Isotopic Labeling of Red Cabbage Anthocyanins with Atmospheric $^{13}C$O$_2$ in Closed Environments

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Introduction

Controlled environment agriculture can produce crops with defined phytochemical composition for nutrition research.

Labeling foods to follow the uptake and metabolism of phytochemicals is a specialized example.

Intrinsic labeling is required if the influence of the food matrix is an issue and stable isotopes are preferred for human studies. There are several approaches:

1. Can be supplied via fertilizer if a compound of interest contains N.

D$_2$O can be used in the irrigation water, but isotope effects often limit the proportion of D$_2$O to 30% to prevent extreme growth inhibition. The resulting range of isotopomers make it hard to track labeled compounds for long durations.

As an alternative, we developed a system to incorporate $^{13}$CO$_2$ photosynthetically using $^{13}$CO$_2$ at ca. 99% isotope enrichment that resulted in almost 99% labeling in kale (see Fig. 1; Kurilich et al. J. Agric. Food Chem. 51: 4877, 2003).

This system was modified to label anthocyanins, flavonoid compounds, that contribute color, photoprotection, and antioxidant activity in plants (see Fig. 5 for structure). Anthocyanins may also provide health benefits in humans, but bioavailability is poor and may be adversely affected by substituents including sugars and aliphatic or aromatic acyl groups (e.g., Charron et al. J. Agric. Food Chem. 55: 5354, 2007). The possibility that anthocyanin breakdown products and/or metabolites are involved in health-promotion needs to be studied. Red cabbage was chosen because it has a large number of variously substituted cyanidins.

Conclusions

We describe a system for isotopic labeling of leafy vegetables with $^{13}$CO$_2$ and demonstrate successful incorporation of $^{13}$C into anthocyanins of preharvest red cabbage (Brassica oleracea L. var. capitata).

Analysis of red cabbage shoot tissue by high performance liquid chromatography/tandem mass spectrometry (HPLC-MS/MS) indicated the presence of 37 anthocyanins, of which fourteen have not previously been described.

36 of 37 compounds were based on cyanidin, but 2 anthocyanins incorporating delphinidin were identified.

Mass shifts representing $^{13}$C incorporation into anthocyanins were evident in mass spectra of anthocyanins from labeled tissue and demonstrate successful isotopic labeling into nearly 100% of all carbons.

In some cases (peaks 12, 20, 21, 32, and 35), mass shifts provided unambiguous identification of all carbons.