Fluorescence Fingerprints as a Rapid Predictor of the Nutritional Quality of Processed and Stored Foods

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Abstract: Foods are complex mixtures of macro- and micronutrients, which interact leading to oxidation, glycation and hydrolysis upon heating (sterilization, cooking) and storage. The nutritional quality and safety is consequently affected justifying the need for accurate monitoring of the evolution of the food composition during processing and in product shelf life. Classical chromatographic analysis as well as newly proposed rapid methods based on fluorescence spectrometry analyses are applied in the present study on (i) fresh and stored carrots, (ii) infant formula resembling model, (iii) heated rapeseed oil, and (iv) wheat biscuits. Fluorescence fingerprints addressing modifications in the product composition during processing were recorded and analyzed by means of chemometric methods. Fluorescence, recorded in a front-face mode on intact and crushed food, or product extracts, is very sensitive to pertinent physicochemical changes induced by heat treatment or storage. Results show the potential of non-destructively applied fluorescence spectrometry for measuring vitamin E in carrots, carboxymethyllysine in powdered infant formula models, polar compounds in rapeseed oil and hydroxymethylfurfural in biscuits. This paper presents the potential of fluorescence as a global approach of the quality of processed food.

Keywords: fluorescence; vitamins; Maillard reaction; processing; storage

INTRODUCTION

The new awareness that a food is an unstable mixture of chemical constituents interacting throughout processing and storage causes a growing need for rigorously controlling the nutritional quality and safety. A loss of the nutritional quality will occur, if the labile constituent is a vitamin (e.g. vitamin E and C), or an essential fatty acid or amino acid (e.g. w3-PUFA, lysine). The safety of the product is questioned, if food processing induces the accumulation of neo-formed compounds (acrylamide, oxidized lipids, carboxymethyllysine) with possible hazardous biological effects. Storage of these processed foods further enhances such development. Indeed the quality of a food depends on each step in the food chain (e.g. quality of ingredients, processing parameters, and storage conditions). Monitoring these various parameters by conventional methods seems very ambitious, not only because of time and cost needed to monitor the various specific reactions possibly taking place in the food product, but also because our knowledge on the chemical reactions in foods is today far from being exhaustive.

In this context, a more global approach regarding the variations in the fluorescence fingerprint may be proposed, which is based on determining characteristic changes occurring during production of foods, and its correlation with pertinent quality indicators. Fluorescence seems a promising technique due to high sensitivity to physicochemical changes in a food product during processing and storage [1, 2]. Indeed, many nutrients are naturally fluorescent, e.g. vitamins, amino-acids, polyphenols, as well as neoformed products developed in Maillard reaction and lipid peroxidation. Fluorescence was already proposed for evaluating the peroxidation in lipid-containing foods [3, 4] or the Maillard reaction in milk products [5, 6]. Front face fluorescence receives a growing interest in food analysis, because of the possibility to

non-invasively recording fluorescence spectra of a food product. Application of multivariate analysis allows extracting the pertinent information of the spectra accounting for the indicator of interest. In the present paper calibration results are shown for predicting indicators of food quality. Various food products were subjected to storage or heat treatment and analyzed by means of commonly used indicators and front face fluorescence spectrometry.

EXPERIMENTAL

All fluorescence and reflectance analysis were performed on a Xenius (SAFAS, Monaco) spectrometer equipped with a Xenon lamp and a double beam excitation and emission.

Carrots (variety Dordogne, France) were stored at 4°C and 16°C for 21 days. Carrot slices were measured directly by means of fluorescence excitation spectra with emission at 330 nm. Vitamin E was analyzed by HPLC-fluorescence in the hexane extract stabilized by BHT, according to the method previously described [7]. Carotenoids were analyzed by HPLC-UV using the same method and the results were used for correcting the fluorescence spectra for interferences.

Commercial rapeseed oil (RO) was heated in a fryer at 190°C for 4 hours. 10 ml aliquots were taken each 30 min for analysis. Emission spectrum at excitation wavelength 298 nm was recorded on a front face mode directly on the oil sample in an acryle 4 face cuvette. The polar compounds (PC) were isolated on a silica gel column, dried and weighted, according to the AOAC method [8]. This polar and the remaining apolar fractions were dissolved in hexane for separately recording the spectra in the same conditions as for the oil.

Infant formula resembling models consisted in whey and hydrolyzed whey, were provided by Armor Protéine (France) and mixed with lactose (80 g/l) and various levels of a vitamin C-iron mixture (0/0; 4/0.4; 8/0.8 mg/g protein). The samples were incubated in the presence of different water contents, 4, 6 and 8% at 60°C for 48 h. Fluorescence excitation at emission 450nm and reflectance were directly recorded on the powders. CML levels were analyzed by ELISA (Microcoat Biotechnologie, D-Bernried).

Biscuits (BI) were prepared from wheat floor as (60%), sucrose syrup (30%) and palm fat (10%) and cooked in an oven set at 250 or 300°C. HMF was analyzed by HPLC using a reversed-phase C_{18} column eluted with methanol/sodium acetate 0.04M (10/90) adjusted at pH 3.6 with acetic acid. Fluorescence emission spectra and reflectance at excitation 450 nm were recorded on the powdered biscuit.

Calibration models were built using fluorescence spectra and corresponding molecule of interest. For this purpose partial least square (PLS) regression was applied (Matlab 6.5, The Mathworks, USA) and the number of latent variables (LV) was chosen regarding the first minimum of the root mean square error of cross-validation (RMSECV). Calibration statistics are given in the figures accounting the models dimensionality (#LV), coefficient of deter-

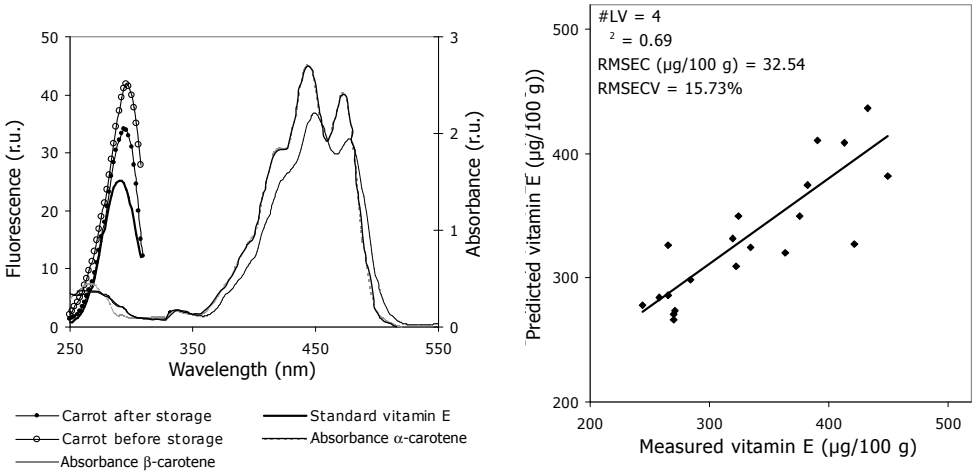

Figure 1. Excitation fluorescence of carrot slices (λ em 330 nm) corrected for carotenoid absorbance and vitamin E calibration

mination (R^2), root mean square error of calibration (RMSEC), and percent of RMSECV.

RESULTS AND DISCUSSION

In Figure 1 fluorescence spectra obtained on carrot slices are shown. Comparing carrot spectra and fluorescence of vitamin E (vitE) standard in hexane reveals a slight blue shift in carrots. This is possibly caused by the different media, cell membrane in carrots and hexane for the standard, but also, to a slight screen effect by carotenoids present at high concentrations in carrots. Fluorescence data were therefore corrected for the samples carotenoid contents improving the quality of PLS regression between fluorescence and vitE (Figure 1). The vitE variation between samples was due to the sample heterogeneity, accounting the distribution in the carrot tissue as well as between carrots, and to the effect of storage. VitE increased after storage at 4°C (347 ± 16 μg/100 g FW) and decreased (225 ± 25 μg/100 g FW) after storage at 16°C as compared to initial levels (245 ± 11 μg/100 g).

Rapeseed oil exhibited various changes in the composition when heated at 189°C. Alpha and gamma tocopherols were degraded exponentially, whereas conjugated dienes, hydroperoxides and malondialdehyde were produced and then degraded in more complex monomeric and dimeric compounds (data not shown). All these oxidative products were formed from PUFA thermo-oxidation [9] and were globally quantified by the content of polar compounds (PC). PC increased linearly with the heat impact (sum of temperatures) from an initial level of 10% to 30 % after 4.5 hours heating at 189°C. Fluorescence spectra changed proportionally to the heating

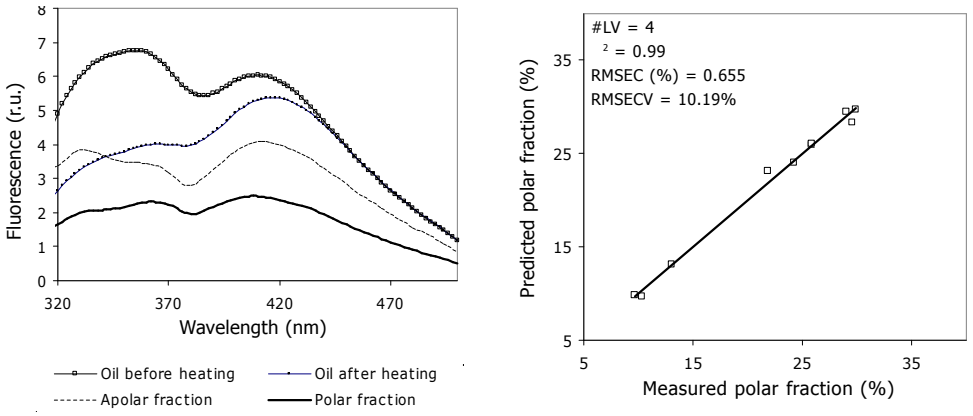

Figure 2. Fluorescence emission (λ exc 298 nm) of rapeseed oil before and after heating and of the polar and apolar islolated fractions and calibration of polar compounds

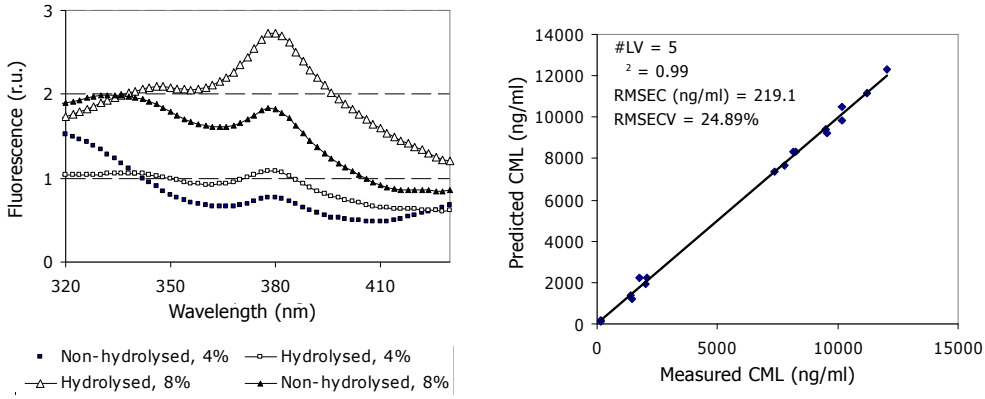

Figure 3. Excitation fluorescence spectra (λ em 450 nm) of hydrolyzed and non hydrolyzed infant formula models as a function of water content (4% and 8%) and CML calibration

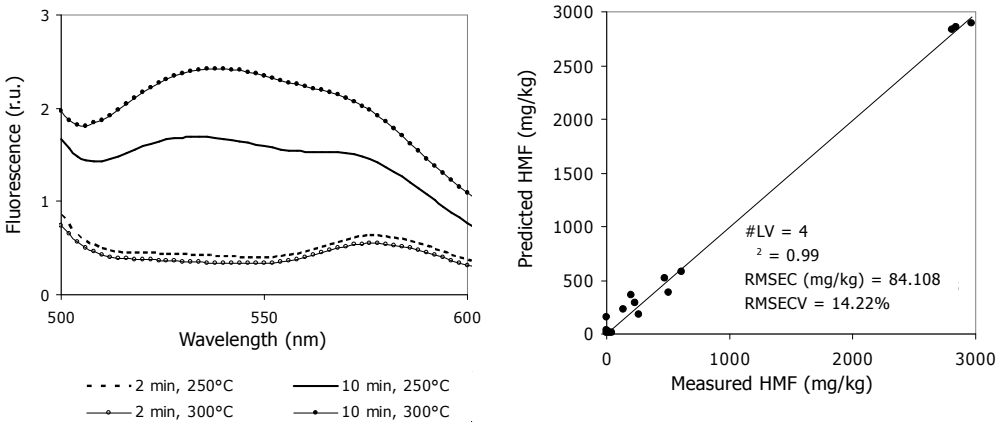

Figure 4. Emission fluorescence spectra (λ exc 450 nm) of biscuits cooked at 250°C and 300°C and HMF calibration

time and temperature. The fluorescence emission spectra (excitation wavelength 298 nm) were well correlated to the PC content (Figure 2).

Powdered infant formula models developed a brown colour upon incubation, resulting from reaction of lactose and oxidation products of vitamin C as reported elsewhere [10], which was proportional to the water content. Browning was more intense when whey proteins were hydrolyzed. CML levels were sensitive to the same parameters. Up to 10 times more CML was found in hydrolyzed formulas as compared to non hydrolyzed formulas with similar composition. The characteristic fluorescence of Maillard products appeared in all samples with a maximal excitation at 380 nm. After correction for the reflectance, this fluorescence was highly correlated to CML contents (Figure 3).

Degradation products of sucrose during biscuits generated HMF after 4–6 min cooking at 300°C and 250°C, respectively, confirming previous observations [11]. HMF accumulation was well correlated to a fluorescence appearing in biscuits at 530 nm (excitation 450 nm) during the cooking process (Figure 4).

CONCLUSION

The fluorescence fingerprints of the food products were generally more sensitive to the impact of food processing and shelf life conditions as did classical chemical indicators, partly because of a greater error of reproducibility in the latter and spectra analysis by chemometric tools. By these examples, we demonstrate the potential of a global approach based on fluorescence techniques for monitoring the nutritional quality and safety of processed foods. Additionally the method presented is rapid, applicable to the food without preparation, and not expensive.

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