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Melatonin and its derivatives in red wine: contribution of fermenting microorganisms

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INTRODUCTION

- ✓ Melatonin (N-acetyl-5-methoxytryptamine; MEL) is an indoleamine produced mainly by the pineal gland in vertebrates [1].
- ✓ MEL carries out several functions: modulation of the circadian and circannual rhythms, reproductive function, bone metabolism and turnover via cell-receptor-mediated mechanisms. It shows an antioxidant activity directly scavenging the free radical species and stimulating the activity of antioxidant enzymes [2].
- ✓ MEL has been found in plant foods, such as seeds, fruits and fermented beverages, wine included [3-5].
- ✓ The circulating levels of MEL are very low (about 0.2 ng/mL at the maximum night peak and lower than 0.01 ng/mL during the day) [6] compared to MEL in grape products (about 1 ng/g in berry skin and 0.5 ng/mL in wine) and, therefore, the intake of grape products is of particular interest [4,7].
- ✓ The content of MEL increases during the fermentation step of the winemaking and other fermented beverages meaning the yeast plays a significant role in its biosynthesis [8-9].
- ✓ MEL isomers were also detected in wine and one of them was recently identified as tryptophan-ethyl ester (TEE) [10-11].

AIMS

Set up of a sample preparation procedure and the validation of an analytical method for the simultaneous detection of MEL, TEE and tryptophan (TRP).

MATERIALS AND METHODS

Solid Phase Extraction (SPE) procedure

The final protocol included four steps:

- Sample loading (fraction A).
- Washing with formic acid 0.1% [v/v] (fraction B).
- Washing with methanol 40% [v/v] (fraction C).
- Elution with methanol 100% (fraction D) which was evaporated under vacuum.
- Re-suspension in methanol 10% acidified with formic acid 0.1% [v/v] corresponding to a concentration folds of 10.

Analytical method development and performances

The detection of the three compounds was carried out by HPLC coupled with both fluorescence (FL) and mass spectrometry (MS) detectors.

The validation was carried out in terms of selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability and recovery for both model wine solution (tartaric acid 5 g/L, ethanol 12% [v/v], pH 3.2) and red wine.

In order to prove the suitability of the method red wine samples (n=8) were analyzed.

RESULTS

Compound	Concentration range added (µg/L)	LOD (µg/L) (n=3)	LOQ (µg/L) (n=3)	Recovery (%) (n=6)		Repeatability (%RSD)	
				SWS	Spiked red wine	SWS	Spiked red wine
TRP	0.11-5500	0.75	1.25	89	84	9.1	10.5
TEE	5-250	0.038	0.12	88	76	6.5	7.9
MEL	5-250	0.0023	0.018	86	79	4.6	5.4

Table 1: Limits of detection (LOD) and quantification (LOQ), recovery (%) and repeatability (as Relative Standard Deviation, %RSD) for the analytical method developed in HPLC-MS.

- ✓ The SPE purification allowed both the removal of compounds potentially interfering the HPLC separation from the wine matrix and the concentration of the analytes of interest making possible their detection due to their presence in low amounts in wine.
- ✓ The analytes were revealed by both the detectors (FL and MS) that were investigated.
- ✓ Linear response was observed for both spiked model wine solution and red wine analyzed by MS detector.
- ✓ No significant difference was found between the two matrices.
- ✓ The repeatability of the SPE purification was evaluated its average values were: 5.4%, 7.4%, 4.0% and 4.2%, 5.2, 5.2% for TRP, TEE and MEL of spiked red wine and model wine solution, respectively.
- ✓ The intra-day precision (n = 12) corresponded to 3.8%, 2.6% and 3.1% for TRP, TEE and MEL, respectively.
- ✓ The developed analytical method resulted suitable for the determination of TRP, TEE and MEL in red wine (Table 2).
- ✓ TEE resulted over one thousand folds highly concentrated than MEL in the Nebbiolo wine samples analyzed.

Sample code	TRP	TEE	MEL
	mg/L	*µg/L*	*µg/L*
Red wine 1	3.85±0.40	172.2±13.6	0.057±0.003
Red wine 2	4.39±0.46	212.0±16.8	0.062±0.003
Red wine 3	1.56±0.16	256.2±20.2	0.063±0.004
Red wine 4	1.02±0.11	223.2±17.6	0.038±0.002
Red wine 5	0.98±0.10	113.0±8.9	0.046±0.003
Red wine 6	0.84±0.09	92.9±7.3	0.054±0.003
Red wine 7	0.44±0.05	71.7±5.7	0.063±0.004
Red wine 8	0.57±0.06	74.4±5.9	0.038±0.001

Table 2: Levels of TRP (tryptophan), TEE (tryptophan ethyl ester) and MEL (melatonin) in Nebbiolo red wine. Data are expressed as mean ± standard deviation.

CONCLUSIONS

- ✓ The proposed analytical method allowed the detection and the reliable quantification of TRP, TEE and MEL simultaneously.
- ✓ Good linearity (r > 0.99), recovery (average 84%) and repeatability (average RSD 7.3%) were achieved.
- ✓ This method represents a useful tool for monitoring the release of MEL and TEE and their fate throughout the wine production and storage.
- ✓ The concentrations of TEE much higher than those of MEL in wine samples confirmed previous outcomes [10,12] even if the origin and nutritional role of this compound still need to be elucidated.

REFERENCES

1. Reiter. The pineal and its hormones in the control of reproduction in mammals. *Endocr Rev* **1980**;1:109–13
2. Reiter et al. Reducing oxidative/nitrosative stress: A newly-discovered gene for melatonin. *Crit Rev Biochem Mol Biol* **2009**;44:175–200.
3. Mercolini et al. HPLC-FL analysis of melatonin and resveratrol isomers in wine using an SPE procedure. *J Sep Sci* **2008**;31:1007–1014.
4. Iriti et al. Melatonin in traditional mediterranean diets. *J Pineal Res* **2010**;49:101–105.
5. Vitalini et al. From vineyard to glass: agrochemicals enhance the melatonin and total polyphenol contents and antiradical activity of red wines. *J Pineal Res* **2011**;51:278-285.
6. Bonnefont-Rousselot et al. Melatonin: action as antioxidant and potential applications in human disease and aging. *Toxicol* **2010**;278:55-67.
7. Iriti et al. Health-promoting effects of traditional mediterranean diets – a review. *Pol. J. Food Nutr Sci* **2012**;62:71-76.
8. Rodriguez-Naranjo et al. Melatonin is synthesised by yeast during alcoholic fermentation in wines. *Food Chem* **2011**;126:1608–1613.
9. Fernandez-Pachon et al. Alcoholic fermentation induces melatonin synthesis in orange juice. *J Pineal Res* **2014**;56:31-38.
10. Gardana et al. "Melatonin isomer" in wine is not an isomer of the melatonin but tryptophan-ethylester. *J. Pineal Res* **2014**;57:435-441.
11. Iriti et al. Tryptophan-ethylester, the false (unveiled) melatonin isomer in red wine. *Int J Tryptophan Res* **2015**;8:1–3.
12. Vigentini et al. Yeast contribution to melatonin, melatonin isomers and tryptophan ethyl ester during alcoholic fermentation of grape musts. *J Pineal Res* **2015**;58:388-396.

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