Innovative methods and metrological aspects applied to food safety: case studies of OTA and SO_2

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During my PhD, I dealt with some chemical and metrological aspects related to the food safety topic working on two categories of chemicals which affect the food safety: food contaminants and food additives. In particular, I faced the study of two main molecules: the ochratoxin A (OTA), which is a food contaminant and in particular a mycotoxin, and the sulfur dioxide (SO_2), which is a food additive and in particular an antimicrobial and antioxidant agent. Furthermore, I synthetized numerous batches of silver nanoparticles (AgNPs) with three nominal lateral sizes. Here, I briefly summarize the results I achieved and the future perspectives and developments.

AgNPs.

I synthetized batches of AgNPs colloidal suspensions with three nominal diameters: 4, 30 and 55 nm. All these types of nanoparticles were characterized in terms of dimension, shape, spectral position of the LSPR and specific surface area. The 4 and 30 nm AgNPs resulted spheroidal and homogeneously distributed, with the LSPR positioned at 391 nm and 397 nm, respectively. Most 55 nm AgNPs were spherical but some of them also had different shapes. Their LSPR was positioned at 417 nm. The specific surface area was higher for the 4 nm AgNPs and lower for 55 nm AgNPs. All these three sizes of AgNPs were tested as a substrate in the innovative in liquid SERS methods developed in my thesis.

Ochratoxin A.

First of all, I used the HPLC-FLD to quantify the OTA concentration in a CRM of red wine. It resulted that, despite it is the election analytical technique, some critical aspects came out. The major issue was related to the solvent evaporation step which led to the crystallization of the toxic solid OTA.

Then, a wide range of pH (from 1 to 12.5) was investigated in order to provide a comprehensive description of the behaviour of OTA in aqueous solutions. This study was divided into two parts. Under acid to neutral condition, the two pK_a of OTA were evaluated by two independent techniques: the UV-Vis spectrophotometry and the fluorescence spectroscopy. They resulted 4.4 ± 0.1 and 7.09 ± 0.01 (pK_{a1}) and 4.2 ± 0.9 and 7.0 ± 0.4 (pK_{a2}), respectively. Moreover, the most probable degradation pathway of OTA in solution under alkaline conditions was identified by experimental fluorescence maps and theoretical calculations. The degradation of OTA conducted to its fragmentation into two molecules: the phenylalanine, an amino acid, and the ochratoxin α, which is much less toxic than its precursor. However, such fragmentation happened only if the degradation process was prolonged over time and under strong alkaline condition. Thus, for this study, the condition of reversibility of the degradation pathway, which conducts again to toxic OTA, was defined. As a result, OTA samples and contaminated glassware should be handled paying greater attention. All the information acquired in this work are useful for future toxicological studies of the various forms of OTA and for the development of CRMs of this mycotoxin.

Despite its high toxicity, a reference Raman spectrum of solid OTA was obtained in a wide spectral range. The corresponding vibrational modes have been carefully attributed to most of the peaks relative to the OTA. Then, an in liquid label-free SERS method with AgNPs as a substrate

was developed for OTA identification. After many tests and parameter optimization, the main peaks identified in the spectrum of solid OTA were found also in the SERS spectrum. The best spectrum was obtained with 30 nm AgNPs and tartaric acid as an aggregating agent. This feasibility study provided a method which presented the advantages of being fast and easy. However, the sensitivity of this method was insufficient for OTA detection at the concentration typical of food matrices.

Lastly, I participated to a Secondment at the NRC – Canada focused on culturing, isolation, and purification of ochratoxin A for reference material production, along with metrological characterization of the material. The first goal of this project was the production of pure solid OTA. I developed my expertise on fundamental techniques in fungal cultures to produce mycotoxins and I learned how to handle biological material. The final goal of the project, which is part of the BIPM's Mycotoxin Metrology Capacity Building and Knowledge Transfer (CBKT) Programme, was the production of CRMs of OTA.

Sulfur dioxide.

I evaluated the recovery of free SO_2 in a wine simulant solution with a gravimetrically known concentration of this additive by applying the OIV-OENO 591A-2018 method. The maximum achieved recovery was of 95.5%. Moreover, a measurement comparison was conducted among nine specialized laboratories with this official method. The first output of the comparison was that this official method suffers of an underestimation of the SO_2 content in wine. Indeed, none of the participants achieved the total recovery of the analyte. The second output regarded the high variability of the results, reported with only their standard deviation, obtained by the laboratories. In this regard, a greater exchange of information and a more homogenous application of the official method is desirable.

I carried out a rigorous evaluation of the measurement uncertainty associated to the free SO_2 concentration achieved by the official method with the INRiM apparatus. For the wine simulant used in the comparison, which presented a concentration of free SO_2 of 150.1 mg/l applying a gravimetric method as a reference, the obtained at INRiM was 143.3 mg/l with an expanded uncertainty of 3.9 mg/l. This work was in support of the official method.

To perform all the above mentioned activities regarding SO_2, including the comparison, I produced samples of wine simulant, which can be considered as a sort of CRM candidates. Indeed, long term stability and homogeneity tests were performed. One of the CRM candidate presented a concentration of free SO_2 of 143.3 mg/l with an expanded uncertainty of 4.8 mg/l (k=2).

In order to develop an in liquid SERS method for the quantification of total SO_2 in wine, I firstly performed tests of interaction between the three sizes of AgNPs and the SO_2 in a hydroalcoholic solution. Once defined the 4 nm AgNPs as the best substrate for this kind of analysis, the same interaction was investigated with the SO_2 in wine simulant. In this matrix, a greater interaction between the two components was observed and the vibrational peaks related to SO_2 were identified. Furthermore, the experimental data were fitted with a Langmuir function, typical of adsorption-desorption phenomena. Six wines (three red and three white) were analysed by this optimized in liquid SERS method. The results achieved by SERS were compared with the ones obtained by applying the official OIV-OENO 591B-2018 method on the same samples. The agreement between the results provided the validation of this innovative SERS technique. This method could be used for *in situ* analysis in the future.