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USE OF SUPRAMOLECULAR SOLVENTS IN THE DETERMINATION OF ANNATTO IN DIFFERENT FOODSTUFFS

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Natural as well as synthetic food colorants are widely used by food manufacturers to attract the attention of consumers. Annatto food colour is one of the oldest known natural colouring agent used as a food additive in the European Union and elsewhere. It is obtained from the outer layer of the seeds of the tropical tree *Bixa Orellana*. Main components of annatto are the carotenoids bixin and norbixin. Both, bixin and norbixin are designated as E 160b and give colour shades in the range red-orange-yellow in different foods such as dairy products, flour confectionary, fish, soft drinks, meat products, snack foods, and dry mixes. The use of food colours in the European Union is controlled by the Directive 94/36/EC, which contains a list of permitted colours, a list of foodstuffs to which these colours may be added and, where appropriate, maximum limits on the level of addition. So, the determination of bixin and norbixin is required for the control of the compliance of current legislation.

Quantification of bixin and norbixin in food has been frequently performed by liquid chromatography with UV-VIS diode array detection. Sample treatment is always the bottleneck of these methods since tedious and solvent-consuming procedures are used.

Supramolecular solvents are an environmental friendly alternative to traditional organic solvents for analytical extractions. They have exceptional properties for extraction processes, which derive from the special structure of the supramolecular assemblies making them up. The different regions of polarity and acidity present in the three-dimensional structure of aggregates offer various types of binding to solutes, which results in very high extraction efficiencies.

In this work, the octanoic acid reverse micelle-based supramolecular solvent has been proposed for the extraction of bixin and norbixin from different foodstuffs prior to its determination by liquid chromatography and photometric detection. The procedure involved the extraction of minute quantities (200-350 mg) of homogenized food sample with 1200 μL of supramolecular solvent in a single step. The overall sample treatment, which included extraction and centrifugation, took about 20 min, and several samples could be simultaneously treated using conventional lab equipment. The precision of the method, expressed as relative standard deviation, was about 1.5 % and the quantitation limits for bixin and norbixin were around 0.29 mg Kg^{-1} and 0.34 mg Kg^{-1}, respectively, which were far below the maximum limits on the level of addition permitted. The method was successfully applied to the determination of bixin and norbixinin in different kind of samples (snacks, mimolette cheese, caramel cream and ice-cream) with recoveries that ranged between about 78 and 113%.