



SUGARCANE WAX EXTRACTION USING HEXANE AND LIMONENE MIXTURES

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ABSTRACT

Hexane is the most widely solvent used in the lipids extraction process, as the case of the sugarcane wax. However, the use of this solvent is highly harmful to the environment and to human health. Limonene is a monoterpene found in the citrus peel, with great potential for use as a green solvent. In this study, the partial and total substitution of hexane by limonene was performed in the process of the sugarcane peel wax extraction to evaluate the effect of this substitution on the physicochemical characteristics of the wax. The extracted samples were compared with a commercial wax sample (carnauba) using the Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC) and Infrared by Fourier Transform (FTIR) analyses. Through this study, we can conclude that the waxes obtained from the use of the hexane and limonene mixture solvents presented similar physicochemical characteristics to those found in commercial waxes. Thus, the total and/or partial substitution of the hexane by solvents less harmful to health and the environment, such as limonene, can be an alternative in the wax extraction process.

Palavras-chave:

Cera da Cana-de-açúcar
Resíduos Agroindustriais
Mistura de Solventes
Solventes Verdes
Limoneno

EXTRAÇÃO DA CERA DA CANA-DE-AÇÚCAR USANDO MISTURAS DE HEXANO E LIMONENO

RESUMO

Hexano é o solvente mais utilizado no processo de extração de lipídios, como é o caso da cera de cana-de-açúcar. Entretanto, a utilização deste solvente é altamente prejudicial ao meio ambiente e à saúde humana. Limoneno é um monoterpene encontrado na casca dos citrinos, com grande potencial de utilização como solvente verde. Neste estudo, foi realizada a substituição parcial e total do hexano por limoneno no processo de extração da cera da casca da cana-de-açúcar para avaliar o efeito desta substituição sobre as características físico-químicas da cera. As amostras extraídas foram comparadas com uma amostra comercial de cera (carnaúba) a partir da Análise termogravimétrica (TGA), Calorimetria Exploratória Diferencial (DSC) e Infravermelho por Transformada de Fourier (FTIR). Por meio deste estudo, podemos concluir que as ceras obtidas a partir da utilização da mistura de solventes formada por hexano e limoneno apresentaram características físico-químicas semelhantes às encontradas nas ceras comerciais. Assim, a substituição total e/ou parcial do hexano por solventes menos nocivos à saúde e ao meio ambiente, como o limoneno, pode ser uma alternativa no processo de extração das ceras.

INTRODUCTION

Wax extracted from sugarcane peel is a lipidic mixture composed of hydrocarbons, wax esters, sterol esters, ketones, aldehydes and fatty acids and alcohols (ASIKIN *et al.*, 2012). The characteristics of the wax obtained can vary according to the type of raw material, climatic conditions, extraction temperature and type of solvent used (GEORGES *et al.*, 2006).

Hexane is generally used as a solvent in lipid extraction processes, such as waxes. However, its use can cause harmful effects due to its high toxicity, flammability, and damage to health and the environment, which makes extra expenses with the security necessary. An alternative that can be proposed is the substitution of hexane by an alternative solvent that is not as harmful to health and the environment, such as the limonene. Limonene is a green solvent extracted from the peel of citrus fruits, which has been tested as a substitute for solvents in lipid extraction processes, showing good results due to its high solvency power. However, limonene is a solvent with a high boiling point (176 °C), which requires a greater amount of energy for its recovery (MAMIDIPALLY & LIU, 2004; VIROT *et al.*, 2008).

Thus, this study aimed to evaluate the effect of partial and total substitution of hexane used in the sugarcane wax extraction by limonene on the physicochemical properties of the obtained waxes.

MATERIAL AND METHODS

Plant Material and Solvents

Sugarcane peels (variety RB 96 6928) were collected from a sugarcane juice processing farm (Rio Claro, SP, Brazil). The material presented particle size between 0.19 and 0.36 mm, moisture content of $8.0 \pm 0.2\%$, and total lipid content of $7.6 \pm 0.5\%$, determined according to the methodology proposed by Bligh and Dyer (1959). These analyses were performed in triplicates.

Hexane analytical grade was purchased from Anidrol (Brazil) and D-Limonene (94%) was donated by Cutrale LTDA (Brazil).

Extraction and Purification Procedure

The extractions of crude wax were performed using a Soxhlet apparatus during 1 h, with approximately 10 g of biomass and 200 mL of the mixture by limonene and hexane in different proportions (0, 10, 30, 50, 70 and 100%). The solvents were recovered by evaporation under reduced pressure. In order to ensure that residual solvent did not interfere with the physicochemical analysis results, the extracts obtained with hexane were placed in Petri dishes and dried under room temperature to remove the residual solvent. The extracts obtained with limonene were more difficult to dry because of the higher normal boiling point of limonene (176 °C) compared to that of hexane (68 °C). For this reason, these extracts were placed in Petri dishes and oven-dried for 20 hours at 90 °C.

After drying the crude wax, the sample was purified according to VIEIRA (2003), in order to remove the dark color, sugars, beyond resins and some impurities that can promote undesirable properties to raw wax. For this purpose, the raw wax was homogenized in hot hexane (1:20 w/v), cooled to 10 °C (during 1 h), centrifuged and the hexane was discarded. The extract was then washed with acetone (2:1 v/w) at 10 °C for the crystal's formation, filtered under vacuum, and dried at room temperature.

The high melting temperature of these waxes results in the formation of sediments that can be easily separated by crystallization using selective solvents at low temperatures, such as acetone (BALCH, 1953; GUNAWAN *et al.*, 2006). The use of acetone promotes rapid solubilization of soluble lipids and enables that the insoluble hard wax portion can be separated by filtration or centrifugation (PATURAU, 1989). The schematic representation of the purification process is shown in Figure 1.

Wax characterization

Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC), and Infrared by Fourier Transform (FTIR) were performed to characterize the obtained waxes. The results obtained were compared with those of the commercial wax from Carnauba (Multiceras - Mexico).

SUGARCANE WAX EXTRACTION USING HEXANE AND LIMONENE MIXTURES

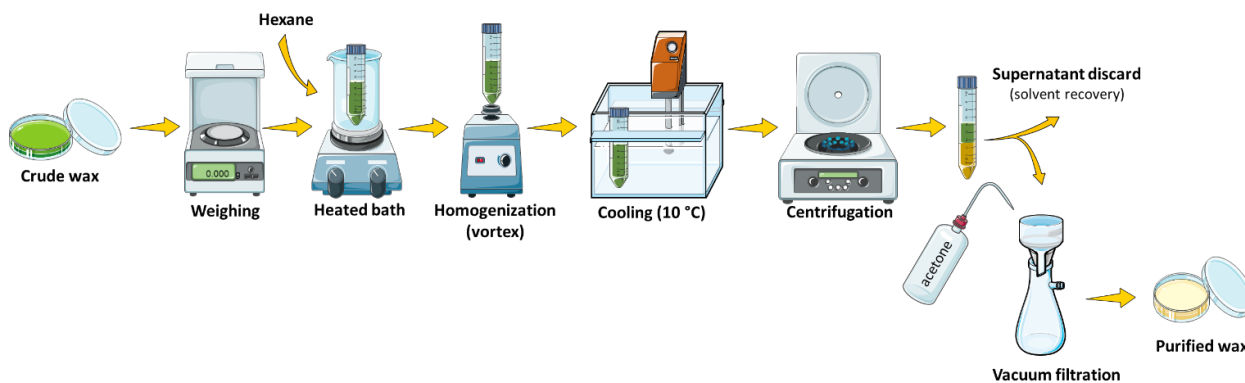


Figure 1. Crude wax purification procedure

Waxes thermal resistances were measured using a thermogravimetric analyzer TGA-50M (Shimadzu, Japan). About 5 mg of wax was heated from 25 to 600 °C at a rate of 10 °C.min⁻¹, and using a flow rate of 50 mL.min⁻¹ of synthetic air. A differential exploratory calorimeter (DSC1, Mettler Toledo, Switzerland), with aluminum crucible heating from 25 to 105 °C (at 10 °C.min⁻¹) and cooling to -10 °C (at 10 °C.min⁻¹), was used to determine the waxes melting temperatures. After standing for 1 min at -10 °C, the system was reheated to 105 °C (at 10 °C.min⁻¹).

FTIR spectra of the waxes' extracts were recorded at 25 °C using a Nicolet 6700 spectrometer (Thermo Scientific, USA) with attenuated total reflectance using Smart Omni Sampler (ATR accessory). The FTIR spectrometer was scanned over the frequency range of 4000-675 cm⁻¹ at a resolution of 4 cm⁻¹. The spectrum was collected

by using OMNIC 8.0 software.

RESULTS AND DISCUSSION

The visual appearance and texture of the waxes extracted with hexane (0% of limonene) and 100% of limonene are presented in Figure 2. The waxes extracted with limonene presented a slightly darker color compared to the waxes extracted with hexane, as already reported by other authors (CASCANT *et al.*, 2017; MAMIDIPALLY and LIU, 2004; VIROT *et al.*, 2008).

The results of the TGA, DSC, and FTIR analysis are shown in Figure 3. From the thermogravimetric analysis performed and represented in Figure 3.a, the commercial carnauba wax presents greater thermal stability than the other samples, since its decomposition started at 300 °C approximately, while for the other samples, the decomposition started at 200 °C approximately. The same

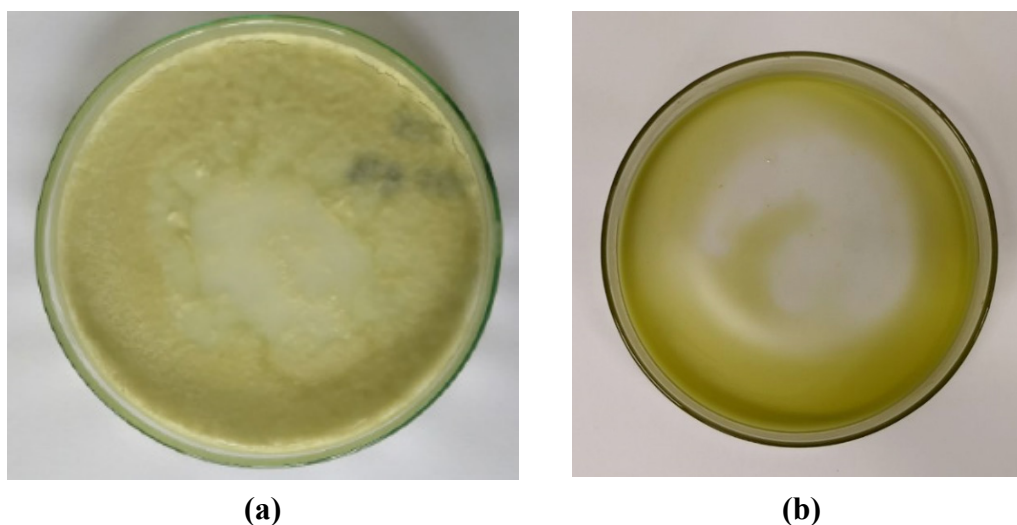


Figure 2. Waxes extracted with (a) hexane (0% of limonene) and (b) 100% of limonene

behavior was observed by OLIVEIRA (2018) when analyzing sugarcane waxes extracted with hexane and limonene. The author observed a greater thermal stability of carnauba wax followed by sugarcane wax extracted with hexane and then limonene.

Upon reaching a temperature of 600 °C, the samples extracted with proportions of limonene greater than 50% did not completely degrade, suggesting that it would be necessary to perform the analysis at a higher temperature. The sample extracted with 50% of limonene showed the least degradation and loss of mass under these conditions.

When analyzing the DSC thermogram (Figure 3.b), melting peak of the wax extracted with hexane

(0% of limonene) is more defined and longer than the others extracted with solvents mixtures, indicating that this mixture is more homogeneous in terms of composition than the other waxes. It is also noticed that the melting temperature of the wax extracted only with hexane is 77.81 °C, being closer to the melting point of the commercial carnauba wax, which has a melting point of 82.62 °C, and to the sugarcane waxes melting point values related by others authors, ranging from 72 °C to 75 °C (PHUKAN & BORUAH, 1999), from 77° C to 78 °C (GARCÍA *et al.*, 1999), 79 °C (PATURAU, 1989), 81 °C (VIEIRA, 2003), from 79 °C to 81 °C (BALCH, 1953) and from 79 °C to 83 °C (AZZAM, 1984,1986).

The melting behaviors were also similar to those

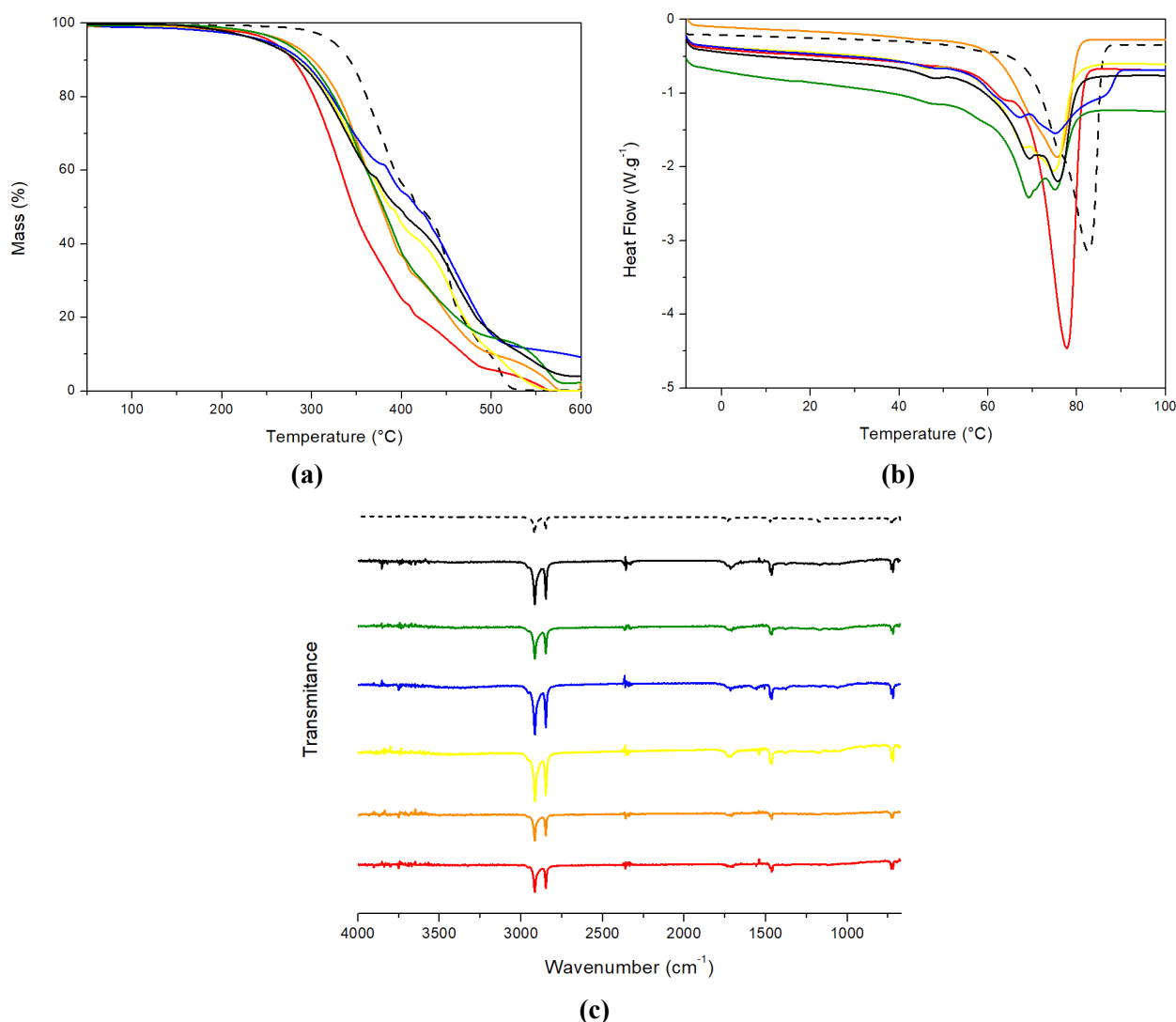


Figure 3. (a) TGA, (b) DSC thermogram and (c) FTIR spectra of carnauba (- -) and purified waxes extracted with 0% (—), 10% (—), 30% (—), 50% (—), 70% (—), 100% (—) of limonene

found by OLIVEIRA (2018) when evaluating the sugarcane wax extraction using different solvents. The author verified the melting temperatures of 77.5 °C, 76.9 °C and 71.9 °C when using hexane, limonene and turpentine, respectively.

The FTIR spectra (Figure 3.c) show that all samples of purified waxes have the same functional groups present in commercial carnauba wax and other natural waxes such as beeswax (KNUUTINEN & NORRMAN, 2000), flax straw (ATHUKORALA *et al.*, 2009), bagasse (QI *et al.*, 2017) and sugarcane peel (INARKAR & LELE, 2012; OLIVEIRA, 2018), which indicates the presence of structural characteristics similar to those found in oils and fats (GUILLÉN & CABO, 1997; ALBUQUERQUE *et al.*, 2003).

The bands between 2915 and 2860 cm⁻¹ are characteristic of the -CH₂ group and represent the aliphatic fraction of the wax. The bands between 2360 and 2320 cm⁻¹ indicate the presence of CO₂ in the air, while the bands between 1715 and 1730 cm⁻¹ are characteristic of -C=O stretch, which indicates the presence of esters or fatty acids. The bands between 1460 and 1465 cm⁻¹ indicate the presence of methylene groups, while the band at 720 cm⁻¹ indicates the presence of long or very long chain groups (ATHUKORALA *et al.*, 2009; QI *et al.*, 2017).

CONCLUSIONS

- In this study, the partial and total substitution of hexane by limonene in sugarcane peel wax extraction was performed to evaluate the effect of this substitution on the physicochemical characteristics of the wax. Waxes obtained from mixtures of hexane and limonene solvent showed physicochemical behavior similar to commercial carnauba wax, thus enabling the total and/or partial substitution of the hexane by solvents less harmful to health and the environment.

AUTHORSHIP CONTRIBUTION STATEMENT

SILVA, G.J.: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft; **HENRIQUES, J.D.O.:** Conceptualization,

Investigation, Methodology, Supervision, Writing – review & editing; **MARTINEZ, P.F.M.:** Conceptualization, Resources, Supervision, Visualization, Writing – review & editing.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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