Simultaneous measurement of tocopherols and carotenoids in oils using reversed-phase high-performance liquid chromatography

Q Su, M Daskalakis, NDH Balazs

Biochemistry Unit, Southern Cross Pathology Australia, Monash Medical Centre, Melbourne, VIC, 3168

Fresh vegetables and fruit are major sources of the antioxidant carotenoids, vitamin C and folate, while olive oil and other vegetable oils are the main source of vitamin E. Evidence is mounting for the potential protective role of such antioxidant vitamins and carotenoids in the development and progressions of cancer, occlusive vascular disease, diabetes, cataract formation and age-related macular degeneration. Absorption efficiency of carotenoids is known to be affected by formation of dietary fat, protein and bile salt concentrations. Various methods have reported determination of tocopherols in oils by using thin layer chromatography followed by spectrophotometry (1), gas chromatography (2) or HPLC (3). Several studies have reported simultaneous determination of α-tocopherol and β-carotene in olive oils (3,4).

We developed a rapid, direct HPLC method for simultaneous measurements of γ-tocopherol, α-tocopherol, lutein, lycopene, α-carotene and β-carotene in oils. Pretreatment of samples for these measurements was not required. The chromatographic system comprised a Waters 2690 separations module, 996 Photodiode Array Detector and a Spherisorb ODS-2 column (250 × 4.6 mm, 5 µm, 'Goldpak', UK). The mobile phase consisted of methanol-acetonitrile-chloroform and run as a gradient at 1.0mL/min. The methanol and acetonitrile contained 0.05% ammonium acetate and 0.1% triethylamine respectively. Run time was 26 minutes. α- and γ-Tocopherols and carotenoids were monitored at 292nm and 450nm respectively. The coefficients of variation (CV) were 6.5% for α-tocopherol, 3.4% for α-carotene and 5.9% for β-carotene. The detection limits were 0.8ng for carotenoids and 15ng for tocopherols. Oil samples were obtained from various sources.

Concentration of α–, and γ–tocopherols, lutein and β–carotene in oils (nmol/g)

Type of oil	γ–tocopherol	α–tocopherol	lutein	α–carotene	β–carotene	13–β–carotene	lycopene
Marine mussel oil	562	3079	5.1	16.9	32.1	32.8	22.8
Sunflower oil	60	3256	–	–	–	–	–
Extra light olive oil	9.5	957	–	–	1.2		–
Extra virgin olive oil	33	1016	0.1	–	–	10.4	–

The content of α- and γ-tocopherols and β-carotene in marine, olive and seed oils were very different. Sunflower oil had the highest levels of α-tocopherol and marine oil had higher γ-tocopherol, α-carotene, β-carotene and lycopene concentrations than all the other oils. The main advantage of the method described is its speed and the ability to simultaneously determine a number of lipid-soluble antioxidant compounds.

References

1. Ranalli A, Sgaramella A, Surricchio G. The new 'Cytolase 0' enzyme processing aid improves quality and yields of virgin olive oil. Food Chem 1999; 66: 443–454.
2. Giacometti J. Determination of aliphatic alcohols, squalene, alpha-tocopherol and sterols in olive oils: direct method involving gas chromatography of the unsaponifiable fraction following silylation. Analyst 2001; 126: 472–5.
3. Gimeno E, Calero E, Castellote AI, Lamuela-Raventoes RM, de la Torre MC, Lopez-Sabater MC. Simultaneous determination of alpha-tocopherol and beta-carotene in olive oil by reversed-phase high-performance liquid chromatography. J Chromatogr A 2000; 881: 255–9.
4. Su Q, Rowley KG, Itsiopoulos C, O'Dea K. Identification and quantitation of major carotenoids in selected components of the Mediterranean diet: green leafy vegetables, figs and olive oil. Eur J Clin Nutr (in press).

Acknowledgement: We thank Dr Kevin Rowley and Professor Andrew Sinclair for suggestions in preparation of this manuscript and the provision of the oils.
Key words: carotenoids, HPLC, measurement