

Characterization and evaluation of the chemical profile by LC-HRMS of pumpkin flowers from varieties of agronomic importance and comparison with a biofortified variety with beta-carotene

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The BioFORT-EMBRAPA project aims to consolidate research on biofortified foods for nutritional improvements of staple foods. In this sense, pumpkin varieties (*Cucurbita spp.*) with increased beta-carotene contents were selected to improve vitamin A nutritional status of people at risk for deficiency. However, genetic variants might present differences in the chemical profile of relevant metabolites. In this study, the chemical profile of two variants of *Cucurbita* genus pumpkin flowers (*C. moschata* and *C. maxima*), one commercial *C. moschata* and one biofortified *C. moschata* variety with beta-carotene all from Recife-PE, were analyzed via an untargeted metabolomic analysis based on liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). The four freeze-dried flowers groups (15 mg, each) provided by EMBRAPA, were mixed in vortex for 30 s with 500 μ L of methanol:water (80:20; v/v). Then, the mixture was taken to an ultrasonic bath for 15 min followed by incubation at 4°C for 15 min and centrifugation at 9000 \times g for 15 min. The supernatant (120 μ L) was collected and analyzed by LC-HRMS. A total of 64 pumpkin flower metabolites were annotated (level 2 of identification) on negative and positive modes of analysis. Among these metabolites, amino acids, fatty acids, and flavonoids were the most abundant compounds. The PCA score plot showed a clear separation between the commercial and *C. maxima* from Recife-PE groups, and an overlap between the *C. moschata* from Recife-PE and biofortified variety groups considering three main components (PC1, PC2, and PC3) with an accumulated variance of 55.2%. The influence of the plant's position in the plant-bed (middle row or lateral ends) on the chemical profile of the flowers was also investigated. The PCA score plot showed a group overlap indicating a lack of difference in the metabolic integrity of the species concerning the planting position. In conclusion, the multivariate analysis allowed the discrimination commercial and *C. maxima* varieties from Recife-PE. This result appears scientifically sound, as the commercial and *C. maxima* from Recife-PE samples belong to different species. Moreover, the overlap observed in PCA plot between the *C. moschata* from Recife-PE and biofortified *C. moschata* variety indicates that the biofortification process have not altered the pumpkin flower metabolic profile. In addition, the planting cultivation position in the plant-bed has not influenced the metabolic integrity of the species. The overlap in PCA plot of the biofortified variety can be considered a positive result because the biofortification process is aimed at a specific nutrient.

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