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Grapes Enrichment with Zinc for Vinification: Mineral Analysis with Atomic Absorption Spectrophotometry, XRF and Tissue Analysis †

Diana Daccak [1,2,*], Ana Coelho [1,2], Ana Marques [1,2], Inês Luís [1,2], Cláudia Pessoa [1,2], Maria Manuela Silva [2,4], Mauro Guerra [3], Roberta Leitão [3], José Ramalho [2,5], Manuela Simões [1,2], Fernando Reboredo [1,2], Maria Pessoa [1,2], Paulo Legoinha [1,2], Paula Scotti-Campos [2,6], Isabel Pais [2,6] and Fernando Lidon [1,2]

[1] Earth Sciences Department, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal
[2] GeoBioTec Research Center, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal
[3] LIBPhys-UNL, Physics Department, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal
[4] ESEAG/Grupo Universidade Lusófona, Lisboa, Portugal
[5] PlantStress & Biodiversity Lab, Centro de Estudos Florestais, Instituto Superior Agronomia, Universidade de Lisboa, Oeiras, Portugal
[6] INIAV, Instituto Nacional de Investigação Agrária e Veterinária, Oeiras, Portugal
* Correspondence: d.daccak@campus.fct.unl.pt; Tel.: +351212948573

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Abstract: Micronutrient deficiency affects individuals all around the world, being a public health problem. To minimize this problem, several alternatives are being developed, namely agronomic biofortification, to increase the amount of nutrients in food crops. In this context, Zn is one of the most relevant micronutrients for the human body, displaying catalytic, structural and regulatory properties. Considering that Zn deficiency leads to health diseases (namely, neurological disorders, autoimmune, degenerative diseases related to age, Wilson's disease, cardiovascular problems, and diabetes mellitus), a technical itinerary for biofortification was outlined in a field grapes located in Palmela (Portugal), aiming to optimize Zn contents for the Syrah variety. Biofortification was performed with foliar spraying with zinc oxide (ZnO) and zinc sulfate ($ZnSO_4$) throughout the production cycle (at concentrations of 0%, 30% and 60%—0, 450 and 900 g ha^{-1}). Zinc biofortification index increased about 59% and 45%, with OZn60 and SZn60, whereas its deposition in the flesh of the grapes increased 2.41 and 2.37 fold and in the seeds *ca.*1.76 and 2.19 fold (in OZn60 and SZn60, respectively). After vinification, wine significant increases of Zn contents were also found (1.92 and 1.77 fold) yet, considering the amount of this nutrient in grapes, it is concluded that vinification must also be optimized.

Keywords: biofortification; Syrah variety; wine; zinc oxide; zinc sulfate

1. Introduction

The deficit of micronutrients affects more than two billion of individuals worldwide, which becomes a serious problem to public health [1]. Zn is a relevant micronutrient in the human physiology, with catalytic, structural and regulator properties, namely critical roles in homeostasis, immunologic function, oxidative stress, and regulation of apoptosis [2,3]. Low levels of Zn can also lead to appearance and worsening of diseases such as, neurological disorders, autoimmune, degenerative diseases related to age, Wilson's disease, cardiovascular problems, and diabetes mellitus [3]. To minimize these health problems, itineraries for Zn biofortification of edible plants can be developed, which consists in increasing the amount of nutrients with agronomic practices [4,5]. To perform agronomic biofortification, foliar application seems to stimulate a more efficient capture and allocation of nutrients than soil application [5]. In 2008, the International Program HarvestPlus and its subproject HarvestZinc, lead to an elevated interest in increasing Zn in food crops, being demonstrated that, relatively to soil fertilization, foliar application was more efficient for wheat, rice and corn [6]. Studies in Anatólia Central and India also showed an increase in Zn concentrations with soil and/or foliar application [7]. Although Portugal isn't one of the countries that exports the most, it has distinguished itself with quality wines and a reputation both nationally and internationally [8]. Besides, various researchers also link moderate wine consumption to health benefits namely with, cardiovascular diseases, in the prevention of various cancers, liver diseases and senility [9]. Considering the physiological importance of Zn in the human body and the importance of wine comsumption worldwide, this work aimed to increase the content of Zn in the grapes of Syrah variety for vinification.

2. Experiments

2.1. Experimental Field

A vineyard located in Palmela, Portugal (38° 35′23.629″ N; 8° 51′ 46.208″ W), with a *Vitis vinífera* L. variety Syrah, having an irrigation system was used for biofortification. The itinerary for biofortification with Zn, was performed between 16 June and 25 September, in 2018. Foliar spraying was carried out with zinc sulfate ($ZnSO_4$) and zinc oxide (ZnO), at concentrations of 0%, 30% and 60% (0, 450 and 900 g ha^{-1}). Harvest was carried out 11 October of 2018. During the production cycle, the weather conditions were characterized by a maximum average temperature of 28 °C and minimum average of 16.6 °C.

2.2. Total Soluble Solids

Total soluble solids (°Brix) was measured in three randomized grapes per treatment, using a digital refractometer Atago (Atago, Tokyo, Japan).

2.3. Quantification of Zn in Grapes and Accumulation at Tissue Level

Zinc contents in grapes were analyzed at harvest using XRF analyzer (model XL3t 950 He GOLDD+) under He atmosphere, to determine Zn content [11]. They were cut, dried (at 60 °C, until constant weight), grounded and proceed into pellets.

At harvest, to map Zn in tissues (skin and seeds), a Micro-energy X-ray Dispersion Fluorescence-μ-EDXRF (M4 Tornado™, Bruker, Germany) siytem was used [10]. The X-ray was operated at 50 kV and 100 μA, without application of filters, to enhance the ionization of low-Z elements. For a better quantification of Zn, a set of filters among the X-ray tube and the sample, composed of three foils of Al/Ti/Cu (with a thickness of 100/50/25 μm, respectively) was further used. The measurements with filters were performed with 600 μA current. Detection of fluorescence radiation was carried out by an energy-dispersive silicon drift detector, XFlash™, with 30 mm^2 sensitive area and energy resolution of 142 eV for Mn Kα. The measurements were made under 20 mbar vacuum conditions, and the point spectra were acquired during 200 s.

2.4. Zn Quantification in Wine

In wine, Zn contents was measured using an atomic absorption spectrophotometer model Perkin Elmer AAnalyst 200 fitted with a deuterium background corrector, with the AA WinLab software program. Before the wine was analyzed it was filtrated.

2.5. Statistical Analysis

Data were statistically analyzed using a One-Way ANOVA ($p \leq 0.05$), to evaluate differences. Using the results, a Tukey's for mean comparison was performed (95% confidence level).

3. Results

3.1. Total Soluble Solids

Total soluble solids were determined, using random grapes, and the results showed that SZn60 had higher °Brix values. Relatively to the control, all treatments increased significantly, with a 1.16 and 1.38 fold increases being found in OZn60 and SZn60, respectively (Table 1).

Table 1. Average content ± S.E. (n = 3) of °Brix in fruits at harvest of *Vitis vinifera* L., variety Syrah. The letters a, b, c indicate significative differences of Zn content among treatments ($p \leq 0.05$). Treatments OZn30, OZn60, SZn30 e SZn60 indicate the following concentrations for zinc oxide (ZnO) or Zinc sulfate ($ZnSO_4$): 0%, 30%, 60%. (i.e., 0, 450 e 900 g ha^{-1}).

Syrah Variety	**Total Soluble Solids (°Brix)**	
	Mean	**SE**
Control	13.10 c	±0.31
OZn30	16.83 ab	±0.36
OZn60	16.33 ab	±0.07
SZn30	15.13 b	±0.22
SZn60	18.10 a	±0.48

3.2. Quantification of Zn in Grapes and Accumulation in the Flesh and Seeds

Zinc contents in grapes treated with ZnO and $ZnSO_4$ showed, relatively to the control, significant increases (Table 2), with OZn60 and SZn60 displaying the highest increase (59% and 45%, respectively).

Table 2. Average content ± S.E. (n = 3) of Zn in fruits at harvest of *Vitis vinifera* L. variety Syrah. Different letters (a, b) indicate significant differences among treatments ($p \leq 0.05$). Treatments OZn30, OZn60, SZn30 e SZn60 indicate the following concentrations for zinc oxide (ZnO) or Zinc sulfate ($ZnSO_4$): 0%, 30%, 60%. (i.e., 0, 450 e 900 g ha^{-1}).

Syrah Variety	**Zn (ppm$_{DW}$)**	
	Mean	**SE**
Control	13.460 b	± 0.876
OZn30	18.843 ab	± 1.799
OZn60	21.400 a	± 0.892
SZn30	19.287 a	± 1.487
SZn60	19.580 a	±0.800

At a tissue level two regions were defined in the grapes, the grape flesh and the seeds (Table 3). Relatively to the control, grapes flesh showed fold 2.41 and 2.37 increases in OZn60 and SZn60, whereas for the seeds, 1.76 and 2.19 fold increases were measured, respectively (Table 3). The grape flesh showed, relatively to the seeds, a consistent higher increase of Zn.

Table 3. Average content (n = 3) of Zn in grapes flesh and seeds (after dehydration) in Syrah grapes, at harvest, an the respectively degree of uncertainty reported by the technique of μ-EDXRF Tornado. Letters a, b indicates the presense of significative differences of Zn content between treatments ($p <$ 0.05). Treatments OZn30, OZn60, SZn30 e SZn60 indicate the following concentrations for zinc oxide (ZnO) or Zinc sulfate ($ZnSO_4$): 0%, 30%, 60%. (i.e., 0, 450 e 900 g ha^{-1}).

Variety Syrah	Grapes Flesh Zn (ppm_{Dw})		Seeds Zn (ppm)	
	Mean	SE	Mean	SE
Control	13.3 b	±0.66	8.74 b	±0.44
OZn30	33.4 a	±1.67	14.8 ab	±0.74
OZn60	32.1 a	±1.61	15.4 a	±0.77
SZn30	28.0 a	±1.40	17.1 a	±0.85
SZn60	31.5 a	±1.58	19.1 a	±0.95

3.3. Quantification of Zn in Wine

Zn- treated grapes triggered an increasing accumulation of this nutrient in the produced wine (Table 4). Spraying with OZn30 and SZn60 triggered the best responses (relatively to the control, 1.92 and 1.77 fold increases, respectively.

Table 4. Average content ± S.E. (n = 3) of Zn in monocast wine produced with Syrah grapes. Different letters (a, b) indicates ignificant differences ($p \leq 0.05$). Treatments OZn30, OZn60, SZn30 e SZn60 indicate the following concentrations for zinc oxide (ZnO) or Zinc sulfate ($ZnSO_4$): 0%, 30%, 60%. (i.e., 0, 450 e 900 g ha^{-1}).

Syrah	Zn Contentes in Wine (μg L^{-1})	
	Mean	SE
Control	0.730 b	±0.088
OZn30	1.398 a	±0.153
OZn60	1.074 ab	±0.135
SZn30	1.289 a	±0.041
SZn60	1.295 a	±0.104

4. Discussion

The amount of total soluble solids (°Brix) is an important parameter for vinification, because it influences the final quality of wine. Grapes must have a sufficient quantity of sugar to ensure a high fermentation perform. Indeed, insufficient time of maturation leads to watery wines with low alcohol concentration and, after its ideal point, it leads to a wine rich in alcohol, but with low acidity [12]. Accordingly, perfect time to harvest depends on the country or region of production, the type of wine and the natural conditions of the environment [13–15]. In this context, our data (Table 1) showed an increase of the amount of soluble solids in Zn-treated grapes, ranging between ca. 13.13–18.10 °Brix, which favored our vinification process.

It has also been reported [18,19] that Zn biofortification, through soil or foliar spraying, might affect yield parameters, grain quality, higher land and water productivity. Yet, $ZnSO_4$ is the most widely applied fertilizer due to its high solubility and low cost [20], but ZnO has shown to be effective too in sunflower plants, increasing the amount of Zn in all plants, dry weight, leaf area and photosynthesis parameters [21]. Relating the effectiveness of Zn enrichment in Syrah grapes, it was possible to verify that OZn60 demonstrated better results (relatively to SZn60). Besides, Zn accumulation prevailed in the flesh of the grapes, surpassing 30% above de control, which revealed the effectiveness of the biofortification [16,17]. Indeed, a higher biofortification index was found (Table 3).

In general, Portuguese wines have low amounts of Zn (between 0.16–1.96 mg L^{-1}) [22]. Similarly to other metals, the amount of Zn in wines depends on the intensity of maceration, extraction and solubilization during fermentation, since it is preferentially situated in the grape peel and seeds [22]. Comparing the content of Zn in grapes and wine, strong losses occurred during vinification, which indicates that this process requires optimization. Nevertheless, a significant yield of Zn was obtained in the produced wine.

5. Conclusions

Biofortification with Zn in Syrah grapes increase the total soluble solids, but once the climatic conditions have an influence in the content, more assays must be carried out.

OZn has led to better results but, in general, although biofortification has proved to be effective in increasing Zn content of grapes and wine, the vinification process needs to be optimized.

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Abbreviations

OZn10	Foliar application of zinc oxide with a concentration of 10% (150 g ha^{-1})
OZn 30	Foliar application of zinc oxide with a concentration of 30% (450 g ha^{-1})
OZn60	Foliar application of zinc oxide with a concentration of 60% (900 g ha^{-1})
SZn10	Foliar application of zinc sulfate with a concentration of 10% (150 g ha^{-1})
SZn30	Foliar application of zinc sulfate with a concentration of 30% (450 g ha^{-1})
SZn60	Foliar application of zinc sulfate with a concentration of 60% (900 g ha^{-1})

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