Melatonin and Serotonin Content of the Main Sour Cherry Varieties and Commercially Produced Sour Cherry Concentrates

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ABSTRACT

Melatonin and serotonin are hormones, antioxidants and important phytochemicals that have immunoactive and neuroactive biological properties. Melatonin and serotonin have an effect against oxidation, ageing, stress and have an important function as a neuromodulator in the central nervous system. For the first time, melatonin and serotonin have been detected in the main sour cherry varieties grown in Turkey and sour cherry juice concentrates. Melatonin content of sour cherries was between 0.14 and 13.8 ng/mL. The highest melatonin content was found in the Montmorency variety. The serotonin content of sour cherry fruit samples was between 1.17 and 2.13 ng/mL. The highest serotonin content was found in the Kütahya variety. Pressing methods and supplement of vitamin C in juices did not affect the melatonin and serotonin content of samples. In addition, the serotonin content of sour cherry concentrate ranged between 1.07 and 1.38 ng/mL but no melatonin was detected in these samples.

Keywords : melatonin; serotonin; sour cherry; sour cherry concentrate; LC/MS/MS

Chemical compounds studied in this article :

Melatonin (PubChem CID : 896); Serotonin (PubChem CID : 5202); Acetonitrile (PubChem CID : 6342); Methanol (PubChem CID : 887); Formic acid (PubChem CID : 284); Hydrochloric acid (PubChem CID : 313)

1. INTRODUCTION

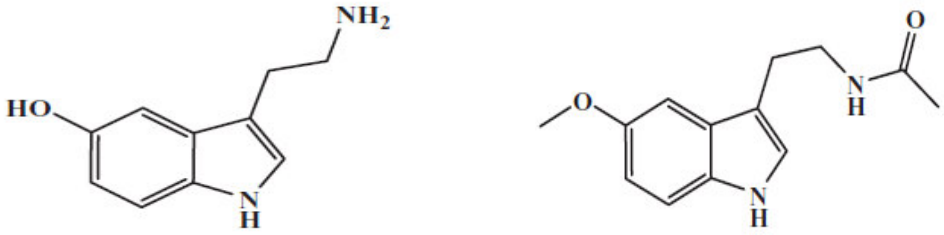
Epidemiological studies show that the consumption of fruits and vegetables reduces the risk of several degenerative and chronic diseases such as certain cancers and cardiovascular diseases (Doll 1990, Dragsted et al., 1993, Ames et al., 1993, Willett 1994, Burkhardt et al., 2001). This is attributed to their bioactive compounds. These compounds have the ability to protect against reactive oxygen species. Recently, attention has been focused on melatonin (N-acetyl-5-methoxytryptamine) and serotonin (5-hydroxytryptamine) as bioactive components (Figure 1). These molecules are indoleamines, which perform an important role in the regulation of the circadian rhythm (Srinivasan et al., 2010, Stürtz et al., 2011).

Melatonin is synthesized from the metabolism of L-tryptophan via serotonin. Melatonin is a neurohormone and is produced mainly by the pineal gland in vertebrates (Reiter 1991). It is a free radical scavenger and a broad-spectrum antioxidant, and also performs an important role in the regulation of the circadian rhythm and modulation of the sleep-wake cycle (Tan et al., 1993, Reiter 1991, Reiter 1998, Burkhardt et al., 2001, Hardeland et al., 2006, Baker and Driver, 2007, Gonzalez-Gomez et al., 2009, Srinivasan et al., 2010).

Serotonin is a heterocyclic amine that is a principal intermediate in melatonin biosynthesis. Serotonin plays an important role as a neurotransmitter, and also controls mood behaviours and body temperature (Gonzalez-Gomez et al., 2009).

There are several studies in literature on the melatonin and serotonin content of plants such as tomatoes, cucumbers, sunflowers seeds, bananas and pineapples (Cao et al., 2006, Dubbels et al., 1995, Manchester et al., 2000, Harumi and Matsushima, 2000, Van Tassel and O'Neill, 2001, Reiter et al., 2000; 2007, Hardeland and Poeggeler, 2003, Kolar and Machackova, 2005, Posmyk and Janas, 2009, Garcia-Parilla et al., 2009). Sour cherry is one fruit whose melatonin and serotonin content have been investigated (Kirakosyan et. al., 2009).

Although Turkey is one of the major producers of sour cherries, there is no study about their melatonin and serotonin content. In this study, the melatonin and serotonin content of the main cherry varieties grown in Turkey are investigated.



5- hydroxytryptamine
Serotonin

N-acetyl-5-methoxytryptamine
Melatonin

Figure 1. The chemical structure of 5- hydroxytryptamine (serotonin) ve N-acetyl-5-methoxytryptamine (melatonin)

2. MATERIAL AND METHOD

2.1. Materials

2.1.1. Sour cherry:

For this study, nine different (Montmorency, 1350, 1353, 1408, Oblacinska, Early Richmond, Rubin, Macar and Kütahya) sour cherry varieties were obtained in 2011. Montmorency, 1350, 1353, 1408, Oblacinska, Early Richmond and Rubin varieties were harvested from Yalova Atatürk Central Horticultural Research Institute; Macar and Kütahya varieties were harvested from the Ankara University Faculty of Agriculture, Research and Application Institute.

2.1.2. Sour Cherry Juice:

The Montmorency variety, which contains the maximum amount of melatonin, was selected to determine the effect of the process on the melatonin and serotonin content of the cherry. Sour cherry juices were obtained by different pressing methods (hot and cold pressing) with and without vitamin C supplement and then analysed.

2.1.3. Sour Cherry Juice Concentrate:

Commercially produced concentrated cherry juices (three different brands) in 2010 and 2011 were investigated for the presence of melatonin and serotonin.

2.1.4. Sample Preparation :

Sour cherry samples (except leaf, stem and seed) were homogenized using ultratorax (Heidolph Silent Crusher M) and 5 g of each sample was dried under nitrogen gas. The grounded samples were accurately weighed to 0.3 g and 10 mL aliquots of methanol were added to each sample. Extraction was completed by sonication in an ultrasonic bath (Falc) for 45 min in darkness. The supernatant was decanted and centrifuged (Hettich Zentrifugen) at 4500×g for 10 min. The resulting extract was filtered through a 0.45 µm filter (Millex syringe filter) and combined with the filtered supernatant of two subsequent methanol washes of the tissue residue. The entire extract was dried to complete drying with nitrogen gas in total darkness and resuspended in 1 mL methanol for injection. Finally, 50 µL of sample was injected into LC/MS/MS.

2.2. Method

2.2.1. Chemicals:

Certified analytical standards of melatonin and serotonin were used. Methanol (High-performance liquid chromatography (HPLC)-grade) used for the extraction, acetonitrile (HPLC purity), analytical-reagent grade formic acid and HCl were purchased from Sigma Aldrich (St. Louis, MO, USA). Ultra-pure water obtained by an ultra pure water-producing device (TKA Gmbh) was used for all analyses. Stock solutions of melatonin and serotonin (0.001 mg/mL) were prepared by dissolving the standards in ultra-pure water. To prevent degradation, stock and working solutions were stored at 4°C, avoiding direct light exposure.

2.2.2. Instrumentation :

All separations were performed with an Agilent Tecnology 6460 Triple Quadrupole LC/MS/MS. Agilent ChemStation software was used for data acquisition. SB-C18 (HT 2.1x50 mm, 1.8 µm) column was used for analysis.

2.2.3. LC/MS/MS analysis:

To determine the amount of melatonin and serotonin found in plants, the LC/MS/MS instrument is used in many studies (Boccalandro et. al. 2011, Rodriguez-Naranjo et. al. 2011, Stürtz M. et. al. 2011, Cao et. al. 2006). First, we compared these methods for the analysis of melatonin and serotonin and decided that the analysis method reported by Cao et al. (2006) was the most appropriate method for the LC/MS/MS instrument used for our study, and hence, we adopted this method.

3. RESULTS AND DISCUSSION

Melatonin and serotonin content of different varieties of sour cherry fruits are given in Table 1. According to the findings, the concentration of melatonin in sour cherry fruit samples, except Macar and Kütahya varieties, were between 0.14 and 13.8 ng/mL. No melatonin was detected in Macar and Kütahya varieties. Montmorency cherries contained the highest melatonin level (13.80 ng/mL). As seen in Table 1, the serotonin content of sour cherry fruit samples was between 1.17 and 2.13 ng/mL. Kütahya cherries contained the highest melatonin level (2.13 ng/mL).

According to the study of Burkhardt et al. (2001), the melatonin concentrations in Balaton tart cherries (2.06 ng/g) were six times lower than in Montmorency cherries. In addition, the highest levels (13.46 ng/g) were detected in the Montmorency variety. In the study by Kirakosyan et al. (2009), melatonin appears to be low in tart cherries, as compared to previously reported results by Burkhardt and co-workers (2001). In addition, Montmorency tart cherry contained about four times higher amounts of melatonin, as compared to the amounts found in Balaton tart cherries (Kirakosyan et. al 2009).

The study by Feldman and Lee (1985) reported that cherries had a low (<0.1 µg/g) serotonin concentration.

Melatonin and serotonin content of sour cherry juices that were obtained by different pressing methods (cold and hot pressing) with and without vitamin C supplement are given in Table 2.

As seen in Table 2, there were no differences between the amount of melatonin and serotonin in sour cherry juices obtained by cold and hot pressing and with/without vitamin C supplement. According to the findings, melatonin was also not detected in pressed juices obtained by cold and hot pressing, and the amount of serotonin was not affected by the pressing methods. In addition, a supplement of vitamin C in juices did not affect the melatonin and serotonin content of the samples.

The melatonin and serotonin amount of sour cherry juice concentrate samples that were processed industrially is given in Table 3.

According to the findings, the serotonin content of concentrate samples ranged between 1.07 and 1.38 ng/mL and no melatonin was detected in the concentrate samples. Similarly, Kirakosyan et al. (2009) also could not detect melatonin in concentrated samples, and their explanation for this observation is that melatonin is very unstable and may be degraded in these samples after processing and storage.

Table 1. Content of melatonin and serotonin in different varieties of sour cherry fruits. Values presented are mean ± SD (n=3).

Sour Cherry Variety	Melatonin (ng/mL)	Serotonin (ng/mL)
Oblacinska	0.14±0.040	1.32±0.046
Rubin	3.56±0.344	1.17±0.066
1350	3.35±0.312	1.18±0.053
1353	3.68±0.475	1.18±0.017
1408	5.39±0.128	1.30±0.044
Early Richmond	0.70±0.020	1.29±0.017
Montmorency	13.80±0.345	1.17±0.044
Macar	-	1.25±0.036
Kütahya	-	2.13±0.027

Table 2. Melatonin and serotonin content of sour cherry juices obtained by cold and hot pressing and with/without vitamin C supplement. Values presented are mean ± SD (n=3).

Pressing	Vitamin C Supplement (mg/kg)	Melatonin (ng/mL)	Serotonin (ng/mL)
Cold pres	-	-	1.08±0.044
Cold pres	400	-	1.08±0.027
Hot press*	-	-	1.16±0.027
Hot press*	400	-	1.05±0.027

*Before pressing samples were heated 90 ^{o}C.

Table 3. Melatonin and serotonin content in sour cherry juice concentrates supplied from market. Values presented are mean ± SD (n=3).

Sour Cherry Concentrates	Melatonin (ng/mL)	Serotonin (ng/mL)
A2010	-	1.24±0.046
A2011	-	1.28±0.044
B2010	-	1.33±0.035
B2011	-	1.38±0.035
C2010	-	1.08±0.049
C2011	-	1.07±0.046

4. CONCLUSION

A melatonin dose of 1.0–1.5 ng/g is sufficient to raise daytime blood melatonin levels to high values normally seen at night in humans (Vakkuri et al. 1985). In addition, for industrial purposes, it will be important to know the distribution of melatonin and serotonin for the preparation of natural dietary supplements. On the basis of the current research, sour cherries, which contain substantial amounts of melatonin and serotonin, may have a great number of health benefits and should be an important part of a healthy diet.

The purpose of this research was to determine the presence of melatonin and serotonin in the main sour cherry varieties grown in Turkey and the commercially produced sour cherry juice concentrates. This research is important to understand the importance of sour cherry in diet, the proper selection of appropriate raw materials for sour cherry juice production, improvement of the process and the development of a quality and healthy diet.

References

Ames, B. M.; Shigenaga, M. K.; Hagen, T. M. (1993). Oxidants, antioxidants and the degenerative diseases of aging. *Proceedings of the National Academy Science, U.S.A. 221*: 1256-1263.

Baker, F. C. and Driver H. S. (2007). Circadian rhythms, sleep and the menstrual cycle. *Sleep Medicine*, 8: 613-622.

Boccalandro, H.E., Gonzalez, C.V., Wunderlin D.A. and Silva, M.F. (2011). Melatonin levels, determined by LC-ESI-MS/MS, fluctuate during the day/night cycle in *Vitis vinifera cv* Malbec: evidence of its antioxidant role in fruits. *Journal of Pineal Research.* 51: 226-232.

Burkhardt, S., Tan, D. X., Manchester, L. C., Hardeland, R. and Reiter, R. J. (2001). Detection and quantification of the antioxidant melatonin in Montmorency and Balaton tart cherries (*Prunus cerasus*). *Journal of Agricultural and Food Chemistry*, 49: 4898-4902.

Cao, J., Murch S. J., O'Barien, R. and Saxena, P.K. (2006). Rapid method for accurate analysis of melatonin, serotonin and auxin in plant samples using liquid chromatography-tandem mass spectrometry. *Journal of Choromatography A*, 1134: 333-337.

Doll, R. (1990). An overview of the epidemiological evidence linking diet and cancer. *Proceedings of Nutrition Society, 49*: 119-131.

Dragsted, L. D.; Strube, M.; Larson, J. C. (1993). Cancerprotective factors in fruits and vegetables: biochemical and biological background. *Pharmacology Toxicology. 72*: 116-135.

Dubbels, S. R., Reiter, R. J., Klenke, E., Goeble, A., Schnakenberg, E., Ehlers, C., Schiwara, H. W., and Schloot, W. (1995). Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass-spectrometry. *Journal of Pineal Research.* 18: 28- 31.

Feldman, J. M. and Lee, E. M. (1985). Serotonin content of foods: effect on urinary excretion of 5-hydroxyindoleacetic acid. *American Journal of Clinical Nutrition,* 42 (4): 639-643

Garcia-Parilla, M. C., Cantos, E. and Troncoso, A. M. (2009). Analysis of melatonin in foods. *Journal of Food Composition and Analysis*, 22: 177-183.

Gonzalez-Gomez, M.L., Lazona, M., Fernandez-Leon, M. F., Ayuso, M. C., Bernalte, M. J. and Rodriguez, A. B. (2009). Detection and quantification of melatonin and serotonin in eight sweet cherry cultivars (Prunus avium L.). *European Food Research and Technology*., 229: 223-229.

Hardeland R. and Poeggeler B. (2003). Non-vertebrate melatonin. *Journal of Pineal Research*, 34: 233–241.

Hardeland, R., Pandi-Perumal, S. R. and Cardinali, D. P. (2006). Melatonin. *The International Journal of Biochemistry and Cell Biology*, 38: 313-316.

Harumi T and Matsushima S. (2000). Separation and assay methods for melatonin and its precursors. *Journal of Chromatography B,* 747: 95–110.

Kirakosyan, A., Seymour, E. M., Llanes, D. E. U., Kaufman, P. B. and Bolling, S. F. (2009). Chemical profile and antioxidant capacities of tart cherry products. *Food Chemistry*, 115: 20-25.

Kolar J, Machackova I. (2005). Melatonin in higher plants: occurrence and possible functions. *Journal of Pineal Research,* 39: 333–341.

Manchester, L. C., Tan, D. X., Reiter, R. J., Park, W., Monis, K., and Qi, W. (2000). High levels of melatonin in the seeds of edible plants: possible function in germ tissue protection. *Life Sci*ence, 67: 3023-3029.

Posmyk MM, Janas KM. (2009). Melatonin in plants. *Acta Physiologia Plantarum,* 31: 1–11.

Reiter, R.J., Tan D.X., Manchester, L.C., Simopoulos, A.P., Maldonado, M.D., Flores, L.J., Terron, M.P. (2007). Melatonin in edible plants (phytomelatonin): identification, concentrations, bioavailability and proposed functions. *World Review of Nutrition and Dietetics,* 97: 211–230.

Reiter, R. J. (1991). Pineal melatonin: cell biology of its synthesis and of its physiological interactions. *Endocrine Reviews, 10*: 151-180.

Reiter, R. J. (1998). Oxidative damage in the central nervous system: Protection by melatonin. Progress in Neurobiology, 56: 359-384.

Reiter, R. J., Tan, D. X., Osuna, C., Gitto, E. (2000). Actions of melatonin in the reduction of oxidative stress. *Journal of Biomedical Science*, 7: 444-458.

Rodriguez-Naranjo, M.I., Gil-Izquierdo, A., Troncoso, A.M., Cantos, E. and Garcia-Parrilla, M.C. (2011). Melatonin: a new bioactive compound in wine, *J. Compos. Anal*. 24:603-608.

Srinivasan, V., Singh, J., Pandi-Perumal, S. R., Brown, G. M., Spence, D. W., and Cardinali, D. P. (2010). Jet lag, circadian rhythm sleep disturbances, and depression: The role of melatonin and its analogs. *Advances in Therapy*, 27(11): 796–813.

Stürtz, M. Cerezo, A. B. Cantos-Villar, E. and Garcia-Parrilla, M. C. (2011). Determination of melatonin content of different varieties pf tomatoes (Lycopersicon esculentum) and strawberries (Fragaria ananassa). *Food Chemistry*, 127: 1329-1334.

Tan, D. X., Chen, L. D., Poeggeler, B., Manchester, L. C. and Reiter, R. J. (1993). Melatonin: a potent, endogenous hydroxyl radical scavenger. *Endocrine Journal* (Basingstoke, U.K.,), 1: 57-60.

Vakkuri, O., Leppaluoto, J. and Kauppila, A. (1985). Oral administration and distribution of melatonin in human serum, saliva and urine. *Life Science*, 37: 489-495.

Van Tassel, D.L., O'Neill, S.D. (2001). Putative regulatory molecules in plants: evaluating melatonin. *Journal of Pineal Research*, 31: 1–7.

Willett, C. W. (1994). Micronutrients and cancer risk. *American Journal of Clinical Nutrition, 59*: 162-165.