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Determination of serotonin in nuts and nut containing products by liquid chromatography tandem mass spectrometry

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ABSTRACT

An ultra performance liquid chromatography-mass spectrometry (UPLC-MS/MS) method was developed for the determination of serotonin in raw and roasted nuts (almond, Brazil nut, cashew, chestnut, coconut, hazelnut, Macadamia nut, pecan, peanut, pine nut, pistachio and walnut) as well as nut products (nut containing snack bars, chocolate and spreads) for the first time. Water extraction without prior defatting was performed to leach serotonin from complex matrices of nuts. Mean recoveries ranged from 64.2 ± 9.6 to 94.7 ± 20.1%. Limit of detection and limit of quantification were between 0.4 and 2.3 and 1.0–7.4 ng/g, respectively. Repeatability and reproducibility values were below 2%. Serotonin content of nuts ranged from 0.05 ± 0.01 (pine nut) to 155 ± 57.0 μg/g (walnut) in raw nuts while it was between 0.03 ± 0.00 (Macadamia nut) and 15.3 ± 1.27 μg/g (pecan) in roasted nuts. Serotonin in nut products was found to range from 0.09 ± 0.00 to 8.99 ± 0.92 μg/g, depending on the nuts they contain.

1. Introduction

Serotonin, known also as 5-hydroxytryptamine, is a neurotransmitter derived through serotonin pathway from tryptophan (Guillen-Casla, Rosales-Conrado, Leon-Gonzalez, Perez-Arribas & Polo-Diez, 2012). It is found in the serotonergic neurons in the central nervous system and mostly synthesized in the enterochromaffin cells of the gastrointestinal tract (Watanabe et al., 2010). Regulation of appetite, anxiety, sleep, mood and blood pressure are some of the important roles of serotonin (Voigt & Fink, 2015; Young & Leyton, 2002; Leonard 1996; Watts, Morrison, Davis & Barman, 2012). Decreasing levels or depletion of its synthesis might cause several diseases, including depression, obesity and schizophrenia (Meltzer et al., 1998; Spadaro, Naug, Du Toit, Donner & Colson, 2015; Bleich, Brown, Kahn, & van Praag, 1988; Bleich, Brown, Kahn, & van Praag, 1988).

Serotonin is found not only in humans but also in different parts of the plants such as leaves, roots, flowers, fruits and seeds (Grosse, 1982; Kang & Back, 2006; Kang, Kang, Lee & Back, 2007). Its synthesis depends on two enzymatic stages. Initially, tryptophan is converted to tryptamine by tryptophan decarboxylase, and then tryptamine is hydroxylated to form serotonin by tryptamine 5-hydroxylase (Kang et al., 2007). Serotonin has many physiological and developmental functions in plants, such as production and growth of shoot, germination of pollen, protection of germ tissues and decreasing of leaf senescence (Erland, Murch, Reiter & Saxena, 2015). Moreover, studies have shown that synthesis of serotonin in plants is related to a detoxification mechanism, which protects the plant from toxic ammonia concentrations (Schroder, Abele, Gohr, Stuhlfauth-Roisch & Grosse, 1999).

Edible nuts, some of which are almond, Brazil nut, cashew, hazelnut, Macadamia nut, pecan, peanut, pine nut, pistachio, and walnut, are globally popular and commercially important. They are usually consumed either raw or roasted with or without their skins. Additionally, they are important ingredients of processed foods, especially bakery and confectionery products. There are many studies showing that nuts are rich sources of unsaturated fatty acids, and many fat-soluble bioactive compounds like tocopherols, tocotrienols, and phytosterols as well as phenolic compounds (Kornsteiner, Wagner & Elmadfa, 2006; Alasalvar & Pelvan, 2011; Maguire, O'Sullivan, Galvin, O'Connor & O'Brien, 2004). Benefits of nut consumption on risk of coronary heart disease, diabetes, and cardiovascular risk factors have been attributed to this unique composition rich in bioactive nutrients and phytochemicals (Ros, 2010). Although health-related compounds of nuts have been comprehensively reviewed in recent years (Alasalvar & Bolling, 2015; Chang, Alasalvar, Bolling & Shahidi, 2016), there is limited information about the profile of neuroactive compounds found in nuts. The studies in the literature are only restricted with hazelnut and walnut varieties (Feldman & Lee, 1985; Lavizzari, Veciana-Nogues, Bover-Cid, Marine-Font & Vidal-Carou, 2006; Tapia et al., 2013).

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However, no systematic and comprehensive study about the serotonin content of raw and roasted edible nuts has been performed.

There are different procedures in the literature for the extraction of serotonin from various food matrices. Serotonin and other eleven biogenically active amines from vegetal origins were extracted by 0.6 M perchloric acid after homogenization (Lavizzari et al., 2006). Perchloric acid was also used to get serotonin from biological materials (Pussard, Guigueno, Adam & Giudicelli, 1996). In the extraction of serotonin from coffee, trichloroacetic acid was used which was followed by an ion-pair clean-up procedure and a derivatization step (Casal, Oliveira & Ferreira, 2002). Serotonin was extracted from freeze-dried vegetable tissues by using methanol and the extracts were cleaned-up after passing through C18 cartridges (Ly et al., 2008). To date, capillary electrophoresis, spectrophotometry, immunoassay, amperometric sensors and liquid chromatography have been used to determine serotonin in biological materials (Benturquia et al., 2005; Chauveau, Fert, Morel & Delaage, 1991; Pussard et al., 1996; Ferry, Gifu, Sandu, Denoroy & Parrot, 2014). Determination of serotonin in foods have been generally performed by using high-performance liquid chromatography (HPLC) methods with ultraviolet, fluorescence and mass spectrometry detection (Mazzucco et al., 2010; Adao & Gloria, 2005, Tapia et al., 2013; Gonzalez-Gomez et al., 2009).

Reliable analysis of serotonin in nuts and nut products is important from many aspects as discussed above. The complex oily matrix of nut and nut products could probably be one of the major challenges in the extraction as well as the analysis. Therefore, this study aimed to develop an analytical method for precise and accurate quantitation of serotonin in various raw and roasted edible nuts by means of liquid chromatography tandem mass spectrometry.

2. Material and methods

2.1. Chemicals and consumables

Acetonitrile (HPLC grade), serotonin hydrochloride (≥98%), L-theanine (≥98%) and ammonium formate (LC-MS grade) were obtained from Sigma-Aldrich (Steinheim, Germany). Syringe filters (nylon, 0.45 µm) and Atlantis HILIC Silica column (150 × 2.1 mm i.d., 3 µm) were supplied by Waters Corp. (Milford, MA, USA). Deionized water (5.6 µS/m) was used throughout the experiments.

2.2. Samples and sample preparation

Raw (almond, cashew, chestnut, coconut (dried), hazelnut, peanut, pecan, pine nut, pistachio and walnut) and roasted nuts (almond, Brazil nut, cashew, hazelnut, Macadamia nut, peanut, pecan, pistachio), and food products containing different nuts were purchased from several local markets in Turkey. One sample from each raw and roasted nut was assessed for the analysis. Selected food products containing various nuts were snack bars, chocolate, and spreads which were also supplied from local markets in Turkey. The ingredients found in these food products were presented in Table 1.

Nuts and nut products were ground homogenously until to obtain a paste by using a lab-scale grinder before extraction. In order to understand the effect of oil on extraction efficiency of serotonin, a defatting procedure was applied to ground hazelnut sample. A portion of the sample was mixed with hexane (1:20, w/v) and vortexed for 10 min. The oil-hexane mixture was removed after centrifugation (4650*g* for 3 min). Then, this procedure was repeated for five times and the ground defatted hazelnut was dried for 24 h at room temperature.

2.3. Extraction of serotonin

Water alone, mixture of methanol:water (1:1, v/v), and mixture of acetonitrile:water (1:1, v/v) were tested as extraction solvents. Roasted hazelnut was used to understand the effect of different solvents on serotonin extraction. Ground samples (1 g) were extracted with one of these solvents in three consecutive stages (5, 2.5, 2.5 mL). After vortexing (3 min) and centrifugation (4650*g* for 3 min) in each stage, supernatants were collected in a test tube and combined. Combined extract (0.6 mL) was mixed with acetonitrile (0.9 mL) in another tube to make final acetonitrile concentration 60% (v/v). Then, a centrifugation step (4650*g* for 3 min) was applied to precipitate co-extracted constituents. A theanine solution at a concentration of 2.5 µg/g was prepared in acetonitrile-water (60:40, v/v) as an internal standard. Clear supernatants (0.9 mL) were mixed with the internal standard (0.1 mL) and filtered through a 0.45 µm syringe filter into an autosampler vial prior to analysis. The extraction procedure was performed twice for each sample.

2.4. LC-MS/MS analysis of serotonin

Serotonin was determined by an ultra high-performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS) (Waters Corp., Milford, MA, USA). Chromatographic separation was performed on Atlantis HILIC column (150 × 2.1 mm i.d., 3 µm) by using a mixture of 2.5 mM ammonium formate in water (A) and 2.5 mM ammonium formate in acetonitrile-water mixture (B) (90:10, v/v). An isocratic elution of the A and B solvents (10:90, v/v) was carried out at a flow rate of 0.3 mL/min at 40 °C. The injection volume was 3 µL. Total chromatographic run time was 12 min. The electrospray source had the following settings: capillary voltage of 0.8 kV; cone voltage of 20 V; extractor voltage of 4 V; source temperature of 120 °C; desolvation temperature of 300 °C; desolvation gas (N_2) flow of 700 L/h and cone gas (N_2) flow of 50 L/h. MS source was used in the positive ionization mode and serotonin was identified by multiple reaction monitoring (MRM). The flow rate of the collision gas (Ar) was 0.21 mL/min. The molecular ion and fragment ion of serotonin were *m/z* 177 and *m/z* 160, respectively. The cone voltage and collision energy were 15 V and 10 V, respectively. The molecular ion of internal standard, theanine, was *m/z* 175 and its fragment ions were *m/z* 46, 129 and 158. The cone voltages and collision energies for theanine were found as 20 V and 12 V (each transition), respectively. The quantifier ions were *m/z* 160 and 158 for serotonin and theanine, respectively.

Table 1
Concentration of serotonin in nut products and ingredients declared on the labels.

Nut products	Ingredients	Serotonin (µg/g)
Product 1	Hazelnut, sugar, vanilla	0.76 ± 0.04
Product 2	Hazelnut, honey, vegetable oil, non-fat milk powder, vanillin	0.09 ± 0.00
Product 3	Hazelnut, glucose syrup, raisin, sultana, oats, puffed raise, sugar, honey, vegetable oil, maltodextrin, soya lecithin	0.32 ± 0.05
Product 4	Pistachio, sugar, cocoa butter, milk powder, cocoa, sunflower lecithin, vanillin	0.60 ± 0.07
Product 5	Pistachio, glucose syrup, raisin, sultana, oats, puffed raise, sugar, honey, vegetable oil, maltodextrin, soya lecithin	0.32 ± 0.11
Product 6	Hazelnut, peanut, almond, oat, olive oil, wheat grain, dates, apple juice concentrate, butter, salt	0.39 ± 0.03
Product 7	Date, cashew, almond, buckwheat flour, hazelnut, goji berry, blueberry, cranberry, walnut, Himalayan salt	8.99 ± 0.92

Data are expressed as mean ± standard deviation. All analytical measurements were performed duplicate from two separate extracts of the sample.