Washing methods for removal of residues of 44 mineral elements from grapes

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Abstract Several elements of anthropogenic and natural origin can settle on the grape berry skin. In this study, the effectiveness of 3 washing methods (using ultrapure water, 0.2% acetic acid and 0.2% citric acid) in removing contaminants was tested on laboratory scale, in comparison with an analytical washing preparation of reference with nitric acid 1%.
Forty-four macro, micro and trace elements were quantified in the solutions after berry washing treatment. The analysis was carried out with an ICP-MS.
The citric acid solution provided comparable or only slightly less effective results in comparison with HNO_3-wahing method, whereas acetic acid and water removed on average only 45% of the contaminants removable using the reference method. The technological removal of pollutant elements using washing solution needs further research as regards environmental sustainability because of the consumption of drinking water.
Keywords Acetic acid; grape berry; citric acid; trace elements; drinking water

INTRODUCTION

Several mineral and trace elements can accumulate on the external surface of grape berries during development and maturation. These elements, of anthropogenic origin (e.g. phytosanitary treatments, car or factory emissions) or coming from natural sources (soil dust), may lead to significant concentrations on the berry skin, even exceeding the endogenous content of grapes. The main concern is usually directed at elements such as Cu and Fe, due to the technological implications, at other so-called heavy metals such as Cd, Pb, Ni, and also at As, for toxicological reasons (Tesseidre *et al.*, 1994; Larcher and Nicolini, 2008 and cited literature).

In this study, the effectiveness of 3 washing methods (using ultrapure water, 0.2% acetic acid and 0.2% citric acid) in removing contaminants was tested on laboratory scale, in comparison with an analytical washing preparation of reference with nitric acid 1%.

MATERIALS AND METHODS

At harvest, 20 bunches were collected at 3 different vineyards situated in areas without industrial pollution far away from main roads. The grapes were characterised by different levels of phytosanitary treatments or soil dust residues. The berries collected in each area were carefully randomised in order to obtain homogeneous and representative subsamples.

Initially, the depletion capability of successive HNO_3-washing steps was studied. For this purpose, a subsample from each vineyard was washed 3 times in sequence with a HNO_3 1% aqueous solution. In detail, 50 berries were put in a PTFE vessel, adding 50 ml of washing solution and carefully shaking in an end to end shaker for 5 minutes. After every step, the washing solution was collected and analysed in order to quantify 44 macro, micro and trace elements.

The different effectiveness of 3 washing methods (one washing step) in removing contaminants was tested on subsamples of grapes collected in one vineyard. The washing solutions compared were: ultrapure water, 0.2% acetic acid, 0.2% citric acid, 1% nitric acid. As specified above, 50 ml of washing solution was added to 50 berries and shaken for 5 minutes; the solutions were then collected and analysed.

Mineral element analysis was carried out with a mass spectrometer (ICP-MS Agilent 7500ce, Tokyo, Japan) equipped with a collision/reaction cell.

All the material used was previously washed with an HNO_3 5% solution and rinsed with ultrapure water.

RESULTS AND DISCUSSION

Depletion capability of 3 washing steps. The first HNO_3 washing step removed at least 70% of external contaminants, with the exception of B, P, K and Rb (Table 1). For Ag, As, Bi, Cd, Dy, Er, Eu, Fe, Ga, Gd, Ge, Hg, Ho, Nd, Pd, Pr, Se, Sm, Sn, Th, Tm, U, V, Yb this first step could completely remove the superficial fraction. After the second washing step 82-100% of the total contaminants had been removed. Lead was shown to be easily solubilisable, confirming the observations of Tesseidre *et al*. (1994).

Table 1. Depletion capability (% of total) of 3 successive washing steps. Values are means of 3 replications ± standard error.

element	Al	B	Ba	Ca	Ce	Cs	Cu	K	La	Li
1st washing step	95 ± 2	68 ± 4	94 ± 6	86 ± 2	95 ± 3	86 ± 14	99 ± 1	51 ± 3	95 ± 3	85 ± 15
2nd washing step	5 ± 2	22 ± 5	4 ± 4	11 ± 2	5 ± 3	9 ± 9	1 ± 1	31 ± 6	5 ± 3	8 ± 8
3rd washing step	0	11 ± 2	2 ± 2	3 ± 1	0	6 ± 6	0	17 ± 3	0	7 ± 7

element	Na	Mg	Mn	P	Pb	Rb	Sb	Sr	Y	Zn
1st washing step	80 ± 4	74 ± 4	91 ± 2	65 ± 3	98 ± 1	50 ± 3	90 ± 5	84 ± 8	79 ± 19	77 ± 5
2nd washing step	16 ± 4	18 ± 4	7 ± 2	23 ± 5	2 ± 1	32 ± 6	10 ± 5	16 ± 8	20 ± 18	15 ± 4
3rd washing step	4 ± 2	8 ± 1	2 ± 1	11 ± 2	0	18 ± 3	0	0	1 ± 1	8 ± 2

Comparison of different washing methods. In general, the 1% HNO_3 washing solution was shown to be more effective in removing contaminants (Table 2). The concentrations quantified in the HNO_3 washing solution were significantly higher (at least $p<0.05$, Tukey's test) for most of the mineral elements. Nevertheless, the citric acid solution often provided comparable or only slightly less effective results. On average, citric acid removed 78% of the residues removable with nitric acid. In a few cases - notably for Se and Sn – the citric acid solution could remove a higher fraction of external contaminants. On the other hand, acetic acid and water removed on average only 45% of the contaminants removable using HNO_3. Washing effectiveness was especially low for Ba, Bi, Ca, Cd, Fe, Mn, Pb, Sr, U, V and rare earth elements.

The percentage removable with the 4 washing processes as compared to the content of unwashed grapes is also shown in Table 2. Most (>50%) of the Pb, Bi, V, Cu and Cd quantified in unwashed grapes was deposited on the surface and removable with all the tested solutions, whereas less than 10% of the total content of Ag, B, Ba, Ca, Cs, Eu, Fe, Ga, Ge, Hg, K, Li, Mg, Na, P, Pd, Rb, Se, Sr and Th was removed with the washing processes described. For the other elements, Al, As, Ce, Dy, Er, Gd, Ho, La, Mn, Nd, Pr, Sb, Sm, Sn, Tm, U, Y, Yb, Zn (and Eu in the case of HNO_3 solution) the percentage content removed ranged from 10 to 49%.

Table 2. External contaminants (μg/kg berries) in the washing solutions and percentage content of residues removed by the different washing process out of the total content of unwashed grapes. Different letters indicate statistically different mineral content in the 4 washing solutions (Tukey's test, $p<0.05$).

washing solution	1% HNO_3			ultrapure H_2O			0.2% citric acid			0.2% acetic acid		
elements	% removed on total	μg/kg berry (N=3)	sign.	% removed on total	μg/kg berry (N=3)	sign.	% removed on total	μg/kg berry (N=3)	sign.	% removed on total	μg/kg berry (N=3)	sign.
Ag	<1	<0.02		<1	<0.02		<1	<0.02		<1	<0.02	
Al	18	83.46	a	11	45.68	b	15	68.01	ab	10	43.38	b
As	37	0.304	ab	30	0.226	b	45	0.423	a	33	0.252	b
B	1	22.58	a	<1	12.94	b	1	21.23	ab	<1	12.58	b
Ba	7	31.19	a	2	11.00	b	4	17.71	b	2	8.14	b
Bi	100	0.008	ab	100	0.003	b	100	0.012	a	100	0.003	b
Ca	4	14222	a	1	4995	c	3	9857	b	1	5261	c
Cd	81	0.200		52	0.053		60	0.072		51	0.051	
Ce	21	0.299	a	7	0.078	b	11	0.137	b	7	0.086	b
Cs	5	0.017	a	2	0.008	b	3	0.009	b	2	0.009	b
Cu	84	4025		74	2231		83	3768		77	2656	
Dy	43	0.031	a	16	0.008	b	27	0.016	b	17	0.009	b
Er	45	0.014	a	18	0.004	c	32	0.008	b	20	0.004	c
Eu	17	0.009	a	5	0.002	c	9	0.004	b	6	0.003	bc
Fe	5	49.24	a	2	17.95	b	4	37.42	a	2	19.45	b
Ga	<1	<0.028		<1	<0.028		<1	<0.028		<1	<0.028	
Gd	42	0.042	a	<1	<0.010	b	24	0.018	b	<1	<0.010	b
Ge	<1	<0.010		<1	<0.010		<1	<0.010		<1	<0.010	
Hg	<1	<0.16		<1	<0.16		<1	<0.16		<1	<0.16	
Ho	41	0.005	a	12	0.001	b	27	0.003	b	13	0.001	b
K	<1	11959		<1	8086		<1	11566		<1	8409	
La	24	0.155	a	7	0.038	b	12	0.065	b	8	0.042	b
Li	3	0.073	a	2	0.051	b	3	0.063	ab	2	0.046	b
Mg	2	2083	a	1	1119	b	1	1678	ab	1	1008	b
Mn	30	449	a	17	215	bc	26	368	ab	16	197	c
Na	6	113.0	ab	5	92.14	b	8	151.0	a	6	114.6	ab
Nd	25	0.180	a	8	0.044	b	14	0.086	b	8	0.047	b
P	1	985		<1	807		1	1109		<1	821	
Pb	100	3.625	a	100	1.366	b	100	2.631	ab	100	1.272	b
Pd	<1	<0.006		<1	<0.006		<1	<0.006		<1	<0.006	
Pr	24	0.041	a	7	0.009	b	13	0.019	b	8	0.011	b
Rb	1	1.711		<1	1.177		1	1.705		<1	1.176	
Sb	20	0.087	a	13	0.054	bc	19	0.082	ab	12	0.047	c
Se	5	0.026	b	5	0.025	b	8	0.040	a	5	0.027	b
Sm	27	0.038	a	7	0.008	b	15	0.017	b	8	0.009	b
Sn	21	0.691	b	<1	<0.67	b	34	1.333	a	<1	<0.67	b
Sr	3	21.80	a	1	9.198	bc	2	14.41	b	1	8.536	c
Th	<1	<0.068		<1	<0.068		<1	<0.068		<1	<0.068	
Tm	43	0.0015	a	<1	<0.0010	b	26	<0.0010	b	<1	<0.0010	b
U	48	0.022	a	<1	<0.001	c	31	0.011	b	21	0.006	bc
V	93	0.240	a	76	0.053	b	92	0.194	a	76	0.052	b
Y	49	0.129	a	19	0.032	c	35	0.073	b	21	0.036	c
Yb	48	0.012	a	<1	<0.005	b	33	0.006	b	<1	<0.005	b
Zn	21	100.6		12	51.34		18	80.12		12	48.49	

Larcher and Nicolini (2008) reported that a washing procedure could significantly reduce the metal content - in particular Sn, Cu, Al and Pb - of grapes by 2-21 times, whereas Teissedre *et al*. (1994) measured a mean external lead content equal only to 8% of the total content of berries.

Cavazza *et al*. (2007) have recently pointed out that the depletion of metal content (Cu, Fe, Pb, Zn) in grapes before crushing could reduce the stress of yeast during vinification and favour *Saccharomyces* against wild non-*Saccharomyces* populations. Other less known trace elements could also have a biochemical catalytic or toxic effect during the wine-making process.

CONCLUSION

This research was realised within the context of a PhD project on Viticulture, Oenology and Marketing at the University of Padova. It has shown that, of the technologically utilisable methods tested, the washing process for berries with a 0.2% citric acid solution gave the best performance, removing an amount of mineral elements comparable to that removable using the analytical reference method. In the conditions studied, with berries collected in unpolluted areas, vigorous washing with ultrapure water could also remove a significant fraction of some metals such as Cu, Pb and Cd. Acetic acid solution did not significantly improve the removal of mineral elements achievable with water.

Despite the results obtained, a washing process aiming at technological removal of pollutant elements does not appear completely convincing from the environmental point of view. Using the tested methods, for 100 kilograms of grapes, almost 75 litres of water are necessary, and the residual process waters - containing amounts of metal (notably Cu and Pb) close to or higher than the legal limit for drinking water - had to be treated.

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