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Evaluation of carbon, nitrogen, and oxygen isotope ratio measurement data for characterization of organically and conventionally cultivated spring barley (*Hordeum vulgare* L.) grain

L. Buša[1,*], M. Bērtiņš[1], A. Vīksna[1], L. Legzdiņa[2] and D. Kobzarevs[1]

[1]University of Latvia, Faculty of Chemistry, Department of Analytical Chemistry, Jelgavas street 1, LV-1004 Riga, Latvia
[2]Institute of Agricultural Resources and Economics, Priekuli Research Centre, 2 Zinatnes street, LV-4126 Priekuli, Latvia
[*]Correspondence: lauma.busa@lu.lv

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Abstract. With the growing interest of public in the quality of their consumed food, organic produce has been steadily gaining an important place in everyday menus of our society. The growing demand has boosted organic farming and we have also seen the price difference between organic products and their conventional counterparts. It is important to the public to have security, that the food labelled 'organic' has really been grown according to the good practices of organic farming and that it has not received any chemical pesticides, herbicides, and synthetic fertilizers. Stable isotope ratios of crops from different crop management systems can help to answer these questions, as these values depend on the growing conditions, fertilizers used etc. In this study, 10 barley grain samples from conventional and organic crop management systems have been studied. Carbon, nitrogen and oxygen isotope ratios have been determined and the element content in the samples has been calculated. Student's t-test has been performed to evaluate whether the differences between various parameters are significant. For potential clustering and discrimination of organic and conventional grains principal component analysis has been carried out. The PCA showed that no significant clustering can be observed, however the Student's t-test for $\delta^{15}N$ values confirmed that barley grown with green-manure fertilizers are significantly ($p < 0.01$) enriched with the heavier nitrogen isotope. Furthermore, it has been concluded that the total element content of carbon and nitrogen in barley grains does not correlate with the stable isotope ratios and cannot help with discriminating of these samples.

Key words: barley, organic farming, grain, principal component analysis, stable isotopes, stable isotope ratio mass spectrometry.

INTRODUCTION

With the growing world population, an important matter is providing enough food for affordable price. Cereal crops are a major food group that on average provides up to 50% of total everyday calorie intake worldwide (Hawkesford, 2014). To ensure the price remains low enough, the crop yield needs to be elevated. This can be accomplished by developing new ways of fertilization of the land, in order to increase the yield (Zhang et al., 2017). The easiest way to achieve elevated crop yields is to use specifically designed fertilizers that contain all the key nutrients plants need. However, there is a growing demand for organically grown food, especially in wealthy countries. This growing interest in organic food has spiked both organic food production and its prices (Pawlewicz, 2020). From these factors comes also the interest of consumers to have confidence in the fact that the products being marketed as organically grown, really have not received any unwanted chemical fertilizers and pesticides. Therefore, novel analytical techniques are being used to characterize and differentiate between the conventionally and organically grown crops (Laursen et al., 2013).

Barley (*Hordeum vulgare* L.) is a member of the grass family and belongs to one of the most important groups of plants in the world, the *Triticeae* tribe, along with other important crops, such as wheat, rye and triticale (Von Bothmer & Komatsuda, 2011). Barley is one of the top five crops in the world, with the average production reaching over 140 million tons per year (Shewry & Ullrich, 2014). In the EU the barley production has been stable at around 60 million metric tons per year (European Union, 2021). Barley can be considered one of the most versatile crops, as it can be grown in areas with moderate average temperatures and precipitation and is considered to be a very tolerant crop towards drought (Shewry & Ullrich, 2014). Barley can be grown using different farming approaches, with the most popular being conventional farming where synthetic fertilizers, containing nutrients essential for plants, are used and organic farming where soil fertilization can be ensured in various ways. In this study, in the organic crop management system, green manure of legumes has been used. Because of the legumes ability to fix atmospheric nitrogen via roots and their substantial biomass, they are widely used as plants for green manure. The use of green manure can increase crop productivity and growth, along with reducing nitrate-nitrogen leaching risk (Dabin et al., 2016).

A very promising novel analytical technique used for the identification of organic production in the last decades has been stable isotope ratio mass spectrometry (SIRMS), as it allows to evaluate the input of fertilizers on the plants. Analysis of bulk $\delta^{15}N$ in organically grown crops has shown the capability to discriminate between crops from conventional and organic farming (Novak et al., 2019). The $\delta^{15}N$ values in fertilizers depend on their way of production. The synthetic fertilizers have nitrogen isotope ratios close to 0 ‰ since they have been produced from atmospheric nitrogen. While the nitrogen isotope ratios of various organic fertilizers (animal manure, compost etc.) are within the range of 4–6‰. Subsequently, the $\delta^{15}N$ values change in the crops and can be determined using SIRMS (Mihailova et al., 2014). Previous studies have shown that depending on the fertilizer used, the stable isotope ratios of plants reflect the fertilizer $\delta^{15}N$ values (higher ^{15}N content in the fertilizer results in higher nitrogen isotope ratios of plants). However, the previous studies have mainly focused on the differences between synthetic and animal origin fertilizers (Bol et al., 2005; Mihailova et al., 2014). In this research, the organic produce was fertilised with green-manure (peas) that is less enriched with ^{15}N if compared to animal manure, compost, etc.

In this study barley grain samples from conventional and organic crop management systems have been analysed to evaluate the possibility to discriminate between organically and conventionally grown grain based on their stable isotope ratio values and total carbon, nitrogen and oxygen content.

MATERIALS AND METHODS

Description of samples

The barley grain samples were cultivated and collected at Institute of Agricultural Resources and Economics, Priekuli Research Centre, during the year 2017. The field trials were established in two crop management systems - organic and conventional (hereinafter - B and C, respectively) in four replicates, the cultivation plots were positioned in a lattice pattern with an area of 12 m^2 each. Sowing rate was 400 germinating seeds per m^2. The organic cultivation plot used in this study was established in the year 2015 when all use of synthetic fertilizers and pesticides was discontinued. In 2015 the organic cultivation plot was left fallow, followed by cultivation of rye as the forecrop and peas for green manure in 2016. No other types of fertilizers have been used in the organic cultivation plot. The conventional cultivation plot has been established for several decades. To characterise the soil, the pH_{KCl} was measured, available potassium and phosphorus content assessed, and soil granulometric composition determined. The soil characteristics, forecrops and applied fertilizers are summarized in Table 1.

Table 1. Summary of soil characteristics and fertilizers used in the conventional and organic barley farming experimental fields at Priekuli Research Centre

Parameter	Crop management system	
	Conventional	Organic
pH_{KCl}	5.3	5.9
Organic matter content, %	1.8	2.3
Available potassium (K_2O), mg kg^{-1}	143	167
Available phosphorus (P_2O_5), mg kg^{-1}	120	177
Soil group, granulometric composition	Sod-podzolic, sandy loam	Sod-podzolic, sandy loam
Forecrop	Potatoes	Peas for green manure
Complex fertilizer	N15-P15-K15	–
Nitrogen fertilizer	N33-P3	–
Nutrient doses in pure substance, kg ha^{-1}	N108-P70-K66	–

Altogether 5 conventionally grown barley samples (BarlA-C to BarlE-C) and 5 organically grown barley samples (BarlA-B to BarlE-B) were analysed, where A, B, C, D, E is the same genotype (variety, mixture or population). A pooled sample of 100 grams was obtained by mixing equal parts of barley grains collected from each of four subplots of the crop management system. Before analysis, the samples were air-dried at 60 °C for 24 hours until constant mass of the samples was achieved. Five grams of each barley grain sample (including husk and bran) were dry ground using mixer mill (MM-400, Retsch, Germany) with steel balls at the frequency of 15 s^{-1} for 5 minutes.

Stable isotope analysis

All samples were analysed by elemental analyser-continuous flow-stable isotope ratio mass spectrometry (EA-CF-SIRMS) using a Nu Horizon (Nu Instruments, United Kingdom) stable isotope mass spectrometer interfaced with an EA3024 (EuroVector, Italy) elemental analyser to determine carbon and nitrogen isotope ratios. For the determination of oxygen isotope ratios, the elemental analyser was additionally interfaced with HT-PyrOH unit (EuroVector, Italy).

1 mg of the milled barley grains were weighed into a small tin capsule (5 mm × 3.5 mm). Afterwards, the capsule was folded and compressed to a ball shape, in order to contain the sample and minimise air presence. An auto-sampler was used to introduce the prepared sample into elemental analyser. The stable carbon, nitrogen and oxygen isotopic composition were measured relative to the Vienna Pee Dee Belemnite (VPDB), atmospheric air (AIR) and Vienna Standard Mean Ocean Water (VSMOW) standards respectively. Each sample was analysed in duplicates and the average is reported.

The reference materials used for $\delta^{13}C$ and $\delta^{15}N$ measurements were L-glutamic acid USGS-40 ($\delta^{13}C_{VPDB}$ = -26.39‰; $\delta^{15}N_{AIR}$ = -4.52‰) and USGS-41 ($\delta^{13}C_{VPDB}$ = 37.63‰; $\delta^{15}N_{AIR}$ = 47.57‰), and benzoic acid IAEA-601 ($\delta^{18}O_{VSMOW}$ = 23.14‰) and IAEA-602 ($\delta^{18}O_{VSMOW}$ = 71.28‰) for the $\delta^{18}O$ analysis.

Determination of carbon, nitrogen and oxygen content

In order to determine the weight fraction of carbon, nitrogen and oxygen in barley grain samples, a calibration curve was constructed using the laboratory standards with known elemental composition: L-glutamic acid (w_C% = 40.82%; w_N% = 9.52%) and sucrose (w_O% = 51.41%). The mass of an element in a sample is directly correlated with the peak area of gas produced during the combustion (CO_2, N_2 and CO for carbon, nitrogen and oxygen respectively). Calibration curves and their respective equations are summarized in Fig. 1.

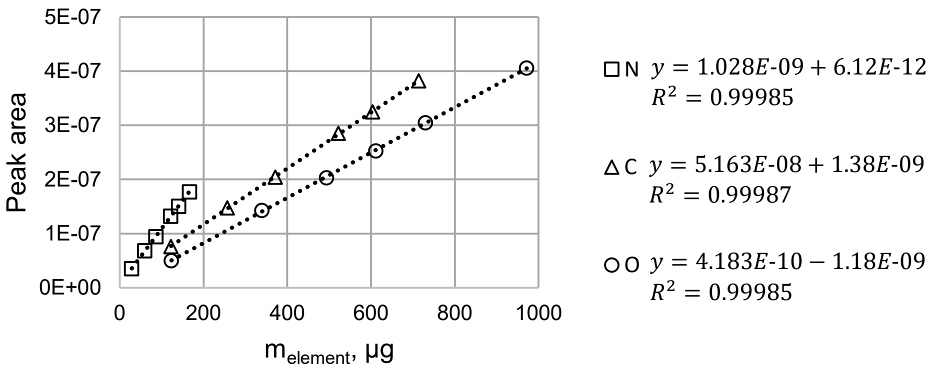

Figure 1. Calibration curves and their respective equations for determination of carbon, nitrogen and oxygen weight fractions in cereal grains using data from EA-CF-IRMS.

Student's t-tests (two-pair two-tailed t-test) were conducted to determine whether the differences of various parameters are significant. The principal component analysis (PCA) was applied to the obtained results using CAT software (Chemometric Agile Tool) in order to describe the probable clustering of samples from different crop management systems.

RESULTS AND DISCUSSION

The obtained values of stable isotope ratios and weight fractions of carbon, nitrogen and oxygen content in barley samples from different crop management systems are summarized in Tables 2 and 3.

Table 2. Stable carbon, nitrogen and oxygen isotope ratios (δ) of barley grain from conventionally and organically cultivated land

Crop management system	Sample	$\delta^{13}C_{VPDB}$, ‰	$\delta^{15}N_{AIR}$, ‰	$\delta^{18}O_{VSMOW}$, ‰
Conventional	BarlA-C	-28.24 ± 0.03	2.36 ± 0.16	13.0*
	BarlB-C	-28.33 ± 0.08	2.28 ± 0.06	13.47 ± 0.06
	BarlC-C	-28.75 ± 0.04	1.6 ± 0.3	14.6 ± 1.5
	BarlD-C	-28.72 ± 0.09	2.03 ± 0.06	16.0 ± 0.6
	BarlE-C	-27.99 ± 0.03	2.0 ± 0.3	16.24 ± 0.17
Organic	BarlA-B	-27.464 ± 0.026	4.01 ± 0.16	17.21 ± 0.19
	BarlB-B	-28.499 ± 0.017	3.63 ± 0.06	16.47 ± 0.16
	BarlC-B	-28.48 ± 0.02	3.27 ± 0.06	17.8 ± 0.4
	BarlD-B	-28.26 ± 0.05	2.7 ± 0.4	17.0 ± 0.4
	BarlE-B	-28.190 ± 0.007	3.5 ± 0.2	17.8 ± 0.4

mean ± SD, ‰; * – a single measurement was performed.

The $\delta^{13}C$ values show that for barley grain samples from both conventional and organic farming systems the values are within the range from -28.75‰ to -27.46‰ and do not differ significantly for grains from different crop management systems ($p > 0.05$). That corresponds with previous research stating that barley is a C_3 crop and the $\delta^{13}C$ values for this type of plants can vary from -37‰ to -20‰ (Kohn, 2010). The reason for the broad $\delta^{13}C$ value range is the effect of different factors on the photosynthesis taking place in C_3 plants, for example, the water use efficiency, isotopic composition of the atmospheric CO_2 etc. (Vogado et al., 2020). Therefore, the $\delta^{13}C$ values alone are not suitable for discrimination between barley grains from different crop management systems.

Table 3. The nitrogen, carbon, and oxygen weight fraction in barley grain from conventionally and organically cultivated land

Crop management system	Sample	w_C, %	w_N, %	w_O, %
Conventional	BarlA-C	41.6 ± 1.0	1.25 ± 0.05	7.8*
	BarlB-C	43.0 ± 1.3	1.57 ± 0.04	40.3 ± 1.3
	BarlC-C	42.0 ± 0.4	1.3 ± 0.03	42.5 ± 0.5
	BarlD-C	44 ± 4	1.6 ± 0.2	42 ± 2
	BarlE-C	41 ± 2	1.53 ± 0.16	42.5 ± 1.4
Organic	BarlA-B	42.0 ± 0.7	1.02 ± 0.18	41.7 ± 1.3
	BarlB-B	41.0 ± 0.4	1.18 ± 0.06	43.4 ± 1.0
	BarlC-B	41.0 ± 0.4	1.15 ± 0.02	40 ± 2
	BarlD-B	42.9 ± 0.5	1.21 ± 0.19	41.2 ± 1.7
	BarlE-B	42.1 ± 0.2	1.29 ± 0.02	41.55 ± 0.04

mean ± SD, %; * a single measurement was performed.

When considering the $\delta^{15}N$ values measured in this study, a difference between the mean values for conventionally grown (2.1 ± 0.3‰) and organically grown barley (3.4 ± 0.5‰) grains can be observed. When performing the Student's t-test on $\delta^{15}N$ values of organic and conventional barley grains, the difference shows to be significant ($p < 0.01$). The main reason for the difference is the types of fertilizers used. For the conventional crop management system, a synthetic complex fertilizer (NPK) was used, while for the organic crop management system the nitrogen content in soil was increased with the help of green manure - peas (Table 1), which is a plant of the legume family with high natural capacity to bind atmospheric nitrogen due to the rhizobia bacteria hosted on the roots of these plants. Previous research has shown that synthetic fertilizers are more depleted in ^{15}N isotope if compared to the fertilizers that can be used in organic farming systems (Bateman & Kelly, 2007). However, it should be taken into consideration that legumes used as green-manure bind the atmospheric nitrogen and therefore their stable nitrogen isotope ratios should be close to the $\delta^{15}N_{AIR}$ of 0‰ (Choi et al., 2017). Our study shows that the mean δ^{15}N values of grains fertilized with green manure are lower than the values of other fertilizers permitted in organic cultivation systems (Bateman & Kelly, 2007). Therefore, additional research should be carried out to understand the fractionation processes taking place during the atmospheric nitrogen fixation and transfer to different parts of cereal crops. The plot of organic crop management system used in this study was established only two years prior to the harvest of samples and therefore the effects of green-manure use might be hindered by the effect of previous land use.

Stable oxygen isotope ratio in plants can change due to various reasons, for example, the variation of source water δ^{18}O values, the different leaf water evaporation rate, etc (Barbour et al., 2005). For the barley grain from different crop management systems the mean δ^{18}O values are higher for organically grown grain (17.3 ± 0.6‰) if compared to the conventionally grown (14.7 ± 1.4‰). When performing Student's *t*-test on δ^{18}O values of organic and conventional barley grains, the difference shows to be not significant ($p > 0.05$). Mainly the non-significant difference can be explained by the natural oxygen isotope abundance that is related to the global water cycle (Gat, 2010). Stable oxygen isotope ratio values in cereal grains are influenced by many factors, the geographic location and water contribution being more significant than fertilizer, growing condition and other effects. As the organic and conventional plots used in this study were situated near each other, the δ^{18}O values are not significantly different.

Fig. 2. displays the relationship between nitrogen and oxygen or carbon stable isotope ratio values. As discussed previously, the grains from different crop management systems can be distinguished based on the δ^{15}N values, while δ^{18}O and δ^{13}C values do not depend on the type of fertilizers used in this study.

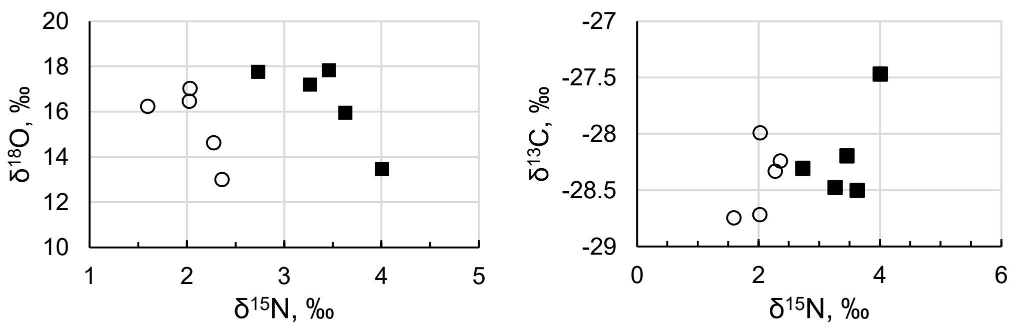

Figure 2. Relationship between $\delta^{15}N/\delta^{18}O$ and $\delta^{15}N/\delta^{13}C$ values of barley grains from different crop management systems (white circles - conventional, black squares - organic).

When evaluating the possible discrimination between organic and conventional barley grain samples in this study, it is important to bear in mind that organic produce is characterized not only by the use of appropriate fertilizers but also by the absence of any pesticides used. The results of this study classify the crop management system used only based on the fertilizers. In case of investigation of an unknown sample, additional methods for pesticide residue testing must be applied (Laursen et al., 2013).

When considering the total carbon, nitrogen and oxygen content in the barley samples, it clearly shows that the element content is not dependent on whether the grains have been grown using conventional or organic fertilizer. The carbon content in barley samples varies between 41.0–43.0%, the nitrogen content between 1.0–1.6‰ and the oxygen content between 37.8–43.4%.

To differentiate barley grain from conventional and organic crop management systems, principal component analysis (PCA) was carried out, based on the stable isotope ratios and element weight fractions in the samples analysed. The results of PCA are shown in Fig. 3.

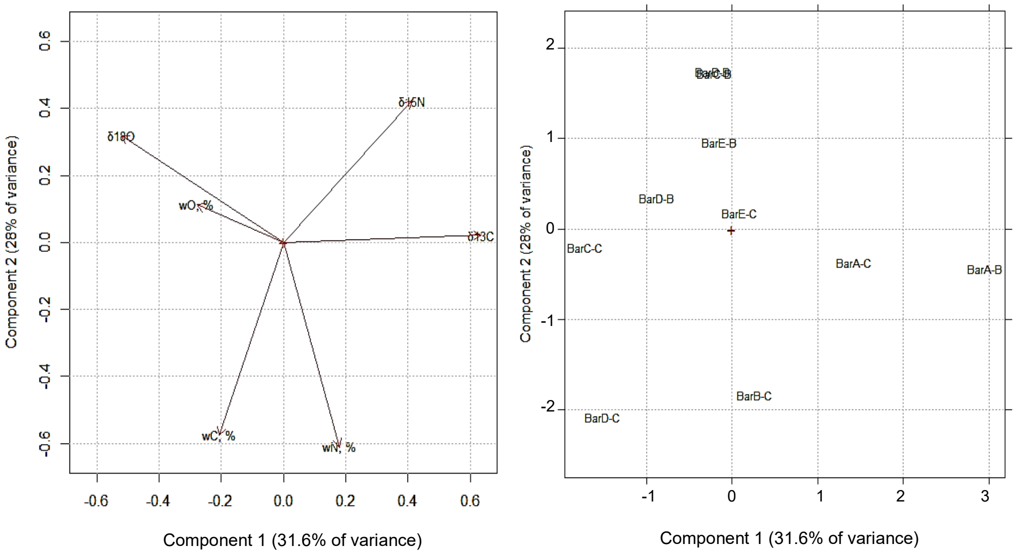

Figure 3. Two-dimensional PCA Loading and Score plots for conventional and organic barley grain, total variance explained by PC1 and PC2 is 59.6%.

The loading plot of PCA shows that 31.6% of the total variance is explained by principal component 1 (PC1) and 28% by principal component 2 (PC2). The highest loadings along the PC1 are the ones of δ^{13}C and δ^{18}O, while along the PC2 the highest ones are of δ^{15}N, δ^{18}O and total nitrogen and carbon content. The loading plot also gives us insight into the correlation between the variables. The δ^{18}O values are positively correlated with total oxygen content in the samples, while in esvery other case - both for carbon and nitrogen, the stable isotope ratios of these elements are not correlated with the total content of the element in the barley grain samples.

In the score plot of the PCA four out of five organically grown barley grain samples are located closer to each other (Fig. 2). These samples are distributed along the axis of PC2 which means that the main variables to be considered when distinguishing between conventional and organic barley grains are the stable isotope ratios of oxygen and nitrogen, as discussed before. One of the organically grown barley grain samples BarlA-B is situated to the right, along the axis of PC1. The reason for this is the different $\delta^{13}C$ value of this sample. The small number of analysed samples and the weak clustering of both organic and conventional samples indicates that stable isotope ratios and total element content do not provide sufficient data for discrimination between barley grains produced using synthetic and green-manure fertilizers. Additional variables must be evaluated for better distinguishing between conventional and organic barley grain samples.

CONCLUSIONS

The study showed that $\delta^{13}C$ of barley grains do not differ between samples from different crop management systems, agreeing with previous research that fractionation of stable carbon isotopes is not dependent on the fertilization of the cereals, but only dependent on the photosynthetic pathway characteristic for barley.

The stable isotope ratios of nitrogen and oxygen vary depending on the crop management system of barley. The barley grains from organic (green-manure) crop management system have significantly ($p < 0.01$) higher $\delta^{15}N$ values than the grains from conventional (synthetic fertilizer) crop management system.

The PCA of obtained data showed that with two principal components 59.6% of the total variance can be explained. When using principal components 1 and 2, the main loading to components is from $\delta^{13}C$ and $\delta^{18}O$ for the component 1 and from $\delta^{15}N$, $\delta^{18}O$ and total nitrogen and carbon content for component 2, respectively.

When plotting PC1 against PC2, four out of five organic barley samples are located closer to each other, but no real clustering is observed. The authors conclude that additional variables on a bigger sample group should be measured and incorporated in the multivariate model to improve the ability to distinguish between barley grains from different crop management systems.

REFERENCES

Barbour, M.M., Cernusak, L.A. & Farquhar, G.D. 2005. Factors Affecting the Oxygen Isotope Ratio of Plant Organic Material. In *Stable Isotopes and Biosphere - Atmosphere Interactions: Processes and Biological Controls*, edited by Flanagan, L.B., Ehleringer, J.R. and Pataki, D.E. Elsevier, pp. 9–28.

Bateman, A.S. & Kelly, S.D. 2007. Fertilizer Nitrogen Isotope Signatures. *Isotopes in Environmental and Health Studies* **43**(3), 237–47. doi: 10.1080/10256010701550732.

Bol, R., Eriksen J., Smith, P., Garnett, M.H., Coleman, K. & Christensen, B.T. 2005. The Natural Abundance of ^{13}C, ^{15}N, ^{34}S and ^{14}C in Archived (1923-2000) Plant and Soil Samples from the Askov Long-Term Experiments on Animal Manure and Mineral Fertilizer. *Rapid Communications in Mass Spectrometry* **19**(22), 3216–26. doi: 10.1002/rcm.2156.

Von Bothmer, R. & Komatsuda, T. 2011. Barley Origin and Related Species. In *Barley: Production, Improvement, and Uses*. Wiley-Blackwell, pp. 14–62.

Choi, W.J., Kwak, J.H., Lim, S.S., Park, H.J., Chang, S.X., Lee, S.M., Arshad M.A., Yun, S.I. & Kim, H.Y. 2017. Synthetic Fertilizer and Livestock Manure Differently Affect Δ^{15}N in the Agricultural Landscape: A Review. *Agriculture, Ecosystems and Environment* **237**, 1–15. doi: 10.1016/j.agee.2016.12.020

Dabin, Z., Pengwei, Y., Na, Z., Changwei, Y., Weidong, C. & Yajun, G. 2016. Contribution of Green Manure Legumes to Nitrogen Dynamics in Traditional Winter Wheat Cropping System in the Loess Plateau of China. *European Journal of Agronomy* **72**, 47–55. doi: 10.1016/j.eja.2015.09.012.

European Union (EU-27) Barley Production by Year (1000 MT). *https://www.indexmundi.com/agriculture/?country=eu&commodity=barley&graph=production.* Accessed 22.02.2021.

Gat, J.R. 2010. The Isotopes of Oxygen and Hydrogen. In *Isotope Hydrology: A Study of the Water Cycle*, edited by Wei, T.K. Imperial College Press, pp. 9–21.

Hawkesford, M.J. 2014. Reducing the Reliance on Nitrogen Fertilizer for Wheat Production. *Journal of Cereal Science* **59**(3), 76–83. doi: 10.1016/j.jcs.2013.12.001

Kohn, M.J. 2010. Carbon Isotope Compositions of Terrestrial C3 Plants as Indicators of (Paleo)Ecology and (Paleo)Climate. *Proceedings of the National Academy of Sciences of the United States of America* **107**(46), 19691–95. doi: 10.1073/pnas.1004933107

Laursen, K.H., Mihailova, A, Kelly, S.D., Epov, V.N., Berail, S., Schjoerring, J.K., Donard, O.F.X., Larsen, E.H., Pedentchouk, N., Marca-Bell, A.D., Halekoh, U., Olesen, J.E. & Husted, S. 2013. Is It Really Organic? - Multi-Isotopic Analysis as a Tool to Discriminate between Organic and Conventional Plants. *Food Chemistry* **141**(3), 2812–20. doi: 10.1016/j.foodchem.2013.05.068

Mihailova, A., Pedentchouk, N. & Kelly, S.D. 2014. Stable Isotope Analysis of Plant-Derived Nitrate – Novel Method for Discrimination between Organically and Conventionally Grown Vegetables. *Food Chemistry* **154**, 238–45. doi: 10.1016/j.foodchem.2014.01.020

Novak, V., Adler, J., Husted, S., Fromberg, A. & Laursen, K.H.. 2019. Authenticity Testing of Organically Grown Vegetables by Stable Isotope Ratio Analysis of Oxygen in Plant-Derived Sulphate. *Food Chemistry* **291**, 59–67. doi: 10.1016/j.foodchem.2019.03.125

Pawlewicz, A. 2020. Change of Price Premiums Trend for Organic Food Products: The Example of the Polish Egg Market. *Agriculture (Switzerland)* **10**(2), 14–18. doi: 10.3390/agriculture10020035

Shewry, P.R. & Ullrich, S.E. 2014. *Barley : Chemistry and Technology, Second Edition*. Elsevier Science & Technology.

Vogado, N.O., Winter, K., Ubierna, N., Farquhar, G.D. & Cernusak, L.A. 2020. Directional Change in Leaf Dry Matter δ^{13}C during Leaf Development Is Widespread in C3 Plants. *Annals of Botany* **126**(6), 981–90. doi: 10.1093/aob/mcaa114

Zhang, M., Tian, Y., Zhao, M., Yin, B. & Zhu, Z.. 2017. The Assessment of Nitrate Leaching in a Rice–Wheat Rotation System Using an Improved Agronomic Practice Aimed to Increase Rice Crop Yields. *Agriculture, Ecosystems and Environment* **24**, 100–109. doi: 10.1016/j.agee.2017.03.002