IMPREGNATION OF AÇAÍ RESIDUE EXTRACTS IN SILICA-AEROGEL

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ABSTRACT. Açaí (Euterpe Oleracea mart.) is a black-purple berry, typically found in Amazon Rainforest. E. Oleracea product is a natural phytochemical source, which shows a high content of polyphenols and flavonoids. It also presents remarkable properties as antioxidant and natural dye. The antique population of South America used Euterpe Oleracea palm as medicine to treat different diseases and some health problems. At present, scientists know that its properties are related to the high amount of bioactive compounds, and efforts are focused on the extraction of these compounds, in order to be able to study the individual effect and advantages of each bioactive substance in vivo and in vitro and its application in pharmaceutical, cosmetic and alimentary products. This work studied the formulation of bioactive compounds extracted from pulp, slurry, and seeds of E. Oleracea fruit, by impregnation in silica-aerogel. Maceration, Microwave Assisted Extraction (MAE) and pressurized MAE (PMAE) were applied as extraction technique and as pre-treatment for extraction, respectively. Ethanol, water and ethanol/water (1:1) were used as solvents for the extraction, determining the efficiency and selectivity of the extraction for each solvent at temperatures of 60ºC and 80ºC. Extracts were characterized in terms of extraction yield, total polyphenols content (TPC), total Anthocyanins contents (TAC), and antioxidant activity by oxygen radical absorbance capacity (ORAC). For a typical extraction, solid/liquid (S/L) ratio was kept constant at 0.33 g/mL for slurry fraction, 0.4 g/mL for seed, and at 0.02 g/mL for pulp extraction. The main parameter used to compare the results of extraction was TPC. For both fractions of residue, the highest TPC values were found using ethanol/water (1:1) as solvent and performing extraction at 60ºC. Applying MAE (300W, 30seconds) as a pre-treatment of slurry material promoted an increase of TPC values, from 269 mgGAE/L to 735 mgGAE/L, after 30 minutes of extraction. Extraction of seeds was not affected by MAE (300W, 30seconds) pre-treatment. Nevertheless, seeds show higher potential as polyphenols source than slurry, in terms of TPC values (29057 mg GAE/L in the case of seeds compared to 735 mgGAE/L in slurry, at the same extraction conditions). In contrast, slurry shows potential as anthocyanins (Peoniding and Cyanidin) source, compounds that are not present in seeds extract. Moreover it is important to preserve the properties of extract to formulate the extracted material. For this purpose, silica gels prepared from tetramethyl ortosilicate (TMOS) precursors were then impregnated with different extracts obtained by wet impregnation, and the impregnated gels were then dried with supercritical CO_2. The aerogels thus produced were characterized considering their surface and morphology properties and the yield of impregnation of the extract.

Key-words. Intensification, green process, supercritical CO_2 dryer, formulation of natural product.

INTRODUCTION

Phytochemicals are biologically active chemical compounds, present in some plants, and a very attractive and studied area of modern chemistry because of their health benefits. Polyphenols are secondary metabolites of plants with remarkable properties as antioxidant and/or natural dye activities, and they are the subject of intense research because of their potential application in pharmaceutical, cosmetic and food products. Some of these properties are their antioxidant, anti-inflammatory and antimicrobial functions[1]. However, these types of compounds have complex structures. This implies that their synthesis reactions are complicated, expensive, and have a low selectivity leading to undesired by-products. In this situation, a viable alternative is extraction from natural products, and *Açaí* berry shows interesting characteristics as a potential source of polyphenols, fatty acids and essential oil.

Açaí is a black-purple berry obtained from *Euterpe Oleracea mart.* palm, typically found in Amazon Rainforest and strongly present in the diet of Brazilians, especially in north and northeast regions. The name comes from the Tupian word ïwaca'I – fruit that cries – in a Brazilian Portuguese adaptation. Although the fruit provides health benefits, it also causes environmental problems. During the season, a processing company produce 40 tons of residue per day. During the pulping process, pulp is separated from seeds (first fraction of residue), in a second step pulp is clarified by a filter where slurry (second fraction of residue) is separated, and finally pulp is characterized to certify that is safe for consumption. When a pulp batch is considered inappropriate for consumption, the batch is destined to residue in its totality (third fraction of residue). All fractions of products can be observed in the Figure 1.

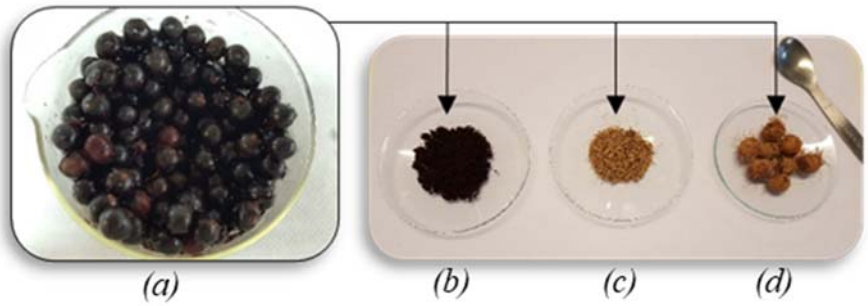

Figure 1 – *(a)* E. Oleracea (*Açaí*) *in nature*; *(b)* seed; *(c)* dry-slurry; and, *(d)* dry-pulp.

The main interesting compounds are identified in the pulp fraction. According to literature, in the pulp of Euterpe Oleracea mart. berry some important compounds are present, such as anthocyanins (Cyanidin, Peonidin, Pelargonin), flavonoids (Homo-Orientin, Orientin, Catequin Epicatechin, p-cumárico), prothocyanidins, and some other interesting products (quercetin [2], vanilic, ferulic, and gallic acid, and resveratrol[3]). These compounds, present in natural pulp, are expected in different concentration in *açaí* slurry. Moreover, each fraction has an oil content that can promote some impedance during the extraction of phenolic compounds if it is not previously removed. These oils also have commercial interest, especially the oil obtained from pulp, because of its high content of unsaturated fatty acids and essential oil. The main property of this oil is the antidiarrheal effects[4]. However, all studies about characterization of Açaí berry are based on pulp or seeds content, reason for which it is necessary perform a specific detailed characterization of its by-product.

Maceration is a simple and classic methodology that has been successfully applied to obtain bioactive compounds when combined with Microwave-Assisted Extraction (MAE) or pressurized MAE (PMAE) intensified extraction. Maceration is

normally performed using a dried plant as a matrix. In the case of large-scale matrix, the current literature recommends to decrease the size of granules to obtain a more homogeneous raw material. This is important especially for extraction from seeds. The starting material is placed in the extractor glass to soak with a mixture of water and organic solvent. The process takes place under room temperature, an advantage when it is compared with other classic extraction methods, which requires elevated temperature for a large period of time. However, the maceration procedure requires many hours to obtain a low yield of extract by itself. For this reason, microwave energy can be applied as a previous step in this traditional extraction process to enhance solid-liquid diffusion. MAE and PMAE are efficient pre-treatments to reduce the time spent in the maceration procedure because the act on the slow diffusion step of the extraction process[5]. Microwave provides energy able to heat the intracellular water; this hot water increases the pressure inside of vegetal-cell and causes the cell-wall rupture, finally releasing the internal content, and drastically accelerating the diffusion step. Evidences of these advantages have been observed in treatment of by-products of wine: skin and seeds of grapes; by MAE the time needed to extract the maximum amount of polyphenols decreased to only 3 minutes, against 60 min without MAE[6]. The increased of temperature provided by microwave energy change the dielectric constant and it also helps to reduce the time for extraction[7], contributing to reduce the degradation of the compounds during the extraction.

Recovery of these compounds would add additional value to the process; it helps to improve their applicability in products and provides protection against early degradation[8-9]. Silica gels prepared from tetramethyl ortosilicate (TMOS) precursors are porous materials which are very suitable for impregnation of compounds presents in extract promoting the slowly release of it in an application product. Wet impregnation followed by supercritical CO_2 was proposed as an efficient method in order to preserve the structure of the gels [10]. During the wet impregnation, the gels are immersed in the solvent, and after the ageing process, they are dried with supercritical CO_2.

MTERIALS AND METHODOS

Sample. Sample preparation is specific for each fraction of residue: first fraction (seeds) has an average diameter size of 1.2 cm, it needs to be milded at 5mm (knife-mill Retsch SM100) and then dried in an oven by 48 h at 45ºC. Second and third fractions (slurry and Pulp, respectively) are frozen at -80ºC and then lyophilized (Telstar LyoQuest) during 72 h. Finally, all fractions are ready to extract the oil content. For this, a known mass of each fraction is placed in an extraction thimble and placed at a Soxhlet apparatus, wherein hexane is applied as solvent to remove the oil content in each fraction. After extraction, hexane is evaporated under vacuum. Known initial and final mass used in the process make possible to determine the percentage of oil content in each fraction.

Extraction. Maceration and Pressurized Microwave Assisted Extraction (PMAE) were applied as extraction technique and as pre-treatment for extraction, respectively. Ethanol/Water (1:1) is used as solvent for the extraction, and citric acid as pH regulator. Extracts were characterized in terms of extraction yield, total polyphenols content (TPC), total Anthocyanins contents (TAC), and antioxidant activity by oxygen radical absorbance capacity (ORAC). HPLC is used to determine the extract composition. Hidroethanolic extracts are purified using Dioxan HB20 activated by methanol (1% HCl). Hexane extracts was purified helped by a vacuum drier (all hexane was recovered).

Preparation of aerogel monoliths. Silica gels were prepared by the use of tetramethyl ortosilicate (TMOS) as precursor. TMOS and methanol are mixed in a safe recipient where ammonium hydroxide-water was added droplet by droplet. Then, 1 mL of solution was transferred to cylindrical moulds and covered with a film for the properly gelification. The process of gelification is fast, and in few minutes alcohol-gels are ready to start the ageing process, where they will keep under solvent for 128 hours in order to strength its structure, washing them with new solvent every 24 hours in order to remove any trace of unreacted water. (*molar ratio:* 1 TMOS : 3 MeOH : 4 H2O : $5x10^{-3}$ NH4OH). [11]

Wet impregnation - indirect. After ageing process, alcohol-gels are transferred to different impregnation solutions containing different fractions of extraction, keeping them there for 72 hours, during which solution is replaced every 24 hours. Moreover, ethanol and methanol were tested as impregnation solvent, individually, and oil extract from the pulp by hexane was compared with an oil sample extracted from the pulp by supercritical carbon dioxide.

Wet impregnation - direct. Extract obtained was insert directly in sol-gel preparation, adding it to the solvent employed during the process.

CO_2 supercritical Drying. Drying process took place in a semi-closed circuit, including a buffer of CO_2, the chamber where monoliths were charged and a pump for CO_2 recycling. Initially, chamber is isolated from the rest of system and then charged with pure solvent, keeping monoliths submerged in the solvent to avoid damages in their structure. In a second step, the buffer is loaded with CO_2, that is compressed at 110 bar and heated at 40ºC. When these conditions are achieved, chamber is opened and CO_2 starts to flow through the system thanks to a pump. Supercritical CO_2 recycling is maintained during 1h, thus reaching saturation of CO_2 by the solvent. Then, the chamber is again isolated, CO_2 from the buffer is released in order to introduce fresh CO_2 into the system. Operating in this way, 3 cycles are performed in order to have completely dried silica aerogel. With this procedure, at the same time that silica are dried, as the impregnated compound is not soluble in CO_2, it is precipitated in the pores of the support by an antisolvent process[12].

Aerogel characterization. Previous to characterization aerogels were milded and degasified using temperature at vacuum. The specific surface area and average pore diameter was determined by sorption-adsorption of N_2 at low temperature method, and calculated by BET (Brunauer, Emmett, Teller) method. Average pore volume was described by desorption curve of N_2. Microporous material was calculated by Langmuir equation. Chemical bond-structures were analysed by FTIR (Bruker Platino-ATR).

RESULTS

Sample. Soxhlet extraction make possible to determine the percentage of fixed oil in each fraction of sample. Knowing the initial and final mass used in the process, it is possible to determine the percentage of oil content in each fraction. Results show that seeds have 7.5±0.1%, slurry has 6.8±0.1% and dry-pulp has 42.0±0.1% of oil content.

Extraction. Pure ethanol and pure water were also tested as solvent but results showed that water is not a good option solvent because polyphenols affinity and any residual oil content can make difficult the penetration of this solvent in the matrix. However, it was possible to identify the improvement caused by MAE pre-treatment in slurry and seeds. In the case that MAE was applied, the time to achieve the maximum extraction yield (MEY) was reduced to 10 min instead of 30min which is the minimum in any conventional treatment. Pure ethanol has potential as selective extraction solvent, 0.107(%) against 0.188(%) found for conventional Sohxlet extraction for slurry fraction.

Anyway, a non-specific solvent, as ethanol/water, shows more efficiency especially when the objective is anthocyanins content. Ethanol/water at 60ºC was the best extraction condition for slurry matrix, with a yield of 0.614(%) (MAE) against 4.428 (%) (Sohxlet characterization). In this case, the maximal yield extract was achieved using MAE as pretreatment. Experiments also show that temperature is a relevant parameter to be studied in extraction condition, as when it is increased by 20ºC the time required to achieve the MEY decrease in 30 minutes. Results do not show any substantial difference between maceration extraction and macerations assisted by MAE. However, the sample treated with PMAE yielded better results attributed to the associated fast increase in temperature. Ethanol/water at 60ºC was also the best extraction condition for pulp matrix and the maximal yield extract were achieved using PMAE as pre-treatment.

Wet impregnation. Initially, wet impregnation were performed just by indirect impregnation process and with low content of oil obtained from the three different residual fractions. Results in table 1 show a soft decrease of specific surface area for aerogels impregnated, taking pure silica as reference. This support is a mesoporous material of type IW according to IUPAC distribution[13]. It is possible to observe a small variation in surface area that might indicate that oil content has filled some pores. Also, taking this value as evaluation parameter, it is possible to conclude that ethanol works better as solvent for impregnation than methanol. Probably, it is related with low solubility of this oil in methanol. It was not found any substantial difference in density of oil obtained from pulp fraction by hexane extraction and supercritical extraction. However oil obtained by hexane extraction had less impedance to self-place inside the pores than oil obtained by supercritical oil extraction. In aerogel, it was not observed any substantial visual change in colour or opacity.

Table 1 – Specific Surface area (m^2/g), Average pore volume (cm^3/g), and Average pore diameter (Å) of Silica (pure and impregnated) using ethanol or methanol as solvent maturation.

Aerogels	Specific Surface area (m2/g)	Average pore volume (cm3/g)	Average pore diameter (Å)
Silica Blank	910	3.04	110
Silica + pulp/hex (methanol)	817	2.65	109
Silica + pulp/CO2 (methanol)	831	2.63	104
Silica + pulp/CO2 (ethanol)	768	2.55	111
Oil Slurry/Hex (ethanol)	835	2.84	112
Oil Seed/hex (ethanol)	859	2.91	110

Oil obtained from slurry and from seed was impregnated following the best result obtained from pulp. As it can be seen in Table 1, pore volume showed a lower reduction when it was impregnated with these oils than when it was impregnated with pulp oil, suggesting a lower impregnation yield, which may be related to differences in the composition of the three oil fractions.

According to table 1, after impregnation there was still a large number of pores that were not impregnated by oil. Then, in a second approach, monoliths were impregnated with a high charge of oil by direct and indirect wet impregnation. After Supercritical drying, monoliths impregnated show visual modifications (figure 2). When

oil was introduced directly in the process, aerogels become opaque and lightly green (figure 2(b). In another hand, gels submitted to indirect impregnation showed no opacity and a strong green colour (Figure 2(c)).

Figure 2 – Dry monoliths of silica gel impregnated by oil extracted from pulp by hexane: (a) as blank; (b) after direct impregnation; and, (c)after indirect impregnation.

According to results in Table 2, direct impregnation yielded a higher average pore diameter but did not interfere in pore volume, these patrons were observed for oil obtained from pulp by hexane and by supercritical CO_2 extraction. Moreover, the addition of oil in the alcohol used in the gel formation interfere in gelification time, because it acts changing the components ratio. Indirect wet impregnation did not interfere in average pore diameter, but showed a bigger decrease in specific surface area (table 2). The variation in pore diameter can indicate a change in mesoporous structure but also a low impedance to occupy the small pores that can increase the average result. The increasing of oil concentration had no effects under specific surface area, it can be related to the capacity of SC CO_2 in oil extraction. However, SC CO_2 has no affinity to polyphenols present in oil, which is a main source of the colour of the oil.

Table 2 – Specific Surface area (m^2/g), Average pore volume (cm^3/g), and Average pore diameter (Å) of Silica (pure and impregnate) using ethanol or methanol as solvent maturation.

Extract	Impregnation process	Dry Process	Specific Surface area (m^2/g)	Average pore volume (cm^3/g)	Average pore diameter (nm)
Blank	-	SC	910	3.04	110
Oil Pulp/hex	Indirect Wet	SC	848	2.89	109
Oil Pulp/hex	Direct Wet	SC	892	2.98	134
Oil Pulp/CO2(*)	Direct Wet	SC	827	3.06	155
Oil Pulp/CO2 (**)	Direct Wet	SC	887	2.91	126
Pulp/extract	Indirect Wet	air	830	0.26	36
Pulp/extract	Indirect Wet	SC	856	1.80	91
Pulp/extract	Direct Wet	Air	595	0.04	3
Pulp/extract	Direct Wet	SC	-	-	-

(*) high concentration (1400uL) (**) low concentration (200uL)

In a typical experiment of drying pure silica-alcogel, it is possible to note the effects of drying process on the structure. If gels air drying is used (Figure 3(a)), pores collapse, causing an important shrinkage of the monoliths.. In contrast, those monoliths committed to SC CO_2 drying keep their structure (Figure 3(b)). The same was observed with gels impregnated with pulp extract. The batch of these gels directly impregnated was divided in two parts: (1) dried by SC CO_2, and (2) dried by air in an open vessel. It is possible to observe how the second fraction (figure 3(c)) gets smaller when compared to blank dried by SC CO_2 (Figure 3(b)), but still bigger than a non-impregnated monolith dried by air (Figure 3(d)). This happens because of extract content that occupied the pores before drying collapsed, decreasing average pore volume to 0.26

(cm3/g) (Table 2). In both cases, extract interfered in the monoliths structure became brittle. Extract confer a purple colour in the monolith, the colour is due to anthocyanins present in extract, and the intensity of colour decreases after drying. Notwithstanding, aerogels obtained by SC CO_2 got successful impregnated. It is shown in the reduction of average pore volume and in the decreasing of pore diameter, which also suggested that bigger pores were occupied first. It is also detachable that pulp extract had less impedance compared to oil extract, more significant decreasing in value.

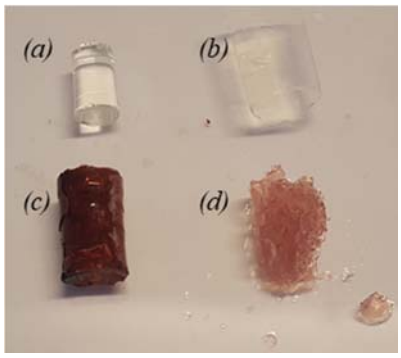

Figure 3 – Dry monoliths of silica gel impregnated by extractfrom pulp by ethanol/water: (a) blank, dried by air; (b) blank, dried by supercritical CO_2; (c) pulp extract, dried by air; and, (d) pulp extract, dried by supercritical CO_2.

When extract was added directly during alcogel preparation, the material did not gelify even after several hours. When the resulting solution was dried with air, a microporous structure with a small pore volume was achieved as presented in Table 2. This was due to the mesoporous structure collapsed during aird drying process. This confirmed that supercritical CO_2 extraction is a good technique to preserve the structure of aerogel even after impregnation process.

CONCLUSION

Extraction oil represented 7.5±0.1% on seeds, 6.8±0.1%, and 42.0±0.1% on dry-pulp. The use of MAE for the extraction of polyphenols was effective to increase total polyphenol content (TPC) when it was applied under slurry fraction where maximum TPC achieved change from 0.82 mgGAE/g of dry material to 2.23 mgGAE/g dm. MAE was not effective when it was applied to seeds or dry-pulp, but both fractions were affected by increment of temperature. PMAE was applied as pre-treatment in extraction using seed and dry-pulp, in order to proportionate a bigger incensement of temperature. Seeds have a high potential as polyphenol source which maximum TPC value was 72.64 mg GAE/g. Using PMAE as pre-treatment under dry-slurry was possible obtaining an extract rich in polyphenols and anthocyanins, 43.43 mg GAE/g and 2.67 mg AE/g for Peoniding and Cyanidin respectively. Fast increase and decrease of temperature played an important role in order to protect extract against early degradation.

Direct impregnation could not be applied using pulp extract because it obstructs the gelification process. Oil extract could be used in this type of impregnation despite of changes in porous size and opacity. On the other hand, indirect impregnation shows better results, especially for pulp extract by ethanol/water. Mesoporous materials were successfully used to impregnate extracts from Euterpe Oleracea Mart. (Açaí) residue extracts. However, additional experiments are required to confirm this conclusion and to maximize the amount of impregnated oil. In addition, in future work releases test will

be done in order to characterize the variation of the release oil and polyphenols after impregnation in silica aerogels as support.

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