A PILOT STUDY (FIRST RESULTS FROM VINTAGE 2015): ISOTOPE INVESTIGATION OF APRICOTS FROM THE WACHAU/LOWER AUSTRIA ("WACHAUER MARILLE") TO CONTROL THE DECLARED GEOGRAPHIC ORIGIN

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25 apricot samples were investigated, 19 of them from the Austrian Wachau region and 6 samples from other areas or of unknown/uncertain geographic origin. The isotope composition of the elements hydrogen (H), carbon (C), nitrogen (N) and oxygen (O) of fruit pulp (H, C, N, O), fruit stone (H, C, O) and fruit juice (O) was analysed to find appropriate parameters for the differentiation of different geographic origin. The first results of a pilot study are presented, showing the successful discrimination of apricots from the Wachau from samples of other origin, and giving promising prospects for the possibility of control of apricots with declared origin of the Lower Austrian Wachau region.
Keywords: origin, carbon, nitrogen, hydrogen, oxygen, apricot, water, drought stress, fertilizer

Pilotstudie (erste Ergebnisse zur Ernte 2015): Isotopenuntersuchungen von Aprikosen aus der Wachau/Niederösterreich ("Wachauer Marillen") zur Kontrolle der deklarierten Herkunft. Von den 25 Marillen-(Aprikosen-)proben, die im Rahmen der Studie untersucht wurden, stammen 19 authentische Proben aus der Wachau. Sechs Proben haben andere Herkunft oder sind von unsicherer Herkunft. Die Isotopenverhältnisse der Elemente Wasserstoff (H), Kohlenstoff (C), Stickstoff (N) und Sauerstoff (O) wurden im Fruchtfleisch (H, C, N, O), Marillenkern (H, C, O) und im Marillensaft (O) untersucht, um geeignete Parameter für die Unterscheidung von Marillen verschiedener geografischer Herkunft zu finden. Die ersten Ergebnisse dieser Pilotstudie werden hier präsentiert und zeigen eine erfolgreiche Unterscheidung zwischen Wachauer und "nicht-Wachauer" Proben. Sie geben einen vielversprechenden Ausblick für eine mögliche Kontrolle der deklarierten Herkunft von Marillen, deren Herkunft als "aus der Wachau stammend" angegeben wurde.
Schlagwörter: Herkunft, Kohlenstoff, Stickstoff, Wasserstoff, Sauerstoff, Marille, Wasser, Trockenstress, Dünger

Consumers are willing to pay higher prices for products of certain, especially valued, geographic origin (Chung et al., 2009). Thus, as the incorrect labelling of geographic origin can increase profit significantly, the control of declaration of geographic origin is necessary. Conventionally, such controls are done by checking the accompanying paperwork of foodstuff, however, it has been shown that such documents can be mixed up, exchanged or manipulated. The preferred method of control of geographic origin is the stable isotope analysis, and already numerous studies have been carried out on a large variation of food, demonstrating its high potential as control method (e.g. Rossmann et al., 2000; Camin et al., 2007; Schellenberg et al., 2010; Horacek et al., 2010). However, to my knowledge, so far no study on geographic origin of apricots has been published.

The principle of application of stable isotope analysis for control of geographic origin is based on the influence of the environmental conditions on the isotope values of the investigated elements, as hydrogen, carbon, nitrogen and oxygen. With respect to plants, their carbon isotope values are mainly influenced by drought stress (limited availability of water; Ballantyne et al., 2011), plant metabolism (Suits et al., 2005) and available CO_2. Nitrogen isotopes are dominantly influenced by the way of uptake of nitrogen (leguminous or non-leguminous plants) and the isotopic composition of plant-available nitrogen (Bateman and Kelly, 2007). Hydrogen and oxygen isotopes are mainly influenced by the isotopic composition of the plant-available water (Bowen and Revenaugh, 2003), air humidity and plant transpiration (Krull et al., 2006).

The apricots grown in the Wachau (Lower Austria) region (named "Wachauer Marille") are famous beyond Austria and highly sought-after. They have been listed as PDO product by the EU. With respect to the EU-listing the Wachau area was defined by the municipalities of Aggsbach-Markt, Albrechtsberg, Bergern im Dunkelsteinerwald, Droß, Dürnstein, Furth, Gedersdorf, Krems, Maria Laach, Mautern, Mühldorf, Paudorf, Rohrendorf bei Krems, Rossatz-Arnsdorf, Senftenberg, Spitz, Stratzing, Weinziel am Wald, Weißenkirchen, Schönbühel-Aggsbach and Emmersdorf (http://www.wachauermarille.at/marille.php; accessed 04. 09. 2017). As repeated suspicion that significant amounts of apricots that have been grown elsewhere are sold under the label of "Wachau area geographic origin" has been voiced (e. g. noe.orf.at; accessed 31. 05. 2017; Zahrl, 2015), there exists the need to control the declared origin of the Wachau area apricot. However, despite claims (e. g. Sailer, 2015), no study about the control of geographic origin of the "Wachauer Marille" has been published to my knowledge so far. Earlier tries to control the geographic origin of the Wachauer Marille (unpublished material by the author) have shown that the differentiation of apricots from the Wachau area from other regions is very challenging, as the Wachau area is geologically, geographically and hydrologically very diverse and complicated. The pilot-study presented below aims to identify parameters for the differentiation of apricots from the Wachau area from apricots of other geographic origin.

MATERIALS AND METHODS

25 apricot samples of about 1 kg weight each (amounts to about 10 to 25 fruits) were investigated in this project. 19 samples were collected by handpicking fruits from different trees in the respective orchard in 2015. One sample comes from one tree in Wieselburg (Lower Austria), one sample from one tree in Amstetten (Lower Austria) and one sample was bought at the local market in Szombathely (Hungary) with declared origin Hungary. Two additional samples were bought in the supermarket in Wieselburg, one with declared origin France and one without geographic indication, but assumed origin from the Wachau area. One sample was bought at a small apricot vendor stall in the Wachau area.

The fruit samples were packed in plastic bags, which were tightly sealed and cooled until reaching the laboratory the same day, where the samples were freeze-stored.

SAMPLE PREPARATION

The apricot samples were defrosted and the stones removed. The apricot pulp was homogenized in a kitchen blender and afterwards centrifuged (Multifuge 1 L-R; Heraeus, Hanau, Germany) to separate pulp and juice. The juice was kept frozen until analysis. The pulp was freeze-dried and homogenized with mortar and pestle. Small aliquots of the samples were weighed in small tin and silver capsules for C- and N- and H- and O-isotope analysis, respectively. The apricot stones were cleaved to remove the seed. Afterwards all stones of each sample were homogenized in a ball-mill (MM200; Retsch, Haan, Germany), but for a few selected samples each stone was milled separately for individual analysis. Small aliquots of the apricot-stone powder were weighed in tin and silver capsules for C- and H- and O-isotope measurements, respectively.

For C- and N-isotope analysis the sample aliquots wrapped in tin foil were inserted in a zero-blanc autosampler (Thermo, Bremen, Germany) mounted on a Thermo elemental analyser (Bremen, Germany). During measurement, as described e. g. in Horacek et al. (2010), the samples are burned at 900 °C and the produced gases are carried by He-continuous flow through a gas-chromatic column, were the gas phases are separated and via a ConFlo (Thermo, Bremen, Germany) into a Delta V isotope ratio mass spectrometer (IRMS, Thermo, Bremen, Germany).

For H- and O-isotope analysis the sample aliquots in the silver capsules are inserted in a zero-blanc autosampler (Thermo, Bremen, Germany) mounted on a Thermo (Bremen, Germany) elemental analyser in pyrolysis mode. During measurement, as described e. g. in Horacek et al. (2015), the samples are pyrolized at 1450 °C and the produced gases are carried by He-continuous-flow through a gas-chromatographic column, where the gases are separated and via a ConFlo (Thermo, Bremen, Germany) into a Delta V IRMS (Thermo, Bremen, Germany). There, the isotope ratio is analysed and presented in the conventional delta-notation (δ) in ‰ deviation to an international accepted reference, V-PDB (Vienna-PeeDee Belemnite) for carbon, N-Air for nitrogen, V-SMOW (Vienna Standard Mean Ocean Water) for hydrogen and oxygen. Reproducibility is better than ± 0,2 ‰ for carbon and nitrogen isotopes (1 σ), better than ± 3 ‰ for hydrogen and ± 0,4 ‰ for oxygen isotopes (1 σ). For partially exchangeable hydrogen measurements were corrected for hydrogen isotopes by measurement of cellulose standard IAEA C3 (δ^2H: -33,5 ‰ V-SMOW, δ^{18}O: 32,5 ‰ V-SMOW). All apricot pulp and stone samples were measured for H- and O-isotopes within a one-week interval, thus resulting in identical influence of ambient laboratory conditions.

For measurement of O-isotopes of the water in the apricot juices the juice samples are biologically inactivated by adding small amounts of benzoe-acid. 0.5 ml of juice are filled into small glass vials, which are inserted in a heating block of a Gas bench (Thermo, Bremen, Germany) set to 20 °C. During measurement the headspace of the vials are flushed with a He-CO_2 gas-mixture and the samples equilibrate with the CO_2-gas for 24 hours. Afterwards the gas is flushed by He-continuous flow into the Delta V IRMS (Thermo, Bremen, Germany). There the O-isotope ratio is analysed and presented in δ-notation as explained above. Reproducibility is better than ± 0,2 ‰ for oxygen isotopes (1 σ).

RESULTS

The results are listed in Table 1 and illustrated in Figures 1 to 8 ("Wachau (shop)": apricots bought in a supermarket indicated as of Wachau origin; "vendor booth Wachau": apricots bought from a street vendor booth in the Wachau). Summing up, the results are as follows:

APRICOT PULP

The apricot samples from the Wachau range from -26,4 to -23,2 ‰ for carbon isotopes versus V-PDB, from 1,0 to 4,8 ‰ for nitrogen isotopes versus N-Air, from -54 to -36 ‰ for hydrogen and 28,6 to 31,3 ‰ for oxygen, the latter both versus V-SMOW. The isotope signature of the Hungarian sample is -26,3 ‰, -0,5 ‰, -41 ‰ and 31,0 ‰ for carbon, nitrogen, hydrogen and oxygen, respectively.

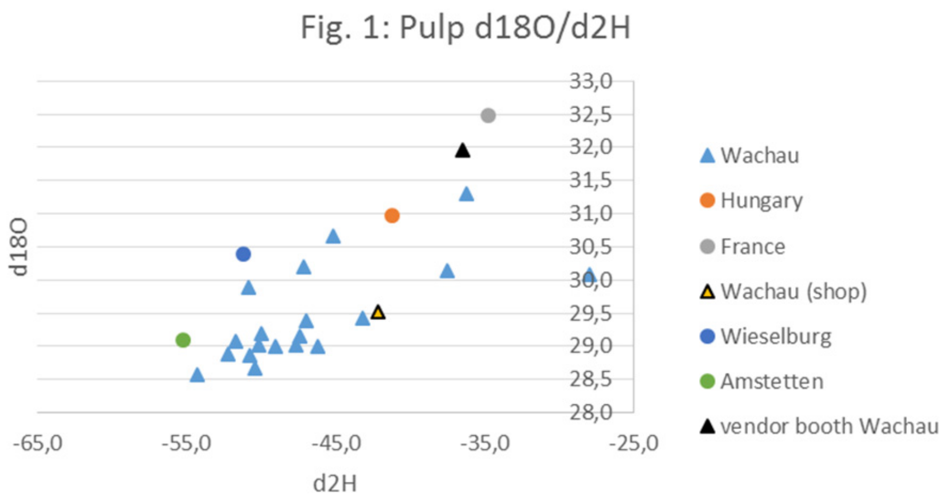

Fig. 1: H- versus O-isotopes of pulp samples

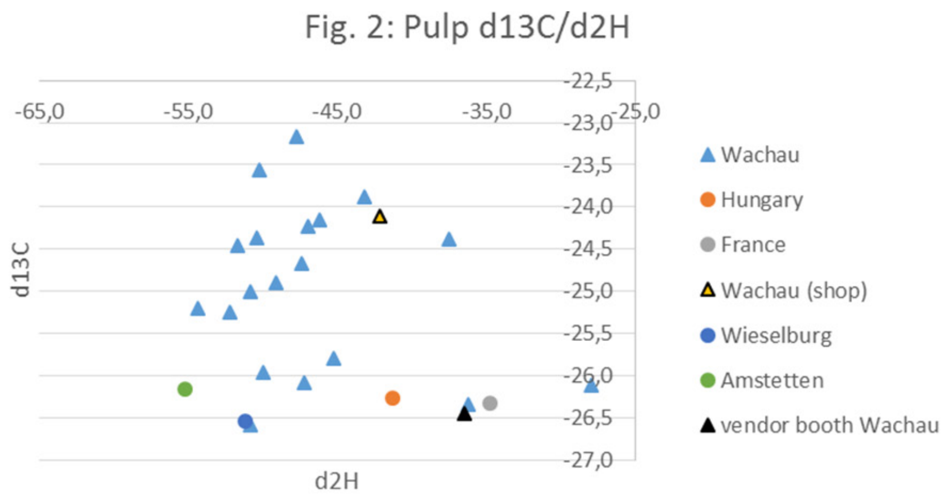

Fig. 2: H- versus C-isotopes of pulp samples

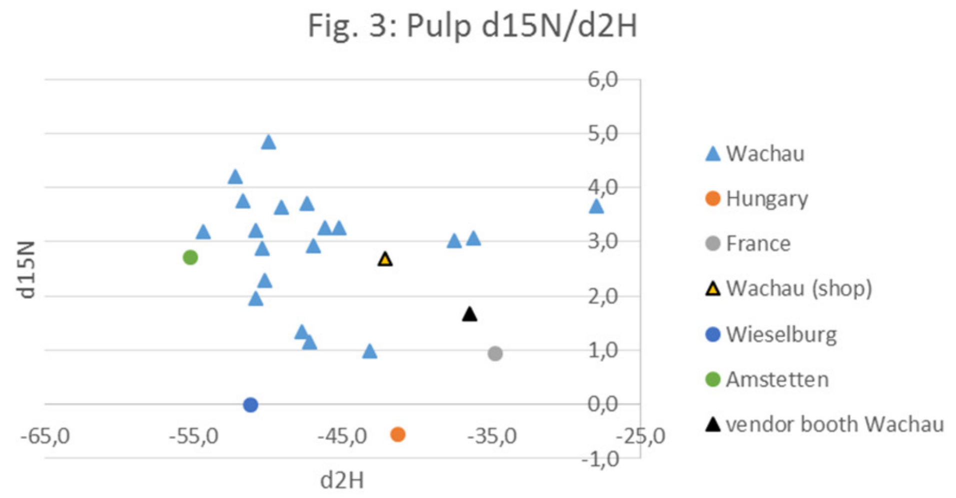

Fig. 3: H- versus N-isotopes of pulp samples

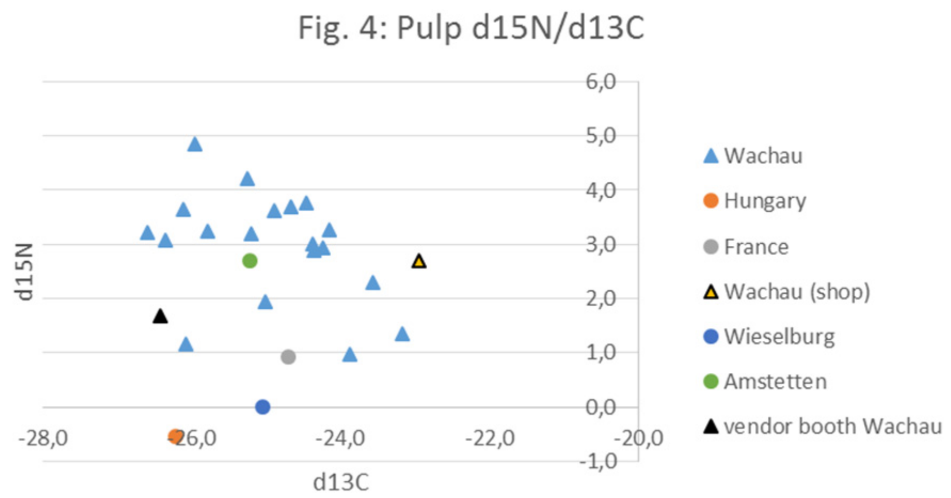

Fig. 4: C- versus N-isotopes of pulp samples

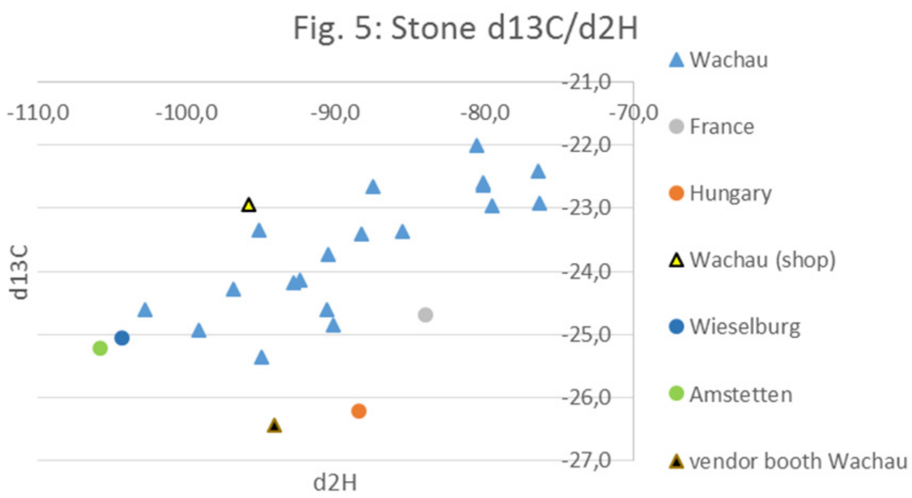

Fig. 5: H- versus C-isotopes of apricot stone samples

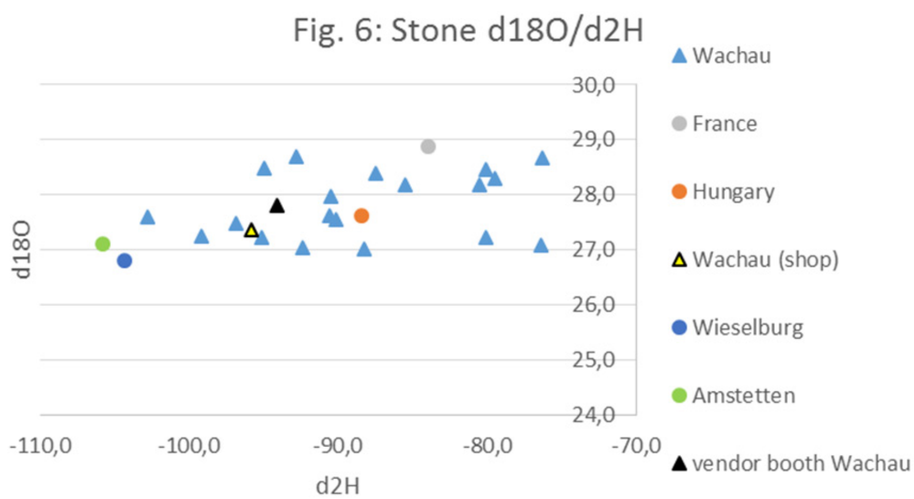

Fig. 6: H- versus O-isotopes of apricot stone samples

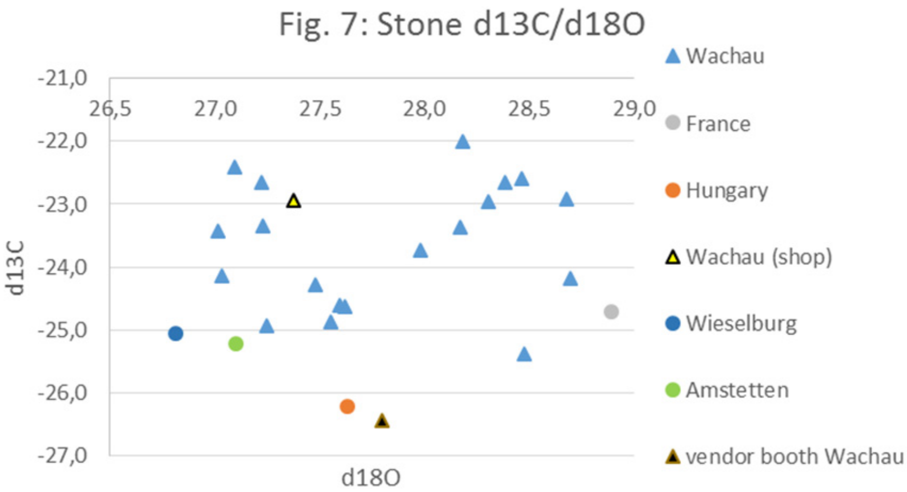

Fig. 7: C- versus O-isotopes of apricot stone samples

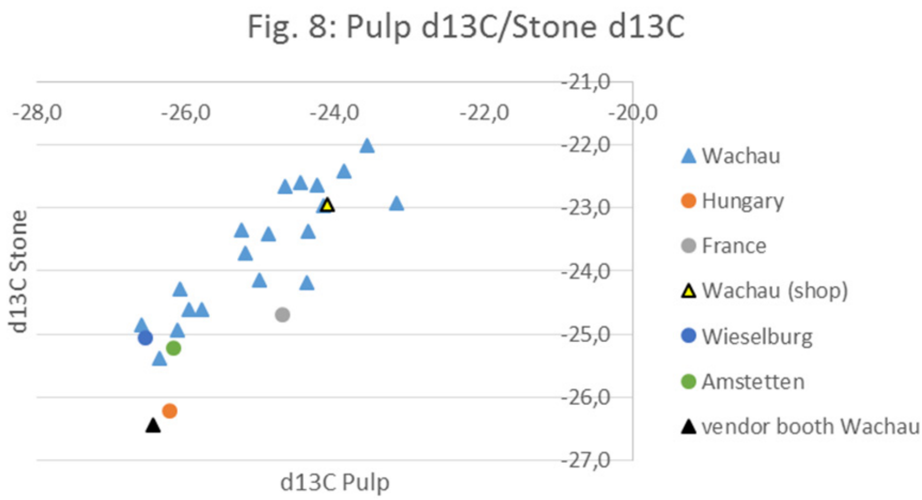

Fig. 8: C-isotopes of apricot pulp versus stone samples

The French sample has the isotope values -26,3 ‰, 0,9 ‰, -35 ‰ and -32,5 ‰ for carbon, nitrogen, hydrogen and oxygen, respectively. The sample from Wieselburg (Lower Austria) has an isotope signature of -26,5 ‰, 0,0 ‰, -51 ‰ and 30,4 ‰ for carbon, nitrogen, hydrogen and oxygen, respectively. The sample from Amstetten shows the values -26,2 ‰, 2,7 ‰, -55 ‰ and 29,1 ‰ for carbon, nitrogen, hydrogen and oxygen, respectively. The apricot sample without geographic indication possesses the values -24,1 ‰, 2,7 ‰, -42 ‰ and 29,5 ‰ for carbon, nitrogen, hydrogen and oxygen, respectively. The sample from the vendor stall in the Wachau has the following isotope pattern: -26,4 ‰, 1,7 ‰, -36 ‰ and 32 ‰ for carbon, nitrogen, hydrogen and oxygen, respectively.

APRICOT STONES

The apricot samples from the Wachau range from -25,4 to -22,0 ‰ for carbon isotopes versus V-PDB, from -103 to -76 ‰ for hydrogen and 27,0 to 29,0 ‰ for oxygen, the latter both versus V-SMOW. The isotope signature of the Hungarian sample is -26,2 ‰, -94 ‰ and 27,8 ‰ for carbon, hydrogen and oxygen, respectively. The French sample has the isotope values -24,7 ‰, -84 ‰ and 28,9 ‰ for carbon, hydrogen and oxygen, respectively. The sample from Wieselburg (Lower Austria) has an isotope signature of -25,0 ‰, -104 ‰ and 26,8 ‰ for carbon, hydrogen and oxygen, respectively. The sample from Amstetten shows the values -25,2 ‰, -106 ‰ and 27,1 ‰ for carbon, hydrogen and oxygen, respectively. The apricot sample without geographic indication possesses the values -22,9 ‰, -96 ‰ and 27,4 ‰ for carbon, hydrogen and oxygen, respectively. The sample from the vendor stall in the Wachau has the following isotope pattern: -26,4 ‰, -94 ‰ and 27,8 ‰ for carbon, hydrogen and oxygen, respectively. For two samples the stones were separated and analysed individually to investigate the range within a sample. Sample 17 gives results varying within 2,1 ‰ for carbon isotopes, 8 ‰ for hydrogen isotopes and 2,5 ‰ for oxygen isotopes. Sample 18 gives results varying within 2,1 ‰ for carbon isotopes, 8 ‰ for hydrogen isotopes and 3,8 ‰ for oxygen isotopes.

Table 1: Isotope results of the investigated apricot samples (PU = pulp, ST = stone, J = juice)

LaborNr	Sample	Origin	δ^{13}C-PU	δ^{15}N-PU	δ^{2}H-PU	δ^{18}O-PU	δ^{13}C-ST	δ^{2}H-ST	δ^{18}O-ST	δ^{18}O-J
15-0453	1	Wachau	-26,1	3,6	-28,0	30,1	-24,9	-99	27,2	4,5
15-0454	2	Wachau	-26,4	3,1	-36,2	31,3	-25,4	-95	28,5	4,6
15-0455	3	Wachau	-26,6	3,2	-50,8	29,9	-24,9	-90	27,6	2,4
15-0456	4	Wachau	-24,2	2,9	-47,0	29,4	-22,6	-80	27,2	2,9
15-0457	5	Wachau	-23,2	1,4	-47,7	29,0	-22,9	-76	28,7	0,8
15-0458	6	Wachau	-24,2	3,3	-46,2	29,0	-23,0	-79	28,3	2,0
15-0459	7	Wachau	-23,6	2,3	-50,2	29,0	-22,0	-81	28,2	0,5
15-0460	8	Wachau	-25,0	2,0	-50,8	28,9	-24,1	-92	27,0	1,8
15-0461	9	Wachau	-24,9	3,6	-49,1	29,0	-23,4	-88	27,0	1,5
15-0462	10	Wachau	-24,5	3,8	-51,7	29,1	-22,6	-80	28,5	0,8
15-0463	11	Wachau	-24,4	2,9	-50,4	28,7	-23,4	-85	28,2	0,7
15-0464	12	Wachau	-26,0	4,8	-50,0	29,2	-24,6	-91	27,6	2,0
15-0465	13	Wachau	-25,3	4,2	-52,2	28,9	-23,4	-95	27,2	0,9
15-0466	14	Wachau	-24,7	3,7	-47,4	29,1	-22,7	-87	28,4	2,2
15-0467	15	Wachau	-25,2	3,2	-54,3	28,6	-23,7	-90	28,0	1,9
15-0468	16	Wachau	-25,8	3,3	-45,2	30,7	-24,6	-103	27,6	3,6
15-0469	17	Wachau	-24,4	3,0	-37,5	30,1	-24,2	-93	28,7	2,2
15-0470	18	Wachau	-23,9	1,0	-43,2	29,4	-22,2	-76	27,1	1,7
15-0471	19	Wachau	-26,1	1,2	-47,2	30,2	-24,3	-97	27,5	2,4
15-0472	20	vendor booth Wachau	-26,4	1,7	-36,5	32,0	-26,4	-94	27,8	5,4
15-0473	21	Hungary	-26,3	-0,5	-41,3	31,0	-26,2	-88	27,6	0,1
15-0474	22	Wachau? (shop)	-24,1	2,7	-42,1	29,5	-22,9	-96	27,4	0,6
15-0475	23	France (shop)	-26,3	0,9	-34,8	32,5	-24,7	-84	28,9	3,5
15-0527	24	Wieselburg	-26,5	0,0	-51,2	30,4	-25,1	-104	26,8	3,0
15-0528	25	Amstetten	-26,2	2,7	-55,2	29,1	-25,2	-106	27,1	0,5

APRICOT JUICE WATER

The $\delta^{18}O$ values of the apricot samples from the Wachau region range from 0,5 to 4,6 ‰ V-SMOW. The Hungarian sample has 0,1 ‰, the French 3,5 ‰, the Wieselburg sample 3,0 ‰, the Amstetten sample 0,5 ‰, the sample without geographic indication 0,6 ‰ and the sample from the vendor booth 5,4 ‰ V-SMOW.

DISCUSSION

If only the pulp isotope values are taken into account, no satisfying differentiation of the origin is possible. The point cloud of the apricot samples from the Wachau area also includes most of the samples of other geographic origin (Fig. 2 to 4). The best separation is achieved by using nitrogen isotopes, as the sample from Wieselburg and from Hungary possess significantly lower nitrogen isotope values than the Wachau area ones (Fig. 4). However, nitrogen isotopes are mainly influenced by fertilization and agricultural practice, therefore this parameter can quickly change and be altered. Potentially oxygen isotopes separate the French and the sample from the vendor booth from the authentic samples from the Wachau area (Fig. 1), but the distance to the Wachau samples is rather small. The use of the isotope data of the apricot stones (Fig. 5 to 7) enhances the possibility for differentiation of geographic origin. Fig. 5 (H- vs. C-isotopes) shows the samples from Hungary, France and the sample from the vendor booth separated from the samples from the Wachau area, and Figure 7 and Figure 8 also illustrate the separation of the Hungarian and the vendor booth samples from the Wachau samples due to lower $\delta^{13}C$ values of pulp and stone of these two samples. Potentially also the samples from Wieselburg and Amstetten can be separated from the Wachau area samples, as the latter ones possess slightly higher $\delta^{2}H$ values. However, as the difference is small it is regarded as insufficient for discrimination.

The fruit juice results show a variation of 4 ‰ within the Wachau area samples and all of the non-Wachau samples besides the Hungarian and the sample bought at the vendor stall in the Wachau region lying within the Wachau sample range. The Hungarian sample has a slightly lower value, whereas the sample bought at the vendor stall in the Wachau region shows the highest value. One might argue that the latter is a further indicator for a non-Wachau geographic origin of the vendor booth sample, but another, also plausible explanation is an increase in δ18O due to evaporation of fruit juice water (Horacek et al., 2009) during (non-cooled) storage in the vendor stall.

CONCLUSIONS AND OUTLOOK

This is the first published pilot study aiming to control the declared geographic origin of apricots fom the Wachau area (Lower Austria), by comparing a limited number of authentic samples with single "non-Wachau" samples of vintage 2015. It is demonstrated that the introduction of the fruit stones as an additional sample commodity increases the possibility of differentiating between various geographic origins for the available sample-set. As several investigated parameters of the sample bought at a vendor stall in the Wachau area (sample 20) deviate from the authentic sample range, a geographic origin of this sample outside of the Wachau area is quite likely. The origin of the sample bought at a shop without geographic indication (but assumed Wachau origin) in fact seems to be from the Wachau area, as its isotope signature always conforms to the Wachau area isotope pattern. Oxygen isotope values from the fruit juice might be influenced by post-harvest evaporation of water from the fruits, which has to be taken into account for the interpretation of the data. Analysis of further vintages is required to study the potential of the fruit stones as newly introduced sample commodity and the annual isotope variations in apricots due to weather conditions, and to evaluate, if identified differences between origins persist over years/vintages. Also the investigation of apricots from other non-Wachau area localities will be necessary, to learn about the potential and limitations of the isotope method for geographic discrimination of apricots.

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