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Report of Alberto Rumignani's STSM at AgroParisTech, Centre de Massy

Department of Science & Engineering of Food and Bioproducts

From October 9th to 30th, 2011

Title: Effect of lipid structure on α-tocopherol stability

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Cost Action FA1001(Pr Laura Piazza, coordinator)

Purpose of the STSM

Monoglycerides are among the most promising polar lipid compounds able to bring new or improved functionalities to foods since they can form self-assembly structures. In particular, saturated monoglycerides, once added to oils, have the ability to form networks entrapping oil creating viscoelastic materials. The resulting system is called organogel (Pernetti et al., 2007). In recent years a number of different applications have been proposed for organogels, such as saturated fat substitution, oil migration inhibitor, vehicles for delivery and protection of lipophilic bioactives (Hughes et al., 2009).

In the attempt to use organogels in food formulations, it is essential to know how these structures affect lipid stability. In previous works, it has been highlighted that the oil structurization properties could affect the oxidation stability of cod liver oil (Da Pieve et al., 2011). Structuring resulted quite ineffective in slowing down the first steps of oxidation reactions (formation of hydroperoxides), but a significant reduction of secondary product formation has been observed. It is likely that oil structurization does not affect the diffusion of oxygen in the matrix and thus the hydroperoxide

formation. On the contrary, it could influence the further oxidation steps that require the encountering among reactants.

This hypothesis could be confirmed by studying the antioxidant activity of chain breaking molecules in structured oils as compared to that in bulk oils. In particular, if the structuration effectively affects reactant mobility, the antioxidant efficiency would be reduced in the organogel as compared to the bulk oil.

On the other hands, previous studies showed that degradation of α-tocopherol, the main component of vitamin E, can be delayed differently when incorporated in lipid droplets depending on their fatty acid composition and also their crystalline fat content and polymorphisms [Relkin et al., 2009].

As α-tocopherol oxidation takes place mainly during propagation phase, after formation of hydroperoxides, we proposed in the present collaborative research to evaluate changes in the stability of α-tocopherol when incorporated in an organogel structured system with monoglycerides , compared to the liquid bulk fat before structuration. To this purpose, organogels were prepared by mixing saturated monoglycerides with cod liver oil added with α-tocopherol. The kinetics of α-tocopherol degradation was evaluated during storage at room temperature. Bulk oil added with α-tocopherol was used as a control. In the light of STSM duration, cod liver oil has been chosen as target oil due to its higher susceptibility to oxidation.

Description of the work carried out

Sample preparation

Cod liver oil (Marco Viti Farmaceutici SpA, VI, Italy) added with 3.1 g/L of α-tocopherol (Sigma-Aldrich, Co.) and 64.8 g/L of Myverol™ (Kerry Bio-Science, Bristol, UK) was heated at 70 °C in a water bath to obtain the complete melting of monoglycerides. Next, 10 g of samples were inserted in 15 mL capacity vials, sealed with plastic snap-cups. Samples were cooled down at room temperature under static conditions, then stored at 20 °C until analysis. The same procedure was applied also to cod liver oil/α-tocopherol mixture in order to avoid a possible effect of preparation conditions on oxidation rate.

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Figure 1. Samples of oil (left) and organogel (right) added with α-tocopherol.

Figure 1 shows fresh prepared samples (24 hours after preparation) of oil and organogel, both added with α-tocopherol. Overturned samples well show the structuring effect of monoglycerids

α-tocopherol determination

α-tocopherol was extracted from bulk oil or organogel using ethanol and hexane as solvents (1:1, by vol). following the methodology of Vatassery and Mortenson (1972), and its concentration was determined using a spectrofluorimetric and spectrofluorimetric method as described in Shukat & Relkin, 2011. Briefly, the concentration was determined by UV-spectrophotometry at 295 nm, and spectrofluorimetry at 295 and 340 nm for excitation and emission wavelength, respectively. The α-tocopherol content was determined using a calibration curve ([α-tocopherol]=(FI+3.7067)/3987.3, R^2=0.9936) obtained from freshly prepared solutions of α-tocopherol in ethanol-hexane (1:1, by vol.) mixture at concentration ranging from 0.001 to 0.07 g/L. Fluorescence index (FI) was computed as shown in the following equation :

$$IF = IF_{measured} \times 10^{\left(\frac{Abs_{295}+Abs_{340}}{2}\right)}$$

Where, Abs_{295} and Abs_{340} are the values of absorbance measured at 295 nm and 340 nm respectively. Uncertainties were deduced from three replication of each sample measurement.

Main results obtained

The first part of the STSM work was dedicated to the set-up of the methodology that allows α-tocopherol concentration to be detected in oil and organogel systems. Following the methodology proposed by Vatassery and Mortenson (1972), samples for spectrofluorimetric analysis were obtained by adding 5 mL of ethanol and 5 mL of hexane to 5mL of oil or organogel previously heated to 70 °C. After 1 minute of manual shaking, an homogeneous mixture was obtained. The α-tocopherol concentration was determined using an excitation wavelength of 295 nm and an emission wavelength of 340 nm.

In these conditions of dilution, $IF_{measured}$ values were closed to zero even for fresh prepared samples, not allowing the α-tocopherol determination. Probably, the quantity of the sample in the solvent mixture is too high and interferes with the capacity of α-tocopherol to show fluorescence. Thus, the samples were diluted.

In particular, 100 µL of oil or organogel were added to 9.9 mL of ethanol-hexane mixture (1:1, by vol.). Results highlight that this sample concentration allows the α-tocopherol to be detected.

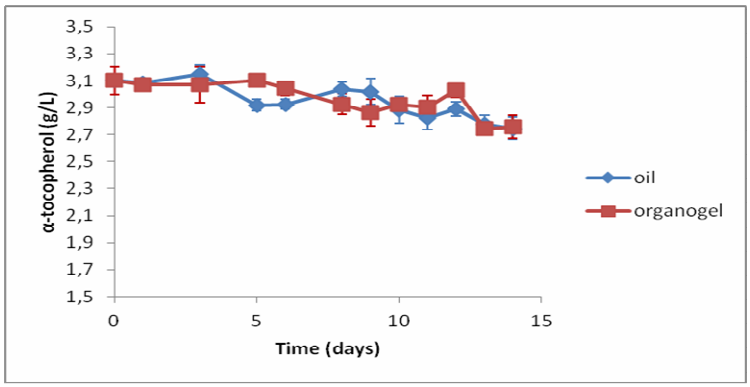

Figure 2. Concentration of α-tocopherol in oil and gel system as a function of storage time. Uncertainty is given by bars (standard error).

Afterwards, the experimental work was dedicated to the evaluation of α-tocopherol concentration in oil and organogels during storage at 20 °C. Figure 2 shows the result obtained. It can be noted that slight changes of α-tocopherol concentration were detected and no significant differences between structured and bulk oil were observed. It is likely that the oxidative reactions are yet in the initial stages and thus it would be necessary to continue the experiments for longer time to draw a conclusion.

Future collaboration with host (if applicable)

The present STSM has opened collaboration between the AgroParisTech, Centre de Massy and the Department of Food Science of the University of Udine on the study of the effect of food structure on lipid oxidation kinetics.

In the short term, AgroParisTech proposes to continue α tocopheriol determination in the same structured oganogels and in the bulk liver oil for longer time of storage.

In longer term, it should be very interesting to perform other research activities to better understand results obtained during the STSM.

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Confirmation of the host institution of the execution of the STSM

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