product, and numerous analytical attempts, based on the measurement of multiple markers or complex chemical/physical profiles, have been tested over recent years. However, the high variability of these measurements due to weather condition factors reduces their efficiency and limits their use. In this sense, stable isotopes have emerged as an analytical technique with great potential for the authentication of organic agricultural products, due to their lower dependence on weather conditions and capability to reflect the origin of plant nitrogen, in the case of stable nitrogen isotopes. In this work, the feasibility was assessed using stable isotopes of bulk nitrogen for the organic authentication of four important horticultural crops (zucchini, cucumber, tomato, and pepper) produced in Almeria, southern Spain, which is the largest producing region with the highest export levels in Europe. To this end, 360 samples of vegetables were collected and their δ¹⁵N values were determined by combustion coupled to stable isotope ratio mass spectrometry (EA/IRMS). The results allowed an authentication framework to be established based on three ranges delimited by δ¹⁵N = 2‰ and δ¹⁵N = 5‰, which made it possible to detect with a high degree of confidence vegetables produced under proper organic practices (δ¹⁵N > 5‰), conventional practices (δ¹⁵N < 2‰), and samples that should be tracked over time to be considered organically produced (middle range). The results of this study demonstrated the potential of using δ¹⁵N as a single measure to authenticate organic vegetables, providing official bodies with a tool to make decisions about the organic accreditation of regularly inspected farmers.

Keywords: organic crops; IRMS; stable isotopes; authentication; vegetables; fertilization; Almeria

1. Introduction

The demand for organic food products has increased in recent years, mainly due to consumer interest in the quality and safety of food, and the perceived healthier, safer, and more sustainable characteristics of organic foods, compared with their conventional counterparts [1,2]. This has prompted consumers to pay higher prices for these organic food products, which are on average between 20–50% more expensive than non-organic products. However, depending on the type of foodstuff, country of origin, and elaboration process, these price increases can even reach about 100–180% [3]. This has driven the organic food industry to reach an estimated global market of 106.4 billion euros in 2019 [4].
Organic agriculture can be defined as a farming technique that aims to promote the responsible use of natural resources, biodiversity, and soil fertility [5]. In the European Union, organic production, labelling, and controls have been regulated since 1991 through different European Council Regulations. Thus, Regulation (EC) No 2018/848 defines the aims, objectives, and principles for organic production, while Regulations (EC) No 2021/1165 and 2021/2306 detail the organic production, control, labelling, and import rules. These regulations generally prohibit the use of pesticides and/or synthetic fertilisers in organic agricultural systems, aiming to maintain soil fertility using green manures, composts, animal manures, etc.

To assure compliance with the rules, farmers require organic production accreditations and are regularly inspected by official bodies. However, the complexity of these systems and global food supply chains makes the authentication of organic food products difficult using only these methods, raising the need for the implementation of analytical methodologies to solve this issue [3].

The authentication of organic foodstuffs is a great analytical challenge and, consequently, numerous attempts have been tested in recent years. Many research papers, reports, and databases from research institutes and governments have addressed the determination of nutrients and pesticide residues in different organically and conventionally grown food matrices. A previous study [2] performed a meta-analysis of the available literature to find evidence of differences in nutrients and pesticide residues of organic and conventional vegetables and potatoes. Organic plants were found to systematically display lower contents of contaminants, although the limited number of pesticides tested in the literature, compared to the large number of existing contaminants, did not ensure the successful authentication of organic production practices. Meanwhile, the study [6] did not find evidence of differences in nutrient contents between organically- and conventionally-produced food products, after a systematic review.

In recent years, mass spectrum-based metabolomics techniques have demonstrated great potential to address organic food authentication [3,7,8], thanks to advantages such as high sensitivity and selectivity for metabolite determination and multianalyte capabilities in one single run [9]. This promising approach is based on the comprehensive characterisation of plant metabolites, since their pattern of variation may reflect the influence of external factors such as organic crop practices. However, plant metabolites can show huge variations between years due to fluctuations in weather conditions or different agricultural management from different operators, reducing their efficiency and limiting the use of these techniques. This explains why, to date, metabolomics-based methods are not used by regulatory entities, and their application for food authentication is still at an early stage that requires more in-depth research with larger datasets [7].

Vibrational spectroscopy platforms combined with chemometrics are rapid and non-destructive analytical techniques that can be used routinely. This, linked to the low implementation costs make them attractive for real applications in the industry, being addressed by many studies [10–12]. However, these techniques are in general less powerful in complex authentication problems and, similar to mass-spectrum-based techniques, depend on the chemical structure of the samples and may show important variation between years.

Stable isotope ratio mass spectrometry currently constitutes one of the most promising and widely used techniques for organic food authentication, most of the studies having investigated the nitrogen isotope composition ($\delta^{15}$N) of bulk matter as a marker for organic cultivation [8,11,13–17]. This approach is based on the differences in the $\delta^{15}$N value of organic and synthetic (not allowed in organic production) fertilizers, values that are reflected in the crops [18]. Synthetic nitrogen-based fertilizers are produced through the Haber-Bosh process by atmospheric nitrogen ($\delta^{15}$N$_{\text{air}} = 0\%$) and display $\delta^{15}$N values around 0%, Meanwhile, organic fertilizers (compost, animal manures, vegetable byproducts, etc.) are more enriched in $^{15}$N, with values generally varying between 8% to even more than 35%, due to the preferential volatilization of $^{15}$N-depleted ammonia in the field, or during storage [13]. However, this technique is rather limited in terms of differentiating organically or
conventionally grown crops that are not plants that fix nitrogen from the air ($\delta^{15}\text{N}_{\text{air}} = 0\%$), since this factor reduces their isotopic differences. The literature reported several attempts to authenticate organic food using this technique. A previous study [19] addressed the feasibility of $\delta^{15}\text{N}$ to discriminate between commercial organic and conventional grown vegetables in New Zealand, reporting good results in fast-growing crops (less than 80 days) such as tomato, broccoli, and zucchini. However, the discrimination was less satisfactory for slow-growing vegetables (more than 80 days), hypothesizing that $^{15}\text{N}$ could be taken by soil microorganisms in situ, reducing the $\Delta^{15}\text{N}$ between the organic and conventional samples. Another study [20] examined soil, irrigation water, and nitrogen fertilizers used in different conventionally and organically grown crops to determine their effects on the nitrogen stable isotopes of spinach, lettuce, broccoli, and tomatoes. The authors of this study found differences in the $\delta^{15}\text{N}$ of these cultivars according to their organic or conventional management. However, despite the previous authors promising results, with regard to organic vegetable authentication, other studies [21] reported overlapping ranges in the $\delta^{15}\text{N}$ values of commercial organic and conventional vegetables, including parsley, sweet pepper, tomato, onion, kohlrabi, cauliflower, carrot, and garlic. These results may be influenced by the uncertainty about the origin of the commercial samples analysed, since several of the organically labelled samples could have been produced misleadingly, with conventional practices. Therefore, further approaches with controlled experimental designs are still needed to understand the plausibility of using stable isotopes of nitrogen as an authentication technique by inspection bodies and importers of organic food products.

The aim of this study was to demonstrate the potential of stable Isotopes of nitrogen for the authentication of the main exports in organic horticultural crops (zucchini, cucumber, tomato, and pepper) collected under a controlled experimental design. Samples from different farmers were assessed along the full plant life cycle and three ranges of $\delta^{15}\text{N}$ values were proposed and discussed to be used as a tracking method to support the inspection bodies.

2. Materials and Methods

2.1. Samples

A total of 360 samples of vegetables were collected from four horticultural crops (zucchini, cucumber, tomato, and pepper) and different farmers in the province of Almeria (Andalusia) (a summary is given in Table 1). These horticultural crops were chosen based on their importance in terms of exportation within the European market, these being the four main exported products. Thus, the Spanish authorities require strict controls to prevent the introduction of questionable organic products in the international market, something that could seriously damage the image of this producer zone.

Table 1. Summary of the samples collected for each agricultural crop and regime (organic or conventional).

<table>
<thead>
<tr>
<th>Crop</th>
<th>Regime</th>
<th>$N_{\text{farmers}}$</th>
<th>$N_{\text{samples}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zucchini</td>
<td>Organic</td>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>Cucumber</td>
<td>Organic</td>
<td>5</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>4</td>
<td>33</td>
</tr>
<tr>
<td>Tomato</td>
<td>Organic</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td>Pepper</td>
<td>Organic</td>
<td>6</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>4</td>
<td>48</td>
</tr>
</tbody>
</table>

All the vegetables were sampled at commercially mature stages along different dates, during the complete horticultural life cycle. Several samples from different horticultural crops were collected from the same farmers (Figure 1) to evaluate the impact of the farmers’ practices on the final $\delta^{15}\text{N}$ values. The vegetables were cleaned with water, the seeds were
removed and crushed, freeze-dried, and homogenized by grinding and milling, before analysis. The powdered samples were then placed into 2 mL Eppendorf tubes and stored at −18 °C until analysis.

Table 1. Summary of the samples collected for each agricultural crop and regime (organic or conventional).

<table>
<thead>
<tr>
<th>Crop Regime</th>
<th>N farmers</th>
<th>N samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zucchini</td>
<td>Organic</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>3</td>
</tr>
<tr>
<td>Cucumber</td>
<td>Organic</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>4</td>
</tr>
<tr>
<td>Tomato</td>
<td>Organic</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>3</td>
</tr>
<tr>
<td>Pepper</td>
<td>Organic</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>4</td>
</tr>
</tbody>
</table>

Figure 1. Boxplots of the δ¹⁵N values measured for the samples from different horticultural crops ((a): zucchini; (b): cucumber; (c): tomato; (d): pepper). CF (conventional farmers) and OF (organic farmers). Results from the ANOVA are given for each cultivar.

2.2. Determination of δ¹⁵N/¹⁴N Isotope Ratio by EA-IRMS and Statistical Analyses

About 1.5 mg of freeze-dried zucchini, 2 mg of cucumber, 1.8 mg of tomato, and 1.3 mg of pepper were weighed into tin capsules (3.3 × 5 mm, IVA Analysentechnik e. K., Dusseldorf, Germany) for the determination of δ¹⁵N values. The ¹⁵N/¹⁴N ratios were determined using a delta V Advantage Isotope Ratio Mass Spectrometer from Thermo Fisher Scientific (Bremen, Germany). This system was equipped with a ConFlo IV Universal Interface for continuous flow analysis and a Flash EA elemental analyzer. ISODAT Software (v. 3.0 from Thermo Scientific, Bremen, Germany) was used to acquire and process the signal.
obtained by the IRMS instruments. The nitrogen isotope ratios were expressed relative to the international standards ratio and denoted in delta notation, although milliurey (mUr) can also be used to comply with the International System of Units (SI), according to the formula given in Brand (et al.) [22]:

$$\delta^{15/14}N = \delta^{15/14}N_{\text{P}} - \delta^{15/14}N_{\text{Ref}}$$

where superscripts denote the highest and the lowest atomic mass number of nitrogen, respectively, and $P$ and $\text{Ref}$ indicate the ratio between the heavier and the lighter isotope in the sample and reference material, respectively.

The samples were analyzed in duplicate, and the instrument was calibrated with the following reference standards: USGS 40 (IAEA-International Atomic Energy Agency, Vienna, Austria), EBD-23 (cow horn), and Wheat-Flour. To control and ensure the quality of the analyses, working standards were included in each sequence analyzed, for every ten samples. The analytical uncertainty (2 std dev) was 0.3‰.

Differences in the $\delta^{15}N$ values of the samples were assessed by means of the analysis of variance (ANOVA) test. Since this is a parametric statistic, Levene’s and Shapiro Wilk’s tests were used to respectively check the normality and heteroscedasticity. Statistically significant differences were considered at a $p$-value $\leq 0.05$. Then, Box and whisker plots were performed using the ggplot2 library, to assess the distribution of the $\delta^{15}N$ values in the samples from the different farmers. Both statistics were performed using the statistical software R v. 4.2.2 (R Core Team, Vienna, Austria).

3. Results & Discussion

3.1. Effect of Fertilization Regime on the Nitrogen Isotope Composition of Vegetables

To assess the impact of the different crop managements, namely conventional farming (CF) and organic farming (OF), vegetables from two horticultural crops of Cucurbitaceae (zucchini and cucumber) and Solanaceae (tomato and pepper) were collected from different farmers, some of them providing samples from two or three horticultural cultivars, which revealed useful information to understand the impact of the crop management on the final $\delta^{15}N$ values. The use of conventional or organic fertilizers led to clearly different $\delta^{15}N$ values in the vegetable samples analysed, as observed in the results from the one-way analysis of variance (ANOVA; Figure 1). Supplementary Tables S1–S4 show the main nitrogen fertilizers used by each farmer and their $\delta^{15}N$ values measured. Since the samples in this study were collected from real farmers, it was impossible to calculate the amount applied. Several organic farmers used fertilizers with low $\delta^{15}N$ values, but the organic vegetable samples displayed higher $\delta^{15}N$ values overall than the conventional ones (as shown in the following sections). This could be partially explained by the fractionation occurring during the slower assimilation of complex chemical-form nitrogen, available in organic fertilizers. Meanwhile, ammonium and nitrates from inorganic fertilizers are rapidly incorporated by the plant, resulting in lower $\delta^{15}N$ values. The $\delta^{15}N$ values of the soil are also given in this Supplementary Material, with clear differences not being observed between the $\delta^{15}N$ values of the soil from the organic and conventional farming systems.

3.1.1. Cucurbitaceae

Results from the stable nitrogen isotopes of zucchini and cucumber are shown in Table 2. The organic and conventional zucchini samples showed an average $\delta^{15}N$ value of 5.54 $\pm$ 1.10‰ and 2.14 $\pm$ 1.64‰, respectively, a higher range of variation being observed for the organically grown vegetables (Table 2). The results from the conventional cultivars were in accordance with those found by previous authors [19,20], who reported respective mean $\delta^{15}N$ values of 2.32 and 2.40‰, but differed from the values obtained for the organic zucchini samples (above 9‰ in both studies). However, despite the lower differences in the $\delta^{15}N$ composition between the organic and conventional samples found in our study,
we observed statistically significant differences between both cultivars’ regimes, although supported by a lower \( p \)-value (0.01 > \( p \) > 0.05) than those found in the remaining vegetables analysed (\( p < 0.001 \)). These results were explained by the samples collected from two farmers (OF1 and OF7), which showed \( \delta^{15}N \) values that could be confounded with the conventional zucchini samples. This was especially noteworthy for the OF1 samples, with low \( \delta^{15}N \) values of between 1.1–1.3‰, similar to those found in the conventional crops (0.4–5.0‰; Figure 1a). Meanwhile, the values from the OF7 samples ranged between 2.9–3.7‰, only being confounded with the CF1 samples, while showing higher \( \delta^{15}N \) values than the other conventionally grown vegetables. This could be linked to the addition of synthetic fertilizers. The nitrogen isotopic composition of the samples collected from the two remaining organic farmers (OF3 and OF4) ranged between 6.0–12.0‰, in line with the high values found for organic zucchini in the abovementioned literature, while most of the CF5 and CF6 (conventional producers) samples were below 2‰.

Table 2. Summarized data, including mean ± standard deviation, minimum, and maximum stable nitrogen isotope values of the different vegetables analysed from organic and conventional regimes.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Regime</th>
<th>( \delta^{15}N )</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zucchini</td>
<td>Organic</td>
<td>5.54 ± 1.10</td>
<td>1.10</td>
<td>11.97</td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>2.14 ± 1.64</td>
<td>0.37</td>
<td>4.98</td>
</tr>
<tr>
<td>Cucumber</td>
<td>Organic</td>
<td>7.00 ± 3.81</td>
<td>1.34</td>
<td>12.06</td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>1.83 ± 1.87</td>
<td>−0.76</td>
<td>5.25</td>
</tr>
<tr>
<td>Tomato</td>
<td>Organic</td>
<td>7.08 ± 2.77</td>
<td>2.69</td>
<td>11.39</td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>0.94 ± 1.48</td>
<td>−1.92</td>
<td>3.42</td>
</tr>
<tr>
<td>Pepper</td>
<td>Organic</td>
<td>6.99 ± 3.89</td>
<td>0.26</td>
<td>11.66</td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>2.51 ± 1.53</td>
<td>0.44</td>
<td>5.10</td>
</tr>
</tbody>
</table>

Cucumber was the second Cucurbitaceae crop analysed in this study and showed similar results to zucchini. The organic samples analysed from this crop showed \( \delta^{15}N \) values of 7.00 ± 3.81‰, compared with the lower 1.83 ± 1.87‰, recorded for the conventional ones. These results were in agreement with those found by previous authors [20], who reported respective mean \( \delta^{15}N \) values of 1.02 and 8.59‰ for conventional and organic commercial samples, and found differences in the samples on the basis of their fertilization regime (organic or conventional). In our case, we observed the largest range of variation in the organic cucumbers compared to the conventional ones, due to the low isotopic values found for the samples from two farmers. The samples from OF1 showed values ranging from 1.3–2.4‰, while the OF7 samples varied in a slightly higher range between 2.3–4.3‰ (Figure 1b). Interestingly, these organic farmers were the same that produced the zucchini samples with low isotopic values, leading to similar results regardless of the cultivar type (Figure 1a,b). The results found for OF1 clearly fit the data found for conventionally grown cucumber and the values found for OF7 could lead to the conclusion of the recurrent addition of synthetic fertilizers to the crops.

3.1.2. Solanaceae

Two horticultural crops were assessed from the Solanaceae family: tomato and pepper. The mean stable isotope values of bulk nitrogen for both the organic and conventional samples are given in Table 2. With regard to the tomato samples, statistically significant different \( \delta^{15}N \) values were observed for their organic or conventional origin (\( p < 0.001 \); Figure 1c), displaying respective mean values of 7.08 ± 2.77 and 0.94 ± 1.48‰ (Table 2). These results are in line with previous studies [13,19–21] that reported mean values between 5.94 and 8.1‰ for organic tomatoes and from −1.2 to 4.4‰ for conventional ones. In these studies, the authors reported significant differences related with the growing regime, except Šturm et al. [21], who found overlapping results between the samples analysed from both regimes. The authors suggested that these differences did not necessarily mean mislabeling
of these commercial tomatoes, since several factors can induce a decrease in $\delta^{15}$N values in organic crops, such as the incorporation of N-fixing crops in rotation systems, fertilization with seaweed-based amendments or protein hydrolysed fertilizers of leguminous plants, and the use of green manures and, therefore, these aspects should be investigated in the future. In our study, we observed good overall separation between the organic and conventional samples (Figure 1c). The samples collected from the three conventionally grown crops presented $\delta^{15}$N values between $-1.92$ to $3.42\%$, while the organic counterparts varied between $2.69$ to $11.39\%$. The overlap between both regime types was due to the tomatoes analyzed from OF8, which displayed $\delta^{15}$N values between $2.69$ to $3.45\%$, while almost all the remaining organic samples were above $6\%$.

The last horticultural crop analysed in this study was pepper, showing $\delta^{15}$N mean values of $6.99 \pm 3.89$ and $2.51 \pm 1.53\%$ (Table 2) for the organic and conventionally grown samples, respectively, meaning significant differences at a $p < 0.001$ (Figure 1d). Previous studies have reported values for the nitrogen isotope composition of organic and conventionally grown peppers in line with our results. Some authors [20,23] observed clear differences in the $\delta^{15}$N composition of peppers related to their growth regime, the second study reporting differences at a $p < 0.001$, even between organic and mixed conventional crops, involving the application of organic matter to the soil (i.e., mixed crop). Meanwhile, Šturm et al. [21] reported no differences due to the growing regime in the case of the tomato. In our study, we observed statistically significant differences in the $\delta^{15}$N values of peppers according to their fertilization regime, although several samples were not well-separated. This was the case of the samples from OF1 and OF5 (Figure 1d). The $\delta^{15}$N values of the samples from these farmers were in the same variation range ($0.26$ to $2.78\%$) as the conventional samples. Similar results were obtained for the OF1 samples in zucchini and cucumber (Figure 1a,b), highlighting again the impact of the likely fraudulent agricultural practices performed by this farmer on the final isotopic values of vegetables. Surprisingly, the OF5 produced tomatoes with high $\delta^{15}$N values, well-separated from the conventional samples (ranging around $8\%$), while in the case of peppers, the OF5 samples ranged around $2\%$. Therefore, these results may be solely associated with agricultural management.

3.2. $\delta^{15}$N Decision Limits for the Dynamic Tracking of Organic Horticultural Crops: A “Traffic Light” Framework

As reviewed in the Introduction, several studies have previously approached the feasibility of using stable isotopes of bulk nitrogen for organic authentication, showing good results overall for fast-growing crops with high nitrogen requirements [19,24]. In 2007, a study [13] conducted a survey and established a $\delta^{15}$N threshold value of $1.7\%$ to differentiate organically produced tomatoes ($>1.7\%$) from conventional ones ($<1.7\%$). Based on the statistical results, the authors assigned a low probability of $2.5\%$ of mislabeling an organic tomato as non-organic. Due to the need for the organic food industry, and specifically the main European importers, to have analytical procedures to be able to assure the authenticity of organic food products, this value has been often rounded down to $2\%$ and extended as an authentication method for different horticultural crops. This measure had a strong impact on the largest horticultural crop zone in Europe (Almeria), the farmers being increasingly concerned about using proper organic management methods to comply with this rule imposed by European importers.

However, this method presents several drawbacks. In all the reviewed studies dealing with vegetables, and the results of the present study, several conventionally grown samples displayed $\delta^{15}$N values above $2\%$. This could be partially explained by the increasing awareness of the population of more sustainable economies, this also being reflected in the field of agriculture. Thus, conventional farming is moving towards the use of more sustainable practices, the use of organic fertilizers in conventional growing regimes, and mixed conventional systems being reported [14,24,25], in addition to optimizing the amounts of synthetic nitrogen inputs to avoid the aquifer contamination. Another drawback is that the use of a single threshold value does not allow tracking mechanisms
to be established by inspection bodies, to take decisions about the use of good organic practices, since as long as the vegetables tracked have displayed a $\delta^{15}N$ value above $2\%_o$, the product is assumed to be organic.

Based on our results, from previous research papers reviewed in the present work, and demands by the organic horticultural industry (with special focus on the region of Almeria), we proposed a tracking framework based on two $\delta^{15}N_{bulk}$ decision limits to address the authentication of organic vegetables. In this study, we successfully analysed four different crops (zucchini, cucumber, tomato, and pepper), although the proposed methodology could be extended to others. As shown in Figure 1, the two $\delta^{15}N$ decision limits were proposed to be used as indicators of organic practices in all the crops. This framework was based on a decision range governed by the $\delta^{15}N$ values of 2 and $5\%_o$, shown in further detail for the case of the cucumber, in Figure 2, which could be interpreted as an inverted “traffic light”. Thus, the samples showing $\delta^{15}N$ values above $5\%_o$ (green zone) are considered to comply with correct organic managements. As Figure 1 shows, this decision interval made it possible to unequivocally identify almost all the samples above $\delta^{15}N > 5\%_o$ as organic. Meanwhile, those samples displaying $\delta^{15}N$ values below $2\%_o$ (red zone) are highly unlikely to have been grown without the use of synthetic fertilizers and, therefore, cannot be considered as organic samples. Finally, an intermediate range remains between 2–5\% (orange zone), which corresponds with a crop that would need to be tracked by the inspection bodies, possibly requiring the farmer to implement proper organic practices, such as improving the soil conditioning, use of organic manures, etc.

![Boxplot of the $\delta^{15}N$ values measured in cucumber including three operation ranges. The green range ($>5\%_o$) indicates good organic practices, the orange range indicates (values between 2–5\%o) organic crops that need to be supervised, and the red range ($<2\%_o$) indicates conventional crop systems.](image)

This proposed “traffic light” framework can be taken into consideration depending on two possible scenarios. A conventional farmer that decides to make the transition from conventional to organic production, and an organic farmer that uses questionable organic practices and records decreasing $\delta^{15}N$ values over time (as in the cases of farmers OF5 (pepper), OF7 (zucchini and cucumber) and OF8 (tomato) of our study, Figure 1). In the
first case, the orange zone could be considered as a transitioning step from conventional to organic practices that the inspection bodies should follow during the transition period, prior to a complete certification as an organic farmer. Meanwhile, in the second scenario, this would imply a more comprehensive tracking stage where the farmer would need to be paid more attention to by the inspection bodies, including an exhaustive examination of the fertilizers used, and increasing inspection and monitoring visits. An “improvement” (increase) in the $\delta^{15}\text{N}$ values of the farmer’s crop should be expected over time to comply with organic practice requirements. Therefore, in cases where a farmer with a crop allocated in the orange zone does not transition towards the green zone during an established period of time (or even cross to $\delta^{15}\text{N}$ values below 2‰), the official inspection bodies would take a decision about the organic accreditation of this farmer. This deeper inspection mechanism could have a great impact on the move towards more conscientious organic agriculture, favouring the improvement of soil management and a sustainable intensiveness of the productions.

4. Conclusions

This study assessed the feasibility of using stable isotopes of bulk nitrogen for the organic authentication of four important horticultural crops produced in Almería (zucchini, cucumber, tomato, and pepper). The results demonstrated the potential of using this single measure to establish a framework for official control bodies that is based on three ranges delimited by $\delta^{15}\text{N} = 2$‰ and $\delta^{15}\text{N} = 5$‰ and may be interpreted as an inverted “traffic light”. This scheme made it possible to detect with a high degree of confidence whether a sample was produced under proper organic practices ($\delta^{15}\text{N} > 5$‰) and confirmed that the samples with $\delta^{15}\text{N} < 2$‰ should not be considered organic (threshold value widely used by the main European importers). Finally, the middle zone allocated between both limit values opened the possibility of establishing tracking mechanisms by official control bodies, conferring them the authority to take decisions over time for the tracked organic farmers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13010131/s1, Table S1: Fertilization used by each farmer and the $\delta^{15}\text{N}$ values for both fertilizers and soil. Table S2: Fertilization used by each farmer and the $\delta^{15}\text{N}$ values for both the fertilizers and soil: cucumber. Table S3: Fertilization used by each farmer and the $\delta^{15}\text{N}$ values for both the fertilizers and soil: tomato. Table S4: Fertilization used by each farmer and the $\delta^{15}\text{N}$ values for both the fertilizers and soil: pepper.

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References


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