AN ETHANOL-BASED PROCESS TO SIMULTANEOUSLY EXTRACT AND FRACTIONATE CAROTENOIDS FROM MAURITIA FLEXUOSA L. PULP

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ABSTRACT - *Mauritia vinifera* (buriti) is a palm tree that grows wild in different areas of Brazil, particularly in the Amazonian region. The buriti oil is rich in carotenoids, especially in β-carotene. The growing interest in other natural sources of β-carotene has stimulated the industrial use of buriti as a raw material for pulp oil extraction. Most processes are based on the conventional technologies, involving drying and pressing the pulp for oil recovery and further separation of carotenoids in a liquid phase using organics solvents. In the present work, the ethanol-based process was evaluated for simultaneous carotenoids recovering and fractionating from buriti pulp. The raw material and ethanol, 1:4 ratio, were placed in an erlenmeyer flask and maintained at 30rpm for 1 hour in a temperature-controlled bath at 65°C. The mixture was filtered under vacuum and cooling at 10°C to allow for the separation of the solvent in two phases. Carotenoids composition, determined by HPLC, has indicated a β-carotene concentration about 12 times greater in the lower phase than in the upper phase. The profile of the carotenoids in the denser phase is quite similar to that of raw buriti oil, and the concentration of total carotenoids is 40% higher than that of the original raw oil, making the ethanol-based process particularly attractive for industrial applications.

Index Terms: Beta-carotene, vegetable oil, ethanol, buriti.

USO DO ETANOL COMERCIAL PARA EXTRAÇÃO E FRACIONAMENTO SIMULTÂNEO DE CAROTENÓIDES DE MAURITIA FLEXUOSA L. PULP

RESUMO-Mauritia vinifera (buriti) é uma palmeira nativa de diferentes regiões do Brasil, particularmente na região Amazônica. O óleo de buriti é rico em carotenóides, especialmente em β-caroteno. A demanda por fontes naturais de β-caroteno tem contribuído para aumentar a industrialização do fruto de buriti pelas usinas de extracção de óleos vegetais. O processo mais adotado baseia-se em tecnologias convencionais envolvendo as etapas de despolpamento, secagem e prensagem da polpa para extração do óleo, seguida da separação dos carotenoides em uma fase líquida usando solventes orgânicos. Neste trabalho, foi avaliado o uso do etanol comercial para extração e fracionamento simultâneo de carotenoides a partir da polpa de buriti. A matéria-prima foi misturada com etanol, na proporção 1:4 solvente/substrato, e mantida em banho termoestatizado a 60°C, por 1 hora, sob agitação constante de 30 rpm. A mistura foi filtrada sob vácuo e resfriada a 10°C resultando na formação de duas fases. A composição de carotenoides foi determinada por CLAE e indicou uma concentração de β-caroteno 12 vezes maior na fase mais densa que na fase leve. O perfil de carotenoides foi similar ao obtido no óleo bruto de buriti, porém a concentração de carotenoides totais foi 40% maior, indicando que o processo tecnológico avaliado é particularmente promissor para aplicação industrial.

Termos para Indexação: β-caroteno, óleo vegetal, etanol, buriti.
INTRODUCTION

The industrial processing of oleaginous raw materials is one of the major activities of Brazilian agro-business due to the wide usage of its products in foodstuffs, cosmetics and pharmaceuticals. However, the vegetable oils demand for energy purposes has increased the world’s interest on improving oil extraction processes but also on the search for new sources of oil (Li et al, 2009).

The conventional process for vegetable oil production includes raw material pressing, followed by solid-liquid extraction of the press cake using n-hexane to extract the residual oil. In a typical oil processing unit, each ton of processed seeds corresponds to a loss of 2L of hexane to the atmosphere. The vegetable oil processing units are, as a consequence, considered by EPA (Environmental Protection Agency) as one of the major responsible for greenhouse gases emission (EPA, 2000).

Due to Environmental Public Programs, recent interest in developing clean technologies has increased sharply, as an attempt to eliminate toxic solvents in industrial processes. Sustainable options using enzyme-based or ethanol-based processes have been recently reported in the literature (JOHNSON;LUSAS, 1983, COURI;FREITAS, 2001, FREITAS et al, 2007). One of the first studies using ethanol as a more safe solvent, carried out in pilot scale for soybean oil extraction, was proposed by Rittner (1991) based on the many economical and environmental advantages of this process. Among the many advantages, ethanol can be obtained from renewable sources as a clean and low cost solvent in Brazil, and may therefore be used in “green” technologies. According to Rittner, ethanol could represent an alternative to reduce the dependency on oil-based products such as n-hexane, besides being friendly to the environment. Despite of ethanol advantage as oil solvent, the Brazilian vegetable processing plants still use n-hexane in this process (FREITAS et al, 2007; FERREIRA-DIAS et al, 2003; FREITAS;LAGO, 2007).

Many vegetable oils present interest in amounts of carotenoids, phytosterols, tocopherols and phenolic compounds, known as the unsaponifiable matter (Table 1). The antioxidant properties and the many health benefits resulting from the use of unsaponifiable matter and its derivatives in foods and health supplements have raised an immense amount of interest in the industry (GUNSTONE;PADLEY, 1997; RODRIGUEZ-AMAYA, 2001; GUNSTONE, 2002; GÜÇLÜ-ÜSTÜNĐAG;TEMELLİ, 2004).

Buriti exhibits a fairly high yield of pulp oil, ranging from 9 to 19% (MARIATH et al, 1989; SANTOS, 2005). Despite a relevant amount of unsaturated fatty acids (approximately 75% of oleic acid), buriti oil shows high stability over oxidation, due to the presence of phytochemicals, particularly tocopherols and phytosterols, and is valuable for cosmetics and pharmaceuticals uses. Buriti oil is rich in carotenoids, 70% of which is β-carotene, exceeding reported values to palm oil (RIBEIRO, 2008).

β-carotene is the main source of provitamin A, and is widely used as a food colorant and a nutritional supplement. The global market for carotenoids is estimated to surpass US$1 billion in 2009, β-carotene being responsible for almost 30% of this market. Most of the β-carotene sold in the world is produced by chemical synthesis from β-ionone, but a small amount is manufactured using biotechnological processes from different microorganisms or by extraction of vegetables sources (FRASER;BRAMLEY, 2004; DUFÖSSE et al, 2005; RIBEIRO, 2008).

The high-temperature required to solvent-based oil extraction modifies the carotenoids profile, yielding several degradation products. The objective of the present work was to evaluate the technical viability of the ethanol-based process for simultaneous recovery and fractionating of preserved carotenoids present in buriti pulp.

MATERIAL AND METHODS

Raw material

Buriti pulp (Mauritia flexuosa L.) was supplied by Beraca Sabará® Company. In order to reduce fungi growth, the raw material was autoclaved at 2 atm, cooled, packed in plastic bags, protected of light and stored at 22°C.

Extraction and fractionating

The buriti pulp was dried in an oven equipped with convective air at 60°C until constant weight for about 6h and then milled to 1mm to 3mm of particle size. The oil extraction has been carried out using 4:1 ethanol/substrate ratio (w/w). The mixture was maintained for 1 hour in a temperature-controlled bath at 60°C and 30 rpm speed. The mixture was filtered under vacuum. The ethanol extract was then cooled to 10°C and further centrifuged (at 3000 rpm, during 15 minutes) at 10°C yielding two liquid phases. The lower phase (low ethanol content) was dried using cold convective air, while the upper phase (high ethanol content) was evaporated through filter paper, under vacuum, at 55°C. In Figure 1, it is shown a simplified flow-diagram of the extraction process.
Analysis

The oil content was determined in a soxhlet apparatus under reflux with petroleum ether at 45°C for 16 hours, according to AOCS (2004). The pulp moisture content was measured by gravimetric analysis, by drying the pulp at 90°C for 90 minutes (AOAC, 2000).

Carotenoids were identified by HPLC. The separation was carried out with a 100x4.6 i.d. mm YMC-Pack ODS-A column (5 µm particle size), using acetonitrile/methanol/THF (50/45/5 v/v/v, with addition of 0.05% triethylamine to methanol, as mobile phase. The flow rate was 1.0mL/min and the absorbance was detected on 450nm. The standards used were obtained by open column chromatography separation, in which an activated magnesium oxide and diatomaceous earth mix was employed. Initially, the unsaponifiable fraction of raw buriti oil was added to the column and then eluted with a combination of petroleum ether and diethyl ether in concentrations varying from 4 to 20% of diethyl ether. The fractions obtained were α-carotene, β-carotene, and γ-carotene. The 10-apo--β--carotenal was obtained by partition in ethanol from refined buriti oil, and then purified by HPLC. The structural identification of those carotenoids was confirmed by their UV-Vis spectrum (GODOY; RODRIGUEZ-AMAYA, 1995; RODRIGUEZ-AMAYA, 2001; RIBEIRO, 2008).

RESULTS AND DISCUSSION

The raw material oil content, in dry basis, was 9 ± 1%. This value has been similar to reported data by Mariath et al (1989) and Santos (2005. A drying step prior to ethanol-based extraction was necessary due to the high moisture content of commercial buriti (32 to 48%). The equilibrium moisture content in the dry cake at 65°C was about 8%.

The average composition of the upper phase was 95 ± 1% of ethanol and 4 ± 1% of oil, and the lower phase contained 50% of ethanol and 50% oil. The oil partition coefficient, measured by the ratio between the oil weight in the upper phase and in the lower phase was 2 ± 0.3 (Figure 2A). The ethanol partition coefficient, however, measured by the ratio between the solvent weight in upper and in the lower phase was 14 ± 1 (Figure 2B).

The oil extraction yield was 78 ± 5%, inferior to the typical values of conventional n-hexane-based process (99%). The yield of carotenoids extraction, however, was around 94%. The carotenoids profile, determined by HPLC, has indicated a total carotenoids concentration about 12 times greater in the lower than in the upper phase, which, on the other hand, contains 10 times more apocarotenoids (Table 2 and Figures 3A and 3B). The apocarotenoids exhibit less polyenic structures than a carotenoid (ranging between 20 and 30 carbon atoms) and a carboxylic group (aldehyde or ketone), which increases its solubility in polar solvents like ethanol and may have been formed by thermal degradation during oil extraction and evaporation (temperatures greater than 50°C). The lower phase has presented a carotenoids composition very similar to raw buriti oil, but with higher (40%) total carotenoids content (RIBEIRO, 2008).

| TABLE 1 | Unsaponifiable matter of some vegetable oils. |
|---------|----------------------------------------------|
| Oils    | Unsaponifiable (%) | Phytosterols (ppm) | α-Tocopherol (ppm) | Carotenoids (ppm) |
| Cotton  | 1,5 – 2,0          | 2700 – 5900        | 389                 | -                  |
| Peanut  | 0,4 – 1,0          | 900 – 2900         | 130                 | -                  |
| Rice    | 3 – 5              | 1800               | 800                 | -                  |
| Buriti  | 1,2 – 2,0          | 800 - 1600         | 700 - 800           | 1150 - 3380        |
| Canola  | 1,2 – 2,0          | 4800 – 11300       | 210                 | 130                |
| Coconut | 0,8 – 1,5          | 500 – 1100         | 5                   | -                  |
| Sunflower| 1,5               | 2400 – 4500        | 487 – 608           | 1,0 – 1,5          |
| Corn    | 1,3 – 2,8          | 7900 – 22100       | 112 – 134           | 1,2 – 3,6          |
| Olive   | 0,5 – 1,5          | 1200 – 2700        | 119                 | 1 – 20             |
| Palm    | 1,2               | 400 – 500          | 130 – 260           | 500 – 700          |
| Palm kernel | 0,1 – 0,8      | 800 – 1400         | 62                  | 4,3 – 11,8         |
| Soya    | 0,5 – 1,6          | 1800 – 4100        | 75 - 117            | -                  |

Fonte: Gunstone and Padley, 1997
TABLE 2 - Comparison of carotenoids composition in lower and upper phases

| Carotenoids     | % in lower phase | % in upper phase |
|-----------------|------------------|-----------------|
| β-carotene      | 1904.1 ± 10.2    | 157.9 ± 0.7     |
| α-carotene      | 237.1 ± 2.5      | 19.2 ± 0.2      |
| γ-carotene      | 99.4 ± 2.5       | 2.2 ± 0.2       |
| Apocarotenoids  | 24.7 ± 1.8       | 22.0 ± 2.6      |
| Others          | 282.9 ± 10.2     | 18.9 ± 0.4      |
| Total (ppm)     | 2549 ± 30        | 220.3 ± 7       |

FIGURE 1 - Flowsheet of ethanol-based process for oil and carotenoids extraction and fractionating.
FIGURE 2- (A) Oil distribution in all streams; (B) Solvent distribution in all streams.
CONCLUSIONS

The use of ethanol from sugar cane fermentation to extract and concentrate carotenoids from buriti pulp is very promising. The alpha, beta and gamma-carotene were transferred predominantly to the lower, oil-rich phase. This fraction may be potentially used in food products, supporting many health benefit claims. The apocarotenoids-rich fraction, from the thermal degradation of carotenoids during drying and ethanol-based extraction, was concentrated in the upper, ethanol-rich phase. This fraction is being evaluated for new uses and applications.

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