Original

Correlation, by multivariate statistical analysis, between the scavenging capacity against reactive oxygen species and the bioactive compounds from frozen fruit pulps[3]

Correlação, por análise estatística multivariada, entre desativação de espécies reativas de oxigênio e compostos bioativos de polpas de frutas congeladas

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Abstract

The contents of total phenolic compounds (TPC), total flavonoids (TF), and ascorbic acid (AA) of 18 frozen fruit pulps and their scavenging capacities against peroxyl radical ($ROO^{\bullet}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) were determined. Principal Component Analysis (PCA) showed that TPC (total phenolic compounds) and AA (ascorbic acid) presented positive correlation with the scavenging capacity against $ROO^{\bullet}$, and TF (total flavonoids) showed positive correlation with the scavenging capacity against $^{\bullet}OH$ and $ROO^{\bullet}$ However, the scavenging capacity against H_2O_2 presented low correlation with TF (total flavonoids), TPC (total phenolic compounds), and AA (ascorbic acid). The Hierarchical Cluster Analysis (HCA) allowed the classification of the fruit pulps into three groups: one group was formed by the açai pulp with high TF, total flavonoids, content (134.02 mg CE/100 g pulp) and the highest scavenging capacity against $ROO^{\bullet}$, $^{\bullet}OH$ and H_2O_2; the second group was formed by the acerola pulp with high TPC, total phenolic compounds, (658.40 mg GAE/100 g pulp) and AA , ascorbic acid, (506.27 mg/100 g pulp) contents; and the third group was formed by pineapple, cacao, caja, cashew-apple, coconut, cupuaçu, guava, orange, lemon, mango, passion fruit, watermelon, pitanga, tamarind, tangerine, and umbu pulps, which could not be separated considering only the contents of bioactive compounds and the scavenging properties.

Keywords: *bioactive compounds; antioxidant capacity; fruit pulp.*

Resumo

Os teores de compostos fenólicos totais (CFT), flavonoides totais (FT) e ácido ascórbico (AA) foram determinados em 18 polpas de frutas congeladas, assim como a capacidade antioxidante na desativação do radical peroxila ($ROO^{\bullet}$), peróxido de hidrogênio (H_2O_2) e radical hidroxila ($^{\bullet}OH$). De acordo com a Análise de Componentes Principais (ACP), CFT e AA apresentaram correlação positiva com a capacidade de desativar o $ROO^{\bullet}$ e o teor de FT apresentou correlação positiva com a capacidade de desativar o $^{\bullet}OH$ e $ROO^{\bullet}$. Entretanto, a capacidade de desativar o H_2O_2 apresentou baixa correlação com FT, CFT e AA. A Análise Hierárquica de Agrupamentos (HCA) permitiu a separação das polpas de frutas em três grupos: o primeiro formado pela polpa de açaí, com elevado teor de FT (134,02 mg EC/100 g polpa) e maior desativação do $ROO^{\bullet}$, $^{\bullet}OH$ e H_2O_2; o segundo pela polpa de acerola, com elevado teor de CFT (658,40 mg EAG/100 g polpa) e de AA (506,27 mg/100 g polpa); e o terceiro grupo formado pelas polpas de abacaxi, cacau, cajá, caju, coco, cupuaçu, goiaba, laranja, limão, manga, maracujá, melancia, pitanga, tamarindo, tangerina e umbu, que não puderam ser separadas considerando apenas os teores dos compostos bioativos e a propriedades antioxidantes.

Palavras-chave: *compostos bioativos; capacidade antioxidante; polpa de fruta.*

1 Introduction

Results of epidemiological studies showed an inverse association between a diet rich in fruits and vegetables and the decreased risk of developing chronic degenerative diseases. This relationship is attributed to the presence of bioactive compounds, including phenolic compounds, carotenoids, and vitamins C and E (GARCÍA-ALONSO et al., 2004). These compounds are able to protect biomolecules, such as lipids and proteins, against the oxidative damage induced by reactive oxygen (ROS) and nitrogen species (RNS).

In most of the studies, the antioxidant capacity of fruits is determined using methods based on the deactivation of non-biological radicals, such as 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical ($ABTS^{\bullet+}$) and 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) (KUSKOSKI et al., 2006; MELO et al., 2008a, b; SANTOS et al., 2008, 2010; BARRETO; BENASSI; MERCADANTE, 2009; ROP et al., 2010; CONTRERAS-CALDERÓN et al., 2011; MEDINA et al., 2011; FU et al., 2011), which are not present in the biological system and have different properties as compared to the ROS produced in the human body. There are some data available in the literature concerning the scavenging capacity of fruit pulps against peroxyl radicals ($ROO^{\bullet}$) (WANG; CAO;

Received 2/8/2012
Accepted 4/10/2012 (00Q5812)
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PRIOR, 1996; WU et al., 2004); however, there is no information regarding the scavenging capacity of frozen fruit pulps against other ROS with physiological importance, such as hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$).

Brazil is the third largest producer of fruits in the world. In 2010, the production reached over 42 million tons (KIST et al., 2012), from which about 53% were destined for processing and 47% for fresh consumption. Currently, the Brazilian production is focused on tropical, subtropical, and temperate fruits due to the large territorial extension of the country, as well as the soil and favourable climatic conditions (XEYLA, 2009). Fruits are perishable; consequently they deteriorate quickly, within a few days, making their *in natura* trading over long distances difficult. Therefore, the production of frozen fruit pulps has become an option to reap the benefits of fruits, to facilitate trading them in places where there is no production, and to preserve them for a longer period after harvest. Therefore, the contents of total phenolic compounds, total flavonoids, and ascorbic acid of 18 frozen fruit pulps were determined, and these values were correlated applying multivariate statistical analysis to the antioxidant capacity against the oxidizing effects of $ROO^{\bullet}$, H_2O_2 and $^{\bullet}OH$.

2 Materials and methods

2.1 Chemicals

Folin-Ciocalteu reagent, catechin (96.0%), gallic acid (98.0%), AAPH (α,α'-azodiisobutyramidinedihydrochloride), ascorbic acid (99.0%), lucigenin, fluorescein, luminol, and Tris-(hydroxymethyl) aminomethane were purchased from Sigma-Aldrich (Steinheim, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (99.5%) was purchased from Fluka ChemieGmbH (Steinheim, Germany). Monosodium phosphate (NaH_2PO_4), disodium phosphate (Na_2HPO_4), hydrogen peroxide (H_2O_2), aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), sodium nitrite ($NaNO_2$), and DMSO (dimethyl sulfoxide) were purchased from Synth (São Paulo, Brazil). DCFI (2-6-dichlorophenolindophenol) was purchased from Nuclear (São Paulo, Brazil) and sodium carbonate (Na_2CO_3) from Ecibra (São Paulo, Brazil).

A microplate reader (Synergy Mx, Biotek, Vermont, USA) for fluorescence, UV-vis and, chemiluminescence measurements, equipped with a thermostat, was used for all the assays. All measurements were performed in triplicate.

2.2 Material and fruit pulp preparation

Two batches of each the frozen fruit pulp (100 g) (Table 1) were acquired from the same manufacturer in Campinas, São Paulo (Brazil) and stored at –80 °C. The samples were prepared by mixing four packages of 100 g of fruit pulp from the same batch, followed by homogenisation with water in the proportion of 100 g of pulp and 250 mL of ultrapure water (1:2.5, w/v) in a domestic blender (according to the manufacturer instructions), and centrifuging at 6450 *g* for 20 minutes at 20 °C. The supernatant (aqueous extract) was used to analyze both the bioactive compounds and antioxidant capacity.

2.3 Bioactive compounds determination

Total phenolic compounds

The content of total phenolic compounds (TPC) of each frozen fruit pulp was determined using the Folin-Ciocalteu colorimetric method, according to Singleton and Rossi (1965) adapted to microplate reader. The reaction mixture (300 µL) contained the Folin-Ciocalteu reagent, the fruit pulp extracts at different concentrations (1 to 83 mg/L), and Na_2CO_3 (7%). The quantification was performed at 765 nm from an analytical curve

Table 1. Common and scientific names, fruit origin, and composition of the frozen fruit pulps analyzed, commercialized in Brazil.

Common name	Scientific name	Fruit origin	Composition
Açai	*Euterpe oleracea* Mart	North Brazil	açai and water
Acerola	*Malpighia emarginata* DC	West Indies	whole acerola
Cacao	*Theobroma cacao* L.	Amazon	whole cacao
Caja	*Spondias lutea* L.	North and Northeast Brazil	whole caja
Cashew-apple	*Anacardium occidentale* L.	North eastern coast	whole cashew-apple
Coconut	*Cocus nucifera* L.	Archipelagos of the Pacific Ocean	whole coconut
Cupuaçu	*Theobroma grandiflorum* Schum	Amazon Forest	whole cupuaçu
Guava	*Psidiumgua java* L.	Central America	whole guava
Lemon	*Citrus* spp.	Southeast Asia	whole lemon
Mango	*Mangifera indica* L.	India	whole mango
Orange	*Citrussinensis* Osbeck	SoutheastAsia	whole orange
Passionfruit	*Passiflora edulis* Sims	Northeast Brazil	whole passion fruit
Pineapple	*Ananas comosus* (*L.*) Merrill	Central America/Northeast Brazil	whole pineapple
Pitanga	*Eugenia uniflora*L.	Brazil	whole pitanga
Tamarind	*Tamarindus indica* L.	India	whole tamarind
Tangerine	*Citrus reticulata* Blanco	Southeast Asia	whole tangerine
Umbu	*Spondias tuberosa* Arr.Cam	Northeast Brazil	whole umbu
Watermelon	*Citrullus vulgaris* Schrad	India	whole watermelon

(measurements in duplicate) using gallic acid as a standard with concentrations ranging from 12.5 to 150 mg/L. The analytical curve was linear ($r^2 = 0.997$), the limit of detection was 8 mg/L, and the limit of quantification was 25 mg/L. The results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of pulp. The contribution of AA to the Folin-Ciocalteau reaction was not evaluated in the present study.

Total flavonoids

The quantification of total flavonoids (TF) was carried out using the colorimetric assay described by Zhishen, Mengcheng and Jianming (1999), with adaptations for the microplate reader. The reaction mixture (300 µL) contained at final concentrations the fruit pulp extracts at different concentrations (1.2 to 100 mg/L), $NaNO_2$ (5%), $AlCl_3$ (10%), and NaOH (1 M). The quantification was performed at 510 nm from an analytical curve (measurements in duplicate) using catechin as a standard with concentrations ranging from 12.5 to 200 mg/L. The analytical curve was linear ($r^2 = 0.998$), the limit of detection was 10 mg/L, and the limit of quantification was 30 mg/L. The results were expressed as milligrams of catechin equivalent (CE) per 100 g of fruit pulp.

Ascorbic acid

The ascorbic acid (AA) contents were determined using the Tillman's method (INSTITUTO..., 2004). An oxalic acid solution (1%) was added (50 mL) to the fruit pulp extract and kept in the dark for 15 minutes. The quantification was performed by titration with 0.2% DCFI solution and compared to the AA standard titration (50 mg/100 mL). The results were expressed as milligrams of ascorbic acid (AA) per 100 g of fruit pulp.

2.4 Antioxidant capacity against reactive oxygen species (ROS)

The assays were carried out using a microplate reader (Synergy Mx, BioTek, Vermont, USA) for fluorescence, UV-vis, and luminescence measurements, equipped with a thermostat set at 37 °C and dual reagent dispenser. The results of H_2O_2 and •OH were expressed as IC_{50} (µg/mL), *i.e.*, concentration necessary to inhibit oxidation by 50%, calculated using non-linear regression analysis and the GraphPad Prism 5 software.

Peroxyl radical (ROO•) scavenging assay

The ROO• scavenging capacity was determined by monitoring the decay of fluorescence of fluorescein as result of ROO•–induced oxidation of fluorescein (HUANG et al., 2002). The ROO• species were generated by thermo-decomposition of AAPH at 37 °C. The reaction mixtures in the sample wells contained the following reagents at the indicated final concentrations (final volume of 200 µL): AAPH (19 mM), fluorescein (61.2 mM), and the extract of fruit pulp at different concentrations (6.3 to 125 mg/L) and diluted in phosphate buffer (75 mM, pH 7.4). The fluorescence signal was monitored every minute using excitation and emission wavelengths at 485 nm 528 nm, respectively, for 60 minutes. Trolox was used as positive control (Net area (2 µM) = 7.4 ± 0.9; Net area (4 µM) = 13.1 ± 1.1, and Net area (8 µM) = 22.6 ± 1.4) (RODRIGUES et al., 2012). The results were expressed as µmol trolox equivalent and were obtained from the analytical curve of trolox with concentration varying from 12 to 94 µM.

Hydrogen peroxide (H_2O_2) scavenging assay

The H_2O_2 scavenging capacity was measured by monitoring the effect of the extracts of fruit pulps on the H_2O_2-induced oxidation of lucigenin (GOMES et al., 2007). Reaction mixtures contained the following reagents at final concentrations (final volume of 250 µL): 50 mM Tris–HCl buffer pH 7.4, lucigenin (0.8 mM) dissolved in the buffer solution, extract of fruit pulps at different concentrations (10 to 200 mg/L) and completely dissolved in Tris-HCl buffer, and 1% H_2O_2. The assays were performed at 37 °C. The chemiluminescence signal was detected using the microplate reader after 10 minutes of incubation. Ascorbic acid was used as the positive control (IC_{50} = 171 µg/mL) (RODRIGUES et al., 2012).

Hydroxyl radical (•OH) assay

The •OH scavenging capacity was measured by monitoring the effect of the extracts of fruit pulps on the •OH-induced oxidation of luminol (GOMES et al., 2006). The •OH was generated by the Fenton reaction ($FeCl_2$-EDTA-H_2O_2). The reaction mixtures contained the following reagents at final concentrations (final volume of 250 µL): luminol (20 µM), extracts of fruit pulps in different concentrations (5 to 100 mg/L), diluted in carbonate buffer (pH 10), $FeCl_2$ – EDTA (25 µM and 100 µM, respectively), and H_2O_2 (3.5 µM). The assays were performed at 37 °C. The chemiluminescence signal was detected using the microplate reader after 5 minutes of incubation. Gallic acid was used as the positive control (IC_{50} = 0.11 µg/mL) (RODRIGUES et al., 2012).

2.5 Statistical analysis

The contents of bioactive compounds and antioxidant capacity values obtained (mean ± standard deviation) were analyzed using the Statistica 6.0 software (Statsoft Inc., 2001). Two multivariate exploratory techniques, Principal Components Analysis (PCA) and Hierarchical Cluster Analysis (HCA), were applied to correlate and classify the extracts of frozen fruit pulps using the Statistica 6.0 software. For PCA, the bioactive compounds (total phenolic compounds, total flavonoids, and ascorbic acid) and the scavenging capacity against the reactive oxygen species (ROO•, H_2O_2 and •OH) were used as variables in the derivation of the principal components. The HCA hierarchical tree was obtained considering the same variables of PCA, and the extracts were grouped by unweighted pair-group average as the linkage rule considering the Euclidian distance as the coefficient of similarity.

In order to successfully perform the PCA and HCA, some considerations were applied. When ascorbic acid was not detected in the extracts of fruit pulps (açai, cacao, watermelon,

and umbu), the value was considered as 0 (zero). When the IC_{50} value was not reached at the highest tested concentration in the H_2O_2 scavenging assay, the IC_{50} values were estimated after fitting the curves of concentration *versus* inhibition percentage, as did for coconut and watermelon. In addition, the IC_{50} values obtained in the H_2O_2 and $^{\bullet}OH$ scavenging assays were used as the inverse of IC_{50} ($1/IC_{50}$) in the data matrix to facilitate the graphical analysis of PCA and HCA.

3 Results and discussion

3.1 Bioactive compounds in the frozen fruit pulps

A wide variation was found in the contents of: total phenolic compounds, from 6 (watermelon) to 658 (acerola) mg GAE/100 g pulp of; total flavonoids, from 1 (coconut and watermelon) to 134 (açai) mg CE/100 g pulp; and ascorbic acid from not detected levels to 506 (acerola) mg of ascorbic acid/100 g of pulp (Table 2).

The TPC contents of frozen fruit pulps were similar to other values reported in the literature (KUSKOSKI et al., 2006; EVANGELISTA; VIEITES, 2006; SANTOS et al., 2008, 2010; GENOVESE et al., 2008; MEZADRI et al., 2008; DANTAS et al., 2010), except for the contents found by Melo et al. (2008a), who used catechin as the analytical standard. The contents of bioactive compounds in açai frozen pulp were related to the protective effects of the pulp against doxorubicin-induced in vivo DNA damage in liver and kidney cells (RIBEIRO et al., 2010).

With regard to ascorbic acid, the contents found for the frozen fruit pulps in the present study were higher than those found by Canuto et al. (2010) for the pulps of acerola (378.5 mg AA/100 g pulp), caja (0.3 mg AA/100 g pulp), cashew-apple (12.4 mg AA/100 g pulp), and tamarind (0.1 mg AA/100 g pulp); while lower than those of açai (10.1 mg AA/100 g pulp) and of cupuaçu pulps (3.3 mg AA/100 g pulp). Ascorbic acid was not detected in açai pulp by Hassimotto, Genovese and Lajolo (2005) either; however, the values for frozen pulps of guava (49.9 mg AA/100 g pulp), cashew-apple (195 mg AA/100 g pulp), and acerola (885 mg AA/100 g pulp) were higher than those found in the present study.

In general, the great variation in the contents of bioactive compounds between the analyzed frozen fruit pulps can be associated to intrinsic (species, cultivar, and maturity stage) and extrinsic characteristics (environmental conditions, storage, and processing) of each fruit pulp and can lead to the variability in the composition of both the fresh fruit and processed products, including the frozen fruit pulp (KING; YOUNG, 1999; TOMÁS-BARBERÁN; ESPÍN, 2001; BALASUNDRAM; SUNDRAM; SAMMAM, 2006). Furthermore, the difference between the contents of bioactive compounds in the frozen fruit pulps found in this study and the contents reported in the literature may be explained due to the different extraction methods of these bioactive compounds.

3.2 Antioxidant capacity against the reactive oxygen species (ROS)

When the antioxidant capacity of bioactive compounds is evaluated, an important factor to be considered is the scavenging mechanism against the specific analyzed reactive species. The $ROO^{\bullet}$ in the presence of an antioxidant, for example, is mainly scavenged by the hydrogen atom transfer; the scavenging mechanism against H_2O_2 probably occurs by the addition of this reactive species to the system of conjugated double bonds

Table 2. Contents of total phenolic compounds (TPC), total flavonoids (TF), and ascorbic acid (AA) from the frozen fruit pulps analyzed.

Fruit pulp	TPC (mg GAE/100 g)	TF (mg CE/100 g)	AA (mg/100 g)
Açai	251 ± 64 (26%)	134 ± 44 (33%)	nd
Acerola	658 ± 179 (27%)	28 ± 11 (40%)	506 ± 54 (11%)
Cacao	29 ± 5 (17%)	10 ± 3 (27%)	nd
Caja	51 ± 4 (7%)	11 ± 3 (24%)	1 ± 0 (28%)
Cashew-apple	95 ± 13 (14%)	4 ± 0.5 (11%)	167 ± 24 (14%)
Coconut	10 ± 4 (45%)	1 ± 1 (78%)	7 ± 3 (42%)
Cupuaçu	80 ± 1 (1%)	29 ± 2 (6%)	1 ± 0 (0%)
Guava	88 ± 39 (45%)	25 ± 4 (16%)	29 ± 3 (9%)
Lemon	8 ± 2 (18%)	2 ± 0.5 (21%)	1 ± 0 (0%)
Mango	38 ± 6 (15%)	6 ± 1 (21%)	18 ± 1 (6%)
Orange	66 ± 4 (7%)	10 ± 2 (18%)	62 ± 9 (15%)
Passion fruit	26 ± 2 (6%)	10 ± 2 (20%)	7 ± 4 (59%)
Pineapple	27 ± 0.5 (2%)	5 ± 2 (35%)	6 ± 3 (49%)
Pitanga	73 ± 5 (7%)	10 ± 0.5 (6%)	12 ± 4 (31%)
Tamarind	56 ± 2 (3%)	24 ± 3 (11%)	1± 0 (0%)
Tangerine	46± 15 (33%)	9± 0 (1%)	58 ± 9 (15%)
Umbu	20 ± 11 (56%)	6 ± 0.5 (9%)	nd
Watermelon	6 ± 0 (0,5%)	1 ± 0 (5%)	nd

The values correspond to the mean ± standard deviation (relative standard deviation) of two batches (each batch in triplicate); GAE = gallic acid equivalent. CE = catechin equivalent. nd = not detected by the Tillman's method.

of the antioxidant compound; while the •OH can be scavenged either by its addition to the conjugated double bonds or by hydrogen atom transfer of the bioactive compound (HUANG; OU; PRIOR, 2005; ANOUAR et al., 2009). All bioactive compounds determined in this study (TPC, TF, and AA) have the necessary structural characteristics to scavenge the analysed ROS, such as, hydroxyl groups and conjugated double bonds. In fact, in general, frozen fruit pulps presenting the highest contents of TPC, or TF, or AA are expected to be the most efficient scavengers of ROS.

The aqueous extracts of the 18 frozen fruit pulps also exhibited great variation in the values of antioxidant capacity against the analyzed ROS (Table 3).

The açai and acerola pulps, which had the highest TPC and TF contents (Table 2) among all pulps analyzed, were the most efficient scavenger of ROO• (7498 and 6773 µmol trolox/100 g pulp, respectively). The values of scavenging capacity against the ROO• found in this study were in the same range as that reported for açai pulp (9970 µmol trolox/100 g pulp) (SCHAUSS et al., 2006), but higher than those found for acerola pulp (3458-5883 µmol trolox/100 g pulp) (MEZADRI et al., 2008). All other fruit pulps can be ranked according to the decreasing efficiency of ROO• scavenging capacity, as follows: tangerine, cupuaçu, orange, guava, cashew-apple, tamarind, pitanga, passion fruit, caja, cacao, mango, pineapple, umbu, lemon, watermelon, and coconut (Table 3).

With regard to the scavenging capacity against H_2O_2, the pulps of lemon, açaí, passion fruit, umbu, and pitanga had the highest efficiencies, *i.e.*, the lowest IC_{50} values, varying from 143 (lemon) to 467 µg/mL (pitanga). On the other hand, the pulps of coconut and watermelon did not reach the IC_{50} value for H_2O_2 scavenging, showing efficiency of only 20% (coconut) and 27% (watermelon) at the highest tested concentrations (13847 and 16864 µg/mL, respectively).

The pulps of açai, cupuaçu, acerola, guava, tamarind, and pitanga exhibited the lowest IC_{50} values of scavenging against •OH, varying from 3 (açai) to 89 µg/mL (pitanga), and thus they were the best scavengers of this species among the samples analyzed (Table 3).

As for the other frozen fruit pulps, the comparison with the literature data concerning the scavenging capacity against the analyzed ROS could not be made since either the applied methodology or the reactive species was different from those used in the present study

3.3 Correlation and classification of frozen fruit pulps by multivariate statistical analysis

In the PCA, the first two principal components (PC1 and PC2) accounted for 85% of the total explained variance (Figure 1).

According to the PCA, a positive correlation was observed between the scavenging capacity against ROO•, and the contents of TPC (r = 0.82), TF (r = 0.81) and AA (r = 0.58) (Figure 1). These correlation values show that the TPC, including the TF, make the highest contribution to the antioxidant capacity against ROO•, probably due to the excellent capacity of this class of bioactive compounds to transfer hydrogen atom, whilst AA presented the lowest contribution. Similar results were previously reported for 12 different fruits, with high positive correlation between the TPC and the antioxidant

Table 3. Scavenging capacities of frozen fruit pulps against peroxyl radical (ROO•), hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH).

Fruit pulp	ROO• (µmol trolox/100g)[1]	H_2O_2 (IC_{50} = µg/mL)[2]	•OH (IC_{50} = µg/mL)[2]
Açai	7498 ± 2509 (33%)	259 ± 102 (39%)	3 ± 1 (36%)
Acerola	6773 ± 74 (1%)	531 ± 126 (24%)	45 ± 4 (10%)
Cacao	859 ± 109 (13%)	2049 ± 250 (12%)	150 ± 48 (32%)
Caja	883 ± 190 (22%)	526 ± 50 (10%)	116 ± 27 (23%)
Cashew-apple	1568 ± 383 (24%)	2049 ± 1038 (51%)	207 ± 13 (6%)
Coconut	166 ± 43 (26%)	13847 ± 1664 (12%)[3]	447 ± 74 (16%)
Cupuaçu	1935 ± 144 (7%)	700 ± 81 (12%)	37 ± 15 (41%)
Guava	1575 ± 249 (16%)	1454 ± 148 (10%)	65 ± 19 (29%)
Lemon	240 ± 3 (1%)	143 ± 2 (1%)	206 ± 23 (11%)
Mango	635 ± 50 (8%)	3318 ± 88 (3%)	415 ± 11 (3%)
Orange	1678 ± 52 (3%)	1552 ± 402 (26%)	164 ± 43 (26%)
Passion fruit	923 ± 125 (14%)	390 ± 49 (12%)	205 ± 4 (2%)
Pineapple	626 ± 254 (41%)	1631 ± 74 (5%)	187 ± 66 (35%)
Pitanga	953 ± 166 (17%)	467 ± 20 (4%)	89 ± 9 (10%)
Tamarind	1164 ± 132 (11%)	572 ± 14 (2%)	75 ± 5 (7%)
Tangerine	1940 ± 487 (25%)	1366 ± 301 (22%)	139 ± 14 (10%)
Umbu	486 ± 58 (12%)	418 ± 86 (21%)	206 ± 19 (9%)
Watermelon	173 ± 24 (14%)	16864 ± 2819 (17%)[4]	313 ± 34 (11%)

The values correspond to the mean ± standard deviation (relative standard deviation) from two batches (each batch in triplicate). [1]Trolox equivalent; [2]IC_{50} = Inhibitory concentration necessary to decrease 50% the oxidative effect of reactive species in the tested media; [3]the inhibition at the highest tested concentration (13846.50 µg/mL) was 20%; [4]the inhibition at the highest tested concentration (16863.50 µg/mL) was 27%.

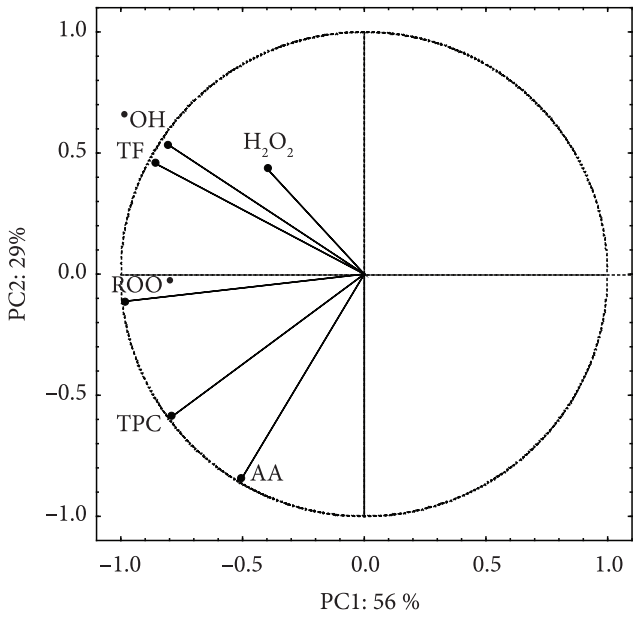

Figure 1. Variable projections in the PCA analysis obtained from the contents of bioactive compounds and the values of scavenging capacity against ROS for the 18 frozen fruit pulps analyzed.

capacity against ROO• (also determined by ORAC assay) and low contribution of AA to the scavenging capacity against the oxidizing effect of ROO• (WANG; CAO; PRIOR, 1996).

With regard to the scavenging capacity against H_2O_2, no significant correlation was observed for TF (r = 0.37), TPC (r = 0.12), and AA (r = –0.06). Therefore, the bioactive compounds considered in this study do not contribute greatly to the scavenging capacity of this specific ROS. On the other hand, carbohydrates, proteins, and amino acids are also components of the extracts and therefore can play a great hole in its scavenging capacity against H_2O_2 since these compounds have been previously reported as efficient scavengers of ROS (PATTISON; DAVIES, 2006). For example, annatto seed extracts, which have both phenolic compounds (minor compounds) and carotenoids (major compounds) exhibited higher scavenging capacity against H_2O_2 (IC_{50} from 11 to 47 µg/mL) (CHISTÉ et al., 2011) than those of the 18 frozen fruit pulps analyzed in this study. Moreover, H_2O_2 is rather inert at low concentrations, and its oxidation power is believed to be observed in combination with Fe(II) through Fenton reaction (HUANG; OU; PRIOR, 2005).

As for the scavenging capacity against •OH, a high positive correlation was observed with TF (r = 0.97), and no significant correlation was found for TPC (r = 0.31) and AA (r = –0.06). In

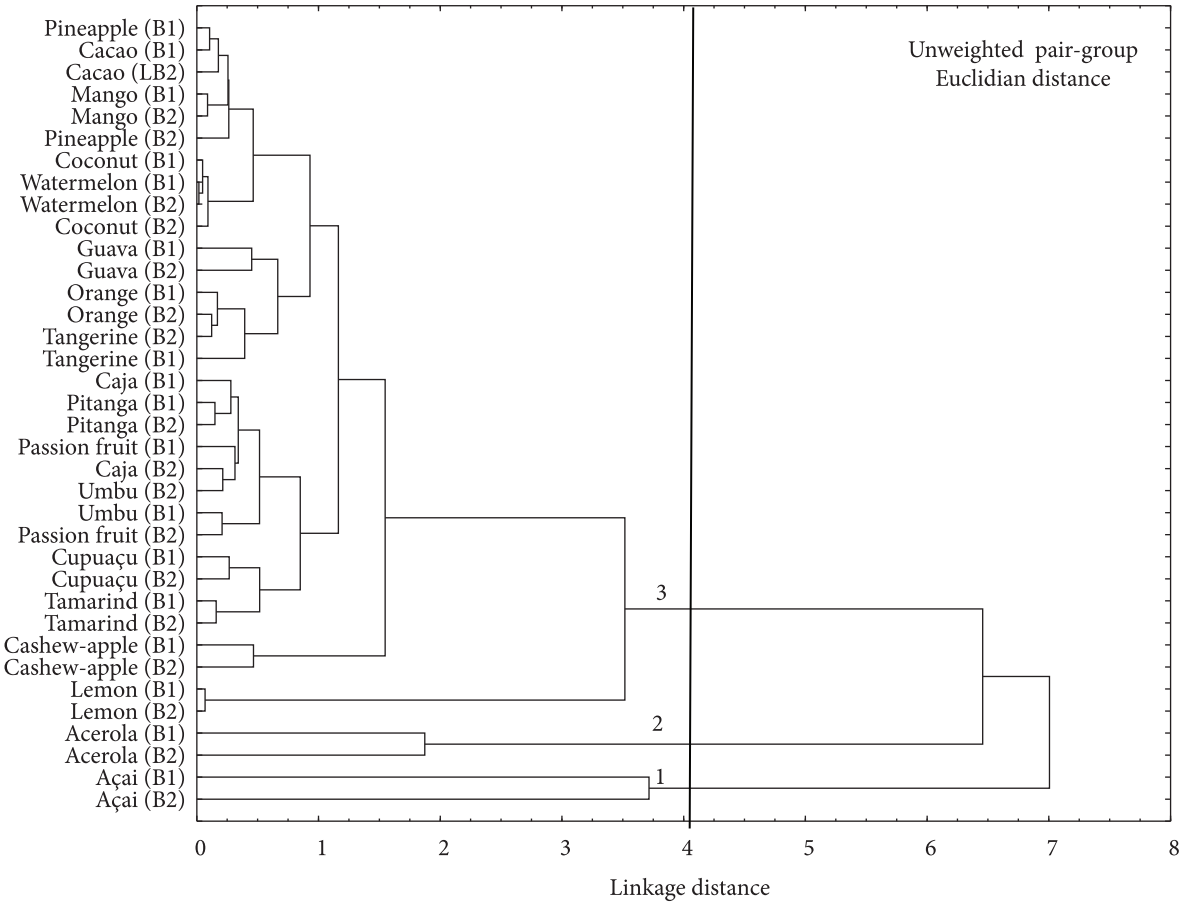

Figure 2. Dendrogram obtained by HCA analysis, considering the content of bioactive compounds and the values of scavenging capacity, against the tested ROS, for the 18 frozen fruit pulps analyzed in two different batches (B1 e B2).

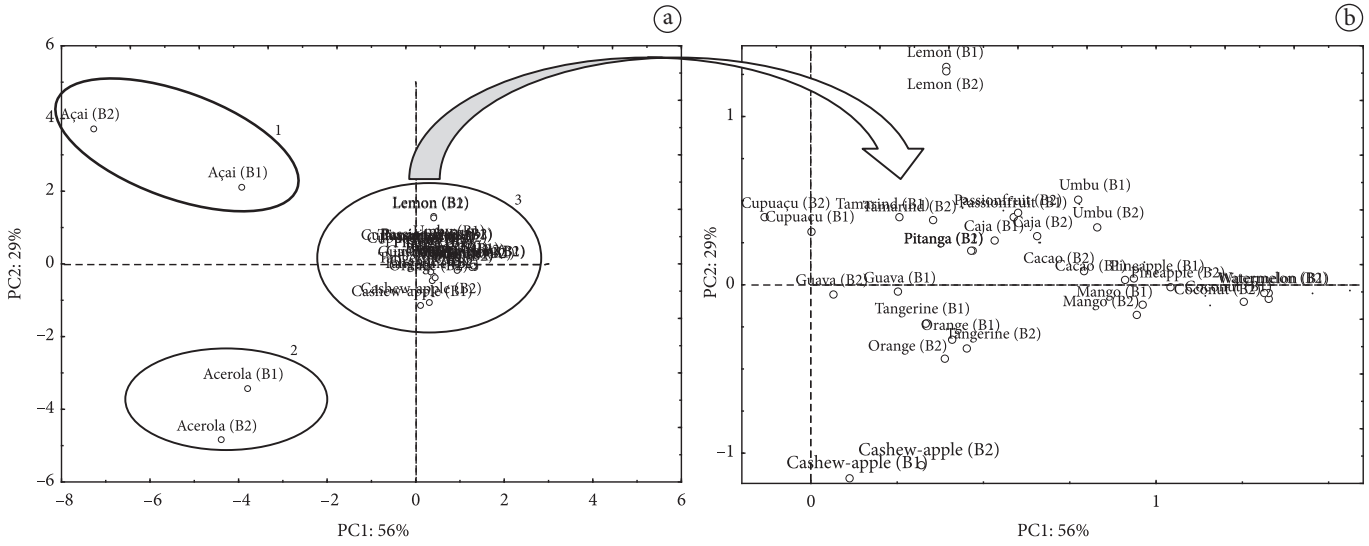

Figure 3. a) Scatterplot for samples by PCA analysis with suggested drawn grouping ellipses (by HCA) and b) expansion of the third group, both obtained from the results of bioactive compounds and scavenging capacity against the ROS for the different extracts of frozen fruit pulps of two different batches (B1 e B2).

this study, the $FeCl_2$-EDTA-H_2O_2 (Fenton reaction), the most used system to generate •OH, was also used. However, this generation mode has disadvantages because many antioxidants, such as some classes of flavonoids, are also metal chelators. When the antioxidant is mixed with Fe (II), it may alter the activity of Fe(II) by chelation, and as a result, it is impossible to distinguish whether the antioxidants are simply good metal chelators or •OH scavengers (HUANG; OU; PRIOR, 2005). This fact can explain the high correlation found between the contents of TF and the values of scavenging capacity against •OH since flavonoids are known to be good metal chelators. In addition, some compounds in foods, such as ascorbic acid, may act as pro-oxidants by reducing Fe(III) to Fe(II) and make the •OH generation catalytic (HUANG; OU; PRIOR, 2005).

The dendrogram obtained when HCA analysis was applied, allowed the classification of the 18 frozen fruit pulps into three groups, considering the contents of TPC, TF, AA, and the scavenging capacities against the different ROS. In addition, similarities between the two batches (B1 and B2) for all pulps were observed (Figure 2). The three groups could be clearly observed in PCA scatterplot (Figure 3).

The first group was formed by the açai pulp, with the highest content of TF (134.02 mg CE/100 g pulp) and the highest scavenging capacities against ROO• and •OH; the second one was formed by the acerola pulp, due to the high contents of TPC (658.40 mg GAE/100 g pulp) and AA (506.27 mg/100 g pulp); and the third group was formed by the other fruit pulps which could not be separated considering only the contents of bioactive compounds and the values of scavenging capacities against the tested ROS (Figures 2 and 3, Table 2).

Considering the third group, the lemon and cashew-apple pulps showed a slight tendency to be separated from the other fruit pulps in this group, most probably due to the high scavenger capacity against H_2O_2 (IC_{50} = 142.70 µg/mL) of lemon pulp and to the high content of AA (166.56 mg AA/100 g pulp) found in the cashew-apple pulp (Figures 2 and 3, Table 2 and 3).

4 Conclusions

The frozen fruit pulps contain bioactive compounds which are *in vitro* scavengers of reactive oxygen species of biological importance, namely the ROO•, H_2O_2, and •OH. In general, the scavenging capacities against ROO•, H_2O_2 and •OH species, as well as the concentrations of bioactive compounds, varied widely among the 18 different fruit pulps. This study confirmed the positive correlation between high levels of phenolic compounds, especially flavonoids, and the capacity to scavenge ROO•. Furthermore, the results indicated that the flavonoids show high scavenging capacity against •OH, but they are less efficient against H_2O_2. In addition, ascorbic acid showed little contribution against all of the ROS studied. Among the 18 frozen fruit pulps evaluated, açai can be considered the best ROS scavenger.

Acknowledgements

The authors are grateful for the financial support provided by the foundations CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo).

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