



Proceeding Paper

# Natural Mineral Enrichment in *Solanum tuberosum* L. cv. Agria: Accumulation of Ca and Interaction with Other Nutrients by XRF Analysis <sup>†</sup>

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Abstract: Calcium is a crucial nutrient for bone development and the normal functioning of the circulatory system, whereas its deficiency can trigger the development of osteoporosis and rickets. On the other hand, Solanum tuberosum L. is one of the most important staple food crops worldwide and is a primary component of the human diet. Accordingly, using this staple food, this study aims to develop a technical itinerary for Ca biofortification of cv. Agria. As such, an itinerary of Ca biofortification was promoted throughout the respective production cycle. Seven foliar sprays with CaCl<sub>2</sub> or chelated calcium (Ca EDTA) were used at concentrations of 12 and 24 kg ha<sup>-1</sup>. The index of Ca biofortification and the related interactions with other chemical elements in the tuber were assessed. It was found that, relative to the control at harvest, Ca content increased 1.07-2.22 fold (maximum levels were obtained with 12 kg ha<sup>-1</sup> Ca-EDTA). Ca(EDTA) at a concentration of 24 kg ha<sup>-1</sup> showed the secondhighest levels in Ca, S and, P content. By adding CaCl<sub>2</sub>, it was also possible to identify a tendency of increasing contents (in Ca, K, S, and P) when the spraying concentration increased (12 kg ha<sup>-1</sup> to 24 kg ha<sup>-1</sup>). Outside of the Ca higher content, dry weight, height, diameter, and the colorimetric parameter L of the tubers did not vary significantly, but minor changes occurred in the colorimetric parameters Chroma and Hue. It was concluded that Ca(EDTA) could trigger a more efficient Ca biofortification of Agria potato tubers with the additional enrichment of K, S, and P.

**Keywords:** calcium accumulation; calcium biofortification; *Solanum tuberosum* L.



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# 1. Introduction

After rice, wheat, and maize [1–4], *Solanum tuberosum* L. is one of the most important staple food crops worldwide [1]. The potato is a primary component of the human diet [5] and can provide 5–15% of dietary calories [6], minerals, vitamins, and carbohydrates [7]. It is rich in K, vitamin C, and B6 [1] and phytochemicals, such as phenolics and carotenoid

compounds [8]. Additionally, due to its major consumption all over the world, enrichment of potato tubers with different minerals, such as selenium [9–11] or zinc [12,13], has been carried out [14]. In this context, agronomic biofortification is frequently used to increase the content of different minerals in edible plants through foliar fertilization, which is faster and more cost-effective [15].

Studies with apples [16], peaches [17], potatoes [18], and other vegetables [19] have shown a higher Ca content after foliar spraying. Calcium has a vital role in the anatomy, physiology, and biochemistry of organisms [20]. It is essential for plants (required as Ca<sup>2+</sup>), as it has a central role in stress responses [21] and acts as a signal transduction agent [22]. Further, it is needed as a cofactor by enzymes taking part in the catabolism of ATP and phospholipids [23] and provides integrity and stability to cell walls [22]. In the human body, it is also a crucial nutrient for bone development and the normal functioning of the circulatory system [24–27]. Ca deficiency can trigger osteoporosis [20] and rickets [25]. In this context, to minimize Ca deficiency in the human population, the aim of this study is to develop an itinerary for Ca biofortification of potato tubers. Regarding the importance of this staple food for agro-industrial processing, the Agria variety was used as a test system because of its range of uses, such as french fries and starch/flakes [28].

### 2. Experiments

An experimental potato field located in western Portugal was used to grow cv. Agria (*Solanum tuberosum* L.). During the growing period, from 15 March (planting date) to 29 July 2019 (harvest date), air temperatures reached a daily average of 21.9 °C and 13.8 °C (with maximum and minimum values of 34.8 °C and 4.7 °C, respectively). The average rainfall was 0.51 mm, with a daily maximum of 10.4 mm. After the beginning of tuberization, seven foliar sprayings (with a 6–8 day interval) were performed with CaCl<sub>2</sub> (12 and 24 kg ha $^{-1}$ ). Because Ca(EDTA) might become highly toxic to plants, only one foliar application of 24 kg ha $^{-1}$  with Ca(EDTA) was carried out, whereas seven spraying applications were performed with 12 kg ha $^{-1}$ . Control plants were not sprayed at any time with CaCl<sub>2</sub> or Ca(EDTA). All treatments were performed in quadruplicate on plots that measured 20  $\times$  24 m.

Calcium, K, S, and P content were determined in randomized tubers after being cut, dried (at 60 °C until constant weight), and ground using an XRF analyzer (model XL3t 950 He GOLDD+) under He atmosphere, according to [29].

Height, diameter, and dry weight were measured considering four randomized tubers per treatment. Colorimetric parameters, using fixed wavelength, followed [30]. Brightness (L) and chromaticity parameters (a\* and b\* coordinates) were obtained with a Minolta CR 400 colorimeter (Minolta Corp., Ramsey, NJ, USA) coupled to a sample vessel (CR-A504). Using the illuminant  $D_{65}$ , the system of the Commission Internationale d'Éclaire (CIE) was applied. The parameter L represents the brightness of the sample, indicating the variation of the tonality between dark and light, with a range between 0 (black) and 100 (white). Parameters a\* and b\* indicate color variations between red (+60) and green (-60) and between yellow (+60) to blue (-60), respectively. The approximation of these coordinates to the null value translates neutral colors like white, gray, and black. Chroma is the relationship between the values of a\* and b\*, where the real color of the analyzed object is obtained. Hue is the angle formed between a\* and b\*, indicating the saturation of the object's color. To calculate Chroma (C), Equation (1) was used, and to calculate Hue-Angle (H), Equation (2). Measurements were carried out in quadruple in the pulp of fresh tubers at harvest.

$$C^* = \sqrt{a *^2 + b *^2} \tag{1}$$

$$H^* = \operatorname{arctg} \frac{b^*}{a^*} \tag{2}$$

Data were statistically analyzed using a One-Way ANOVA to assess differences among treatments in cv. Agria, followed by a Tukey's for mean comparison. A 95% confidence level was adopted for all tests.

#### 3. Results

After harvest, Ca, K, S, and P accumulation in the tubers was assessed in cv. Agria, (Table 1). Relative to the control, the content of Ca was significantly higher in all treatments (except in  $CaCl_2$ –12 kg  $ha^{-1}$ ), with an increase in Ca content ranging between 1.07 and 2.22 fold (maximum levels obtained with 12 kg  $ha^{-1}$  Ca-EDTA). Considering all the four macronutrients analyzed, the treatment of 12 kg  $ha^{-1}$  Ca(EDTA) showed the maximum content, with significant differences regarding the control, whereas the control showed the lowest content (Table 1). Regarding both fertilizers, the highest content prevailed in the treatments of Ca(EDTA), despite only one application for treatment with 24 kg  $ha^{-1}$  being carried out. Ca(EDTA) at a concentration of 24 kg  $ha^{-1}$  showed the second-highest levels in Ca, S, and P content. Regarding only the treatments applied with CaCl<sub>2</sub>, it is possible to identify a tendency of increasing content (in Ca, K, S, and P) when spraying concentration is increased (12 kg  $ha^{-1}$  to 24 kg  $ha^{-1}$ ).

**Table 1.** Mean values  $\pm$  S.E. (n = 4) of Ca, K, S, and P in tubers of *Solanum tuberosum* L., cv. Agria, at harvest. Different letters indicate significant differences of each parameter between treatments ( $p \le 0.05$ ). Foliar spraying was carried out with two concentrations (12 and 24 kg·ha<sup>-1</sup>) of CaCl<sub>2</sub> and Ca(EDTA). Control was not sprayed.

Treatments	Ca	K	S	P
	$ m g~kg^{-1}$			
Control	$0.57~{ m d}\pm0.01$	$30.73 \text{ e} \pm 0.19$	$1.13 c \pm 0.06$	$0.80 d \pm 0.05$
$CaCl_2$ (12 kg $ha^{-1}$ )	$0.61 d \pm 0.02$	$31.57 d \pm 0.08$	$1.15~c\pm0.01$	$0.62~\mathrm{e}\pm0.01$
$CaCl_2$ (24 kg $ha^{-1}$ )	$0.72 c \pm 0.00$	$35.40 \text{ b} \pm 0.02$	$1.24~c\pm0.00$	$1.00 c \pm 0.00$
$Ca(EDTA)$ (12 kg $ha^{-1}$ )	$1.27~\mathrm{a}\pm0.01$	$41.23~a\pm0.15$	$2.07 a \pm 0.03$	$1.72~\mathrm{a}\pm0.01$
Ca(EDTA) (24 kg ha <sup>-1</sup> )	$1.07~b\pm0.00$	$32.28 c \pm 0.09$	$1.49\mathrm{b} \pm 0.01$	$1.34~\text{b}\pm0.01$

Independently of the Ca higher content, dry weight, height, and diameter of the tubers did not vary significantly (Table 2). However,  $24 \text{ kg ha}^{-1} \text{ CaCl}_2$  tubers showed the lowest dry weight comparing the applied treatments. Also, treatment with  $12 \text{ kg ha}^{-1} \text{ CaCl}_2$  showed the highest percentage of dry weight.

**Table 2.** Mean values  $\pm$  S.E. (n = 4) of dry weight, height, and diameter in tubers of *Solanum tuberosum* L., cv. Agria, at harvest. Letter a indicates no significant differences of each parameter between treatments ( $p \le 0.05$ ). Foliar spraying was carried out with two concentrations (12 and 24 kg·ha<sup>-1</sup>) of CaCl<sub>2</sub> and Ca(EDTA). Control was not sprayed.

Treatments	Dry Weight (%)	Height (cm)	Diameter (cm)
Control	$17.12~a\pm0.69$	$8.20~a\pm0.49$	$7.57~{ m a}\pm0.48$
$CaCl_2$ (12 kg $ha^{-1}$ )	$21.89 \text{ a} \pm 0.89$	$10.10~\mathrm{a}\pm1.01$	$8.03~{ m a}\pm0.52$
$CaCl_2$ (24 kg $ha^{-1}$ )	$16.77~a\pm 2.52$	$9.20~{ m a}\pm0.96$	$6.63~a\pm0.52$
Ca(EDTA) (12 kg ha <sup>-1</sup> )	$20.97~a\pm1.87$	$8.20~{ m a}\pm0.61$	$6.60~{ m a}\pm0.42$
$Ca(EDTA)$ (24 kg $ha^{-1}$ )	$18.99 \text{ a} \pm 0.44$	$12.67~a\pm 2.27$	$7.97~a \pm 0.38$

Considering the colorimetric parameters in the fresh tubers of cv. Agria, it was found that (Table 3) the brightness/luminosity had no significant changes. However, the Chroma parameter (saturation) did vary significantly, with the more intense color obtained in 12 kg ha<sup>-1</sup> Ca(EDTA) treatment. The control and 24 kg ha<sup>-1</sup> Ca(EDTA) treatment showed similar values of Chroma. Concerning the Hue parameter, only 12 kg ha<sup>-1</sup> Ca(EDTA) showed significant differences regarding the remaining treatments and control.

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**Table 3.** Mean values  $\pm$  S.E. (n = 4) of colorimetric parameters (L, Chroma, and Hue) in fresh tubers of *Solanum tuberosum* L., cv. Agria, at harvest. Letters a and b indicate significant differences of each parameter between treatments (statistical analysis using the single factor ANOVA test,  $p \le 0.05$ ). Foliar spraying was carried out with two concentrations (12 and 24 kg·ha<sup>-1</sup>) of CaCl<sub>2</sub> and Ca(EDTA). Control was not sprayed.

Treatments	L	Chroma	Hue
Control	$62.88 \text{ a} \pm 1.36$	$22.76 \text{ b} \pm 0.37$	$105.8~{ m a}\pm0.2$
$CaCl_2$ (12 kg $ha^{-1}$ )	$62.74~a \pm 2.03$	$24.18 \ \text{a,b} \pm 0.89$	$104.9~\mathrm{a}\pm0.4$
$CaCl_2$ (24 kg $ha^{-1}$ )	$63.51 \text{ a} \pm 0.74$	$25.09 \text{ a,b} \pm 0.38$	$108.5~\mathrm{a}\pm0.1$
Ca(EDTA) (12 kg ha <sup>-1</sup> )	$62.92~a\pm0.71$	$30.47~a \pm 2.91$	$102.3 \text{ b} \pm 1.1$
Ca(EDTA) (24 kg $ha^{-1}$ )	$64.98~a \pm 3.12$	$23.27 \text{ b} \pm 0.82$	$105.3~\mathrm{a}\pm0.2$

#### 4. Discussion

Calcium accumulation in potato tubers relies upon the interaction of different factors, such as the development of the tuber, phloem and xylem delivery, and other chemical interactions within the tuber [31]. In fact, Ca depends on its delivery via the xylem because, in the phloem, it is almost immobile [32]. Different types of cultures provided with Ca, showed an increase in this mineral content, mainly using CaCl<sub>2</sub> [33]. However, despite that, Ca-EDTA is not usually used. There were studies carried out, namely with sweetcorn [34] and apples [35], that applied this type of Ca chelate. In this context, CaCl<sub>2</sub> and Ca(EDTA) were applied in similar concentrations in the tuber plants cv. Agria. Yet, despite just one foliar application with 24 kg ha<sup>-1</sup> Ca(EDTA), it showed the second-highest Ca content regarding the remaining treatments. In fact, the two concentrations applied with Ca(EDTA) showed higher Ca content, as with the two treatments of CaCl<sub>2</sub> (Table 1). Comparing the number of foliar applications of both treatments with Ca(EDTA), it was possible to verify that treatment with 24 kg ha<sup>-1</sup> (applied only once) presented just less (15.75%) Ca content than the treatment with 12 kg ha<sup>-1</sup> (that was applied seven times). However, as seen in tomato plants, Ca(EDTA) is toxic to plants when applied repeatedly [36]. Regarding the nutrient content (Table 1), cv. Agria varied among the treatments. Potassium is one of the main minerals present in tubers [37], and the contents of Ca and P obtained were higher compared to another study that used the same cultivar [38]. Also, it can be seen that, with the increase in Ca content, S content also increased, which was reported previously by [39]. On the other hand, higher content of S can also improve the absorption of K and P [39], as found in our study (Table 1).

We considered the importance of the dry matter content since it is an important characteristic for industrial processing and one criterion for the classification of potato tubers [40]. It was possible to verify any significant differences compared to the control. Also, the industry has a requirement for potatoes to have a dry matter content higher than 20% (which is the case of 12 kg  $ha^{-1}$  CaCl<sub>2</sub> and Ca(EDTA) treatments (Table 2)), since higher dry matter content reduces fat absorption during the frying process, producing more crispy chips [40]. A positive relationship between Ca application and the hardness of fries was found in other potato varieties, improving this quality parameter [41]. Considering the dry matter obtained in this study, it was further possible to verify a similarity to the values obtained by other authors for the same variety [42,43]. Regarding the height and diameter of tubers, there was no interference relatively to the Ca-biofortification process (Table 2), maintaining its industrial characteristics. According to Portuguese law, the tubers' caliber should be higher than 3.5 cm [44], which agrees with our data for cv. Agria, and therefore, the biofortified potatoes are suitable for industrial processing [45]. Also, the diameter of tubers acquired in this study is in accordance with values obtained by other authors for the same variety [46,47].

The perception of color as a definition of quality for agricultural products, such as in coffee [30,48], strawberries, grapes, plums [49], sweet potatoes [50], apples [51,52], and potatoes [1,8], is very important to consumers [53]. Regarding the L parameter, the data obtained showed lower values compared to other studies for the same cultivar [47,54–56].

Also, the Chroma parameter showed lower values compared to other studies for the same cultivar (except in Chroma—12 kg ha<sup>-1</sup> Ca(EDTA) treatment) [5,55]. However, it showed a higher Hue value compared to other authors [5]. In this context, there were minor effects among the different Ca treatments; for example, the treatment that showed higher Ca content (Table 1) showed the maximum value for Chroma and the lowest value for Hue (Table 3).

#### 5. Conclusions

In all treatments, pulverized with CaCl<sub>2</sub> and Ca(EDTA), *S. tuberosum* cv. Agria showed a significant increase in Ca contents. Nevertheless, for both applied concentrations of Ca(EDTA), a higher Ca content was found relative to CaCl<sub>2</sub> treatments (being 12 kg ha<sup>-1</sup> Ca(EDTA) treatment, the one that showed the higher Ca biofortification). Additionally, Ca biofortification did not trigger any changes in the dry matter, height, diameter, or L parameter of color. However, minor changes occurred in the colorimetric parameters Chroma and Hue.

**Supplementary Materials:** The poster presentation is available online at https://www.mdpi.com/article/10.3390/IECPS2020-08709/s1.

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

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