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Proceeding Paper

Effect of $CaCl_2$ Enrichment on Fatty Acid Profile in Rocha Pears †

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Abstract: Human malnourishment is a current problem of society, and agronomic biofortification is a procedure that wishes to tackle these mineral deficits in human diets by increasing a specific nutrient in the edible part of food crops. Calcium is an important mineral element that performs structural functions and thus can help prevent the development of pathologies such as osteoporosis. Thereby, this work aims to study the impact of calcium enrichment on fatty acid (FA) content in Rocha pears. Thus, an agronomic enrichment workflow with seven foliar sprays of $CaCl_2$ (with concentrations between 4–8 kg/ha) was performed in an orchard located in the western region of Portugal. Besides Ca enrichment assessment in fruits (with a portable X-ray fluorescence analyzer) at harvest, fatty acids quantification and FA profile (acquired with a gas–liquid chromatograph, coupled to a flame ionization detector (GC-FID)), double bond index (DBI), and lipoperoxidation values (with a spectrophotometer) were also attained. Increases of Ca in sprayed fruits reached 7.6% to 44.3%. For FA-related parameters, no significant differences were observed, suggesting that Ca sprays did not impact these parameters. Total fatty acids (TFA), DBI, and lipoperoxidation values varied between 0.72–0.74 g/100 g FW, 8.13–9.83 and 2.23–3.18 μM/g FW, respectively. The following FA profile was attained: C18:2 > C16:0 > C18:3 > C18:0 > C18:1 > <C16:0. No significant differences were observed. In summary, $CaCl_2$ can be used to increase Ca levels in fruits, allowing the production of fruits with prophylactic characteristics, while the concentrations from this study did not impact their FA content. Overall, this suggests that cell compartmentation and membranes' regular functioning were maintained, suggesting the absence of lipid decay and avoiding a potential increase in storage losses.

Keywords: agronomic Ca enrichment; Ca content in fruit; DBI; fatty acids profile; *Pyrus communis* L.; lipoperoxidation; TFA

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1. Introduction

Mineral deficits in human diets are a current problem that can promote health issues [1]. Among the different minerals, Ca deficits can lead to bone deformations or lower mass density, affecting both growth and locomotion ability, which ultimately can increase the occurrence of fractures [2]. In this regard, agronomic biofortification can be a strategy to acquire foods with higher contents of a selected mineral after the application of fertilizers (directly to the soil or to the aerial part of plants via foliar sprays) [3].

In Portugal, Rocha pear is a valuable fruit that contributes to the country's economy since over half of its production is exported [4]. In 2021, over 225,000 tons were produced from a little over 10,000 ha [5]. Although lipids are present in low quantities on some fruits such as pears [6], they can not only act as energy storage molecules but also contribute to the maintenance of cellular compartmentalization [7], and the modification of these structures can be related to the development of diseases in post-harvest [8].

Thus, since agronomic biofortification with $CaCl_2$ has increased Ca levels in another pear variety [9], this study aimed to test the efficiency of this fertilizer application on the Rocha pear variety while simultaneously monitoring any impact on the fatty acids (FA) content of sprayed fruits.

2. Materials and Methods

2.1. Enrichment Workflow

In an orchard located in the West region of Portugal, a total of three tree rows were selected. One was kept as the control, while the remaining two were sprayed with $CaCl_2$. One row was sprayed seven times with 4 kg/ha (T1), while the second row was initially sprayed three times with 4 kg/ha, followed by four sprays with double the concentration (8 kg/ha) (T2).

2.2. Calcium Assessment in Fruits

The calcium content of fruits at harvest was assessed using an X-ray fluorescence system as described in [10]. For sample preparation, fruits were firstly washed and then cut, being later put to dry (60 °C) until constant weight.

2.3. Fatty Acids Content and Lipoperoxidation Assessment

Total fatty acids (TFA), FA profile, and double bond index (DBI) of fruits at harvest were attained as described in Pessoa et al. (2023) [11].

Membrane lipoperoxidation was also determined by quantifying the production of malondialdehyde (MDA). Thus, lipid oxidation was estimated based on Hodges et al. (1999) [12], with some modifications. Rocha pear samples previously peeled were weighted (400 mg, n = 3) and macerated in 3000 µL of 0.1% trichloroacetic acid (TCA). After centrifugation (13,000× g, 10 min, 4 °C), the supernatant (750 µL) was withdrawn into a test tube, followed by the addition of 0.5% thiobarbituric acid (2250 µL). For the blank, the supernatant amount was replaced by 0.1% TCA. All samples were transferred to a water bath (20 min, 90 °C) and then placed on ice. A spectrophotometer (SPECORD 50 PLUS, Analytik Jena, Jena, Germany) and WinASPECT PLUS software (version 4.2) were used to obtain the spectrum between wavelengths 450–620 nm, and the absorbance was recorded at 532 nm. The determination of MDA was performed considering the molar extinction coefficient of ε_{532} = 155 mM^{-1} cm^{-1}.

2.4. Statistical Analysis

A One-way ANOVA ($p \leq 0.05$) was performed to compare tree rows, and a Tukey test was conducted, considering a 95% confidence level.

3. Results

At harvest, lower Ca contents were reported in the control (Table 1), while T2 presented significantly higher values than the other treatments. Within this framework, the

biofortification index of Ca ranged from 7.6% to 44.3%. For the remaining parameters (TFA, DBI, and MDA), no significant differences were reported. Total fatty acids (Table 1) varied between 0.72 and 0.74 g/100 g FW, DBI (Table 1) between 8.13 and 9.83, and MDA values (Table 1) ranged between 2.23 and 3.18 µM/g FW.

Table 1. Mean values (n = 4) and standard error of Ca content, TFA, DBI, and MDA of Rocha pear fruits at harvest. Letters a and b represent significant differences between treatments for each parameter ($p \leq 0.05$).

Treatment	Ca (%)	TFA (g/100 g FW)	DBI	MDA (µM/g FW)
Control	0.131 b ± 0.017	0.72 a ± 0.10	9.09 a ± 0.88	2.91 a ± 0.11
T1	0.141 b ± 0.002	0.74 a ± 0.05	8.13 a ± 0.29	3.18 a ± 0.05
T2	0.189 a ± 0.002	0.74 a ± 0.10	9.83 a ± 1.47	2.39 a ± 0.33

The profile of FA (Table 2) was characterized by the highest abundance of linoleic acid (C18:2), followed by palmitic acid (C16:0) and linolenic acid (C18:3). Stearic (C18:0) and oleic (C18:1) acids were the least abundant, while there was also a small percentage of FA with C chains lower than C16 (<C16:0). No significant differences were observed.

Table 2. Mean values (n = 4) and standard error of FA profile of Rocha pear at harvest. Letter a indicates the absence of significant differences between treatments in the different parameters ($p \leq 0.05$).

Treatment	mol %					
	<C16:0	C16:0	C18:0	C18:1	C18:2	C18:3
Control	1.76 a ± 0.27	13.13 a ± 1.25	5.37 a ± 1.31	3.60 a ± 0.89	66.76 a ± 1.33	9.31 a ± 1.68
T1	1.21 a ± 0.07	15.97 a ± 0.49	4.07 a ± 0.19	2.34 a ± 0.12	67.47 a ± 1.24	8.75 a ± 0.83
T2	1.76 a ± 0.23	14.87 a ± 1.75	2.96 a ± 0.54	2.82 a ± 0.22	69.08 a ± 1.99	8.34 a ± 0.62

4. Discussion

Similarly, in another study [9], Ca levels in Rocha pears at harvest increased with the applied concentration of $CaCl_2$. Higher concentrations at later stages of development provided better results. However, the use of higher concentrations should be carefully monitored since a study reported damage in leaves [9]. Since pear trees are a permanent culture, in order to maintain yields during the following years, toxicity levels must thus be avoided to prevent damage to photosynthetic systems.

Lipids constitute only 0.4 g/100 g of edible portion of pears [13]. In agreement, our TFA values were similar to this reference, although slightly higher. This can be due to a variety of variability or edaphoclimatic characteristics since changes in FA composition can be related to geographical parameters [14]. Nevertheless, pears are considered fruits with low lipid content (<10%) [6].

The FA profile was identical in all fruits (C18:2 > C16:0 > C18:3 > C18:0 > C18:1). Similar profiles were observed in pears [15] and apples [6]. Indeed, in two different pear varieties, the predominant FAs were C18:2 > C16:0 > C18:1 > C18:3 [15]. In turn, in apple pulp, the following profile was attained: C18:2 > C16:0 > C18:3 > C18:1 > C18:0 [6]. In comparison to these other studies, only oleic acid (C18:1) was present in a different proportion, namely with inferior values. This may be related not only to variety variability but also possibly to adaptative responses to environmental stresses (such as temperature, rainfall, or hours of sun exposure), which can lead to modifications in the proportions of unsaturated FAs [16]. Our data are also in agreement with PortFIR [13] when it mentions a predominance of linoleic acid (0.1 g/100 g of edible portion) and consequent higher content of unsaturated

FAs over saturated ones. Furthermore, fruits that are more resistant to low temperatures tend to show a higher presence of unsaturated FAs than saturated ones [17], being in accordance with the storage temperatures (−0.5 to 1 °C) sustained using Rocha pear when in conservation chambers [18].

Regarding the impact of foliar sprays, Ca did not appear to impact the TFA and FA profile at harvest due to the absence of significant differences. In fact, part of the Ca present in plant tissues is located in the cell wall, providing strength and rigidity to the structure [19]. In addition, Ca can help to maintain membrane integrity by acting on the binding of anionic groups of lipids and proteins and can promote membrane fusion [19]. Thus, in plants subjected to stress and deficient in Ca, solute leakage and disintegration of the cell structure/compartmentalization are expected. In fact, enzyme activity can lead to changes in the lipid composition of cell membranes and cause irreversible membrane damage [20,21].

The DBI is an indicator of the level of unsaturation since it refers to the average number of double bonds in the FAs. Thus, the absence of significant differences in this parameter in comparison to the control suggests that membrane fluidity was not affected by Ca, indicating no ion losses or compromises in cell compartmentalization [22]. This is in agreement with the impact of Ca on cell membranes mentioned by Deng (2008) [23], namely on their integrity, which may be related to the stabilizing effect of Ca on the lipid bilayer via its binding to phospholipids.

Malondialdehyde is an aldehyde resulting from lipid oxidation, a process associated with changes in sensory attributes (such as color, texture, and flavor) and nutritional attributes (affecting not only vitamins but also essential FAs) [24]. The absence of significant differences in this analysis can be related to the same absence of differences in the former lipid parameters. This suggests that the concentrations of $CaCl_2$ did not affect the FA content of fruits, indicating lipid membrane well-functioning and good prospects for less storage losses.

5. Conclusions

The concentrations of $CaCl_2$ used in this study led to increases in Ca content in Rocha pear fruits, namely after increased spray concentration at later stages of the production cycle. Foliar applied concentrations did not affect the FA content of fruits, suggesting that the membrane was well-functioning and cell compartmentation was well-kept, indicating fewer prospects for storage losses.

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