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Development of a Method of Analysis for Phenolic Acids in Milk

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Phenolic compounds are secondary metabolites of plants generally involved in defence against ultraviolet radiation or aggression by pathogens. Among the different classes of these compounds, defined according to their molecular structure, very important are the phenolic acids, constituted of

a phenolic ring bonded to a short acidic chain and one or more –OH and $–OCH_3$ groups. These compounds can be distinguished in two classes, the derivatives of benzoic acid and the derivatives of cinnamic acid. Their presence was highlighted generally in plants but also in some foods such as drinks, jam and bakery products.

The aim of this work was to develop and validate a method of analysis for these compounds in milk in which have never been determined before.

The analytical procedure includes four steps: 1) extraction of phenolic acids with acetone and acetate buffer (pH = 4,6); 2) hydrolysis of the conjugated phenolic acids using β-glucuronidase at 37°C; 3) clean up, with a solid-phase extraction to remove co-extracted materials with a DPA-6S cartridge and finally 4) analysis with reversed phase liquid chromatography and an UV diode array detector equipped with a 5 cm length cell, operating in full scan modality.

The recovery and the repeatability of the method has been evaluated according to European current guidelines for trace methods then L.O.D. and L.O.Q. of 19 phenolic acids have been determined.

Analysis of milk samples produced in highland and lowland pastures were performed with this new method and results showed significant differences in phenolic acid composition due to the different feeding of the cows.

Keywords: phenolic acids, milk, liquid chromatography, traceability