MELATONIN AND PHYTOMELATONIN USED AS BIOACTIVE SUBSTANCES IN ANIMAL AND HUMAN NUTRITION

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Abstract

Nutrition and diet are two essential components for maintaining optimal health and life for the entire body, and proper nutrition, combined with good quality sleep, are two factors generally associated with promoting a healthy and balanced lifestyle. Sleep is a natural physiological phenomenon that serves to maintain the entire body in a state of rest necessary for recuperation and for the synchronization of most physiological, metabolic, and biological processes, which generally occur at a lower or higher intensity under the influence of the photoperiod. Melatonin is a biochemical compound produced by the pineal gland of vertebrates, with the purpose of acting as a biological signal, informing the body about the type of photoperiod in the external environment, and stimulating the onset of sleep. In this review paper, we conducted a detailed analysis of melatonin, based on a selection of a total of 80 bibliographic sources from the specialized literature, starting with the first evidence reported in 1917, which provided clues about the existence of this substance, and ending with the most recent information documented up to 2025, which highlights the importance of melatonin in nature through the roles and functions it performs in living organisms.

Keywords: *nutrition, diet, sleep, melatonin, biological signal*

INTRODUCTION

Melatonin, scientifically known as N-acetyl-5-methoxytryptamine [1-3], is a natural hormone [4,5] considered to be an idioamine, which is present in most forms of life on Earth. The presence of melatonin has been confirmed in vertebrates such as mammals, fish, and birds [6-9] and invertebrates [10-12], as well as in other life forms such as plants [13,14] and various species of microorganisms [15,16]. Melatonin has the chemical formula $C_{13}H_{16}N_2O_2$ [17] and is mainly known for its role in initiating sleep, regulating the circadian rhythm [18,19], and improving sleep quality in vertebrates [20], but it also has the ability to perform multiple other roles, depending on the organism in which it is present, such as antioxidant [21,22], anti-inflammatory [23], anticarcinogenic [24], antiapoptotic [25], anti-aging [26], and various other functions, such as regulating the reproductive system, the immune system, etc.

Melatonin is considered to be an idoalamine, classified as an idoalamine compound due to the fact that it is derived from tryptophan, and the chemical structure of indole is functionalized with a 3-amino group and a 5-alkoxy group, which give this molecule amphiphilic character [27].

Difficulties in achieving good quality sleep, generally manifested by the excessive amount of time an individual needs to fall asleep (the time required to initiate sleep),

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followed by difficulties in remaining asleep for a sufficient period of time for the sleep achieved to be considered good quality (approximately 8 hours per day), are mainly due to an inadequate diet and the stressful situations that are increasingly common in 21st-century society. Lack of sleep and difficulties in achieving good quality sleep affect an increasing number of individuals [28,29], so that melatonin (the main promoter that ensures both the initiation of sleep and the maintenance of this physiological state) has come to be widely used worldwide as a dietary supplement [30] to compensate for melatonin deficiencies suffered by various individuals.

MATERIALS AND METHODS

In this review paper, we presented the physiology and functions of the pineal gland; the differences between the synthesis processes of melatonin produced by vertebrates and melatonin produced by plant organisms; the level of knowledge about melatonin based on data available in the scientific literature from 1917 to 2025, through the analysis of a total of 80 scientific papers; various laboratory methods and techniques used to determine melatonin in various plant and animal raw materials and in different foods; nutritional sources of melatonin for animals and humans; as well as the effects of exogenous melatonin administration in humans.

The search for bibliographic sources was conducted in the digital databases Web of Sciences, Scopus, Google Academic, and Multidisciplinary Digital Publishing Institute (MDPI). The search for scientific articles was conducted based on keywords and keyword combinations: melatonin, phytomelatonin, pineal gland, melatonin determination techniques, dietary sources of melatonin, roles of melatonin in animals, roles of melatonin in human health, effects of exogenous melatonin in humans.

Searches based on keywords and keyword combinations generated a total of 67,287 scientific papers. Of the 67,287 papers identified as a result of searches in digital databases, 51,793 scientific papers were found based on the keyword "melatonin"; 155 scientific papers were found based on the keyword "phytomelatonin"; 11,889 scientific papers were found based on the keyword "pineal gland"; 359 scientific papers were found based on the keyword combination "melatonin determination techniques"; 209 scientific papers were found based on the keyword combination "melatonin food sources"; 1,949 scientific papers were found based on the keyword combination "roles of melatonin in animals"; 472 scientific papers were found based on the keyword combination "roles of melatonin in human health"; 461 scientific papers were found based on the keyword combination "effects of exogenous melatonin in humans".

For this review, 80 scientific papers were selected from the 67,287 papers found in the searches. The selection of scientific papers that make up the bibliography of this review was made based on the following inclusion and exclusion criteria, as applicable:

1. Duplicate works and those presenting identical or similar information to other works included in the bibliography were excluded;
2. Articles presenting information on the administration of exogenous melatonin to animals were excluded, due to European Union regulations prohibiting the use of hormones in farm animal husbandry;
3. Only studies reporting concrete results and presenting few limitations in terms of the conduct of the experiments and the accuracy of the results obtained were included.

PINEAL GLAND: LOCATION, PHYSIOLOGY, AND FUNCTIONS

The pineal gland, also known as the epiphysis, is a complex organ located on the sagittal line of the brain, connected to the third ventricle, shaped like a pine cone and reddish-gray in color. Anatomically, the pineal gland varies in size and weight between 5 and 9 mm in length, 2 and 5 mm in width, and 100 and 180 mg in weight [31-33]. The main function of the pineal gland is to produce melatonin, the hormone responsible for regulating circadian rhythms and controlling the physiological state of sleep by initiating, maintaining, and improving sleep quality [33,34]. The pineal gland is differently affected by photostimuli from the external environment, depending on the organism in which it is located, and can receive information about the intensity of light in the environment, either directly or indirectly.

In humans and higher vertebrates, evolutionary processes have led to the loss of the pineal gland's ability to directly detect light in the environment, as a result of the pineal gland's location at the base of the brain and the protection provided by the skull and the tissues covering it, which do not allow light to penetrate inside the skull. This aspect led to the evolution of the channel for perceiving and transmitting information about light intensity from the retina to the pineal gland, representing the indirect perception of light. In contrast, in amphibians and some species of fish, there is a thinner area of the skull and the tissue covering it, located at the back of the head, which allows light to penetrate and the light beam to be detected, directly or indirectly, by the pineal gland. Depending on the organism, there are two channels of light perception, separate from interaction with the eyeball.

Fish and some species of amphibians have a photosensitive pineal organ, called the parietal eye, which directly perceives light entering through the more transparent area at the front of the skull, converts it into an electrical signal, and transmits it to the pineal gland, thus informing the body about the type of photoperiod in the natural environment. Other amphibian species do not have such a photosensitive pineal organ, but they do have photosensitive cells integrated directly into the pineal gland, and the light that penetrates the skull is perceived directly by the pineal gland through the photosensitive cells [7,10]. The pineal gland is a highly vascularized organ, ensuring a blood flow of approximately 4 milliliters of blood per minute per gram. It is located outside the blood-brain barrier (BBB), more precisely between the superior colliculi of the tectum, in the posterior part of the wall behind the third ventricle. Thus, based on these characteristics, the pineal gland can accumulate large amounts of fluoride and calcium throughout life [33].

Fluoride has a very limited safety margin for the human body, so even in small doses it can cause various disorders or even toxicity. The level of safety and potential toxicity of fluoride on animal and human organisms depends on the dose and long-term intake of fluoride into the body. Naturally, vertebrates have developed and adapted several defense mechanisms against fluoride poisoning, but these mechanisms are quite limited, due to the fact that excessive doses of fluoride cannot be properly managed by the body, which can ultimately lead to poisoning. Among the mechanisms used by animals and humans to utilize fluoride, the most useful in preventing poisoning are fluoride homeostasis with other minerals such as calcium, magnesium, sodium, and potassium, and the deposition of fluoride in various resistant tissues such as bones and teeth. Other mechanisms that protect animals and humans against fluoride poisoning include the elimination of fluoride through urine and the protection of the brain provided by the blood-brain barrier, which prevents excessive amounts of fluoride from passing from the bloodstream to the brain. Fluoride can also be deposited in the pineal gland as a result of calcium deposits at the pineal level and due to fluoride's increased affinity for calcium [34].

Figure 1 shows the morphology of the pineal gland, while Figure 2 shows the mechanisms of light perception depending on the vertebrate organism and the morphology of the skull.

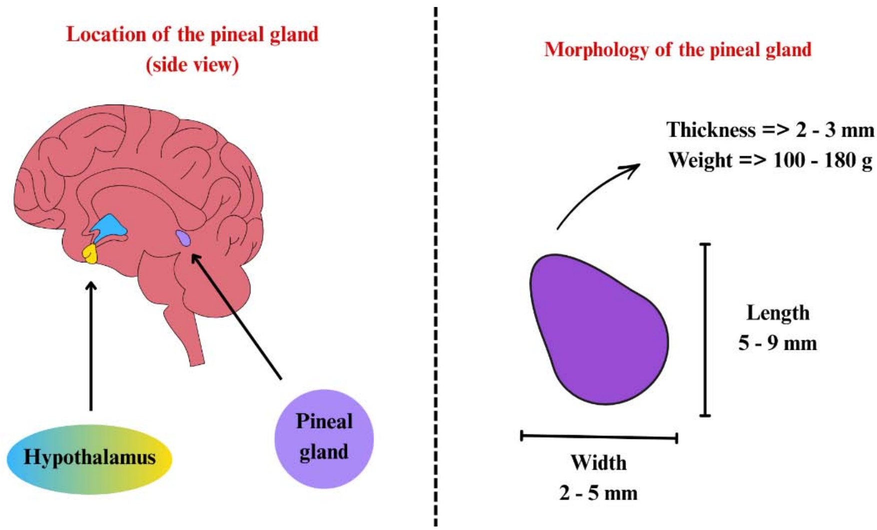

Fig. 1 Morphological characterization of the pineal gland

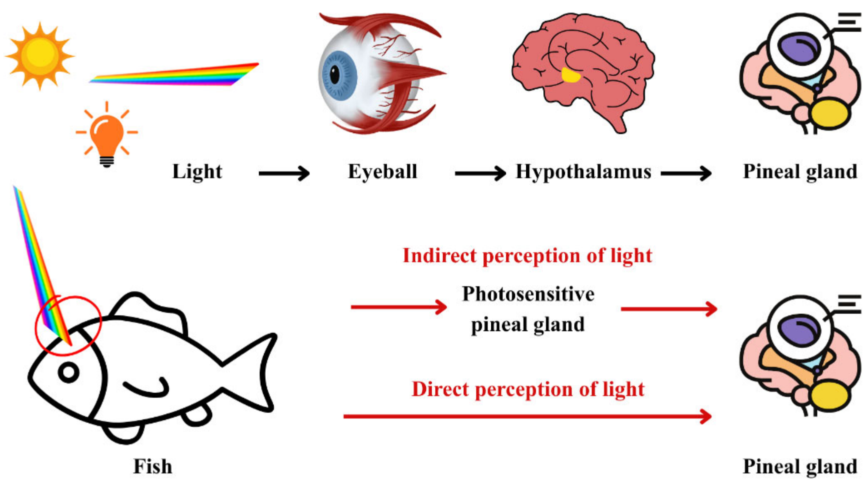

Fig. 2 Mechanisms of light perception in the pineal gland

An undesirable process affecting the pineal gland is pineal calcification, which is generally associated with aging and involves excessive calcium accumulation in the pineal gland, specifically in the apatite structure.

Numerous specialized studies have reported that the process of calcium accumulation in the pineal gland begins in childhood in newborns, but increases with age [33,35].

MELATONIN AS A PINEAL AND EXTRAPINEAL HORMONE

Melatonin is a molecule that was discovered in extracts from the pineal gland of cattle by Lerner A.B. and his colleagues in 1958 [36]. At that time, Lerner A.B. et al. relied on a study published in 1917 by

McCord C.P. and Allen F.P., who reported at the time that there were one or more substances in the pineal gland that had the ability to act on the reproductive system of amphibians [37]. Based on this observation, Lerner A.B. and his colleagues discovered in 1958 a new substance derived from the pineal gland of cattle that had the ability to whiten the skin of amphibians, which they named melatonin, but they were unable to fully characterize it chemically in 1958. In 1959, the presence of melatonin in the human body was confirmed [38], and in 1960, melatonin was fully characterized chemically [39]. The name melatonin was given to this substance as a result of the first observed effect of this molecule, which was to light up the skin of amphibians. Initially, after its discovery, it was observed that melatonin has the ability to lighten the skin of amphibians, as well as some species of fish, through the phenomenon of aggregation of melatonin granules found in the dermal melanosomes around the skin nucleus of frogs and fish, thus giving the skin a lighter shade [40,41]. Thus, based on this discovery, melatonin was subsequently used on human patients to reduce skin pigmentation in certain areas that were hyperpigmented, but this method proved ineffective, as no effects on skin lightening were observed in humans [42]. This is because mammalian melanosomes are permanently dispersed in smaller or larger proportions compared to amphibian melanosomes, so melatonin has a reduced effect on the phenomenon of pigment aggregation in mammalian skin [42]. The antioxidant role of melatonin was discovered in 1993 [43], but according to subsequent research and discoveries, melatonin was initially produced by bacteria and primitive eukaryotes about 2.5-3.5 billion years ago, precisely to neutralize free radicals, the antioxidant role being the first function performed by this substance in living organisms [44].

Extra-pineal melatonin is produced in vertebrates both centrally, by the brain, and peripherally by other tissues and organs such as the skin, retina, liver, kidneys, thyroid gland, cochlea, testicles, ovaries, and gastrointestinal tract [45].

PHYTOMELATONIN

Melatonin was first discovered in plants in 1995, simultaneously by three different groups of researchers [45-47], who identified melatonin in various plant materials. Melatonin from plants is generically called phytomelatonin (a name proposed in 2004 in order to differentiate plant-derived melatonin from other types of melatonin), and has a synthesis pathway similar to that of melatonin produced by the pineal gland of vertebrates, but which may follow a different synthesis pathway, depending on the need of plant organisms for phytomelatonin [48,49]. Phytomelatonin is produced exclusively by plants and algae.

The synthesis of phytomelatonin begins with the amino acid tryptophan, similarly to vertebrates, but in plants, tryptophan is produced directly by the plant organism, through the shikimate pathway [48]. Tryptophan is then converted into tryptamine by the enzyme tryptophan decarboxylase (TDC), and tryptamine is converted by the enzyme tryptamine 5-hydroxylase (T5H) into serotonin, also known by its scientific name 5-hydroxytryptamine. The next step in obtaining phytomelatonin is the process of N-acetylation of serotonin, carried out by the enzyme N-acetyltransferase (SNAT), thus obtaining N-acetylserotonin, which is subsequently methylated by acetylserotonin O-methyltransferase (ASMT), an enzyme in the hydroxyindole O-methyltransferase enzyme category, through which phytomelatonin is generated [30]. The process of obtaining phytomelatonin differs from the synthesis of melatonin in vertebrates in the way its precursors are synthesized. For example, after the action of N-acetyltransferase, serotonin can be converted to 5-methoxytryptamine by

ASMT or by caffeic acid O-methyltransferase (COMT). Another example is the methylation process of N-acetylserotonin, which can also be performed by COMT [30,50,51], a class of enzymes that can act on a wide variety of substrates, including caffeic acid [30]. Specialized studies conducted in the field of phytomelatonin have shown that in plants, the cellular compartments where the highest amounts of melatonin are found are chloroplasts and mitochondria (and in vertebrates, mitochondria are also important sources of melatonin production) [52]. The shikimate pathway is a large and complex process involving seven different steps, through which all aromatic amino acids in plants, including tryptophan, are synthesized [53]. The first step in obtaining tryptophan via the shikimate pathway involves the enzyme 3-Deoxy-D-arabinoheptulosonate-7-phosphate synthase (EC 2.5.1.54) converting phosphoenolpyruvate (PEP) and erythrose-4-phosphate into 3-Deoxy-D-arabinoheptulosonate-7-phosphate(DAHP). The next step is achieved by cyclizing DAHP into 3-dehydrochimate (DHQ) due to the action of DHQ synthase (EC 4.2.3.4). In the third step, shikimate is obtained, which is synthesized through dehydration and catalyzed dehydrogenation processes by DHQ dehydratase (EC 4.2.1.10) and shikimate dehydrogenase (EC 1.1.1.25). Subsequently, shikimate is phosphorylated by shikimate kinase (EC 2.7.1.71), forming shikimate-3-phosphate, which is converted by EPSP synthase (EC 2.5.1.19) into 5-enolpyruvylshikimate-3-phosphate. The next step is achieved through the formation of chorismate, which occurs through chorismate synthesis (EC 4.2.3.5), which converts EPSP into chorismate. In the next step, chorismate (the main intermediate in tryptophan biosynthesis) is converted to anthranilate by anthranilate synthase (EC 4.1.3.27), which is condensed with phosphoribosyl pyrophosphate (PRPP), ultimately generating phosphoribosyl anthranilate (PRA). The ribose ring added in the previous reaction is opened by PRA isomerase (PRAI; EC 5.3.1.24), which is then subjected to reductive decarboxylation to form indole-3-glycerol phosphate, which is spontaneously converted to the indole skeleton. In the final stage of the shikimate pathway, tryptophan is obtained through the reaction of indole with serine and the action of tryptophan synthase (TPS; EC 4.2.1.20) [48]. Melatonin produced by vertebrates and phytomelatonin have some differences in terms of synthesis pathways, the enzymes involved in the processes of converting tryptophan into serotonin and subsequently into melatonin, as well as in the order of decarboxylation and hydroxylation of the amino acid tryptophan. In vertebrates, tryptophan is first hydroxylated and converted to 5-hydroxytryptophan (5-HTP), after which 5-HTP undergoes decarboxylation to be converted to serotonin. However, in plant organisms, tryptophan is first decarboxylated to obtain tryptamine, after which tryptamine is hydroxylated to obtain serotonin. To obtain melatonin and phytomelatonin, the main precursor involved in the synthesis of this substance is the amino acid tryptophan, and the intermediate substance obtained in both synthesis processes is serotonin. Thus, tryptophan is the starting point in the creation of a metabolic pathway that supports the health of living organisms [2].

Figure 3 and Table 1 show a schematic and comparative representation of the synthesis processes of melatonin (in vertebrates) and phytomelatonin.

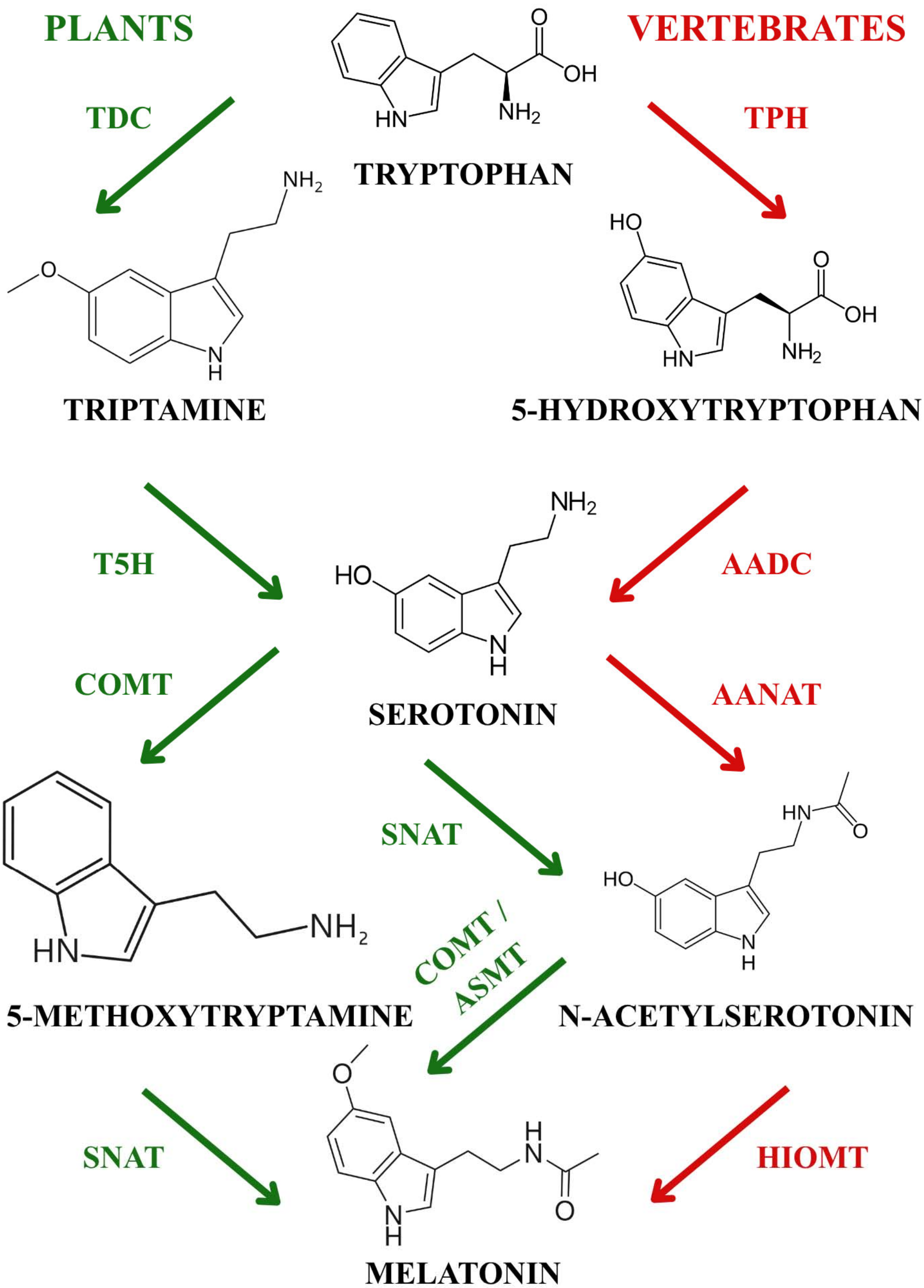

Figure 3 Schematic representation of the synthesis processes of melatonin and phytomelatonin

Table 1 Description of the stages of melatonin and phytomelatonin synthesis

Substance of origin	The enzyme that acts	Process name	Resulting substance	References
Melatonin				
Tryptophan	Tryptophan hydroxylase	Hydroxylation	5-hydroxytryptophan	[3]
5-hydroxytryptophan	5-Hydroxytryptophan Decarboxylase	Decarboxylation	Serotonin	
Serotonin	Serotonin-N-acetyltransferase	Acetylation	N-acetylserotonin	
N-acetylserotonin	Hydroxyindole-O-methyltransferase	Methylation	Melatonin	
Phytomelatonina				
Tryptophan	Tryptophan decarboxylase	Decarboxylation	Tryptamine	[44]
Tryptamine	5-hydroxylase tryptamine	Hydroxylation	Serotonin	
Serotonin	Serotonin-N-acetyltransferase	Acetylation	N-acetylserotonin	[48]
N-acetylserotonin	Hydroxyindole-O-methyltransferase	Methylation	Phytomelatonină	

In plant organisms and microorganisms, the synthesis pathway of melatonin can be accelerated under stressful conditions in order to neutralize reactive oxygen species (ROS) and reactive nitrogen species (RNS). In general, the final step involved in melatonin synthesis is methoxylation by the enzyme acetylserotonin O-methyltransferase (ASMT), but it has been observed that under certain conditions in which plant organisms are subjected to additional stress, the step in which acetylation is carried out by serotonin N-acetyltransferase (SNAT) becomes the final step in melatonin synthesis. This phenomenon occurs in order to accelerate melatonin production, to more effectively protect the body from oxidative damage caused by ROS and RNS [51,53].

METHODS FOR DETERMINING MELATONIN LEVELS IN DIFFERENT FOODS

Melatonin is a substance found in most biological fluids in the animal and human body, such as blood, cerebrospinal fluid, saliva, urine, and milk. Numerous researchers have reported the presence of melatonin in a wide range of plant and animal raw materials, as well as in several finished food products that have undergone various processing steps.

The determination of melatonin content can be achieved by several methods, depending on the type of raw material or food product being analyzed. Through several specialized studies, the level of melatonin in various plant and animal products has been determined using specific detection methods. Based on research conducted up to 2025 on melatonin, it has been demonstrated that the most useful techniques for determining the melatonin content in various biological materials are high-performance liquid chromatography (HPLC), tandem electrospray ionization mass spectrometry (EIS-MS-MS), radioimmunoassay, and ELISA [54-58].

The presence of melatonin in plant organisms provides humans and animals with an additional source of melatonin, apart from that produced naturally by vertebrates.

Examples of organic materials of plant and animal origin with a high melatonin content, as well as the laboratory techniques used to determine melatonin in these organic materials, are presented in Table 2.

Table 2 Melatonin levels determined in various raw materials of plant and animal origin and in various food products

Product name	Melatonin level	N	Determined by	References
Cereals				
Whole yellow corn	1.0 ng/g FW	5	HPLC	[59]
Corn germ flour	1.3 ng/g FW	5	HPLC	
Black rice	212.01 ng/g FW	9	PLE HPLC-FD	
Red rice	182.04 ng/g FW	9	PLE HPLC-FD	
Purple wheat	4.0 ng/g FW	3	HPLC-UV	
Fruit				
Apples (*Malus domestica Borkh.* Cv. Red Fuji)	5 ng/g FW	3	HPLC	[59]
Strawberries (*Fragaria ananassa* L. cv. Festival)	11.26 ng/g FW	3	HPLC	[60]
Cherries (*Prunus avium* L. cv. Hongdeng)	10-20 ng/g FW	3	SPE HPLC	[59]
Cranberry (*Vaccinium vitis-idaea* L.)	25 ug/g DW	5	UPLC-MS	[61]
Grapes + peel (*Vitis vinifera* L. cv. Malbec)	1.2 ng/g	5	CEC	[59]
Vegetables				
Tomatoes (*Solanum lycopersicum* L. cv. Optima)	14.77 ng/g FW 249.98 ng/g DW	4	UHPLC-MS/MS	[61]
Tomatoes (*Solanum lycopersicum* L. cv. Ciliegia)	0.64 ng/g FW 7.47 ng/g DW	4		
Pepper (*Capsicum annuum* L. cv. F26)	11.9 ng/g FW 93.4 ng/g DW	4		
Meat				
Cattle	2.1 ng/g	5	HPLC	[59]
Pork	2.5 ng/g	5		
Lamb	1.6 ng/g	5		
Chicken (meat with skin)	2.3 ng/g	5		
Fish (Salmon)	3.7 ng/g	5		
Milk				
Raw cow's milk	1.3 ÷ 25 pg/mL	10	ELISA	[62]
Raw cow's milk (night milk)	14.87 ± pg/mL^{-1}	30	ELISA	[63]
Skim milk	18.41 pg/mL^{-1}	6	LC-MS/MS	[59]
UHT milk	4.16 pg/mL	12	ELISA	[63]
UHT milk	4.5 pg/mL	20	ELISA	[62]
HTST milk	7.6 pg/mL	21		
ESL-HE milk	7.0 pg/mL	19		
ESL-MF milk	6.5 pg/mL	20		
NTMP milk	12.4 pg/mL	20		
Eggs				
Raw eggs, whole (egg white + egg yolk)	1.54 ng/g **	5	HPLC	[59]
Solid, dry egg	6.1 ng/g	5		

Abbreviations used: N - Number of Tests Performed; **FW** - Fresh Weight; **DW** - Dry Weight; **HPLC** - High Performance Liquid Chromatography; **PLE HPLC-FD** - Pressurized Liquid Extraction Followed by High Performance Liquid Chromatography with Fluorescence Detection; **HPLC-UV** - High Performance Liquid Chromatography - Ultraviolet detection; **SPE HPLC** - Solid Phase Extraction High Performance Liquid Chromatography; **UPLC-MS** - High Performance Liquid Chromatography coupled with Mass Spectrometry; **CEC** - Capillary Electrochromatography; **UHPLC** - Ultra-Efficient High Performance Liquid Chromatography; **UHT** - Ultra-High Temperature; **HTST** - High Temperature Pasteurized Milk; **ESL-HE** - Pasteurized Milk with Extended Shelf Life; **ESL-MF** - Microfiltered Milk with Extended Shelf Life; **NTMP** - Night Milk Powder.

High Performance liquid chromatography

High-performance liquid chromatography, abbreviated HPLC, is a laboratory technique characterized by high precision in the accuracy of the results obtained, short laboratory analysis times, and a wide range of analysis methods derived from this technique. Basically, HPLC is a type of column chromatography that involves two phases, one mobile and one stationary. As a result of adding the solvent to the sample, a solution of known concentration and volume is obtained, which is added to the top of the column. The separation of the mixture in the sample is due to dissolution and absorption processes, which depend on the stationary phase. HPLC allows the detection of melatonin concentrations in small samples of biological material, up to 20 microliters [64].

ELISA method

The ELISA (enzyme-linked immunosorbent assay) method is a laboratory technique based on the reaction between an antigen and an antibody, which allows the determination of melatonin concentrations in various biological materials such as blood, serum, saliva, urine, and milk. ELISA is a laboratory technique that can be used to quantitatively determine the level of melatonin in a sample, based on the principle of competitive reaction between a specific antibody and melatonin [64].

Radioimmunoassay

Radioimmunoassay, abbreviated RIA, is a laboratory technique widely used in the 21st century to determine melatonin concentrations in various biological materials such as blood, saliva, and urine. The method combines biochemical and statistical principles and involves measuring the radioactivity of a radioactive isotope attached to the antigen. Three types of substances are used in the laboratory test: the melatonin antibody, unlabeled melatonin, and labeled melatonin. The antibody for melatonin and labeled melatonin are added in known concentrations, and unlabeled melatonin is that which comes from the analyzed biological material. Once the laboratory sample is mixed and the reaction is started, the labeled melatonin and unlabeled melatonin compete to bind to the specific antibodies. After reaching reaction equilibrium, the antigen-antibody complexes are separated from the free melatonin, and the radioactivity of the bound fraction is measured. The measured radioactivity is inversely proportional to the concentration of unlabeled melatonin, so that the higher the radioactivity level of the sample, the lower the level of unlabeled melatonin [64].

THE BENEFITS OF MELATONIN IN ANIMAL ORGANISMS

Nutrition is a key factor in obtaining high-quality and high-quantity animal production [65], and the nutritional intake of plants/plant by-products rich in phytomelatonin can support and even improve animal health.

In farm animals, melatonin generally performs roles and functions similar to those performed in the human body. However, the use of hormones in livestock farming technologies is prohibited at European Union level [66], so that the nutritional intake of phytomelatonin remains the only source of exogenous melatonin for farm animals.

Peña-Delgado V. et al. [67] reported in a study published in 2023 that enriching the diets of Aragonesa rams with various plant by-products rich in melatonin improved the quality of the rams' semen, thus proving the powerful antioxidant role of melatonin in protecting sperm against free radicals and the impact of this molecule in regulating the reproductive system of seasonally breeding animals. The plant by-products rich in phytomelatonin that were introduced into the experimental group's diet were grape pulp, which had a melatonin content of

45.94 ± 4.19 ng/g, pomegranate pomace with a melatonin content of 35.81 ± 0.4 ng/g, and tomato pomace with a melatonin content of 23.76 ± 1.37 ng/g [67].

In the case of animals, for which European Union legislation and regulations do not allow the administration of exogenous hormones during their rearing [66], in order to obtain animal products with high levels of melatonin, additional stimulation of the pineal gland is necessary to produce larger quantities of N-acetyl -5-methoxytryptamine, which enters the bloodstream and is subsequently released into the production of milk, meat, eggs, etc.

The concentrations of melatonin present in various raw materials of plant and animal origin, as well as in various finished food products, vary widely depending on several factors external to the body, such as climatic and environmental conditions in the case of plant organisms, or nutritional and technological factors in the case of farm animals. The impact of nutritional and technological factors, when managed correctly on livestock farms, on melatonin concentrations in cow's milk is a topic we addressed in a previous paper [68]. The bioavailability of tryptophan and the duration of exposure of the eyeball to intensities greater than 30 lux during the day are the main factors influencing the pineal gland's ability to produce and secrete melatonin into the blood, which is then released into the milk of mammals.

The influence of milk processing on melatonin concentrations in finished products is also a topic of interest for the development of functional foods that improve sleep quality. In studies conducted up to 2025 on melatonin concentrations determined in various finished dairy products, there is a lack of traceability of raw materials (there are no studies comparing melatonin levels in the same batch of milk before and after processing).

Schaper S. et al. (2015) [62] investigated and compared melatonin concentrations in raw milk from 10 Holstein cows, collected twice with a two-week interval between samples, and melatonin concentrations in five different types of processed milk (the results of that study on melatonin content in raw milk and processed milk are presented in Table 2). Significant differences were reported between all types of milk analyzed by Schaper S. et al. (2015) [62], with a difference primarily observed in the melatonin content determined in the 20 raw milk samples collected from 10 Holstein cows (melatonin concentrations in raw milk ranged from 1.3 to 25.0 pg/mL, with an average of 13.6 pg/mL for the 20 samples analyzed), and secondly between the average of the raw milk samples and the results obtained for the 5 types of processed milk (UHT, HTST, ESL-HE, ESL-MF, NTMP) [62].

Melatonin concentrations in different types of dairy products were also determined by Romanini E.B. et al. (2019) [63], who determined the melatonin content in three different sources of milk samples (raw milk collected individually from 30 Holstein cows, raw milk collected from 16 raw material storage tanks, and UHT heat-treated milk from 12 different producers in the Castro region, Brazil). The results obtained by Romanini E.B. et al. (2019) [63] suggest that the UHT heat treatment process does not significantly influence melatonin concentrations in finished products, as observed by the similar melatonin values determined in the three types of milk analyzed (6.98 pg/mL for raw milk collected individually from 30 Holstein cows; 4.71 pg/mL in raw milk collected from storage tanks; and 5.62 pg/mL in UHT milk).

Correlating the results obtained for melatonin content determined in UHT milk by Schaper S. et al. (2015) [62] with the results obtained by Romanini E.B. et al. (2019) [63], in which the melatonin concentration was 4.5 pg/mL [62] and 4.16 pg/mL [63], respectively, suggests that there is a negative effect caused by heat treatment performed at extreme temperature parameters.

MELATONIN USED AS A FOOD SUPPLEMENT

In humans, melatonin is used as a dietary supplement to alleviate difficulties caused by a lack of naturally produced melatonin, mainly to improve sleep quality. Several clinical studies have reported that melatonin is not toxic when administered in high doses. Synthetic melatonin can be administered intravenously, subcutaneously, or orally.

The impact of diets rich in dairy products with high tryptophan and/or melatonin content, as well as the impact of Mediterranean dietary patterns, on sleep quality in healthy people of different ages, is a topic that we have presented in detail in a previous paper [69].

The multiple roles and functions performed by melatonin in the animal and human body make this compound a complex substance that can act as a hormone that initiates and maintains sleep (in the case of pineal melatonin), as a vitamin with antioxidant properties, and also has autacoid and paracoid properties [70].

The decrease in the pineal gland's ability to synthesize melatonin in relation to each individual's age, combined with the difficulties increasingly encountered by 21st-century society, in achieving good quality sleep, lead to an increasingly pronounced need to consume exogenous melatonin in order to improve sleep quality and reduce the risk of diseases or conditions generally associated with disturbances in the physiological state of sleep. Melatonin can be introduced into the human body exogenously, either through the consumption of foods with a high melatonin content or through the direct consumption of melatonin-based pharmaceutical supplements.

Consuming orange or pineapple juice and eating two bananas for breakfast have been shown to be important sources of phytomelatonin, which increased plasma melatonin levels in healthy adults. Sae-Teaw M. et al. (2013) [71], reported that orange juice consumption increased serum melatonin levels from 40 to 151 pg/mL, pineapple juice consumption increased plasma melatonin levels from 48 to 146 pg/mL, and fresh bananas increased levels from 30 to 140 pg/mL. Another aspect observed by Sae-Teaw M. et al. (2013) [71] was the time taken for serum melatonin concentrations to increase and the half-life. According to the data reported by the authors of that study, plasma melatonin levels reached their maximum concentration two hours after consumption of plant products, after which they fell to less than 50 pg/mL between two and three hours after consumption, thus indicating the rapid metabolism of melatonin [71,72].

Supplementing diets with melatonin in tablet or capsule form can be an effective strategy for treating sleep-related disorders, but it is not a universal solution [73]. Melatonin is synthesized in varying amounts by the pineal gland, depending on the age of each individual. Numerous specialized studies have shown that supplemental exogenous melatonin intake in humans does not appear to have negative effects such as toxicity or dependence on melatonin intake [74].

Chemically produced melatonin has both advantages and disadvantages in terms of its use in clinical research and as a dietary supplement in humans. Synthetic melatonin can be produced through several chemical synthesis pathways, starting from different molecules, such as 5-methoxytryptamine; phthalimide, also known as 1,3-dihydro-1,3-dioxoisonidol; 5-methoxy-3-(2-nitroethyl)-indole; 5-methoxy-3-indoleacetonitrile. This aspect, in direct correlation with the processes of obtaining and purifying synthetic melatonin, can lead to the formation of residues in the resulting melatonin mass, which can have toxic effects on the human body if consumed. According to Minich D.M. et al. (2022) [16], common contaminants in synthetic melatonin preparations are represented by:

- ✓ 3-(phenylamino)-alanine;
- ✓ 1,1'-ethylidene bis-(tryptophan), also called E-tip;
- ✓ 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid;
- ✓ 2-(3-indolylmethyl)-tryptophan;
- ✓ Formaldehyde-melatonin condensation products;
- ✓ Hydroxy-bromo-propylphthalimide;
- ✓ 5-hydroxy-tryptamine derivatives;
- ✓ 5-methoxy-tryptamine derivatives;
- ✓ Hydroxymelatonin isomers;
- ✓ N-acetyl and diacetyl indole derivatives;
- ✓ Chloropropylphthalimide;
- ✓ 1,3-diphtalimidopropane.

Wang Y. et al. [75] conducted a study in which they presented the beneficial effects of administering an encapsulated tablet containing fast-release vitamin B6 and controlled-release melatonin. The difficulties in obtaining good quality sleep have generated increasing interest among the human population in the global consumption of melatonin, with the aim of reducing the consequences caused by the lack of melatonin in the human body. Due to melatonin's short half-life, the pineal gland continuously produces this hormone throughout the night to help the body maintain sleep. For these reasons, it is necessary that melatonin introduced into the body exogenously, through dietary supplements, has a controlled release rate that allows the body to be supplied with melatonin throughout the entire duration of the dark photoperiod.

According to Wang Y. et al. [75], in addition to the very low toxicity of mematonin, oral administration of melatonin has other advantages, such as a hypnotic effect at very low doses, endogenous action through regulation of the endocrine system, and the existence of its own metabolic pathway, through which the accumulation of the drug and its metabolites in the body is not promoted. Due to the short half-life, melatonin is completely eliminated from the body, so there is no possibility of various residual concentrations of melatonin accumulating in the body and affecting energy levels the next day.

The bilayer tablet produced by Wang Y. et al. [75] showed high resistance to elevated temperature and humidity, as well as increased resistance to direct light exposure. However, the authors recommended that the encapsulated product be stored in cool, dry environments. The release time of the two layers of the tablet was 10-15 minutes for the complete release of vitamin B6 and 8 hours for the release of approximately 90% of the melatonin substrate.

The use of synthetic melatonin as a supplement in combating sleep-related disorders appears to be a useful long-term solution due to several characteristics of melatonin, such as the effects of small amounts of melatonin in the human body, the lack of toxicity of this substance, and the lack of dependence on this substance. However, a relevant question in this field, the answer to which highlights the effectiveness of naturally produced melatonin in living organisms, is the following: "Can synthetic melatonin have the same effects and intensity on living organisms as vertebrate melatonin or phytomelatonin?". To answer this question, we analyzed several papers in the literature that studied and compared the effects of synthetic melatonin with those of melatonin produced naturally by living organisms.

Kukula-Koch W. et al. [76] reported in a paper published in 2021 that phytomelatonin has anti-inflammatory and antioxidant effects that are much stronger than those of synthetically produced melatonin. The results reported by Kukula-Koch W. et al. (2021) [76], who conducted the experiment on human cell lines, it was found that phytomelatonin has a 646% higher efficacy in inhibiting cyclooxygenase-2 (COX-2), a 267-470% higher antioxidant effect, and a ROS neutralization power that is 100% higher compared to synthetic melatonin [76].

Consuming foods with levels of melatonin is a useful method for combating sleep-related disorders, as most of the melatonin ingested through diet enters the bloodstream [77].

The use of melatonin as a nutraceutical is an area that has been the subject of numerous scientific papers. Nutraceuticals are defined as foods or parts of certain foods that can have beneficial effects on human health. Numerous authors have reported in various specialized studies that melatonin introduced into the body exogenously, from various nutritional sources or through synthetic melatonin intake, is effectively absorbed by the human body. However, it has been shown that the level of absorption of exogenous melatonin and the rate of elimination through urine vary from one individual to another [78,79]. Andersen L.P.H. et al. (2016) [80] conducted a cross-over cohort study in which they reported that melatonin administered orally to healthy male patients had a higher absorption rate in the human body compared to exogenous melatonin administered intravenously.

Anderson L.P.H. et al. (2016) [80] administered melatonin in doses of 10 mg/day and studied the pharmacokinetics of orally and intravenously administered melatonin by determining the time to maximum concentration (t_{max}) and maximum plasma concentrations of melatonin (C_{max}). The time to maximum melatonin concentration was 41 minutes for orally administered melatonin. Maximum plasma melatonin concentrations varied between individuals, and the bioavailability of orally administered melatonin was found to be only 3% higher than that of intravenously administered melatonin.

CONCLUSIONS

Melatonin is a complex substance produced widely by most life forms on Earth. In relation to the physiology of the pineal gland, melatonin synthesized by the epiphysis is produced in varying amounts, depending on the age of each individual, showing an upward trend in synthesis from childhood to around the age of 30, followed by a period in which melatonin is produced relatively constantly in the body (30-50), after which the process of melatonin synthesis gradually decreases until it is almost completely absent around the age of 75. Nutrition can be a supporting factor in compensating for the natural lack of melatonin, through an adequate nutritional intake of foods that have a high chemical content of N-acetyl-5-methoxytryptamine. Another approach proposed in this field is the use of synthetic melatonin, administered orally in capsule form, or in injectable form, subcutaneously or intravenously. However, melatonin produced naturally by living organisms appears to have stronger effects in performing certain functions, such as antioxidant and anti-inflammatory, compared to synthetic melatonin.

In the case of farm animals, additional amounts of melatonin can be introduced into the body by supplementing their feed with various plant products or by-products from other plant processing industries that contain large amounts of phytomelatonin.

Melatonin is not toxic to the human body when administered in large doses, nor is it addictive. However, synthetic melatonin tablets may contain certain protective coatings made up of substances that could make the dietary supplement potentially addictive, along with other secondary substances.

REFERENCES

1. Biggio, G; Biggio, F; Talani, G; Mostallino MC; Aguglia, A; Aguglia, E; Palagini, L Melatonin: From Neurobiology to Treatment. *Brain Sciences*. **2021**; *11(9)*, 1121. https://doi.org/10.3390/brainsci11091121.

2. Sharif, R; Xie, C; Zhang, H; Arnao, MB; Ali, M; Ali, Q; Muhammad, I; Shalmani, A; Nawaz, MA; Chen, P; Li, Y. Melatonin and Its Effects on Plant Systems. *Molecules* **2018**, *23(9)*, 2352. https://doi.org/10.3390/molecules23092352.
3. Miranda-Riestra, A; Estrada-Reyes, R; Torres-Sanchez, ED; Carreño-García, S; Ortiz, GG; Benítez-King, G. Melatonin: A Neurotrophic Factor? *Molecules* **2022**, *27(22)*, 7742. https://doi.org/10.3390/molecules27227742.
4. Zhong, J; Lu, Z; Zhou, Z; Ma, N; Li, YJ; Hu, JJ; Wan, B; Lu, W Melatonin biosynthesis and regulation in reproduction. *Front. Endocrinol.* **2025**, *16*, 1630164. https://doi.org/10.3389/fendo.2025.1630164.
5. Reiter, RJ; Sharma, R; Tan, D-X; Chuffa, LGdA; da Silva, DGH; Slominski, AT; Steinbrink, K; Kleszczynski, K Dual sources of melatonin and evidence for different primary functions. *Front. Endocrinol.* **2024**, *15*, 1414463. https://doi.org/10.3389/fendo.2024.1414463.
6. Acuña-Castroviejo, D; Escames, G; Venegas, C; Díaz-Casado, ME; Lima-Cabello, E; López, LC; Rosales-Corral, S; Tan, D-X; Reiter, RJ Extrapineal melatonin: sources, regulation, and potential functions. *Cell. Mol. Life Sci.* **2014**, *71*, 2997–3025. https://doi.org/10.1007/s00018-014-1579-2.
7. Li, J; You, X; Bian, C; Yu, H; Coon, SL; Shi, Q Molecular Evolution of Aralkylamine N-Acetyltransferase in Fish: A Genomic Survey. *Int. J. Mol. Sci.* **2016**, *17(1)*, 51. https://doi.org/10.3390/ijms17010051.
8. Aranda-Martínez, P; Fernández-Martínez, J; Ramírez-Casas, Y; Guerra-Librero, A; Rodríguez-Santana, C; Escames, G; Acuña-Castroviejo, D The Zebrafish, an Outstanding Model for Biomedical Research in the Field of Melatonin and Human Diseases. *Int. J. Mol. Sci.* **2022**, *23(13)*, 7438. https://doi.org/10.3390/ijms23137438.
9. Esteban, MÁ; Cuesta, A; Chaves-Pozo, E; Meseguer, J Influence of Melatonin on the Immune System of Fish: A Review. *Int. J. Mol. Sci.* **2013**, *14(4)*, 7979–7999. https://doi.org/10.3390/ijms14047979.
10. Falcón, J; Torriglia, A; Attia, D; Viénot, F; Gronfier, C; Behar-Cohen, F; Martinsons, C; Hicks, D Exposure to Artificial Light at Night and the Consequences for Flora, Fauna, and Ecosystems. *Front. Neurosci.* **2020**, *14*, 602796. https://doi.org/10.3389/fnins.2020.602796.
11. Maciel, FE; Geihs, MA; Cruz, BP; Vargas, MA; Allodi, S; Marins, LF; Nery, LEM Melatonin as a Signaling Molecule for Metabolism Regulation in Response to Hypoxia in the Crab *Neohelice granulata*. *Int. J. Mol. Sci.* **2014**, *15(12)*, 22405-22420. https://doi.org/10.3390/ijms151222405.
12. Keklikoglou, K; Mandalakis, M; Fanouraki, E; Anastasiou, TI; Kagiampaki, E; Vasileiadou, K; Pavloudi, C; Arvanitidis, C; Pavlidis, M Disrupted darkness: the impact of anthropogenic light at night on melatonin secretion of *Hermodice carunculata* (Polychaeta, Annelida). *Photochem. Photobiol. Sci.* **2025**, *24*, 1031–1040. https://doi.org/10.1007/s43630-025-00744-5.
13. Arnao, MB; Hernández-Ruiz, J The Potential of Phytomelatonin as a Nutraceutical. *Molecules* **2018**, *23(1)*, 238. https://doi.org/10.3390/molecules23010238.
14. Feng, X; Wang, M; Zhao, Y; Han, P; Dai, Y Melatonin from different fruit sources, functional sources, and analytical methods. *Trends Food Sci. Technol.* **2014**, *37(1)*, 21–31. https://doi.org/10.1016/j.tifs.2014.02.001.
15. Bonmatí-Carrión, M-Á; Rol, M-A Melatonin as a Mediator of the Gut Microbiota–Host Interaction: Implications for Health and Disease. *Antioxidants* **2024**, *13(1)*, 34. https://doi.org/10.3390/antiox13010034.
16. Minich, DM; Henning, M; Darley, C; Fahoum, M; Schuler, CB; Frame, J Is Melatonin the "Next Vitamin D"?: A Review of Emerging Science, Clinical Uses, Safety, and Dietary Supplements. *Nutrients* **2022**, *14(19)*. https://doi.org/10.3390/nu14193934.
17. Andrani, M; Dall'Olio, E; De Rensis, F; Tummaruk, P; Saleri, R Bioactive Peptides in Dairy Milk: Highlighting the Role of Melatonin. *Biomolecules* **2024**, *14(8)*, 934. https://doi.org/10.3390/biom14080934.
18. Juliana, N; Maluin, SM; Effendy, NM; Abu, IF; Azmani, S Cortisol Detection Methods and the Hormone's Role in Evaluating Circadian Rhythm Disruption. *Int. J. Mol. Sci.* **2025**, *26(18)*, 9141. https://doi.org/10.3390/ijms26189141.
19. Megha, KB; Arathi, A; Shikha, S; Alka, R; Ramya, P; Mohanan, PV Significance of Melatonin in the Regulation of Circadian Rhythms and Disease Management. *Mol. Neurobiol.* **2024**, *61*, 5541–5571. https://doi.org/10.1007/s12035-024-03915-0.
20. Fatemeh, G; Sajjad, M; Niloufar, R; Neda, S; Leila, S; Khadijeh, M Effect of melatonin supplementation on sleep quality: a systematic review and meta-analysis of randomized controlled trials. *J. Neurol.* **2022**,

269, 205–216. https://doi.org/10.1007/s00415-020-10381-w.
21. Monteiro, KKAC; Shiroma, ME; Damous, LL; Simões, MdJ; Simões, RdS; Cipolla-Neto, J; Baracat, EC; Soares-Jr., JM Antioxidant Actions of Melatonin: A Systematic Review of Animal Studies. *Antioxidants* **2024**, *13(4)*, 439. https://doi.org/10.3390/antiox13040439.
22. Tan, D-X; Manchester, LC; Esteban-Zubero, E; Zhou, Z; Reiter, RJ Melatonin as a Potent and Inducible Endogenous Antioxidant: Synthesis and Metabolism. *Molecules* **2015**, *20(10)*, 18886-18906. https://doi.org/10.3390/molecules201018886.
23. Chitimus, DM; Popescu, MR; Voiculescu, SE; Panaitescu, AM; Pavel, B; Zagrean, L.; Zagrean, A-M Melatonin's Impact on Antioxidative and Anti-Inflammatory Reprogramming in Homeostasis and Disease. *Biomolecules* **2020**, *10(9)*, 1211. https://doi.org/10.3390/biom10091211.
24. Wang, X The antiapoptotic activity of melatonin in neurodegenerative diseases. *CNS neurosci. ther.* **2009**, *15(4)*, 345–357. https://doi.org/10.1111/j.1755-5949.2009.00105.x.
25. Talib, WH; Alsayed, AR; Abuawad, A; Daoud, S; Mahmod, AI Melatonin in Cancer Treatment: Current Knowledge and Future Opportunities. *Molecules*, **2021**, *26(9)*, 2506. https://doi.org/10.3390/molecules26092506.
26. Martín Giménez, VM; de las Heras, N; Lahera, V; Tresguerres, JAF; Reiter, RJ; Manucha W Melatonin as an Anti-Aging Therapy for Age-Related Cardiovascular and Neurodegenerative Diseases. *Front. Aging Neurosci.* **2022**, *14*, 888292. https://doi.org/10.3389/fnagi.2022.888292.
27. Omar, R; Koparir, P; Ahmed, L; Koparir, M Computational and spectroscopy study of melatonin. *Indian J. Chem. B* **2021**, *60*, 732–741. DOI:10.56042/ijcb.v60i5.40996.
28. Benjafield, AV; Sert Kuniyoshi, FH; Malhotra, A; Martin, JL; Morin, CM; Maurer, LF; Cistulli, PA; Pépin, J-L; Wickwire, EM; medXcloud group. Estimation of the global prevalence and burden of insomnia: a systematic literature review-based analysis. *Sleep Med Rev.* **2025**, *82*, 102121. https://doi.org/10.1016/j.smrv.2025.102121.
29. van Straten, A; Weinreich, KJ; Fábián, B; Reesen, J; Grigori, S; Luik, AI; Harrer, M; Lancee, J The Prevalence of Insomnia Disorder in the General Population: A Meta-Analysis. *J. Sleep Res.* **2025**, *34(5)*, e70089. https://doi.org/10.1111/jsr.70089.
30. Salehi, B; Sharopov, F; Fokou, PVT; Kobylinska, A; Jonge, Ld; Tadio, K; Sharifi-Rad, J; Posmyk, MM; Martorell, M; Martins, N; Iriti, M Melatonin in Medicinal and Food Plants: Occurrence, Bioavailability, and Health Potential for Humans. *Cells* **2019**, *8(7)*, 681. https://doi.org/10.3390/cells8070681.
31. Arnao, MB; Giraldo-Acosta, M; Castejón-Castillejo, A; Losada-Lorán, M; Sánchez-Herrerías, P; El Mihyaoui, A; Cano, A; Hernández-Ruiz, J Melatonin from Microorganisms, Algae, and Plants as Possible Alternatives to Synthetic Melatonin. *Metabolites* **2023**, *13(1)*, 72. https://doi.org/10.3390/metabo13010072.
32. Macchi, MM; Bruce, JN Human pineal physiology, and functional significance of melatonin. *Front. Neuroendocrinol.* **2004**, *25(3-4)*, 177–195. https://doi.org/10.1016/j.yfrne.2004.08.001.
33. Peruri, A; Morgan, A; D'Souza, A; Mellon, B; Hung, CW; Kayal, G; Shin, H; Nguyen, K; Zahed, M; Yount, M; Ellis, R; Wynne, T; Fritz, V; Simmons, Z; Roballo, KCS Pineal Gland from the Cell Culture to Animal Models: A Review. *Life* **2022**, *12(7)*, 1057. https://doi.org/10.3390/life12071057.
34. Chlubek, D.; Sikora, M. Fluoride and Pineal Gland. *Appl. Sci.* **2020**, *10(8)*, 2885. https://doi.org/10.3390/app10082885.
35. Reiter, RJ; Mayo, JC; Tan, D-X; Sainz, RM; Alatorre-Jimenez, M; Qin, L Melatonin as an antioxidant: Under promises but over delivers. *J. Pineal Res.* **2016**, *61(3)*, 253–278. https://doi.org/10.1111/jpi.12360.
36. Lerner, AB; Case, JD; Takahashi, Y; Lee, TH; Mori, W Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J. Am. Chem. Soc.* **1958**, *80(10)*, 2587.
37. McCord, CP; Allen, FP Evidence associating pineal gland function with alterations in pigmentation. *J. Exp. Zool.* **1917**, *23(1)*, 207–224. https://doi.org/10.1002/jez.1400230108.
38. Lerner, AB; Case, JD Pigment cell regulatory factors. *J. Investig. Dermatol.* **1959**, *32(2)*, 211–221. https://doi.org/10.1038/jid.1959.38.
39. Lerner, AB; Case, JD; Takahashi, Y Isolation of Melatonin and 5-Methoxyindole-3-acetic Acid from Bovine Pineal Glands. *J. Biol. Chem.* **1960**, *235(1)*, 1992–1997. https://doi.org/10.1016/S0021-9258(18)69351-2.

40. Lerner, AB; Case, JD; Mori, W; Wright, MR Melatonin in Peripheral Nerve. *Nature* **1959**, *183*, 1821. https://doi.org/10.1038/1831821a0.
41. Tan, D-X; Zheng, X; Kong, J; Manchester, LC; Hardeland, R; Kim, SJ; Xu, X; Reiter, RJ, Fundamental Issues Related to the Origin of Melatonin and Melatonin Isomers during Evolution: Relation to Their Biological Functions. *Int. J. Mol. Sci.* **2014**, *15(9)*, 15858–15890. https://doi.org/10.3390/ijms150915858.
42. Nordlund, JJ; Lerner, AB The effects of oral melatonin on skin color and on the release of pituitary hormones. *J. Clin. Endocrinol. Metab.* **1977**, *45(4)*, 768–774. https://doi.org/10.1210/jcem-45-4-768.
43. Hardeland, R; Reiter, RJ; Poeggeler, B; Tan, DX The significance of the metabolism of the neurohormone melatonin: Antioxidative protection and formation of bioactive substances. *Neurosci. Biobehav. Rev.* **1993**, *17(3)*, 347–357. https://doi.org/10.1016/S0149-7634(05)80016-8.
44. Mannino, G; Pernici, C; Serio, G; Gentile, C; Bertea, CM Melatonin and Phytomelatonin: Chemistry, Biosynthesis, Metabolism, Distribution and Bioactivity in Plants and Animals-An Overview. *Int. J. Mol. Sci.* **2021**, *22(18)*, 9996. https://doi.org/10.3390/ijms22189996.
45. Kolar, J; Machackova, I; Illnerova, H; Prinsen, E; van Dongen, W; van Onckelen, H, Melatonin in Higher Plant Determined by Radioimmunoassay and Liquid Chromatography-Mass Spectrometry. *Biol. Rhythm Res.* **1995**, *26*, 406–409.
46. Hattori, A; Migitaka, H; Iigo, M; Itho, M; Yamamoto, K; Ohtani-Kaneko, R; Hara, M; Suzuki, T; Reiter, RJ, Identification of Melatonin in Plants and Its Effects on Plasma Melatonin Levels and Binding to Melatonin Receptors in Vertebrates. *Biochem. Mol. Biol. Int.* **1995**, *35(3)*, 627–634.
47. Dubbels, R; Reiter, RJ; Klenke, E; Goebel, A; Schnakenberg, E; Ehlers, C; Schiwara, HW; Schloot, W, Melatonin in Edible Plants Identified by Radioimmunoassay and by high performance liquid chromatography-mass spectrometry. *J. Pineal Res.* **1995**, *18(1)*, 28–31. https://doi.org/10.1111/j.1600-079X.1995.tb00136.x.
48. Murch, SJ; Erland, LAE A Systematic Review of Melatonin in Plants: An Example of Evolution of Literature. *Front. Plant Sci.* **2021**, *12*. https://doi.org/10.3389/fpls.2021.683047.
49. Back, K; Tan, DX; Reiter, RJ Melatonin Biosynthesis in Plants: Multiple Pathways Catalyze Tryptophan to Melatonin in the Cytoplasm or Chloroplasts. *J. Pineal Res.* **2016**, *61(4)*, 426–437. https://doi.org/10.1111/jpi.12364.
50. Tan, DX; Hardeland, R; Back, K; Manchester, LC; Alatorre-Jimenez, MA; Reiter, RJ On the Significance of an Alternate Pathway of Melatonin Synthesis via 5-Methoxytryptamine: Comparisons across Species. *J. Pineal Res.* **2016**, *61(1)*, 27–40. https://doi.org/10.1111/jpi.12336.
51. Kanwar, MK; Yu, J; Zhou, J Phytomelatonin: Recent advances and future prospects. *J. Pineal Res.* **2018**, *65(4)*, e12526. https://doi.org/10.1111/jpi.12526.
52. Mannino, G; Gentile, C; Ertani, A; Serio, G; Bertea, CM Anthocyanins: Biosynthesis, Distribution, Ecological Role, and Use of Biostimulants to Increase Their Content in Plant Foods-A Review. *Agriculture* **2021**, *11(3)*, 212. https://doi.org/10.3390/agriculture11030212.
53. Laurindo, LF; Simili, OAG; Araújo, AC; Guiguer, EL; Direito, R; Valenti, VE; de Oliveira, V; de Oliveira, JS; Yanaguizawa Junior, JL; Dias, JA; Maria, DA; Rici, REG; Bueno, MdS; Sloan, KP; Sloan, LA; Barbalho, SM Melatonin from Plants: Going Beyond Traditional Central Nervous System Targeting—A Comprehensive Review of Its Unusual Health Benefits. *Biology* **2025**, *14(2)*, 143.https://doi.org/10.3390/biology14020143.
54. Yin, B; Li, T; Li, Z; Dang, T; He, P Determination of Melatonin and Its Metabolites in Biological Fluids and Eggs Using High-Performance Liquid Chromatography with Fluorescence and Quadrupole-Orbitrap High-Resolution Mass Spectrometry. *Food Anal. Methods* **2016**, *9*, 1142-1149. https://doi.org/10.1007/s12161-015-0288-2.
55. Yang, S; Zheng, X; Xu, Y; Zhou, X Rapid determination of serum melatonin by ESI–MS–MS with direct sample injection. *J. Pharm. Biomed. Anal.* **2002**, *30(1)*, 781–790. https://doi.org/10.1016/S0731-7085(02)00387-4.
56. Riga, P; Medina, S; Garcia-Flores, LA; Gil-Izquierdo, A Melatonin content of pepper and tomato fruits: Effects of cultivar and solar radiation. *Food Chem.* **2014**, *156*, 347–352.

https://doi.org/10.1016/j.foodchem.2014.01.117.
57. Stege, PW; Sombra, LL; Messina, G; Martinez, LD; Silva, MF, Determination of melatonin in wine and plant extracts by capillary electrochromatography with immobilized carboxylic multi-walled carbon nanotubes as stationary phase. *Electrophoresis* **2010**, *31(13)*, 2242–2248. https://doi.org/10.1002/elps.200900782.
58. Eremia, SAV; Albu, C; Radu, GL; Ion, M Different Extraction Approaches for the Analysis of Melatonin from Cabernet Sauvignon and Feteasca Neagra Wines Using a Validated HPLC-FL Method. *Molecules* **2023**, *28(6)*, 2768. https://doi.org/10.3390/molecules28062768.
59. Meng, X; Li, Y; Li, S; Zhou, Y; Gan, R-Y; Xu, D-P; Li, H-B, Dietary Sources and Bioactivities of Melatonin. *Nutrients* **2017**, *9(4)*, 367. https://doi.org/10.3390/nu9040367.
60. Stürtz, M; Cerezo, AB; Cantos-Villar, E; Garcia-Parrilla, MC Determination of the melatonin content of different varieties of tomatoes (*Lycopersicon esculentum*) and strawberries (*Fragaria ananassa*), *Food Chem.* **2011**, *127(3)*, 1329–1334. https://doi.org/10.1016/j.foodchem.2011.01.093.
61. Brown, PN; Turi, CE; Shipley, PR; Murch, SJ Comparisons of large (*Vaccinium macrocarpon* Ait.) and small (*Vaccinium oxycoccos* L., *Vaccinium vitis-idaea* L.) cranberry in British Columbia by phytochemical determination, antioxidant potential, and metabolomic profiling with chemometric analysis. *Planta Med.* **2012**, *78(6)*, 630–640. DOI:10.1055/s-0031-1298239.
62. Schaper, C; Koethe, M; Braun, PG Comparison of melatonin concentrations in raw and processed cow's milk. *J. Food Saf. Food Qual. (JFSFQ)* **2015**, *66(5)*, 149-153. https://doi.org/10.2376/0003-925X-66-149.
63. Romanini, EB; Marchi Volpato, A; dos Santos, JS; de Santana, EHW; de Souza, CHB; Ludovico, A Melatonin concentration in cow's milk and sources of its variation, *J. Appl. Anim. Res.* **2019**, *47(1)*, 140–145. https://doi.org/10.1080/09712119.2019.1583570.
64. Rzepka-Migut, B; Paprocka, J Melatonin-Measurement Methods and the Factors Modifying the Results. A Systematic Review of the Literature. *Int. J. Environ. Res. Public Health* **2020**, *17(6)*, 1916. https://doi.org/10.3390/ijerph17061916.
65. Simeanu, D; Radu-Rusu, R-M; Macri, AM; Mierliță, D Animal Nutrition and Productions: Series II. *Agriculture* **2024**, *14(3)*, 448. https://doi.org/10.3390/agriculture14030448.
66. Council Directive 96/22/EC of 29.4.1996 concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of ß-agonists, and repealing Directives 81/602/EEC, 88/146/EEC and 88/299/EEC.
67. Peña-Delgado, V; Carvajal-Serna, M; Fondevila, M; Martín-Cabrejas, MA; Aguilera, Y; Álvarez-Rivera, G; Abecia, JA; Casao, A; Pérez-Pe, R Improvement of the Seminal Characteristics in Rams Using Agri-Food By-Products Rich in Phytomelatonin. *Animals* **2023**, *13(5)*, 905. https://doi.org/10.3390/ani13050905.
68. Andronachi, V-C; Simeanu, C; Matei, M; Radu-Rusu, R-M; Simeanu, D Melatonin: An Overview on the Synthesis Processes and on Its Multiple Bioactive Roles Played in Animals and Humans. *Agriculture* **2025**, *15(3)*, 273. https://doi.org/10.3390/agriculture15030273.
69. Andronachi, V-C; Matei, M; Radu-Rusu, R-M; Lup, FG; Simeanu, D impact of diets rich in dairy foods on circulating melatonin levels and sleep quality. *Sci. Study Res.: Chem. Chem. Eng. Biotechnol. Food Ind.* **2025**, *26(3)*, 377–400. DOI:10.29081/ChIBA.2025.639.
70. Tan, D-X; Manchester, LC; Hardeland, R; Lopez-Burillo, S; Mayo, JC; Sainz, RM; Reiter, RJ, Melatonin: A hormone, a tissue factor, an autocoid, a paracoid, and an antioxidant vitamin. *J. Pineal Res.* **2003**, *34(1)*, 75–78. https://doi.org/10.1034/j.1600-079X.2003.02111.x.
71. Sae-Teaw, M; Johns, J; Johns, NP; Subongkot, S Serum melatonin levels and antioxidant capacities after consumption of pineapple, orange, or banana by healthy male volunteers. *J. Pineal Res.* **2013**, *55(1)*, 58–64. https://doi.org/10.1111/jpi.12025.
72. Kolupaev, YE; Yemets, A; Yastreb, TO; Blume, Y Functional interaction of melatonin with gasotransmitters and ROS in plant adaptation to abiotic stresses. *Front. Plant Sci.* **2024**, *15*, 1505874. https://doi.org/10.3389/fpls.2024.1505874.
73. Minari, TP; Pisani, LP Melatonin supplementation: new insights into health and disease. *Sleep Breath* **2025**, *29*, 169. https://doi.org/10.1007/s11325-025-03331-1.

74. Vasey, C; McBride, J; Penta, K Circadian Rhythm Dysregulation and Restoration: The Role of Melatonin. *Nutrients* **2021**, *13(10)*, 3480. https://doi.org/10.3390/nu13103480.
75. Wang, Y; Xu, J; Gao, N; Lv, H; Sun, M; Zhang, P Controlled release of bilayer tablet comprising vitamin B6 rapid-release layer and melatonin sustained-release layer. *Pharm. Sci. Adv.* **2023**, *1(2), 100008*. https://doi.org/10.1016/j.pscia.2023.100008.
76. Kukula-Koch, W; Szwajgier, D; Gaweł-Bęben, K; Strzępek-Gomółka, M; Głowniak, K; Meissner, HO Is Phytomelatonin Complex Better Than Synthetic Melatonin? The Assessment of the Antiradical and Anti-Inflammatory Properties. *Molecules* **2021**, *26(19)*, 6087. https://doi.org/10.3390/molecules26196087.
77. Ali, MAA; Nasser, MA; Abdelhamid, AN; Ali, IAA; Saudy, HS; Hassan, KM Melatonin as a key factor for regulating and relieving abiotic stresses in harmony with Phytohormones in horticultural plants-A review. *J. Soil Sci. Plant Nutr.* **2024**, *24*, 54–73. https://doi.org/10.1007/s42729-023-01586-9.
78. Kamfar, WW; Khraiwesh, HM; Ilie, MO; Qadhi, AH; Azhar, WF; Ghafouri, KJ; Alhussain, MH; Aldairi, AF; AlShahrani, AM; Alghannam, AF; Abdulal, RH; Al-Slaihat, AH; Qutob, MS; Elrggal, ME; Ghaith, MM; Azzeh, FS Comprehensive review of melatonin as a promising nutritional and nutraceutical supplement. *Helyon* **2024**, *10(2)*, e24266.https://doi.org/10.1016/j.heliyon.2024.e24266.
79. Sejbuk, M; Siebieszuk, A; Witkowska, AM The Role of Gut Microbiome in Sleep Quality and Health: Dietary Strategies for Microbiota Support. *Nutrients* **2024**, *16(14)*. https://doi.org/10.3390/nu16142259.
80. Andersen, LPH; Werner, MU; Rosenkilde, MM; Harpsøe, NG; Fuglsang, H; Rosenberg, J; Gögenur, I Pharmacokinetics of oral and intravenous melatonin in healthy volunteers. *BMC Pharmacol. Toxicol.* **2016**, *17*, 8. https://doi.org/10.1186/s40360-016-0052-2.