Characterization of eucalyptus and citrus monofloral honey in São Paulo State by pollen and physical –chemical analysis.

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Considering the characterization of monofloral honeys as a worldwide tendency, this study presents the development and optimization of methodologies for carbohydrate analysis by HPLC, the kind of standardization for lamina preparations for pollen analysis and the determination of moisture content in order to characterize eucalyptus and citrus monofloral honey samples from some regions in São Paulo State. The modified method of Iwama and Melhem (1979) was employed for pollen analysis pollen cleaning. For lamina preparation it a comparison study was made between the Iwana and Melhem (1979) method and the proposed method (Louveaux modified) through two proportion tests. A comparison for moisture content was made between two official methods (AOAC and EHC) through a factorial desing and a study of variability through hierarchical desings. For the carbohydrate content analysis an optimization of analysis conditions for HPLC was made using mixture desing and a linear regression analysis for calibration curves, a *t-test* for a recovery study and a variance analysis (ANOVA) to compare the carbohydrate contents of both floral origins. In pollen analysis one can observe that the proposed Louveaux modified method was the most adequate since it showed a bigger distribution of less frequently occcoring families. With the pollen spectrum (dominant pollen) from honey samples, the monoflorals of eucalyptus and citrus, can be classified. And by complete pollen spectrum analysis one can observe that citrus monofloral honeys contain more diversified families relative to eucalyptus monofloral honeys, suggesting these honeys have a bigger variation of nectars and pollen grains in their formation. This fact can be related to regions of plantation. The factorial desing 2^2 in moisture content suggests that the crystallized samples interfere in refractive index measurements. The (EHC) sample pre-treatment led to lower moisture contents of crystallized samples. When this pre-treatment was used for liquid samples no significant differences were observed concerning moisture content. Therefore it can be suggested that the EHC refractrometric method is more appropriate to use for liquid and crystallized samples. The study of moisture content variability through hierarchical desing and variance analysis indicates significant differences among floral sources and moisture content of honey samples. The use of a aminopropil column of smaller size (15,0 cm x 4,5 cm) and a temperature of 32 ^{o}C in the column and 35,5 ^{o}C for the refractive index detector and a flow rate of 1.2 ml/min were the best experimental conditions chosen to determine the carbohydrates in honey by HPLC. For the mobile phase the mixture desing indicated that the best combination was 50: 10: 40(acetonitrile, water, ethyl acetate). The calibration curves of the carbohydrates (glucose, fructose, sucrose, turanose, maltose) were linear, with high R^2 and had acceptable accuracy for carbohydrate quantification. Both the detection capacity and quantification capacities were determined the former being (0.2 – 0.4%) and the latter (0.7 – 1.3 %) for sucrose, turanose and maltose. The mean recovery study of carbohydrates suggested that the calibration curves are reliable to determine carbohydrate contents. The evaluation among the mean concentrations of individual carbohydrates by ANOVA and t-test at the 95% confidence level of eucalyptus and citrus monofloral honeys suggested that there are significant differences in glucose, sucrose and turanose concentration in these honey samples. In this way eucalyptus and citrus monofloral honeys can be classified by the pollen spectrum. Therefore, it was concluded that the eucalyptus and citrus monofloral honeys can be classified by moisture content determination and/or carbohydrate (glucose, sucrose and turanose) determination.

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