*proceedings*

*Proceedings*

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

**Ana Coelho [1,2,*], Cláudia Pessoa [1,2], Ana Marques [1,2], Inês Luís [1,2], Diana Daccak [1,2], Maria Manuela Silva [2,3], Manuela Simões [1,2], Fernando Reboredo [1,2], Maria Pessoa [1,2], Paulo Legoinha [1,2], José Ramalho [2,4], Paula Scotti-Campos [2,5], Isabel Pais [2,5] 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 ESEAG—Grupo Universidade Lusófona, Lisboa, Portugal
4 PlantStress & Biodiversity Lab, Centro de Estudos Florestais, Instituto Superior Agronomia, Universidade de Lisboa, Oeiras, Portugal
5 INIAV, Instituto Nacional de Investigação Agrária e Veterinária, Oeiras, Portugal
† Presented at the 1st International Electronic Conference on Plant Science, 1–15 December 2020; Available online: https://iecps2020.sciforum.net/.
* Correspondence: arf.coelho@campus.fct.unl.pt; Tel.: +35-121-294-8573

Published: 1 December 2020

**Abstract:** Calcium is a very crucial nutrient for bone development and 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, being a primary component of 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 Ca biofortification was promoted throughout the respective production cycle. Seven foliar sprays with $CaCl_2$ or, alternatively, chelated calcium (Ca EDTA) were used at concentrations of 12 and 24 kg $ha^{-1}$. The index of Ca biofortification and the related interactions with other chemical elements in the tuber were assessed. It was found that, relatively to the control, at harvest, Ca content increase 1.07–2.22 fold (maximum levels obtained with 12 kg $ha^{-1}$ Ca-EDTA). Besides, Ca(EDTA) at a concentration of 24 kg $ha^{-1}$ showed the second highest levels in Ca, S and P content, but with $CaCl_2$ was also possible to identify a tendency of increasing contents (in Ca, K, S and P) when spraying concentration increased (12 kg $ha^{-1}$ to 24 kg $ha^{-1}$). Independently of the Ca higher content, dry weight, height, diameter and the colorimetric parameter L of tubers did not varied significantly, but minor changes occurred in the colorimetric parameters Chroma and Hue. It is concluded that Ca(EDTA) can trigger a more efficient Ca biofortification of Agria potato tubers, with additional enrichment of K, S and P.

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

## 1. Introduction

After rice, wheat and maize [2–4], *Solanum tuberosum* L. is one of the most important staple food crops worldwide [1]. 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]. Besides, due to the major consumption all over the world, enrichment of potato tubers with different minerals, such as selenium [9–11] or zinc [12,13] as been carried out [14]. In this context, agronomic biofortification is frequently used to increase different minerals content in the edible part of plants, being through foliar fertilization, a more faster and cost-effetive way [15].

Some studies with apples [16], peach [17], potato [18] and some vegetables [19] showed a higher Ca content after foliar spraying. Calcium has a vital role in the anatomy, physiology and biochemistry of organisms [20], being essential for plants (required as $Ca^{2+}$), as it has a central role in stress responses [21] and acts as a signal transduction agent [22]. It is further 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 also is a very crucial nutrient for bone development and normal functioning of the circulatory system [24–27]. However, Ca deficiency can trigger osteoporosis [20] and rickets [25]. In this context, to minimize Ca defiency 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, Agria variety was used as a test system because of the range of uses, such as french fries and starch/flakes [28].

## 2. Experiments

The experimental potato field, located in the Western of Postugal, was used to growth cv. Agria (*Solanum tuberosum* L.). During the agricultural period, from 15th March (planting date) to 29th July of 2019 (harvest date), air temperatures reached an average daily 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 spraying (with 6-8 days interval) were performed with $CaCl_2$ (12 and 24 kg $ha^{-1}$). As Ca(EDTA) might become highly toxic to plants only one foliar application of 24 kg $ha^{-1}$ with Ca(EDTA) was carried out, whereas with 12 kg $ha^{-1}$ seven spraying applications were performed. Control plants were not sprayed at any time with $CaCl_2$ or Ca(EDTA). All treatments were performed in quadruplicate in plots of 20 × 24 m.

Calcium, K, S and P content were determined in randomized tubers after being cut, dried (at 60 °C, until constant weight) and grounded, using a 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 quadrupled in the pulp of fresh tubers at harvest.

$$C^* = \sqrt{\boldsymbol{a} *^2 + \boldsymbol{b} *^2} \quad (1)$$

$$H^* = \mathbf{arctg}\, \frac{\boldsymbol{b}*}{\boldsymbol{a}*} \quad (2)$$

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). Relatively 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 1.07–2.22 fold (maximum levels obtained with 12 kg $ha^{-1}$ Ca-EDTA). Besides, considering all the four macronutrients analyzed, the treatment of 12 kg $ha^{-1}$ Ca(EDTA) showed the maximum contents with significant differences, regarding the control, whereas the control showed the lowest contents (Table 1). Regarding both fertilizers, the highest content prevailed in the treatments of Ca(EDTA), in spite of only one application for treatment with 24 kg $ha^{-1}$ were carried out. Actually, 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 $CaCl_2$, is possible to identify a tendency of increasing contents (in Ca, K, S and P) when spraying concentration increased (12 kg $ha^{-1}$ to 24 kg $ha^{-1}$).

**Table 1.** Mean values ± S.E. (n = 4) of Ca, K, S and P in tubers of *Solanum tuberosum* L., cv. Agria, at harvest. Different Letters indicates significant differences, of each parameter, between treatments ($P \leq 0.05$). Foliar spray was carried out with two concentrations (12 and 24 kg·$ha^{-1}$) of $CaCl_2$ and Ca(EDTA). Control was not sprayed.

| **Treatments** | **Ca** | **K** | **S** | **P** |
|---|---|---|---|---|
| | **g kg⁻¹** | | | |
| Control | 0.57d ± 0.01 | 30.73e ± 0.19 | 1.13c ± 0.06 | 0.80d ± 0.05 |
| $CaCl_2$ (12 kg $ha^{-1}$) | 0.61d ± 0.02 | 31.57d ± 0.08 | 1.15c ± 0.01 | 0.62e ± 0.01 |
| $CaCl_2$ (24 kg $ha^{-1}$) | 0.72c ± 0.00 | 35.40b ± 0.02 | 1.24c ± 0.00 | 1.00c ± 0.00 |
| Ca(EDTA) (12 kg $ha^{-1}$) | 1.27a ± 0.01 | 41.23a ± 0.15 | 2.07a ± 0.03 | 1.72a ± 0.01 |
| Ca(EDTA) (24 kg $ha^{-1}$) | 1.07b ± 0.00 | 32.28c ± 0.09 | 1.49b ± 0.01 | 1.34b ± 0.01 |

Independently of the Ca higher content, dry weight, height and diameter of tubers did not varied significantly (Table 2). However, 24 kg $ha^{-1}$ $CaCl_2$ tubers showed a lowest dry weight comparing to the applied treatments. Also, treatment with 12 kg $ha^{-1}$ $CaCl_2$ showed the highest percentage of dry weight.

**Table 2.** Mean values ± 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 ($P \leq 0.05$). Foliar spray was carried out with two concentrations (12 and 24 kg·$ha^{-1}$) of $CaCl_2$ and Ca(EDTA). Control was not sprayed.

| **Treatments** | **Dry Weight (%)** | **Height (cm)** | **Diameter (cm)** |
|---|---|---|---|
| Control | 17.12a ± 0.69 | 8.20a ± 0.49 | 7.57a ± 0.48 |
| $CaCl_2$ (12 kg $ha^{-1}$) | 21.89a ± 0.89 | 10.10a ± 1.01 | 8.03a ± 0.52 |
| $CaCl_2$ (24 kg $ha^{-1}$) | 16.77a ± 2.52 | 9.20a ± 0.96 | 6.63a ± 0.52 |
| Ca(EDTA) (12 kg $ha^{-1}$) | 20.97a ± 1.87 | 8.20a ± 0.61 | 6.60a ± 0.42 |
| Ca(EDTA) (24 kg $ha^{-1}$) | 18.99a ± 0.44 | 12.67a ± 2.27 | 7.97a ± 0.38 |

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

**Table 3.** Mean values ± 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 indicates significant differences, of each parameter, between treatments (statistical analysis using the single factor ANOVA test, *P* ≤0.05). Foliar spray was carried out with two concentrations (12 and 24 kg·ha⁻¹) of $CaCl_2$ and Ca(EDTA). Control was not sprayed.

| Treatments | L | Chroma | Hue |
|---|---|---|---|
| Control | 62.88a ± 1.36 | 22.76b ± 0.37 | 105.8a ± 0.2 |
| $CaCl_2$ (12 kg $ha^{-1}$) | 62.74a ± 2.03 | 24.18a,b ± 0.89 | 104.9a ± 0.4 |
| $CaCl_2$ (24 kg $ha^{-1}$) | 63.51a ± 0.74 | 25.09a,b± 0.38 | 108.5a ± 0.1 |
| Ca(EDTA) (12 kg $ha^{-1}$) | 62.92a ± 0.71 | 30.47a ± 2.91 | 102.3b ± 1.1 |
| Ca(EDTA) (24 kg $ha^{-1}$) | 64.98a ± 3.12 | 23.27b ± 0.82 | 105.3a ± 0.2 |

## 4. Discussion

Calcium accumulation in potato tubers rely upon the interaction of different factors, as such 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 phoem it is almost immobile [32]. Different types of cultures provided with Ca, showed an increased of this mineral content, mainly using $CaCl_2$ [33]. However despite that, Ca-EDTA is not usually used, there were some studies carried out, namely with sweetcorn [34] and apples [35], that applied this type of Ca chelate. In this context, $CaCl_2$ and Ca(EDTA) were somewhat applied in the same concentrations in tuber plants of cv. Agria. Yet, despite of just one foliar application with 24 kg $ha^{-1}$ Ca(EDTA), it showed the second highest Ca content regarding the remain treatments. In fact, the two concentrations applied with Ca(EDTA) showed higher Ca content likening with the two treatments of $CaCl_2$ (Table 1). Comparing the number of foliar applications of both treatments with Ca(EDTA), it was possible to verify that treatment with 24 kg $ha^{-1}$ (applied only once) presented just less 15.75 % Ca content than the treatment with 12 kg $ha^{-1}$ (that was applied seven times). However, as seen in tomato plants, Ca(EDTA) is toxic to plants when applied repeatedly and several times [36]. Regarding the nutrient content (Table 1) cv. Agria varied among the treatments. Potassium is one of the main mineral 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 been seen that, with the increase in Ca content, S content also increased, as being reported previsioly by [39]. On the other hand, higher contents of S can also improve the absorption of K and P [39], as found in our study (Table 1).

Considering the importance of the dry matter content, being a relevant characteristics for industrial processing and one criteria for the classification of potato tubers [40], it was possible to verify no significant differences regarding the control. Also, the industry considers a requirement for potatoes to have a dry matter content higher than 20% (which is the case of 12 kg $ha^{-1}$ $CaCl_2$ and Ca(EDTA) treatments—Table 2), since higher dry matter content reduces fat absorption during the frying process, producing more crispy chips [40]. Actually, it was found out (in other potato varieties) a positive relationship between Ca application and the hardness of fries, 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 not any interference relatively to the Ca-biofortificantion process (Table 2), maintaining its industrial characteristics. According to the Portuguese law, tubers caliber should be higher than 3.5 cm [44], being in agreement 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 authores 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 potato [50], apples [51,52] and potatoes [1,8] is very important to consumers [53]. Regarding L paramenter, the data obtained showed lower values compared to other studies for the same cultivar [47,54–56]. Also, Chroma parameter, showed lower values compared to other studies for the same cultivar (except in Chroma—12 kg $ha^{-1}$ Ca(EDTA) treatment) [5,55]. However, it showed a higher Hue value compared to other authors [5]. In this context, there was minor effects among the different Ca treatments, as such 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 $CaCl_2$ and Ca(EDTA), *S. tuberosum* cv. Agria showed a significant increase of Ca contents. Nevertheless, for both applied concentrations of Ca(EDTA) an higher Ca content was found relatively to $CaCl_2$ treatments (being 12 kg $ha^{-1}$ 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 and in L parameter of color. However, minor changes occurred in the colorimetric parameters Chroma and Hue.

**Author Contributions:** F.L conceived and designed the experiments; A.C., A.M., C.P., I.L., and D.D. performed the experiments; A.C. and F.L. analyzed the data; M.M.S., M.S, F.R, M.P. J.R., A.R., A.R., P.S.-C., I.P., J.S. and F.L. contributed reagents/materials/analysis tools; A.C. and F.L. wrote the paper.

**Acknowledgments:** The authors thanks to Eng. Nuno Cajão (Cooperativa de Apoio e Serviços do Concelho da Lourinhã- LOURICOOP) for technical assistance in the agricultural parcel as well as to project PDR2020-101-030719—for the financial support. We also thanks to the Research centres (GeoBioTec) UIDB/04035/2020, and (CEF) UIDB/00239/2020.

**Conflicts of Interest:** The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

## References

1. Xiao, Q.; Bai, X.; He, Y. Rapid Screen of the Color and Water Content of Fresh-Cut Potato Tuber Slices Using Hyperspectral Imaging Coupled with Multivariate Analysis. *Foods* **2020**, *9*, 94, doi:10.3390/foods9010094.
2. FAO (Food and Agriculture Organization). Potato world: Production and Consumption—International Year of the Potato 2008. Available online: http://www.fao.org/potato-2008/en/world/ (accessed on 3 November 2020).
3. CIP—International Potato Center. 2018**.** Potato Facts and Figures. Available online: https://cipotato.org/crops/potato/potato-facts-and-figures/ (accessed on 4 November 2020).
4. Muthoni, J.; Mbiyu, M.; Nyamongo, D. A review of potato seed systems and germplasm conservation in Kenya. *J. Agric. Food Inf*. **2010**, *11*, 157–167, doi:10.1080/10496501003680565.
5. Yang, Y.; Achaerandio, I.; Pujolà, M.. Classification of potato cultivars to establish their processing aptitude. *J. Sci. Food Agri*. **2016**, *96*, 413–421, doi:10.1002/jsfa.7104.
6. Thompson, M.; Thompson, H.; McGinley, J.; Neil, E.; Rush, D.; Holm, D.; Stushnoff, C. Functional food characteristics of potato cultivars (*Solanum tuberosum* L.): Phytochemical composition and inhibition of 1-methyl-1-nitrosourea induced breast cancer in rats. *J. Food Compos. Anal*. **2009**, *22*, 571–576, doi:10.1016/j.jfca.2008.09.002.
7. Ali, M.; Nasiruddin, K.; Haque, M.; Faisal, S. Virus elimination in potato through meristem culture followed by thermotherapy. *SAARC J. Agric*. **2014**, *11*, 71–80, doi:10.3329/sja.v11i1.18376.
8. Cima, F.F.; Pereira, E.D.S.; Schiavon, M.V.; Munhoz, P.C.; Lenz, E.A.; Wolter, D.D.; Pereira, A.D.S. Bioactive compounds, processing quality and yield of colored flesh potato clones. *Horti. Bras*. **2020**, *38*, 139–145, doi:10.1590/s0102-053620200205.
9. Poggi, V.; Arcioni, A.; Filippini, P.; Pifferi, P. Foliar application of selenite and selenate to potato (*Solanum tuberosum*): Effect of a ligand agent on selenium content of tubers. *J. Agr. Food Chem*. **2000**, *48*, 4749–4751, doi:10.1021/jf000368f.
10. Oliveira, V.; Faquin, V.; Andrade, F.; Carneiro, J.; Júnior, E.; Souza, K.; Pereira, J.; Guilherme, L. Physiological and physicochemical responses of potato to selenium biofortification in tropical soil. *Potato Res*. **2019**, *62*, 315–331, doi:10.1007/s11540-019-9413-8.
11. Zhang, H.; Zhao, Z.; Zhang, X.; Zhang, W.; Huang, L.; Zhang, Z.; Yuan, L.; Liu, X. Effects of foliar application of selenate and selenite at different growth stages on Selenium accumulation and speciation in potato (*Solanum tuberosum* L.). *Food Chem*. **2019**, *286*, 550–556, doi:10.1016/j.foodchem.2019.01.185.

12. Mousavi, S.; Galavi, M.; Ahmadvand, G. Effect of zinc and manganese foliar application on yield, quality and enrichment on potato (*Solanum tuberosum* L.). *Asian J. Plant Sci*. **2007**, *6*, 1256–1260, doi:10.3923/ajps.2007.1256.1260.
13. White, P.; Thompson, J.; Wright, G.; Rasmussen, S. Biofortifying scottish potatoes with zinc. *Plant Soil* **2016**, *411*, 151–165, doi:10.1007/s11104-016-2903-4.
14. Díaz-Gómez, J.; Twyman, R.M.; Zhu, C.; Farré, G.; Serrano, J.C.E.; Portero-Otin, M.; Muñoz, P.; Sandmann, G.; Capell, T.; Christou, P. Biofortification of crops with nutrients: Factors affecting utilization and storage. *Curr. Opin. Biotech.* **2017**, *44*, 115–123, doi:10.1016/j.copbio.2016.12.002.
15. Alshaal, T.; El-Ramady, H. Foliar application: From plant nutrition to biofortification. *EBSS* **2017**, *1*, 71–83, doi:10.21608/jenvbs.2017.1089.1006.
16. Sen, F.; Karacali, I.; Irget, M.E.; Elmaci, O.L.; Tepecik, M. A new strategy to enrich calcium nutrition of fruit: Synergistic effects of postharvest foliar calcium and boron sprays. *J. Plant Nutr*. **2010**, *33*, 175–184, doi:10.1080/01904160903434246.
17. Elmer, P.A.G.; Spiers, T.M.; Wood, P.N. Effects of pre-harvest foliar calcium sprays on fruit calcium levels and brown rot of peaches. *Crop Prot*. **2007**, *26*, 11–18, doi:10.1016/j.cropro.2006.03.011.
18. McGuire, R.G.; Kelman, A. Calcium in potato tuber cell walls in relation to tissue maceration by Erwinia carotovora pv. atroseptica. *Phytopathology* **1986**, *76*, 401–406.
19. D'Imperio, M.; Renna, M.; Cardinali, A.; Buttaro, D.; Serio, F.; Santamaria, P. Calcium biofortification and bioaccessibility in soilless "baby leaf" vegetable production. *Food Chem.* **2016**, *213*, 149–156, doi:10.1016/j.foodchem.2016.06.071.
20. NIH (National Institutes of Health). Available onlie: https://pubchem.ncbi.nlm.nih.gov/compound/calcium (accessed on 4 November 2020).
21. Hocking, B.; Tyerman, S.; Burton, R.; Gilliham, M. Fruit calcium: Transport and physiology. *Front. Plant Sci.* **2016**, *7*, 569, doi:10.3389/fpls.2016.00569.
22. Wei, S.; Qin, G.; Zhang, H.; Tao, S.; Wu, J.; Wang, S.; Zhang, S. Calcium treatments promote the aroma volatiles emission of pear (*Pyrus ussuriensis* 'Nanguoli') fruit during post-harvest ripening process. *Sci. Hortic-Amst.* **2017**, *215*, 102–111, doi:10.1016/j.scienta.2016.12.008.
23. Taiz, L.; Zeiger, E. *Plant Physiology*, 3rd ed.; Sinauer Associates, Inc. Publishers: Sunderland, UK, 2002; p. 665.
24. Peacock, M. Calcium metabolism in health and disease. *Clin. J. Am. Soc. Nephrol.* **2010**, *5*(Suppl. 1), S23–S30, doi:10.2215/cjn.05910809.
25. IOM—Institute of Medicine. *Dietary Reference Intakes for Calcium and Vitamin D*; The National Academies Press: Washington, DC, USA, 2011; ISBN 978-0-309-16395-8.
26. Buchowski, M.S. Calcium in the context of dietary sources and metabolism. In *Calcium: Chemistry, Analysis, Function and Effects*; Food and Nutritional Components in Focus—Book series; Chapter 1; Preedy, V.R., Ed.; 2015; pp. 3–20, doi:10.1039/9781782622130-00003, ISBN: 978-1-78262-213-0.
27. Sharma, D.; Jamra, G.; Singh, U.; Sood, S.; Kumar, A. Calcium biofortification: Three pronged molecular approaches for dissecting complex trait of calcium nutrition in finger millet (*Eleusine coracana*) for devising strategies of enrichment of food crops. *Front. Plant Sci.* **2017**, *7*, 2028, doi:10.3389/fpls.2016.02028.
28. AHDB—Agriculture and Horticulture Development Board. Available online: http://varieties.ahdb.org.uk/varieties/view/Agria (accessed on 4 November 2020).
29. Pelica, J.; Barbosa, S.; Lidon, F.; Pessoa, M.F.; Reboredo, F.; Calvão, T. The paradigm of high concentration of metals of natural or anthropogenic origin in soils—The case of Neves-Corvo mine area (Southern Portugal). *J. Geochem. Explor*. **2018**, *186*, 12–23, doi:10.1016/j.gexplo.2017.11.021.
30. Ramalho, J.C.; Pais, I.P.; Leitão, A.E.; Guerra, M.; Reboredo, F.H.; Máguas, C.M.; Carvalho, M.L.; Scotti-Campos, P.; Ribeiro-Barros, A.I.; Lidon, F.J.C.; et al. Can elevated air [CO2] conditions mitigate the predicted warming impact on the quality of coffee bean? *Front. Plant Sci*. **2018**, *9*, 287, doi:10.3389/fpls.2018.00287.
31. Subramanian, N.K.; White, P.J.; Broadley, M.R.; Ramsay, G. The three-dimensional distribution of minerals in potato tubers. *Ann Bot*. **2011**, *107*, 681–691, doi:10.1093/aob/mcr009.
32. Weinl, S.; Held, K.; Schlücking, K.; Steinhorst, L.; Kuhlgert, S.; Hippler, M.; Kudla, J. A plastid protein crucial for Ca2+-regulated stomatal responses. *New Phytol*. **2008**, *179*, 675–686, doi:10.1111/j.1469-8137.2008.02492.x.

33. Dayod, M.; Tyerman, S.; Leigh, R.; Gilliham, M. Calcium storage in plants and the implications for calcium biofortification. *Protoplasma* **2010**, *247*, 215–231, doi:10.1007/s00709-010-0182-0.
34. El-Yazied, A.; Ragab, M.E.; Ibrahim, R.E.; El-Wafa, A. Effect of nitrogen fertigation levels and chelated Calcium foliar application on the productivity of sweet corn. *AJS* **2007**, *15*, 131–139, doi:10.21608/AJS.2007.14681.
35. Rasouli-Sadaghiani, M.; Moghaddas Gerani, M.; Ashrafi Saeidlou, S.; Sepehr, E. Effect of Different Calcium Sources Application on Antioxidant, Enzymatic Activity and Qualitative Characteristics of Apple (Malus domestic). *JCPP* **2017**, *7*, 73–87, doi:10.18869/acadpub.jcpp.7.2.73.
36. Alonso, T.A.; Barreto, R.; Prado, R.; Souza, J.; Carvalho, R. Silicon spraying alleviates calcium deficiency in tomato plants, but Ca-EDTA is toxic. *J. Plant. Nutr. Soil Sci*. **2020**, doi:10.1002/jpln.202000055.
37. Navarre, D.A.; Goyer, A.; Shakya, R. Nutritional value of potatoes. *Adv. Potato Chem. Technol.* **2009**, *4*, 395–424, doi:10.1016/B978-0-12-374349-7.00014-3.
38. Rusinovci, I.; Aliu, S.; Fetahu, S.H.; Kaçiu, S.; Salihu, S.; Zeka, D.; Berisha, D. Contents of mineral substances in the potato (*Solanum tuberosum* L.) tubers depending on cultivar and locality in the agro-ecological conditions of Kosovo. *Acta Hortic.* **2012**, *960*, 289–292, doi:10.17660/ActaHortic.2012.960.41.
39. Aulakh, M.S.; Dev, G.Interaction effect of calcium and sulphur on the growth and nutrient composition of alfalfa (*Medicago sativa* L. pers.), using$^{35}$S. *Plant Soil* **1978**, *50*, 125–134, doi:10.1007/BF02107162.
40. Braun, H.; Fontes, P.; Finger, F.; Busato, C.; Cecon, P. Carboidratos e matéria seca de tubérculos de cultivares de batata influenciados por doses de nitrogênio. *Ciência e Agrotecnologia* **2010**, *34*, 285–293, doi:10.1590/s1413-70542010000200003.
41. Murayama, D.; Sakashita, Y.; Yamazawa; Nakata, K.; Shinbayashi, Y.; Palta, J.; Tani, M.; Yamuchi, H.; Koaze, H. Effect of calcium fertilization on processing properties and storability of frozen French fries. *Food Sci. Technol. Res.* **2016**, *22*, 451–459, doi:10.3136/fstr.22.451.
42. Romano, A.; D'Amelia, V.; Gallo, V.; Palomba, S.; Carputo, D.; Masi, P. Relationships between composition, microstructure and cooking performances of six potato cultivars. *Food Res. Int.* **2018**, *114*, 10–19, doi:10.1016/j.foodres.2018.07.033.
43. Arvanitoyannis, I.; Mavromatis, A.; Vaitsi, O.,; Korkovelos, A.; Golia, E. Effect of genotype and geographical origin on potato properties (physical and sensory) for authenticity purposes. *J. Agric. Sci.* **2012**, *4*, 1916–1960, doi:10.5539/jas.v4n4p63.
44. *Portaria n.º 587/87 de 9 de Julho. Diário da República n.º 155/1987, Série, I. Ao abrigo do disposto no n.º1 do artigo 4.º do Decreto-Lei n.º 512/85, de 31 de Dezembro*; Ministérios da Agricultura, Pescas e Alimentação e da Indústria e Comércio: Lisboa, Portugal.
45. Amaral, A.; Militão, J. Efeito de doses e modo de fraccionamento de potássio na batata de indústria "VR0808". *Revista da Unidade de Investigação do Instituto Politécnico de Santarém* **2015**, *3*, 118–130. Available online in: http://hdl.handle.net/10400.15/1569 (assessed on 5 November 2020).
46. Ahangarnezhad, N.; Najafi, G.; Jahanbakhshi, A. Determination of the physical and mechanical properties of a potato (the Agria variety) in order to mechanise the harvesting and post-harvesting operations. *Res. Agric. Eng*. **2019**, *65*, 33–39, doi:10.17221/122/2017-rae.
47. Mesías, M.; Holgado, F.; Márquez-Ruiz, G.; Morales, F.J. Impact of the characteristics of fresh potatoes available in-retail on exposure to acrylamide: Case study for French fries. *Food Control* **2017**, *73*, 1407–1414, doi:10.1016/j.foodcont.2016.11.005.
48. Bicho, N.C.; Leitao, A.E.; Ramalho, J.C.; Lidon, F.C. Application of colour parameters for assessing the quality of Arabica and Robusta green coffee. *Emir. J. Food Agric*. **2014**, *26*, 9–17, doi:10.9755/ejfa.v26i1.17190.
49. Hernández-Herrero, J.A.; Frutos, M.J. Colour and antioxidant capacity stability in grape, strawberry and plum peel model juices at different pHs and temperatures. *Food Chem*. **2014**, *154*, 199–204, doi:10.1016/j.foodchem.2014.01.007.
50. Cevallos-Casals, B.A.; Cisneros-Zevallos, L. Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants. *Food Chem*. **2004**, *86*, 69–77, doi:10.1016/j.foodchem.2003.08.011.
51. Kus, Z.A.; Demir, B.; Eski, I.; Gurbuz, F.; Ercisli, S. Estimation of the colour properties of apples varieties using neural network. *Erwerbs-Obstbau* **2017**, *59*, 291–299, doi:10.1007/s10341-017-0324-z.
52. Kumar, N.; Sarnagat, V.S. A Study on Colour and Dimensional Assessment of Different Apple cultivars present in Domestic Fruit Market of NCR Region. *J. Trop. Agric*. **2017**, *35*, 849–856, ISSN:0254-8755.

53. Coleman, W. Comparative performance of the L* a* b* colour space and North American colour charts for determining chipping quality in tubers of potato (*Solanum tuberosum* L.). *Can. J. Plant Sci.* **2004**, *84*, 291–298, doi:10.4141/p02-164.
54. Cantos, E.; Tudela, J.A.; Gil, M.I.; Espín, J.C. Phenolic compounds and related enzymes are not rate-limiting in browning development of fresh-cut potatoes. *J. Agric. Food Chem*. **2002**, *50*, 3015–3023, doi:10.1021/jf0116350.
55. Cabezas-Serrano, A.B.; Amodio, M.L.; Cornacchia, R.; Rinaldi, R.; Colelli, G. Suitability of five different potato cultivars (*Solanum tuberosum* L.) to be processed as fresh-cut products. *Postharvest Biol. Technol*. **2009**, *53*, 138–144, doi:10.1016/j.postharvbio.2009.03.009.
56. Picouet, P.A.; Gou, P.; Pruneri, V.; Diaz, I.; Castellari, M. Implementation of a quality by design approach in the potato chips frying process. *J. Food Eng*. **2019**, *260*, 22–29, doi:10.1016/j.jfoodeng.2019.04.013.