

# Tissue Accumulation and Quantification of Zn in Biofortified *Triticum aestivum* Grains—Interactions with Mn, Fe, Cu, Ca, K, P and S <sup>†</sup>

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**Abstract:** Zinc has a fundamental role at the regulatory, functional and structural levels, yet its deficiency leads to loss of brain function, changes in growth and weakening of the immune system. In this context, biofortification, which is a process in which there is an enrichment of both content and the bioavailability of micronutrients in edible tissues of staple foods, may be used to overcome Zn deficiency. Considering that *Triticum aestivum* L. is a staple food largely used for flour production, an itinerary for Zn biofortification was implemented in two cvs (Roxo and Paiva), produced in an experimental cereal field production located in Alentejo, Portugal. These cvs were submitted to three different treatments (control—without foliar spraying, 6.3 and 12.6 kg ha<sup>-1</sup> of Zn-EDTA pulverization), being applied three zinc foliar application at booting, heading and grain milk stages. The accumulation of Zn, Mn, Fe, Cu, Ca, K, P and S in bread wheat was investigated, being found that, in general, maximum contents occurred in the embryo and vascular bundle. Besides, although Zn increased in the wheat grain, specially the higher concentration, did not markedly affected the other minerals concentration. It was concluded that whole wheat flour biofortified in Zn is a more suitable option for a healthier diet, rich in minerals and leading to the creation of an added value product useful to decrease micronutrient deficiency.

**Keywords:** grain minerals location; minerals quantification; *Triticum aestivum*; Zn biofortification

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## 1. Introduction

About 3 billion people suffer from malnutrition. Malnutrition can be divided into undernutrition, overnutrition and hidden hunger (or micronutrient deficiency) [1]. The hidden hunger affects approximately 2 billion people [2]. The world population is expected to reach more than 8 billion in 2030 and in 2050 it is estimated to range between 9.4 and 10.1 billion [3]. In this way,

it is vital to find new strategies to increase food production as well as to diminish nutrient deficiencies. In this context, Zn deficiency is considered the fifth major cause of disease and mortality in developing countries and can lead to a weakening of the immune system, loss of brain functions and changes in physical growth [4].

Biofortification can be a strategy to be implemented, mainly in developing countries, as a mean of tackling nutrient deficiencies. Indeed, biofortification is a strategy of enrichment of whether the content or the nutrient bioavailability in edible parts of staple crops, during the plant growth [5,6]. In this context, as cereal crops, like *Triticum aestivum* L. are considered to be one of the staple foods which can provide a considerable source of carbohydrates in human diet, besides, it is consumed in large scale around the world [7], it is largely prone to biofortification. Bread wheat is a cereal crop with low content of Zn and Fe [8,9]. Due to this fact, its biofortification in zinc can be a good approach to invest in the enhancement of Zn.

Zinc accumulates preferably in the aleurone and the embryo in wheat grain, nonetheless, it accumulates in minor predominance in the endosperm [10]. Zinc also interacts with a whole myriad of enzymes and proteins, playing a fundamental role at regulatory, functional, and structural levels in the human body [8].

This work aims to quantify and locate, at the tissue level of Zn biofortified bread wheat grains of cvs Roxo and Paiva, Mn, Fe, Cu, Ca, K, P and S, to select the most suitable wheat flour with a higher nutritional value.

## 2. Experiments

### 2.1. Experimental Field

*Triticum aestivum* L. cv Roxo and cv Paiva, obtained from the national breeding programme carried out on the National Institute for Agriculture and Veterinary Research (INIAV), located in Elvas, Portugal, were cultivated in a cereal production field located at 38°01'52.38" N; 7°52'53.72" W, in Beja, Portugal. The field was sown at 30 December of 2018, with a rate of 350 seeds m<sup>-2</sup>, and the experimental period finished with the harvest taking place at 27 June of 2019. This period was characterized by maximum and minimum average temperatures of 22 °C and 11 °C, with a maximum temperature of 39 °C and a minimum of 0 °C, respectively. During this experimental period, maximum and minimum air humidity were 100% and 0%, respectively (with an average ranging between 69% and 11% for the maximum and minimum, respectively). The total rainfall accumulation was about 5.43 mm (with a daily maximum of 1.85 mm), corresponding to an average rainfall of 0.03 mm. The agronomic biofortification of these cvs comprised three zinc foliar application on three different moments (booting, heading and grain milk stages), in April and May. The experimental cereal field was divided in two parts, where both bread wheat cvs (Roxo and Paiva) were submitted to three different treatments (control—without foliar spraying, 6.3 and 12.60 kg ha<sup>-1</sup> of Zn-EDTA pulverization) and also with 46% urea. The control plots were not sprayed at all with the fertilizer. Before sowing, the field was fertilized with 50 kg Zn ha<sup>-1</sup> and with NPK fertilizer. The experimental field was sown in a randomized block design with four repetitions, comprising 24 plots with an area of 9.6 m<sup>2</sup> (8 m × 1.2 m), with 0.4 m rows between plots and 2 m between repetition.

### 2.2. Tissue Location and Quantification of Nutrients in Wheat Grain

Zinc location in grain tissues collected at harvest was determined using the *micro-Energy Dispersive X-Ray Fluorescence system* ( $\mu$ -EDXRF) (M4 Tornado™, Bruker, Germany), according to [11]. The X-ray generator was operated at 50 kV and 100  $\mu$ A without the use of filters, to enhance the ionization of low-Z elements. To a better quantification of the element, a set of filters between the X-ray tube and the sample, composed of three foils of Al/Ti/Cu (with a thickness of 100/50/25  $\mu$ m, respectively) was used. All the measurements with filters were performed with 600  $\mu$ A current. Detection of fluorescence radiation was performed by an energy-dispersive silicon drift detector, XFlash™, with 30 mm<sup>2</sup> sensitive area and energy resolution of 142 eV for Mn K $\alpha$ . To better measure the distribution mapping of zinc, the grains were cut in half longitudinally, along the crease tissue,

with a stainless-steel surgical blade. Measurements were carried out under 20 mbar vacuum conditions and performed directly on one side of the grains. These point spectra were acquired during 200 s.

### 3. Results

In overall, the macroelements P, S, K and Ca, as well as, the microelements Mn, Fe, Cu and Zn are preferably located in the embryo and in the vascular bundle (Tables 1 and 2). Relatively to S, Ca, Mn and Zn, in the grains P, K, Mn and Fe prevailed with higher values. Besides, for all mineral mentioned, both varieties tended to present similar values. Relatively to P, with the increasing foliar application of Zn-EDTA a slight rise in the four zones of the grain occurred (particularly for the embryo). There were no differences in S contents when applying higher concentrations of the fertilizer. A minor rise in K and Ca values was observed between the control and Zn-EDTA concentration 6.3 kg ha<sup>-1</sup>, but with 12.6 kg ha<sup>-1</sup> both minerals decrease in every zone.

**Table 1.** Quantification of P, S, K and Ca in Paiva and Roxo varieties of bread wheat grains, in the control (C0) and after pulverization with Zn-EDTA (C1—6.3 kg ha<sup>-1</sup> and C2—12.6 kg ha<sup>-1</sup>). Grain quantification was divided into four zones of the grain (embryo; endosperm; vascular bundle and whole grain) and each side of the grain was quantified separately.

		Microelements (ppm)					
Fertilizer	Variety	Treatment	Zone	Mn	Fe	Cu	Zn
Zn-EDTA	Paiva	C0	Embryo 1	437	308	47.3	257
			Embryo 2	210	155	27.2	159
			Endosperm 1	23.5	25.7	5.95	21.7
			Endosperm 2	29.3	29.7	14.8	20.9
			Vascular bundle 1	287	44.04	25.3	109
			Vascular bundle 2	604	127	61.6	116
		Whole grain 1	114	68.6	11.8	54.3	
		Whole grain 2	107	67.03	14.3	53.1	
		C1	Embryo 1	343	241	22.2	271
			Embryo 2	331	244	31.6	235
			Endosperm 1	24.3	37.3	17.97	31.99
			Endosperm 2	33.3	22.4	10.5	20.3
	Vascular bundle		432	81.8	25.8	134	
	Whole grain 1		149	81.2	12.5	76.7	
	Roxo	C2	Whole grain 2	124	87.8	13.8	72.4
			Embryo 1	491	266	36.99	375
			Embryo 2	289	196	40.1	211
			Endosperm 1	26.1	19.5	12.6	34.8
			Endosperm 2	14.2	28.3	11.4	21.1
			Vascular bundle	339	106	23.1	187
		C0	Whole grain 1	161	98.2	14.1	117
			Whole grain 2	112	76.5	12.7	85.7
			Embryo 1	297	208	40.2	190
			Embryo 2	175	146	26.4	110
Endosperm 1			14.6	36.5	14.8	24.6	
Endosperm 2			12.1	31.1	11.7	30.9	
			Vascular bundle 1	84.7	74.7	20.5	60.7
			Vascular bundle 2	184	131	31.4	107

C1	Whole grain 1	86.7	78.2	14.2	52.2
	Whole grain 2	72.5	65.2	10.99	44.8
	Embryo 1	552	265	36.4	329
	Embryo 2	233	162	27.9	139
	Endosperm 1	39.5	69.5	16.5	47.8
	Endosperm 2	58.96	26.9	18.2	50.04
	Vascular bundle 1	519	193	39.8	193
	Vascular bundle 2	296	87.7	34.7	154
	Whole grain 1	93.5	76.99	12.6	69.7
	Whole grain 2	165	97.3	14.9	99.3
C2	Embryo 1	337	233	28.04	192
	Embryo 2	472	249	27.9	326
	Endosperm 1	57.7	38.4	14.3	59.2
	Endosperm 2	48.4	34.3	16.6	47.3
	Vascular bundle 1	304	113	28.1	171
	Vascular bundle 2	273	77.4	27.3	158
	Whole grain 1	137	71.4	12.4	87.4
	Whole grain 2	147	81.95	13.6	110

There was a rise in Mn contents with the gradual increase of Zn-EDTA pulverization, being more pronounced in the endosperm of Roxo variety. A sharp value of Fe was observed in the embryo, relatively to the other zones in the grain. Cu poor contents spreaded through the different grain areas, in an apparently uniform way, with low values. There was a gradual rise of Zn levels with increasing concentration of Zn-EDTA in all the grain zones, with Paiva displaying slightly higher values than Roxo.

**Table 2.** Quantification of Mn, Fe, Cu, Zn, in Paiva and Roxo varieties of bread wheat grains, in the control (C0) and after pulverization with Zn-EDTA (C1—6.3 kg ha<sup>-1</sup> and C2—12.6 kg ha<sup>-1</sup>). Grain quantification was divided into four zones of the grain (embryo; endosperm; vascular bundle and whole grain) and each side of the grain was quantified separately.

Fertilizer	Variety	Treatment	Macroelements (%)					
			Zone	P	S	K	Ca	
Zn-EDTA	Paiva	C0	Embryo 1	1.97	0.405	2.79	0.301	
			Embryo 2	1.37	0.295	1.33	0.14	
			Endosperm 1	0.158	0.2001	0.217	0.0235	
			Endosperm 2	0.183	0.24	0.209	0.0314	
			Vascular bundle 1	0.196	0.205	1.08	0.162	
			Vascular bundle 2	0.854	0.317	1.88	0.435	
			Whole grain 1	0.454	0.21	1.17	0.1403	
			Whole grain 2	0.449	0.193	1.53	0.1605	
			C1	Embryo 1	1.92	0.444	2.56	0.185
				Embryo 2	2.17	0.446	2.7	0.359
		Endosperm 1		0.164	0.211	0.313	0.052	
		Endosperm 2		0.165	0.213	0.349	0.058	
		Vascular bundle		0.607	0.323	2.47	0.387	
		Whole grain 1		0.673	0.214	2.02	0.206	
		Whole grain 2		0.785	0.223	2.51	0.238	
		C2		Embryo 1	2.13	0.489	2.72	0.193
			Embryo 2	2.22	0.44	1.84	0.148	

		Endosperm 1	0.161	0.173	0.144	0.0186
		Endosperm 2	0.121	0.137	0.0962	0.0127
		Vascular bundle	0.333	0.248	1.35	0.122
		Whole grain 1	0.569	0.229	1.66	0.102
		Whole grain 2	0.952	0.241	1.83	0.104
		Embryo 1	1.92	0.532	2.84	0.192
		Embryo 2	1.81	0.417	1.88	0.191
		Endosperm 1	0.0978	0.184	0.137	0.0278
		Endosperm 2	0.141	0.203	0.121	0.0286
	<b>C0</b>	Vascular bundle 1	0.529	0.261	0.872	0.0753
		Vascular bundle 2	0.436	0.22	1.2	0.121
		Whole grain 1	0.63	0.198	1.87	0.112
		Whole grain 2	0.966	0.226	1.98	0.131
		Embryo 1	3.07	0.519	2.64	0.269
		Embryo 2	1.47	0.457	1.36	0.179
		Endosperm 1	0.185	0.317	0.156	0.0382
		Endosperm 2	0.157	0.27	0.0895	0.0291
	<b>C1</b>	Vascular bundle 1	0.435	0.451	1.48	0.2199
		Vascular bundle 2	0.294	0.333	1.14	0.188
		Whole grain 1	0.532	0.253	1.4	0.114
		Whole grain 2	0.592	0.251	1.83	0.138
		Embryo 1	1.95	0.582	2.27	0.35
		Embryo 2	3.14	0.675	2.46	0.255
		Endosperm 1	0.12	0.284	0.344	0.0517
		Endosperm 2	0.181	0.42	0.185	0.0444
	<b>C2</b>	Vascular bundle 1	0.374	0.313	1.12	0.133
		Vascular bundle 2	0.227	0.266	2.04	0.397
		Whole grain 1	0.552	0.284	1.43	0.151
		Whole grain 2	0.886	0.317	1.87	0.174

#### 4. Discussion

In bread wheat minerals do not accumulate throughout the different zones in a similar manner, prevailing specific accumulation zones. The preferable accumulation of minerals in the embryo and the vascular bundle is consistent with previous studies of our research team [12], where P, S, K, Ca, Mn, Fe, Cu and Zn presented higher concentrations in the embryo scutellum, embryo radicle and vascular bundle, of biofortified *Triticum aestivum* cv. Roxo (with a solution containing ZnSO<sub>4</sub>·4H<sub>2</sub>O). However, Ca, Mn, Fe, Cu and Zn were mostly located in the vascular bundle. Also, compared to the control, Fe and Cu accumulation was slightly higher in the embryo in biofortified grains. Biofortification also increased Zn values, specially in the vascular bundle. Zinc fertilization is well known to increase grain and, consequently, the whole flour zinc concentration in wheat, either by soil or foliar application or by combining zinc soil and foliar applications [13,14,15]. The presence of minerals in this particular zones of grain reveals that, at an industrial level, when preferentially refined wheat flour is used, in which the outermost layers of the grain are removed, the products become less nutritionally rich. It was also found that Zn further interacts with the biochemical processes of some minerals, displaying antagonistic interactions, namely with Cu, Fe and Ca. Nevertheless P and Zn might reveal both interactions of antagonistic and synergistic interactions [16]. In this study Ca appears to have an antagonistic interaction with Zn, but the same effect was not observed in Fe and Cu.

#### 5. Conclusions

Through Zn-EDTA foliar spraying, in general, accumulation of Zn, Mn, Fe, Cu, Ca, K, P and S in bread wheat prevails in the embryo and vascular bundle. Through the applied biofortification itinerary of both cvs, Zn increased in the wheat grain, specially the higher concentration, but did not affected markedly the other minerals concentration in the grain, which suggests that the whole wheat flour biofortified in Zn is a more suitable option for a healthier diet rich in minerals and leading to the creation of an added value product useful to decrease micronutrient deficiency.

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## Abbreviations

The following abbreviations are used in this manuscript:

- C0 Control
- C1 corresponds to three foliar sprays of Zn-EDTA with the concentration of 6.3 kg ha<sup>-1</sup>
- C2 corresponds to three foliar sprays of Zn-EDTA with the concentration of 12.6 kg ha<sup>-1</sup>

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