FREE RADICAL SCAVENGING CAPACITY OF CHLOROGENATE ESTERS: A QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS STUDY

L. J. LOPEZ-GIRALDO[a], M. LAGUERRE[a], J. LECOMTE[a], M. C. FIGUEROA-ESPINOZA [a,b], J. WEISS [c], E.A. DECKER [c] , Y. HIROMI [c] and P. VILLENEUVE [a]

[a] CIRAD, UMR IATE, Lipotechny Laboratory, 34398 Montpellier, France
[b] IRC Montpellier SupAgro, 1101 avenue d'Agropolis CS 24501, 34093 Montpellier, France
[c] Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA.

Scholarship N° E05D055786CO

Chlorogenic acid (5-CQA) is phenolic acid exhibiting antioxidant properties. However, due to its hydrophilicity, application of this compound in oil-based food processing and cosmetics have been limited. Therefore, we tried to enhance its hydrophobicity by enzymatic lipophilization, which corresponds to the grafting of an aliphatic chain through lipase-catalyzed acylation. In this way, 5-CQA (C_0) was esterified in a two-step chemoenzymatic reaction with various aliphatic primary alcohols (C_1-C_{16}) [1]. The free radical scavenging capacity of native and lipophilized molecules was then evaluated by the DPPH [2] test and by our new conjugated triene assay [3] (CTA).

1.1 DPPH test. The free radical scavenging capacity of each chlorogenate ester was measured by DPPH assay and expressed as antiradical power (ARP). The lipophilization induced a significant increase of ARP with a maximum for an alkyl chain lenght ranging from C4 and C8 (Fig. 1).

1.2 QSAR. The QSAR analysis showed that the molecular volume (V_M), the moment of inertia (I) and the dipole moment are closely linked to the ARP, as shown by the following equation :

$$ARP = 50.41 - 0.062 * V_M - 3321.54 * I + 3.83 * \mu$$

(n = 6 ; r^2 = 0.902 ; SD = 1.33)

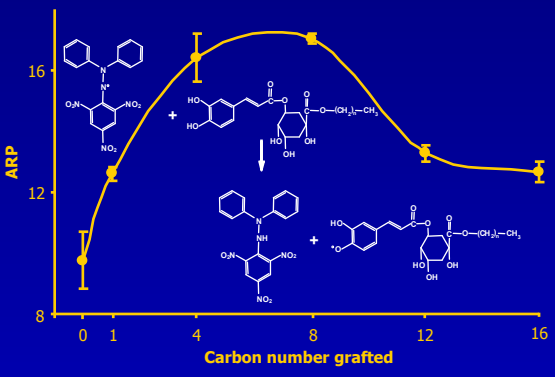

Figure 1. Effect of grafted alkyl chain lenght on ARP

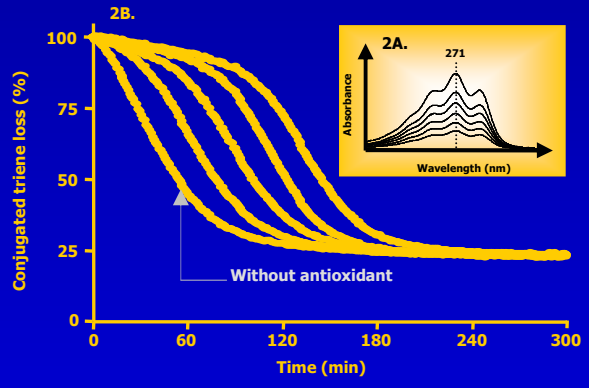

Figure 2. (A) UV spectra evolution of purified TO and (B) kinetics of conjugated triene degradation in presence of butyl chlorogenate.

2.1 Conjugated Triene Assay (CTA). This new *in vitro* test developed in our laboratory is based on spectral properties of conjugated triene (CT) fatty acids naturally present in tung oil (TO). The AAPH-induced oxidation leads to a degradation of CTs and to a bleaching at 271 nm (Fig. 2A). From these UV spectra, kinetics of CTs loss were established with and without alkyl chlorogenates as antioxidants (Fig. 2B). The experimental conditions (200 µL) were: purified tung oil (115 µM), AAPH (1000 µM) and 5-CQA fatty esters (0.2-0.8 µM in PBS).

$$\text{CTA Value} = \left[\frac{AUC_{Sample} - AUC_{Blank}}{AUC_{Trolox} - AUC_{Blank}}\right] \times \left[\frac{\text{moles Trolox}}{\text{moles sample}}\right]$$

Finally, via the classical AUC (Area Under the Curve) calculation, the CTA value was expressed in Trolox equivalent. The results (Fig. 3) show that the grafting of a C_{12}-chain induced a great enhancement of antioxidant activity, that could be explained by a near localization of this molecule towards oxidizable substrate.

2.2 QSAR. In the equation underneath, CTA value was correlated with the same parameters used in QSAR DPPH with, in addition, log P as a position descriptor:

$$CTA\ (value) = 325.7 - 0.968 * V_M - 3694.81 * I + 9.06 * \mu + 37.89 * logP$$

(n = 6 ; r^2=0.909 ; SD = 0.315)

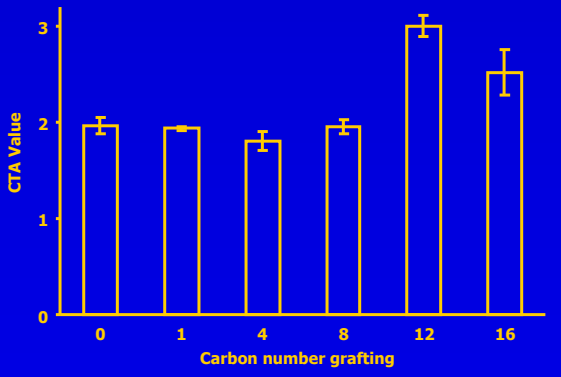

Figure 3. Effect of grafted alkyl chain lenght on CTA value.

Conclusions

From QSAR analysis it is likely that the observed dispersion interactions were responsible of the radical scavenging capacity (DPPH test) in homogeneous media. In heterogeneous media (CTA), these interactions, together with the antioxidant position (towards substrate), are both the factors that best correlate with the CTA value. However, further investigations with a larger set of lipophilized molecules are necessary to validate a general model.

References.

[1] **Lopez Giraldo L.J.** *et al., Enz. Microb. Tech 41,721-726,2007.* [2] **Williams W.B.** et al., Lebensm.-Wiiss. U-Technol., 28, 25-30, 1995. [3] **Laguerre M.** *et al.,* 5th EuroFed Lipid Congress, 16-19 September, Gothenburg, Sweden