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Griffonia simplicifolia seed-based supplements: how stable isotope ratio analysis can help in commercial samples authentication

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Summary: *L-5-hydroxytryptophan-based supplements can be obtained either from the natural source seeds of Griffonia simplicifolia or it can be produced (bio)synthetically, the latter resulting in a higher profit. Natural products must therefore be protected against economical frauds. Stable isotope ratio analysis can serve this purpose, discriminating effectively between natural and (bio)synthetic products.*

Keywords: *Griffonia simplicifolia, stable isotope ratio analysis, authentication*

Introduction

The global trade in seed material from *Griffonia simplicifolia*, a tropical shrub of West Africa, has been boosted in the last few years because of its content of the free amino acid L-5-hydroxytryptophan (5-HTP) [1]. This active ingredient has been interesting consumers since it is a precursor of both serotonin and melatonin. Since *Griffonia simplicifolia* is reach in this amino acid (up to 20%), its cultivation has been increasing in the last years to face consumer's request [2].
On the other hand, L-5-hydroxytryptophan can also be obtained through several synthetic and biosynthetic pathways, which involve cheaper starting materials and result in a more cost-effective procedure than the extraction from the seed [2].
It is well known that where money goes, adulteration follows, and it is also sure that natural supplements have higher prices than (bio)synthetic ones. But when consumers buy a natural-labelled supplement, how can they be 100% sure that it is natural? It is mandatory to have a method to characterise the authentic natural L-5-hydroxytryptophan extracted from *Griffonia simplicifolia.*
In the last decades, stable isotope ratio analysis (SIRA) proved to be a powerful tool for this purpose, figuring as a technique able to discriminate between natural, synthetic and biosynthetic products.

Experimental

In the present work, 2 samples of synthetic 5-HTP, 4 samples of biosynthetic 5-HTP, 15 samples of commercial *Griffonia simplicifolia* extract supplements and 8 samples of *Griffonia simplicifolia* seeds have been considered. To extract 5-HTP from the seeds and from the commercial samples, an ethanol extraction was carried out, followed by purification through preparative HPLC.
Both high-purity and purified samples were therefore analysed through EA-IRMS (Elemental Analysis-Isotope Ratio Mass Spectrometry) to measure both carbon ($\delta^{13}C$, ‰ vs V-PDB) and nitrogen ($\delta^{15}N$, ‰ vs Air) isotopic ratios.

Results

The isotopic results for natural, synthetic and biosynthetic 5-HTP are reported in Figure 1. While synthetic and biosynthetic values are quite close to each other in the biplot, the natural samples are statistically different (p<0.001) from the rest of the dataset.

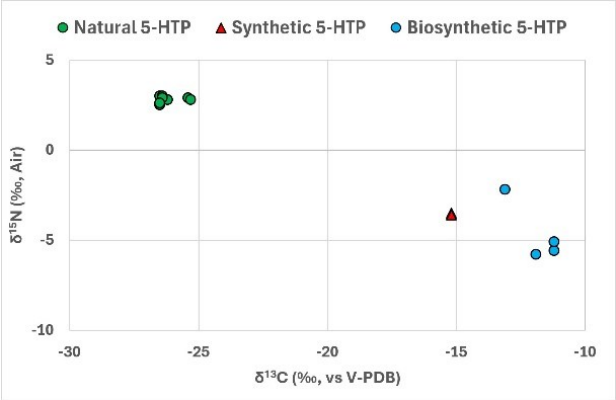


Fig. 2. *Nitrogen and carbon isotopic ratios for natural, synthetic and biosynthetic 5-HTP samples.*