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ARTICLE

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Acai fruit is recognized for its health promoting properties. However, there is still a need to address the effects of industrial processing on this fruit. In this study, phenolic content, anti-inflammatory and dermal wound repair properties of 20 acai samples, before and after industrial processing, from various Amazon regions were investigated. Acai pulp was rich in total phenolics (18.9-58.8 mg g^{-1}) and proanthocyanins (9.8-43.1 mg g^{-1}), but contained trace anthocyanins (up to 0.1 mg g^{-1}). Industrially processed samples lost substantial amounts of proanthocyanidins (up to 83.2%), while the anthocyanins inherently present were greatly enriched after processing (20-fold higher). Non-processed acai pulp extracts protected against early inflammation response which was correlated with proanthocyanidins, by significantly inhibiting nitric oxide production and suppressing pro-inflammatory genes expression including interleukin-1β, cyclooxygenase-2, nitric oxide synthase, and interleukin-6. The promotion of dermal wound repair of acai seed and pulp extracts was mainly contributed by anthocyanins and other bioactive compounds. The anti-inflammatory effect was diminished but wound healing effect was retained after pulp processing, suggesting the processing technology needs to be improved to maintain biological properties of acai fruit.

Introduction

Acai (*Euterpe oleracea* Mart.) is a berry-like fruit native to the Amazon region, which has received increasing attention in recent years due to its high content of antioxidants.[1] Several acai varieties have been reported which were differentiated based mainly on the color of the fruits. The purple and white fruits are the main commercially available types of *E. oleracea*.[2] Acai is consumed by locals of the northern Brazilian populations and accounts for up to 42% of the Amazon community diet.[3] Nowadays, its consumption has been extended to the southern and south-eastern regions of Brazil, and in many other areas as well, such as Europe, United States, Japan, and China.[1, 3] The consumed part of acai is mainly the pulp which produces different types of foods and is commercialized as frozen pulp, juice, beverages, powder, jelly, liqueur, among others.[4] The seed, accounting for 80 to 95% of the volume of acai fruit, is usually treated as pig food, natural fertilizer or waste.[5, 6]

In Latin America, many geographically isolated communities with limited access to medical care, traditionally use the acai stem or leaf to treat snake bites and muscular aches and leaves to relieve chest pain. A dark green oil produced from the seeds is used as an antidiarrheal agent. In many parts of South America, the roots are used against malaria and to alleviate strong muscle, back or sciatic pains or even kidney and liver disease and all skin ulceration healing problems. Roots are also used as a subcutaneous treatment against leishmaniasis, and diarrhea treatment.[1]

Recently, acai has received much attention as a functional food due to its chemical composition and health related bioactivities, which have led to its extensive study.[2, 7, 8] The health benefits associated with acai are mainly attributed to the presence of high levels of various phenolic compounds including phenolic acids, anthocyanins, proanthocyanidins, and other flavonoids.[9] Among the various biological activities of acai, the high antioxidant capacity, and anti-inflammatory activities are the most described.[1, 10, 11] Acai was reported to be a potential cyclooxygenase (COX)-1 and COX-2 inhibitor but showed a weak effect on nitric oxide (NO) production in lipopolysaccharide (LPS)-induced mouse splenocytes.[12] The unique flavonoid velutin isolated from acai pulp exhibited strong anti-inflammatory activity by reducing proinflammatory cytokine tumor necrosis factor (TNF)-α and interleukin (IL)-6 production. It also inhibited nuclear factor (NF)-κB activation as well as mitogen-activated protein kinase p38 and JNK

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phosphorylation in lipopolysaccharide-induced RAW 264.7 peripheral macrophages and mice peritoneal macrophages.[13]

Other bioactivity mechanisms may also contribute to the overall health benefits of acai. To maintain skin integrity and protect the body from infection, effective wound healing mechanisms are essential. The efficiency of these mechanisms decreases with age and chronic wounds, which is a worldwide problem.[14] There are a variety of topical treatments in the market that are commonly used for wound healing, such as antibiotic ointment, immunosuppressive agents, and antiseptics. However, many of them contain fusidic acid sodium or triamcinolone acetonide which have side effects, are costly, or inefficient.[15, 16] Natural plant extracts are an effective and economical means to maintain and improve skin health, so it is important to discover natural remedies with effective wound healing properties.[17, 18] Recently, wound healing effects of acai berry water extract were studied, and a possible underlying mechanism involved was confirmed by using *in vitro* human normal fibroblast cells model, and *in vivo* model based on macroscopic and histopathological observation on skin and oral mucosa of Sprague-Dawley rats.[16, 19]

Due to its various health benefits, the Amazonian acai has been investigated for the food, cosmetic and pharmaceutical industries.[1] The effects of different processing technologies on bioactive content of acai and other exotic fruits and their products have been reviewed and summarized.[20] In addition, different processed acai products, such as frozen pulp, spray-dried and freeze-dried powders were investigated.[21] Fibigr and co-works developed a rapid UHPLC-UV method to determine anthocyanins from different acai supplements.[22] A recent study determined the anthocyanins and other flavonoids of twenty acai supplements in a variety of forms (capsule, powder, frozen pulp, and liquid). The data indicated that over half of the supplements contained little or no acai fruit, while many had sufficient amounts of water to substantially lower the concentration of acai chemical components.[23] Moreover, two of the investigated acai supplements contained unlisted ingredients that greatly altered the product's chemical properties.[23] The results showed great variability in the anthocyanin content of the tested processed samples.[23]

The aim of this study was to comparatively analyze the non-processed and industrially processed acai fruit for phenolic content and composition using spectrophotometric assays and chromatography techniques, and to test their anti-inflammatory and wound healing properties *in vitro*. The relationship between polyphenolic content and bioactivities within different varieties, seed, and pulp of acai from different Amazonian regions in Brazil has been statistically analyzed using Pearson's correlation analysis and principal component analysis. To the best of our knowledge, this study was the first to compare the differences between the phenolic content of non-processed and industrially processed acai products from different sources and to compare their bioactivities.

Materials and methods

Acai collection and preparation

Fresh acai samples were collected from a local market in Belém (Pará State, Brazil) where the acai is harvested by native residents from different areas of Amazon forests or islands of Ilhas or along Amazonian rivers with the exception of samples PA-IC-P and PA-IM-P, which were obtained from Point do Acai and Acai Santa Helena, respectively. Industrially processed acai pulps were obtained from three companies. The sample code, variety, origin, type, location obtained, and harvest time information are shown in **Table 1**.

Fresh acai berries from the local market were washed and softened in a tank of water, and the pulps (the outer layer of the fruit) were separated from seeds by hand. The seeds of PA-IC-P and PA-IM-P samples were removed mechanically by a pulping machine in the industry. The rest of the acai fruits from different companies, after machine removal of their seeds, had further been processed to the final pulp products - fluid (moisture content 92%), medium (moisture content 89%), thick (moisture content 86%), and freeze-dried. All acai seeds and pulps (from the fresh market or industrially processed) were freeze-dried, ground into powder, and subjected to an extraction procedure as follows.

Acai extraction

Extraction of acai freeze-dried materials was conducted following our previous procedure with minor modification.[24] The lyophilized berry pulp and seed powders (0.5 g, in duplicate) were weighed into 15-mL centrifuge tubes and extracted with 8 mL of 80% aqueous methanol (0.5% acetic acid), sonicated for 10 min and centrifuged (Sorvall RC-6 plus, Asheville, NC, USA) for 10 min at 3452 g-force at 4 °C. The supernatant was transferred into a 25-mL volumetric flask. The residue was then extracted two more times as above, and supernatants were collected all together and brought to a final volume of 25 mL. The hydro-methanol extract was filtered (PTFE syringe filter, 0.2 µm pore size, Thermo Scientific) and used for all polyphenol investigations. An aliquot was evaporated to a dry residue, made to the desired concentration with 80% hydro-ethanol and used for *in vitro* cell-based assays.

Total phenolic content

Total phenolic content (TP) was determined calorimetrically by a modified Folin-Ciocalteu procedure in microplates.[25] Briefly, in a 96 well-plate, 75 µL of distilled water were added, followed 25 µL of the diluted samples or standards (in triplicate), and 25 µL of Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) (diluted with distilled water 1:1 v/v). After 6 minutes, 100 µL of 7.5% sodium carbonate solution were added to the mixture. The plate was then left in the dark for 90 minutes and read using a UV-Vis plate reader (SpectraMax® M3, Sunnyvale, CA, USA) at 765 nm. The UV-vis measurements were calibrated against gallic acid (Sigma-Aldrich, St. Louis, MO, USA) and the TP Results were presented as mg of gallic acid equivalent.

Anthocyanin content

Anthocyanin (ANC) profiles were analyzed by HPLC-DAD using an Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with a photodiode array detector (DAD) set

at 520 nm. The chromatographic separation was performed on an RP Supelcosil-LC-18 column, 250 mm × 4.6 mm × 5 µm (Supelco, Bellefonte, PA, USA) according to our previously described method.[26] Anthocyanin content was quantified by cyanidin-3-glucoside or peonidin-3-glucoside (ChromaDex Inc., CA, USA) standards which have the same aglycone.

Proanthocyanidin content

Proanthocyanidins (PAC) were separated using an Agilent 1200 HPLC with fluorescence detector (FLD) (excitation at 230 nm and emission at 321 nm) and a Develosil Diol normal phase column (250 mm × 4.6 mm × 5 µm, Phenomenex, Torrance, CA, USA), as described earlier.[26] Total PAC concentration and different degrees of polymerization (DPs) content were calculated based on a procyanidin B1 standard reference (ChromaDex).

Table 1 Variety, origin, type information, sampling location and harvest time of acai samples.

#	Code*	Variety	Origin	Original materials before lab freeze-drying		Sampling Location	Date harvested
				Type	Moisture content (%)		
	Non-processed Seed						
1	WB-GE-S	White Bacapa	Genipauba	Seed	46.4	Local market	4/9/2018
2	PB-GE-S	Purple Bacapa	Genipauba	Seed	39.4	Local market	4/9/2018
3	PA-IL-S	Purple Acai	Ilhas	Seed	47.5	Local market	4/9/2018
4	PA-MA-S	Purple Acai	Macapa	Seed	54.9	Local market	4/5/2018
5	PA-AN-S	Purple Acai	Anajas	Seed	50.2	Local market	4/6/2018
	Non-processed Pulp						
6	WB-GE-P	White Bacapa	Genipauba	Pulp	27.9	Local market	4/9/2018
7	PB-GE-P	Purple Bacapa	Genipauba	Pulp	32.2	Local market	4/9/2018
8	PA-IL-P	Purple Acai	Ilhas	Pulp	29.6	Local market	4/9/2018
9	PA-MA-P	Purple Acai	Macapa	Pulp	30.6	Local market	4/5/2018
10	PA-AN-P	Purple Acai	Anajas	Pulp	31.7	Local market	4/6/2018
11	PA-IC-P	Purple Acai	Ilha-Combu	Pulp	33.2	Point do Acai	4/10/2018
12	PA-IM-P	Purple Acai	Igarape-Miri	Pulp	35.5	Acai Santa Helena	4/10/2018
	Industrially processed pulp						
13	PA-IC-PM	Purple Acai	Ilha-Combu	Pulp (medium)	89.0	Point do Acai	4/10/2018
14	PA-AB-PL	Purple Acai	Abaetetuba	Pulp (fluid)	92.0	Acai Santa Helena	2/11/2018
15	PA-IM-PL	Purple Acai	Igarape-Miri	Pulp (fluid)	92.0	Acai Santa Helena	4/10/2018
16	PA-IM-PM	Purple Acai	Igarape-Miri	Pulp (medium)	89.0	Acai Santa Helena	3/2/2018
17	PA-PA-PT	Purple Acai	Paragominas	Pulp (thick)	86.0	Acai Santa Helena	1/2/2018
18	PA-OB-PM	Purple Acai	Obidos	Pulp (medium)	89.0	Acai Amazonas	--
19	PA-OB-PT	Purple Acai	Obidos	Pulp (thick)	86.0	Acai Amazonas	--
20	PA-OB-PD	Purple Acai	Obidos	Pulp (freeze-dried)	0	Acai Amazonas	--

*The codes of acai berry samples: first two letters for the variety, second two letters for the origin, last letter(s): S for seed, P for pulp, PL for fluid pulp, PM for medium pulp, PT for thick pulp, and PD for freeze-dried pulp.

Cell culture

Murine macrophage RAW 264.7 cells (ATCC®, Rockville, MD, WI, USA) were maintained in Dulbecco's modified Eagle's medium (DMEM Life Technologies, Grand Island, NY) with 100 IU mL^{-1} penicillin, 100 µg mL^{-1} streptomycin (Thermo Fisher Scientific, Waltham, MA), and 10% fetal bovine serum (FBS, Life Technologies, Long Island, NY). Human dermal fibroblasts cells isolated from adult skin (HDFa, Invitrogen C-013-5C) were cultured in Medium 106 (Invitrogen M-106-500) with low serum growth supplement (LSGS, Invitrogen S-003-10) supplemented with 100 IU mL^{-1} penicillin, 100 µg mL^{-1} streptomycin according to the previously reported protocol.[27]

Cell viability assay

Viability tests for both cell lines were determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] assay in triplicate and quantified spectrophotometrically at 550 nm using a microplate reader SynergyH1 (BioTek, Winooski, VT, USA).[27]

Anti-inflammatory *in vitro* assay

The anti-inflammatory activity of samples was evaluated according to our previously reported protocol.[27] The RAW 264.7 cells were seeded into 24-well plates and treated with 100 µg mL^{-1} of acai berry extracts and stimulated by 10 ng mL^{-1} of lipopolysaccharides (LPS, from Escherichia coli O127:B8), then incubated for 18 h. 10 µM of dexamethasone (DEX) was used as positive control.

Nitric oxide radical inhibition assay

The accumulated nitric oxide (NO) production in culture medium supernatant was determined by a colorimetric assay using the Griess reagent system (Promega Corporation, WI, USA) according to manufacturer protocol. The absorbance was recorded at 540 nm. NO levels were calculated based on a calibration curve built with serial concentrations of sodium nitrite. Results were expressed as NO production (%) relative to LPS induction.

Biomarkers of inflammation by gene expression analysis

The RAW 264.7 culture medium supernatant was removed, then the cells were harvested in TRIzol reagent for total RNA isolation, and cDNA synthesis. Pro-inflammatory gene

expression analysis was conducted by a real time quantitative PCR method, adopting a previously reported method.[27] Results were expressed as mRNA fold ratio relative to LPS induction.

Skin fibroblast migration assay

The dermal wound repair properties of acai berries were evaluated with the *in vitro* skin fibroblast migration assay in 96-well Oris plates (Platypus Technologies, LLC, Madison, WI, USA) according to the manufacturer protocol. HDfa cells were seeded into 96-well plates (with stoppers firmly sealed against the bottom for wound induction) and cultured to permit cell attachment, after which the HDFa cells were labeled with fluorescent dyes (NucBlue Live Cell Stain and CellTracker Red CMTPX, at 1 µM). Once confluence was reached, well inserts and growth media were removed to expose a central, cell-free zone initiating cell migration. After washing by sterile PBS (phosphate-buffered saline) and adding fresh growth medium, cells were treated with 125 µg mL^{-1} of acai extracts, vehicle (80% ethanol) as blank control, and a positive control (10% FBS). Fully confluent cells and cell-free wells served as references. Cell migration into the central zone was quantitatively assessed at 0 and 48 h by cell-specific fluorescence measurement (Synergy H1, Biotech, Winooski, VT, USA), at excitation/emission wavelengths of 360/460 and 577/605 nm. Fluorescence images of wound area were photographed by the EVOS FL Auto Cell Imaging System (Life Technologies).

Statistical analysis

Data were analyzed by one-way ANOVA in order to assess the significance of the acai preparation. Post hoc analyses of differences between experimental groups and Pearson's correlation analysis were conducted by Tukey's multiple comparison test; differences between LPS or CON and tested groups were assessed by Dunnett's multiple comparison tests; and heat map was performed using software Prism 8.1.1 (GraphPad Software, San Diego, CA, USA). Principal component analysis (PCA) was carried out on the quantitative data for total phenolics, individual and total anthocyanins, total and different degrees of polymerization (DPs) of proanthocyanidin content. The dataset was organized in a matrix with 20 rows corresponding to the non-processed and industrially processed acai berry in Brazilian Amazon geographical variability and 18 columns corresponding to the concentrations of total phenolics, anthocyanin and proanthocyanidin data, PCA calculations were performed using the software SPSS 26 (SPSS Inc, Chicago, IL, USA).

Results and discussion

Total phenolics, anthocyanins and proanthocyanidins

All acai fruit seeds and pulp extracts were analyzed for their polyphenol content. **Table 2** shows the concentration of phenolic constituents inherent in all extracts, and final concentrations were expressed as mg g^{-1} dry weight (DW).

As recorded in Table 2, non-processed Purple Acai variety pulp extracts group (#7-12) contained the highest TP levels, 28.8 to 58.8 mg g^{-1}. Non-processed White and Purple Bacapa varieties (#6,7) contained lower TP compared to Purple Acai variety, 18.9 and 20.2 mg g^{-1}, respectively. Industrially processed Purple Acai variety pulp extracts (#13-20) from the three industrial sources had a variation in TP ranging from 15.6 to 26.6 mg g^{-1}. PA-IC-P (#13) from Point do Acai company and PA-IM-P (#15,16) from Acai Santa Helena lost nearly half TP content after being industrially processed to fluid, pulp or medium pulp products. Acai Santa Helena samples (#14-17) included Purple Acai varieties from Abaetetuba (AB), Igarape-Miri (IM), and Paragominas (PA) (**Table 1**), among which PA samples (#17, 25.4 mg g^{-1}) showed TP in the acai extract significantly higher than in from the other two origins. TP of the acai extract from PA (#17) and from Ilha-Combu (IC, #13) (Point do Acai) (26.6 mg g^{-1}) were not significantly different from non-processed acai pulp extracts from IM (28.8 mg g^{-1}). The TP of the three product extracts of company Acai Amazonas (#18-20), which were from the same area Obidos (OB), were not significantly different. TP from acai seed extracts (#1-5, 5.5-9.9 mg g^{-1}) were about 12.0%-50.0% of the corresponding acai pulp extracts.

In contrast to TP, ANC concentrations were significantly higher in the industrially processed pulp extracts group, which varied from 0.60 to 3.43 mg g^{-1} (**Table 2**). ANC of non-processed pulp extracts (0-0.11 mg g^{-1}), showed no significant differences with the values in seed extracts (0.03-0.17 mg g^{-1}), regardless the variety. The ANC of #6-8 and #12 samples were not detected. Among the anthocyanins, cyanidin-glucoside (Cyn-glu) and cyanidin-rutinoside (Cyn-rut) were identified as the major constituents (**Figure 1A**) in agreement with previous reports work.[28] Trace amounts of peonidin-glucoside (Peo-glu) and peonidin-rutinoside (Peo-rut) were also quantified in some tested samples, especially in the industrially processed acai pulp extracts. Comparing individual and total ANC of the acai pulp extracts from the same source before and after industrial processing, PA-IC-P (#13) was 17.5-fold enriched in Cyn-glu, 32-fold enriched in Cyn-rut, 7-fold enriched in Peo-glu, 10-fold enriched in Peo-rut, and 20-fold enriched in total ANC compared to #11. The ANC in PA-IM-P (#12) was under the quantification limit, but was able to be quantified for the two product extracts after industrial processing (#15,16). Interestingly, ANC of acai seed extracts (#1-5) were higher than those from acai pulp extracts from the same fruits (#6-12).

From **Table 2**, the contribution of compounds in the PAC group dominated the phenolic content of acai over the ANC group in all tested acai extracts, especially in non-industrially processed seed and pulp extracts, whose PAC constituted 31.0% to almost 100% of TP. Similar to TP, the

highest PAC level was in the non-processed Purple Acai (PA) variety pulp extracts group, 23.2-43.1 mg g^{-1} (#8-12), 2 to 4-fold the PAC value in non-processed White and Purple Bacapa variety pulp extracts (#6,7). PAC values in all industrial pulp extracts were not significantly different from each other, 5.2-7.9 mg/g (#13-20). PA-IC-P and PA-IM-P lost about 83.2% and 73.5% of PAC content during industrial processing, respectively. Compounds were separated according to their degree of polymerization (DP), and a representative compositional profile of PAC with various DPs for each group is presented in **Figure 1B**. The non-industrially processed pulp extracts showed clearly identifiable signals for oligomers with DP between 2-10, while polymers with DP > 10 were not resolved and co-eluted as a wide peak at retention times between ca.40 and 45 min. However, only monomer and polymers with DP < 10 were clearly shown in acai seed extracts and industrially processed pulp extracts. Concentrations of PAC, according to their DPs, were calculated as proanthocyanidin B1 equivalent, and the results are presented in **Figure 1C.** Polymers with DP > 10 dominated in the non-processed Bacapa variety seed and pulp extracts, 78.6% and 57.3% of total PAC, respectively. For non-processed Purple Acai variety (PA), average concentrations of monomer and polymers with DP > 10 represented 53.3% and 31.6% of total PAC in seed extracts, respectively, and 15.9% and 37.9% of total PAC in pulp extracts, respectively. DP1-DP10 of non-processed pulp extracts also constituted 2.4%-9.2% of total PAC. DP1 and DP2 of industrially processed pulp extracts constituted 67.1% and 14.0% of total PAC, respectively, while DP3-DP10 and polymers with DP > 10 presented 0%-7.1%. After industrial processing, contents of all DP groups decreased for PA-IC-P; whereas for PA-IM-P, DP2-DP10 and polymers with DP > 10 decreased, and DP1 increased 1.3-fold. These results would suggest that PAC may have degraded and lost during industrial processing.

Table 2 Polyphenol content, anthocyanins and proanthocyanins of acai samples.

#	Sample[1]	Total phenolics[2]	Anthocyanins[3]					Total proanthocyanidins[4]
			Cyn-glu	Cyn-rut	Peo-glu	Peo-rut	Total	
	Non-processed Seed							
1	WB-GE-S	9.45±1.23^{f}	0.02±0.00^{f}	0.01±0.00^{h}	ND[5]	ND	0.03±0.00^{g}	3.98±0.46^{ef}
2	PB-GE-S	5.47±0.36^{f}	0.03±0.00^{f}	0.07±0.00^{g}	ND	ND	0.10±0.00^{g}	4.77±0.31^{ef}
3	PA-IL-S	6.88±1.11^{f}	0.07±0.00^{f}	0.04±0.00^{h}	ND	ND	0.11±0.00^{g}	2.35±0.10^{f}
4	PA-MA-S	9.94±0.76^{f}	0.08±0.00^{f}	0.08±0.00^{g}	ND	0.01±0.00^{d}	0.17±0.00^{g}	3.08±0.02^{ef}
5	PA-AN-S	7.25±0.36^{f}	0.07±0.00^{f}	0.06±0.00^{gh}	ND	0.02±0.00^{d}	0.14±0.01^{g}	3.03±0.09^{ef}
	Non-processed Pulp							
6	WB-GE-P	18.91±1.02^{de}	ND	ND	ND	ND	ND	9.84±0.87^{d}
7	PB-GE-P	20.20±2.17^{de}	ND	ND	ND	ND	ND	11.63±1.33^{d}
8	PA-IL-P	57.57±1.19^{a}	ND	ND	ND	ND	ND	32.73±0.31^{b}
9	PA-MA-P	31.99±0.47^{b}	0.06±0.00^{f}	0.05±0.00^{gh}	ND	ND	0.11±0.00^{g}	23.19±0.79^{c}
10	PA-AN-P	36.66±0.47^{b}	0.02±0.00^{f}	0.02±0.00^{h}	ND	ND	0.03±0.00^{g}	35.60±1.45^{b}
11	PA-IC-P	58.83±2.18^{a}	0.04±0.00^{f}	0.03±0.00^{gh}	0.01±0.00^{b}	0.01±0.01^{d}	0.09±0.01^{g}	43.09±3.22^{a}
12	PA-IM-P	28.80±4.39^{c}	ND	ND	ND	ND	ND	28.79±2.03^{b}
	Industrially processed pulp							
13	PA-IC-PM	26.60±3.53^{c}	0.70±0.02^{c}	0.96±0.01^{d}	0.07±0.03^{a}	0.10±0.02^{ab}	1.82±0.02^{c}	7.25±0.01^{de}
14	PA-AB-PL	20.40±3.92^{de}	0.66±0.08^{c}	1.03±0.01^{c}	0.08±0.04^{a}	0.12±0.05^{ab}	1.88±0.00^{c}	6.65±0.42^{de}
15	PA-IM-PL	17.15±0.81^{de}	0.25±0.01^{e}	0.30±0.01^{f}	0.02±0.00^{b}	0.03±0.01^{cd}	0.60±0.03^{f}	7.91±0.67^{de}
16	PA-IM-PM	15.63±0.93^{e}	0.26±0.01^{e}	0.29±0.01^{f}	0.03±0.01^{b}	0.06±0.00^{bc}	0.65±0.02^{f}	7.34±0.88^{de}
17	PA-PA-PT	25.43±1.83^{c}	1.08±0.02^{a}	2.18±0.04^{a}	0.08±0.01^{a}	0.09±0.02^{ab}	3.43±0.09^{a}	6.31±0.31^{def}
18	PA-OB-PM	18.97±2.03^{de}	0.33±0.05^{e}	0.44±0.02^{f}	0.05±0.00^{a}	0.05±0.00^{bc}	0.87±0.05^{e}	5.31±0.27^{ef}
19	PA-OB-PT	19.78±0.39^{de}	0.91±0.00^{b}	1.07±0.00^{b}	0.10±0.02^{a}	0.13±0.02^{a}	2.21±0.04^{b}	5.21±0.59^{ef}
20	PA-OB-PD	22.19±2.08^{cd}	0.47±0.04^{d}	0.69±0.02^{e}	0.04±0.00^{b}	0.07±0.02^{ab}	1.28±0.08^{d}	5.35±0.14^{ef}

[1]The acai sample codes: first two letters for variety, second two letters for origin, third letter(s) for seed, pulp, or industrial form of product, please refer to Table 1 for details. [2]Total phenolics quantified by Folin Ciocalteu assay as mg gallic acid equivalent g^{-1}. [3]Anthocyanins were measured by HPLC, Cyn-glu and Cyn-rut were quantified as mg cyanidin-3-glucoside equivalent g^{-1}, Peo-glu and Peo-rut were quantified as mg peonidin-3-glucoside equivalent g^{-1}, Cyn=cyanidin; Peo=peonidin; glu=glucoside; rut=rutinoside. [4]Total proanthocyanidins measured by HPLC, quantified as mg proanthocyanidin B1 equivalents g^{-1}. [5]ND: Not detected. Results were expressed as mean ± SD (n=2). All concentrations were calculated based on dry weight. Means with different superscript letters within the same column are significantly different ($p < 0.05$).

Principal component analysis (PCA) was conducted to assess the association between polyphenolic constituents and determine whether non-processed and industrially processed acai fruits could be grouped according to their polyphenolic content. The loading plot (**Figure 2A**) enables examination of the association among the 18 variables, while the score plot (**Figure 2B**) provides an overview of the differences or classification among the 20 acai samples. Two principal components accounting for 94.2% of the total variation were obtained. Notably, the first component (PC1) accounted for 73.5% of the total data variance, with TP and PAC were the dominant features

(**Figure 2A**). TP, PAC, and content of different DPs were clustered together suggesting they were well positively correlated with each other. A significant positive correlation between TP and PAC could also revealed by Pearson's correlation analysis (**Figure 2C**) with correlation coefficient (r) =0.8866, p<0.0001. Total ANC content and individual ANC compounds dominated in the second component (PC2), which describes 20.7% of the total variability (**Figure 2A**), and were not correlated with TP. When the scores of each acai extract sample were examined in a two-dimensional plot (PC1 vs PC2, **Figure 2B**), a certain separation of samples into three groups was observed. The first group included the industrial acai pulp extracts group, in which the separation was attributable to ANC and DP1; the second group gathered non-processed Purple Acai variety pulp extracts in which the PAC and TP were the predominant; and finally in the 3rd group non-processed Bacapa variety pulp extracts and all seed extracts whose scores were low in both components. Meanwhile, the non-processed acai pulp extracts from

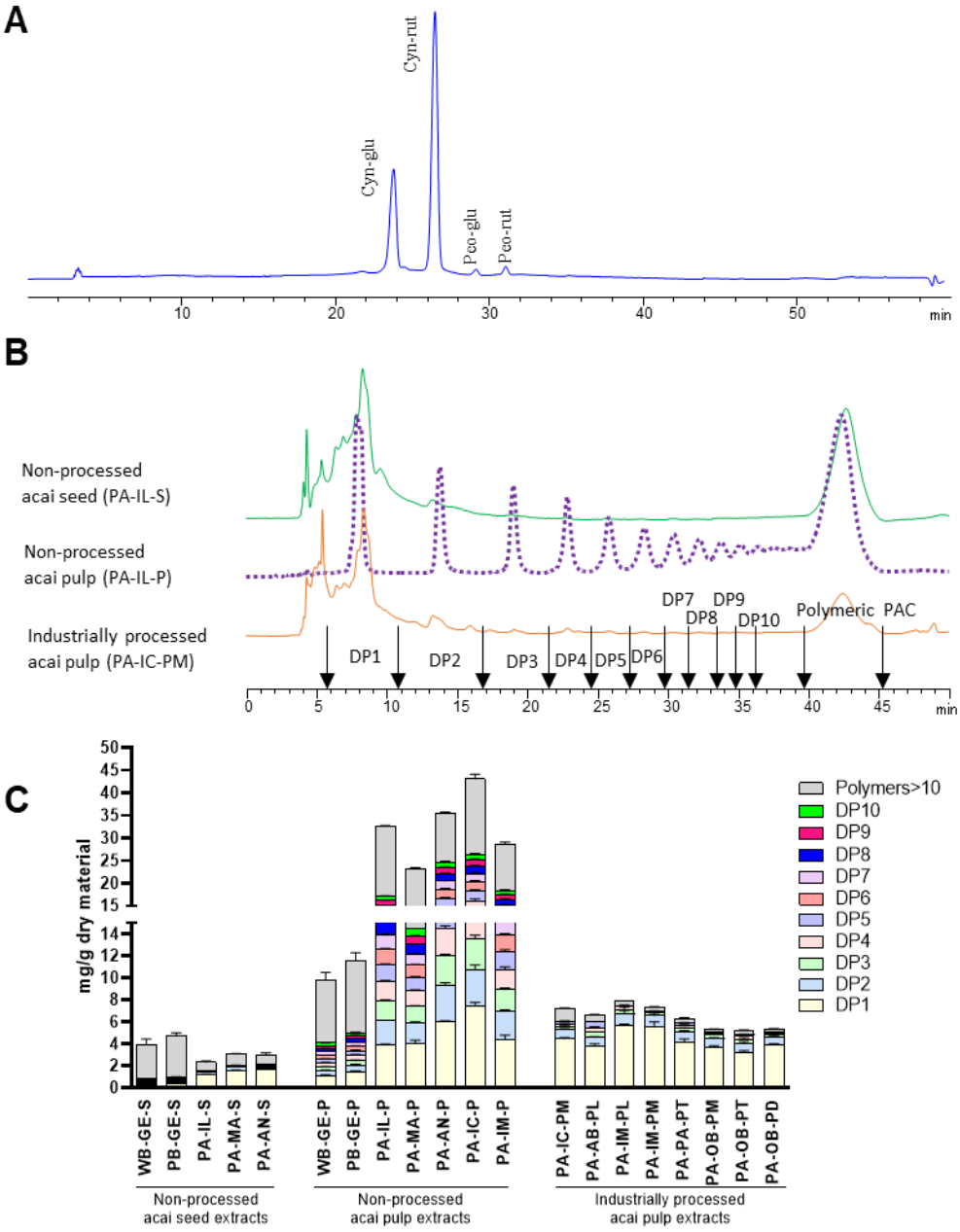

Figure 1 Representative HPLC chromatograms for anthocyanin (ANC), recorded at 520 nm, Cyn=cyanidin; Peo=peonidin; glu=glucoside; rut=rutinoside (A), HPLC–fluorescence detection (FLD) profiles for proanthocyanidin (PAC) compositions (excitation, 230 nm; emission, 320 nm) (B), degree of polymerization (DP), quantification was based on peak area and expressed as proanthocyanidin B1 equivalents (C). The sample code is in format of: first two letters for variety, second two letters for origin, third letter(s) for seed, pulp or industrial product form, please refer to Table 1 for details.

different Amazon regions mainly differed in PAC, especially from Genipauba (GE) where White and Purple Bacapa varieties were obtained. The differences in phytochemicals of the same variety from different regions may be due to such factors as the age of the palm tree, the maturation or the fruit exposure to sunlight.[29]

Overall, the total decrease in polyphenol and proanthocyanidin levels in industrially processed samples

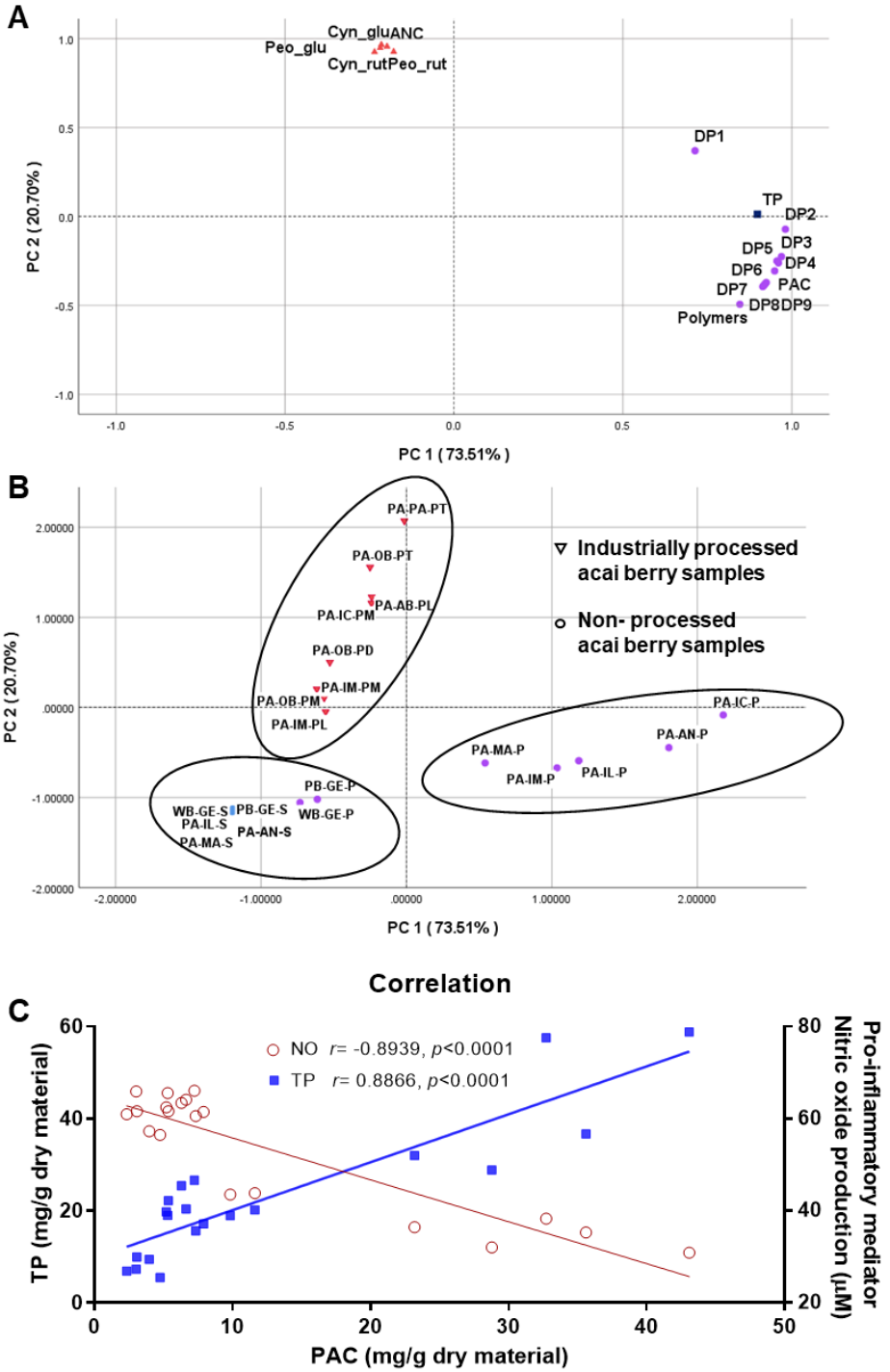

Figure 2 Principal components analysis: loading plot **(A)** and score plot **(B)** applied to non-processed and industrially processed acai fruit from Brazilian Amazon geographical variability. A 2D projection is presented using total phenolics, anthocyanin and proanthocyanidin compounds as variables. The acai samples is in format: first two letters for variety, second two letters for origin, third letter(s) for seed, pulp or industrial product form, please refer to Table 1 for details. Correlation between proanthocyanins (PAC), total phenolics (TP) of acai samples, and nitric oxide (NO) production in the LPS-stimulated RAW 264.7 macrophage cells pre-treated by acai berry extracts **(C)**.

may suggest that they were lost and degraded during the processing of the acai fruit. The same was observed in samples sourced from the same region and processed by the same company suggesting that changes of TP and PAC might be due to the delayed processing of the materials after harvest, washing and pulp softening, pasteurization or other processing steps. On the other hand, the increase in anthocyanin levels suggested they were enriched during some steps of industrial processing. However, because of the proprietary reasons, the details of the industrial processing steps were not disclosed. Therefore, further investigation would be necessary in order to provide a plausible explanation to the relationship between decreased proanthocyanidins and increased anthocyanins.

Effects of acai extracts on cell viability

Cytotoxicity of acai berry extracts to RAW 264.7 and HDFa cells at different treatment concentrations was evaluated through the MTT assay after 24 h of exposure. Non-cytotoxicity results to the cells was considered in the range 50-125 μg mL^{-1} (data not shown), concentrations that are easily achievable in the gastrointestinal tract after consumption of berries.[30]

Impacts on LPS-stimulated inflammatory response variables

Inflammation has been considered as a common immune and physiological response to various diseases.[31] To determine the anti-inflammatory activity, extracts from acai seed, industrial and non-processed acai pulp were pre-treated with RAW 264.7 macrophages at 50 μg mL^{-1} before LPS-activation. None of the samples suppressed the production of NO (data not shown). Therefore, a higher concentration at 100 μg mL^{-1} was applied to allow the detection of the activity.

Acai seed extracts and industrial acai pulp extracts still showed no inhibitory effect to NO production at 100 μg mL^{-1} (**Figure 3A**). However, all non-processed acai pulp extracts showed significant suppression activity by inhibiting 26%-48% NO production. Compared to acai seed extracts and industrial acai pulp extracts, non-processed acai pulp extracts contained higher PAC levels,

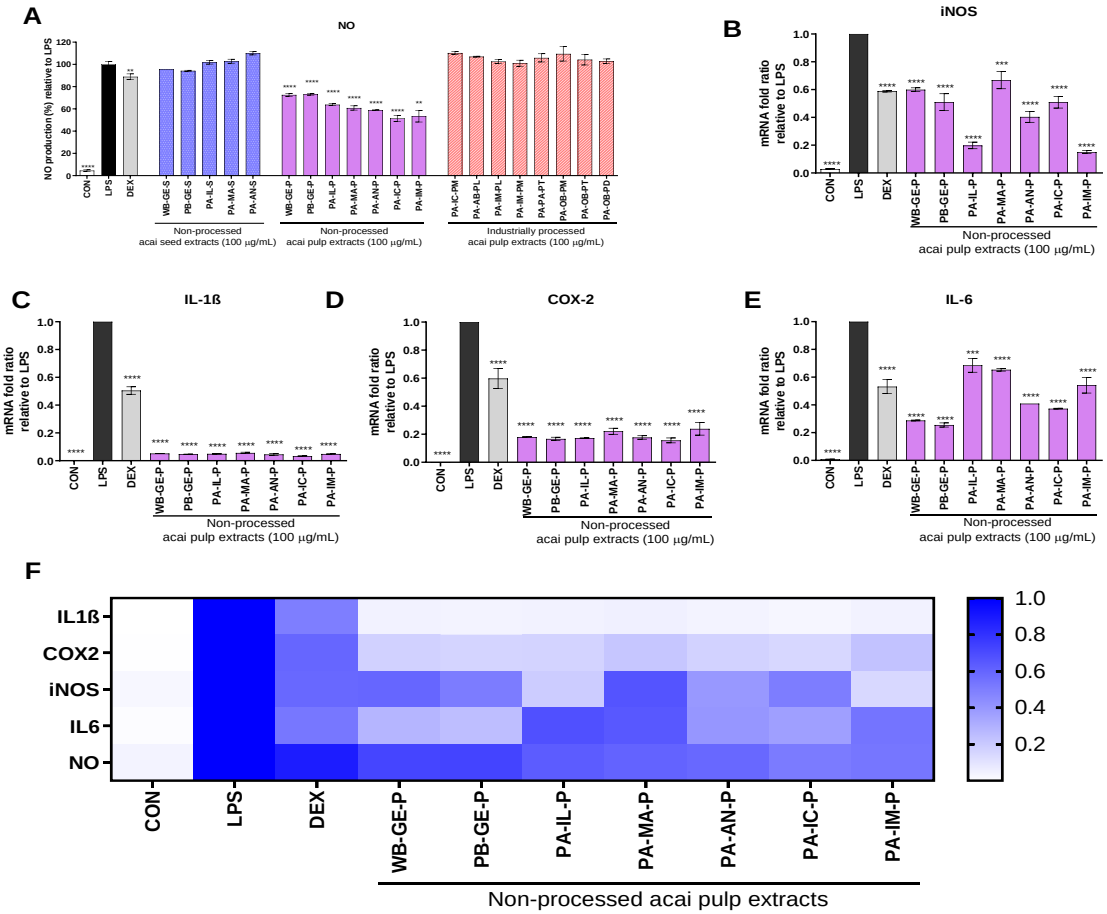

Figure 3 Effect of acai extracts on nitric oxide (NO) production **(A)**, and on proinflammatory gene expression **(B-E)** in the lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells. Heat map of NO production fold to LPS and gene expression from four genetic inflammatory biomarkers **(F)**. Cells were treated with acai extracts at concentration 100 μg mL^{-1}. Control (CON) is the absence of both LPS and acai extracts. Dexamethasone (DEX) was used as positive control. Results were expressed as means ± SEM, n=2. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ vs. the LPS treated group. One-way ANOVA, Dunnett's post hoc test. The acai samples were coded in the format: first two letters for variety, second two letters for origin, third letter(s) for seed, pulp, or industrial product form, please refer to Table 1 for details.

and there was a significantly negative correlation observed between the PAC level and LPS-mediated NO production in RAW 264.7 cells for all tested samples (r=-0.8939, p<0.0001). This may indicate that PAC was a significant contributor to the anti-inflammatory capabilities of acai extracts. In agreement, previous research also reported that the anti-inflammatory activity was partially due to the presence of PAC, or the combination with other phenolic compounds in the pistachio polar extracts.[32] PAC from purple maize were able to extremely inhibit pro-inflammatory Inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) gene expression, and moreover, proanthocyanidin enriched extracts from strawberry and blackberry exerted higher anti-inflammatory activity than anthocyanin enriched extracts.[18, 33]

Exposure of mammalian cells to LPS can lead to the release of proinflammatory cytokines and consequently activate inflammatory cascades.[31] Among the pro-inflammatory cytokines, interleukin-1β (IL-1β) produced by macrophages, induces early responses against infection or injury.[34] COX-2 is an inducible early response gene and derives prostaglandin E2 which is associated with increased inflammation. Inducible iNOS contributed to the NO production and can function as an upstream enhancer of inflammatory response.[35] Interleukin-6 (IL-6) can facilitate autoimmune phenomena, amplify acute inflammation, and promote the evolution into a chronic inflammatory state.[36]

As shown in **Figure 3B-E**, all non-processed acai pulp extracts had strong or moderate suppression effects on the four common genetic biomarkers (p<0.01). Expressions of IL-1β were almost attenuated to basal level. Expressions of COX-2 were also inhibited 76.1%-84.4%. The heat map of the relative mRNA expression of the four genes and NO production shows an overall anti-inflammatory effectivity of non-processed acai pulp extracts (**Figure 3C**). Note that color shade in **Figure 3C** indicates normalization of mRNA expression relative to LPS-induced cells, thus the lighter the color, the greater the decrease in expression relative to the positive (LPS) control. According to the distribution of colors, non-processed acai pulp extracts had a better protective effect against acute early inflammatory response. Moreover, Bacapa pulp extract had better potential to protect against the transition from acute to chronic inflammation by inhibiting IL-6. Inhibition of NF-κB activation and MAPK pathway has been reported as one potential mechanism to explain the anti-inflammatory activity of the acai polyphenols in the LPS-induced RAW 264.7 peripheral macrophages model.[13] Another study on anti-inflammatory action of acai PAC in acute lung inflammation induced by cigarette smoke in the mouse model, attributed the activity of acai to its capacity to reduce the expression of adhesion molecules.[37]

Impacts on HDFa migration in exclusion zone wound healing

Wound healing and skin repair are primary therapeutic targets of regenerative and cosmetic interventions designed to facilitate restoration of normal tissue architecture and function.[38] Three overlapping stages occur during the wound healing process, including the inflammatory, proliferation, and remodeling stages.[18] Cell migration plays a central role during wound healing. An

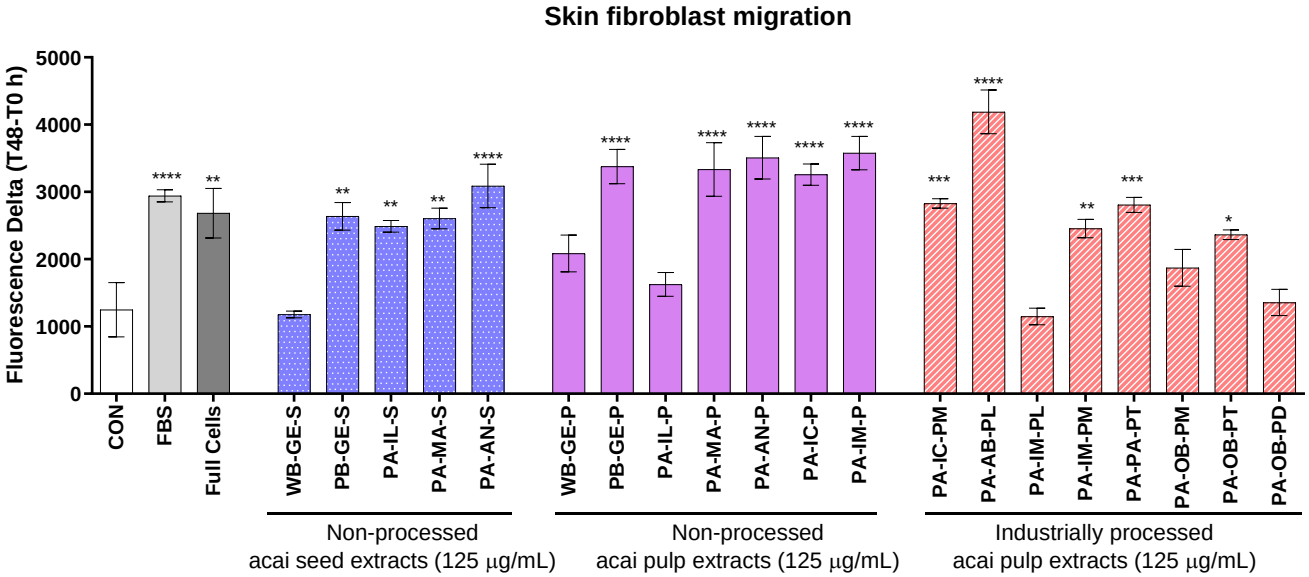

Figure 4 Effects of acai extracts on skin fibroblast migration. Cells were treated with acai extracts at final concentration 125 μg mL^{-1}, vehicle (80% ethanol) as blank control (CON), 10% fetal bovine serum as positive control. Full cells means no stopper used. Results were expressed as means ± SEM, n=4. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 vs. the CON group. One-way ANOVA, Dunnett's post hoc test. The acai samples were coded in the format: first two letters for variety, second two letters for origin, third letter(s) for seed, pulp or industrial product form, please refer to Table 1 for details.

Oris™ assay was conducted using silicone-based stoppers to create a central cell-free detection zone in each well of a 96-well plate to study the effect of acai extracts on HDFa. In comparison with conventional scratch wounding (mechanical wound), the exclusion zone (non-mechanical) assay has the advantages of better reproducibility, and it also avoids mechanical damage to cells or cell death after wounding. According to the results of the cell viability (cell proliferation) assay induced by different treatment concentrations of acai extracts, a concentration of 125 μg mL^{-1} was chosen to perform the HDFa migration assay.

Figure 4 shows that acai seed extracts accelerated fibroblast cell migration about 2-fold compared with the treatment vehicle after 48 h, with the only exception of the White Bacapa variety. The wound healing effects of acai seed extracts had a strong positive correlation with ANC (r=0.735, p<0.05). Although acai seed extract did not inhibit NO, it exhibited an effect on fibroblast cell migration. In addition, an *in vitro* cell bioassay study suggested acai seed extract effectively decreased human lung carcinoma cells at the concentration of 200 μg mL^{-1}.[39] Unlike for the anti-inflammatory activity, non-processed acai pulp extracts showed a slightly stronger wound repair property than the other two groups. Except for WB-GE-P and PA-IL-P, the other samples were able to accelerate fibroblast cell migration about 2.7-fold. Correlations between wound healing effects with TP, ANC, PAC for non-processed acai pulp extracts were not observed, suggesting that their activity is partially due to the presence of polyphenols. Other compounds in the acai pulp, still need to be identified, may also contribute to their bioactivity.[1] Industrially processed acai showed variable wound healing effects. While PA-IM-PL, PA-OB-PM and PA-OB-PD did not enhance the cell migration, the samples caused an enhancement in fibroblast migration, about 2 to 3-fold higher than the treatment vehicle. The wound repair capability of samples PA-IC-P and PA-IM-P decreased after processing by the respective companies. A moderate positive correlation between the wound healing effects of industrially processed acai pulp extracts and the ANC was found (r=0.504, p<0.05), indicating that ANC in combination with other phenolic compounds may be associated with their wound repair property. Wound repair capability of strawberry and blackberry were previously reported to be linked to their ANC.[18]

Some studies suggested that the mechanisms underlying the wound healing process are associated with antioxidant, anti-inflammation, growth factor and cytokines. Chronic inflammatory state experienced with age results in reactive oxygen species accumulation which damages the cell and proteins of the extracellular matrix and subsequently activates inflammatory cytokines and proteases, ultimately hindering wound healing.[40] Potent antioxidants can reduce reactive *oxygen* species (ROS) and NO to normal levels, decrease wound chronicity, and accelerate the healing process in animal models[41]. It was reported that anthocyanins potentiate cell migration patterns of fibroblasts and keratinocytes by promoting vascular endothelial growth factor production to enhance angiogenesis, besides suppressing the ROS generation and inflammatory response.[42] In addition, increased expression of fibronectin, which plays an important role in coagulation, epithelial cell movement, cell differentiation, collagen matrix assembly, and wound contraction in the wound healing process, was observed in human normal fibroblast cells when treated with acai berry water extract.[19]

Conclusions

Acai pulp was rich in TP, showing levels of PAC one or two orders of magnitude higher than ANC. Industrial processing, resulted in significant losses of TP and PAC, and higher degree of PAC polymers were substantially degraded, while causing an increase of the ANC. Acai seeds contained lower TP and PAC than the pulp. Polyphenol contents of acai pulp varied according to the source's geographical regions. However, differences in the composition between acai varieties were more obvious than the geographical variability. More sampling would be needed to assess regional effects. *In vitro* cell bioassays for anti-inflammatory activities showed that non-processed acai pulp significantly inhibited NO production, but no inhibitory effect observed after industrial processing. Non-processed acai pulp had a protective effect against acute early inflammatory response by significantly suppressing transcriptional levels of inflammatory regulatory genes including IL-1β, COX-2, iNOS, and IL-6. The anti-inflammatory effect of acai fruit may be due to their high level of PAC. Acai seed and pulp had a potential would healing property, and their effect may be contributed by ANC and the combination with other bioactive components. There is a strong need for natural products purposed for wound healing without side effects.[16] The results presented here show that acai seed (a by-product of acai processing) can become a valuable natural source, due to its benefit to skin wound repair and has potential for the future development of a wound healing agent.

Conflicts of interest

There are no conflicts to declare.

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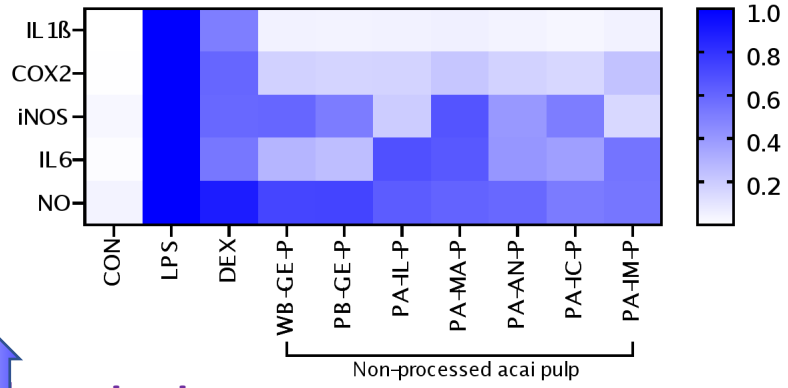
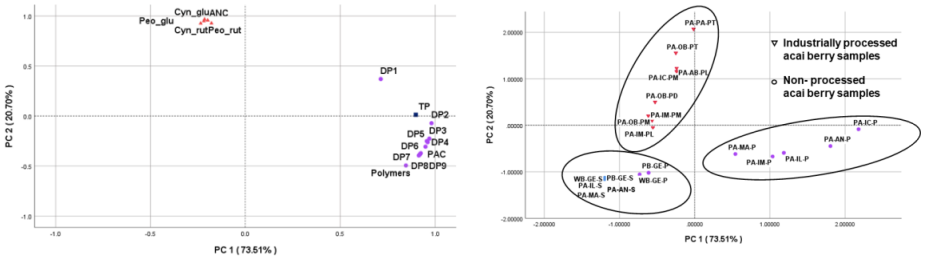
Industrial processing affected polyphenol content, anti-inflammatory, and dermal wound repair properties of Brazilian Amazon Acai extracts

Anti-inflammatory property



Principal component analysis for polyphenol content



Dermal wound repair property