Determining the sugar content in grape juice by HPLC-RID

Anaïs STEMMELEN

Introduction

As part of a biological control project, a study was conducted to evaluate the impact and the effects of pre-harvest treatments on ochratoxinogenic contaminant of grapes. The grape was collected in the experimental station INRA-Pech-Rouge by the "Institut Français de la Vigne et du Vin (IFV)" in Narbonne (Languedoc-Roussillon). Around the Mediterranean Sea, grapes tend to be contaminated by Aspergillus carbonarius, a fungal species, which produces a mycotoxin called Ochratoxin A (OTA). This toxin, carcinogen and nephrotoxic, is a real problem for our consumer society. According to the EU regulation, OTA is regulated at 2 µg/L in commercialised wine and grape juice.
After determining the impact of the treatments on the OTA content (not treated is this paper), the aim of this work was to determine the effect of these treatments on the sweet taste of grapes.

Materials methods

After artificial contamination by Aspergillus carbonarius, several biological and chemical treatments were carried out on plots of the "mourvèdre" variety, which is sensitive to fungal contamination:

- One chemical treatment: fungicide, Scala®.
- Three biological treatments: a fungal antagonist Trichoderma atroviride species, a yeast antagonist Saccharomyces cerevisiae species, Stifénia® FEN560, a natural product developed from fenugrec seeds as elicitor.

Two plots were used as control:

- Control n°1: not contaminated and not treated.
- Control n°2: contaminated and not treated.

According to the « Recueil des Méthodes d'analyses de l'Organisation Internationale de la Vigne et du Vin (OIV) », glucose and fructose (main sugars in grapes) were analyzed using high-performance liquid chromatography (HPLC) with a refractometric detector (RID).
The different grape juices were collected with a press laboratory and filtered with a filter of cellulose nitrate (pore diameter 0.45 µm) and a syringe Mini-Sart, Sartorius®. Then, juices were diluted in order to have a concentration included in the standard curve calibration.
The chromatographic system was composed of a Shimadzu LC-6A pump coupled to a Shimadzu RID-6A refractometer and to a Shimadzu CTO-10AC column oven at 40°C. This system was used with the following parameters:

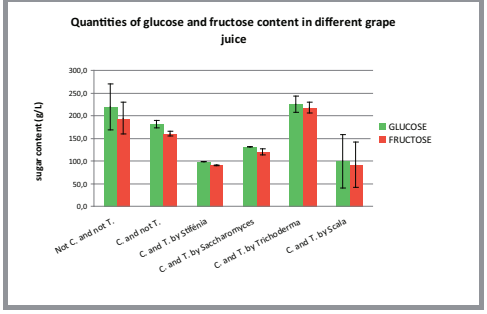
Column:

- length	30 cm
- internal diameter	7.8 mm
- particle diameter	9 µm
- Injection ring	20 µL
- Mobile phase	solution of 0.5% sulphuric acid
- Flow rate	0.6 mL/min

Results

For each sample, the analysis demonstrated that the retention time for glucose was 13.9 min and 15.1 min for fructose.
The results of the sugar content in grape juice are given in Fig 1. For treated grape juice, glucose was present in a quantity slightly greater than fructose.

Figure 1: Content of glucose (green) and fructose (red) for grape juice determined by HPLC-RID (C. means "contaminated" and T. means "treated"). Error bars are also represented.



Some significant differences were noted between treatments with ranges from 98.5 ± 0.6 g/L (Stifénia) to 225.7 ± 18.1 g/L (Trichoderma sp.) for glucose and 90.8 ± 0.7 g/L (Stifénia) to 217.9 ± 11.9 g/L (Trichoderma sp.) for fructose. All contaminated and treated juices had less sugar than those from the control samples, except for Trichoderma sp. juice, which was sweeter than the juice treated chemically. The elicitor treatment induced grapes with reduced contents of reduced sugars. This could act directly on the growth and the toxigenesis of contaminants. Effectively, results of OTA contents of juice (not given here) showed reduced contents of OTA for grapes treated with Scala, yeasts and especially Stifénia corresponding to samples with lowest reduced sugar content (fig.1).

Conclusion

A conclusion could not be established on the unique study of the sugar content in order to determine the most adapted treatment. It is necessary to consider other parameters : the most important is the decrease of toxin in grapes but also other markers of grape quality and markers of stimulation of plant defence such as the content of acids or polyphenols.

UMR QUALISUD
Place Eugène Bataillon
34095 MONTPELLIER Cedex