biology and life sciences forum

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Proceeding Paper

Increase of Calcium in 'Rocha' Pear (*Pyrus communis* L.) for Development of Functional Foods †

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† Presented at the 1st International Electronic Conference on Plant Science, 1–15 December 2020; Available online: https://iecps2020.sciforum.net/.

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Citation: Pessoa, C.C.; Coelho, A.R.F.; Marques, A.C.; Luís, I.C.; Daccak, D.; Silva, M.M.; Ramalho, J.C.; Simões, M.; Reboredo, F.H.; Pessoa, M.F.; et al. Increase of Calcium in 'Rocha' Pear (*Pyrus communis* L.) for Development of Functional Foods. *Biol. Life Sci. Forum* **2021**, *4*, 6. https://doi.org/10.3390/IECPS2020-08668

Published: 1 December 2020

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Abstract: The food industry will face determinant challenges in the future, namely, feeding a growing population, set to reach up to 9 billion people by 2050, while maintaining food quality, in circumstances of resource limitations and sustainable use. In this outlook, minimizing mineral deficits in the human diet can prevent health diseases. Calcium is one of the most abundant minerals in human organisms, performing both structural and signaling functions, its deficits being associated with the development of osteoporosis and other pathologies. To minimize this issue, foliar spraying of edible plants can increase the amount of minerals, triggering additional value in unprocessed food products. Following this assumption at an orchard of Rocha pears located in the West region of Portugal, seven foliar sprays were carried out with calcium chloride (4% corresponds to the exclusive use of $CaCl_2$ 4 kg ha^{-1}; 8% corresponds to the use of $CaCl_2$ 4 kg ha^{-1} (for the first three sprays) and $CaCl_2$ 8 kg ha^{-1} (for the four remaining sprays)). At harvest, calcium levels increased about 12.2–38.3%, whereas significant differences in physicochemical parameters occurred only in malic acid and total soluble solids. In conclusion, calcium levels increased in fruits after foliar spraying, but quality parameters only revealed minor changes, enabling the production of biofortified Rocha pears.

Keywords: biofortification; calcium; *Pyrus communis* L.

1. Introduction

In the human body, calcium (Ca) is the most abundant mineral, performing structural and signaling roles, mainly being found in the bones (99%) and teeth (1%), but also acting as an intracellular messenger necessary for regulation of metabolic processes (such as muscle and vascular contraction, vasodilation blood clotting and transmission of nerve impulses) [1,2].

The world's population is expected to reach 9.1 billion by 2050, increasing mainly in developing countries [3], therefore putting pressure on agro-industries to meet the population's demands, while managing the limited available resources. Calcium, out of the

16 essentials nutrients, is present in limited quantities in foods; therefore, a monotonous diet can lead to nutritional deficiencies [4]. For Ca, deficits can occur in developing or developed countries, and it can be associated with pathologies such as hypertension, osteoporosis, rickets and infantile bone deformity [1,5,6].

An average daily intake of 800–1300 mg (depending on the age and gender of individuals, and the specific situation, such as pregnancy and lactation), helps to avoid Ca malnutrition situations [6,7]. However, while dairy products are the main source of this nutrient, intolerances to dairy products or dietary choices can limit the intake of this mineral, leaving plant products as an option [5]. Although Ca bioavailability remains lower in plant products, due to anti-nutrients (such as phytate and oxalate), fruits, vegetables and nuts have higher contents of Ca than cereals [5].

Strategies to avoid mineral malnutrition can pass by biofortification, consisting in the increase of a target mineral and their bioavailability in the edible part of crops [4]. The use of fertilizers to supply Ca to food crops, such as foliar spray, is an option for breeding or genetic approaches [5,8]. Although it requires regular physical application in every crop season, it can be rather inexpensive and simple to implement when compared to genetic or breeding programs. Furthermore, organic forms of minerals are easily absorbed by humans in comparison to inorganic forms, and are less excreted [8].

Calcium biofortification of Golden and Jonagold apples using foliar sprays (of different formulations with $CaCl_2$ and $Ca(NO_3)_2$) showed increases of this mineral in fruits, while it maintained or enhanced quality and physicochemical attributes in both varieties [9,10]. In pears, pre-harvest foliar sprays with Ca promoted its accumulation in fruits [11,12], but some leaf damages were observed for concentration of 10–25 kg ha^{-1} [12]. Benefits were further reported in the post-harvest phase, such as a decrease of development of hard end disorder in *Pyrus pyrifolia* 'Whangkeumbae' [13] and positive changes in organoleptic properties, such as aroma in *Pyrus ussuriensis* 'Nanguoli', by an increase in the content of volatile aromatic substances [14].

Rocha pear (*Pyrus communis* L.) is a typical Portuguese variety, mainly produced in the West region of the country [15], producing about 200,000 tons each year [16], which is largely exported to the EU. Considering the importance of this fruit, this study aimed to develop an agronomic itinerary for Ca biofortification of Rocha pears, to add nutritional value through this mineral enrichment. Physicochemical parameters were further measured to monitor fruit quality.

2. Experiments

2.1. Orchard

An orchard (coordinates 39°29′52.641″ N; 9°1′19.604″ W) located in the West region of Portugal was selected, being sprayed seven times, with 15 days spacing between each, from 23 April to 9 August 2019. A total of three rows were monitored, keeping one row between each of them to prevent contaminations. One row was kept as control (i.e., without Ca applications), whereas in the remaining two rows, $CaCl_2$ (4 kg ha^{-1}) was applied three times in 24 trees (12 per row) and thereafter, for the last four sprays, the concentration in one of the rows was increased to 8 kg ha^{-1}. The harvest was performed on 3 September 2019. During the agricultural period, from April to September, air temperatures, humidity and total precipitation varied between 8–33 °C, 11–100% and 0–11.68 mm.

2.2. Calcium Content in Fruits

In randomized fruits, Ca contents were determined at harvest (3 September 2019) using an XRF analyzer (model XL3t 950 He GOLDD+) under He atmosphere [17]. The fruits were first cut and dried (at 60 °C, until constant weight), then grounded and processed into pel.

2.3. Physicochemical Parameters of Fruits

Diameter (mm) and dry weight (%) were measured considering four randomized fruits per treatment. Total soluble solids (°Brix) were measure in fruit juice, using a digital refractometer Atago (Atago, Tokyo, Japan). Acidity (grams of malic acid per liter) was quantified in the juice of 5 randomized fruit sample (4 juice samples per treatment), by acid-base titration and using 0.1 N sodium hydroxide and a pH meter (Jenway, 350 model, Chelmsford, England) [18].

Colorimetric parameters were measured in the epidermis (peel) of four randomized fruits per treatment, using a Minolta CR 300 colorimeter (Minolta Corp., Ramsey, NJ, USA), with D65 as illuminant, coupled to a sample vessel (CR-A504) [19].

Brightness (L), which can vary between 0 (black) and 100 (white), indicates the variation of the tonality between dark and light. Chromaticity parameters (a* and b* coordinates) relate to variations between green (−60) and red (+60), or between blue (−60) and yellow (+60), respectively. Neutral colors such as white, black or gray correspond to the proximity of these coordinates to the null value.

2.4. Statistical Analysis

Statistical analysis was carried out using a One-Way ANOVA ($p \leq 0.05$), to assess differences, then a Tukey's for mean comparison was performed, considering a 95% confidence level.

3. Results

At harvest, Ca content (Table 1) increased in all sprayed fruits, with 8% treatment being significantly different from the control. Hence, the use of $CaCl_2$ resulted in Ca biofortification of Rocha pear fruits, varying between 12.2–38.3%.

Table 1. Average ± SE (n = 4) of Ca mineral content, diameter, dry weight, total soluble solids and malic acid of fruits from *Pyrus communis* L., variety Rocha pear, at harvest. Letters a, b indicate significant differences ($p \leq 0.05$), for each parameter, between treatments. Ctr = Control; 4% corresponds to seven foliar sprays of $CaCl_2$ (4 kg ha^{-1}); 8% corresponds to three foliar sprays of $CaCl_2$ (4 kg ha^{-1}) and four foliar sprays of $CaCl_2$ (8 kg ha^{-1}).

Treatments	Ca Contents (%$_{Dw}$)		Diameter (mm)		Dry Weight (%)		Total Soluble Solids (°Brix)		Malic Acid (g/L)	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Ctr	0.107b	±0.010	63.3a	±1.9	12.9a	0.6	9.9b	±0.1	1.53a	±0.02
4%	0.120ab	±0.003	63.5a	±1.9	14.4a	0.4	11.6a	±0.6	1.41ab	±0.07
8%	0.148a	±0.008	65.0a	±1.1	12.7a	0.4	11.1ab	±0.2	1.32b	±0.06

Regarding physicochemical parameters (Table 1), no significant variations in diameter and dry weight were observed, the values ranging between 63.3–65.0 mm and 12.7–14.4%, respectively. Diameter of all fruits ranged between 55–65 mm, which is the typical range for this variety [16]. Furthermore, water is a major part of pear fruits composition, reaching up to 85.5 g/100 g of the edible portion [16,20], justifying the dry weight values obtained.

Sprayed fruits showed higher °Brix and a decrease in malic acid (Table 1), with 4% and 8% treatment being significantly different from the control, respectively. Both parameters can be used to determine the harvest time, and thus, values should vary among 11–13 °Brix and 2–3 g/L malic acid [21].

Total soluble solid values (Table 1) of sprayed fruits were within the ideal interval for harvest of this pear variety. Malic acid, citric acid and tartaric acid are three organic acids usually present in fruits, which are responsible for their acidity [22], and in pears, 0.1 g/100 g of edible portion are organic acids [20], and malic acid is the most abundant [22]. These results (Table 1) indicate that pre-harvest Ca sprays may affect Rocha pear fruit acidity, slightly augmenting its pH. Looking at both analyses, the control displayed the highest malic acid value and the lowest °Brix value, while the sprayed fruits that displayed higher °Brix had lower malic acid values, indicating a relation between both parameters.

Different pear fruit varieties can present different colors such as green ('Anjou'; 'Concorde'), yellow ('Abate Fetel'), or brown ('Bosc') [23]. Rocha pears are characterized by having a yellow or light green epidermis [16,18]. At harvest, no significant differences were observed in colorimetric parameters (Table 2) in the peel of fruits. The coordinates indicate a prevalence of green (a* coordinate: negative values) and yellow (b* coordinate: positive values).

Table 2. Average ± SE (n = 4) of colorimetric parameters of the peel of fruits from *Pyrus communis* L., variety Rocha pear, at harvest. Letter a indicates no significant differences ($p \leq 0.05$). Ctr = Control; 4% corresponds to seven foliar sprays of $CaCl_2$ (4 kg ha^{-1}); 8% corresponds to three foliar sprays of $CaCl_2$ (4 kg ha^{-1}) and four foliar sprays of $CaCl_2$ (8 kg ha^{-1}).

Treatments	**L**		**a***		**b***	
	Mean	**S.E.**	**Mean**	**S.E.**	**Mean**	**S.E.**
Ctr	68.6a	±0.8	−16.7a	±0.6	44.8a	±0.5
4%	70.3a	±1.0	−17.4a	±0.6	45.3a	±0.5
8%	67.6a	±0.6	−17.6a	±0.6	43.0a	±0.4

4. Discussion

In the pre-harvest phase, $CaCl_2$ foliar sprays increased Ca content in all pulverized fruits (Table 1), and an increase of concentration in the later sprays resulted in higher Ca values. Additionally, regarding physicochemical characteristics, chemical parameters were more affected than morphometric and colorimetric. These results are in agreement with a previous study on 'Conference pears', where pre-harvest foliar spray of $CaCl_2$ did not affect negatively fruit appearance [12]. Furthermore, although post-harvest applications were made, Ca increases of 17% to 35% were attained, with the higher concentrations resulting in higher Ca content in fruit [12]. Our results followed the same tendency with the 8 kg ha^{-1} treatment showing higher values than 4 kg ha^{-1}. Moreover, other studies also revealed Ca increases when using $CaCl_2$ [11,24,25], reinforcing the use of $CaCl_2$ in agronomic biofortification of pears. Thus, agronomic biofortification can become an option for the agro-industrial sector, by being a rather quick way to increase Ca contents in the Rocha pear when compared to other biofortification techniques like conventional breeding or genetic modification [8]. On the other hand, Ca is important in fruits for quality and storability, helping in the post-harvest phase by reducing decay and thus promoting an increase in the shelf-life of fruits [14]. This is important since physiologic changes occur during fruits' maturation, making then highly perishable [22]. Even considering this characteristic, though, Rocha pear fruits are commercialized almost all year round, mainly due to their conservation capacity and storage in controlled atmosphere conditions [16].

Morphological (diameter and dry weight) parameters indicate that Rocha pears development was not affected by Ca foliar sprays, with all fruits reaching ideal calibers for either industrial processing or commercialization. Besides, water content was within the expected values, meaning that essential physiological activities in fruits and their respective storage period were not affected [26]. This also indicated that dry matter contents were not significantly affected, namely, carbohydrates, since they are the second major components of pears after water [16,27].

In climacteric fruits, such as pears, physicochemical changes happen in the post-harvest phase, namely, increase of total soluble solids, decrease in acid content and color development [22]. Besides, acidity and total soluble acids influence fruits' flavor [27]. These parameters indicated that Ca can have an effect on organoleptic characteristics such as flavor, which has already been confirmed regarding the aroma in *Pyrus ussuriensis* 'Nanguoli' [14]. Nevertheless, malic acid delays bacterial deterioration by decreasing pH in fruits [27], and although there was a slight decrease in values from Ca sprayed fruits, it does not arise major concerns.

This study supports that pre-harvest sprays of Ca do not affect colorimetric parameters, and the same was observed in 'Conference' pears like those previously mentioned [12]. Additionally for 'Rocha' pears stored for 2 to 5 months, L ranged from 66.69 to 79.55, and a* and b* coordinates varied between −12.87 to 5.64 and 39.17 to 45.52, respectively [28]. The values from our study fall within these ranges, especially considering that during storage, pear color can change from a prominence of green to yellow [16,18], explaining the a* coordinate difference. This is important because visual appearance (such as color and size) becomes relevant, not only for consumers [22], but also for agro-industries due to long storage periods of this variety.

5. Conclusions

Foliar sprays of $CaCl_2$ (8 kg ha^{-1}) increase Ca content in Rocha pear fruits. From the physicochemical parameters analyzed, only chemical parameters had minor changes. Morphological and colorimetric aspects of fruits were not affected, allowing commercialization to the consumer, storage or further processing by agro-industry to obtain new products with added value. Accordingly, agronomic biofortification of the Rocha pear can be used to enrich Ca content, allowing its prophylactic consumption to face osteoporosis and other physiological diseases triggered by Ca malnutrition.

Supplementary Materials: The poster presentation is available online.

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

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors thank Tiago Peralta (Ribamaior–Produção e Comércio de Frutas Lda) for technical assistance in the orchards as well as the project PDR2020-101-030734 for the financial support. We also thank the Research centers (GeoBioTec) UIDB/04035/2020 and (CEF) UIDB/00239/2020 for support facilities.

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.

Abbreviations

The following abbreviations are used in this manuscript:

Ctr	Control
4%	corresponds to seven foliar sprays of $CaCl_2$ (4 kg ha^{-1})
8%	corresponds to three foliar sprays of $CaCl_2$ (4 kg ha^{-1}) and four foliar sprays of $CaCl_2$ (8 kg ha^{-1})

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