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Proceeding Paper

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

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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 disorders (namely, neurological disorders, autoimmune and degenerative diseases related to age, Wilson's disease, cardiovascular problems, and diabetes mellitus), a technical itinerary for biofortification was outlined in a field of grapes located in Palmela (Portugal), aiming to optimize Zn content for the Syrah variety. Biofortification was performed with foliar spraying of 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}). The zinc biofortification index increased about 59% and 45%, respectively, with OZn60 and SZn60 (i.e., concentrations of 60% with treatment ZnO and $ZnSO_4$ respectively), whereas its deposition in the flesh of the grapes increased 2.41- and 2.37-fold and in the seeds by approx. 1.76- and 2.19-fold (with OZn60 and SZn60, respectively). After vinification, significant increases in Zn content in the wine were also found (1.92- and 1.77-fold); however, considering the amount of this nutrient in grapes, it was concluded that vinification must also be optimized.

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

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1. Introduction

Micronutrient deficiencies affect more than two billion individuals worldwide and are a serious public health issue [1]. Zn is an important micronutrient in human physiology, with catalytic, structural, and regulatory properties as well as critical roles in homeostasis,

immunologic function, oxidative stress, and the regulation of apoptosis [2,3]. Low levels of Zn can lead to the onset and worsening of a variety of disorders, such as neurological disorders, autoimmune and degenerative diseases related to age, Wilson's disease, cardiovascular problems, and diabetes mellitus [3]. To minimize these health problems, schedules for Zn biofortification of edible plants can be developed to increase the amounts of this nutrient through agronomic practices [4,5]. For agronomic biofortification, foliar application seems to stimulate more efficient capture and allocation of nutrients than soil application [5]. In 2008, the International Program HarvestPlus and its subproject HarvestZinc fueled elevated interest in increasing Zn in food crops by demonstrating that relative 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 applications [7]. Although Portugal is not a major wine exporter, it has distinguished itself both nationally and internationally with a reputation for quality wines [8]. Moreover, some researchers have linked moderate wine consumption to health benefits associated with the prevention of cardiovascular diseases, various cancers, liver diseases, and senility [9]. Considering the physiological importance of Zn in the human body and the importance of wine consumption worldwide, this work aimed to increase the content of Zn in grapes of the 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), cultivated with the *Vitis vinífera* L. variety Syrah and equipped with an irrigation system, was used for biofortification. The schedule for biofortification with Zn was implemented between 16 June and 25 September 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}). The harvest was conducted on 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) were measured in 3 randomized grape samples per treatment, using an Atago digital refractometer (Atago, Tokyo, Japan).

2.3. Quantification of Zn in Grapes and Accumulation Level in Grape Tissues

The zinc content in grapes was analyzed at harvest using an XRF analyzer (model XL3t 950 He GOLDD+) under He atmosphere [10]. The grapes were cut, dried (at 60 °C, until constant weight), ground, and processed into pellets.

To map Zn in tissues (skin and seeds) at harvest, a Micro-energy X-ray Dispersion Fluorescence (μ-EDXRF) (M4 Tornado™, Bruker, Berlin, Germany) system was used [11]. The X-ray system was operated at 50 kV and 100 μA, without application of filters, to enhance the ionization of low-Z elements. For better quantification of Zn, a set of filters between the X-ray tube and the samples, composed of 3 foils of Al/Ti/Cu (with a thickness of 100, 50, and 25 μm, respectively) was further used. The measurements with filters were performed with 600 μA current. Detection of fluorescence radiation was carried out with 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 in 200 s.

2.4. Zn Quantification in Wine

The Zn content in wine was measured using a Perkin Elmer AAnalyst 200 atomic absorption spectrometer equipped with a deuterium background corrector and the AA WinLab software program. The wine was filtrated before analysis.

2.5. Statistical Analysis

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

3. Results

3.1. Total Soluble Solids

Total soluble solids were determined using random grape samples, and the results showed that the SZn60 application yielded higher °Brix values. Relative to the control, all foliar treatments increased Zn content significantly, with 1.16- and 1.38-fold increases found for 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 significant differences of Zn content among treatments ($p \leq 0.05$). Treatments OZn30, OZn60, SZn30, and SZn60 indicated the following concentrations for zinc oxide (ZnO) or zinc sulfate ($ZnSO_4$): 0%, 30%, 60%. (i.e., 0, 450, and 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 content in grapes treated with ZnO and $ZnSO_4$ showed significant increases compared to the control (Table 2), with the OZn60 and SZn60 foliar treatments achieving the highest increases (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, and SZn60 indicate the following concentrations for zinc oxide (ZnO) or zinc sulfate ($ZnSO_4$): 0%, 30%, 60%. (i.e., 0, 450, and 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 the tissue level, two regions were defined in the grapes: the grape flesh and the seeds (Table 3). Compared to the control, grape flesh showed 2.41- and 2.37-fold increases in Zn content with the OZn60 and SZn60 treatments, respectively, whereas for the seeds, 1.76- and 2.19-fold increases were measured, respectively (Table 3). Compared to the seeds, the grape flesh showed a consistently higher increase in Zn content.

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

Variety Syrah	Grape 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 yielded an increasing accumulation of this nutrient in the produced wine (Table 4). Spraying with OZn30 and SZn60 yielded the best responses (relative 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) indicate significant differences ($p \leq 0.05$). Treatments OZn30, OZn60, SZn30, and SZn60 indicate the following concentrations for zinc oxide (ZnO) or zinc sulfate ($ZnSO_4$): 0%, 30%, 60%. (i.e., 0, 450, and 900 g ha^{-1}).

Syrah	Zn Content 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 the wine. Grapes must have a sufficient quantity of sugar to ensure a high fermentation rate. Indeed, insufficient time to maturation leads to watery wines with low alcohol concentration. Additionally, grapes that are harvested after the optimal time point produce a wine rich in alcohol but with low acidity [12]. Accordingly, the perfect timing for harvest depends on the country or region of production, the type of wine, and natural environmental conditions [13–15]. In this context, our data (Table 1) showed an increase of the amount of soluble solids in Zn-treated grapes, ranging between approx. 13.13–18.10 °Brix, which favored our vinification process.

It has also been reported [16,17] that Zn biofortification, through soil application or foliar spraying, might affect yield parameters, grain quality, and land and water productivity. $ZnSO_4$ is the most widely applied fertilizer due to its high solubility and low cost [18], but ZnO has also been shown to be effective in sunflower plants, increasing Zn content in all plants and improving dry weight, leaf area, and photosynthesis parameters [19]. Regarding the effectiveness of Zn enrichment in Syrah grapes, it was possible to verify that OZn60 demonstrated better results (compared to SZn60). Moreover, Zn accumulation prevailed in the flesh of the grapes, surpassing 30% compared to the control, thus revealing the effectiveness of biofortification [20,21]. Indeed, a higher biofortification index was achieved (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 because Zn is preferentially concentrated in the grape peel and seeds [22]. Comparing the content of Zn in grapes and wine, significant 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 increases the total soluble solids, but additional assays should be performed when climatic conditions have a stronger influence on the content.

Biofortification in general proved to be effective in increasing the Zn content of grapes and wine, and OZn yielded the best results, but the vinification process itself needs to be optimized.

Supplementary Materials: The poster presentation is available online at https://www.mdpi.com/article/10.3390/IECPS2020-08718/s1.

Author Contributions: M.G., J.C.R., P.S.C. and F.C.L. conceived and designed the experiments; C.C.P., A.R.F.C., A.C.M., I.C.L., D.D., P.S.C. and I.P.P. performed the experiments; C.C.P., P.S.C., I.P.P. and F.C.L. analyzed the data; M.M.S., M.G., R.G.L., M.S., J.C.R., F.H.R., M.F.P., P.S.C., I.P.P., P.L. and F.C.L. contributed reagents/materials/analysis tools; D.D. and F.C.L. wrote the paper. All authors have read and agreed to the published version of the manuscript.

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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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