

Melatonin is a phytochemical in olive oil

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Abstract

Given the numerous observations regarding the positive effects of olive oil consumption and the presence of melatonin in edible plants, we addressed for the first time the question of melatonin determination in virgin olive oil. All the extra virgin olive oil registered designation of origins from Spain and commercial samples of refined olive and sunflower oil were used. Immunoprecipitation and ELISA were combined for melatonin determination. Melatonin is present in olive oil at higher levels in extra virgin olive oil than in refined olive or sunflower oil samples. We concluded that melatonin is part of the phytochemical profile of the olive oil. Particularly, extra virgin olive oil had almost double the melatonin contents of the other refined oils analysed. Thus, melatonin may account for the healthy effects of the Mediterranean diet in which olive oil is the main source of fat.

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1. Introduction

Mediterranean diet has long been known for contributing to a healthy life and preventing disease (Alexandratos, 2006; Serra-Majem, Roman, & Estruch, 2006). Virgin olive oil constitutes one of the characteristics of this antique diet (Stark & Madar, 2002). Numerous studies have demonstrated that it has relevant properties which convey important benefits for individual health, when taken as part of a habitual diet (Perez-Jimenez, 2005).

Virgin olive oil contains trace amounts of a wide variety of substances such as different polyphenols and tocopherols, which have proven to exert beneficial effects on health, such as reduction of risk of coronary heart disease (CHD) (Giugliano & Esposito, 2005; Perona, Cabello-Moruno, & Ruiz-Gutierrez, 2006), prevention or reduction of risk of

certain cancers (Perez-Jimenez, 2005; Wahle, Caruso, Ochoa, & Quiles, 2004), as well as immunomodulatory effects and the ability to partially reverse some inflammatory conditions (Bogani, Galli, Villa, & Visioli, 2006; de la Puerta, Martinez-Dominguez, & Ruiz-Gutierrez, 2000; Miles, Zoubouli, & Calder, 2005). Due to the presence of these minor constituents and the health benefits exerted by them, virgin olive oil is considered as a functional food (Stark & Madar, 2002), since it complies with all the requirements for this nutritional definition (Roberfroid, 2002).

To further support the functional value of virgin olive oil, we have determined whether melatonin is present in it. Melatonin, an indolamine produced by the pineal gland, and which until a decade ago was thought to be only present in the animal kingdom, has also been detected in vegetable tissues (Reiter, Tan, Burkhardt, & Manchester, 2001). Moreover, its presence in edible plants, such as bananas, cherries, white sprouts, and cucumbers (Reiter & Tan,

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2002) has evidenced that there is a correlation between dietary vegetable intake and blood levels of melatonin (Reiter, Manchester, & Tan, 2005; Reiter et al., 2001), demonstrating that this molecule is well absorbed and it readily raises blood plasma concentration of melatonin (Nagata, Nagao, Shibuya, Kashiki, & Shimizu, 2005; Reiter et al., 2001). This indolamine has long proven to be a radical-scavenger and a powerful antioxidant which also exerts other physiological functions (e.g. immunomodulatory and anti-inflammatory) (Reiter, 2003). In the present study we aim to detect the presence of significant amounts of melatonin in virgin olive oil and to compare these amounts with any that might also be present in refined olive oil and sunflower oil. Thus, since melatonin has long proved to confer beneficial effects on health, as mentioned above, the presence of this indolamine in virgin olive oil would constitute an additional functional value of this Mediterranean food.

To our knowledge, this is the first time melatonin has been determined in virgin olive oil. For this purpose, we have performed an extraction of melatonin from this oil and have measured its content. The result was partly validated by performing an immunoprecipitation of melatonin from the same oil samples as were being tested.

2. Materials and methods

2.1. Chemicals

Organic solvents were from Panreac Chemical Company (Barcelona, Spain). Extra virgin olive oils, refined olive oil and sunseed oil were commercially available. Control quality requirements were fulfilled under national and EU olive oil registered designations of origin (RD 308/1983; EC 2472/97; EC 1176/2003).

2.2. Melatonin extraction from extra virgin olive oil and measurements

A combined method was used for melatonin extraction, based on sequential methanol and chloroform extraction. A 500 μ l volume of extra virgin olive oil was mixed with an equal volume of methanol for 20 min at room temperature in a rotary shaker. The homogenates were centrifuged at 10,000g at 4 °C for 20 min. The supernatants were collected into new Eppendorf tubes and evaporated under vacuum for 30 min. Residues were resuspended in 500 μ l of PBS (BioWhittaker, Vervier, Belgium) and mixed with 1 ml of chloroform for 20 min at room temperature in a rotary shaker. After centrifugation at 1000g at 4 °C for 5 min, the water phase was discarded and the organic phase was evaporated under vacuum. Residues were dissolved in deionised water and melatonin levels were determined by a competitive enzyme immunoassay kit (Melatonin ELISA, IBL-Hamburg, Germany) according to the manufacturer's instructions. Briefly, samples, standards and controls were measured in duplicate using 96 well microtitre plates coated with captured antibody goat anti-rabbit Ig. Each microtitre

plate was filled, either with 50 μ l of blank reagent, calibrators, extracted samples or standard solutions containing 0, 3, 10, 30, 100 and 300 pg/ml of melatonin. After that, 50 μ l of melatonin biotin and 50 μ l of rabbit-antiserum were added into each well, shaken carefully, sealed with adhesive foil and incubated over night (14–20 h) at 2–8 °C. After washing, 150 μ l of anti-biotin conjugate to alkaline phosphatase were added to each well and these were incubated for 2 h at RT. The reaction was developed using *p*-nitrophenyl phosphate and optical densities were determined at 450 nm in an automatic microplate reader. The sensitivity of the melatonin assay was 3.0 pg/ml. Both the intra- and inter-assay coefficients of variation (CV) were less than 10%.

2.3. Melatonin immunoprecipitation

Extracted samples were incubated in PBS with anti-melatonin antibody (Biogenesis, Poole, England) (5 μ g/ml) or Rabbit IgG control Ab (Sigma–Aldrich, St. Louis, MO, USA) for 1 h at 4 °C in a rotary shaker. Samples were incubated with protein A-Sepharose beads (Amersham Biosciences) for 30 min at 4 °C. After PBS washing, the immunoprecipitated complexes were discarded and the supernatants recovered in ELISA buffer for melatonin measurement.

3. Results and discussion

The melatonin extraction, based on the sequential methanol and chloroform extraction used in this report, was confirmed by immunoprecipitation (Fig. 1). The melatonin values in the case of the extra virgin olive oil samples were roughly almost double those of both the refined olive and sunflower oil samples (Table 1). Only the samples from the D.O. Bajo Aragón showed melatonin levels (71.5 ± 15.1 pg/ml) similar to one of the refined olive oils analysed (74.8 ± 6.92 pg/ml). Generally, the higher melatonin levels are in those samples with no heat-treatment or chemical processing, i.e. extra virgin olive oil.

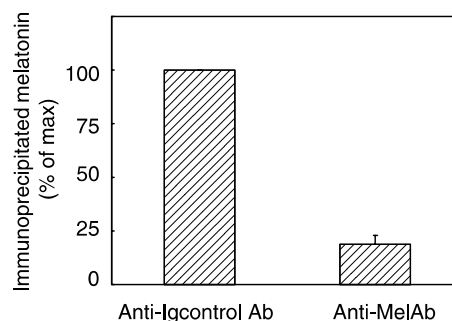


Fig. 1. Determination of melatonin content by ELISA after immunoprecipitation. To validate the specificity of the ELISA method, melatonin was determined after immunoprecipitation with anti-melatonin antibodies (Mel I.P.) or isotype control antibodies (Control I.P.). The experiment is representative of another three.

Table 1

Melatonin values (pg/ml) in refined olive oil and extra virgin olive oil registered designations of origin (D.O.)

Oil sample	Mean \pm S.E.M
D.O. Sierra Mágina	107 \pm 36.2
D.O. Siurana	95 \pm 20.3
D.O. Bajo Aragón	71 \pm 15.1
D.O. Montes de Toledo	108 \pm 9.76
D.O. Baena	119 \pm 2.93
D.O. Sierra de Segura	89 \pm 2.93
D.O. Les Garrigues	98 \pm 3.89
D.O. Toscano	108 \pm 17.3
Refined olive oil sample 1	53 \pm 5.6
Refined olive oil sample 2	75 \pm 6.92
Refined sunflower oil sample	50 \pm 12.2

Epidemiological studies provide evidence to support the concept that a Mediterranean diet has a beneficial influence on diseases associated with oxidative damage, such as coronary heart disease, cancer, cardiovascular and neurodegenerative diseases (Alexandratos, 2006). A common key in the dietary habits of Mediterranean populations is the consumption of olive oil (Gerber, 2006; Scali, Richard, & Gerber, 2001). Growing experimental evidence demonstrates that olive oil phytochemicals, both *in vitro* and *in vivo*, are potent bioactive compounds responsible for the functional properties of olive oil, particularly extra virgin olive oil (Stark & Madar, 2002). Bearing in mind the antioxidant capacity of melatonin (Reiter, 2003), its presence in edible plants (Hattori et al., 1995; Reiter et al., 2005; Reiter & Tan, 2002) and its role in key physiological responses with therapeutic implications (Carrillo-Vico et al., 2006; Pandi-Perumal et al., 2006), we wanted to determine whether melatonin was present in olive oil. The highest levels of melatonin in plants are in Chinese herbal products (Chen et al., 2003; Murch, Simmons, & Saxena, 1997). It is notable that purslane (*Portulaca oleracea*), which is the most important source of terrestrial α -linolenic acid in edible wild plants (Simopoulos & Salem, 1986), is also rich in melatonin, as has recently been reported (Simopoulos, Tan, Manchester, & Reiter, 2005). Moreover, it is eaten as a leaf vegetable as part of the Mediterranean diet (Simopoulos, 2001). Unfortunately, there is no such systematic evaluation of melatonin levels in plant materials, the walnut being the first common tree nut in which melatonin has been studied from a nutritional perspective (Reiter et al., 2005).

The role of melatonin in plants remains to be elucidated. However, experimental data indicate a role similar to the auxin molecule (Hernandez-Ruiz, Cano, & Arnao, 2004, 2005) and a protective effect in germ cells has been proposed (Manchester et al., 2000).

Recent studies have demonstrated that dietary combinations of phytochemicals show enhancing health benefits by additive and synergistic effects (Jacobs & Steffen, 2003; Liu, 2004). It is possible that melatonin and other new bioactive compounds, previously found in other physiological contexts, account for the positive effects of Mediterranean die-

tary habits. This is an additional argument for the adoption of healthy and diversified eating habits rather than the use of dietary supplements.

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