biology and life sciences forum

Proceeding Paper

Antioxidants Were Efficient in Reducing Browning and Increasing the Shelf Life in Minimally Processed Arracacha (*Arracacia xanthorrhiza* Bancroft) †

Aline Priscilla Gomes da Silva [1,*], Jaqueline Visioni Tezotto-Uliana [2], Thais Helena de Araujo [3], William Gustavo Sganzerla [4] and Ricardo Alfredo Kluge [5]

1 Department of Biosystems and Agricultural Engineering, Michigan State University, 469 Wilson Road, East Lansing, MI 48824, USA
2 College of Higher Education Santa Bárbara, Tatuí 18277-000, São Paulo, Brazil; jaqueline.tezotto@usp.br
3 State University of Minas Gerais, Passos 37900-106, Minas Gerais, Brazil; thais.helena@uemg.br
4 School of Food Engineering, University of Campinas (UNICAMP), Monteiro Lobato St., N. 80, Campinas 13083-862, São Paulo, Brazil; sganzerla.william@gmail.com
5 Department of Biological Sciences, Escola Superior de Agricultura 'Luiz de Queiroz'-University of Sao Paulo, Pádua Dias 11, Piracicaba 13418-900, São Paulo, Brazil; rakluge@usp.br
* Correspondence: gomesdas@msu.edu
† Presented at the 1st International Electronic Conference on Horticulturae, 16–30 April 2022; Available online: https://sciforum.net/event/IECHo2022.

Abstract: Arracacha (*Arracacia xanthorrhiza* Bancroft) is a nutritional crop with high energy value and good digestibility. However, this plant has a short shelf life due to excessive weight loss, browning, and disease incidence. Therefore, the aim of this study was to investigate the effect of antioxidant compounds in the preservation of minimally processed arracacha during storage. The roots were harvested, sanitized, and cut into 10 mm slices (±1 mm). The slices were subjected to treatments with citric acid (CA) 1%, ascorbic acid (AA) 1%, ethylenediaminetetraacetic acid (EDTA) 1%, and distilled water (control). Weight loss, color parameters (L*, a*, and b*), visual appearance, pH, total soluble solids, total sugars, polyphenoloxidase (PPO), peroxidase (POD), and phenylalanine ammonia-lyase (PAL) were analyzed. Hence, the samples were discriminated by multivariate analysis to determine the effect of antioxidants during the storage time. The results showed that antioxidants controlled the fresh mass loss of arracacha. On the sixth day of storage, arracachas treated with EDTA had the highest b* and L* color parameters, indicating that they were more yellow and brighter when compared with the other treatments. The visual notes (appearance and decay) for the antioxidant treatments were better than the control (without antioxidants). The influence of antioxidants on PPO and POD enzyme activity was very similar, and both enzymes showed high activity for the control treatment (2-fold higher than the samples with antioxidants). Using the multivariate approach, samples were discriminated mainly into two groups. The first major group corresponded to the initial day of the experiment, and the second major group was associated with the last day of storage. The appearance and color parameters were the most crucial factors for sample discrimination in the first group, while for the second group, PAL, weight loss, and sugars were the variables responsible for the multivariate discrimination. In conclusion, the use of EDTA is the most indicated antioxidant to delay the browning reactions and increase the shelf life of minimally processed arracacha.

Keywords: shelf life; postharvest; color; antioxidants; citric acid; ascorbic acid; ethylenediaminetetraacetic acid; anti-browning agents

check for updates

Citation: da Silva, A.P.G.; Tezotto-Uliana, J.V.; de Araujo, T.H.; Sganzerla, W.G.; Kluge, R.A. Antioxidants Were Efficient in Reducing Browning and Increasing the Shelf Life in Minimally Processed Arracacha (*Arracacia xanthorrhiza* Bancroft). *Biol. Life Sci. Forum* **2022**, *16*, 23. https://doi.org/10.3390/IECHo2022-12489

Academic Editor: Carmit Ziv

Published: 15 April 2022

1. Introduction

Arracacha (*Arracacia xanthorrhiza* Bancroft) is a vegetable remarkable by its high nutritional and energetic value, rich in minerals, vitamins, and fibers, and highly appreciated for its characteristic flavor and aroma. However, arracacha roots are a highly perishable product, and their shelf life is very limited [1]. In recent years, there has been a great demand for minimally processed products due to some characteristics, such as being available in smaller portions, ease of use or preparation, and physicochemical qualities. However, minimal processing operations cause mechanical damage to the tissue of the products, which often accelerates senescence and deterioration, leading to discoloration and nutritional value losses. Hence, it is possible to extend the postharvest life of minimally processed products by implementing low temperatures, packaging, and adequate postharvest handling [2]. For instance, refrigeration slows respiration rate and decreases water loss and biochemical reactions that are dependent on higher temperatures. In addition, when associated with another postharvest method, there is an even greater increase in the shelf life of vegetables [3].

The literature reports that the use of antioxidants, such as citric acid (CA), ascorbic acid (AA), and ethylenediaminetetraacetic acid (EDTA), emerges as an alternative that can prolong the shelf life of perishable products [4,5]. CA is one of the main natural organic acids in fruits, and it is responsible for the prevention of enzymatic browning by its action on polyphenoloxidases and peroxidases. It is also used to potentiate other antioxidants by synergistic action [6]. On the other hand, AA stands out for its vitamin C activity, together with its oxidized form, dehydroascorbic acid, which is a potent antioxidant responsible for the inhibition of free oxygen radicals in food and the human body. AA is a cofactor in numerous physiological reactions, and it also has a high redox potential, alone and coupled with other antioxidants [7]. Some natural antioxidant compounds (i.e., CA and AA) have the ability to reduce the quinones formed by the action of oxidases, preventing the formation of darkened products, in addition to being able to act as inhibitors of oxidative enzymes [8]. EDTA is another well-tested antioxidant, acting by complexing copper and iron ions through an unconjugated pair of electrons in their molecular structures [9]. The recent interest in the role of antioxidants on vegetables and on specific food components is necessary to acquire valuable data on their postharvest behavior to facilitate future studies related to the nutritional values of foods, aiming at making recommendations on their consumption. Therefore, the aim of this study was to investigate the effect of antioxidant compounds (CA, AA, and EDTA) in the postharvest preservation of minimally processed arracacha during storage under refrigeration.

2. Material and Methods

2.1. Plant Material

The roots of arracacha (cv. *Amarela de Senador Amaral*) were harvested in commercial cultivation in the region of Pouso Alegre (Minas Gerais State, Brazil). The roots were manually harvested, classified, washed, dried, and packed in 50 kg boxes. The day after harvesting and processing, the roots were brought to the laboratory by dry load and were subsequently minimally processed.

2.2. Processing of Arracacha and Antioxidant Treatments

The roots were initially immersed for 5 min in chlorinated water (200 mg L^{-1} of active chlorine) cooled at 10 °C. After that, the barks were removed and rewashed, and the roots were cut into 10 mm slices (±1 mm). The slices were sanitized for 5 min in chlorinated water (200 mg L^{-1} of active chlorine), then drained to remove the excess water. The slices were submitted to the following treatments: (i) citric acid (CA) 1%; (ii) ascorbic acid (AA) 1%; (iii) ethylenediaminetetraacetic acid (EDTA) 1%; and iv) control (distilled water). The immersion was carried out in $50 \times 36.5 \times 26.5$ cm^3 plastic boxes for 5 min. After the treatments, centrifugation was performed at 2200 rpm for 40 s to remove the excess moisture. Then, 180 g of the minimally processed product was placed in polyethylene bags and manually sealed. The packages were stored in a chamber at 5 ± 1 °C (90 ± 5% RH) for six days. Samples were analyzed immediately after processing (initial characterization) and every two days during the established storage time.

2.3. Analytical Determinations in Minimally Processed Arracacha

Physicochemical and enzymatic analyses were carried out in minimally processed arracacha to determine the effect of antioxidants in postharvest conservation. The weight loss was determined by initial and final weighing of the trays, with the results expressed in %. The color parameters (L*, a*, and b*) were assessed with a Croma Meter CR-400 colorimeter (Ramsey, NJ, USA), where the L* corresponds to brightness, a* represents the transition from green (−a*) to red (+a*), and b* represents the transition from blue (−b*) to yellow (+b*). The visual appearance was recorded through a hedonic scale, ranging from zero (dislike extremely) to five (liked extremely). The rot notes ranged from zero (absence of rot) to five (high incidence of rot). The pH was measured in the arracacha extract, prepared at the concentration of 10% (w/v). The total soluble solids (TSS) content was determined in a digital refractometer, with automatic temperature correction to 20 °C, and the results were expressed in °Brix. The content of total sugars was determined according to the methodology described by Somogy and Nelson [10,11], and the results were expressed in g glucose 100 g^{-1}. The enzymatic activity of polyphenoloxidase (PPO), peroxidase (POD), and phenylalanine ammonia-lyase (PAL) were assessed. The PPO extraction and determination were performed according to the method proposed by Daiuto et al. [12]. The extraction and determination of POD activity were performed according to the method proposed by Cano et al. [13]. The PAL was performed based on the methodology by Peixoto et al. [14]. Enzymatic activity of PPO, POD, and PAL were expressed as U min^{-1} g^{-1} (fresh weigh, f.w.), defined as enzyme content that produces an increase in absorbance per min.

2.4. Statistical Analysis

The experimental design was completely randomized in a factorial scheme (6 replicates × 4 treatments). The results obtained were submitted to analysis of variance (ANOVA), and the means were compared using Tukey's test ($p \leq 0.05$). Cluster analysis (CA) was used to identify similarities between the dataset. Principal component analysis (PCA) was applied to observe interrelationships among the samples. All the statistical analysis was developed using Statistica 10.0 software (StatSoft Inc., Tulsa, OK, USA).

3. Results and Discussion

The results showed that antioxidants controlled the weight loss of arracacha (Figure 1a). The mass loss was significant only in the last two days of storage, and on the sixth day, the arracacha treated with AA showed higher weight loss (0.6%), while the treatment with EDTA showed the lowest weight loss (0.3%). Additionally, on the sixth day of storage, arracachas treated with EDTA had the highest b* and L* color parameters, indicating that they were more yellow and brighter than the other treatments (Figure 1b–d). In addition, the visual notes (appearance and decay) for the antioxidant treatments were better than those compared with the control (without antioxidants) (Figure 1e). No treatment showed wilting, confirming the fact that the treatments were able to prevent the dehydration of minimally processed arracacha. Both the drop in the grades during the storage period and the lower grade of the control on these days are due to browning, the presence of visible pathogens, and cell leakage, which left a sticky appearance around the slices. Thus, it is evident that the antioxidant treatment can also help to reduce the rot rate. This is probably due to the change in pH around the slices, which makes it difficult for some types of pathogens to proliferate (Figure 1f). The TSS contents were significantly influenced by the storage time and antioxidants. Arracacha treated with citric acid had higher TSS contents. The increase in TSS was probably due to the breakdown of starch into soluble sugars and also the loss of mass during the storage period (Figure 1g). The pH showed a significant interaction between the different antioxidants and storage days (Figure 1h). For total sugars (Figure 1i), the control treatment showed distinct behavior, increasing the total sugar mainly after the second day of storage. Nevertheless, the treatment composed of antioxidants showed similar contents for the total sugar at the end of storage (14.59–18.20 g glucose 100 g^{-1}).

The treatment containing EDTA showed the lowest sugar content. This behavior may be due to a decrease in metabolism and, consequently, in the consumption of soluble solids, the main substrates of respiration, since the physical actions of minimal processing induce an increase in respiration, which will quickly use the reserve substrates [15]. Additionally, tissue stress after processing can explain these sugar content fluctuations during storage. Under stress conditions, starch decomposes into soluble sugars, causing an increase in total carbohydrate levels. This process is also related to enzymes responsible for starch degradation and reduction of respiratory activity. This change in tissue physiology results in the accumulation of these carbohydrates [16].

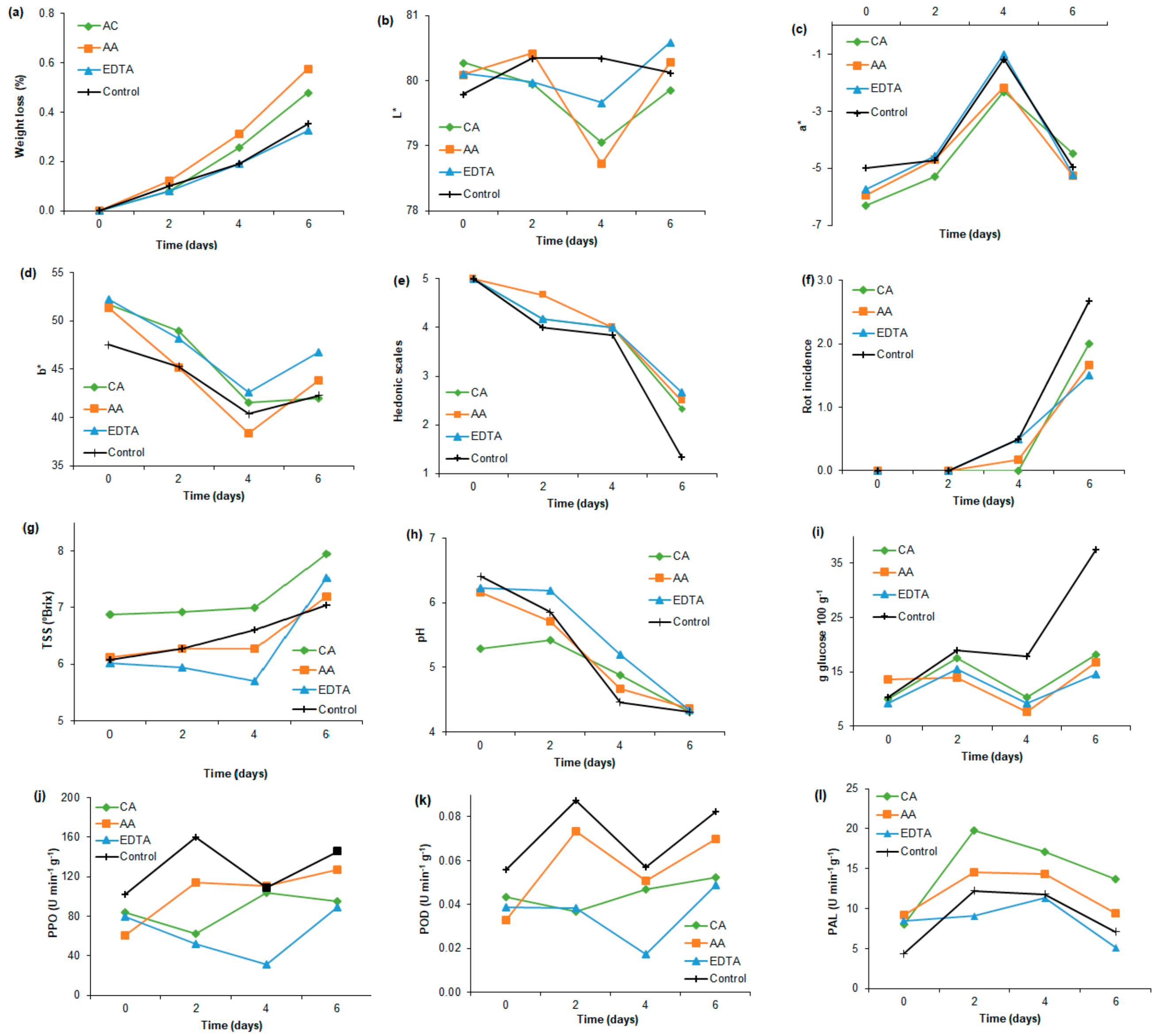

Figure 1. Analytical determinations in minimally processed arracacha treated with antioxidants and stored in a cold room (5 ± 1 °C, 90 ± 5% RH) for six days. (**a**) Weight loss; (**b**) L*; (**c**) a*; (**d**); b*; (**e**) visual notes; (**f**) rot incidence; (**g**) TSS; (**h**) pH; (**i**) total sugar; (**j**) PPO activity; (**k**) POD activity; and (**l**) PAL activity.

Regarding the enzymatic activity of the minimally processed arracacha treated with antioxidants, PPO is the most important enzyme in minimally processed fruits and vegetables. PPO activity was significant for the interaction of different antioxidants and storage days (Figure 1j). The activity of this enzyme was higher in the control treatment at the beginning of storage (102.01 U min^{-1} g^{-1}) and lower in those treated with CA (60.31 U min^{-1} g^{-1}). At the end of storage, it can be observed that the treatment with EDTA (89.48 U min^{-1} g^{-1}) was more satisfactory in delaying enzymatic browning. Both POD and PAL enzymes participate in the browning of minimally processed vegetables and are related to physiological processes [17]. Due to the presence of phenolic compounds immediately after cutting, the pulp darkens, and the activity of oxidative enzymes (PPO and POD) increases [18]. The POD activity (Figure 1k) was higher in the EDTA treatments treated roots on the first day of storage (132.27 U min^{-1} g^{-1}) and lower in those treated with AA (56.63 U min^{-1} g^{-1}). At the end of storage, it can be seen that the control and AA treatments showed high POD activity (Figure 1k). The activity of PAL (Figure 1l) at the beginning of storage was less accentuated in the arracacha from the control treatment (4.36 U min^{-1} g^{-1}) and more highlighted in the roots submitted an antioxidant treatment. However, on the sixth day of storage, the fresh cut treated with EDTA (5.10 U min^{-1} g^{-1}) showed the lowest activity. This enzyme is closely linked with essential functions in the plant. Among them is the mechanical support of the plant provided by lignin, and substances that act as protectors against abiotic stresses such as antioxidants stand out. In addition to being important in the plant's normal development, it is also a key enzyme indicator of plant stress [19].

For multivariate analysis, the dataset recorded on the first and last day of storage was used to evaluate the classification by cluster analysis and the discrimination by PCA. The cluster analysis can be used for initial evaluation of the information in data matrices, finding sample groupings, using criteria developed from the data themselves, grouped according to similarities [20,21]. In this study, a high similarity was observed between CA and EDTA initial and CA and EDTA final (Figure 2a). By multivariate approach, the graphic projection of samples demonstrates that the samples can be discriminated into two major groups composed of five minor groups (Figure 2c). The PCA explained 72.32% of the data variability (PC1 58.06% and PC2 14.26%). The first major group corresponded to the initial day of the experiment, and the second major group was associated with the sixth last day of storage. The appearance and color parameters were the most crucial factors for sample discrimination in the first group, while for the second group, PAL, weight loss, and sugars were the variables responsible for the discrimination of the second group (Figure 2b).

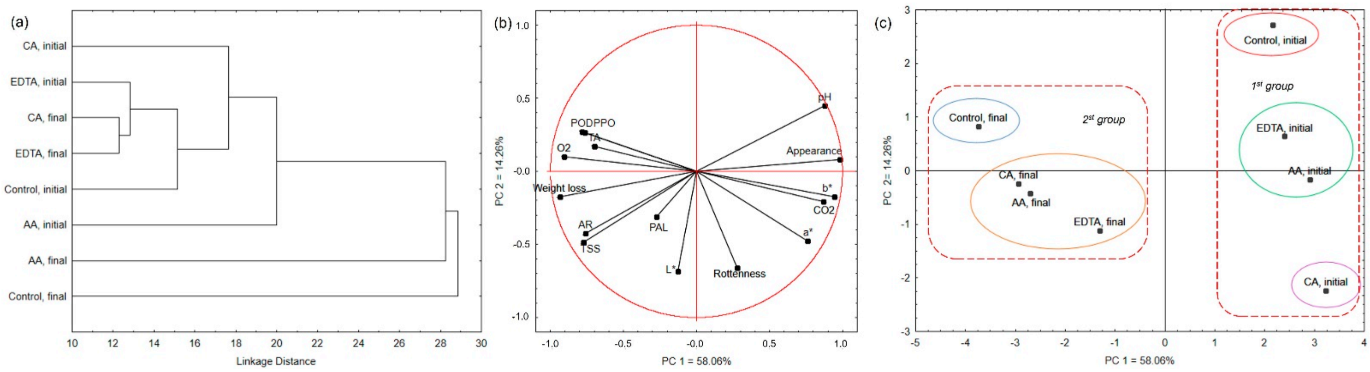

Figure 2. (**a**) Classification using cluster analysis (Euclidean distances); (**b**) projection variable distribution for discrimination by PCA; and (**c**) graphic projection of samples.

4. Conclusions

This study evaluated the effect of antioxidant compounds in the preservation of minimally processed arracacha. The roots treated with this antioxidant showed lower weight loss, better visual grades, and lower rot rates. In addition, this treatment also led to the retention of PPO, POD, and PAL activity. Through multivariate analysis, the variables that characterized the beginning of the experiment were appearance and color parameters, while for the end of the storage, PAL, weight loss, and sugars were responsible for the discrimination, indicating that the application of antioxidants affects the postharvest quality of minimally processed arracacha. Finally, from the results obtained, EDTA is the most indicated antioxidant to delay the browning reactions and increase the shelf life of minimally processed arracacha.

Supplementary Materials: The presentation material can be downloaded at: https://www.mdpi.com/article/10.3390/IECHo2022-12489/s1.

Author Contributions: Conceptualization, A.P.G.d.S. and J.V.T.-U.; data curation, A.P.G.d.S. and T.H.d.A.; formal analysis, A.P.G.d.S., J.V.T.-U. and T.H.d.A.; investigation, A.P.G.d.S., J.V.T.-U. and T.H.d.A.; methodology, W.G.S.; supervision, R.A.K.; validation, W.G.S.; writing—original draft, A.P.G.d.S., J.V.T.-U. and T.H.d.A.; writing—review and editing, A.P.G.d.S., W.G.S. and R.A.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Buso, E.K.R.P.D.M.; Clemente, E.; Estrada, K.R.F.S.; Zárate, N.A.H.; Oliveira, J.S.B. Comportamento pós-colheita de mandioquinha-salsa revestida com quitosana. *Cienc. Agron.* **2014**, *45*, 850–855. [CrossRef]
2. Odoch, M.; Buys, E.M.; Taylor, J.R. Effects of vacuum packaging storage of minimally processed cassava roots at various temperatures on microflora, tissue structure, starch extraction by wet milling and granule quality. *J. Sci. Food Agric.* **2021**, *101*, 6347–6354. [CrossRef] [PubMed]
3. Petriccione, M.; Mastrobuoni, F.; Pasquariello, M.S.; Zampella, L.; Nobis, E.; Capriolo, G.; Scortichini, M. Effect of chitosan coating on the postharvest quality and antioxidant enzyme system response of strawberry fruit during cold storage. *Foods* **2015**, *4*, 501–523. [CrossRef] [PubMed]
4. Soares, C.D.F.; Martin, J.G.P.; Berno, N.D.; Kluge, R.A. Antioxidant chemical treatment affects physiology and quality of minimally-processed escarole. *Horticulturae* **2019**, *5*, 75. [CrossRef]
5. Rodríguez, G.M.; Sibaja, J.C.; Espitia, P.J.; Otoni, C.G. Antioxidant active packaging based on papaya edible films incorporated with Moringa oleifera and ascorbic acid for food preservation. *Food Hydrocoll.* **2020**, *103*, 105630. [CrossRef]
6. Guo, S.; Yan, Z.; Yuan, S.; Geng, W. Inhibitory effect and mechanism of l-ascorbic acid combined with tea polyphenols on coal spontaneous combustion. *Energy* **2021**, *229*, 120651. [CrossRef]
7. Attia, M.; Essa, E.A.; Zaki, R.M.; Elkordy, A.A. An overview of the antioxidant effects of ascorbic acid and alpha lipoic acid (in liposomal forms) as adjuvant in cancer treatment. *Antioxidants* **2020**, *9*, 359. [CrossRef]
8. Moon, K.M.; Kwon, E.B.; Lee, B.; Kim, C.Y. Recent trends in controlling the enzymatic browning of fruit and vegetable products. *Molecules* **2020**, *25*, 2754. [CrossRef]
9. Guiamba, I.R.; Svanberg, U. Effects of blanching, acidification, or addition of EDTA on vitamin C and β-carotene stability during mango purée preparation. *Food. Sci. Nutr.* **2016**, *4*, 706–715. [CrossRef]
10. Somogyi, M. Notes on sugar determination. *J. Biol. Chem.* **1952**, *195*, 19–23. [CrossRef]
11. Nelson, N. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* **1944**, *153*, 375–380. [CrossRef]
12. Daiuto, É.R.; Vieites, R.L.; Simon, J.W.; de Carvalho, L.R.; Pegoretti, C. Avaliações sensoriais, bioquímicas e microbiológicas do guacamole, um produto à base de abacate, sob armazenamento a frio e com adição de ácido ascórbico. *Semin. Cienc.* **2011**, *32*, 599–611. [CrossRef]
13. Cano, M.P.; de Ancos, B.; Matallana, M.C.; Cámara, M.; Reglero, G.; Tabera, J. Differences among Spanish and Latin-American banana cultivars: Morphological, chemical and sensory characteristics. *Food Chem.* **1997**, *59*, 411–419. [CrossRef]
14. Peixoto, P.H.P.; Cambraia, J.; Sant'Anna, R.; Mosquim, P.R.; Moreira, M.A. Aluminum effects on lipid peroxidation and on the activities of enzymes of oxidative metabolism in sorghum. *Braz. J. Plant Physiol.* **1999**, *11*, 137–143.
15. Carvalho, A.V.; Lima, L.C.D.O. Qualidade de kiwis minimamente processados e submetidos a tratamento com ácido ascórbico, ácido cítrico e cloreto de cálcio. *Pesqui. Agropecu. Bras.* **2002**, *37*, 679–685. [CrossRef]
16. Ribeiro, R.A.; Finger, F.L.; Puiatti, M.; Casali, V.W.D. Vida útil e metabolismo de carboidratos em raízes de mandioquinha-salsa sob refrigeração e filme de PVC. *Pesqui. Agropecu. Bras.* **2007**, *42*, 453–458. [CrossRef]
17. Nunes, E.E.; Vilas Boas, E.V.D.B.; Piccoli, R.H.; Xisto, A.L.R.; Vilas Boas, B.M. Efeito de diferentes temperaturas na qualidade de mandioquinha-salsa minimamente processada. *Hortic. Bras.* **2010**, *28*, 311–315. [CrossRef]
18. Daiuto, É.R.; Vieites, R.L. Atividade da peroxidase e polifenoloxidase em abacate da variedade Hass, submetidos ao tratamento térmico. *Rev. Iberoam. Tecnol. Postcosecha* **2008**, *9*, 106–112.

19. Ritter, H.; Schulz, G.E. Structural basis for the entrance into the phenylpropanoid metabolism catalyzed by phenylalanine ammonia-lyase. *Plant Cell* **2004**, *16*, 3426–3436. [CrossRef]
20. Sganzerla, W.G.; Beling, P.C.; Ferreira, A.L.A.; Azevedo, M.S.; Ferrareze, J.P.; Komatsu, R.A.; Nunes, M.R.; de Lima Veeck, A.P. Geographical discrimination of uvaia (*Eugenia pyriformis* Cambess) by principal component analysis. *J. Sci. Food Agric.* **2019**, *99*, 6778–6787. [CrossRef]
21. da Silva, A.P.G.; Spricigo, P.C.; Purgatto, E.; de Alencar, S.M.; Sartori, S.F.; Jacomino, A.P. Chemical composition, nutritional value and bioactive compounds in six uvaia accessions. *Food Chem.* **2019**, *294*, 547–556. [CrossRef] [PubMed]