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Melatonin is synthesised by yeast during alcoholic fermentation in wines

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ABSTRACT

Melatonin (*N*-acetyl-5-methoxytryptamine) is a neurohormone produced in the pineal gland. Its biological properties are related to the circadian rhythm. Recently, the European Food Safety Authority (EFSA) accepted the health claim related to melatonin and the alleviation of subjective feelings of jet lag. This molecule has been detected in some foods. In this work, 13 grape varieties were studied; 7 monovarietal wines were produced in an experimental winery under strictly controlled conditions and were sampled in different steps. The grape varieties used to make the wines were: *Cabernet Sauvignon, Merlot, Syrah, Tempranillo, Tintilla de Rota, Palomino Fino* and *Alpha red*. Liquid chromatography tandem mass spectrometry (LC-MS/MS) unequivocally confirmed the presence of melatonin in wines. The main contribution of this paper is the results that clearly show that melatonin is synthesised during the winemaking process, specifically after the alcoholic fermentation. Indeed, melatonin is absent in grapes and musts and is formed during alcoholic fermentation.

1. Introduction

The determination of the chemical composition of wines aims to improve the winemaking process, establish a relationship with the sensory properties of wines, enable the study of the health benefits or facilitate characterisation. During the last two decades, research in the field has encompassed the development of new, more sophisticated, more sensitive analysis methods (Pereira, Câmara, Cacho, & Marques, 2010; Saurina, 2010), a full description of chemical composition (Polaskova, Herszage, & Ebeler, 2008) and the targeting of molecules with an impact on organoleptic properties (Vilanova, Genisheva, Masa, & Oliveira, 2010). Additionally, a large effort has been made in determining the bioactivity of compounds present in wine and in describing new molecules with biological activity.

The study of the health benefits of wine has focused on polyphenols. Studies have reviewed their content in grapes as a source of bioactive compounds (Chira, Suh, Saucier, & Teissèdre, 2008; Nassiri-Asl & Hosseinzadeh, 2009), their role in the winemaking process (Pérez-Serradilla & Luque de Castro, 2008), their reactivity and influence on the organoleptic properties (Paixão, Perestrelo, Marques, & Câmara, 2007). The described biological activities are varied and include antioxidant, cardioprotective, anti-inflammatory, anti-ageing and antimicrobial properties, among others (Xia, Deng, Guo, & Li, 2010). Bertelli (2007) discussed the possible health benefits of wine consumption on the basis of the concentration of bioactive compounds in wine and the dose needed to achieve the different activities. Indeed, these considerations should be taken into account when evaluating the possible effect of a certain bioactive compound.

In addition to phenolic compounds, indoleamines possess antioxidant activity and are naturally present in wines (Rodriguez-Naranjo, Gil-Izquierdo, Troncoso, Cantos, & Garcia-Parrilla, 2010). Indeed, melatonin has already been reported in grapes and wines (Iriti, Rossoni, & Faoro, 2006; Mercolini et al., 2008). Melatonin (*N*-acetyl-5-methoxytryptamine) (MEL), a neurohormone discovered in the pineal gland, is also produced as secondary metabolite in plants. The amino acid tryptophan is the precursor of all 5-methoxytryptamines (or indoleamines), including MEL, and the biosynthetic pathway is via serotonin (5-hydroxytryptamine) in the case of yeasts, plants and mammals (Chattoraj, Liu, Zhang, Huang, & Borjigin, 2009; Posmyk & Janas, 2009; Sprenger, Hardeland, Fuhrberg, & Han, 1999). MEL has been detected in the roots, leaves, seeds and fruits of a considerable variety of plants (Paredes, Korkmaz, Manchester, Tan, & Reiter, 2009). The role of this molecule in plants is as a phytohormone regulated by light, as an UV irradiation protector and as an antioxidant (Paredes et al., 2009; Posmyk & Janas, 2009). Like other secondary metabolites, MEL has antioxidant properties as a direct free radical scavenger and by stimulating

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antioxidant enzymes (Hardeland & Pandi-Perumal, 2005; Reiter, Tan, & Maldonado, 2005). Moreover, the biological activities of MEL metabolites (N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) and N^1-acetyl-5-methoxykynuramine (AMK)) have been described previously (Guenther et al., 2005; Schaefer & Hardeland, 2009; Than, Heer, Laatsch, & Hardeland, 2006).

MEL has been reported to be present in foods (De la Puerta et al., 2007; Iriti et al., 2006; Maldonado, Moreno, & Calvo, 2009). The EFSA has recently accepted the link between MEL and the alleviation of subjective feelings of jet lag based on a list of health claims in relation to MEL (EFSA, 2010). The MEL dose should be between 0.5 and 5 mg per day (EFSA, 2010). However, there are not enough scientific data on the MEL content in foods to evaluate dietary intakes. Previous work on MEL in foods includes analysis of beer, cherries, tomatoes, rice and olive oil (De la Puerta et al., 2007; González-Gómez et al., 2009; Maldonado et al., 2009; Paredes et al., 2009).

Research of the MEL in grapes is in its beginning as it has only just been detected by ELISA. However, its distribution in grapes and its synthesis during ripening needs to be studied. To the best of our knowledge, the influence of the winemaking process on MEL content in wines has not yet been studied from the perspective of oenology, even though it is essential to do so to understand why MEL is present in wines. As a starting point, reliable analytical methods are required to obtain these data (Garcia-Parrilla, Cantos, & Troncoso, 2009).

This paper aims to determine the presence and content of MEL in different parts of the grapes (skin, pulp and seeds) and monitor the winemaking process (initial must for all wines, pressed and racked for red wines, and dejuice for the white wine) to check the influence of these steps on the content of MEL throughout the winemaking process.

2. Materials and methods

2.1. Chemicals and reagents

N-acetyl-5-methoxytryptamine standard was purchased from Sigma (Ref. M5250), L-tryptophan from Fluka (Ref. 93659), methanol from Merck (Darmstadt, Germany) and formic acid from Panreac (Barcelona, Spain). Solutions were prepared by diluting with Milli-Q water. All reagents were of analytical grade. Pectolytic enzymes (Vinozym® Vintage FCE, Novozymes, Bordeaux, France) and *Saccharomyces cerevisiae* yeast (Actiflore® F5, LAFFORT, France) were used for winemaking.

2.2. Grape samples

The grapes used for this study were from experimental cultivars or the winery at the Rancho de la Merced research centre (Instituto de Investigación y Formación Agraria y Pesquera, IFAPA, Jerez de la Frontera, Spain). Grapes were taken from the 2008 vintage and harvested at their optimum ripeness. Winemaking grapes and table grapes were collected to study the presence of MEL in different parts of the grape. The winemaking grape varieties studied were as follows: *Cabernet Sauvignon*, *Merlot*, *Nebbiolo*, *Palomino Fino*, *Pedro Ximénez*, *Petit Verdot*, *Syrah*, *Tempranillo* and *Tintilla de Rota*. The table grape varieties were also included: *Flame Seedless*, *Moscatel Itálica*, *Red Globe* and *Superior Seedless*.

2.3. Winemaking procedure. Pilot and laboratory assays

Pilot red winemaking: *C. Sauvignon*, *Merlot*, *Syrah*, *Tempranillo* and *Tintilla de Rota* grapes were de-stemmed, crushed and placed in a 50 litre steel vessel. Peptolitic enzymes (3 g/100 kg, Vinozym Vintage FCE, Novozymes, Spain) and sulphur dioxide (70 mg/kg) were added to maximise extraction and to protect the must. One day later, the fermentation was started after yeasting (Actiflore F5, Laffort, Spain), and the temperature was maintained at 27 °C ± 1 during alcoholic fermentation. As soon as the tumultuous fermentation had finished (density 999 g/l), the wine was pressed (Willmes, Germany). For the malolactic fermentation, lactic bacteria *Oenococus lacti* (100 g/l, Challenge Easy ML, Sepsa-Enartis, Spain) and nutrients (2 kg/l Nutriferm ML, Sepsa-Enartis, Spain) were used. When this stage of the process was finished, the wines were racked and clarification (1 kg/l egg white albumen, Laffort, Spain) was performed. Finally the wines were bottled. A diagram of the process is shown in Fig. 1.

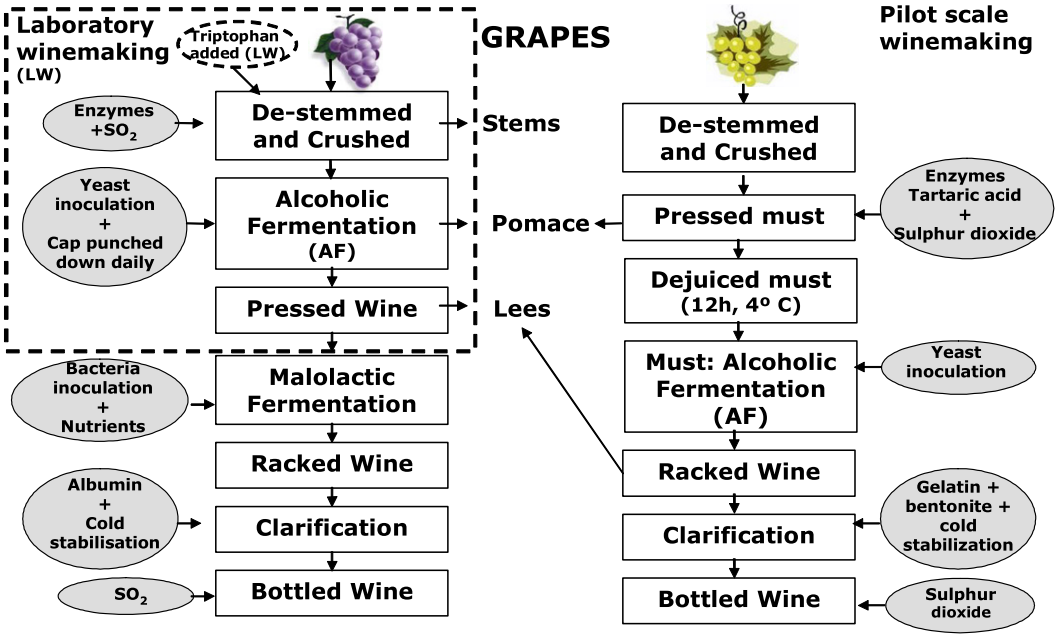

Fig. 1. Pilot and laboratory winemaking procedure scheme following the traditional methods for red and white wines. Solid white line: winemaking procedures at pilot scale; broken white line: laboratory winemaking assay.