Metabolic Profiles of Pumpkin Flowers from Agronomically Important Varieties in the Agreste Region of Pernambuco by GC-MS

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Embrapa leads the project Rede BioFORT, which aims to biofortify foods in Brazil, focusing on reducing malnutrition and ensuring greater food and nutritional security. Among the projects developed by Rede BioFORT, the improvement of pumpkin seeds (Cucurbita spp.) through crossbreeding is prominent. In this context, it is relevant to precisely determine the actual levels of metabolites in biofortified pumpkin flowers. This study aimed to investigate the metabolic profile of four samples: two varieties of local pumpkin flowers (Recife, PE), one from the species *C. moschata* and another from *C. maxima* (species 1 and species 2, respectively); one commercial variety and one biofortified variety, both from the species *C. moschata*. The influence of the position where the seeds were planted in the plant-bed (center or extremities) on their metabolic profile was also investigated.

The freeze-dried flowers (15 mg), provided by Embrapa, were homogenized in 500 µL of methanol:water (80:20, v/v), sonicated, incubated at 4 °C and centrifuged at 9000 x*g* for 15 min. The supernatant (120 µL) was collected and analyzed by LC-HRMS (data presented in another abstract at BrProt). The residual material from the first extraction (remaining in the centrifugation tube after aliquot removal for LC-HRMS analyses) was dried in a Speed Vac and subsequently suspended in 1000 µL of methyl tert-butyl ether (MTBE). Then, after mixing and centrifugation, 600 µL from this nonpolar organic solution were collected, dried under nitrogen flow, and the extract was stored at -20°C until derivatization by silylation, being analyzed by gas chromatography coupled with mass spectrometry (GC-MS). Xcalibur (Thermo Scientific) and NIST MS Search 2.0 softwares were used for peak integration and peak annotation. A commercial mixture of hydrocarbons (Supelco, saturated alkanes C7-C30) was used to assist peak annotation via calculation of their linear retention indices (LRI). Peak annotation was achieved by comparing the fragmentation profile of the obtained mass spectra (electron ionization, 70 *e*V) with those available in the NIST 2020 library, with compatibility above 800 and a percentage difference in the linear retention index presented by the analyte of the sample lower than 15% when compared to compound databases (NIST library, HMDB, PubChem).

Metabolic profile of pumpkin flowers showed their composition in fatty acids, such as palmitic acid, stearic acid, and alpha-linolenic acid, and sugars such as fructose and glucose. Phenolic compounds like 4-hydroxybenzyl alcohol were also detected. Additionally, statistical analyses showed that biofortification and the plant\'s position in the planting ground did not influence pumpkins' flowers metabolic integrity.

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