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Pineapple by-products as a source of bioactive compounds with potential for industrial food application†

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Pineapple is a tropical fruit consumed fresh or processed into various food products. However, the peel and crown of this fruit are not industrially exploited, thus generating tons of by-products that represent an economic and environmental concern. In order to promote the upcycling of these by-products, this work aimed to characterize the phenolic profile of its hydroethanolic extracts obtained from pineapple peel and crown leaves and to evaluate their *in vitro* bioactivity. The HPLC-DAD-ESI/MS analysis allowed the identification of 25 phenolic compounds, including phenolic acids and flavonoids. The antioxidant, cytotoxic, and antimicrobial activity assays highlighted the peel extract as the most promising and, therefore, it was incorporated into a traditional Portuguese pastry cake as a functional ingredient. The nutritional parameters of the developed food were not affected by the incorporation of the extract, but it promoted the antioxidant activity during its shelf-life. Overall, pineapple peel and crown appeared as promising by-products to be exploited by the food industry, which can be achieved through a circular economy approach.

1. Introduction

In recent years, the beneficial effects shown by bioactive compounds present in different plant matrices have aroused great interest of the world scientific community.

As a result of this scientific evidence, these compounds have been incorporated into the formulation of new food products in order to offer more natural and healthier choices for consumers who are increasingly concerned about their health and well-being.[1] The benefit of these bioactive compounds upon frequent food consumption or even with direct supplementation of these compounds generated multiple health effects, such as anti-aging effects, protection against cardiovascular diseases, control and prevention of metabolic diseases, prevention and treatment of cancer, and also protection against neurodegenerative diseases.[2] Concerning bioactive compounds, phenolic compounds are one of the main groups within the phytochemicals existing in medicinal and aromatic plants.[3] These compounds are secondary metabolites, which have a very diverse chemical structure and activity, and some of these phenolic compounds can contribute to colour, flavour and astringency which are the typical organoleptic characteristics of foods.[4]

Being found in foods such as fruits, cereals, vegetables and food processing by-products, bioactive ingredients preserve their characteristics even after extraction.[5] Pereira-Netto[6] and Cádiz-Gurrea *et al.*[7] reported several tropical fruits as having a wide variety of unique characteristics such as shapes, size, flavour, colour, and texture, but especially high contents of bioactive compounds with functional properties for the prevention or *improvement* of some diseases. Among them is pineapple (*Ananas comosus* (L.) Merr.), one of the most important and cultivated tropical fruits worldwide, followed by avocado, mango, and papaya.[8] According to Alexandre *et al.*[9] pineapple can be used for both fresh consumption and industrial use; since most of the fruit is not consumed, generating by-products, found in the crown, peel, bottom, stem, and trimmings, representing almost 60% of the total fresh weight.[10,11]

In order to add value these products, has economic and environmental interest, requiring scientific and technological research, allowing efficient, economical, and safe use.[12] In

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addition, the demand for products containing natural additives that promote health rather than synthetic substances has been increasingly growing over the past few years in the food, cosmetic and pharmaceutical industries.[13] Therefore, this work intends to valorise pineapple by-products, aiming at its application as a potential natural ingredient for the food industry, thus promoting an improvement in human health, as well as a reduction of the impact on the environment.

2. Materials and methods

2.1. Preparation of the samples

Pineapple (*Ananas comosus* (L.) Merr.) peel and crown by-products were kindly provided by the company ALITEC, S.A. from Valado dos Frades, Portugal. All the by-product samples were the result of fruit processing for second-range products. Both samples were frozen and lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA), reduced to a fine powder, and stored protected from moisture and light until further analysis.

2.2. Extracts preparation

Each lyophilized sample obtained in section 2.1. (2 g) was subjected to maceration with 50 mL of ethanol/water (80 : 20, v/v) for 1 h at room temperature with stirring at 150 rpm (x). Subsequently, the mixture was filtered through filter paper (Whatman No. 4) and the process was repeated. The ethanolic fraction of the combined extracts was evaporated under reduced pressure (100 rpm, 40 °C; rotary evaporator, Heidolph, Schwabach, Germany) and the aqueous phase was frozen and lyophilized (Labconco Freeze Zone 6, USA) for further analysis.

2.3. Identification and quantification of phenolic compounds

For phenolic profile analysis, the extract described was redissolved in 2 mL of EtOH/H_2O solution (20 : 80, v/v). The phenolic compounds' composition was determined by high-performance liquid chromatography coupled with photodiode array detection (280, 330 and 370 nm as preferred wavelengths) and mass spectrometry with electrospray ionization (HPLC-DAD-ESI/MS), previously described by the authors Bessada *et al.*[14] and Gonçalves *et al.*[15] The identification of compounds was based on the comparison with commercial standards and the available literature information. The quantification was performed using the calibration curve of the most similar available standard. The results were expressed as mg g^{-1} of extract.

2.4. Bioactivity evaluation

2.4.1. Evaluation of antioxidant activity. The lipid peroxidation inhibition was evaluated by the extract's capacity to inhibit the formation of thiobarbituric acid reactive substances (TBARSs) using porcine (*Sus scrofa*) brain tissues as oxidizable substrates, according to the methodology described by Barros *et al.*[16] The results were expressed as IC_{50}, which corresponds to the extract concentration responsible for inhibiting 50% of oxidation (μg mL^{-1}). Trolox was used as a positive control.

The antihaemolytic activity of the extracts was evaluated as previously described by Lockowandt *et al.*[17] This assay was performed using sheep blood erythrocytes and the results were expressed as IC_{50} values (μg mL^{-1}) for Δt of 60 and 120 min, which translate the extract concentration needed to protect 50% of the erythrocyte population from the haemolytic action of 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) for specific time periods. Trolox was used as a positive control.

2.4.2. Evaluation of cytotoxic activity. For the cytotoxicity activity, the effect of the extracts (8 mg mL^{-1}) on the growth of human tumour cell lines was evaluated by sulforhodamine B (SRB) assay to determine cell growth inhibition.[18] Four tumour cell lines were used in this assay: MCF-7 (breast carcinoma), NCI-H460 (lung carcinoma), AGS (gastric adenocarcinoma) and CaCo-2 (colon adenocarcinoma). The non-tumour cell line VERO (fibroblast-like kidney cell line from African green monkey) was also tested. Ellipticine was used as a positive control and the results were expressed as GI_{50} values (μg mL^{-1}), which correspond to the extract concentration that inhibits 50% of cell growth.

2.4.3. Evaluation of antimicrobial activity. The methodology of antibacterial activity was previously described by Carocho *et al.*[19] The extracts at a concentration of 20 mg mL^{-1} were evaluated against six strains of bacteria, three being Gram-positive: *Staphylococcus aureus* (ATCC 11632), *Bacillus cereus* (food isolate), *Listeria monocytogenes* (NCTC 7973) and three Gram-negative: *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 35030) and *Salmonella typhimurium* (ATCC 13311). The minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined using two food additives, sodium sulphite (E221) and potassium metabisulphite (E224), as a positive control. The results were expressed in mg mL^{-1}.

For antifungal activity, the methodology described by Carocho *et al.*[19] was followed. Six fungal strains were used: *Aspergillus fumigatus* (ATCC 9197), *Aspergillus versicolor* (ATCC 11730), *Aspergillus niger* (ATCC 6275), *Penicillium funiculosum* (ATCC 36839), *Penicillium aurantiogriseum* (food isolate), and *Trichoderma viride* (IAM 5061). These organisms were acquired in the Mycology Laboratory of the Department of Plant Physiology of the Institute of Biological Research "Siniša Stanković" at the University of Belgrade in Serbia. The MIC and minimum fungicidal concentration (MFC) were determined and compared with the values of commercial food preservatives sodium sulphite (E221) and potassium metabisulphite (E224).

2.5. Formulation of "súplicas", a pastry food product

The "súplicas" are a typical pastry food product from Bragança (Portugal) and were prepared following the traditional recipe. The dough was prepared by mixing 8 eggs, 1 yolk, 600 g of wheat flour, and 500 g of sugar. The dough was then divided into two groups, one without addition of extract (control) and other for incorporation of pineapple peel extract. The amount

of lyophilized extract for addition to the dough was determined from the tests previously performed to achieve 50% of the bioactivity effectiveness. Thus, 2.8 g of extract were added to the dough to prepare the "súplicas". Thereafter, the doughs were used to prepare small dumplings that were baked in the oven at 180 °C for 12 min.

2.6. Evaluation of quality parameters of "súplicas" during shelf-life

The prepared "súplicas" were lyophilized, finely crushed, and analysed in triplicate immediately after cooking (T0) and after three (T3) and seven (T7) days of storage at room temperature.

2.6.1. Colour parameters. The surface colour of the "súplicas" was measured in triplicate by each group of samples, at three different points using a colorimeter (model CR-400, Konica Minolta Sensing Inc., Tokyo, Japan). Illuminant C and an 8 mm diaphragm aperture were used, with previous calibration on a white tile.[20]

2.6.2. Proximate composition. The nutritional profile of the "súplicas" was determined according to the official food analysis methodologies.[21] The quantification of macronutrients was done analysing the content of proteins, fats, and carbohydrates, as well as the amounts of ash, moisture and total energy. The total protein content ($N \times 5.70$) was calculated as the nitrogen content by the Kjeldahl method (AOAC 991.02).

2.6.3. Free sugars. A high-performance liquid chromatography (HPLC) system coupled to a refraction index (RI) detector was used to determine the soluble sugar composition, following a previously described procedure.[22] Melezitose was used as the internal standard at 25 mg mL^{-1}. For the identification of the compounds, Clarity 2.4 Software DataApex 4.0 Software (Prague, Czech Republic) was used, from which the relative retention times of the sample peaks were compared with known patterns. The results were obtained by the internal pattern method and expressed in gram of composed of 100 g of fresh weight (fw).

2.6.4. Fatty acids. Fatty acids were determined by gas chromatography with flame ionization (GC-FID) design, as previously described by Pereira *et al.*[23] The identification and quantification were performed by comparing the relative retention times of FAME peaks from samples with standards (standard mixture 47885-U, Sigma, St Louis, USA) and results were recorded and processed using the Clarity software and expressed in relative percentage of each fatty acid.

2.6.5. Antioxidant activity. The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity was evaluated according to a methodology described by Barros *et al.*[24] The extract concentration providing 50% of radicals scavenging activity (RSA) was calculated by interpolation from the graph of RSA percentage against extract concentration, and the results were expressed as EC_{50} values (mg mL^{-1}). Trolox was used as a positive control.

The reducing power was evaluated according to a methodology described by Barros *et al.*[25] This methodology was performed using a microplate reader and by measuring the absorbance at 690 nm. The different concentrations of the extract or Trolox (0.5 mL) were mixed with sodium phosphate buffer (200 mmol L^{-1}, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL) and the mixture was incubated at 50 °C for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.6 mL) was poured in a 48-well plate, as also ferric chloride (0.1% w/v, 120 μL) and deionized water (0.8 mL). The results were expressed as EC_{50} values (mg mL^{-1}).

2.7. Statistical analysis

The results are expressed as mean values ± standard deviation (SD). Furthermore, to better understand the effects of the pineapple peel extract incorporation (*I*) and the storage time (ST) on "súplicas" quality parameters, a two-way ANOVA with type III sums of squares was applied using SPSS Software, version 25. All analyses were carried out using a *p*-value of 0.05.

3. Results and discussion

3.1. Phenolic composition of pineapple peel and crown extracts

Fruit by-products are of great importance to provide nutraceutical and biological properties such as anticancer, antimutagenicity, antiallergy and anti-aging activity due to the presence of phenolic compounds, which have been reported both for natural and synthetic antioxidants.[26] According to the literature, pineapple by-products have considerable levels of phenolic compounds, among them gallic, ferulic and caffeic acids, as well as high antioxidant activity and high fibre content, presenting potential to be applied as a food ingredient in the development of new affordable products.[27]

In this way, it is necessary to search for bioactive molecules, such as phenolic compounds that are present in many agri-food by-products, such as those from pineapple. Twenty-five phenolic compounds were identified in the peel and crown extracts, such as, phenolic acids and glycosidic flavonoids. The main detected compounds were caffeic acid derivatives, namely caffeic acid hexosides and flavones such as apigenin 6,8-*C*-diglucoside. Table 1 shows the phenolic compounds profile details regarding the retention time, maximum absorption wavelength in the UV-Vis region (λ_{max}), pseudomolecular ion ($[M - H]^-$) and molecular ion fragmentation (MS^2), as well as their quantification.

Based on their mass spectra, the peel presented thirteen molecules, nine phenolic acids (peaks **1**, **2**, **3**, **5**, **11**, **15**, **17**, **19** and **21**), one phenylpropane monoglyceride (peak **12**), three flavonoids, among them, one flavan-3-ol (peak **18**) and two flavones (peak **8** and **9**). Peaks **1**, **2**, **3** and **5** ($[M - H]^-$ at *m*/*z* 341), were identified as caffeic acid hexoside; these compounds were described by Steingass *et al.*[28] in *A. comosus*. In another study performed by Lourenço *et al.*,[29] demonstrated that the intensity of all the peaks was higher in the ethanolic extract instead of the water extract. Peak **9** showed a pseudomolecular ion $[M - H]^-$ at *m*/*z* 593, being identified as apigenin 6,8-*C*-diglucoside (vicenin-2); the same was also reported

Table 1 Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, and identification and quantification of phenolic compounds in pineapple peel and crown extracts

Peak	Rt (min)	λ_{max} (nm)	$[M-H]^-$ (m/z)	MS^2 (m/z)	Identification	Content (mg g^{-1} extract)	
						Peel extract	Crown extract
1	4.48	275	341	179(100)	Caffeic acid-*O*-hexoside[1]	0.0009 ± 0.0001	nd
2	4.93	275	341	179(100)	Caffeic acid-*O*-hexoside[1]	0.0034 ± 0.0002	nd
3	5.30	277	341	179(100)	Caffeic acid-*O*-hexoside[1]	0.0294 ± 0.0002	nd
4	5.90	292	451	253(100), 179(3), 161(20), 135(11)	Dicaffeoylglycerol[1]	nd	0.011 ± 0.001
5	6.79	277	341	179(100)	Caffeic acid-*O*-hexoside[1]	0.006 ± 0.0002	nd
6	6.86	321	517	473(100), 269(18)	Apigenin-malonyl-hexoside[2]	nd	1.1 ± 0.1
7	7.86	326	385	209(8), 191(100), 147(5), 129(11)	Feruloyl aldarate[3]	nd	0.035 ± 0.001
8	8.03	332	461	347(100), 299(25)	Diosmetin 8-*C*-glucoside[4]	0.1175 ± 0.0002	nd
9	8.45	328	593	473(100)	Apigenin 6,8-*C*-diglucoside[5]	0.46 ± 0.01	0.56 ± 0.01
11	9.29	274, 332	597	359(17), 295(20), 197(62), 179(56), 135(95)	Yunnaneic acid F[6]	0.41 ± 0.005	nd
12	10.15	275, 327	399	253(100), 235(23), 163(17), 145(9)	*p*-Coumaroyl-caffeoylglycerol[7]	0.25 ± 0.01	nd
13	10.70	326	385	209(6), 191(100), 147(7), 129(15)	Feruloyl aldarate[3]	nd	0.031 ± 0.001
14	12.19	324	593	431(100)	Apigenin 6,8-*C*-diglucoside[5]	nd	0.483 ± 0.005
15	13.05	365	771	609(95), 301(32), 179(12)	3,5-Di-*O*-Caffeoyl-4-*O*-(3-hydroxy, 3-methyl) glutaroylquinic acid[7]	0.18 ± 0.01	nd
16	13.05	315	757	595(21), 449(46), 287(90)	Eriodictyol-4′-*O*-neohesperidoside-7-*O*-glucoside[8]	nd	0.058 ± 0.003
17	13.29	277, 301	337	191(85), 173(100), 155(4)	*p*-Coumaroylisocitrate[7]	0.132 ± 0.005	nd
18	14.06	225, 277	305	261(12), 221(47), 219(56), 179(100), 165(28), 137(30), 125(40)	(Epi)gallocatechin[9]	0.59 ± 0.02	nd
19	14.40	315	537	491(72), 329(100), 209(2), 167(24), 152(4)	Vanilloyl dihexoside[10]	0.118 ± 0.001	nd
20	14.63	322	563	473(100), 443(69), 383(22), 353(28)	Apigenin-6-*C*-hexoside-8-*C*-pentoside[5]	nd	0.462 ± 0.002
21	15.42	225, 274	441	249(100)	*N*-L-γ-Glutamyl-*S*-sinapyl-L-cysteine[11]	0.17 ± 0.02	nd
22	15.60	327	537	375 (100)	Ananaflavoside B[12]	nd	0.468 ± 0.001
23	16.51	350	609	301(100)	Quercetin-3-*O*-rutinoside[12]	nd	0.471 ± 0.001
24	17.09	327	533	311(100)	Apigenin-di-*C*-pentoside[5]	nd	0.467 ± 0.002
25	20.55	327	623	315(100)	Isorhametin rhamosyl-hexoside[12]	nd	0.469 ± 0.001
					Σ Phenolic compounds	2.48 ± 0.03	4.7 ± 0.1

Calibration curves used in the quantification: 1. caffeic acid ($y = 388345x + 406369$; $R^2 = 0.994$; LOD = 0.78 μg mL^{-1}; LOQ = 1.97 μg mL^{-1}); 2. apigenin-7-*O*-glucoside ($y = 10683x - 45\,794$; $R^2 = 0.999$; LOD = 0.10 μg mL^{-1}; LOQ = 0.53 μg mL^{-1}); 3. ferulic acid ($y = 633\,126x - 185\,462$; $R^2 = 0.999$; LOD = 0.20 μg mL^{-1}; 1.01 μg mL^{-1}); 4. taxifolin ($y = 478.06x + 657.33$; $R^2 = 0.999$; LOD = 0.67 μg mL^{-1}; LOQ = 2.01 μg mL^{-1}); 5. apigenin 6-*C*-glucoside ($y = 107025x - 61\,531$; $R^2 = 0.9989$; LOD = 0.19 μg mL^{-1}; LOQ = 0.63 μg mL^{-1}); 6. rosmarinic acid ($y = 191291x - 652\,903$; $R^2 = 0.999$; LOD = 0.15 μg mL^{-1}; LOQ = 0.68 μg mL^{-1}); 7. chlorogenic acid ($y = 168823x - 161\,172$; $R^2 = 0.9999$; LOD = 0.20 μg mL^{-1}; LOQ = 0.68 μg mL^{-1}); 8. naringenin ($y = 18433x + 78\,903$, $R^2 = 0.9998$; LOD = 0.20 μg mL^{-1}; LOQ = 0.64 μg mL^{-1}); 9. epicatechin ($y = 10314x + 147\,331$, $R^2 = 0.9994$; LOD = 0.15 μg mL^{-1}; LOQ = 0.78 μg mL^{-1}); 10. vanillic acid ($y = 197\,337x + 30\,036$; $R^2 = 0.999$; LOD = 0.17 μg mL^{-1}; LOQ = 1.22 μg mL^{-1}); 11. sinapic acid ($y = 197\,337x + 30\,036$; $R^2 = 0.999$; LOD = 0.17 μg mL^{-1}; LOQ = 1.22 μg mL^{-1}); 12. quercetin-3-*O*-glucoside ($y = 34843x - 160\,173$; $R^2 = 0.9998$; LOD = 0.21 μg mL^{-1}; LOQ = 0.71 μg mL^{-1}). nd: not detected.

by Steingass *et al.*[28] in pineapple crown and peel. Peak **8** was identified as diosmetin 8-*C*-glucoside ($[M - H]^-$ at *m*/*z* 461), considering its fragmentation pattern. This compound was also reported in sugarcane leaves[30] and in mandarin juice (*Citrus reticulata* Blanco).[31]

Compound **11** ($[M - H]^-$ at *m*/*z* 597) was identified as yunnaneic acid F that is a biogenetically derivative of yunnaneic acid C and normally detected in *Salvia yunnanensis.*[32] This compound was also found in lemon balm extract (*Melissa officinalis* L.) by Pérez-Sánchez *et al.*[33] Yunnaneic acid is also considered a phenolic compound derived from rosmarinic acid (RA) as lithospermic acid, salvianolic acid, and melitric acid, which have all a diverse biological function.[34] Peak **12** ($[M - H]^-$ at *m*/*z* 399) was identified as *p*-coumaroyl-caffeoylglycerol, this compound was previously found 'Phulae' Pineapple peel.[35] *p*-Coumaroylglycerol and caffeoylglycerol have been described in the pineapples stems and crown leaves, indicating that they were the primary forms of phenolic glycerides.[36,37]

Compound **15** ($[M - H]^-$ at *m*/*z* 771) was identified as 3,5-di-*O*-caffeoyl-4-*O*-(3-hydroxy, 3-methyl) glutaroylquinic acid, this compound was also identified in *Gardenia jasminoides* Ellis fruits by Wang *et al.* (2016).[38] Compound **17** ($[M - H]^-$ at *m*/*z* 337) was identified as *p*-coumaroylisocitrate, based on their UV and mass characteristics as reported by Steingass *et al.*[39] and by Difonzo *et al.*[40] in the pulp and peel of *A. comosus*.

Peak **18** ($[M - H]^-$ at *m*/*z* 305) was identified as (epi)gallocatechin, a flavan-3-ol that has been previously identified in cabbage stalk flour.[41] Peak **19** ($[M - H]^-$ at *m*/*z* 537), was identified as vanilloyl dihexoside (formic acid adduct) being previously identified in pineapple pulp by Steingass *et al.*[28] Compound **21** ($[M - H]^-$ at *m*/*z* 577) was identified *N*-L-γ-glutamyl-*S*-sinapyl-L-cysteine, a sinapyl derivative revealing a standard fragmentation in *m*/*z* 249, this assumption was made taking into account the study performed by Wen *et al.*,[42] which also reported the presence of this molecule in *A. comosus* juice.

Regarding the pineapple crown leaves, twelve phenolic compounds were identified, compromising nine flavonoids and two phenolic acids. Among them two flavonols (peaks number **23** and **25**), one phenylpropane diglyceride, (**4**), two hydroxycinnamic acids (**7** and **13**) and seven flavones (**6**, **9**, **14**, **16**, **20**, **22** and **24**). Peak **4** ($[M - H]^-$ at *m*/*z* 451) was tentatively identified as dicaffeoylglycerol, a glycerol ester, which was previously identified by Steingass *et al.*[28] in pineapple peel, crown, and pulp. Compound **6** ($[M - H]^-$ at *m*/*z* 517) was tentatively identified as an apigenin-*O*-malonyl-hexoside, this flavone was identified taking into account the findings reported in a study regarding cardoon (*Cynara cardunculus* var. *altilis*) bracts describe preciously by Mandim *et al.*[43] Peaks **7** and **13** ($[M - H]^-$ at *m*/*z* 385) were identified as a typical pineapple glycosylated polyphenol, called feruloyl aldarate, found by Steingass *et al.*[28] and Campos *et al.*[44] in the peel and crown of this fruit. Compounds **6**, **9**, **14**, **20** and **24** were identified as apigenin derivatives, linked to different sugars and typical *C*-glycosyl fragments. All the mentioned compounds were tentatively identified as apigenin-malonyl-hexoside (peak **6**; λ_{max}, 321 nm; $[M - H]^-$ at *m*/*z* 517), apigenin-6,8-*C*-diglucoside (peak **9** and **14**; λ_{max}, 328 nm; $[M - H]^-$ at *m*/*z* 593), apigenin-6-*C*-hexoside-8-*C*-pentoside (peak **20**; λ_{max}, 322 nm; $[M - H]^-$ at *m*/*z* 563), and apigenin-*C*-dipentoside (peak **24**; λ_{max}, 327 nm; $[M - H]^-$ at *m*/*z* 533), being all previously found in *Ananas* sp. by Franke *et al.*[45] and Harnly *et al.*[46]

Compound **16** was identified as an eriodictyol-4′-*O*-neohesperidoside-7-*O*-glucoside ($[M - H]^-$ ion at *m*/*z* 757 and MS^2 ions at *m*/*z* 595 (M-glucose), 449 (M-308, neohesperidose), and 287 (M-glucose and neohesperidose). Compound **22** ($[M - H]^-$ ion at *m*/*z* 537) revealed a single fragment ion at *m*/*z* 375, derived from the loss of a dehydrated hexose, and was tentatively identified as ananaflavoside B, sub classified as a metoxylated flavone, that can be synthetized from subsequent hydroxylations, methylations and glycosylation of luteolin.[47] Ananaflavoside B was reported previously by Steingass *et al.*[28] in pineapple crown and peel by-products.

Peak **23** was identified as quercetin-3-*O*-rutinoside (rutin) considering the chromatographic characteristics in comparison to the commercial standard. Two derivatives of quercetin were reported by Steingass *et al.*[28] in pineapple crown, being quercetin dihexoside ($[M - H]^-$ at *m*/*z* 625) and quercetin hexoside ($[M - H]^-$ at *m*/*z* 463). Compound 25 ($[M - H]^-$ at *m*/*z* 623) was identified as isorhamnetin-*O*-rhamosyl-hexoside also reported by Steingass *et al.*[28] in pineapple peel and in the palm fruit peel (*Phoenix dactylifera* L.) by Farag *et al.*[48]

Comparing the total phenolic composition of pineapple crown and peel extracts, the highest content was detected in the crown with 4.7 ± 0.1 mg g^{-1} of extract, with predominance of flavonoids. In contrast, the peel only yielded 2.48 ± 0.03 mg g^{-1} of extract, with similar amounts of phenolic acids and flavonoids. The main compounds in the peel extract were (epi) gallocatechin (0.59 ± 0.02 mg g^{-1}) and apigenin-6,8-*C*-diglucoside (0.46 ± 0.01 mg g^{-1}), while apigenin-*O*-malonyl-hexoside and apigenin-6,8-*C*-diglucoside (1.1 ± 0.1 mg g^{-1} and 0.56 ± 0.01 mg g^{-1}, respectively) were predominant in the crown extract. These compounds may be responsible for the bioactivities found since PCs are considered great antioxidants, decreasing the generation of free radicals that attack the cells and maintaining good health.

The present work presented some phenolic compounds that differ slightly from those described in the literature. This is because fruits and vegetables have different pre-harvest and post-harvest treatments that can change the phenolic compound content, which may induce the accumulation or degradation of these compounds.[49] Conditions such as temperature, soil properties, light irradiation, irrigation, fertilizers, harvesting stage, wounding, modified atmosphere, and elicitor treatments, are known to regulate the content of PCs in fruits and vegetables. According to De la Rosa *et al.*,[49] these technologies induce oxidative stress (formation of reactive oxygen species (ROS) in fruits and vegetables), triggering the plants defense system, which involves the synthesis of antioxidant secondary metabolites such as PCs, and the activation of antioxidant enzymes. In contrast, this highlights that obtaining PC from residues and byproducts of the food production chain, specifi-

cally the pineapple, is a cheap natural source of these compounds. It has no economic value, contributing to reducing waste that represents a significant percentage in industries.

3.2. Bioactive properties of pineapple peel and crown extracts

3.2.1. Antioxidant activity. The antioxidant activity of the hydroethanolic extracts obtained from pineapple peel and crown was evaluated through the inhibition of lipid peroxidation using the TBARS and the oxidative haemolysis inhibition (OxHLIA) assays. The results, expressed as IC_{50} values, are presented in Table 2. Considering that the lower IC_{50} value indicates higher antioxidant activity, the extract that revealed the best activity in both assays was pineapple peel, with an IC_{50} of 4.3 ± 0.1 μg mL^{-1} for the TBARS assay and 190 ± 7 μg mL^{-1} for the OxHLIA assay at a Δt of 60 min. For the TBARS assay, the IC_{50} obtained for the peel extract is lower than that presented by Trolox, making these extracts an important source of compounds with antioxidant potential.

According to Le,[50] lipid peroxidation is considered a biomarker for the evaluation of oxidative stress where free radicals such as radical hydroxyl, being the most reactive form of reactive oxygen species (ROS), can initiate lipid peroxidation by attacking polyunsaturated fatty acids. In the work conducted by Selani *et al.*,[51] the treatment with the by-product of pineapple delayed lipid oxidation in raw hamburgers and cooked in relation to other treatments. This fact is probably due the phenolic compounds composition of pineapple peel (3.78 mg gallic acid equivalent per g peel), with antioxidant activity that could have helped protect hamburgers from lipid oxidation.

According to Gómez and Pablos,[27] pineapple peel extract reduces secondary oxidation formation by 45.92%, showing that polyphenols in extracts reduce the formation of secondary oxidation products, despite the presence of high concentration of primary oxidation products. The significant antioxidant capacity of pineapple extracts has been proven by Jovanović *et al.*,[52] where the highest antioxidant activity was detected in pericarp extract prepared with absolute methanol (IC_{50} = 1.74 ± 0.05 mg mL^{-1}) against the lowest, detected in pineapple juice (IC_{50} = 88 ± 2 mg mL^{-1}). Regarding the DPPH radical scavenging activity assay, the highest percentage of inhibition presented by the pineapple by-products was attributed to the crown extract with 100% ethanol ratio (75.57%), followed by the peel (72.67%) and core (49.14%) extracted with 50% ethanol ratio. Based on the results obtained by this study, it shows that the antioxidant activities showed greater inhibition capacity were in samples with a higher percentage of ethanol, concluding that the solubility of metabolites was lower in water compared to ethanol.[53]

3.2.2. Cytotoxic activity. The results obtained for the cytotoxic activity testing four human tumour cell lines and one non-tumour cell line are also listed in Table 2 and are expressed in values of the concentration of extract responsible for inhibiting cell proliferation by 50% (GI_{50}), values in μg mL^{-1}. The lower GI_{50} values represent a higher cytotoxic potential of the tested samples, the extract that most demonstrated no cytotoxicity was the pineapple crown leaves with the GI_{50} values higher than 400 μg mL^{-1} for all tested cancer cell lines. Both extracts did not express cytotoxicity against non-tumour cell line, VERO (GI_{50} > 400 μg mL^{-1}). Sah *et al.*[54] reported other cytotoxic activity against HT29 colon cancer cells, where probiotic yogurt with pineapple peel powder significantly was higher than in nonsupplemented probiotic yogurt. The pineapple peel powder-supplemented probiotic yogurt presented a stronger cytotoxic activity of 56.36% against the nonsupplemented control probiotic yogurt with 40.52% and plain yogurt with 35.71% after 28 days of refrigerated storage.

3.2.3. Antimicrobial activity

3.2.3.1. Antibacterial activity. The antibacterial activity of hydroethanolic extracts obtained from pineapple peel and crown leaves was tested against a panel of six bacteria, including three Gram-positive bacteria (*Staphylococcus aureus, Bacillus cereus* and *Listeria monocytogenes*) and three Gram-negative bacteria (*Escherichia coli*, *Salmonella* Typhimurium and *Enterobacter cloacae*). Table 3 presents the results obtained for each extract in MIC and MBC values.

The results presented showed that the bacteria *E. coli* was the most sensitive bacteria for both tested extracts with a lower MIC value, followed by *S. aureus* and *E. cloacae*. Both analysed extracts showed similar antibacterial performance for all strains tested; however, it is important to highlight the better performance when compared to positive controls (E211 and E224).

Regarding MBC values, it is possible to highlight *E. coli* and *E. cloacae* as the most sensitive bacteria for both pineapple extracts. In this case, it is interesting to note that the performance of the extracts was more efficient than the E211 control and similar to that presented by the E224 control. These positive controls are used as a food preservative in the food indus-

Table 2 Antioxidant and cytotoxic activities of pineapple peel and leaf crown extracts

		Peel extract	Crown extract	Positive control
Antioxidant activity (IC_{50}, μg mL^{-1})				Trolox
TBARS formation inhibition		4.3 ± 0.1*	6.6 ± 0.3	5.4 ± 0.3
OxHLIA	Δt 60 min	190 ± 7*	395 ± 19	21.8 ± 0.3
	Δt 120 min	333 ± 9*	714 ± 33	43.5 ± 0.8
Cytotoxic activity (GI_{50}, μg mL^{-1})				Ellipticine
Tumour cell lines	AGS	>400	>400	0.9 ± 0.1
	CaCo-2	378 ± 7*	>400	0.8 ± 0.1
	MCF-7	322 ± 3*	>400	1.020 ± 0.004
	NCI-H460	>400	>400	1.01 ± 0.01
Non-tumour cell line	VERO	>400	>400	0.6 ± 0.1

GI_{50} values correspond to the concentration that causes 50% inhibition of cell proliferation; AGS – human gastric adenocarcinoma; CaCo-2 – human colon adenocarcinoma MCF-7 – human breast adenocarcinoma; NCI-H460 – human lung carcinoma. An * in each line corresponds to a significant statistical difference with a p-value of 0.05 using a Student's T test.

Table 3 Antimicrobial activity of pineapple peel and crown extracts against foodborne bacterial and fungal strains

	Peel extract		Crown extract		E211		E224	
Antibacterial	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	1.00	2.00	1.00	1.00	4.00	4.00	1.00	1.00
B. cereus	1.00	2.00	1.00	2.00	0.50	0.50	2.00	4.00
L. monocytogenes	2.00	2.00	2.00	4.00	1.00	2.00	0.50	1.00
E. coli	0.50	1.00	0.50	1.00	1.00	2.00	0.50	1.00
S. Typhimurium	1.00	2.00	1.00	2.00	1.00	2.00	1.00	1.00
E. cloacae	1.00	1.00	1.00	1.00	2.00	4.00	0.50	0.50
Antifungal	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
A. fumigatus	0.50	1.00	0.50	0.50	1.00	2.00	1.00	1.00
A. niger	0.25	0.50	0.25	0.50	1.00	2.00	1.00	1.00
A. versicolor	1.00	2.00	0.50	1.00	2.00	2.00	1.00	1.00
P. funiculosum	0.25	0.50	0.25	0.50	1.00	2.00	0.50	0.50
P. aurantiogriseum	2.00	4.00	1.00	2.00	2.00	4.00	1.00	1.00
T. viride	0.25	0.50	0.25	0.50	1.00	2.00	0.50	0.50

MIC: Minimum inhibitory concentration; MIC: Minimum inhibitory concentration (mg mL^{-1}); MBC: Minimum bactericidal concentration (mg mL^{-1}); MFC: Minimum fungicidal concentration (mg mL^{-1}). Sodium sulphite (E221) and potassium metabisulphite (E224) were used as positive controls.

try, applied in most of the fruits and vegetables to extend the shelf life by shielding them against deterioration caused by microorganisms, being effective bleaching agents, antimicrobials, oxygen scavengers, reducing agents, and enzyme inhibitors.[55–57] The work presented by Wijayati *et al.*[58] showed good results for pineapple peel extract against *E. coli* and *S. aureus*, proving to be effective as an antibacterial agent. According to Wijayati *et al.*,[58] the hand sanitizer formulation with pineapple peel extract at concentrations 0.5%, 1% and 1.5% extract produced an inhibition zone against *E. coli* that is 9 mm, 13 mm, and 15 mm, while against *S. aureus* produced inhibition zone of 10 mm, 15 mm and 15.5 mm. In conclusion, the higher the concentration of extract added, the greater the zone will be.

In a study developed by Goudarzi *et al.*,[59] pineapple peel extract presented the strongest antibacterial activity with highest inhibition zones (30 and 28 mm) and the lowest MIC (1.56 and 6.26 mg mL^{-1}) against *Streptococcus sanguinis* and *S. mutans*, respectively. The hydroethanolic extracts of pineapple peel showed better results for the minimum bactericidal concentration for both bacteria (3.12 mg mL^{-1} for *S. sanguinis*; 12.5 mg mL^{-1} for *S. mutans*) against, 25 mg mL^{-1} (*S. sanguinis*) and 100 mg mL^{-1} (*S. mutans*) for the pulp. This also was confirmed by Ogwu *et al.*[60] and Okoh *et al.*,[61] where the peel extracts revealed stronger antibacterial activity than the pulp against *E. coli*, *S. aureus*, *Streptococcus faecalis* and *Pseudomonas aeruginosa*.

In the work conducted by Punbusayakul,[62] the antibacterial activity of the pineapple peel was tested against four foodborne pathogens, and the results revealed that *B. cereus* exhibited the most sensitive strain with a minimum inhibition concentration (MIC) of 0.0675 g mL^{-1}, followed by *S. aureus* and *E. coli* (0.1349 g mL^{-1}) and *S.* Typhimurium (0.2699 g mL^{-1}), respectively. Regarding pineapple crown leaves, Brito *et al.*[41] reported that the essential oil of pineapple-crown flour showed to be active against *B. cereus, E. coli* and *L. monocytogenes*. The same result obtained by this study, except for *L. monocytogenes* which demonstrated higher or similar indices of MIC and MBC in relation to positive control. In the research presented by Dutta & Bhattacharyya,[63] it was proved that the crown leaves extract possessed bacteriostatic and fungistatic components, exhibiting 70–95% inhibition of microbial growth with a minimum inhibitory concentration range of 1.65–4.95 mg mL^{-1} against *Saccharomyces cerevisiae*, *Escherichia coli*, *Bacillus subtilis*, *Candida albicans* and *Staphylococcus aureus*.

According to Zharfan & Mustika,[64] previous studies suggested that the bioactive compounds that act against Gram-negative bacteria are majorly bromelain and saponin, while flavonoids and polyphenols are more potent in inhibiting Gram-positive bacteria. In the case of flavonoids and polyphenols, they work mainly in the peptidoglycan layer in Gram-positive bacteria by having polar properties.[65]

The bromelain is a proteolytic enzyme, and it is suggested that operate in weakening of the outer membrane of Gram-negative bacteria by proteins, thus leading to cellular damage.[64] On the other hand, saponin causes a change in membrane structure and function, increasing the permeability of the bacterial cell membrane, thus allowing antibacterial substances to easily enter cells, causing an interference in cellular metabolism while denaturing proteins in the membrane so that it is disintegrated.[66]

3.2.3.2. Antifungal activity. The antifungal activity of the hydroethanolic extracts obtained from pineapple peel and crown leaves was tested against a panel of six fungi (*Aspergillus fumigatus*, *Aspergillus niger, Aspergillus versicolor*, *Penicillium funiculosum, Penicillium aurantiogriseum* and *Trichoderma viride*), and the results expressed in MIC and MFC values, being presented in Table 3.

The results obtained demonstrate that pineapple hydroethanolic extracts presented antifungal activity against the six tested fungi. The results presented showed that *A. niger*, *P. funicolosum* and *T. viride* were the most sensitive fungi for

both extracts analysed with lower MIC values. It was even possible to verify that, for most of the fungi tested, the extracts showed better antifungal performance than the used controls (E211 and E224).

A study developed by Olakunle *et al.*,[67] compared the antifungal activity of four fruit peels (banana, pineapple, cashew, and orange) and the results showed a greater antifungal activity of pineapple peel when tested against *A. niger* and *Alternaria alternata*. In turn, Chanda *et al.*[68] intended to verify the influence of different solvents on the antimicrobial capacity of extracts, demonstrating that polar solvents (acetone and methanol) are more effective than nonpolar solvents (hexane and chloroform). In this study methanol extracts of *A. comosus* showed the best results against *Candida albicans*, *C. glabrata*, *Cryptococcus luteoluscom* and *C. tropicalis* with inhibition zones of 12, 11.5, 10.5, and 9.5 mm, respectively. As for nonpolar solvents, only chloroform showed activity against pathogenic fungi.

3.3. Characterization of the developed "súplicas"

3.3.1. Proximate composition and energy. For a better interpretation of the results, the following tables result of a two-way ANOVA, being divided in two sections, the upper represents the incorporation (control and pineapple peel) (*I*) but, included in the standard deviation of each incorporation are both the three storage times (0, 3 and 7 days), and, in the bottom section for each storage time (ST) both incorporations are included. Using this statistical tool, each factor can be analysed independently. When *p*-value $I \times ST > 0.05$, a *post-hoc* classification was used, namely student's *t*-test for *I* and Tukey's test for ST. However, when *p*-value $I \times ST < 0.05$, then, a significant interaction between both factors hinders an independent classification, and thus, for some cases, only general trends can be obtained through the estimated marginal means plots (EMM).

Table 4 shows the influence of incorporation (*I*) and shelf life (ST) in each of the nutritional components analysed in the "súplicas". Carbohydrates were the macronutrients that stood out as the main macronutrient in "súplicas", followed by proteins. Regarding free sugars, only sucrose was detected in the samples, which would be expected considering that sugar was one of the ingredients used in making the cakes. Overall, for all components except moisture, proteins and sucrose, a significant interaction was found for ST and *I*, and thus no *post-hoc* classification could be performed. For moisture, the effect of ST did not induce statistically significant changes, although the incorporation did induce significant changes, revealing a higher amount of moisture in the "súplicas" with pineapple peel, meaning that the peel was responsible for increasing the moisture content in those samples. Regarding proteins, once again the pineapple peel was responsible for a statistically significant increase when compared to the control samples. Once again, ST did not show statistical influence on the protein fraction. Regarding sucrose, the incorporation of the pineapple peel did not show any significant effect, but the passage of time did show a significant statistical decrease in sucrose from

Table 4 Nutritional composition, colour parameters, and antioxidant activity of control and pineapple peel extract functionalized "súplicas". The effects of extract incorporation (I) and storage time (ST) on these quality parameters are demonstrated

	Incorporation (*I*)		Student's *t*-test	Storage time (ST)			Tukey's test	ST × *I*
	Control	Functionalized	*p*-Value ($n = 9$)	0 Days	3 Days	7 Days	*p*-Value ($n = 18$)	*p*-Value ($n = 27$)
Moisture (g per 100 g fw)	6.5 ± 0.2*	7.4 ± 0.2	<0.001	7.0 ± 0.2	6.9 ± 0.5	7.0 ± 0.7	0.089	0.154
Fat (g per 100 g fw)	4.13 ± 0.06	3.56 ± 0.04	<0.001	3.8 ± 0.3	3.9 ± 0.3	3.8 ± 0.3	0.004	0.029
Proteins (g per 100 g fw)	7.80 ± 0.07*	7.90 ± 0.01	0.005	7.92 ± 0.07	8.0 ± 0.1	7.92 ± 0.07	0.050	0.152
Ash (g per 100 g fw)	0.34 ± 0.01	0.36 ± 0.01	<0.001	0.37 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	<0.001	<0.001
Carbohydrates (g per 100 g fw)	80.9 ± 0.2	80.7 ± 0.2	<0.001	80.8 ± 0.1	80.8 ± 0.3	80.8 ± 0.4	0.897	<0.001
Energy (kcal)	393 ± 1	386 ± 1	<0.001	389 ± 2	390 ± 3	390 ± 4	<0.001	<0.001
Energy (kJ)	1645 ± 4	1618 ± 4	<0.001	1630 ± 9	1633 ± 14	1631 ± 19	<0.001	<0.001
Sucrose (g per 100 g fw)	86 ± 13	85 ± 3	0.708	91 ± 2[b]	82 ± 3[a]	82 ± 3[a]	0.003	0.102
C16:0 (relative %)	20 ± 2	23.6 ± 0.4	<0.001	22 ± 2	21 ± 3	22.7 ± 0.6	<0.001	<0.001
C16:1 (relative %)	2.5 ± 0.2	2.96 ± 0.08	<0.001	2.7 ± 0.3	2.6 ± 0.3	2.8 ± 0.2	<0.001	<0.001
C18:1t (relative %)	5.8 ± 0.3	6.3 ± 0.1	<0.001	6.0 ± 0.2	5.9 ± 0.6	6.22 ± 0.08	<0.001	<0.001
C18:1c (relative %)	49 ± 3	44.0 ± 0.7	<0.001	46 ± 3	48 ± 4	45.5 ± 0.9	<0.001	<0.001
C18:2 (relative %)	19.3 ± 0.5	20.0 ± 0.2	<0.001	19.8 ± 0.4	19.4 ± 0.8	19.8 ± 0.2	<0.001	<0.001
SFA (relative %)	21 ± 2	24.0 ± 0.4	<0.001	22 ± 2	21 ± 3	23.1 ± 0.7	<0.001	<0.001
MUFA (relative %)	58 ± 2	53.5 ± 0.7	<0.001	55 ± 2	57 ± 4	54.8 ± 0.7	<0.001	<0.001
PUFA (relative %)	21.3 ± 0.8	22.4 ± 0.3	<0.001	22.0 ± 0.6	21 ± 1	22.1 ± 0.2	<0.001	<0.001
L^* (lightness)	80.4 ± 0.5	80.9 ± 0.6	<0.001	80.9 ± 0.6	80.2 ± 0.6	80.7 ± 0.3	<0.001	0.009
a^* (redness)	1.4 ± 0.2	1.5 ± 0.2	0.196	1.4 ± 0.1	1.4 ± 0.1	1.6 ± 0.2	<0.001	0.040
b^* (yellowness)	32 ± 1	31.7 ± 0.5	<0.001	32.5 ± 0.9	32.4 ± 0.7	31.0 ± 0.5	<0.001	<0.001
DPPH scavenging (EC_{50}, mg mL^{-1})	>400*	134 ± 53	<0.001	237 ± 173	278 ± 125	292 ± 111	<0.001	0.101
Reducing power (EC_{50}, mg mL^{-1})	17 ± 2	20 ± 4	<0.001	17 ± 2	19 ± 4	20 ± 4	<0.001	<0.001

In each row, for *I* and ST, an asterisk (*) or different letters mean significant statistical differences (*p*-value <0.05) between samples, respectively. The presented standard deviations were calculated from results obtained under different operational conditions. Therefore, these values should not be regarded as a measure of precision, rather as the range of the recorded values.

T0 to T3 days, probably due to the breakdown of sucrose into fructose and glucose.

A study developed by Oliveira[69] showed a higher moisture content in the cake incorporated with 80% of pineapple peel flour and 20% of core (27.10%) than in cake with pulp flour (24.38%), this being justified due to the high fibre contents in the peel and core that together preserve the water during the supply in its structure. Likewise, the protein content was also higher in the cake with the pineapple by-products (5.51%) when compared to the cake prepared with pulp flour (5.43%). Silva *et al.*[70] reported a higher moisture content with 25.13% in muffins with pineapple peel, being above the stipulated standard, which should be a maximum of 14%. One of the reasons that may explain the maintenance of moisture in the "súplicas" in storage time, would be that glucose and fructose can absorb more water than sucrose (non-reducing sugars), by breaking the molecules over time.[69] In relation to proteins, Adeoye *et al.*[71] showed the highest protein value in cookies contained 50 and 40% of pineapple peel flour (2.45%) comparing with traditional cookies (2.19%). In the work presented by de Toledo *et al.*,[72] the protein values were higher than this study, containing 8.14% in cookies with 5% of pineapple central axis.

Ash content is considered an important parameter to be analysed in foods, as it is related to the minerals presented in the food composition.[72] Damasceno *et al.*[73] reported a significant difference in ash content when comparing cereals bars containing 3% of pineapple peel flour (3.19%) when compared to traditional bars (2.81%). In turn, Oliveira[69] highlighted a higher ash content in cakes prepared with pineapple peel flour (4.41%) when compared to cakes prepared with pulp flour (2.66%) or with central part of the fruit (2.42%).

Regarding the crude fat content, a study by Reis Junior[74] showed a significant difference in the reduction of lipids in hamburgers containing pineapple peel flour at concentrations of 5, 10, 15, 20 and 25% when compared to the control, concluding that the reduction in lipids was proportional to the addition of flour. Oliveira[75] obtained similar results and conclude that pineapple peel flour could be considered a healthy ingredient that improves the nutritional aspect of hamburgers.

3.3.2. Composition in fatty acids. Table 4 shows the effect of incorporation and storage time on the fatty acid content of the "súplicas". Eleven individual fatty acids were identified and in Table 4 only the majority are presented (with representation greater than 1%). Oleic acid (C18:1) was the most abundant individual fatty acid, followed by palmitic acid (C16:0). Monounsaturated fatty acids (MUFA) prevailed followed by saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) with very similar percentages. For all fatty acids a significant interaction was sought and thus no *post-hoc* classification was performed. Still, some general tendencies can be extracted from the EMM plots, shown in Fig. 1. For C18:1 (Fig. 1A), the amount of oleic acid in the control samples was overall higher than the samples with pineapple peel, probably due to the degradation of fats by enzymes present in the peel. Still, over the first three days there is an increase for the control sample that then decreases from T3 to T7, while all the amount in the incorporated samples remains mostly constant during the whole seven days. Fig. 1B shows the EMM for SFA, revealing that pineapple peel samples showed higher SFA amounts, which gradually decreased over time while the control sample increased from T0 to T3 and then decreased from T3 to T7.

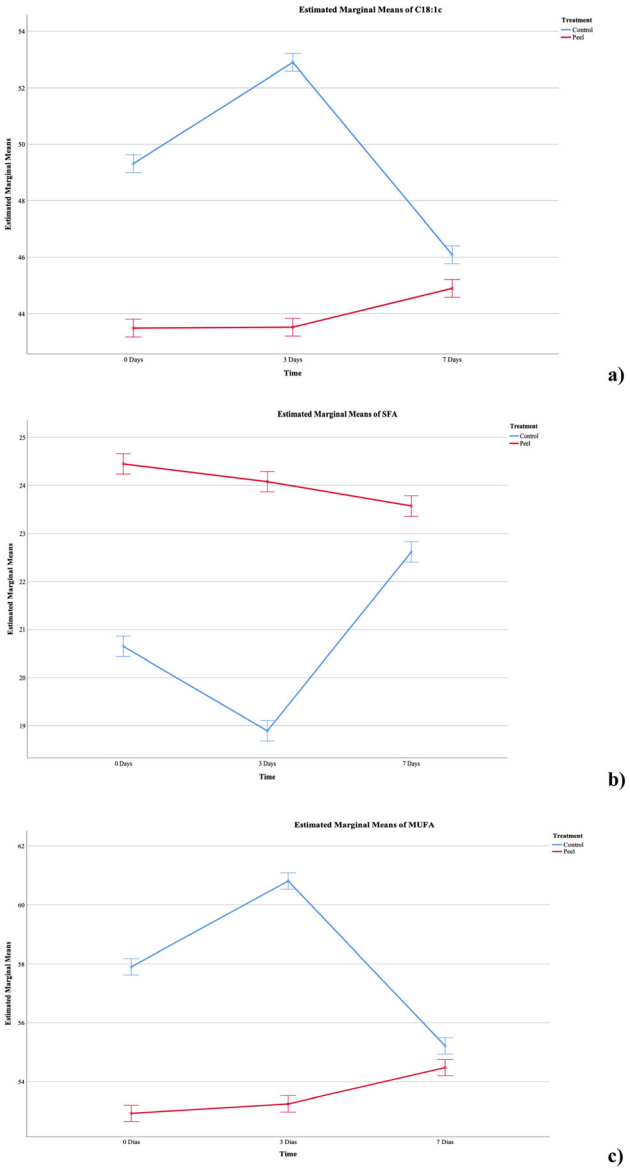

Fig. 1 EMM plots of (a) C18:1, (b) SFA and (c) MUFA.

This variation could be due to oxidation of fats in the control samples which did not have any compounds to protect them (contrarily to the samples with pineapple peel), and thus they decreased from T0 to T3. Then from T3 to T7, the increase could be explained by the degradation of MUFA to SFA by oxidation. Finally, this phenomenon is also reported in Fig. 1C where there is a very high variation of MUFA over time in the control samples, while the samples with pineapple peel show a

slight decreasing tendency. The control samples increased from T0 to T3 probably due to the degradation of PUFA to MUFA and then decrease from T3 to T7, due to the breakdown of MUFA to SFA.

Consisting of fatty acids, lipids are important components in the food industry because they confer desirable sensory characteristics to food, where the conversion of long-chain fatty acids into short-chain organic fatty acids, give unpleasant flavour and odour to products.[70,75] Fats can affect the food texture by forming structures of crystalline networks and by the disruption of structure by interfering with non-fat networks.[75,76] In baked products, fat has numerous functions being responsible for the improve gas retention, lubrication, aeration, heat transfer in dough, and desirable texture in the final product (in breads), incorporation of air, softness, lubrication, mouthfeel, and structural and sensory properties (in cakes and biscuits).[76]

3.3.3. Colour and antioxidant activity. Table 4 shows the results of the effect of extract incorporation and shelf life on antioxidant potential (through DPPH and RP assays) and on colour parameters (defined by CIE Lab coordinates of L^*, a^* and b^*) of the "súplicas". Regarding the antioxidant activity analysis, there was a significant interaction for RP but for DPPH, the pineapple peel did seem to statistically improve the antioxidant activity of the "súplicas" reducing the EC_{50} from over 400 mg mL^{-1} to 134. ST did not show any effects on the antioxidant activity of the snacks, probably due to a very low amount of moisture.

For the colour parameter b^*, the coordinate that defines blue as negative and yellow values for positive values, where, over storage time, a decrease in yellow colour was evidenced in the samples and, for the seventh day, there was an increase in the red colour (a^*) in the "súplicas". Colour is an important feature of pastry products, not only by consumer preference, but also, depends on the physical–chemical characteristics of the mass and processes such as sugars, amino acid content, oven temperature, relative humidity, and pH.[77]

In the work carried out by Oliveira,[69] values of the colour parameters in cakes prepared with peel flour and core pineapple were higher than those presented by cakes prepared with pulp flour. This can be justified due to the high amount of reducing sugars present in the pulp flour when compared to the peel and core. The effects of colour parameters L^*, ($+a^*$) and ($+b^*$) caused differences in behaviour in the cakes during storage possibly as a function of the water content as well as in this work, which could be justified by the association with the levels of sugars present in the flours, which enable greater water absorption and, therefore, the intensification or restriction of colour.[69]

In the research conducted by Silva *et al.*,[70] the muffins prepared with pineapple peel by-products showed a low lightness (38.77) and the yellow intensity (30.87) was out of the intensity of red (11.00), consequently a low chroma index (32.77), meaning that the chroma closer the material is to the gray colour. Adeoye *et al.*[71] reported that the pineapple peel flour gave smooth texture to cookies, but brown colour was imparted, and the colour gets darker as the levels of pineapple peel flour increases. However, in food formulations it is desirable that flour be lighter because flours with dark colours may limit possible food applications.[78] For this reason and all parameters evaluated, the biscuits made from wheat–pineapple (90 : 10) peel flour were indicated having the highest mean scores and were close to 100% wheat cookies.[71]

In relation to the antioxidant analysis, one of the objectives of this work was to add value to a pastry product that normally has no functionality, being able to transfer to it, bioactive compounds of the by-products that present antioxidant activity. The same proposal was done by Toledo *et al.*,[79] where the use of fruit by-products (pineapple central axis, apple endocarp, and melon peels) in the formulation of cookies contributed to an increase of more than 100% in the phenolic compound's contents from 7.80 ± 0.13 up to 16.91 ± 0.19 mg of gallic acid equivalents per g of sample, being the cookies made with apple by-product the most promising formulation. For the DPPH assays, the pineapple central axis values showed great capacity of antioxidant activity, being 7.14 ± 0.34 μmol of Trolox equivalents (TE) per g of sample against 3.94 ± 0.70 μmol TE per g for the cookies control. The ABTS assays also reinforced the promising results, with 7.24 ± 0.16 μmol TE per g for the pineapple by-product and 5.39 ± 0.33μmol TE per g for the control.

Also, Sah *et al.*[80] reported stronger antimutagenic and antioxidant activities (evaluating the reducing power and scavenging capacity of DPPH; 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), and hydroxyl radicals), in crude water-soluble peptide extract of the probiotic yogurt with pineapple peel than control during storage time. All yogurt samples exhibited varying degrees of reducing power, scavenging capacities for DPPH, ABTS, and hydroxyl radicals. On the first day, the sample containing pineapple peel showed a reduction power of 0.39 *versus* 0.36 compared to the control and, for the last day (28th), 0.58 against 0.48 for the control only with starter culture (*Streptococcus thermophilus* + *Lactobacillus bulgaricus*). For the DPPH analysis expressed great results in the first day with the probiotic culture (*Lactobacillus acidophilus* + *Lactobacillus casei* + *Lactobacillus paracasei*), being 43.90 for the pineapple peel against 36.96 evaluated in the control. The results showed that pineapple peel could be used as a prebiotic ingredient in the manufacture of yogurts that would improve food nutrition and functionality.[80]

The work conducted by Barros *et al.*,[81] presented significant values for antioxidant activity in cookies prepared with pineapple peel through ferric reducing antioxidant power assay, where the activity was proportional to the increase in the concentration of the peel flour.

4. Conclusions

In the characterization of pineapple peel and crown leaves, twenty-five phenolic compounds were identified in both extracts divided into phenolic acids and flavonoids. The main

detected compounds were caffeic acid derivatives (namely caffeic acid-*O*-hexoside) and flavones (such as apigenin 6,8-*C*-diglucoside). Eleven phenolic acids and two flavonoids were identified in pineapple peel while on the crown leaves were identified twelve phenolic, nine flavonoids and two phenolic acids. None of the extracts showed toxicity and both showed antioxidant, cytotoxic and antimicrobial potential, with the pineapple peel extract standing out with the best results.

Due to its greater bioactive potential, the pineapple peel extract was selected to incorporate in a typical pastry product from the northeast of Portugal, the "súplicas" in order to study the potential of this residue as a natural ingredient. Thus, the effects of the incorporation of the extract on colour, nutritional profile, content in sugars and fatty acids, as well as the antioxidant activity were evaluated over the shelf life and by comparison with the traditional product. The results obtained show that no significant changes in the profile, only changes in proteins and moisture where, it can be explained by the high fibre content in the peel that preserved that water during the supply in its structure and the breakdown of the molecules in glucose and fructose that can absorb more water than sucrose. In fatty acids the alterations were little noticeable, but the extract allowed reducing the variations between PUFA, MUFA and SFA. At the colour level, the variations were not significant and, as expected, the extract was able to statistically improve the antioxidant activity of the "súplicas" proving that pineapple peel extract can be used in the food industry as a functionalizing agent with antioxidant activity without modifying the nutritional profile, colour, and composition of fatty acids in food.

With the results obtained by this research, we can conclude that many fruit and vegetable by-products have valuable compounds for human health and that could be used to remove them from the environment to reduce the damage caused, adding value and return these wastes back into the economy. The incentive to research, through the dissemination of scientific work in the area, becomes extremely important and can give these wastes that would be discarded, a nutritional and market value, causing an increase in these foods and a greater generation of jobs for families that survive from agriculture.

Conflicts of interest

There are no conflicts to declare.

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