

Proceeding Paper

Assessment of Calcium Content in Pear Fruits under Storage after CaCl₂ Applications during Pre and Post-Harvest Phases †

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† Presented at 1st International Electronic Conference on Horticulturae, 16-30 April 2022.

Citation: Pessoa, C.C.; Marques, A.C.; Coelho, A.R.F.; Daccak, D.; Luís, I.C.; Ramalho, J.C.; Campos, P.S.; Pais, I.P.; Semedo, J.N.; Silva, M.M.; et al. Assessment of Calcium Content in Pear Fruits under Storage after CaCl₂ Applications during Pre and Post-Harvest Phases. *Biol. Life Sci. Forum* **2022**, *2*, x.
<https://doi.org/10.3390/xxxxx>

Academic Editor(s): Carmit Ziv

Received: date

Accepted: date

Published: date

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Abstract: Post-harvest systems are crucial for fruit conservation since it minimizes the waste of such perishable food and allows its marketability to consumers during the year. This study thus aims to assess calcium values in stored fruits, previously sprayed and/or immersed in CaCl₂, and possible implications on quality. Fruits previously sprayed with different concentrations of calcium chloride (0–8 kg·ha⁻¹ CaCl₂) during the productive cycle (pre-harvest phase) were separated into two groups at harvest. One was immediately stored in conservation chambers, while the second group was immersed in 1.3% CaCl₂ (for 10 min at room temperature) in the post-harvest stage. After 4 months of storage, calcium content was evaluated with X-Ray fluorescence analysis and quality parameters, such as total soluble solids, malic acid, hardness, and colorimetric parameters of pulp were also monitored. Overall, calcium content in stored fruits with post-harvest bath were superior, but fruits with the exclusive application of CaCl₂ during the pre-harvest phase were superior to the respective control. Furthermore, treatment T2 (corresponding to the highest concentration of CaCl₂ during pre-harvest, up to 8 kg·ha⁻¹) presented the lowest total soluble solids values. The highest value of malic acid prevailed in fruits with no application of CaCl₂ while it also presented the lowest value of hardness. No significant differences were observed for colorimetric parameters of pulps. In conclusion, the pre-harvest workflow used for this study increased calcium content in fruits at harvest, but post-harvest immersion can be used in complementation to pre-harvest treatments to avoid fruit quality decay. Calcium applications did not compromise its marketability to consumers.

Keywords: calcium; pear storage; pre-harvest and post-harvest calcium treatments; quality parameters

1. Introduction

Agroindustries are investing in ways to increase food productivity to feed the growing global population [1]. However, in meads of hydric and land resource limitations, and

climate changes, minimizing food loss and waste, which claims a major part of agriculture output, could decrease the demand for production increases [1,2]. Furthermore, higher consumptions of vegetables and fruits, in low and middle-income countries, are expected to happen [1]. In this context, due to its perishability, fruit preservation methods during the post-harvest phase become important to avoid the loss of quality characteristics that often lead to discarded products or reduce consumers acceptability [3].

In plants, calcium (Ca) can either act as an intracellular second messenger or perform structural roles [4], making it one of the most important nutrients for fruit quality and respective post-harvest life [5]. Developing tissues such as fruits or young leaves are more prone to Ca deficiencies, due to lower transpiration rates and reduced remobilization of Ca from older tissues [4,6], and bitter pit, a typical disorder in apples has been linked to this mineral's deficit [5,6]. Thus, studies with Ca applications and respective impacts in quality parameters of pears and other fruits during pre-harvest and post-harvest phase have been performed [7–10].

Rocha pear (*Pyrus communis*) is the main pear variety grown in Portugal and over half of its annual total production is exported, benefiting from its resistance to handling and transport [11]. The storage time of these fruits varies according to the type of storage conditions applied [12]. Under controlled atmosphere (often used at an industrial scale), Rocha pear fruits can last between 7 and 8 months, since processes such as ripening and senescence are delayed [11,12].

For this study, Ca content in Rocha pear fruits sprayed with CaCl_2 in the pre-harvest phase, and/or immersed in CaCl_2 at post-harvest was assessed after 4 months of storage under controlled atmosphere. Monitorization of quality parameters such as total soluble solids, acidity, hardness, and color of pulp, was also performed.

2. Materials and Methods

2.1. Pre-Harvest and Post-Harvest Workflow

During the production cycle of 2019, between April and August, pre-harvest foliar sprays were performed in a Rocha pear orchard. For T1, the seven sprays were applied with $4 \text{ kg}\cdot\text{ha}^{-1} \text{ CaCl}_2$, while for T2 the first three sprays were performed with $4 \text{ kg}\cdot\text{ha}^{-1} \text{ CaCl}_2$, following the increase of concentration to $8 \text{ kg}\cdot\text{ha}^{-1} \text{ CaCl}_2$ for the remaining sprays. No sprays were performed on the control (Ctr - $0 \text{ kg}\cdot\text{ha}^{-1} \text{ CaCl}_2$). In September, fruits were harvested and transported to the fructiculture center, where each treatment was then divided into two groups. The first group was immediately taken to storage chambers at temperatures ranging from $-0.5 \text{ }^\circ\text{C}$ to $1 \text{ }^\circ\text{C}$ and humidity of 95%. The second group was then kept on the same storage chamber, but first fruits were immersed in a solution of 1.3% CaCl_2 for 10 min at room temperature. In January fruits were collected from the storage chambers for analysis.

2.2. Mineral Content Analysis

Mineral content in fruits was assessed as described in [13] with slight modifications for fruits. Namely, after random selection of fruits from the different treatments, and brief cleaning with deionized water, fruits were sliced and put to dry at $50 \text{ }^\circ\text{C}$ until constant weight, being then grounded for Ca content analysis.

2.3. Quality Parameters Assessment

Total soluble solids (TSS), acidity, and hardness, expressed in $^\circ\text{Brix}$, g malic acid/L and kg respectively, were assessed in randomized fresh fruits from the different treatments. For TSS and acidity, a refractometer (Atago, Tokyo, Japan), and pH meter (Jenway, 350 model, Chelmsford, UK) were used, and samples prepared as described on the following works [14,15]. For hardness determinations, a manual penetrometer (Bellevue type) with an 8 mm (0.5 cm^2) tip was used [14].

Using a Minolta CR 400 colorimeter (Minolta corp., Ramsey, NJ, USA) as described in [13], the colorimetric parameters of pulp from fresh fruits were determined, namely L (brightness), a* (red and green) and b* (yellow and blue).

2.4. Statistic

For data's statistical analysis, a One-Way ANOVA ($p \leq 0.05$), and Tukey test (95% confidence level) were executed. Letters a to c indicate significant differences between all treatments.

3. Results

3.1. Calcium in Fruits

Calcium content (Figure 1) in fruits immersed in CaCl_2 , were superior to the ones of fruits with pre-harvest applications. Furthermore, when considering only fruits without post-harvest applications (Figure 1), T2 was significantly different from the control.

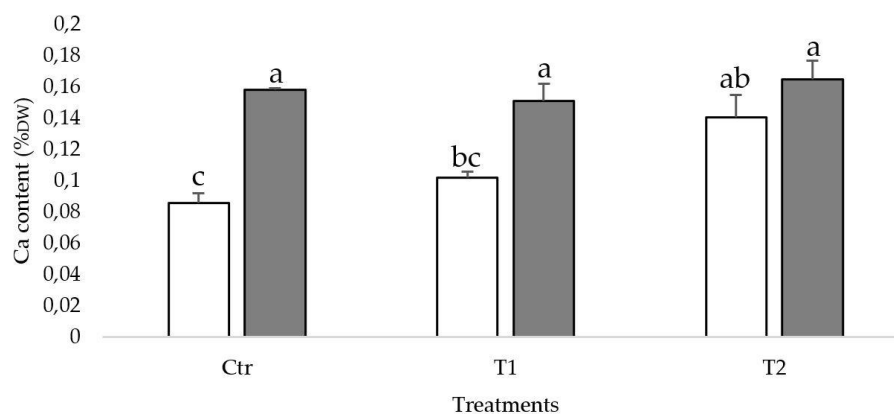


Figure 1. Average values and S.E. of Ca mineral content in pear fruits after 4 months of storage, with (grey) and without (white) post-harvest CaCl_2 immersion. For statistical analysis, letters a to c correspond to significant differences between all treatments (single factor ANOVA test, $p \leq 0.05$). Ctrl = no foliar sprays, $0 \text{ kg} \cdot \text{ha}^{-1} \text{ CaCl}_2$; T1 = foliar sprays with exclusive use of $4 \text{ kg} \cdot \text{ha}^{-1} \text{ CaCl}_2$. T2 = foliar sprays with $4 \text{ kg} \cdot \text{ha}^{-1}$ and $8 \text{ kg} \cdot \text{ha}^{-1} \text{ CaCl}_2$.

3.2. Quality

For quality parameters (Table 1), significant differences were observed for TSS, malic acid and hardness with values ranging between $10.1\text{--}11.3$ °Brix, $0.80\text{--}1.09$ g/L and $5.44\text{--}5.81$ kg respectively. Fruits without any CaCl_2 applications (Ctrl), presented the highest values of TSS and malic acid, but the lowest hardness value.

Table 1. Average values and S.E. of quality parameters of pear fruits stored for 4 months. For statistical analysis, letters a to c, correspond to significant differences between all treatments (single factor ANOVA test, $p \leq 0.05$). Ctrl = no foliar sprays, $0 \text{ kg} \cdot \text{ha}^{-1} \text{ CaCl}_2$; T1 = foliar sprays with exclusive use of $4 \text{ kg} \cdot \text{ha}^{-1} \text{ CaCl}_2$. T2 = foliar sprays with $4 \text{ kg} \cdot \text{ha}^{-1}$ and $8 \text{ kg} \cdot \text{ha}^{-1} \text{ CaCl}_2$. I = Fruits immersed in 1.3% CaCl_2 for 10 min at room temperature.

Treatments	Total Soluble Solids (°Brix)	Malic Acid (g/L)	Hardness (kg)
Ctrl	11.3 ± 0.2 a	1.09 ± 0.09 a	5.44 ± 0.06 c
T1	10.3 ± 0.2 bc	0.80 ± 0.03 b	5.77 ± 0.06 ab
T2	10.1 ± 0.1 c	0.90 ± 0.04 ab	5.51 ± 0.04 bc
I-Ctrl	11.1 ± 0.3 ab	0.92 ± 0.02 ab	5.60 ± 0.06 abc
I-T1	11.3 ± 0.1 a	0.92 ± 0.03 ab	5.81 ± 0.08 a
I-T2	10.7 ± 0.2 abc	0.89 ± 0.03 ab	5.54 ± 0.06 bc

Regarding the colorimetric parameters of pulp, no significant differences were observed for L, a* and b* parameters, predominating a higher contribution of white, green and yellow respectively.

Table 2. Average values and S.E. of colorimetric parameters from pulp of pear fruits stored for 4 months. For statistical analysis, letter a corresponds to the absence of significant differences between all treatments (single factor ANOVA test, $p \leq 0.05$). Ctr = no foliar sprays, 0 kg.ha⁻¹ CaCl₂; T1 = foliar sprays with exclusive use of 4 kg.ha⁻¹ CaCl₂. T2 = foliar sprays with 4 kg.ha⁻¹ and 8 kg.ha⁻¹ CaCl₂. I = Fruits immersed in 1.3% CaCl₂ for 10 min at room temperature.

Treatments	L	a*	b*
Ctr	76.3 ± 2.1 a	-5.4 ± 0.6 a	20.1 ± 1.5 a
T1	78.0 ± 0.6 a	-5.9 ± 0.4 a	20.6 ± 1.5 a
T2	79.8 ± 1.1 a	-6.8 ± 0.1 a	18.8 ± 0.4 a
I-Ctr	79.5 ± 0.3 a	-6.4 ± 0.3 a	19.8 ± 1.0 a
I-T1	78.2 ± 0.4 a	-6.1 ± 0.1 a	19.8 ± 0.5 a
I-T2	79.3 ± 0.2 a	-6.0 ± 0.1 a	18.8 ± 0.3 a

4. Discussion

Storage circumstances were the same for all treatments. Humidity and temperature conditions were accurate for Rocha pear fruits, and since this fruit is not sensible to cold, lower temperatures such as the ones applied can be used without resulting in cold induced damages [12,16].

The enhancement of Ca content in Rocha pear fruits without post-harvest treatment are related to the pre-harvest applications of CaCl₂, and these increases are in accordance with other studies [9,17–20], where foliar application of CaCl₂ increased Ca content in the edible parts of sprayed plants. Additionally, Ca applications are already practiced on the horticultural sector not only during pre, but also during post-harvest phase [6,10,21] since it helps with shelf-life and cell wall integrity, ultimately avoiding quality parameters decay [22]. Immersion in CaCl₂ further increased Ca in pear fruits, being in accordance with the results in two apple varieties [17].

At harvest, Rocha pear values of TSS, acidity and hardness should vary between 11–13 °Brix, 2–3 g/L and 5.5–6.5 kg/0.5 cm² respectively [14,23]. However, pears are climacteric fruits, remaining physiological active while in storage and thus, their physico-chemical properties can change during the conservation stage [12].

Fruits flavor is related to TSS and acidity [16], and while in storage, the internal production of ethylene and respiratory rate can lead to an increase of TSS and a decrease of acidity [12]. Regarding both parameters, only TSS is classified as a fingerprint marker for this variety (stored for 5 months under controlled atmosphere), with values ranging between 10.0–13.8 °Brix [12], in which our values are comprehended. Furthermore, a study [9] using Ca pre-harvest applications ranging from 2 to 25 kg.ha⁻¹ in Conference pears reported no differences of soluble solids concentration after 150 days of storage in similar conditions to our study. For acidity, even though there was a slight decrease in values in comparison to Ctr, the general absence of significant differences suggests that Ca does not influence this parameter.

Overall, hardness values were still within the ideal range set for harvest. For this parameter, higher values are desirable for longer shelf-lives and resistance during transport and storage [16]. However, pre and post-harvest aspects can influence this parameter, such as fertilization during the production cycle or Ca application during post-harvest [16]. Accordingly, immersion in CaCl₂ (during post-harvest) increased Ca values of all treatments, namely for Ctr. Furthermore, when considering the exclusive applications during pre-harvest, sprayed fruits presented higher values than the respective control. These results confirm that Ca applications during pre or post-harvest phases influence hardness of Rocha pear fruits, due to its structural role in cell wall properties [6].

Color changes in fruits can be related to the different maturity stages, such as a prevalence of green on Rocha pears peel at harvest that later transitions to yellow [11,23]. It is thus considered a quality parameter, however, color changes can also be related to quality decay due to physiological changes during storage, that ultimately result in disorders, such as superficial scald or internal browning [24]. Furthermore, this fruit's pulp is white [11], and according to one study [12], parameters L and b* can also be used as fingerprint markers for this variety (for pears stored for 5 months under a controlled atmosphere), with maximum and minimum values ranging between 70.36–87.19 and 6.81–17.68 respectively. The absence of differences in colorimetric parameters indicates that Ca treatments did not affect the color of pulps, and our parameters were in accordance with the fingerprint values, with coordinate b* presenting values slightly higher, confirming the absence of damages in pulp.

5. Conclusions

The application of CaCl₂ during pre-harvest led to Ca content increases in fruits, further enhanced by immersion in CaCl₂. Additionally, taking into consideration fruit processing (where peeling can be performed) and storage time (with fruits remaining physiologically active), further analysis to assess calcium distribution in fruit tissues should be considered.

Quality parameters of fruits were not negatively impacted by CaCl₂ concentrations and storage conditions were adequate, preserving their marketability to consumers or further processing into different food products. Calcium structural role was also confirmed with hardness values of Rocha pear fruits increasing with CaCl₂ applications during pre and post-harvest phases.

Supplementary Materials: Not applicable.

Author Contributions: Conceptualization, P.S.C. and F.C.L.; methodology, P.S.C. and F.C.L.; formal analysis, C.C.P., A.C.M., A.R.F.C., D.D., I.C.L., P.S.C., I.P.P. and J.N.S.; resources, J.C.R., P.S.C., M.M.S., P.L., F.H.R., M.S., M.F.P. and F.C.L.; writing—original draft preparation, C.C.P.; writing—review and editing, C.C.P. and F.C.L.; supervision, F.C.L.; project administration, F.C.L.; funding acquisition, F.C.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by PDR2020, grant number 101-030734. Funding from Fundação para a Ciência e Tecnologia (FCT) UI/BD/150718/2020 is also greatly acknowledged.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thanks to José Henriques (HBio Lda.) and Eng. Ricardo Mendes (Frutalvor—Central Fruteira CRL) for technical assistance on the orchard and storage chambers. We also give thanks to 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 funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. FAO—Food and Agriculture Organization of the United Nations. *The Future of Food and Agriculture—Trends and Challenges*; FAO: Rome, Italy, 2017. ISBN 978-92-5-109551-5.
2. FAO—Food and Agriculture Organization of the United Nations. *The State of Food and Agriculture—Climate Change, Agriculture and Food Security*; FAO: Rome, Italy, 2016. ISBN 978-92-5-109374-0.
3. Shewfelt, R.L.; Prussia, S.E.; Sparks, A.A. Challenges in handling fresh fruits and vegetables. In *Postharvest Handling—A systems approach*, 3rd ed.; Florkowski, W.J.; Shewfelt, R.L.; Brueckner, B.; Prussia, S.E., Eds.; Elsevier: Oxford, UK, 2014; pp. 11–25.
4. Thor, K. Calcium—Nutrient and messenger. *Front. Plant Sci.* **2019**, *10*, 440. <https://doi.org/10.3389/fpls.2019.00440>.

5. Bonomelli, C.; Mogollón, R.; Tonetto de Freitas, S.; Zoffoli, J.P.; Contreras, C. Nutritional relationships in Bitter Pit affected fruit and the feasibility of Vis-NIR Models to determine calcium concentration in 'Fuji' Apples. *Agronomy* **2020**, *10*, 1476. <https://doi.org/10.3390/agronomy10101476>.
6. Hocking, B.; Tyerman, S.D.; Burton, R.A.; Gilliham, M. Fruit calcium: Transport and physiology. *Front. Plant Sci.* **2016**, *7*, 569. <https://doi.org/10.3389/fpls.2016.00569>.
7. 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. <http://doi.org/10.1016/j.scienta.2016.12.008>.
8. Wang, Y.; Zhang, X.; Wang, Y.; Yang, S.; Qu, H. The changes of intracellular calcium concentration and distribution in the hard end pear (*Pyrus pyrifolia* cv. 'Whangkeumbae') fruit. *Cell Calcium* **2018**, *71*, 15–23. <https://doi.org/10.1016/j.ceca.2017.11.002>.
9. Wójcik, P.; Skorupińska, A.; Filipczak, J. Impacts of preharvest fall sprays of calcium chloride at high rates on quality and 'Conference' pear storability. *Sci. Hortic-Amsterdam* **2014**, *168*, 51–57. <https://doi.org/10.1016/j.scienta.2014.01.017>.
10. Gao, Q.; Tan, Q.; Song, Z.; Chen, W.; Li, X.; Zhu, X. Calcium chloride postharvest treatment delays the ripening and softening of papaya fruit. *J. Food Process. Pres.* **2020**, *44*, 1–12. <http://doi.org/10.1111/jfpp.14604>.
11. ANP—Associação Nacional de Produtores de Pera Rocha. Available online: <https://perarocha.pt/anp/> (accessed on 29 September 2021).
12. Pedro, S.I.; Coelho, E.; Peres, F.; Machado, A.; Rodrigues, A.M.; Wessel, D.F.; Coimbra, M.A.; Anjos, O. Physicochemical fingerprint of "Pera Rocha do Oeste". A PDO pear native from Portugal. *Foods* **2020**, *9*, 1209. <https://doi.org/10.3390/foods9091209>.
13. Luís, I.C.; Lidon, F.C.; Pessoa, C.C.; Marques, A.C.; Coelho, A.R.F.; Simões, M.; Patanita, M.; Dôres, J.; Ramalho, J.C.; Silva, M.M.; et al. Zinc enrichment in two contrasting genotypes of *Triticum aestivum* L grains: Interactions between edaphic conditions and foliar fertilizers. *Plants* **2021**, *10*, 204. <https://doi.org/10.3390/plants10020204>.
14. Soares, J.; Silva, A.; Alexandre, J. *O Livro da Pera Rocha, 1º volume—Contributo para uma produção integrada*, 1st ed.; Associação Nacional de Produtores de Pera Rocha: Cadaval, Portugal, 2001; p. 184.
15. 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>.
16. Soares, A.C.A. Avaliação da Qualidade Físico-Química e Sensorial de Frutas Durante o Armazenamento e Comercialização. Master Thesis, Universidade de Aveiro, Aveiro, Portugal, 2015.
17. Lidon, F.; Ribeiro, V.; Reboredo, F.; Pessoa, M.; Santos, M.; Ramos, P.; Sánchez, C. Calcium biofortification of apples: Interaction with Macronutrients. In Proceedings of the COST Action Project FA 0905 Mineral-Improved Crop Production for Healthy Food and Feed, Final Conference Agronomic, Molecular Genetics and Human Nutrition Approaches for Improving the Nutritional Quality and Safety of Food Crops, Ela Quality Resort, Antalya-Belek, Turkey, 17–19 March 2014; pp. 102–103.
18. Coelho, A.R.F.; Lidon, F.C.; Pessoa, C.C.; Marques, A.C.; Luís, I.C.; Caleiro, J.C.; Simões, M.; Kullberg, J.; Legoinha, P.; Brito, G.; et al. Can foliar pulverization with CaCl₂ and Ca(NO₃)₂ trigger Ca enrichment in *Solanum Tuberosum* L. tubers?. *Plants* **2021**, *10*, 245. <https://doi.org/10.3390/plants10020245>.
19. Madani, B.; Mirshekari, A.; Sofo, A.; Mohamed, M.T.M. Preharvest calcium applications improve postharvest quality of papaya fruits (*Carica papaya* L. cv. Eksotika II). *J. Plant Nutr.* **2016**, *39*, 1483–1492. <http://dx.doi.org/10.1080/01904167.2016.1143500>.
20. Tzoutzoukou, C.G.; Bouranis, D.L. Effect of preharvest application of calcium on the postharvest physiology of apricot fruit. *J. Plant Nutr.*, **1997**, *20*, 295–309. <https://doi.org/10.1080/01904169709365251>.
21. Picchioni, G.A.; Watada, A.E.; Conway, W.S.; Whitaker, B.D.; Sams, C.E. Postharvest calcium infiltration delays membrane lipid catabolism in apple fruits. *J. Agric. Food Chem.* **1998**, *46*, 2452–2457. <https://doi.org/10.1021/jf971083e>.
22. Dayod, M.; Tyerman, S.D.; Leigh, R.A.; Gilliham, M. Calcium storage in plants and the implications for calcium biofortification. *Protoplasma* **2010**, *247*, 215–231. <https://doi.org/10.1007/s00709-010-0182-0>.
23. Caderno de Especificações da Pera Rocha D.OP. Available online: http://primofruta.pt/ficheiros/especificacoes/pera_rocha_caderno_especificacoes.pdf (accessed on 19 October 2021).
24. Deuchande, T.; Larrigaudière, C.; Guterres, U.; Carvalho, S.M.P.; Vasconcelos, M.W. Biochemical markers to predict internal browning disorders in 'Rocha' pear during storage under high CO₂. *J. Sci. Food Agric.* **2017**, *97*, 3603–3612. <https://doi.org/10.1002/jsfa.8219>.