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Distribution of rare earth elements in *Vitis vinifera* L. 'Chardonnay' berries

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Summary

The content of 13 rare earth elements (REEs) in the skin, flesh and seeds of 'Chardonnay' berries harvested at technological ripeness in 2 vineyards in Trentino (Italy) with calcareous soils was analysed using inductively coupled plasma mass spectrometry. The total REE content of the berry was 2.1 and 2.7 $\mu\text{g}\cdot\text{kg}^{-1}$ fresh weight (median value, $N = 20$) respectively in the 2 vineyards, La, Ce and Nd making up about 80 %. Despite the significantly different amounts of REEs in the berries of the 2 vineyards, the percentage content of each element as compared to the total amount of REEs and the percentage distribution in the 3 parts (skin, flesh and seeds) were similar. Overall, 57 % of REEs were localized in the skin, 40 % in the flesh and 3 % in the seeds (median value, $N = 40$). Most of the elements prevailed in the skin, while there were negligible amounts in the seeds, where the percentage of Eu was around 20 times higher than in the other parts.

Key words: Rare Earth Elements, berry, seed, skin, ICP-MS, mineral composition.

Introduction

Rare earth elements (REEs) are a homogeneous group of elements including 15 lanthanides plus yttrium and scandium, which have similar properties. They occur in general as trivalent cation with exceptions concerning in particular cerium and europium, also present as Ce^{+4} and Eu^{+2} .

Much research seems to indicate that low amounts of REEs applied to seeds or crops may promote the growth and productivity of various species but the evidence obtained by different authors is often conflicting or not reproducible. REEs have characteristics biologically similar to calcium, affecting the stability and functionality of membranes and seem to be involved in photosynthesis (HU *et al.* 2004, TYLER 2004). As regards REE distribution in plant tissue, decreasing amounts in root > leaf > stem > flower > fruit have usually been reported (HU *et al.* 2004, LIANG *et al.* 2005, DING *et al.* 2006). Little data is available for REEs in *Vitis vinifera* and only recently have ODDONE *et al.* (2006) studied their content in the whole grape berry. The aim of this research was to study REE distribution in the skin, seeds and flesh of 'Chardonnay' grapes.

Material and Methods

Sampling: The grapes belonged to the *Vitis vinifera* L. 'Chardonnay' variety, clone 95 onto rootstock 3309. The vineyards were located in S. Michele all'Adige (A) and in Faedo (B) (Trentino, Italy at 240 and 700 m a.s.l. respectively; Guyot trellising system). Soil A was a silt loam, calcareous, alkaline soil, whereas soil B was a sandy loam, alkaline soil, notably calcareous with low active lime. Both were managed without manure treatments and with grass-covering. In 2006, the vineyards were divided into 4 parcels of 20–50 grapevines each and 300 berries were picked from 60 bunches every week (5 dates of sampling), from 30 to 60 d after veraison, when cell distension was substantially completed.

In both vineyards, 16 samples of soil (depth: 5–60 cm) were randomly collected from the 4 parcels. Each sample was air-dried and the < 2 mm fraction was ground (< 0.2 mm) and analysed.

Sample preparation and analysis: The juice of 100 berries per sample was analysed to determine the basic composition using FT-IR (Grapescan 2000; FOSS, Hillerød, Denmark). The remaining 200 berries were washed with ultrapure HNO_3 1 % (Merck, Darmstadt, Germany): 100 berries were immediately homogenised and used for quantification of the total REE content, whereas the other 100 berries were used to separately obtain skins and seeds. The samples (1.5 g of seeds or skin or 2.5 g of berries) were acid digested (max. temperature 210 °C) using a microwave system (MARS EXPRESS; CEM, Matthews, NC, USA) with 4 ml of ultrapure HNO_3 , 5 ml of ultrapure water and 1 ml of internal standard solution (Re, 800 $\mu\text{g}\cdot\text{l}^{-1}$; Agilent Technologies, Tokyo, Japan). The soil was extracted with aqua regia (ISO 11466/1995) using a microwave system (max. temperature: 175 °C)

REE analysis was performed using an inductively coupled plasma mass spectrometer (ICP-MS, 7500ce; Agilent Technologies) equipped with an Octopole Reaction System using He as collision gas to remove polyatomic interferences for the quantification of Eu. The 13 REEs analysed were quantified as ^{89}Y , ^{139}La , ^{140}Ce , ^{141}Pr , ^{146}Nd , ^{147}Sm , ^{151}Eu , ^{157}Gd , ^{163}Dy , ^{165}Ho , ^{167}Er , ^{169}Tm and ^{171}Yb . The REE content in flesh was roughly estimated as the difference between the whole berry and skin plus seeds. STATISTICA 6.1 (StatSoft Italia srl, Padova, Italy) was used for the statistical treatment of data.

Results and Discussion

All the grape samples had ripeness levels (19.9-24.5 °Brix) consistent with the Trentino Chardonnay Controlled Designation of Origin production regulations. In both vineyards, differences among harvest dates in terms of the amount ($\mu\text{g}\cdot\text{berry}^{-1}$) and concentration ($\mu\text{g}\cdot\text{kg}^{-1}$ fresh weight) of each REE in the whole berry, skin and seeds were not statistically significant or systematic, showing no clear trend (data not shown), so we discuss the data as a whole.

REE content in the whole berry: La, Ce and Nd made up about 80 % of the total REEs, confirming that so-called light REEs with a lower atomic number are generally present in larger amounts (KABATA-PENDIAS, 2001). La, Ce and Nd, with Sc and Y, were also the most plentiful REEs in a German must studied by GOMEZ *et al.* (2004). The amounts of La and Ce observed in our grapes

were lower than those shown by ODDONE *et al.* (2006). The sum of the REE contents was 2.1 and 2.7 $\mu\text{g}\cdot\text{kg}^{-1}$ fresh weight respectively for vineyards A and B. The content of Y, La, Ce, Pr, Nd, Sm, Gd, Dy and Er in the berries of vineyard B was significantly higher than in the samples of vineyard A ($p < 0.05$), in accordance with the higher amounts of these elements measured in the aqua regia extracted soils (median values: 108.6 and 144.7 $\text{mg}\cdot\text{kg}^{-1}$ dry weight in vineyards A and B, respectively). Nevertheless, the percentage profile of the REEs was very similar in both vineyards: only the Eu and Tm percentages were statistically different ($p < 0.05$).

Distribution in the different parts of the berry: The flesh represented about 81.0 %, the skin 15.2 % and the seed only 3.8 % of the fresh weight of the berry (median values). As already observed for the whole berry, the elemental amounts in each part (Tab. 1) were generally higher in the berries produced in vineyard B, but

Table 1

Rare earth element content ($\mu\text{g}\cdot\text{kg}^{-1}$ berry, fresh weight) in the whole berry, skin, seeds and flesh. The percentage of each element as compared to the overall amount of rare earth elements in berry and in skin, seeds and flesh is also given. Y = yttrium; La = lanthanum; Ce = cerium; Pr = praseodymium; Nd = neodymium; Sm = samarium; Eu = europium; Gd = gadolinium; Dy = dysprosium; Ho = holmium; Er = erbium; Tm = thulium; Yb = ytterbium; ns = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; sign. = significance of median contents between vineyards (Mann-Whitney's test)

	vineyard A (N = 20)		vineyard B (N = 20)		sign.	vineyard A (N = 20)		vineyard B (N = 20)		sign.	
	% on total REE	median ($\mu\text{g}\cdot\text{kg}^{-1}$ berry)	% on total REE	median ($\mu\text{g}\cdot\text{kg}^{-1}$ berry)		% on total REE	median ($\mu\text{g}\cdot\text{kg}^{-1}$ berry)	% on total REE	median ($\mu\text{g}\cdot\text{kg}^{-1}$ berry)		
whole berry						skin					
Y	5.3	0.099	5.2	0.133	*	Y	5.4	0.064	5.7	0.093	***
La	18.2	0.369	17.9	0.458	***	La	18.0	0.215	17.7	0.259	***
Ce	39.4	0.869	40.4	1.104	**	Ce	41.8	0.511	43.0	0.655	***
Pr	4.6	0.093	4.7	0.129	**	Pr	4.7	0.058	4.6	0.072	**
Nd	20.7	0.416	20.5	0.561	***	Nd	19.8	0.225	18.9	0.292	**
Sm	3.5	0.068	3.8	0.096	***	Sm	3.2	0.039	3.3	0.052	***
Eu	2.2	0.042	1.5	0.043	ns	Eu	1.2	0.015	0.93	0.015	ns
Gd	2.5	0.053	2.7	0.070	***	Gd	2.6	0.029	2.5	0.038	***
Dy	1.5	0.029	1.4	0.038	**	Dy	1.4	0.018	1.4	0.022	**
Ho	0.30	0.006	0.29	0.008	ns	Ho	0.31	0.004	0.31	0.005	***
Er	0.81	0.015	0.76	0.022	*	Er	0.72	0.009	0.70	0.011	***
Tm	0.23	0.005	0.18	0.005	ns	Tm	0.18	0.002	0.16	0.003	***
Yb	0.76	0.016	0.66	0.017	ns	Yb	0.66	0.008	0.67	0.011	***
flesh						seeds					
Y	4.7	0.041	4.1	0.037	ns	Y	9.7	0.007	9.7	0.008	ns
La	17.8	0.141	17.3	0.159	ns	La	14.5	0.010	15.3	0.012	ns
Ce	38.6	0.333	38.3	0.373	ns	Ce	17.8	0.013	20.2	0.015	*
Pr	4.7	0.034	4.9	0.052	ns	Pr	1.6	0.001	1.9	0.002	*
Nd	23.0	0.180	23.2	0.251	*	Nd	15.3	0.011	19.1	0.014	***
Sm	3.8	0.029	4.6	0.039	*	Sm	4.9	0.004	4.8	0.004	ns
Eu	1.1	0.009	1.3	0.012	ns	Eu	29.6	0.020	21.2	0.017	***
Gd	2.2	0.020	3.0	0.029	*	Gd	2.3	0.001	2.4	0.002	ns
Dy	1.6	0.011	1.3	0.014	ns	Dy	2.0	0.001	2.3	0.002	*
Ho	0.26	0.002	0.25	0.002	ns	Ho	0.14	<0.0001	0.15	0.0001	ns
Er	1.0	0.007	0.79	0.008	ns	Er	1.1	0.0007	1.7	0.0012	***
Tm	0.31	0.002	0.21	0.002	ns	Tm	0.12	<0.0001	0.21	0.0001	**
Yb	0.86	0.008	0.71	0.006	ns	Yb	1.0	<0.0007	1.2	0.0010	ns

the REE percentage profile accumulated in the 3 parts was similar for the 2 areas. Gd, Ho Tm and Yb in seeds were close to or below the detection limit.

Tab. 2 shows the percentage distribution in skin, flesh and seeds, taking into account all the collected samples (N = 40). For most of the elements skin prevailed over flesh and seeds. These last, usually having negligible amounts of REEs, showed a remarkable percentage of Eu. The total REE content was 40 % in the flesh, 57 % in the skin and 3 % in the seeds (median values). This distribution confirms the observations of SUN *et al.* (1994) in wheat and rice, where the external part of the fruit (the husk) contains more REEs than the seeds, and those reported by HU *et al.* (2004) showing higher Nd in the hull than in the grain of wheat. This particular distribution could be explained by the cytolocalization of REEs, which are reported to be mainly aggregated on the outer surface of cytoplasmatic membranes, combined with the cell wall, accumulated in the intercellular space or concentrated in the cell nucleus. Moreover, light REEs are said to be bound to chlorophyll (HU *et al.* 2004) and so they could be a residue of the previous photosynthetic activity of the berry.

The distribution of Eu could be related to its occurrence also as bivalent cation, as for calcium. The seeds accumulated calcium (more than 50 % of the Ca present in the berries in our samples) as previously shown by ROGIERS *et al.* (2006). Eu⁺² is more abundant in Ca-containing minerals and is defined as a moderate reducing element in solution (COTTON and WILKINSON 1984). However, other authors have stated that Eu⁺² is not stable at a low temperature and the reduction of Eu⁺³ to Eu⁺² required extreme reducing conditions not usually observed in plants (DING *et al.* 2006). The accumulation of Eu in seeds could nevertheless be due to the high presence of inorganic phosphorus in seeds, which would retain Eu as the phosphate precipitates, as suggested by DING *et al.* (2006). Indeed, the seeds are richer in P than the flesh and the skin (about 45-50 % of the berry's P was located in the seeds in our samples) as shown also by ROGIERS *et al.* (2006).

To conclude, this paper updates the limited knowledge available on REE composition and distribution in grape berries. Further research is necessary to confirm the elemental distribution here observed in grapes grown on calcareous soil, also in order to better understand the role of REEs in the geographic traceability of wine.

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Table 2

Median (N = 40) of the percentage contents of rare earth elements in skin, seeds and flesh (fresh weight). Different letters indicate significant differences for the Kruskal-Wallis's test, $p < 0.05$

Element	Skin	Flesh	Seeds
Y	63.4 a	29.8 b	6.8 c
La	58.6 a	38.8 b	2.7 c
Ce	61.4 a	37.0 b	1.6 c
Pr	61.6 a	37.1 b	1.3 c
Nd	53.1 a	44.2 b	2.8 c
Sm	52.8 a	43.3 b	3.9 c
Eu	33.5 b	27.0 b	39.5 a
Gd	54.7 a	42.9 b	2.4 c
Dy	59.7 a	35.7 b	4.6 c
Ho	66.8 a	31.6 b	1.5 c
Er	53.0 a	41.8 b	5.2 c
Tm	48.6 a	29.0 a	2.5 b
Yb	52.6 a	43.3 b	4.1 c

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