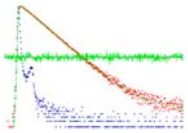

Time-resolved fluorescence for monitoring food composition

The use of time-resolved fluorescence has expanded as the relative cost of instrumentation has decreased in recent years. One area where this is especially true is in the food industry, where time-resolved fluorescence has been applied in the characterisation of food stuffs as well as aspects related to food safety and degradation.

Fluorescence in food

There are a variety of fluorescent substances in food stuffs, ranging from amino acids (Phe,Tyr,Trp) in proteins to vitamins to chlorophyll, for example. Fluorescent substances are therefore contained in both plant and animal derived foods, which means that fluorescence techniques can be widely applied. The principal area of usage includes food safety and storage. The monitoring of contaminants, such as bacteria, toxins and insects, is possible using fluorescence and the technique can also be applied to the development of novel foodstuffs. The versatility of fluorescence is evident from its usage with dairy products, vegetable oils, beers / wines, animal proteins, enzymes and food colourings to name just a few. Although intrinsic fluorophores can be used, at times it can be advantageous to add an extrinsic probe.

In this note a couple of examples will be given, looking at the use of extrinsic probes in food related micro-emulsions and intrinsic fluorescence in a staple foodstuff elucidated using time-resolved fluorescence.

Oil in water microemulsions

Microemulsions have recently been applied to stabilise food colourings and deliver bioactives, as well as being used in low fat spreads. However, there are naturally occurring microemulsions such as milk. There are many dyes available which can either partition to the aqueous or oil phase, or to specific constituents in the emulsion. Fig. 1, recorded with the CCD camera on a *HORIBA Scientific DynaMyc*, is an example of a solution of non dairy creamer with rhodamine B and nile red used as extrinsic dyes. The field of view is approx 1.3 x 1mm.

Part of the reason to study these systems is to ascertain their stability by characterising their make up. Information concerning the membrane structure can be obtained from how the probe molecule locates, ie if there is the formation of lipid rafts or other inhomogeneities. Fig. 2 shows an uneven location of the fluorophore in an oil droplet from a creamer indicating a non homogeneous structure.

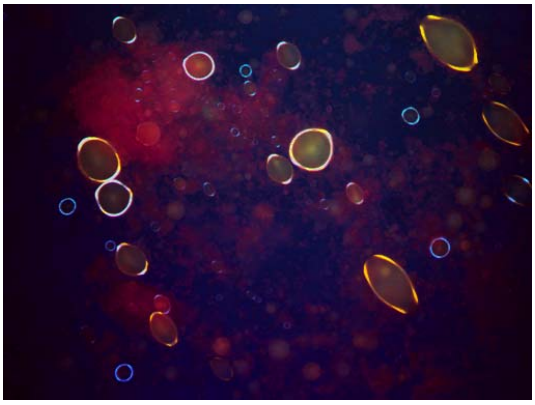

Fig. 1. Camera image using white light illumination and filtercubes with 500nm excitation, >515nm emission.

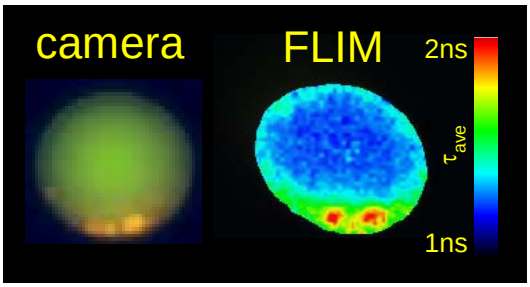

Fig. 2. Fluorescence camera and FLIM image of a stained fat droplet (~15µm in diameter) recorded using a *DynaMyc*.

Anthocyanin form and location

Recently there has been considerable interest in anthocyanins. This is from a health point of view, because of their antioxidant properties, and their potential usage as food colourings. These phenolic compounds contribute to the bright red, blue and purple colours in plants and are responsible for the characteristic autumn leaf colour. Many food crops contain anthocyanins and there has been interest in potatoes rich in these substances. However this class of compound is not very stable and can be affected by temperature, pH and light.

Therefore studies are required to assist in their efficient application, in terms of extraction and stability. Fig. 3 shows a *DynaMyc* image of a variety of purple potato showing the location of an anthocyanin rich area (red in Fig. 3a). The position of starch granules (blue in Fig. 3b) can also be seen.

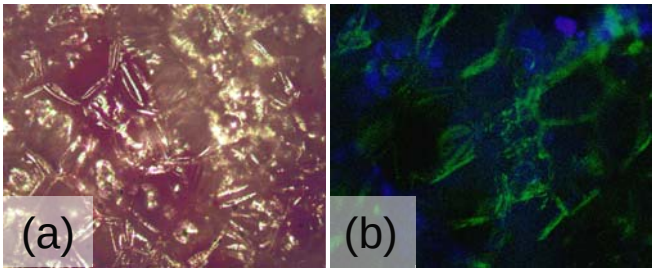

Fig. 3. DynaMyc camera images (a) beam splitter image, (b) composite fluorescence image of a section through an anthocyanin containing potato.

FLIM measurements can provide further information and help provide contrast. As well as *in-situ* measurements, extraction of the anthocyanin permitted a more detailed time-resolved study to be performed. These techniques can also be applied to monitor the effect of different extraction methodologies.

By making use of a *FluoroCube* system time-resolved emission spectra (TRES) were measured. Fig. 4 shows normalised TRES from an extracted sample. A defined peak just after excitation becomes broader at longer times after excitation showing the presence of different emitting species. Global analysis of these data allowed decay associated spectra to be obtained and an example of these are shown in Fig. 5. It is worthwhile to note that in this case, the decay associated spectra exhibit less noise than the TRES as they make use of a larger dataset. The spectral differences and lifetimes allow the form of anthocyanin to be elucidated.

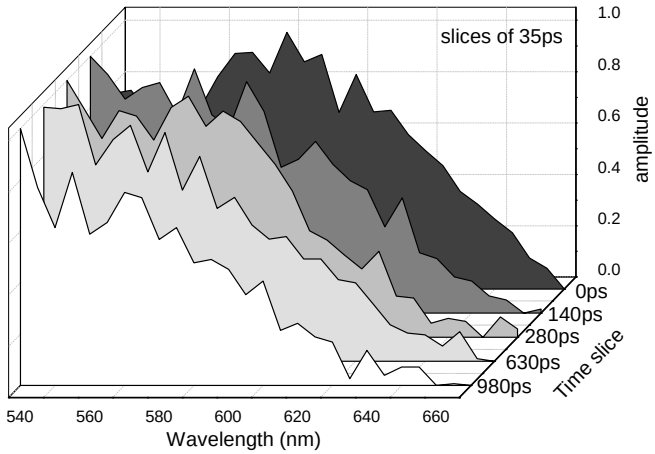

Fig. 4. TRES of anthocyanin extracted from a purple potato.

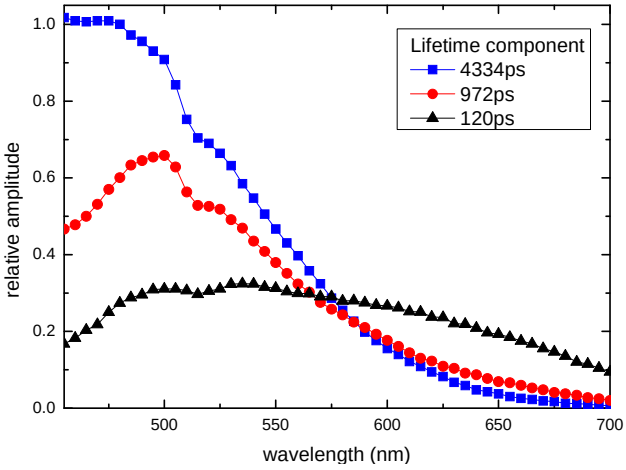

Fig. 5. Decay associated spectra of anthocyanin extracted from a purple potato.

Summary

The note provides two examples relating to the food research where different fluorescence techniques can be employed to help characterise the constituents of foodstuffs.

This note acknowledges the following paper;

M.A. Lemos, M.M. Aliyu and G. Hungerford, ***2012****. Observation of the location and form of anthocyanin in purple potato using time-resolved fluorescence. Food Sci. Emerging Technol.* ***In press***
DOI: 10.1016/j.ifset.2012.04.008