

**Théo EFSTATHIOU , Christian NIO and Stéphanie FOURNY**

Analyses & Recherche, NUTRINOV - Z.A.C. des Trois Marches – 3, impasse de La Jonchée  
35132 VEZIN-LE-COQUET, France

## Purpose of the study

Epidemiological studies on the relationships between diet and cancer suggest that an increase consumption of fruits and vegetables may be directly related with a reduced incidence of some cancers (1). Studies conducted over the past decade have indicated that compounds such as flavonoids, carotenoids and phenolic acids have antioxidant, anti-inflammatory, antiviral, antimutagenic and anticarcinogenic properties (2). The food and pharmaceutical industry, always looking for new high-values products, adds more and more natural extracts in dietary supplements and functional health foods. These extracts contain micro-nutrients with specific physiological properties. The high diversity and increasing number of these micro-nutrients in each food generates a real need for reliable and resolute analytical methods. Bioflavonoids, phenolic acids and carotenoids are characterised and quantified in several compounds, as soya, apples, red clover, wine, citrus fruits (orange, grapefruit) extracts.

## Methods and Materials

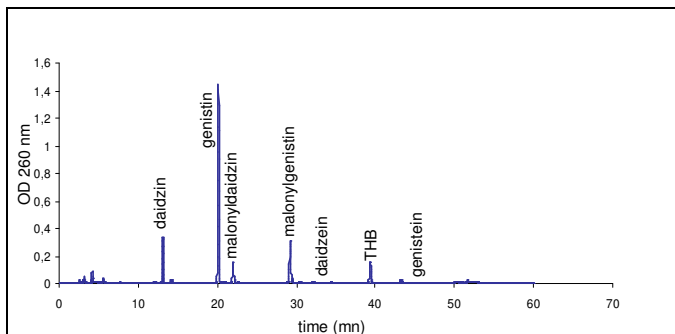
Phenolic acids and flavonoids from soya, wine and apples are characterised and quantified by reverse phase liquid chromatography (HPLC - RP). Analyses were monitored with the diode - array detector at 280 nm. After extraction with or without acid hydrolysis, the sample is injected on a YMC- pack ODS - AM -303 column. The standards for malonyl forms are extracted and purified in our laboratory. We also synthesize the internal standard (trihydroxydebenzoïn). A system combining HPLC and mass spectrometry is used for the characterization. Carotenoids (lycopen,  $\alpha$  and  $\beta$ - caroten, lutein, zeaxanthin, cryptoxanthin, astaxanthin) are evaluated by HPLC with UV detection. After extraction with a binar solvent system (specific for each food matrix), the sample is injected on a reverse phase column.

## Results and discussion

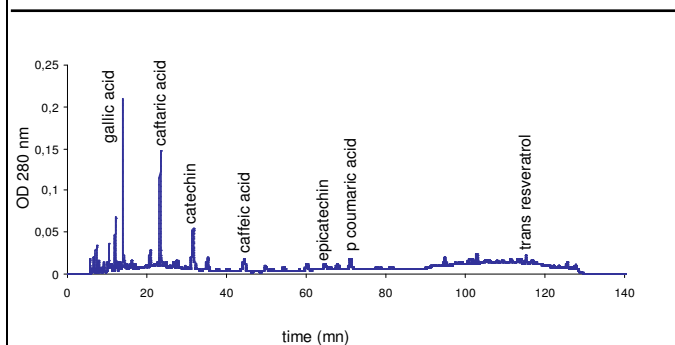
Chromatographs obtained from soya, wine and apples extracts (fig1, 2 and 3 respectively) show a simultaneous and fine partition of phenolic acids and bioflavonoids, allowing their quantification in foods, but also in biological samples. Nevertheless, with a 3- hour duration, the number of samples analysed daily is limited. The chromatographic profile (fig 4) obtained for carotenoids allows a simultaneous assessment of numerous pigments in various food and seaweeds, and in human plasma samples. As carotenoids are very light- and temperature - labile, the extraction conditions have been optimally adapted. This method is very fast (30 mn), specific and reliable (variation coefficient  $\leq 5\%$ ). Those specific analytical methods are very useful, facilitating the formulation of new dietary supplements and functional health food. We also use them to study the micro-nutrient stability during food storage. Last, our analytical methods may be used as an accurate tool of nutrient titration in ingredients and in products.

**References :** 1. Fiala E.S, Reddy B.S, Weisburger J.H. (1985) Naturally occurring anticarcinogenic substances in foodstuffs. *Ann. Rev. Nutr.* 5: 295-321.

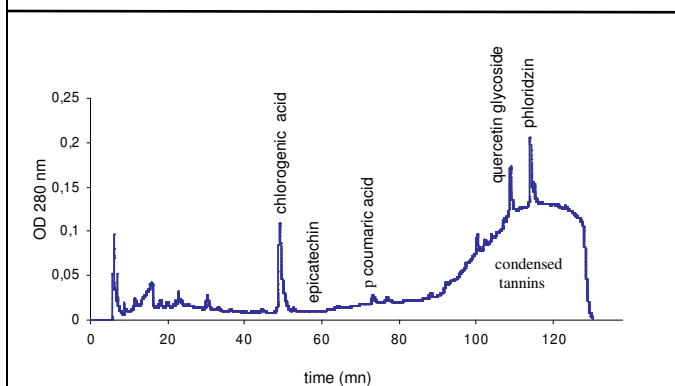
2. Haslam,E. (1996) Natural polyphenols (vegetable tannins) as drugs: possible modes of action. *J. Nat. Prod.* 59: 205-209.



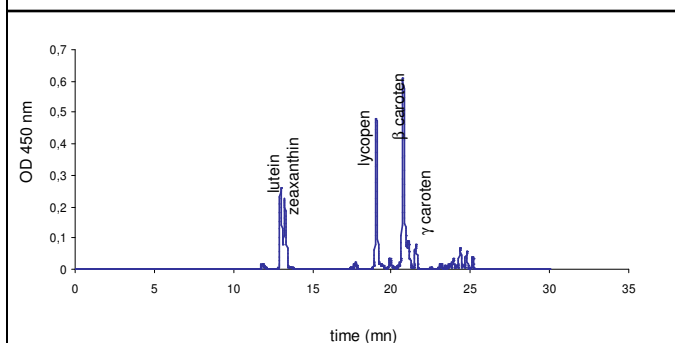
**Figure 1: HPLC Isoflavones profile of extracts from soya**



**Figure 2: HPLC phenolic acids and polyphenols profile of extracts from wine.**



**Figure 3: HPLC flavonoids profile of extract from apples.**



**Figure 4: HPLC carotenoids profile.**