Melatonin in Wine and Beer

Subjects: Biochemistry & Molecular Biology

Contributor: Javier Marhuenda

Melatonin is a hormone secreted in the pineal gland with several functions, especially regulation of circadian sleep cycle and the biological processes related to it. This review evaluates the bioavailability of melatonin and resulting metabolites, the presence of melatonin in wine and beer and factors that influence it, and finally the different benefits related to treatment with melatonin. When administered orally, melatonin is mainly absorbed in the rectum and the ileum; it has a half-life of about 0.45–1 h and is extensively inactivated in the liver by phase 2 enzymes. Melatonin (MEL) concentration varies from picograms to ng/mL in fermented beverages such as wine and beer, depending on the fermentation process. These low quantities, within a dietary intake, are enough to reach significant plasma concentrations of melatonin, and are thus able to exert beneficial effects. Melatonin has demonstrated antioxidant, anticarcinogenic, immunomodulatory and neuroprotective actions. These benefits are related to its free radical scavenging properties as well and the direct interaction with melatonin receptors, which are involved in complex intracellular signaling pathways, including inhibition of angiogenesis and cell proliferation, among others. In the present review, the current evidence on the effects of melatonin on different pathophysiological conditions is also discussed.

Keywords: melatonin ; wine ; beer ; polyphenols ; free radical

1. Introduction

Melatonin (MEL) is a neurohormone (N-acetyl-5-methoxytyramine) from the pineal gland that is produced as secondary metabolite in the plant kingdom. Moreover, MEL synthesis occurs from tryptophan, 5-hydroxytryptophan, serotonin, and ultimately N-acetylserotonin. In addition, MEL can similarly be produced by O-serotonin methylation followed by N-methoxytryptamine acetylation in yeast [1][2].

MEL has been described in seeds such as rice and sweet corn, roots, leaves and fruits of a considerable variety of plants. In fact, it is present in some fruits [3]. The presence of MEL has also been described in olive oil, especially in extra virgin olive oil, and in sunflower oil [4]. In addition, the presence of MEL in grapes and wines has been recently described. Iriti et al. [5] detected various amounts of MEL in different grape varieties. These authors described concentrations from 0.005 ng/g to 0.9 ng/g. Moreover, MEL has been found in even higher concentrations (245–423 ng/mL), in ten single-varietal wines [6]. MEL and its isomers, despite not being present in grape musts, were detected in different finished wines. Finally, some experimental winemaking methods revealed that MEL is formed after inoculation with yeast, with the role of Saccharomyces cerevisiae being crucial [6].

MEL can even exert several beneficial effects for health in humans, demonstrating antioxidant, anticarcinogenic, immunomodulatory and neuroprotective action. On the other hand, the biological activities of the most important MEL metabolites, N-1-acetyl-N-2-formyl-5-methoxyquinuramine (AFMK) and N-1-acetyl-5-methoxyquinuramine (AMK), have also been studied. AFMK is considered a powerful antioxidant, providing protection to DNA and lipids against oxidative damage through diverse metabolic pathways. Instead, AMK is also a potent antioxidant that is able to inhibit the biosynthesis of prostaglandins by binding to diazepam receptors [7].

As is the case with many secondary metabolites, MEL is able to stimulate endogenous antioxidant enzymes and/or counteract free radicals (both in vitro and in vivo). Moreover, MEL is capable of capturing reactive oxygen species, such as peroxynitrite [8] and even hydrogen peroxide in a dose-dependent way [9], as well as demonstrating antioxidant capacity in vitro through the $ABTS^+$ method [10].

Studies in vivo have also demonstrated its antioxidant effect. When administered in mice it was observed that MEL was able to reduce chronic oxidative stress related to aging [11], and that it could even reduce blood pressure in men with chronic hypertension [12].

The role of MEL as a neuroprotective agent is also relevant. It has been successfully tested in sleep disorders, helping to restore circadian rhythm, and is especially effective in patients with neurodegenerative diseases. Several trials have been conducted with the aim of mitigating the consequences of diseases such as Alzheimer's, Parkinson's, Huntington's disease or amyotrophic lateral sclerosis, obtaining satisfactory results. Finally, MEL has also managed to inhibit the fibrinogenesis process significantly [13][14].

The amphipathic character of MEL allows it to cross physiological barriers, being present in the cytosol, mitochondria and different biological membranes [15]. Therefore, MEL provides the antioxidant defense where it is needed.

2. Melatonin, Bioavailability, and Pharmacokinetics

2.1. Bioavailability

MEL absorption shows site dependency in the intestinal tract, being mainly absorbed in the rectum and the ileum. However, the absorption behaviors of MEL are more complicated when simultaneously dosed with excipients. Moreover, the absorption rate of MEL can be affected by the tissue damage, the formation of micellar complexes—characterized by NMR analysis—, the distribution of the particle size. For example, higher sodium cholate and sodium oleate concentrations are able to decrease the absorption of MEL due to the formation of micellar complexes, notwithstanding histological tissue injury. It is important to note that new insights in absorption of MEL and effects of common pharmaceutical excipients could be valuable for oral dosing in clinical pharmacokinetics in addition to stablish new oral formulations.

The elimination stage of orally administered MEL is biphasic, with a half-life of about 0.45-1 h depending on the administered dose, a fact that could be probably explained by the possible saturation of catabolic pathways. The differences observed on elimination half-life regarding age are probably due to the differences in metabolism. Finally, MEL is partially insoluble in water and it could influence the dissolution into the gastrointestinal tract [16].

MEL is inactivated in the liver by 6-hydroxylation, followed by sulfate and glucuronide conjugation, which leads to its main urinary metabolite 6-sulfatoxymelatonin [17]. It is important to note that liver clears more than 90% of the plasmatic MEL and is the primary site for its metabolism. MEL is originally hydroxylated before being excreted in urine as sulfate and, to a minor extent, as glucuronide conjugates.

The excretion of urine 6-sulfatoxymelatonin meticulously matches plasmatic MEL profile and only about 1% of circulating MEL is finally excreted in the urine without changes. 3-Hydroxymelatonin is also detected in urine and could denote the generation of the $OH^{\bullet}$ radical. In addition to reduced pineal secretory activity, subjects with liver cirrhosis show a minor MEL clearance, with the consequent postponed rise of plasmatic MEL peak and augmented daytime concentration of MEL.

The plasmatic profile of MEL shows wide intersubject variation. However, it is actually reproducible daily in the same person on behalf of one of the most robust circadian rhythms. It provides a good evaluation of MEL secretion if absence of renal or hepatic function is normal.

In some people, the nocturnal secretion of MEL is really short or even absent. The concerns of reduced MEL secretion on susceptibility to rhythmic organization and morbidity are still unknown. Plasmatic MEL is mainly bound to albumin (70%) and, to a minor extent, to orosomucoid, reaching every tissue—and is able to cross the blood–brain barrier—and modulating brain activity [18].

Matthews et al. [19] studied MEL metabolism in healthy volunteers. Urinary concentration of 6-sulfatoxymelatonin was similar in men and women (ranging from 6.3 to 30.9 mg/24 h). There was marked rise in nocturnal 6-sulfatoxymelatonin with > 80% of the total 24 h excretion comprised in the first urine sample. Following intravenous administration of MEL (1 mg), 99.7% was excreted within the first 24 h.

2.2. Pharmacokinetics

The bioavailability of MEL has been studied and very well described by the literature, showing an absorption reaching up to 33% after oral administration. However, the efficacy of MEL is still uncertain, needing more studies that to prove the good availability of MEL.

MEL has a noticeable hepatic uptake, reducing bioavailability if it is orally administered. Moreover, MEL was determined for healthy individuals and patients with cirrhosis, establishing a normal MEL production of 28.8 mg/day for the healthy population, and 12.3 mg/day for cirrhotic patients [20]. Kennaway et al. [21] reported the effect of possible structural

modifications of MEL on plasmatic MEL half-life, showing a major uptake of 6-hydroxyMEL sulfate [22]. Golovanov et al. [23] studied the pharmacokinetics of MEL administered orally, intravenously or intramuscularly in rabbits and dogs. The maximum plasmatic concentration was achieved earlier and clearance from the blood was faster in rabbits than dogs for both oral and intravenous MEL. In fact, dogs showed higher area-under-the curve after oral MEL administration than rabbits. Therefore, the bioavailability was bigger for dogs than rabbits. After intramuscular MEL treatment, bioavailability between the species showed to be similar in both cases.

The clearance of MEL has been studied in man after intravenous injection of 5 or 10 mg and after 5 hours infusion of 20 mg, showing biexponential decline [24]. Le Bars et al. [25] reported plasmatic pharmacokinetics of MEL, showing maximum activity in the brain after the injection of 9.5 mg/kg. The results of that study confirmed that MEL freely crosses the blood–brain barrier and that 6-sulfatoxymelatonin is the major plasma metabolite that can be found. Cavallo et al. [26] performed a pilot study on adult males showing dose linearity, absence of saturation kinetics, and unaltered metabolism and urinary excretion for doses of 0.1, 0.5 and 5.0 mg/kg. The results of the pharmacokinetic study confirmed no significant gender differences in adults.

The review of the literature specifies that oral bioavailability of MEL in humans and animal models reveals that the apparent elimination half-life of MEL following intravenous dose of 3 mg/kg was 19.8, 18.6, and 34.2 min, respectively, in rats, dogs, and monkeys [27]. Fluvoxamine is a selective serotonin reuptake inhibitor that is known to increase plasmatic MEL concentration. However, it is not clear whether these effects might be attributed to increased MEL production or decreased removal of plasmatic MEL. The last hypothesis was examined by Hartter et al. [28], who coadministrated fluvoxamine to a 17-fold high serum concentration. Haertter et al. [29] studied the influence of concomitant caffeine intake on the pharmacokinetics of oral MEL, showing a noticeable effect of caffeine on the bioavailability of oral MEL.

MEL shows volatile absorption from the gastrointestinal tract and—as commented before—extensive first-pass hepatic metabolism. Therefore, oral bioavailability of MEL varies widely between different subjects. MEL distribution follows on open one—or two—compartments, while its elimination may be described by biphasic first order kinetics [30].

MEL is typically administered orally at doses of 1–5 mg, which leads to pharmacological concentration in plasma. It has been reported that oral administration of 0.3 mg given 2–4 h before bedtime leads to normal nighttime plasmatic MEL concentration [31]. In addition,180 min after oral administration of 80 mg of MEL, plasma MEL concentration increases with an absorption half-life of 0.4 h and an elimination half-life of 0.8 h [32]. There have been observed huge interindividual variations among subjects for MEL absorption (reaching even 25-fold differences). It is important to note that plasmatic MEL and half-life change depending on the dose, time of administration, and the type of oral preparation used [33]. Moreover, the MEL receptor sensitivity is increased between 17.00h and 20.00 h [34]. The preservation of the high-amplitude circadian rhythm of MEL, with its high nocturnal concentration and low daytime concentration, is critical for the therapeutic treatment with exogenous MEL.

3. Melatonin in Fermented Products

MEL concentration varies from picograms to ng/mL of product in fermented beverages such as wine and beer. Although the concentration is low it seems that these concentrations are sufficient in the dietary intake to measure their effects by different methods. Although the MEL content can vary in different non-fermented products, it seems clear that the alcoholic fermentation process is decisive for the formation of MEL, since it is generated after inoculation with yeasts, the role of Saccharomyces cerevisiae being crucial [35].

3.1. Quantity of MEL in wines

MEL concentration varies with fermentation, presenting its highest value between the first and second day of fermentation. It should be taken into account that several factors can affect the concentration of MEL in red wine, such as agrochemicals used, winemaking practices, fermenting microorganisms or even the composition of the grapes used [36].

Several authors have described the presence of MEL in wines, as shown in Table 1 [37]. Mercolini et al. found values of 0.4 and 0.5 ng/mL in Sangiovese red wines and Trebbiano white wine [38], and found 0.3 and 0.5 ng/mL in varieties of Albana grappa and grape juice [39]. Stege et al. found values of 0.16 ng/mL for Malbec red wine, 0.24 ng/mL for Cabernet Sauvignon red wine and 0.32 ng/mL for Chardonnay white wine [40]. Vitalini et al. [41] values of 4.1 and 8.1 ng/mL for Gropello and Merlot wine varieties, respectively. Rodriguez-Naranjo et al. found values between 74 and 322 ng/mL for Presses wines (Sauvignon, Merlot, Syrah, Tempranillo and Tintillo de Rota) and between 250 and 340 ng/mL for Racked

wines (Sauvignon, Merlot, Syrah, Tempranillo, and Tintilla de Rota) . Vitalini et al. found values between 0.14 and 0.62 ng/mL for varieties of single-variety red wines, 0.05–0.31 ng/mL for polyvarietal red wines, 0.18 ng/mL for white wine and between 0 and 0.31 ng/mL for Dessert wines and 0.11–0.13 ng/mL for Modena balsamic vinegars .

Table 1. MEL content in different types of wines depending on grape variety, country and harvest. Adapted from Meng et al [42].

Wine Variety	Country	Vintage	MEL Content (ng/mL)	Reference
Sangiovese Red Wine	Italy	2005	0.4	[38]
Trebbiano White Wine			0.5	
Albana Grappa	Italy	2009	0.3	[43]
Grape Juice			0.5	
Malbec Red Wine	Argentina	2005	0.16	[40]
Cabernet Sauvignon Red Wine			0.24	
Chardonnay White Wine			0.32	
Gropello	Spain	2009	4.1	[41]
Merlot			8.1	
Presses Wines	Spain	2008	74–322	[6]
Racked Wines	Italy		250–423	
Monovarietal Red Wines	Italy	2009	0.14–0.62	
Polyvarietal Red Wines	Italy	2010	0.05–0.31	
White Wine	Italy	2010	0.18	[36]
Dessert Wines	Italy	2007	0–0.31	
Modena Balsamic Vinegars	Italy	2008	0.11–0.13	

In a study where tryptophan and certain metabolites, including MEL, were simultaneously analyzed in several types or red wine, MEL values ranged from 0.038 ± 0.001 g/L to 0.063 ± 0.004 g/L , data consistent with those shown by Vitalini et al. as 0.05–0.062 g/L .

It should be noted that the presence of MEL in the grape is not always reflected later in the wine, as shown in a study of Gómez et al. [44] where the concentration of MEL of the grape was 120–160 ng/g and yet in the wine of those grapes there was no longer MEL but a MEL isomer that ranged from 18 to 24 ng/g.

It is important to remark that the oral bioavailability of MEL after intake of a glass of wine is not known, which is not the case for polyphenols, perhaps due to the complex food matrix that may influence the absorption of active metabolites, since it is in the form of a supplement that is consumed at high doses and has been known for years [45]. However, the

presence of ethanol seems to improve the amount of MEL, given its solvent ability, by improving the permeability of membranes [43].

Varoni et al. evaluated the serum MEL levels after administering a wine enriched with MEL vs a wine with placebo in humans and it was observed that the maximum MEL concentrations were within 60 minutes, being 8.7 ± 2.2 pg/ min for the MEL group and 6.7 ± 0.6 pg/ min for placebo wine, obtaining an area under the curve of 993 ± 162 vs 745 ± 88 pg/min of the MEL vs placebo group, respectively, without observing significant differences. As for salivary concentration, the peak was reached at 45 min after MEL intake, also without statistical significance, returning after 120 min to placebo levels.

3.2. Quantity of Melatonin in Beer

Beer is part of the usual consumption of a large number of people, and is characterized by having a wide variety of bioactive nutraceutical and phytochemical compounds such as polyphenols and antioxidants presenting B-complex vitamins, ascorbic acid, citric acid, etc. As for the MEL content present in beer, a study of 18 brands of beer present on the market, featuring different alcohol content, showed how all beers featured MEL being directly proportional to the alcohol content at higher MEL content, with values varying from 51.8 ± 2.2 pg/mL—non-alcoholic beer—to 169.7 ± 8.7 pg/mL—beer. Such an effect could be due to the solubility of MEL in alcohol. Another study of 20 varieties of beer showed that MEL content ranged from 58 ± 1.44 pg/mL in beverages with 0–2% volume of alcohol to 169 ± 2.4 pg/mL in beers with 7–7.8% volume of alcohol, data consistent with the previous study. In addition, another study produced beer by hand and measured the concentration in the different manufacturing processes obtaining a final value of 333 pg/mL in a beer of 5% volume of alcohol after the second fermentation, which are values that are three times higher than commercial beers [46]. In terms of composition, high levels of MEL (339 ± 9 pg/mL) were found in barley of concentrated musts versus low amounts in hops 33 ± 10 pg/mL [46]. It seems that what happens in wine, where the concentration of MEL is attributable to the fermentation process rather than the content of the grapes, is different in beer, where it can be attributed to the content in barley.

References

1. Hardeland, R.; Pandi-Perumal, S.R. Melatonin, a potent agent in antioxidative defense: actions as a natural food consti tuent, gastrointestinal factor, drug and prodrug. Nutr. Metab. (Lond). 2005, 2, 22, doi:10.1186/1743-7075-2-22.
2. Sprenger, J.; Hardeland, R.; Fuhrberg, B.; Han, S.-Z. Melatonin and other 5-methoxylated indoles in yeast: presence in high concentrations and dependence on tryptophan availability. Cytologia (Tokyo). 1999, 64, 209–213.
3. Fernández-Mar, M.I.; Mateos, R.; García-Parrilla, M.C.; Puertas, B.; Cantos-Villar, E. Bioactive compounds in wine: Re sveratrol, hydroxytyrosol and melatonin: A review. Food Chem. 2012, 130, 797–813.
4. de la Puerta, C.; Carrascosa-Salmoral, M.P.; García-Luna, P.P.; Lardone, P.J.; Herrera, J.L.; Fernández-Montesinos, R.; Guerrero, J.M.; Pozo, D. Melatonin is a phytochemical in olive oil. Food Chem. 2007, 104, 609–612.
5. Iriti, M.; Rossoni, M.; Borgo, M.; Faoro, F. Benzothiadiazole enhances resveratrol and anthocyanin biosynthesis in grap evine, meanwhile improving resistance to Botrytis cinerea. J. Agric. Food Chem. 2004, 52, 4406–4413, doi:10.1021/jf0 49487b.
6. Rodriguez-Naranjo, M.I.; Gil-Izquierdo, A.; Troncoso, A.M.; Cantos-Villar, E.; Garcia-Parrilla, M.C. Melatonin is synthesi sed by yeast during alcoholic fermentation in wines. Food Chem. 2011, 126, 1608–1613, doi:10.1016/j.foodchem.2010. 12.038.
7. Schaefer, M.; Hardeland, R. The melatonin metabolite N-acetyl-5-methoxykynuramine is a potent singlet oxygen scave nger. J. Pineal Res. 2009, 46, 49–52, doi:10.1111/j.1600-079X.2008.00614.x.
8. Hardeland, R. Aging, Melatonin, and the Pro- and Anti-Inflammatory Networks. Int. J. Mol. Sci. 2019, 20, doi:10.3390/ij ms20051223.
9. Than, N.N.; Heer, C.; Laatsch, H.; Hardeland, R. Reactions of the melatonin metabolite N1-acetyl-5-methoxykynuramin e (AMK) with the ABTS cation radical: identification of new oxidation products. Redox Rep. 2006, 11, 15–24, doi:10.117 9/135100006X100977.
10. Herraiz, T.; Galisteo, J. Endogenous and dietary indoles: a class of antioxidants and radical scavengers in the ABTS as say. Free Radic. Res. 2004, 38, 323–331, doi:10.1080/10611860310001648167.
11. Nogués, M.R.; Giralt, M.; Romeu, M.; Mulero, M.; Sánchez-Martos, V.; Rodríguez, E.; Acuña-Castroviejo, D.; Mallol, J. Melatonin reduces oxidative stress in erythrocytes and plasma of senescence-accelerated mice. J. Pineal Res. 2006, 4 1, 142–149, doi:10.1111/j.1600-079X.2006.00344.x.

12. Scheer, F.A.J.L.; Van Montfrans, G.A.; van Someren, E.J.W.; Mairuhu, G.; Buijs, R.M. Daily nighttime melatonin reduces blood pressure in male patients with essential hypertension. Hypertens. (Dallas, Tex. 1979) 2004, 43, 192–197, doi:10.1161/01.HYP.0000113293.15186.3b.

13. Srinivasan, V.; Pandi-Perumal, S.R.; Maestroni, G.J.; Esquifino, A.I.; Hardeland, R.; Cardinali, D.P. Role of melatonin in neurodegenerative diseases. Neurotox. Res. 2005, 7, 293–318, doi:10.1007/BF03033887.

14. Hardeland, R.; Pandi-Perumal, S.R.; Cardinali, D.P. Melatonin. Int. J. Biochem. Cell Biol. 2006, 38, 313–316, doi:10.1016/j.biocel.2005.08.020.

15. Karbownik, M.; Lewinski, A.; Reiter, R.J. Anticarcinogenic actions of melatonin which involve antioxidative processes: comparison with other antioxidants. Int. J. Biochem. Cell Biol. 2001, 33, 735–753, doi:10.1016/s1357-2725(01)00059-0.

16. Vakkuri, O.; Leppäluoto, J.; Kauppila, A. Oral administration and distribution of melatonin in human serum, saliva and urine. Life Sci. 1985, 37, 489–495.

17. Touitou, Y. Human aging and melatonin. Clinical relevance. Exp. Gerontol. 2001, 36, 1083–1100, doi:10.1016/s0531-5565(01)00120-6.

18. Babu, R.J.; Dayal, P.; Singh, M. Effect of cyclodextrins on the complexation and nasal permeation of melatonin. Drug Deliv. 2008, 15, 381–388.

19. Matthews, C.D.; Seamark, R.F. Pineal Function: Proceedings of the Satellite Symposium, Sixth International Congress of Endocrinology, Melbourne, Australia, February 1980; Elsevier/North-Holland Biomedical Press: Amsterdam, The Netherlands, 1981; ISBN 0444803130.

20. Lane, E.A.; Moss, H.B. Pharmacokinetics of melatonin in man: first pass hepatic metabolism. J. Clin. Endocrinol. Metab. 1985, 61, 1214–1216, doi:10.1210/jcem-61-6-1214.

21. Kennaway, D.J.; Royles, P.; Dunstan, E.A.; Hugel, H.M. Prolactin response in Border-Leicester x merino ewes to administration of melatonin, melatonin analogues, a melatonin metabolite and 6-methoxybenzoxazolinone. Aust. J. Biol. Sci. 1986, 39, 427–433, doi:10.1071/bi9860427.

22. English, J.; Arendt, J.; Poulton, A.; Symons, A.M. Short-term variations of circulating melatonin in the ewe. J. Pineal Res. 1987, 4, 359–366, doi:10.1111/j.1600-079x.1987.tb00875.x.

23. Golanov, I.; Shteinpress, A.; Guskova, T.; Vigdorchik, M.; Aiache, J.; (Eds.), Hirtz, J. Proceedings of 3rd European Congress on Biopharmacology and Pharmacokinetics, 1987; 2, 675.

24. Mallo, C.; Zaĭdan, R.; Galy, G.; Vermeulen, E.; Brun, J.; Chazot, G.; Claustrat, B. Pharmacokinetics of melatonin in man after intravenous infusion and bolus injection. Eur. J. Clin. Pharmacol. 1990, 38, 297–301, doi:10.1007/BF00315035.

25. Le Bars, D.; Thivolle, P.; Vitte, P.A.; Bojkowski, C.; Chazot, G.; Arendt, J.; Frackowiak, R.S.; Claustrat, B. PET and plasma pharmacokinetic studies after bolus intravenous administration of [11C]melatonin in humans. Int. J. Radiat. Appl. instrumentation. Part B, Nucl. Med. Biol. 1991, 18, 357–362, doi:10.1016/0883-2897(91)90132-5.

26. Cavallo, A.; Ritschel, W.A. Pharmacokinetics of melatonin in human sexual maturation. J. Clin. Endocrinol. Metab. 1996, 81, 1882–1886, doi:10.1210/jcem.81.5.8626852.

27. Yeleswaram, K.; McLaughlin, L.G.; Knipe, J.O.; Schabdach, D. Pharmacokinetics and oral bioavailability of exogenous melatonin in preclinical animal models and clinical implications. J. Pineal Res. 1997, 22, 45–51, doi:10.1111/j.1600-079x.1997.tb00302.x.

28. Härtter, S.; Grözinger, M.; Weigmann, H.; Röschke, J.; Hiemke, C. Increased bioavailability of oral melatonin after fluvoxamine coadministration. Clin. Pharmacol. Ther. 2000, 67, 1–6.

29. Härtter, S.; Nordmark, A.; Rose, D.-M.; Bertilsson, L.; Tybring, G.; Laine, K. Effects of caffeine intake on the pharmacokinetics of melatonin, a probe drug for CYP1A2 activity. Br. J. Clin. Pharmacol. 2003, 56, 679–682, doi:10.1046/j.1365-2125.2003.01933.x.

30. Tsakalozou, E.; Markantonis-Kyroudis, S. Pharmakeutike. 2006, 19, 9.

31. Zhdanova, I. V; Wurtman, R.J.; Morabito, C.; Piotrovska, V.R.; Lynch, H.J. Effects of low oral doses of melatonin, given 2-4 hours before habitual bedtime, on sleep in normal young humans. Sleep 1996, 19, 423–431, doi:10.1093/sleep/19.5.423.

32. Waldhauser, F.; Waldhauser, M.; Lieberman, H.R.; Deng, M.H.; Lynch, H.J.; Wurtman, R.J. Bioavailability of oral melatonin in humans. Neuroendocrinology 1984, 39, 307–313, doi:10.1159/000123997.

33. Guardiola-Lemaître, B. Toxicology of melatonin. J. Biol. Rhythms 1997, 12, 697–706, doi:10.1177/074873049701200627.

34. Touitou, Y.; Arendt, J.; Pévet, P. Melatonin and the pineal gland, from basic science to clinical application: proceedings of the International Symposium on Melatonin and the Pineal Gland: From Basic Science to Clinical Application, Paris, 6

-9 September 1992; Elsevier Science & Technology: Amsterdam, The Netherlands, 1993; ISBN 0444895833.

35. Rodriguez-Naranjo, M.I.; Torija, M.J.; Mas, A.; Cantos-Villar, E.; Garcia-Parrilla, M. del C. Production of melatonin by Saccharomyces strains under growth and fermentation conditions. J. Pineal Res. 2012, 53, 219–224, doi:10.1111/j.1600-079X.2012.00990.x.
36. Vitalini, S.; Gardana, C.; Simonetti, P.; Fico, G.; Iriti, M. Melatonin, melatonin isomers and stilbenes in Italian traditional grape products and their antiradical capacity. J. Pineal Res. 2013, 54, 322–333, doi:10.1111/jpi.12028.
37. Kocadağlı, T.; Yılmaz, C.; Gökmen, V. Determination of melatonin and its isomer in foods by liquid chromatography tandem mass spectrometry. Food Chem. 2014, 153, 151–156, doi:10.1016/j.foodchem.2013.12.036.
38. Mercolini, L.; Addolorata Saracino, M.; Bugamelli, F.; Ferranti, A.; Malaguti, M.; Hrelia, S.; Raggi, M.A. HPLC-F analysis of melatonin and resveratrol isomers in wine using an SPE procedure. J. Sep. Sci. 2008, 31, 1007–1014, doi:10.1002/jssc.200700458.
39. Hornedo-Ortega, R.; Cerezo, A.B.; Troncoso, A.M.; Garcia-Parrilla, M.C.; Mas, A. Melatonin and Other Tryptophan Metabolites Produced by Yeasts: Implications in Cardiovascular and Neurodegenerative Diseases. Front. Microbiol. 2015, 6, 1565, doi:10.3389/fmicb.2015.01565.
40. Stege, P.W.; Sombra, L.L.; Messina, G.; Martinez, L.D.; Silva, M.F. Determination of melatonin in wine and plant extracts by capillary electrochromatography with immobilized carboxylic multi-walled carbon nanotubes as stationary phase. Electrophoresis 2010, 31, 2242–2248.
41. Vitalini, S.; Gardana, C.; Zanzotto, A.; Fico, G.; Faoro, F.; Simonetti, P.; Iriti, M. 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, doi:10.1111/j.1600-079X.2011.00887.x.
42. Meng, J.-F.; Shi, T.-C.; Song, S.; Zhang, Z.-W.; Fang, Y.-L. Melatonin in grapes and grape-related foodstuffs: A review. Food Chem. 2017, 231, 185–191, doi:10.1016/j.foodchem.2017.03.137.
43. Mercolini, L.; Mandrioli, R.; Raggi, M.A. Content of melatonin and other antioxidants in grape-related foodstuffs: measurement using a MEPS-HPLC-F method. J. Pineal Res. 2012, 53, 21–28, doi:10.1111/j.1600-079X.2011.00967.x.
44. Gomez, F.J.V.; Raba, J.; Cerutti, S.; Silva, M.F. Monitoring melatonin and its isomer in Vitis vinifera cv. Malbec by UHPLC-MS/MS from grape to bottle. J. Pineal Res. 2012, 52, 349–355, doi:10.1111/j.1600-079X.2011.00949.x.
45. Fracassetti, D.; Vigentini, I.; Lo Faro, A.F.F.; De Nisi, P.; Foschino, R.; Tirelli, A.; Orioli, M.; Iriti, M. Assessment of Tryptophan, Tryptophan Ethylester, and Melatonin Derivatives in Red Wine by SPE-HPLC-FL and SPE-HPLC-MS Methods. Foods (Basel, Switzerland) 2019, 8, doi:10.3390/foods8030099.
46. Varoni, E.M.; Paroni, R.; Antognetti, J.; Lodi, G.; Sardella, A.; Carrassi, A.; Iriti, M. Effect of Red Wine Intake on Serum and Salivary Melatonin Levels: A Randomized, Placebo-Controlled Clinical Trial. Molecules 2018, 23, doi:10.3390/molecules23102474.