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Freezing storage of persimmon: strategy of post-harvest losses prevention aligned to the circular economy precepts

Armazenamento congelado do caqui: estratégia de prevenção de perdas pós-colheita alinhada aos preceitos da economia circular

Abstract

Introduction: Persimmon (*Diospyros kaki*) is an important fruit with high nutritional value and interesting bioactive compounds for the development of new high value-added products; however, this fruit is seasonal and has a significant post-harvest loss. Therefore, it is important to invest in and explore technologies that extend the shelf life of persimmons and make the fruit available out of season. ***Objective:*** To evaluate the effects of frozen storage at -18 °C for three months (P3M) and for one year (P1Y) of persimmon. ***Methods:*** The chemical constituents, volatile profile and potential biological activity of persimmon were analyzed. ***Results:*** There were no differences in ash, crude fat, protein and mineral content for both time points. The decrease in moisture content was observed in P1Y. The ratios between total soluble solids (°Brix) and total titratable acidity (g malic acid $100g^{-1}$) were 48.23 and 57.84 for P3M and P1Y, respectively. The one-year frozen storage influenced the volatile fraction. Tetracosane, 2-ethyl-1-hexanol, 2,4-dimethyl-3-hexanone, o-cymene, α-terpineol and thymol were only found in P3M. The P1Y achieved a significant reduction in total phenolic content and IC50(ABTS) compared to the P3M sample. The autoclave extract of P3M and P1Y showed a cytotoxic effect (30-40% reduction of Alamar Blue®) on MCF-7 and MDA-MB-231 cancer cell lines. ***Conclusion:*** Freezing can preserve the proximate composition, mineral profile and physicochemical properties of persimmon so that it can be used for food production in the off-season.

Keywords: Diospyros kaki.Bioactive compounds. Frozen storage. Innovative ingredients. Sustainable food systems.

Resumo

Introdução: O caqui (*Diospyroskaki*) é uma fruta de elevado valor nutricional e rica em compostos bioativos com potencial para o desenvolvimento de novos produtos de alto valor agregado. No entanto, por ser sazonal, apresenta perdas significativas no período pós-colheita. Diante disso, torna-se essencial investir em tecnologias que prolonguem sua vida útil e possibilitem sua disponibilidade na entressafra. ***Objetivo:*** Avaliar os efeitos do armazenamento

do caqui por congelamento a -18 °C por três meses (P3M) e por um ano (P1Y). *Métodos:* Foram analisadas a composição química, o perfil de compostos voláteis e a potencial atividade biológica do caqui. *Resultados:* Não foram observadas diferenças nos teores de cinzas, lipídios, proteínas e minerais entre os períodos avaliados. Houve redução no teor de umidade em P1Y. As razões entre sólidos solúveis totais (°Brix) e acidez titulável total foram de 48,23 (P3M) e 57,84 (P1Y). O armazenamento por um ano afetou a fração volátil. Compostos como tetracosano, 2-etil-1-hexanol, 2,4-dimetil-3-hexanona, o-cimeno, α-terpineol e timol foram detectados apenas em P3M. Verificou-se também redução significativa no teor de fenólicos totais e na capacidade antioxidante (IC50 – ABTS) em P1Y. Os extratos autoclaves de P3M e P1Y apresentaram efeito citotóxico (redução de 30–40% do Alamar Blue®) nas linhagens celulares MCF-7 e MDA-MB-231. *Conclusão:* O congelamento preserva a composição centesimal, o perfil de mineral e as propriedades físico-químicas do caqui, viabilizando seu uso na entressafra. No entanto, o armazenamento por um ano impacta negativamente os compostos voláteis e antioxidantes, o que limita sua aplicação para a obtenção de aditivos naturais. A extração em autoclave mostrou-se eficaz na eluição de compostos com efeito citotóxico, indicando potencial para investigações futuras.

Palavras-chave: Diospyroskaki. Compostos bioativos. Armazenamento congelado. Ingredientes inovadores. Sistemas alimentares sustentáveis.

INTRODUCTION

Persimmon (*Diospyros kaki L.*) is an important subtropical fruit that is cultivated and appreciated all over the world for its unique taste and excellent nutritional value due to its carbohydrates, fibers and micronutrients. In addition, it is a consistent source of many bioactive substances, such as phenolic, carotenoids and other antioxidant compounds, which have a positive effect on human health and can be used in various industries.[1] The most abundant phenolic compounds found in persimmon include ferulic acid, p-coumaric acid, gallic acid, catechin, epicatechin, catechin epigallo and condensed proanthocyanidins.[2]

China, Korea, Azerbaijan, Japan and Brazil are among the most important countries for growing persimmons. In the last ten years, persimmon cultivation has increased by around 50% worldwide, with a production of 5.1 million tons in 2023.[3,4] However, persimmon fruits are highly perishable, resulting in a short shelf life. In view of these facts, it was not surprising that the development of production was accompanied by a significant increase in volume losses, which in Brazil are estimated at around 20% of the harvested product after the harvest.[3,5]

Several factors and metabolic processes lead to a considerable increase in persimmon losses in the post-harvest phase.[4,6] The perishability of persimmon fruits reduces their quality (softening, weight loss and fungal attack), resulting in significant damage during storage and handling.[6] In addition, the persimmon harvest is short and intensive, resulting in a large supply on the market and a low economic value at harvest time.[1] The loss and wastage of persimmons has implications for the waste of essential nutrients and micronutrients as well as environmental resources (e.g. water, land, energy).[7]

There is an urgent need to avoid post-harvest losses by developing technologies that extend shelf life and allow the fruit to be used throughout the year.[7,8] To minimize deterioration and extend the shelf life of food and its by-products, some studies have been published proposing storage techniques such as drying,[9] refrigeration[10] and freezing.[11] Among these methods, frozen storage is commonly used for long-term food preservation. Basically, freezer storage of fruits is used to delay decay, preserve properties such as color and flavor, and provide better accessibility to consumers.[12] Many fruits are frozen with the aim of extending their post-harvest shelf life and avoiding loss and waste, as found in studies with strawberries,[13] and papaya.[14]

The use of persimmon fruit to develop new value-added products is in line with the principles of the circular economy, which focuses on creating value from natural resources and reducing waste generation in a socially, environmentally and economically sustainable way.[7,15] A better understanding of the chemical profile, bioactive compounds, potential biological activity and their respective changes during off-season storage of persimmon is important to explore strategies to extend shelf life, reduce post-harvest losses and explore new specific uses through technological and biotechnological processes.[16-18] Therefore, the aim of this study was to evaluate the effects of up to one year of freezer storage on the chemical composition, volatile compound profile, and potential biological activity of persimmon.

METHOD

Plant material and sample preparation

The studied persimmon fruits were cultivated in the municipality of Rio de Janeiro, in Vargem Grande (22.97 latitude; 43.49 longitude), state of Rio de Janeiro, Brazil, and harvested in April 2018. The commercially ripe fruit was purchased from a local agroecological producer in Rio de Janeiro, Brazil. The fresh persimmons were washed with tap water and sanitized with a hypochlorite solution (200 ppm). The persimmons were frozen whole (-18ºC) without the addition of water or additives. Part of the persimmons were frozen for three

months (P3M) and analyzed, while another part (P1Y) was analyzed after one year of storage. Prior to analysis, the frozen samples were thawed for one hour at room temperature (20 ± 1°C).

Physicochemical analysis

Moisture, ash, crude fat and protein of the P3M and P1Y samples were analyzed according to the guidelines of the Adolf Lutz Institute.[19] The available carbohydrate content was calculated by difference. Total titratable acidity (TTA), total soluble solids (TSS) and pH were measured using standard methods.[19] Each sample was measured in triplicate (n = 3).

Analysis of mineral element content

To determine the mineral content (calcium, manganese, potassium, sodium, iron, magnesium, zinc and copper), persimmon samples (P3M and P1Y) were calcined and then treated with concentrated nitric acid in an acidic medium. The resulting suspension was filtered through ashless filter paper and analyzed by flame atomic absorption spectrometry (VARIAN, AAS-220).[19] Each sample was measured in triplicate (n = 3).

Isolation, identification and quantification of volatile compounds

The solvent extraction method was based on Radulović, Blagojević & Palić.[20] The whole intact fruits (130 g) were immersed in vessels containing 125 mL of diethyl ether containing 1 ppm BHT as an internal standard in an ultrasonic bath (UNIQUE, UltraCleaner 700)for 15 min at 25ºCThe resulting ether washes were filtered through small columns packed with 1 g Celite (Merck, Germany) to remove all insoluble material and then concentrated to 0.25 mL under N_2 vapor at room temperature prior to chromatographic analyzes. Analyzes (two replicates) were performed using a gas chromatography system coupled to a mass spectrometer (Shimadzu, GC-2010Plus/GCMS-QP2010) (GC/MS) containing a SupelcowaxTM-10 fused silica capillary column (cross-linked poly(ethylene glycol), 20 M, 30 m x 0.25 mm i.d., film thickness 0.25 µm, Supelco, USA). The analysis was performed under the following conditions: initial oven temperature of 50°C, ramped to 200°C at 2°C/min and held for 1 min; then ramped to 230°C at 10°C/min and also held for 1 min; splitless injection mode; injector temperature of 230°C; helium as carrier gas at 1.0 mL min^{-1}; interface temperature of 240°C; ionization voltage of 70 eV; acquisition mass range of 35–500 m/z; scan time of 0.32 s.

Extract components were identified by comparing the mass spectra of the samples with the mass spectral libraries (NIST12.lib and NIST62.lib) and on the basis of the calculated linear retention indices (relative to a C7-C26 alkane mixture - 1,000 µg mL^{-1} of each component in hexane) of reference substances or literature values. Only the compounds that were identified at least by reference compounds and mass spectral data were considered unambiguously identified.

The concentrations of volatile compounds were estimated using the internal standardization method. Calibration curves were generated by analyzing standard solutions at three different concentrations under identical experimental conditions. For the tentatively identified compounds, semi-quantification was performed with respect to the most structurally similar reference compounds available in our laboratory.

Extraction techniques for the analysis of total phenolic compounds and antioxidants

For the extraction, 3 grams of thawed persimmon and 40 mL of a water:methanol (1:1, v/v) solution were homogenized with a vortex (Vixar, VM 3000) and allowed to stand for 60 minutes at room temperature. Then the solution was centrifuged at 3,500 rpm for 15 minutes. Finally, the supernatant was transferred to a 100 mL volumetric flask. For the second extraction, the residue from the previous extraction was used and the same process was carried out with aqueous acetone solution (70%). The supernatant was transferred to the same 100 mL volumetric flask, which was filled with distilled water.[21]

Total phenolic content assay

The total phenolic content (TPC) of the original solution described in the *extraction techniques for the analysis of total phenolic compounds and antioxidants* was evaluated by the Folin-Ciocalteu spectrophotometric method using gallic acid as a reference compound.[22] The total phenolic content was calculated using a calibration curve of gallic acid (1, 5, 10, 25, 50, 100 and 300 mg L^{-1}) and the results were expressed in mg gallic acid equivalents (GAE) L^{-1}. Absorbance was recorded in a digital spectrophotometer (EDUTEC, EEQ-9023) at 760 nm. Each sample was measured in triplicate (n = 3).

ABTS assay

The antioxidant activity was evaluated using the ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] assay.[23] From the original solution described in the *extraction techniques for the analysis of total phenolic compounds and antioxidants*, new solutions were prepared (500 µg mL^{-1}; 1,000 µg mL^{-1}; 2,000 µg mL^{-1}; 3,000 µg mL^{-1}; 10,000 µg mL^{-1}; 20,000 µg mL^{-1}; and 30,000 µg mL^{-1}) to generate the antioxidant curve used to calculate the IC50 of the fruits. Absorbance was recorded in a digital spectrophotometer (EDUTEC- EEQ-9023) at 734 nm. Each sample (P3M and PY1) was measured in triplicate (n = 3).

Extraction techniques for cytotoxic effectson breast cancer cells

The extracts (cold aqueous extract and aqueous autoclave extract) of samples P3M and P1Y were prepared according to Suh, Kim, Yang, Ko & Hong,[24] with modifications. Two extracts were prepared: (1) the cold aqueous extract was prepared by processing P3M or P1Y with water (1:3) in an industrial blender for 1 min at 23,000 rpm. Then the samples were filtered twice under sterile conditions to remove fibers and solid particles. First through a cotton cloth and then through a PTFE membrane with 0.45 µm pores. (2) The aqueous autoclave extract was subjected to the same procedure, but the samples were autoclaved at 121°C and 100 kPa for 15 minutes before filtration.

Cytotoxic effect on breast cancer cells by comparing two different extraction methods

The MCF-7 (wild type p53) and MDA-MB-231 (mutant p53 – p.R280K) human breast epithelial carcinoma cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cell lines were incubated in 10% inactivated fetal bovine serum added to DMEM at 37°C and 5% CO2. Treatment of the cells with the extracts prepared from P3M and P1Y (1, 2, 5 and 10 %) was performed under the same conditions. After the 24-hour treatment, the media were replaced with a 10 % Alamar Blue® solution and kept under the same incubation conditions for 3 hours. The absorbance was read at 570 and 600 nm in a microplate

reader (EZ Read 2000 Monochromator Based; Montreal Biotech; QC; CA). Results were expressed as percent reduction of Alamar Blue® compared to the control.[25]

Statistical analysis

Results for physicochemical properties and minerals and for cytotoxic effect on breast cancer cells were subjected to one-way ANOVA followed by Tukey's test ($p < 0.05$). Data were expressed as means ± standard deviation of three replicates. Volatiles, antioxidant capacity, and TPC were tested for normality using D'Agostino and Pearson's test. For the parameters that passed the normality test, the existence of significant statistical differences ($p < 0.05$) between the groups was assessed using the parametric t-test. For the parameters that did not pass the normality test, the non-parametric Mann-Whitney test was performed to check for significant statistical differences between the groups. Differences were considered statistically significant at $p < 0.05$. Correlations between antioxidant capacity and TPC were determined using the Pearson correlation coefficient test. All statistical analyzes were performed using Graph Pad Prism 6.0 software.

RESULTS AND DISCUSSION

Nutritional and physical chemical characteristics

Table 1 shows the effects of freezing on the proximate composition, mineral content and physico-chemical properties of persimmon fruit. Freezing for one year had no significant effect on ash, crude fat and protein of persimmon. The change in moisture content (decrease) after one year of freezing can be explained by the change in cell structure due to the formation of ice crystals.[26]

Table 1. Proximate composition, mineral content and physicochemical characteristics of persimmon (Diospyros kaki) stored for three months and one year at -18°C [1]. Rio de Janeiro, 2018.

		P3M [2]	P1Y [2]
Proximate composition (g/100g)	Moisture	82.65 ± 0.14 [a]	82.17 ± 0.21 [b]
	Ash	0.31 ± 0.07 [a]	0.45 ±0.1 [a]
	Crude Fat	0.37 ± 0.08 [a]	0.39 ± 0.15 [a]
	Protein	1.99 ± 0.09 [a]	1.48 ± 0.74 [a]
	Carbohydrate[3]	14.68	15.50
Minerals (mg/100g)	Calcium	13.8 ± 1.46 [a]	12.5 ±0.71 [a]
	Manganese	0.55 ± 0.25 [a]	0.1 ±0.00 [a]
	Potassium	108.45 ± 16.88 [a]	135.5 ± 3.54 [a]
	Sodium	14.1 ± 7.09 [a]	13.25 ± 1.91 [a]
	Iron	0.23 ± 0.12 [a]	0.25 ± 0.07 [a]
	Magnesium	5.97 ± 0.33 [a]	6 ±0.00 [a]
	Zinc	0.1 ± 0.00 [a]	0,1 ± 0.00 [a]
	Copper	N.D.	N.D. [2]

Table 1. Proximate composition, mineral content and physicochemical characteristics of persimmon (Diospyros kaki) stored for three months and one year at -18°C [1]. Rio de Janeiro, 2018. (Continues)

		P3M [2]	P1Y [2]
Physico chemical characteristic	Ph	5.21 ± 0.11 [a]	5.53 ± 0.18 [a]
	Total Soluble Solid - TSS (°Brix)	18.57 ±0.71 [a]	16.98 ± 0.35 [b]
	Total titratable acidity - TTA (% malic acid)	0.38 ± 0.16 [a]	0.29 ± 0.11 [a]
	Ratio (TSS/TTA)	48.23	57.84

[1] Values expressed as mean ± SD of 100 g of fruit. The values marked with different letters in the same line was statistically different ($p < 0.05$).
[2] P3M- Persimmon storage for three months; P1Y- Persimmon storage for one year; N.D.- non-detected.
[3] Carbohydrate was determined by difference.

There were no differences in the mineral content of persimmons after one year of frozen storage. Previous studies have reported that changes in mineral content after this preservation method are probably due to the effect of pretreatments (washing and blanching) or damage to plant tissues and cells by the formation of ice crystals.[27,28] The mineral profile of persimmons is particularly noteworthy due to their high potassium content. Potassium is also the predominant element in the macrominerals analyzed in the red-fleshed pitaya (0.681 g/100g), underlining its nutritional importance in both fruits.[29] Persimmon is a valuable source of potassium, an essential mineral for human nutrition.[30] For instance, infant food supplements for children aged 0 to 6 months must contain 400 mg of potassium.[31] Thus, persimmon can be strategically utilized for nutritional enrichment and value addition in food products.[30]

In fruits, pH, TA, soluble solids (°Brix) and organic acids are associated with quality characteristics; they are also important parameters for marketing and use in industrial products.[32] TTA and pH were not affected during frozen storage. Chassagne-Berces, Fonseca, Citeau & Marin[33] also found no change after freezing.

The TSS value decreased significantly after the applied conservation method ($p < 0.05$). The TSS value can be considered as an indirect measure of soluble sugar; however, this measurement can also be related to the concentration of compounds such as organic acids and tannins.[16] The decrease in TSS suggests that the conditions for freezing persimmons for one year did not prevent the occurrence of biochemical processes that metabolize and/or degrade soluble solids.[2,34] In our study, although the TSS content of persimmons decreased statistically ($p<0.05$) from 18.5 to 16.9 °Brix after one year of frozen storage, this parameter is still sufficient for use in the food industry (at least 13 °Brix).[32] The TSS/TTA ratio in P3M (48.23) and P1Y (57.84) was high. This is an interesting result showing a good balance between the total sugar content and organic acids, indicating a high quality of the fruit.[35]

Volatile and antioxidant compounds

Analysis of volatile fraction

The analysis of P3M allowed the identification of 10 volatile compounds, including 1 hydrocarbon, 2 esters, 1 alcohol, 1 ketone, 3 terpenic compounds and 2 miscellaneous (Table 2). The freezing methods for one year affected the profile and concentration of volatile compounds in persimmons. After one year of freezing, six compounds (tetracosane, 2-ethyl-1-hexanol, 2,4-dimethyl-3-hexanone, o-cymene, α-terpineol, and thymol) were no longer found in persimmon fruit and the compounds 4-hydroxy-4-methyl-2-pentanone and benzophenone were found at lower concentrations ($p < 0.05$). Frozen storage of durian pulp for 12

months resulted in changes in some volatile compounds, particularly a decrease in sulfur and ester content and an increase followed by a decrease in the concentration of alcohols.[36]

Table 2. Volatile compounds of persimmon (*Diospyros kaki*) after frozen storage for three months and one year at -18°C. Rio de Janeiro, 2018.

Volatile Compounds	LRI (calculated)	LRI (literature)	P3M (Avg ± SD) ppb	P1Y (Avg ± SD) ppb
Hydrocarbon				
*Tetracosane▲,•,■	2400	2400[2]	23.37 ± 13.45	nd
Esters				
*Methyl hexadecanoate▲,•,■	2163	2170[1]	28.45 ± 3.61[a]	27.53 ± 3.63[a]
*Methyl oleate▲,•,■	2383	2400[1]	56.51 ± 13.85[a]	53.03 ± 9.89[a]
Alcohol				
2-Ethyl-1-Hexanol•,■	1436	1441[1]	[(A)]13.42 ± 1.07	nd
Ketone				
2,4-Dimethyl-3-Hexanone•,■	1213	1178[1]	[(B)]5.61 ± 3.78	nd
Terpenic compounds				
Monoterpene				
o-Cymene•,■	1211	1232[2]	[(C)]27.09 ± 4.94	nd
Oxygenated monoterpenes				
α-Terpineol•,■	1631	1650[1]	[(D)]7.85 ± 1.00	nd
Thymol•,■	2166	2166[1]	[(D)]18.97 ± 1.43	nd
Miscellaneous				
4-hydroxy-4-methyl-2-Pentanone•,■	1339	1366[1]	[(B)]14.44 ± 3.62[a]	[(B)]4.07 ± 0.55[b]
Benzophenone•,■	2403	2410[1]	[(E)]10.28 ± 0.42[a]	[(E)]8.06 ± 0.57[b]

▲Identified by coelution with standard volatile compounds; •Identified by the mass spectra data;■Identified by comparing the calculated KI with the theoretical KI (literature); *compound considered definitely identified (identified at least by coelution with standard volatile compounds and mass spectra data); LRI - modified Kovats index calculated using C7-C26 alkanes (Van den Dool and Kratz, 1963); Avg - average value; SD - standard deviation; nd – non-detected compound;P3M- persimmon frozen for three months; P1Y- Persimmon frozen for one-year; References: 1- NIST; 2 – Pherobase; (A) - concentration given in ppb octanol equivalent; (B) - concentration given in ppb cyclohexen-1-one equivalent; (C) - concentration given in ppb limonene equivalent; (D) - concentration is given in ppb linalool equivalent; (E) - concentration is given in ppb phenol equivalent. In a row when the contents of the compounds were compared, the values marked with the different lowercase letters were considered statistically different ($p < 0.05$).

There are few published studies on volatile organic compounds in persimmon fruit, and there is no common compound found in all fruits.[37] The study conducted with the persimmon 'Rama Forte' allowed the identification of 31 volatile compounds, including 10 terpenes, 10 esters, 5 aldehydes, 3 ketones, 2 carboxylic acids and 1 phenol.[37] One of these compounds (methyl hexadecanoate) was found in our study. In the 'persimmon species 'Fuyu', methyl hexadecanoate, 2-ethyl-1-hexanol and 4-hydroxy-4-methyl-2-pentanone were found as components of the volatile fraction.[38] Methyl oleate and o-cymene were detected in the persimmon of the Mikado variety.[38]

The terpenic compounds identified only in the P3M sample exhibited aromatic notes, namely fruity, leafy, citrus, herbaceous (α-terpineol), solvent, gasoline, citrus (o-cymene) and thyme (thymol).[39-41] Esters (see Table 2) were the main volatile compounds found in both samples (P3M or P1Y). This finding can be explained

by the choice of the isolation procedure used in the present work. This isolation technique, in which the whole fruit comes into contact with the extractant, probably extracted mainly components of the persimmon surface.

TPC and antioxidant capacity

The average TPC of persimmons was about six times lower after freezing from 3 months to one year (Table 3). These results indicate that the freezing conditions used in this work were not sufficient to inhibit the decay process of the phenolic compounds.[42]

Table 3. Total phenolic content and antioxidant capacity of persimmon (Diospyros kaki) after frozen storage for three month and one year at -18°C. Rio de Janeiro, 2018.

Analyses	P3M (Avg ± SD)	PYM (Avg ± SD)
TPC (mg GAE L^{-1})	80.51 ± 7.51^{A}	13.19 ± 2.20^{B}
Antioxidant capacity (IC_{50}) (mg mL^{-1})	1.39 ± 0.68^{A}	19.01 ± 10.5^{B}

P3M- persimmon frozen for three months; P1Y- Persimmon frozen for one-year; TPC – total phenolic content; GAE - gallic acid equivalents; IC50 – the concentration of an antioxidant which reduces the free radical ABTS about 50%; Avg – average value; SD – standard deviation. In a specific row of this table, when the extracts of the P3M were compared with the extracts of the P1Y in relation to a parameter that was measured for both of them, the values marked with different letters were statistically different ($p < 0.05$).

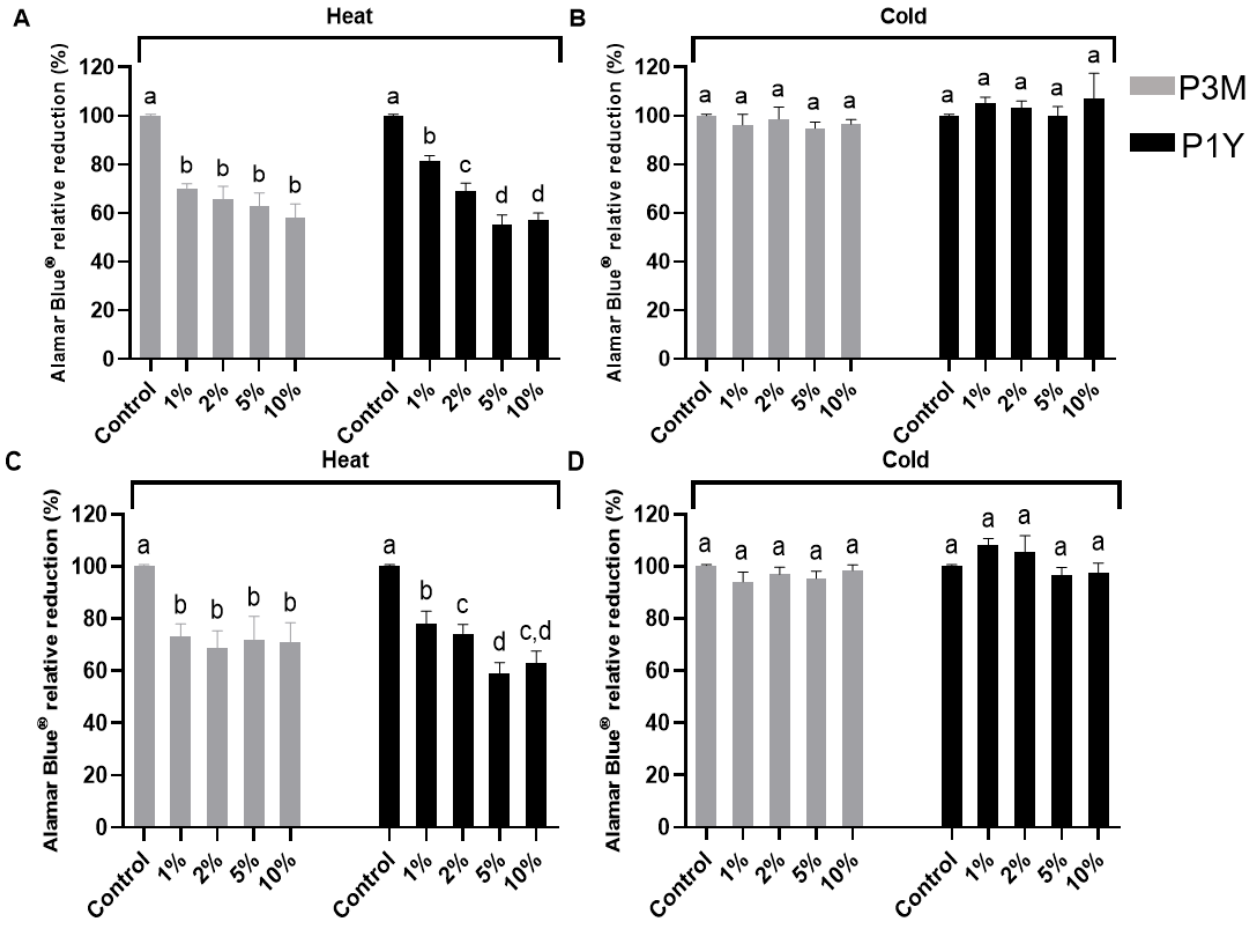
The mean IC50 value found for the extracts of P1Y [(19.01 ± 10.53) mg mL^{-1}] was 13.6 times greater ($p < 0.05$) than that for P3M [IC50 = (1.3 ± 0.68) mg mL^{-1}]. This result is reinforced by the decrease in TPC during freezer storage and the high negative correlation of the TPC of persimmon with the IC50 estimated by the ABTS assay ($r = - 0.8318$, $p < 0.05$). The P3M extracts were more potent antioxidants than the P1Y.

The antioxidant activity of P3M was consistent with the antioxidant activity of four persimmon fruit cultivars, particularly the cultivar Dogeunjosaeng [(IC50(ABTS) = 1,130.23 ± 64.30) µg mL^{-1}].[43] Freezing persimmons for one year affected the decrease in TPC and antioxidant capacity. During freezing and prolonged storage, the formation of ice crystals can disrupt the cell structure and promote oxidation and enzymatic reactions that lead to the degradation of soluble compounds, including soluble tannins. This loss of bioactive compounds can reduce the antioxidant potential of persimmons.[5]

Cytotoxic effect on breast cancer cells

The autoclaved aqueous extract of P3M and P1Y showed a cytotoxic effect on both cancer cell lines ($p < 0.05$). In contrast, the cold aqueous extract showed no effect ($p < 0.05$) (Figure 1). The autoclaved P3M extract at a concentration of 1% was sufficient to reduce cancer cell viability by 30-40 and showed no dose-dependent effect ($p < 0.05$). In contrast, an analysis of autoclaved extracts of persimmon showed a dose-dependent effect after one year of freeze storage, and a cell reduction of about 50% was achieved from an extract concentration of 5%. This cytotoxicity behavior was observed for both MCF-7 and MDA-MB-231 neoplastic cells. Interestingly, there were differences between the two cell lines. While MCF-7 expresses hormone receptors and is susceptible to therapeutic drugs, MDA-MB-231 does not express hormone receptors and is therefore more resistant to treatments.[44,45]

Figure 1. P3M- persimmon frozen for three months; P1Y- Persimmon frozen for one-year;MCF-7 (A, B) and MDA-MB-231 (C, D) cell viability after a 24 h treatment with 1 % to 10 % persimmon extract concentration (n = 3). Untreated cells represented as control in each graph. Cells in (A, C) were treated with autoclaved extract from persimmons (heat) while in (B, D), with the simple aqueous extract (cold). Data are represented as mean ± standard deviation. Different concentrations in the same cell line were analyzed with two-way analysis of variance (ANOVA) with Tukey's post-test, and different lowercase letters represent a significant difference ($p<0.05$).



This study did not investigate which specific compound was responsible for the cytotoxic potential against the MCF-7 and MDA-MB-231 cell lines. In any case, autoclave water extraction was more efficient ($p < 0.05$) than cold water extraction to elute this compound (or compounds) from persimmon (Figure 1). Previous studies have shown that autoclave extraction could be an effective strategy for extracting bioactive compounds. This can be explained by the fact that the harsh environment of the autoclave, which is characterized by high temperature and pressure, can cause the cell membranes to collapse and the bioactive compounds are then eluted more efficiently.[46-49] In addition, some studies have reported that high temperatures increase the efficiency of water in the extraction of compounds by improving their solubility through a reduction in surface tension.[48,50] Further studies should be conducted to investigate which compounds in an autoclaved persimmon extract have anticancer activity and what their mechanism of action might be.

There is evidence that the consumption of bioactive compounds naturally present in fruits and vegetables reduces the risk of chronic diseases.[51–53] Ramadan et al,[54] when evaluating the anticancer activity (for colon and breast cancer) of the ethanolic extract of cape gooseberry fruit (*Physalis peruviana* L.), found that the anticancer potential was more effective in inhibiting colon cell lines. Jo, Lee, Lee & Park[55] demonstrated that methanolic extracts from the persimmon calyx inhibited the growth of colon cancer cells

(HT-29) in a dose-dependent manner and 500 μg mL^{-1} PCE caused 31.1% inhibition of the cell lines. Gloria et al.[56] found that carotenoids (abundant in fruits and vegetables) are potential anticancer compounds; they also reported their effect on cell cycle and cell viability in human breast cancer cell lines.

CONCLUSIONS

After one year of storage of persimmons under freezing conditions, no significant differences in proximate composition, mineral profile or physicochemical properties were observed. Based on these results, freezing as a preservation method can be considered a viable strategy depending on the intended use. In this context, frozen storage of persimmons proves to be a promising approach for the development of innovative high value-added products with applications in food technology (e.g. hot sauces and ketchup), bio-based packaging (including thin and flexible films) and biotechnology (e.g. mineral supplements). This approach also expands the possibilities for using the fruit out of season and supports its integration into more sustainable and diversified production chains.

On the other hand, freezing for one year had a direct impact on the volatile organic compounds and antioxidant properties of the samples. Therefore, this preservation method is not recommended if the aim is to extract volatile compounds and antioxidants to produce natural antioxidant additives from persimmon.

The results of this study also suggest that aqueous extraction of persimmons in combination with autoclaving is an effective strategy for eluting bioactive compounds with cytotoxic effects on cancer cell lines MCF-7 and MDA-MB-231. However, further studies are needed to optimize the extraction conditions, identify the compounds responsible for the potential cytotoxic effects and elucidate their mechanisms of action. In addition, further studies should be conducted to evaluate the effects of different preservation systems on the chemical profile, bioactive compounds and potential biological activity of persimmon.

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Contributors

Maragoni-Santos C collected the data, performed the analysis, wrote the paper, contributed data and analysis tools; Takeyama MM performed the analysis, contributed data and analysis tools; Matheus JRV contributed with analysis tools; Arcanjo ME collected the data and performed the analysis; da Costa DCF and Marques MRC contributed with analysis tools; Moreira RFA performed the analysis, wrote the paper, contributed with analysis tools; Fai AEC conceived and designed analysis, wrote the paper, revision and approval of the final version.

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