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Thermo- and photosensitivity of *Euterpe oleracea* Mart. (Arecaceae) fruit pulp: Consequences for its antioxidant activity under basic storage conditions in the Amazon

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Abstract

Anthocyanins are chemically unstable, casting doubt on the antioxidant activity (AA) of *Euterpe oleracea* Mart. (Arecaceae) fruit pulp during storage. In this study, AA, total monomeric anthocyanin (TMAC), total flavonoid (TFC), and total phenolic contents (TPC) of this preparation after 24 h and 5 days at ambient temperature/ambient light-dark (AT/AL), ambient temperature/continuously dark (AT/CD), and under refrigeration/continuously dark (UR/CD) were compared to those of the fresh preparation. A FRAP and a DPPH assay, and a pH differential, an AlCl₃ colorimetric, and Folin-Ciocalteu's method were used. Associations between pairs of variables were determined by Pearson correlation coefficient (r). Student's t test and one-way ANOVA with Tukey HSD were used to evaluate the data for statistically significant differences (p < 0.05). FRAP and DPPH IC₅₀ values of the fresh preparation were 116 ± 3 μ M Fe²⁺ equivalents/100 μ g, and 57 ± 12 μ g/mL, respectively. They were hardly affected after 24 h, but had decreased 4-fold and increased 2-fold, respectively, after 5 days, the least at UR/CD. TMAC, TFC, and TPC of the fresh preparation were 21 ± 4 mg Cy3GE/mg, 25 ± 4 μ M RE/100 μ g, and 67 ± 2 μ M GAE/100 μ g. The loss of AA after 5 days was accompanied by a 3- to 5-fold TMAC and TFC degradation, and a 1.5-fold TPC decrease. AA correlated better with TMAC and TFC (r ±0.7 - ±1.0) than with TPC (r ±0.4 - ±0.9). These observations are important for communities with only basic facilities for preparing and storing *E. oleracea* fruit pulp.

Keywords: *Euterpe olercaea* Mart. (Arecaceae); Fruit pulp; Antioxidant activity; Total monomeric anthocyanin contents; Total flavonoid contents; Total phenolic contents; Thermostability; Photostability

1. Introduction

The açai palm *Euterpe oleracea* Mart. (Arecaceae) is indigenous to South America including the northerly located Republic of Suriname. The fruit is commonly known as açaí berry and is made up of a hard endocarp that consists of a single large seed of 7 to 10 millimeters in diameter, a fibrous, purple-colored, pulpy mesocarp of about 1 millimeter thick, and a deeply purple-colored exocarp. The pulp prepared from the mesocarp and the exocarp has a high nutritional density, containing, among others, appreciable amounts of carbohydrates, proteins, several types of vitamin, carotenoids, calcium, iron, mono- and polyunsaturated fatty acids, as well as a variety of phenolic compounds [1,2]. Understandably, *E. oleracea* fruit pulp has been consumed for centuries by the Amazon peoples - including those in Suriname's hinterland - as an ingredient of nutritious beverages and meals with, for instance, cassava and fish [1,3].

E. oleracea fruit pulp is also abundantly used as a traditional medicine in Suriname and the rest of South America. A few indications are anemia; hypotension; various types of wounds such as open cuts, scorpion stings, and shot wounds; and as an external contraceptive [4-6]. Pharmacological studies with fruit pulp preparations have shown, among others, antidiarrheal, anti-inflammatory, anti-nociceptive, anti-angiogenic, antimicrobial, antileishmanial, skin-regenerating

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and anti-ageing, cosmeceutical, neuroprotective, anticancer, and antioxidant activities [2,7-10]. These and other findings have led to the rapid commercialization and marketing of a multitude of *E. oleracea* fruit pulp products as health-promoting supplements, nutraceuticals, and functional foods formulated as beverages, frozen pulp, powders, tablets, and capsules, as well as ready-prepared healthy food items such as jams, ice creams and other frozen treats, mousses, cakes, porridges, and bonbons [2,3,11].

The purported health-promoting and disease-preventing properties of *E. oleracea* fruit pulp have particularly been attributed to the powerful antioxidant properties of its relatively high contents of phenolic compounds, most notably the anthocyanins [1,2,6,8,9,12-14]. The main anthocyanins in *E. oleracea* fruit pulp are cyanidin-3-glucoside and cyanidin-3-rutinoside, and these compounds are for an important part responsible for the dark purple color of the fruit's exocarp and endocarp [13-16]. Anthocyanins are ubiquitous in the plant kingdom and presumably help ward off microorganisms and phytopathogens; attract insects, birds, and small mammals for pollination and seed dispersal; and provide protection against the detrimental effects of ultraviolet radiation, high light intensity, drought, low temperatures, water stress, high salinity, and wounding [17].

Anthocyanins derive their antioxidant capacity from their polyhydroxyl structure and strong reducibility, providing them the ability to donate a hydrogen atom or transfer an electron to reactive oxygen species (ROS) [18,19]. In this way, anthocyanins are able to scavenge free radicals, reducing oxidative stress and oxidative damage to cellular constituents [18,19]. For these reasons, anthocyanin-rich diets may be beneficial against diseases associated with oxidative stress such as neoplastic, neurodegenerative, cardiovascular, age-related, cerebrovascular, diabetic, and inflammatory diseases [20,21]. Indeed, the anthocyanins in *E. oleracea* fruit pulp very efficiently scavenged ROS *in vitro* [8,12-14,18-21]; decreased the severity of harmful inflammatory and oxidative events in laboratory models [22-26]; and increased plasma antioxidant capacity [27], serum catalase activity, and serum antioxidant capacity in human subjects while reducing ROS production [28].

However, the high reactivity of anthocyanins also renders them chemically unstable, speeding up their degradation and decreasing their antioxidant activity [29-32]. Indeed, the chemical structure as well as the antioxidant activity of anthocyanins rapidly decrease in response to, among others, changes in light, temperature, and pH, as well as the presence of oxygen, ascorbic acid, other pigments, sulfites, and certain enzymes [29-32]. For these reasons, various approaches have been evaluated with the aim to preserve the stability of *E. oleracea* fruit pulp preparations on an industrial scale, such as hot air drying, vacuum drum drying, freeze drying, snap freezing, and spray drying (see, for instance, references [33,34]).

So far, less attention has been dedicated to the antioxidant stability of the fruit pulp under more basic conditions such as those in Suriname's hinterland and the rest of the Amazon, where adequate electricity service provision and proper refrigeration facilities to extend their shelf-life are scant [35]. Indeed, the few options for these communities are storage at ambient temperature/ambient light-dark, ambient temperature/continuously dark, and in some cases, under refrigeration/continuously dark. Hence the current *in vitro* study, in which the *E. oleracea* fruit pulp prepared according to the traditionally employed method in Suriname, has been assessed for its antioxidant activity as well as its total monomeric anthocyanin contents (TMAC), total flavonoid contents (TFC), and total phenolic contents (TPC) after storage under these conditions for 24 h and 5 days.

2. Material and methods

2.1. Collection of *E. oleracea* fruit and preparation of fruit pulp samples

Fresh *E. oleracea* fruits were collected in the northern-rural Surinamese district of Para (GPS coordinates 5.6303° N, 55.08568° W) at a location that has been free from herbicides and pesticides in the preceding six months. The collections took place in the year 2021 in the month of November, the optimum period for harvesting *E. oleracea* fruit [36]. *E. oleracea* is not on the International Union for Conservation of Nature Red List of Threatened Species [37]. The collected material has been authenticated by the National Herbarium of Suriname and was identical to previously deposited *E. oleracea* samples (voucher number IVL0148).

The *E. oleracea* fruits were first thoroughly washed with tap water, then with distilled water, after which the exocarps and mesocarps were carefully removed using a small knife. The exocarps and endocarps of about ten fruits yielded about 60 mg pulp, which was diluted with 10 mL distilled water. This was based on the Surinamese custom to standardize the preparation of the fruit pulp for consumption by diluting a handful of fruits (which corresponds to about ten fruits and yields about 60 mg fruit pulp) with a tablespoon of water (which corresponds to 10 - 15 mL).

The diluted *E. oleracae* fruit pulp was filtered to remove particles and was divided into several aliquots of 6 mg/mL which were used for the experiments described hereunder. Some of these fruit pulp stock solutions were immediately subjected to the experiments, while others were stored for 24 h or 5 days at ambient temperature/ambient light-dark; at ambient temperature/continuously dark; or under refrigeration/continuously dark, before submitting them to the same experiments as the fresh, unprocessed samples.

2.2. Drugs and chemicals

2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), iron(III) chloride hexahydrate (FeCl₃. 6H₂O), iron(II) sulfate heptahydrate (FeSO₄ . 7H₂O), 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, aluminium chloride hexahydrate (AlCl₃. 6H₂O), rutin, and Folin-Ciocalteu reagent were from Sigma-Aldrich (St. Louis, MO, USA). Sodium acetate (CH3COONa) was from BDH Laboratory Supplies (Poole, UK), absolute ethanol from AppliChem GmbH (Darmstadt, Germany), sodium carbonate (Na₂CO₃) from Merck (Darmstadt, Germany), acetic acid glacial (CH₃COOH) from Solon Industrial Parkway (Solon, Ohio, USA), and potassium chloride from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals used were from our laboratory stock and were of the highest grade available.

2.3. Determination of antioxidant activity using the ferric reducing antioxidant power assay

The antioxidant activity of the *E. oleracea* fruit pulp samples was determined by a ferric reducing antioxidant power (FRAP) assay [38]. The assay involves the donation of an electron (at low pH) by the antioxidant(s) in the fruit pulp to the ferric (Fe³⁺) ion in the colorless Fe³⁺-TPTZ complex, reducing it to a ferrous (Fe²⁺) ion in the Fe²⁺-complex [38]. Thus, 3 mL FRAP reagent consisting of TPTZ 10 mM in HCl, FeCl₃. $6H_2O$ 20 mM, and acetate buffer 300 mM pH 3.6 in the proportion of 1/1/10 (v/v/v), was mixed with 70 µL from each 6 mg/mL-fruit pulp stock solution and 1 mL distilled water to give an amount of fruit pulp of about 100 µg/mL. After thorough mixing and incubation for 30 min in the dark and at room temperature, the absorbance was recorded at a wavelength of 593 nm against a blank consisting of distilled water instead of fruit pulp. The change in absorbance was directly related to the total reducing power of the electron-donating antioxidants present in the fruit pulp. These values were determined from a calibration curve constructed from the absorbance of different concentrations of FeSO₄ at 593 nm, expressed as µm Fe²⁺ equivalents reduced per 100 µg *E. oleracea* fruit pulp, and eventually expressed relatively to the value obtained with the fresh, unprocessed sample.

2.4. Determination of antioxidant activity using the 1,1-diphenyl-2-picrylhydrazyl-assay

The antioxidant activity of the *E. oleracea* fruit pulp samples was also assessed using a DPPH free radical scavenging assay [397]. This assay is based on the donation of an electron or hydrogen radical by the antioxidant(s) in the fruit pulp to the stable and violet-colored DPPH free radical, turning it colorless to pale yellow [39]. Thus, using distilled water, serial dilutions were prepared from each 6 mg/mL-fruit pulp stock solution, and 0.3 mL of each of these dilution was mixed with 3 mL absolute ethanol and 0.5 mL of a DPPH solution of 0.5 mM in ethanol. After 90 min in the dark and at room temperature, the absorbance of the solutions was measured at a wavelength of 517 nm against a mixture of 3.5 mL ethanol and 0.3 mL of the corresponding fruit pulp sample as a blank. The control solution consisted of 3.5 mL ethanol and 0.3 mL DPPH solution.

The percentage antioxidant activity (AA %) of each dilution of each plant extract was determined using the formula:

AA % =
$$100 - ([Abs_{sample} - Abs_{blank}] \times 100) / Abs_{control}$$

Where Abs_{sample} is the absorbance of the *E. oleracea* samples, Abs_{blank} the absorbance of the blank, and $Abs_{control}$ the absorbance of the control. For each *E. oleracea* sample, the absorbance values of the dilutions were plotted against the corresponding concentrations. From the resulting dose-response curve, IC_{50} values were derived, *i.e.*, the concentrations of the *E. oleracea* samples (in µg/mL) accomplishing a 50% decrease in absorbance when compared to untreated samples.

2.5. Determination of total monomeric anthocyanin contents

The TMACs of the *E. oleracea* fruit pulp extracts were determined by means of a pH differential method [40]. This method is based on the occurrence of reversible changes in the structure of monomeric anthocyanins between pH 1.0 and 4.5 (the colored oxonium form predominates at pH 1.0 and the colorless hemiketal form at pH 4.5), and the reflection of the amount of monomeric (or 'pure') anthocyanins in the *E. oleracea* samples by the difference in absorbance at the two pH values measured at a wavelength of 520 nm (the λ_{max} of anthocyanins) [40]. Thus, 0.2 mL of each 6 mg/mL-fruit pulp stock solution (containing about 1 mg fruit pulp) was diluted with either 9.8 mL KCl 1.86 g/L, pH 1.0, or 9.8 mL CH3COONa 32.82 g/L, pH 4.5. After mixing, the 10-mL-samples were incubated for 30 min in the dark

and at room temperature, after which their absorbance was determined at 520 nm and 700 nm, in the latter case to correct for turbidity in the samples. The TMAC of the *E. oleracea* fruit pulp extracts was calculated using the formula:

anthocyanin pigment = A x MW x DF x 1,000 / ϵ x 1

In this formula, A corresponds to ([absorbance at 520 nm - absorbance at 700 nm] at pH 1.0) - ([absorbance at 520 nm - absorbance at 700 nm] at pH 4.5). MW corresponds to the molecular weight of anthocyanin for cyanidin-3-glucoside (449.2 g/mol), the major anthocyanin in most plants including *E. oleracea* [13-16]. DF, 1,000, and 1 correspond to the dilution factor, the conversion factor from g to mg, and the path length in the spectrophotometer in cm. The ε in the formula represent the molar extinction coefficient, which has a value of 26,900 L x mol⁻¹ x cm⁻¹ in aqueous buffer solution. TMACs were expressed in mg cyanidin-3-glucoside equivalents (Cy3GE) per mg fruit pulp.

2.6. Determination of total flavonoid contents

The TFCs of the *E. oleracea* fruit pulp samples were determined using a previously described aluminium chloride (AlCl₃) colorimetric method [41]. This method is based on the formation of acid-stable complexes between AlCl₃ and the hydroxyl groups of flavones and flavonols [41]. Thus, each 6 mg/mL-fruit pulp stock solution was diluted 1:10 with distilled water, and 60 μ L of these samples was further diluted with 0.32 mL distilled water. These 0.5 mL fruit pulp samples contained about 100 μ g fruit pulp, and they were mixed with 0.5 mL AlCl₃ 2% (*w/v*) in absolute ethanol, after which 0.5 mL 1 M potassium acetate and 0.5 mL 1 M HCl were added. The samples were incubated for 10 min at room temperature and the absorbances were measured at 425 nm against blanks of distilled water. A yellow color indicated the presence of flavonoids. TFC of the *E. oleracea* fruit pulp extract was calculated by intrapolation into a standard curve of rutin prepared from serial dilutions of this compound between 0 and 200 μ g/L. Data were expressed as μ M rutin equivalents (RE) per 100 μ g fruit pulp.

2.7. Determination of total phenolic contents

The TPCs of the *E. oleracea* samples were determined using Folin-Ciocalteu's method [42]. The Folin-Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate, and the method is based on the transfer of electrons in alkaline medium from phenolic compounds to the phosphomolybdate/phosphotungstate complex to form a blue chromophore that is spectrophotometrically detectable [42]. Thus, 20 μ L of each fruit pulp sample was added to 0.1 mL Folin-Ciocalteu reagent 1 N, after which each mixture was supplemented with 1.9 mL distilled water. The mixtures then contained about 100 μ g fruit pulp. They were shaken and allowed to react for 5 min at room temperature, after which 1.0 mL of Na₂CO₃ 7% (*w*/*v*) was added. The solutions were adjusted with distilled water to a final volume of 3.4 mL and thoroughly mixed. After incubation for 30 min in the dark, the absorbances were read at 765 nm with respect to blanks containing only Folin-Ciocalteu reagent 1 N and Na₂CO₃ 7% (*w*/*v*). The TPC of each sample was calculated from the linear equation of a standard curve prepared with gallic acid (0 to 200 µg/mL) and expressed as µM gallic acid equivalents (GAE) per 100 µg fruit pulp.

2.8. Data processing and statistics

All experiments have been carried out at least three times in triplicate. Data (means \pm SDs) have been compared using Student's t test or one-way ANOVA with Tukey post hoc test. P values < 0.05 were taken to indicate statistically significant differences. Possible correlations between FRAP values and DPPH IC₅₀ values, and between FRAP values or DPPH IC₅₀ values on the one hand, and TMACs, TFCs, or TPCs on the other hand, were explored by calculating Pearson's correlation coefficient. This value determines the strength of the linear relationship between two variables and can have a value between 0 and \pm 1. Values > 0.5 or < -0.5 indicate a strong correlation, those between \pm 0.30 and \pm 0.49 a moderate correlation, and those between 0 and \pm 0.29 a small correlation.

3. Results

3.1. Antioxidant activity

The FRAP value of the fresh, unprocessed *E. oleracea* fruit pulp was $116 \pm 3 \mu M Fe^{2+}$ equivalents reduced per 100 µg fruit pulp. After storage for 24 h, either at ambient temperature/ambient light-dark, ambient temperature/continuously dark, or under refrigeration/continuously dark, this value had statistically significantly but only slightly decreased (Table 1a; p ≤ 0.01, Student's t-test). The FRAP values found after 24 h under the different conditions did not differ statistically significantly from each other (Table 1a; p ≥ 0.356, ANOVA). After 5 days, the FRAP values under all the storage conditions were roughly four times lower than those found after 24 h (Table 1a; p ≤ 0.0007, Student's t test) with again no statistically significant differences among them (p ≥ 0.623, ANOVA).

The DPPH IC₅₀ values of the fresh, unprocessed *E. oleracea* fruit pulp was $57 \pm 12 \mu g/mL$. As found for the FRAP values after 24 h, the IC₅₀ values under the different storage conditions differed statistically significantly but only slightly from that of the fresh, unprocessed material (Table 1b; $p \le 0.021$, Student's t test) with no statistically significant differences among the different conditions (Table 1b; $p \ge 0.390$, ANOVA). After 5 days, the DPPH IC₅₀ values were also statistically significantly lower than that of the fresh, unprocessed fruit pulp (Table 1b; $p \le 0.0001$, Student's t test) and there were again no statistically significant differences among the different conditions (Table 1 differences among the different conditions (Table 1 b; p = 0.328; Student's t test). However, whereas the FRAP values after 5 days were roughly four times lower than those after 24 h (Table 1a), the DPPH IC₅₀ values after 5 days were about twice higher than those after 24 h (Table 1b; p = 0.013; Student's t test).

Table 1a Ferric reducing antioxidant power (FRAP) values of *E. oleracea* fruit pulp after 24 h and 5 days under various storage conditions relative to that found for the FRAP value of the fresh, unprocessed fruit pulp. The latter value was $116 \pm 3 \mu m Fe^{2+}$ equivalents reduced/100 μg and was set at 100%. Data are expressed in %, and are means \pm SDs of at least three independent experiments performed in triplicate

	After24 h	After 5 days
Ambient temperature/ambient light-dark	88 ± 2ª	20 ± 13 ^{a,b}
Ambient temperature/continuously dark	91 ± 3ª	$27 \pm 6^{a,b}$
Under refrigeration/continuously dark	89 ± 2ª	23 ± 9 ^{a.b}

^aStatistically significantly different from the FRAP value of the fresh, unprocessed fruit pulp ($p \le 0.001$, Student's t-test); ^bstatistically significantly different from the corresponding FRAP value after storage for 24 h ($p \le 0.0007$, Student's t test)

Table 1b 1,1-diphenyl-2-picrylhydrazyl (DPPH) IC₅₀ values of *E. oleracea* fruit pulp after 24 h and 5 days under various storage conditions relative to that found for the DPPH IC₅0 value of the fresh, unprocessed fruit pulp. The latter value was 57 \pm 12 µg/mL fruit pulp and was set at 100%. Data are expressed in %, and are means \pm SDs of at least three independent experiments performed in triplicate

	After24 h	After 5 days
Ambient temperature/ambient light-dark	135 ± 17^{a}	$205 \pm 40^{a,b}$
Ambient temperature/continuously dark	151 ± 13^{a}	226 ± 29 ^{a,b}
Under refrigeration/continuously dark	147 ± 20^{a}	$223 \pm 14^{a,b}$

^aStatistically significantly different from the DPPH IC₅₀ value of the fresh, unprocessed fruit pulp ($p \le 0.022$, Student's t-test); ^bstatistically significantly different from the corresponding DPPH IC₅₀ value after storage for 24 h ($p \le 0.048$, Student's t test)

Table 2 Associations between FRAP and DPPH IC₅₀ values of *E. oleracea* fruit pulp on the one hand, and total monomeric anthocyanin contents (TMAC), total flavonoid contents (TFC), and total phenolic contents (TPC) on the other hand, under various storage conditions up to 5 days, using Pearson's correlation coefficient

	Pearson correlation coefficient		
	Ambient temperature/ ambient light-dark	Ambient temperature/ continuously dark	Under refrigeration/ continuously dark
FRAP vs. DPPH	-1.0	-1.0	-1.0
FRAP vs. TMAC	0.8	0.8	0.9
DPPH vs. TMAC	-0.9	-0.9	-1.0
FRAP vs. TFC	0.7	0.8	0.8
DPPH vs. TFC	-0.8	-0.9	-0.9
FRAP vs. TPC	0.8	0.4	0.4
DPPH vs. TPC	-0.9	-0.7	-0.6

Taken together, the data from the FRAP and DPPH studies suggest that the antioxidant activity of the *E. oleracea* fruit pulp was only minimally affected after 24 h, either at ambient temperature/ambient light-dark, ambient temperature/continuously dark, or under refrigeration/continuously dark, but that it had decreased 2- to 4-fold after 5 days under all the storage conditions. Applying Pearson correlation method, a strong (negative) association was seen between FRAP values and DPPH IC₅₀ values found under the various conditions (Table 2; correlation coefficient of -1.0, $p \ge 0.12$). This suggests that FRAP values and DPPH IC₅₀ values both properly represented the antioxidant activity of the *E. oleracea* fruit pulp.

3.2. Total monomeric anthocyanin contents

The TMAC of the fresh, unprocessed *E. oleracea* fruit pulp was 21 ± 4 Cy3GE per mg fruit pulp. After storage for 24 h, this value had decreased by about two-thirds at ambient temperature/ambient light-dark, by about half at ambient temperature/continuously dark, and by about one-third under refrigeration/continuously dark (Table 3a; p values < 0.0001, < 0.0001, and 0.003, respectively, Student's t test). The TMAC after storage under refrigeration/continuously dark was statistically significantly higher than those found at ambient temperature/ambient light-dark and ambient temperature/continuously dark (Table 3a; p values 0.003 and 0.045, respectively, ANOVA). There were no statistically significant differences between the two latter values (Table 3a; p values 0.121, ANOVA). This suggest that the TMAC of the fruit pulp was more stable when it was stored under refrigeration when compared to ambient temperature.

After 5 days, the TMACs of the samples were also statistically significantly lower (60-75%) when compared to that of the fresh, unprocessed sample (Table 3a; p values < 0.0001, Student's t test). Markedly, as seen after 24 h, the TMAC after 5 days was statistically significantly higher (20-40%) under refrigeration/continuously dark when compared to ambient temperature/ambient light-dark or ambient temperature/continuously dark (Table 3a; p values 0.046 and 0.031, respectively; ANOVA). There were again no statistically significant differences between the two latter conditions (Table 3a; p values 0.445; ANOVA). Thus, the loss of the TMAC of the *E. oleracea* fruit pulp was more pronounced after 5 days than after 24 h, but it might be limited by refrigeration. Furthermore, the comparable courses of TMAC on the one hand, and FRAP and DPPH IC₅₀ values on the other hand suggest that the anthocyanins were for an important part involved in its antioxidant activity.

3.3. Total flavonoid contents

The TFC of the fresh, unprocessed *E. oleracea* fruit pulp was $25 \pm 4 \mu$ M RE per 100 µg fruit pulp. After 24 h, this value had decreased to 30-40% of that of the fresh, unprocessed material under all the storage conditions (Table 2b; p ≤ 0.0004; Student's t test). There were no statistically significant differences among the values found under the different conditions (Table 2b; p ≥ 0.106, ANOVA). After 5 days, the TFCs had further decreased to roughly 20% of that of the fresh, unprocessed material (Table 2a; p < 0.0001; Student's t test), with again no statistically significant differences among the values found under the different storage conditions (Table 2a; p < 0.0001; Student's t test), with again no statistically significant differences among the values found under the different storage conditions (Table 2a; p ≥ 0.624; ANOVA). The TFCs at day 5 were about twice lower than those at 24 h (Table 2b; p ≤ 0.041; Student's t test). Thus, the decrease in antioxidant activity of the *E. oleracea* fruit pulp upon prolongation of the storage time might be associated with the degradation of flavonoids including anthocyanins (as seen in the previous paragraph), but perhaps also non-anthocyanin flavonoids.

3.4. Total phenolic contents

The TPC of the fresh, unprocessed *E. oleracea* fruit pulp was $67 \pm 2 \mu$ M GAE per 100 µg fruit pulp. This value had decreased under all the storage conditions by roughly one-third, either after 24 h or 5 days (Table 3c; $p \le 0.015$; Student's t test). There were no statistically significant differences among the TPCs of the fruit pulp under the different storage conditions, either after 24 h or after 5 days (Table 3c; $p \ge 0.310$; ANOVA). Since the TPC had remained at the same level under all storage conditions, either after 24 h or after 5 days- whereas the FRAP and DPPH IC₅₀ values had decreased - it seemed that the TPC of the *E. oleracea* fruit pulp was not a major determinant of its antioxidant activity.

3.5. Relationships between FRAP values and DPPH IC $_{50}$ values on the one hand, and TMACs, TFCs, and TPCs on the other hand

The relationships between FRAP values or DPPH IC₅₀ values of the *E. oleracea* fruit pulp on the one hand, and TMACs, TFCs, or TPCs on the other hand, were explored by calculating Pearson's correlation coefficient. As shown in Table 2, the time course of the antioxidant activities correlated well with those of the plant substances over the 5-day evaluation period. The strongest correlations under all the storage conditions (ambient temperature/ambient light-dark, ambient temperature/continuously dark, and under refrigeration/continuously dark conditions) were those between pairs of FRAP and DPPH IC₅₀ values on the one hand, and TMACs and TFCs on the other hand, producing correlation coefficients between ± 0.7 and ± 1.0 . There were also strong correlations between FRAP and DPPH IC₅₀ value on the one hand, and TMACs and TPC at ambient temperature/ambient light-dark on the other hand (Table 2; correlation coefficients of 0.8 and -0.9,

respectively). However, the associations between FRAP and DPPH IC₅₀ values and TPCs at ambient temperature/ continuously dark and under refrigeration/continuously dark were lower and even moderate (correlation coefficients of 04 and -0.7, 0.4 and -0.6, respectively) (Table 2). These observations suggest that TMAC and TFC were more important determinants of the antioxidant activity of the *E. oleracea* fruit pulp than TPC in general.

Table 3a Total monomeric anthocyanin contents of *E. oleracea* fruit pulp after 24 h and 5 days under various storage conditions relative to that found for the fresh, unprocessed fruit pulp. The latter value was 21 ± 4 mg cyanidin-3-glucoside equivalents/mg and was set at 100%. Data are expressed in %, and are means \pm SDs of at least three independent experiments performed in triplicate or quadruplicate

	After 24 h	After 5 days
Ambient temperature/ambient light-dark	39 ± 9 ^a	$24 \pm 4^{a,b}$
Ambient temperature/continuously dark	47 ± 8 ^a	$31 \pm 5^{a,b}$
Under refrigeration/continuously dark	69 ± 11 ^{a,c}	$41 \pm 3^{a,b.d}$

^aStatistically significantly different from the TMAC of the fresh, unprocessed fruit pulp ($p \le 0.01$, Student's t-test); ^bstatistically significantly different from the corresponding TMAC after storage for 24 h ($p \le 0.048$, Student's t test); ^cstatistically significantly different from the TMACs at ambient temperature/ambient light-dark (p = 0.003, ANOVA) and ambient temperature/continuously dark after 24 h (p = 0.045, ANOVA); ^dstatistically significantly different from the TMACs at ambient temperature/ambient light-dark (p = 0.046, ANOVA) and ambient temperature/ambient light-dark (p = 0.046, ANOVA) and ambient temperature/ambient light-dark (p = 0.046, ANOVA) and ambient temperature/continuously dark after 5 days (p = 0.031, ANOVA)

Table 3b Total flavonoid contents of *E. oleracea* fruit pulp after 24 h and 5 days under various storage conditions relative to that found for the TPC of the fresh, unprocessed fruit pulp. The latter value was $25 \pm 4 \mu$ M rutin equivalents/100 μ g and was set at 100%. Data are expressed in % and are means \pm SDs of at least three independent experiments performed in triplicate or quadruplicate

	After 24 h	After 5 days
Ambient temperature/ambient light-dark	28 ± 4^{a}	15 ± 3 ^{a.b}
Ambient temperature/continuously dark	37 ± 10^{a}	18 ± 5 ^{a.b}
Under refrigeration/continuously dark	41 ± 3ª	16 ± 5 ^{a.b}

^aStatistically significantly different from the TFC of the fresh, unprocessed *E. oleracea* fruit pulp (p < 0.0001, Student's t-test); ^bstatistically significantly different from the corresponding TFC after storage for 24 h (p ≤ 0.041, Student's t test)

Table 3c Total phenolic contents of *E. oleracea* fruit pulp after 24 h and 5 days under various storage conditions relative to that found for the TPC of the fresh, unprocessed fruit pulp. The latter value was $67 \pm 2 \mu$ M gallic acid equivalents/100 μ g and was set at 100%. Data are expressed in %, and are means \pm SDs of at least three independent experiments performed in triplicate or quadruplicate

	After 24 h	After 5 days
Ambient temperature/ambient light-dark	73 ± 12^{a}	63 ± 6 ^a
Ambient temperature/continuously dark	64 ± 7^{a}	71 ± 6 ^a
Under refrigeration/continuously dark	69 ± 11ª	78 ± 8 ^a

aStatistically significantly different from the total phenolic contents of the fresh, unprocessed *E. oleracea* fruit pulp ($p \le 0.01$, Student's t test)

4. Discussion

In this study, *E. oleracea* fruit pulp has been assessed for its antioxidant activity as well as its TMAC, TFC, and TPC, both immediately after its preparation and 24 h and 5 days later, at ambient temperature/ambient light-dark, ambient temperature/continuously dark, and under refrigeration/continuously dark. Our results showed that the antioxidant activity of the *E. oleracea* fruit pulp was hardly affected under any of the storage conditions after 24 h, but that it had

decreased 2- to 4-fold after 5 days; that the decrease in antioxidant activity was more pronounced at higher temperatures and in the presence of light when compared to refrigeration and in the dark; and that the loss of antioxidant activity seemed particularly associated with the degradation of anthocyanins and flavonoids rather than phenolic compounds in general. These observations are of relevance to communities that rely on *E. oleracea* fruit pulp preparations but lack modern-day processing and storage facilities to extend their shelf-life such as those in the Amazon including Suriname's interior.

The decrease in the antioxidant activity of the *E. oleracea* fruit pulp after 24 h and 5 days is in accordance with its much reported relatively high perishability [29-32]. The fruit pulp has a remarkably high antioxidant activity [1,2,6,8,9,12-14] that has been associated with a relatively high anthocyanin contents [13-16]. These compounds deteriorate relatively easily during prolonged storage, particularly at higher temperatures and/or in light [29-32] which, obviously, goes at the cost of their antioxidant activity and the associated health benefits [29-32]. The relatively high - thermo- and photosensitivity of the anthocyanins in the fruit pulp [29-32] manifest as a (brownish) discoloration in the presence of oxygen during prolonged storage [43]. Indeed, long-term studies on *E. oleracea* anthocyanins showed a decrease in their contents in the fruit pulp with increasing temperature [44]; a 3.5 times faster decline of their stability in whole, semiclarified, and clarified fruit pulp at 20 °C than at 4 °C [45]; and a faster decrease of their stability in powdered fruit pulp juice produced by spray drying at higher temperatures than at lower temperatures [46]

Similarly, the anthocyanins extracted from four *Berberis* species degraded with increasing temperature or exposure to light [47]; about 33% of the anthocyanins in a microencapsulated and spray-dried ethanol extract of black carrots was lost after 64 days of storage at 25 °C versus only 11% at 4 °C [48]; the antioxidant activity and the anthocyanin contents of mulberry fruit extracts substantially decreased upon storage for 10 h at room temperature under fluorescent light when compared to the freshly prepared extract [49]; the half-life of anthocyanins in several berry juices was considerably shorter at room temperature than in cold storage [50]; and nearly half of the anthocyanin contents of grape juice was removed at 20 °C and in the presence of light, whereas only about 30% was destroyed at the same temperature but in the dark [51].

A notable aspect of the time course of the antioxidant activity of the *E. oleracea* fruit pulp observed in the current study, was the (twice) greater reduction of the FRAP value when compared to the DPPH IC₅₀ value after the 5-day storage period. Many studies have reported a high correlation between the antioxidant activities found with both methods (see, for instance, references 52,53]). Nevertheless, assessments of, for instance, 927 freeze-dried vegetable samples [54], a series of carotenes and xanthophylls [55], and the stem extract from the Indonesian medicinal plant *Tinospora crispa* (L.) Hook.f. & Thomson (Menispermaceae) [56] for their antioxidant activity using different methods, resulted in substantial variations in activities and trends depending on the assay used.

These apparent discrepancies as well as that seen in the current study might be attributed to differential affinities of the FRAP and the DPPH assay to the antioxidants in test samples. For instance, the antioxidant mechanism of ascorbic acid and α -tocopherol (which are also present in *E. oleracea* fruit pulp [1,2]) involve hydrogen atom donation [57], and this may render the pulp more susceptible to the DPPH assay (that is also based on the donation by an antioxidant of an electron or hydrogen radical to the stable DPPH free radical [39]) than to the FRAP assay (that is based on the donation by an antioxidant of an electron to a ferrous (Fe²⁺) ion [38]). Clearly, this supposition must be clarified in future studies.

Finally, the strong association seen in the current study between the antioxidant activity of the *E. oleracea* fruit pulp with particularly its TMAC and TFC rather than with its TPC in general, is in accordance with the apparent key role of anthocyanins (such as cyanidin 3-glucoside and cyanidin 3-rutinoside [58-60]) and non-anthocyanin flavonoids (such as the flavones orientin and isoorientin [61,62]) in the antioxidant activity of the plant. These observations may be attributable, among others, to the presence of multiple hydroxyl groups in the chemical structure of anthocyanins and other flavonoids when compared to non-flavonoid phenolic compounds, providing them the capacity to readily eliminate ROS [63]. Contributing to the relatively strong antioxidant activity of flavonoids is their ability to chelate metal catalysts involved in the generation of free radicals [64,65] and to activate antioxidant enzymes and inhibit enzymes involved in the formation of ROS and other reactive species such as those produced in the NADPH oxidase complex [66]. Indeed, TMAC as well as TFC seem to be better predictors of the antioxidant activity of *E. oleracea* fruit pulp than TPC.

Summarizing, the results from the current study indicate that the *E. oleracea* fruit pulp was rather stable after storage for 24 h at ambient temperature/ambient light-dark, retaining almost all of its antioxidant activity and 40-70% of its anthocyanin contents; that the antioxidant activity had decreased 2- to 4-fold and the anthocyanin contents by 60-80% after 5 days; but that the anthocyanin content was then (about twice) more stable under refrigeration and in the dark. These findings are particularly relevant to communities who rely on this preparation as a source of antioxidants but

who lack proper processing and storage facilities to prevent its decay, such as those in Suriname's hinterland and other parts of the Amazon.

However, the relatively small sets of data in the current study (those found for the fresh, unprocessed *E. oleracea* fruit pulp and those found after storage for 24 h and 5 days) makes it difficult to determine the statistical significance of the correlations between pairs of FRAP values, DPPH IC₅₀ values, TMACs, TFFs, and TPCs. Furthermore, *E. oleracea* fruit pulp contains several antioxidants other than phenolic compounds (such as ascorbic acid, tocopherols, carotenoids, and polyunsaturated fatty acids) as well as several nutrients that are less susceptible to degradation [1,2], implying that part of its antioxidant activity and nutritional value may well be preserved after prolonged storage. Furthermore, the antioxidant and anthocyanin stability of *E. oleracea* fruit pulp are also affected by various other common factors besides temperature and light, such as oxygen and pH [29-32]. Therefore, follow-up studies should be carried out that employ more measuring points during prolonged storage periods under different conditions; assess the antioxidant activity and nutritional value (f most of) its anthocyanins, non-anthocyanin flavonoids, and non-flavonoid phenolics have been degraded; and investigate "natural" plant-derived additives from the Amazon which are able to improve its stability.

5. Conclusion

The results from this study indicate that the antioxidant activity as well as TMAC and TFC of *E. oleracea* fruit pulp were rather stable after storage for 24 h under basic storage conditions (ambient temperature/ambient light-dark, ambient temperature/continuously dark, and under refrigeration/continuously dark), but that these features had decreased up to about 20% of those of the fresh, unprocessed material after 5 days. The decrease after 5 days was less by storage under refrigeration/continuously dark. These observations are particularly relevant to communities that are deprived of modern forms of energy and consequently, modern processing and storage facilities such as those in Suriname's hinterland and other parts of the Amazon.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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