

*Title:* Determination of biogenic amines in wines and sparkling wines by liquid chromatography with precolumn derivatization

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Biogenic amines are often present in different types of food, but they are especially abundant in wines, cheeses, meat and fish as well as in spoiled products. Besides, the presence of a high content of biogenic amines in food can be an indicator that it has been processed under poor hygiene conditions. These amines are generated mainly from the decarboxylation of the respective amino acids.

In moderate concentrations, these compounds can be beneficial for the body as they participate in different cellular functions but when the intake of these compounds is too high for the body, toxicological problems such as migraines, headache, hypo- or hypertension, effects on the vascular or nervous system and anaphylactic shocks can result. Therefore, given the harmful effects that biogenic amines can cause in an organism, it is necessary to establish an effective and selective method to determine these amines in food in order to avoid the commercialization of any food with high concentration of these compounds. Consequently, research is also needed to establish legal limits on the contents of different biogenic amines in food products. This last point is more complicated because the content of biogenic amines that an organism can tolerate depends on it, in turn, on the catabolic pathways of each individual. Thus, a given concentration of amines can be tolerated by an organism with a normal catabolism, but it can cause harmful effects on a sensitive organism.

In wines, the most important biogenic amines are histamine, tyramine, putrescine and cadaverine because they are the most abundant and can cause the greatest number of toxicological problems. Among the different types of wines, red wines have the highest content of biogenic amines.

Also, in wines, the presence of ethanol increases the toxicological problems that can cause these biogenic amines because this alcohol inhibits or decreases the activity of aminooxidases, responsible for their metabolism.

In this project, the following biogenic amines have been considered: ethylamine, methylamine, dimethylamine, ethanolamine, butylamine, isobutylamine, isopentylamine and hexylamine.

Although these amines are present at trace level, their study is important as possible descriptors of the health quality of food. To separate and determine them adequately the following studies have been carried out:

- A pre-column derivatization reaction has been performed with the 1,2-naphthoquinone-4-sulfonate reagent (NQS) to attach a chromophore group to the initial amines so that they could be detected with an ultraviolet-visible absorption detector.

- A liquid-liquid extraction process with chloroform as the solvent and subsequent evaporation until dryness has been used to obtain simpler chromatograms removing efficiently amino acid derivatives and other polar compounds that often interfere with the chromatographic separation of biogenic amines, in addition to preconcentrating the extracts obtained.

Combination of both derivatization and extraction procedures before the HPLC separation results in an effective clean-up of samples and affords a sensitive determination of biogenic amines.

- HPLC has been used as a technique for separating different amines under reversed-phase mode, optimizing the efficiency and resolution by modifying some variables such as the chromatographic column, the elution gradient or the separation temperature.

It should be pointed out that due to the consequences of the global pandemic caused by the SARS-CoV-2 virus, significant parts of method optimization and validation studies as well as the analysis of wine and cava samples have not been finished.

**Keywords:** biogenic amines, toxicological problems, wines, derivatization, 1,2-naphthoquinone-4-sulfonate, high performance liquid chromatography