

An antioxidant assay for the real world

Researchers at CIRAD have developed a simple antioxidant assay allowing direct measurement of the antioxidant capacity of food samples. Based on UV absorption, this rapid technique is being performed on an Infinite M1000[®] PRO.



The French Agricultural Research Center for International Development (Centre de coopération internationale en recherche agronomique pour le développement, CIRAD) is a publically-owned industrial and commercial enterprise working with a range of developing countries to further scientific research and support international agricultural development. Its Performance of Tropical Production and Processing Systems Department centers its research on the interaction between biological processes and environmental conditions, providing advice and technical interventions to improve

agricultural practices. Within the Department, the Agropolymer Engineering and Emerging Technologies Research Unit (UMR IATE), based in Montpellier, uses a range of techniques to assess and improve crops and plant biomass derivatives, from increasing the nutritional content of whole plants or active ingredients to modifying the properties of macromolecules and composite foods.

One area currently under investigation within the UMR IATE is the antioxidant capacity of foods. The presence and efficacy of antioxidants within foodstuffs is of ever-increasing interest to nutritionists and consumers alike, yet the assays required to assess antioxidant capacity are generally laborious and ineffective, requiring organic solvents and offering a poor reflection of true *in vivo* activity. To overcome this, the Unit has developed a CAT (conjugated

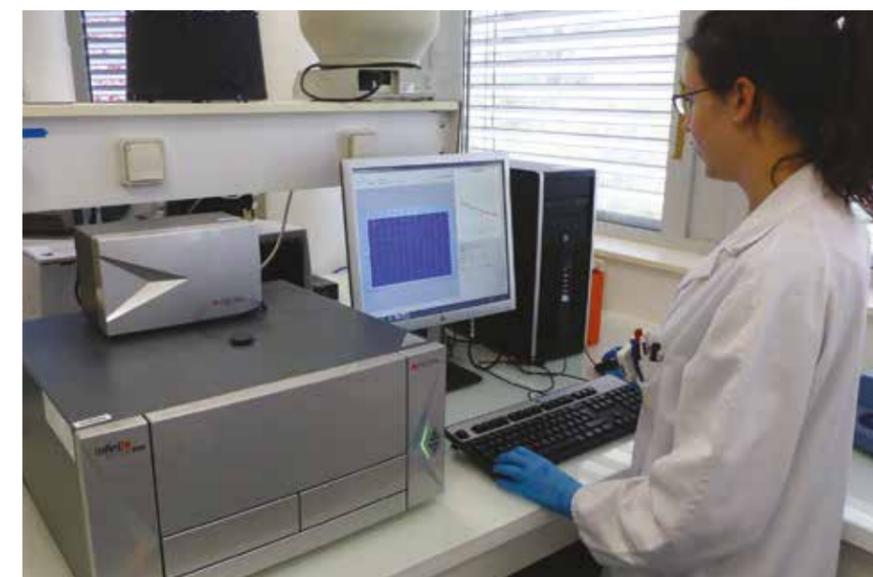
autoxidizable triene) assay¹ which allows routine screening of pure plant extracts for antioxidant activity. Mickaël Laguerre, the researcher who developed the CAT assay as his PhD project, explained: "The aim was to create a simple, relevant method that could be easily transferred to other labs or even used for routine measurements in industry. For this reason, we wanted to develop a high throughput assay using an aqueous medium instead of the organic solvent often used in this situation. We also wanted to employ a lipid-like substrate that would be more representative of *in vivo* activity."

Mickaël and his colleagues developed a microemulsion-based optical method using commercially-available stripped tung oil as an oxidizable substrate. Triacylglycerols (TAGs) from the oil contain eleostearic acid which has conjugated triene groups (three

conjugated double bonds) that exhibit strong UV absorption at 273 nm. Under oxidizing conditions, degradation of conjugated triene TAGs leads to bleaching at 273 nm. This process can be delayed by the presence of antioxidants, enabling the quantification of the antioxidant activity in a sample. Mickaël commented: "A UV absorption method seemed to be the simplest option, but there were several significant obstacles to this approach. The first issue we encountered was that the pro-oxidative compound used to generate peroxy radicals in the assay is extremely temperature sensitive, making it impossible to achieve consistent results in a standard cuvette. As a result, we required a microplate reader with very precise temperature regulation."

"We looked at several options, and the Tecan instrument was the best fit for our needs, combining format flexibility and excellent temperature control with monochromator-based optics. This set-up is ideally suited to assay development, as it allows complete freedom in wavelength selection, and also makes it more versatile for other research activities. The ability to evolve the instrument as your needs change – adding fluorescence, luminescence or polarization functions – is also a significant advantage, as your assay development choices are not limited by the reader."

"The Infinite M1000 PRO is very user-friendly, as is the Magellan™ data analysis software. One feature that I particularly like is the option to perform fully automated data analysis – providing a quick result for screening applications – or export of the data to an Excel® spreadsheet. It's probably true of all researchers, but I like to have the raw data available for more in-depth analysis, and it is very easy to do that with Magellan."



Erika Zago operating the Infinite M1000 PRO

As well as providing the reader, Tecan's application team was instrumental in translating the method from a research project to a routine assay. Mickaël continued: "When I first started, I was using a quartz microplate to allow measurements at 273 nm, which was both tedious to use and very expensive. The Tecan team put me in contact with Greiner Bio-One, as the company was about to launch the UVStar® microplate, and this was a real breakthrough in the development of the assay."

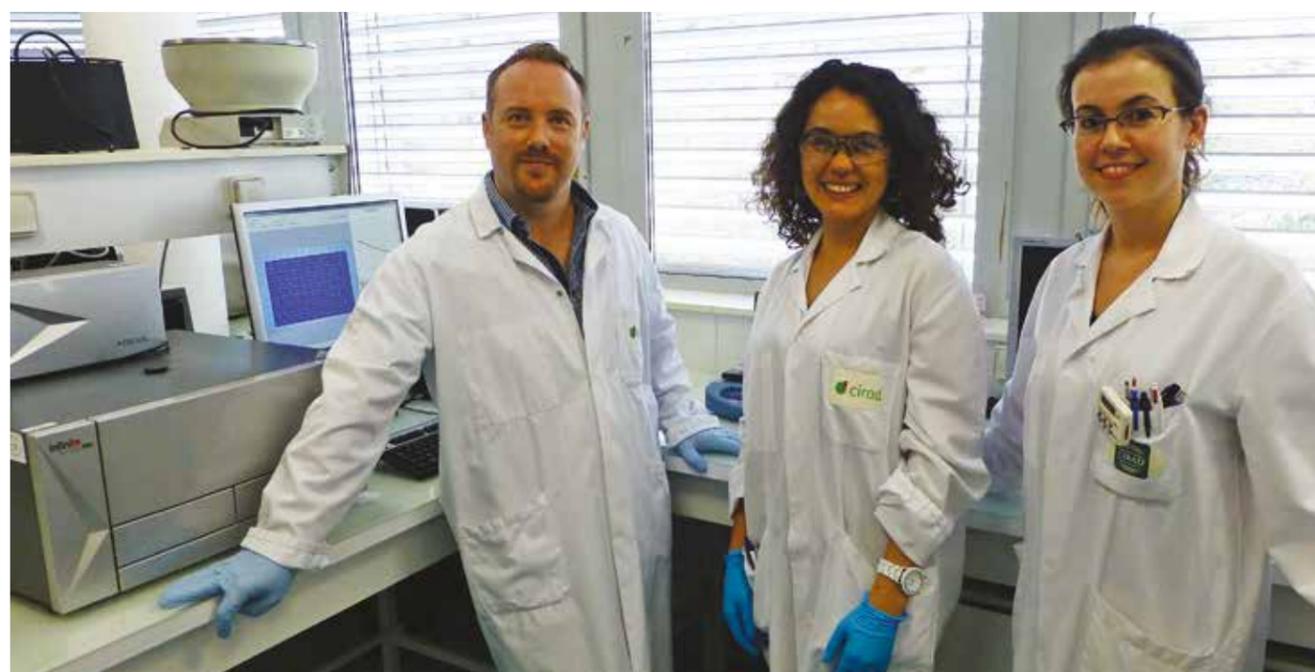
"Since completion of the project, this assay is now in routine use, and we are also employing the Infinite M1000 PRO for a variety of other applications. We perform a number of ORAC assays and fluorescence-based measurements, as well as UV absorbance measurements to follow, for example, the stability of polyphenols. Overall we are very happy with the system."

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¹Laguerre M, López Giraldo LJ, Lecomte J, Baréa B, Cambon E, Tchobo PF, Barouh N, Villeneuve P. Conjugated autoxidizable triene (CAT) assay: A novel spectrophotometric method for determination of antioxidant capacity using triacylglycerol as ultraviolet probe. *Anal Biochem* 2008; 380:282-290.

To find out more on Tecan's Infinite M1000 PRO, visit www.tecan.com/infinitem100opro

To learn more about CIRAD, go to www.cirad.fr



Left to right: Mickaël Laguerre with PhD students Claudia Grajeda and Erika Zago