

Review

## Melatonin in yeast and fermented beverages: analytical tools for detection, physiological role and biosynthesis

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### ABSTRACT

The recently established relation between the metabolism of aromatic amino acids of yeast and the production of different bioactive molecules during fermentation opens up new and interesting research topics. Among these molecules, melatonin has drawn researchers' attention in the last decade given its potential benefits for human health. This review summarizes melatonin production in fermented beverages, and conventional and current methods for detecting melatonin in yeast-derived samples. In addition, the role of melatonin in yeast is discussed and the biosynthetic pathway of melatonin is presented in *Saccharomyces cerevisiae*.

**Keywords:** *Saccharomyces cerevisiae*, fermentation, bioactive, antioxidant, indoleamine serotonin, tryptophan, melatonin.

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### 1. INTRODUCTION

The development of precise analytical tools allows us to detect most food components, even those at very low concentrations, and with much interest shown in their potential bioactivity. Apart from tryptophan being a proteinogenic amino acid, it is a key precursor of the different relevant molecules deriving from its metabolism, such as indole-3-acetic acid (IAA), serotonin, N-acetyl serotonin and melatonin. For example, IAA is known as the major auxin-type phytohormone that regulates many cellular processes and plant development. However, it has been shown that it can act as a signaling molecule in microorganisms, and it exerts tolerance to various toxic compounds and stress conditions in *E. coli* (1), which means it is capable of regulating gene expression. Moreover, the exogenous administration of IAA in *Saccharomyces cerevisiae* leads to the conversion of vegetative cells into their filamentous form (2). Other structurally related molecules like indole derivate molecules, including tryptamine and melatonin, are synthesized through the metabolism of tryptophan in plants, but also by many microorganisms, including bacteria and yeast (3). Although a large proportion of compounds deriving from tryptophan in food is of plant origin, their presence in fermented products can also be related to the microorganisms involved in the fermentation process which, through their metabolism, increase the levels of such molecules of

interest. These examples point out melatonin is no longer exclusively considered as a neurohormone since it is not exclusively produced in the pineal gland.

Melatonin is an indoleamine that not only plays an important role in human health, but also a powerful antioxidant role in protecting DNA against oxidative damage (4). Given its amphiphilic properties, melatonin is able to cross physiological barriers and has been found in all cellular compartments (5). Given the recognized effects and characteristics of melatonin on human health, the presence of this molecule in food is of much interest. In fact, some studies in humans have related rising serum melatonin concentrations to increased antioxidant capacity in serum after the intake of fruits and fruit juices containing melatonin and 6-sulfatoxymelatonin levels, the main urinary metabolite of melatonin in mammals, with total antioxidant capacity in urine (6, 7). In plants, occurrence of melatonin was described in 1995 (8, 9). However, it was not until 2006 and 2007, melatonin was detected in typical Mediterranean foods like wine or olive oil, respectively. It was then when the study of the presence of melatonin in foods considerably increased. It has also been widely reported in different plant parts and in a wide variety of species (13). For more detailed dietary food sources of melatonin and health potential for humans, see the reviews Meng *et al.* (14) and Salehi *et al.* (15).

Increasingly more published articles describe the presence of melatonin in different foods, but the concentrations of indoleamines in foods vastly vary and range from either picograms to micrograms per gram or picograms to micrograms per milliliter. Variability in melatonin concentration in food may be due to different effects. Regarding plant-foods, both endogenous and external factors have been linked and are considered to affect melatonin content. For example, there are several factors that influence final melatonin content, such as the genetic traits of the cultivar, the presence of pathogen infections, meteorological conditions, agronomical practices, phytosanitary treatments, etc. (16). In edible plants, both abiotic and biotic stresses cause endogenous melatonin levels to significantly rise because melatonin acts as an antioxidant and reacts against the free radicals generated during stress (17). The catabolism of melatonin itself is another reason to explain the reported variability. Melatonin can be catabolized both enzymatically and non-enzymatically to give different downstream metabolites, some of which are found at much higher concentrations, which would explain variations in melatonin detection (18–20).

## **2. DEVELOPMENT AND OPTIMIZATION OF ANALYTICAL TECHNIQUES TO DETECT MELATONIN AND OTHER INDOLIC COMPOUNDS**

The main analytical techniques followed to determine melatonin in food, fermented beverages and yeast cells derive from the development or adaptation of existing techniques to determine this molecule in biological tissues. Based on chromatography separation, melatonin has been successfully determined by gas chromatography coupled with mass spectrometry (GC-MS) and by high-performance liquid chromatography (HPLC) coupled with electrochemical, UV and fluorescence detectors (10, 21–23). A combination HPLC with capillary electrochromatography (CE) technique has been proposed and used to determine melatonin in wine, grape skin and plant extracts (24). Thin-layer chromatography (TLC) coupled with densitometric detection has allowed the determination and quantification of five indole compounds, including melatonin, from methanolic extracts of shoot cultures (25).

Currently, the most powerful technique for determining indolic compounds is based on ultra-high performance liquid chromatography coupled with high-resolution tandem mass spectrometry (UHPLC-HRMS/MS). This technique has lowered the limit of detection (LOD) and the limit of

quantification (LOQ) of these tryptophan-derived compounds present at very low concentrations. Regarding these objectives, Fernández-Cruz *et al.* developed and validated a new UHPLC/HRMS method with a LOD below 0.5 ng/mL for the nine analyzed indolic compounds (tryptophan, tryptamine, 5-hydroxytryptophan, serotonin, *N*-acetylserotonin, melatonin, tryptophan ethyl ester, tryptophol and IAA) for both culture medium and fermented products (26). More recently Fraccasetti *et al.* highlighted sample preparation using solid-phase extraction (SPE) prior to chromatographic conditions to detect five melatonin isomers (MIs) simultaneously with melatonin and tryptophan ethyl ester in wine (27). The detection of MIs was not previously possible by other techniques (28–30) and this could lead to co-measure melatonin and its isomers, including tryptophan ethyl ester, a compound with the same molecular weight of melatonin and previously considered as a melatonin isomer. The use of internal standards with an specific LC-MS/MS fragmentation are reported to give a more accurate detection and quantitation (31). For this technique, several authors have reported the importance of the extraction method because inadequate choice could lead to poor melatonin recovery due to its amphipathic nature and solubility (32, 33). Ethanol and methanol in different proportions are the generally preferred extraction solvents, but in-depth recovery studies should be conducted depending on the matrix (34, 35).

Most metabolomic research relies on the analysis of yeast extracellular metabolites, while studies on intracellular metabolic changes are relatively less abundant. The possibility of following the behavior of metabolites during the fermentation process, in parallel to both intracellular and extracellular media, is useful for gaining knowledge of signaling and the metabolomic reaction network. Álvarez-Fernández *et al.* evaluated three different procedures for the intracellular extraction of tryptophan and tyrosine-derived metabolites by UHPLC-HRMS using 3-nitrotyrosine solution as an internal standard. These authors suggested that low-temperature intracellular extraction methods were more suitable for studying melatonin and its related compounds in yeast. After each extraction method they recommend a clean-up and concentration procedure prior to sample injection in order to remove the phospholipids and proteins (36), conversely the use of SPE cartridges for this purpose are reported to severely affect analyte recovery rates (37). Similarly Vitalini *et al.* (37) tested four different extraction procedures and developed a multicomponent analytical method to measure and improve the recovery of 14 tryptophan derivatives in different plant matrices by UHPLC-MS/MS. They found water extraction at room temperature was the most suitable when working with plant foods samples, and they also remark the importance of adapting the election of internal standards according to each compound class (amino acids, indoleamines, *N*-acetyl indoleamines, amino-benzoic, and pyridine derivatives).

Another important milestone reached in recent years has been the development and setup of rapid detection methods of melatonin and other indolic compounds. Even so, faster, simpler and easier-to-adapt routine technique detection methods to widely detect yeast-derived samples are still demanded. Of these techniques, a novel method based on voltammetry of immobilized particles (VIMP) has been proposed and directly applied to yeast cells (38). With this methodology, melatonin has been detected and monitored in different yeast strains. Notwithstanding, voltammetric methodology does not possess the high discriminating capacity of chromatographic techniques. Other alternative rapid techniques are based on the use of specific antibodies against melatonin as an antigen, such as radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) (35, 39). In the case of RIA detection of melatonin, results indicate an overestimation of melatonin concentration when compared to that detected by GC-MS, and

cases of false positives are also reported. ELISA kits for the detection of melatonin are available, especially for biological samples like urine, but their use in food samples like fermented products is still not suitable as they may need further optimization (33, 39). Development and validation of new antibodies for these complex matrices may be critical for ELISA method to be completely suitable. More recently, two different methods that employ mammalian melatonin receptors are worth mentioning. The first consists of engineered yeasts that act as melatonin sensors. This is possible because melatonin receptor MTNR1A is a G-protein coupled receptor (GPCR), just like the yeast pheromone receptor, and downstream signal transduction occurs when yeast GPCR is replaced with MTNR1A. Thus, by using a reporter gene at the end of the pheromone response pathway, a heterologous yeast strain was obtained that acted as a melatonin sensor. By employing the green fluorescent protein as a reporter, the authors detected melatonin production by yeasts using a microplate reader without having to process the sample, but directly from the supernatant (40). The second method was set up to detect melatonin in fermented beverages, and it involved mammalian melatonin receptor MTNR1B and cell lines as a sensor. In this case, the reporter system is different because it uses the  $\beta$ -lactamase enzyme (BLA) and a FRET (Förster resonance energy transfer)-based fluorescent substrate (CCF2/4) that presents green fluorescence when is intact. Thus, only when melatonin is present in the sample does the BLA enzyme cleave CCF2/4 to generate blue fluorescence. The relation between blue and green fluorescence is what is used for melatonin quantification (41). Methodologies like these offer the advantage of not requiring excessive sample processing; e.g. using C18 SepPak cartridges for each analysis, which cuts analysis costs and reduces the complexity of melatonin determination in large detection tests. This would allow to cost-effectively extend melatonin detection under many different conditions and by different strains of various yeast species. However, melatonin can be detected only by these methods, which reduces the understanding of tryptophan metabolism and its derivatives. When this is the study objective, HPLC-MS/MS is the most reliable method because it allows the simultaneous quantitation of such analytes.

### 3. MELATONIN AND MELATONIN ISOMERS IN FOOD, FERMENTED BEVERAGES AND YEAST CELLS

The presence of melatonin in food products derived from fermentation is no exception (Table 1). Several studies have shown the presence of melatonin in wine, beer and bread (28, 39, 45, 46). Human studies relate a rise in serum melatonin levels and antioxidant capacity after drinking beer (50), and more recently red wine (48, 51), supporting the role of wine melatonin in counteracting the physiological decline of the hormone into the bloodstream. The French paradox highlights the inverse relation between moderate alcohol consumption based on wine and coronary heart disease in the French population despite the fact that its diet is rich in saturated fatty acids (52). Initially, the plausible metabolites to attribute the health-promoting properties of wine were mainly polyphenols, but nowadays melatonin is taking their place as a relevant bioactive molecule (48, 53–55). Therefore, the consumption of different food products derived from fermentation represents a daily intake of melatonin with our diet. Thus, it is important to know the recommended and safe dose of dietary melatonin. Seabra *et al.* (56) reported that an oral daily intake of melatonin up to 10 mg did not show toxicological effects. On the other hand, it is important to gain knowledge about stability and longevity of melatonin in food and fermented beverages. Some studies reported that melatonin presents in such food and drinks is liable to be degraded to some extent by free

radicals, light and temperature, being this latter the most important factor affecting its stability (57).

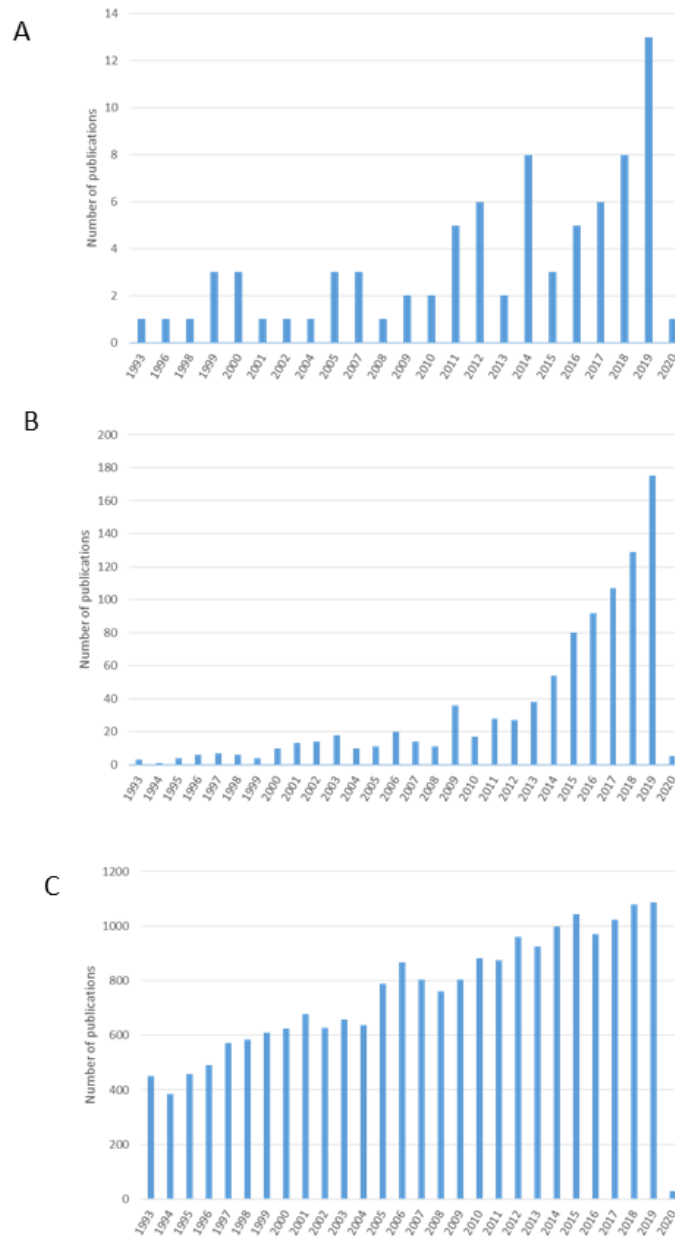
**Table 1: Concentration of melatonin in various fermented food and beverages.**

Fermented product	Melatonin concentration	Analytical method	Ref.
Sg and Tb wine	0.4-0.5 ng/mL	SPE-HPLC-F	(21)
Ch, Mb, CS wine	0.16-0.32 ng/mL	CE+HPLC-UV	(24)
Beer	51.8-169.7 pg/mL	ELISA	(42)
PV, Sh, CS, PP, Tp	140.5-277.5 pg/mL (ELISA); 5.1-129.5 ng/mL (HPLC-MS/MS)	ELISA, HPLC-F and HPLC-ESI-MS/MS	(39)
CS, MI, PF, Syrah, Tp, TR wine	74.13-423.01 ng/mL	HPLC-MS/MS	(43)
Gp, MI wine	5.8-8.1 ng/mL	UHPLC-MS/MS	(16)
Ab, Sg, Tb wine	0.3-1.5 ng/mL	MEPS-HPLC-F	(22)
Pomegranate wine	0.54-5.50 ng/mL	HPLC-ESI-MS/MS	(44)
Red, white and dessert wines	0.05-0.62 ng/mL	UHPLC-MS/MS	(45)
Beer	58-169 pg/mL	ELISA	(46)
Fermented orange juice	3.15-21.80 ng/mL	UHPLC-QqQ-MS/MS	(30)
Beer	94.50 pg/mL	HPLC-MS/MS	(28)
Bread	138.10-341 pg/g	HPLC-MS/MS	(28)
Yogurt	126 pg/g	HPLC-MS/MS	(28)
Mulberry wine	3.41-14.20 ng/mL	HPLC-ESI-MS/MS	(47)
Tp, Gc wine	0.03-161.83 ng/mL	UHPLC-QqQ-MS/MS	(48)
Cr, Ch, Ms, PF, SB, Vj and Tp wine	0.07-322.70 ng/mL	UHPLC/HRMS	(49)
Nb wine	0.038-0.063 ng/mL	SPE-HPLC-FL UHPLC/ESI-QTRAP	(27)

*Sg: Grape varieties represented are Sangiovese, Tb: Trebbiano, Ch: Chardonnay, Mb: Malbec, CS: Cabernet Sauvignon, PV: Petit Verdot, Sh: Syrah, PP: Prieto Picudo, Tp: Tempranillo, MI: Merlot, PF: Palomino Fino, TR: Tintilla de Rota, Gp: Gropello, Ab: Alaban, Gc: Garnacha, Cr: Corredera, Ms: Moscatel, SB: Sauvignon Blanc, Vj: Vijiriega, Nb: Nebbiolo.*

The fact that *S. cerevisiae* is primarily responsible for alcoholic fermentation could imply a role in melatonin synthesis by yeast. This hypothesis was unequivocally demonstrated by Rodriguez-Naranjo *et al.* (43). These authors conducted the winemaking process and monitored melatonin synthesis during alcoholic fermentation. They observed melatonin production from musts that lacked melatonin initially before being converted into wine. In fact melatonin synthesis by *S. cerevisiae* under laboratory conditions has been previously demonstrated by Sprenger *et al.* (58). To date, several studies have ascertained the relevance of yeast as a melatonin producer (43, 59-61) but, despite the crescent number of studies, melatonin in yeast is still a novel topic based on

the number of publications in recent years, which are still scarce compared to melatonin in relation to other organisms (Figure 1).



**Fig. 1. Evolution of publications on related topics compared to melatonin in yeast.**

A. "melatonin yeast", B. "melatonin plants" and "melatonin mammals", C. since 1993, For 2020, the data correspond to the first month of the year. Search results were obtained using Web of Science v5.34 search engine from Clarivate™ Analytics.

It would seem that melatonin synthesis most strongly depends on the yeast strain/species, and also on the cell metabolic state (62), but a clear condition that produces increased melatonin is still unknown. During the alcoholic fermentation of natural grape must, the production of indolic compounds largely depends on the used cultivar and fermentation time (49). It is important to bear in mind that when we talk about melatonin present in fermentation products, bacteria also play an



important role. Recently, the microorganism responsible mainly for malolactic fermentation, *Oenococcus oeni*, has been seen to produce melatonin on both winery and laboratory scales (63).

Regarding MIs, one proposal suggests that more than 40 potential isomers could originate as the indole ring of the molecule nucleus has seven accessible positions at which the 5-methoxy and 3-(*N*-acetylaminoethyl) groups in side chains can be exchanged (64). The nomenclature for these MIs has been proposed, but information about them is limited because their structures and patterns are not commercially available. In 2011, Rodriguez-Naranjo *et al.* (39) found another compound with an identical fragment pattern, but with a different retention time, to melatonin in certain monovarietal wines. It was tentatively considered to be the first melatonin isomer detected in wine. Similarly, other authors have reported the presence of this apparent melatonin isomer in the fermented products of orange juice and grape stuff-derived products (28–30, 65). However, this melatonin-like compound has been evidenced to be tryptophan-ethyl ester instead of a melatonin isomer (66). This compound is formed through the esterification of tryptophan, but what role it can play remains unclear. Several yeast species, including *S. cerevisiae* and non-*Saccharomyces* species, have been reported as being capable of releasing tryptophan ethyl ester under enological conditions, and have been found in different wines (59, 62, 67, 68). Vitalini *et al.* (45) reported the presence of three MIs in wines and balsamic vinegars, while Fracassetti *et al.* (27) more recently identified up to five MIs by UHPLC/ESI-QTRAP. Improvements in detection methods are beneficial for shedding light on MIs. To structurally identify isomers, an X-ray or NMR diffraction spectrum should be performed on the isolated MIs, or perhaps synthesizing them and using them as standards could be a good option (69).

#### 4. PHYSIOLOGICAL ROLE OF MELATONIN IN YEAST

Melatonin's physiological effects have been widely studied in a variety of organisms in animal and plant kingdoms. These studies have shown a vast number of functions for melatonin, ranging from the regulation of circadian and seasonal rhythms to therapeutic effects against cell aging or certain cancer types (34, 70, 71). Unicellular organisms like dinoflagellate *Gonyaulax polyedra* also produce melatonin, which regulate cyst formation. This physiological process occurs according to photoperiodicity, in which melatonin photooxidation plays an important role in metabolic regulation (72). In yeast, circadian behavior has also been reported as a systematic circadian metabolism in response to cyclic environment stimuli (73), but the role of melatonin in metabolic rhythms remain unclear.

Melatonin features antioxidant properties that exert multiple benefits for many organisms. Part of these properties is due to melatonin's chemical nature as it acts as a free radical scavenger, but also acts directly on metabolism at different levels. In yeast, the protective effect of melatonin against oxidative stress has been demonstrated with different approaches, where some authors have reported better growth performance in melatonin-treated cells, lower intracellular reactive oxygen species (ROS) levels, improved cell viability and better respiratory activity after oxidative stress and gene expression modulation for genes related to antioxidant response. This protective feature of melatonin extends to other stresses like UVC light (254 nm) irradiation. Moreover, melatonin significantly modulates gene expression in both unstressed cells and during various stress treatments, and the most enriched functions in the presence of melatonin in H<sub>2</sub>O<sub>2</sub>-stressed cells are antioxidant and oxidoreductase activities, transport and mitochondrial function (74–77). The synthesis or presence of melatonin in the medium also seems to influence some cell parameters, such as modulating cell fatty acid composition, increasing oleic and palmitoleic acids, and leading

to higher UFA/SFA (unsaturated fatty acids/saturated fatty acids) ratios, which have been previously related to greater H<sub>2</sub>O<sub>2</sub> tolerance. This modulation in fatty acid composition is, therefore, related to the antioxidant effect of melatonin in both *Saccharomyces* and non-*Saccharomyces* yeasts (75).

A screening of *Saccharomyces* and non-*Saccharomyces* strains revealed that melatonin synthesis took place at the end of the exponential growth phase, and its presence during fermentation followed a zigzag pattern that appeared and disappeared (59, 78). These results were also corroborated during grape must fermentation (79). The interaction of melatonin with glycolytic proteins in yeast indicates a possible role in melatonin transport through membranes, a feature that has also been reported in mammals, where the relation between melatonin and glucose transporters and glucose metabolism was assessed (80). These findings also support a possible role as a growth signal molecule and its production have been correlated with a yeast-growth phase (81).

## 5. MELATONIN BIOSYNTHESIS IN YEAST

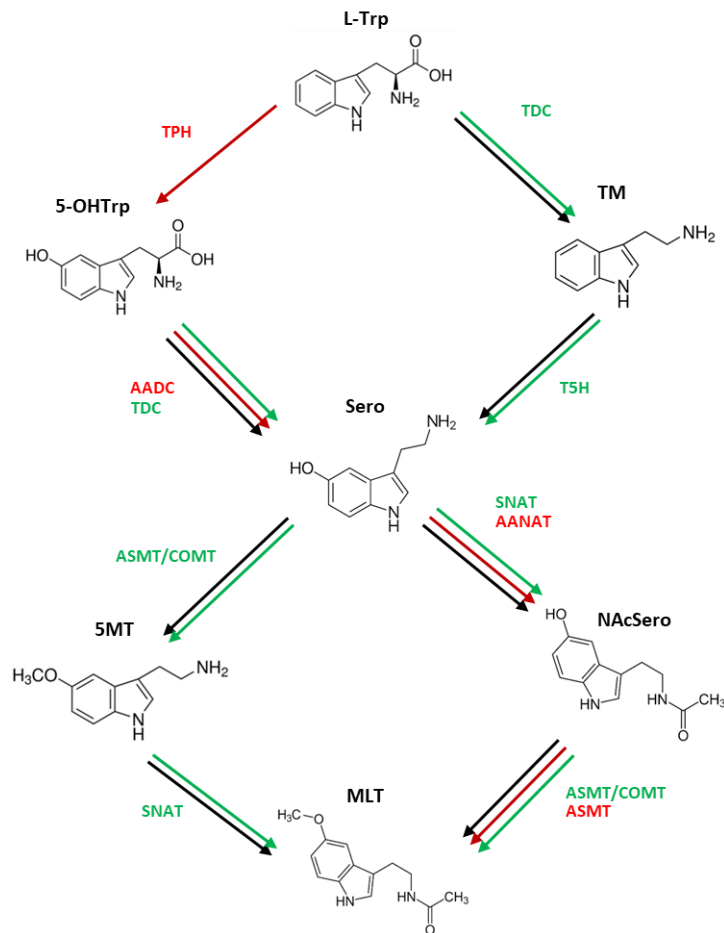
The synthetic pathway of melatonin in vertebrates has been widely studied. The classic melatonin synthetic pathway in animals was first deduced by Axelrod and Weissebach (1960) (82). It starts with tryptophan as a precursor, which is first hydroxylated to form 5-hydroxytryptophan by tryptophan-5-hydroxylase (TPH). Then 5-hydroxytryptophan is decarboxylated to form serotonin (5-hydroxytryptamine) under the catalytic action of an aromatic amino acid decarboxylase (AADC). Serotonin is acetylated to form *N*-acetylserotonin by arylalkylamine *N*-acetyltransferase (AANAT). Finally, *N*-acetylserotonin is methylated to melatonin by *N*-acetylserotonin *O*-methyltransferase (ASMT).

Surprisingly, information about the genes and enzymes in the biosynthetic pathway of melatonin in *S. cerevisiae* is lacking, except for the *PAA1* gene (*YDR071C*). *PAA1* encodes a polyamine acetyltransferase that can acetylate both polyamines and arylalkylamines (58, 83). Compared to the vertebrate AANAT, the *PAA1* enzyme has 47% similarity to the sheep AANAT, but without the N- and C-terminal regulatory flanking regions conserved in all vertebrate AANATs. Regarding the catalytic core, an important structural element is shorter and lacks the proline involved in substrate binding, which could explain the higher specificity and poorer specific activity of the yeast enzyme compared to vertebrates (84). The specificity of *PAA1* for a selection of amines has shown where the best amine substrates followed the order 5-methoxytryptamine, tryptamine and, finally, serotonin (84). This substrate preference backs the hypothesis which states that the last melatonin synthesis step is the acetylation of 5-methoxytryptamine to melatonin (85, 86). The main function of AANAT at the beginning of evolution has been suggested to be detoxification to avoid aldehydes from forming from amines (84), a function that may remain in *S. cerevisiae*.

The first work to show that *S. cerevisiae* was capable of synthesizing melatonin was performed by Sprenger *et al.* (58). In this work, the authors reported that *S. cerevisiae* was able to produce melatonin at high concentrations by feeding yeast cells with either complete growth medium or precursors (L-tryptophan, serotonin, *N*-acetylserotonin and 5-methoxytryptamine) in a saline medium. Recently, a similar strategy was used, but was extended to reveal the putative biosynthetic pathway of melatonin in *S. cerevisiae*. Bioconversion experiments were performed with yeast cells in different growth stages and on several growth media. Samples were analyzed to track and detect the intracellular and extracellular presence of various generated indole compounds (86). In this



case, L-tryptophan, 5-hydroxytryptophan, tryptamine, serotonin, N-acetylserotonin, 5-methoxytryptamine and melatonin were analyzed by UHPLC-MS/MS as precursors and products. Based on the obtained results, serotonin in yeast was formed differently from animals (Figure 2) via tryptophan decarboxylation, followed by tryptamine hydroxylation, as in plants.



**Fig. 2: Illustration of the biosynthetic pathway of melatonin from tryptophan for different organisms.**

*MLT: melatonin, L-Trp: tryptophan, 5-OHTrp: 5-hydroxytryptophan, Sero: serotonin, NAcSero: N-acetylserotonin which are the predominant intermediates in mammalian model whereas additional or alternative steps including TM: tryptamine and 5MT: 5-methoxytryptamine are reported for yeast and plant models. Enzymes represented are TPH: tryptophan hydroxylase, TDC: tryptophan decarboxylase, AADC: aromatic amino acid decarboxylase, T5H: tryptamine 5-hydroxylase, SNAT: serotonin N-acetyltransferase, AANAT: aromatic amino acid N-acetyltransferase, ASMT: N-acetylserotonin methyltransferase and COMT: caffeic acid O-methyltransferase. Red arrows refer to mammalian pathway, green arrows for plants and black arrows for yeast.*

It would also appear that 5-hydroxytryptophan synthesis by tryptophan hydroxylation does not occur in yeasts. Instead tryptophan is decarboxylated into tryptamine as the first step of the route despite it being capable of transforming the 5-hydroxytryptophan present in medium into

serotonin. Finally, serotonin can be either O-methylated by generating 5-methoxytryptamine or N-acetylated by producing N-acetylserotonin. Thus, melatonin can be formed from both of them, with 5-methoxytryptamine being the preferred substrate. Melatonin has been metabolized into N-acetylserotonin and 5-methoxytryptamine. The catabolism route by melatonin deacetylation in 5-methoxytryptamine was in concordance with the results obtained by Sprenger et al. who showed that melatonin was metabolized to 5-methoxytryptamine and also to 5-methoxytryptophol (58). Moreover, the cells supplemented with N-acetylserotonin produced substantial amounts of 5-methoxytryptamine (87). In plants, histone deacetylase genes have been related to conversions of N-acetylserotonin into serotonin and melatonin into 5-methoxytryptamine (88). It remains to be investigated if any *S. cerevisiae* histone deacetylases possess functional deacetylation activity on acetylated amines, such as N-acetylserotonin or melatonin. In animals, melatonin can be metabolized into N-acetylserotonin and 6-hydroxymelatonin (89, 90). As previously mentioned, melatonin O-demethylation into N-acetylserotonin has been reported in yeast, but melatonin hydroxylation has yet to be examined along with other potential melatonin catabolites.

The formation of 5-methoxytryptophol from 5-methoxytryptamine has already been described in vertebrates, and occurs through the catabolism of 5-methoxytryptamine by monoamine oxidase A (MAO A) to 5-methoxyindole-3-acetaldehyde, which is converted by alcohol dehydrogenase into 5-methoxytryptophol. Alternatively, 5-methoxyindole-3-acetaldehyde is catabolized by aldehyde dehydrogenase to 5-methoxyindole-3-acetic acid (18). Both 5-methoxytryptophol and 5-methoxyindole-3-acetic acid have been reported in yeast (58, 91). Therefore, additional studies in which both metabolites are analyzed can provide more information about the prevalent form of the catabolism of 5-methoxytryptamine in *S. cerevisiae*.

## 6. CONCLUSIONS

Yeast's ability to produce melatonin and detailed knowledge of this process have been recently examined. Fermented drinks as wine and beer contain multiple compounds with health-promoting effects, making them a rich product in beneficial compounds. Despite this, care must be taken when categorizing them as functional food as their content in ethanol is a trade-off for their nutritional and nutraceutical value. The presence of melatonin in yeast-fermented beverages and the benefits of the intake of this bioactive compound through diet open interesting ways toward product improvement via cell metabolism optimization to increase the concentration of bioactive molecules (92, 93). It also highlights the need for a better understanding of the physiological role and the biosynthetic pathway of melatonin and its related compounds in yeasts.

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## AUTHORSHIP

All the authors contributed to conception, revised the manuscript critically and approved it. SMC prepared, drafted and edited the manuscript and figures. RB contributed in editing the manuscript and figures. JMG edited the final version of this manuscript.

## CONFLICT INTEREST

The authors declare no conflict of interest.

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