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Simultaneous analysis of serotonin, tryptophan and tryptamine levels in common fresh fruits and vegetables in Japan using fluorescence HPLC

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ABSTRACT

Serotonin is a monoamine neurotransmitter that functions as a hormone, neuromodulator, and psychoactive agent. Peripheral serotonin is involved in lipid and glucose metabolism, and it may prevent the development of metabolic syndrome. Serotonin is biosynthesized from tryptophan and can be obtained from certain foods. However, information regarding the dietary intake of serotonin is scarce. In this study, we determined the levels of serotonin and its precursors, tryptophan and tryptamine, in 38 fruits and vegetables commonly consumed in Japan using HPLC – fluorescence detection. The highest serotonin levels were found in cherry tomato (12.44 ± 0.19 μg/g of fresh weight), and the highest tryptophan and tryptamine levels were detected in potato and kiwi (64.47 ± 1.54 and 6.38 ± 1.24 μg/g of fresh weight, respectively). Thus, these foods may represent excellent dietary sources of serotonin and may be used to develop effective therapeutic strategies.

1. Introduction

Serotonin (also known as 5-hydroxytryptamine [5-HT]) is a well-known neurotransmitter in mammals. Serotonin is also widely distributed in plants (Pelagio-Flores, Ortíz-Castro, Méndez-Bravo, Macías-Rodríguez & López-Bucio, 2011). It was first discovered as a vasoconstrictor that is released from platelets during blood coagulation and later, as a monoamine neurotransmitter in the brain (Mück-Seler and Pivac, 2011). The neurotransmitter activity of serotonin in the central nervous system affects appetite, sleep, anxiety, sexual behaviour, mood, and social interactions (Tecott et al., 1995; Leonard, 1996; Loer and Kenyon, 1993; Young and Leyton, 2002). Nearly 2% of the body's serotonin is found within the central nervous system, while the remaining 98% exists peripherally. Peripheral serotonin is synthesized in the enterochromaffin cells of the gastrointestinal tract, where it acts as a peripheral hormone (Watanabe et al., 2010).

In the human system, serotonin is produced from tryptophan via two steps. Initially, tryptophan is converted to 5-hydroxytryptophan (5-HW) by the action of tryptophan hydroxylase, (EC 1.14.16.4). 5-HW is then decarboxylated by aromatic L-amino acid decarboxylase (EC 4.1.1.28) to form serotonin, a reaction in which tryptophan hydroxylase acts as the rate-limiting enzyme (Veenstra-Vander Weele, Anderson & Cook, 2000).

In plants, serotonin is synthesized via a different pathway, where tryptophan is first converted into tryptamine by tryptophan decarboxylase (TDC; EC 4.1.1.28). Then, tryptamine is converted to serotonin by the action of tryptamine 5-hydroxylase (Schröder, Abele, Gohr, Stuhlfauth-Roisch & Grosse, 1999).

Current methods for the estimation of biogenic amines include thin layer chromatography, radioimmunoassay (RIA), enzyme-linked immunosorbent assay, high-performance liquid chromatography with ultraviolet detection (HPLC – UV), and high-performance liquid chromatography with mass spectrometry (HPLC – MS). However, HPLC with fluorescence detection is still the method of choice because of its high accuracy and sensitivity, relatively low cost of analysis, simple sample pretreatment requirements, and ease-of-use in comparison with HPLC – UV (Wang and Chan, 2014). Serotonin content has been estimated for a variety of plants by using RIA (Feldman and Lee, 1985) and HPLC – UV (Ly et al., 2008); however, to the best of our knowledge, the levels of serotonin and its precursors, tryptophan and tryptamine, in conventional foodstuffs has not been reported. Therefore, the purpose of this study was to precisely determine the levels of serotonin and its precursors, tryptophan and tryptamine, in commonly consumed foods in Japan using the

Abbreviations: 5-HW, 5-hydroxytryptophan; 5-HT, 5-hydroxytryptamine; TDC, tryptophan decarboxylase

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HPLC – fluorescence detection method. In the context of promoting human health, our findings may be used to construct a database for serotonin content in common foods to estimate their potential contribution to dietary requirements.

2. Materials and methods

2.1. Chemicals

All chemicals used were of analytical grade. Serotonin, tryptamine, L-tryptophan, and 5-HW were supplied by Sigma (St. Louis, MO, USA). Perchloric acid (PCA) and formic acid were supplied by Wako Pure Chemical Industries (Osaka, Japan). Acetonitrile and water (HPLC grade) were supplied by Kanto Chemical Co. (Tokyo, Japan).

2.2. Sample preparation

Fresh fruits and vegetables were purchased from local markets at different times. Once purchased, the vegetables and fruits were washed with tap water to eliminate any unwanted debris or other substances. For single food analysis, eight uniform fruits or vegetables were cut, pooled, and thoroughly mixed using a blender. After blending, three 1.0-g aliquots were taken for further analysis. These aliquots were mixed with 50.0 μL of an internal standard (5-HW; 1.0 mg/mL) and 4.0 mL of 0.2 M PCA, and homogenized further by using a polytron homogenizer. A 1.0-mL aliquot was then taken from each sample and centrifuged at 12,000 × *g* for 10 min. After centrifugation, 800 μL of supernatant was stored at −30 °C until further analysis. The entire extraction procedure was carried out protected from light or under dim light and at room temperature. The extracted samples were centrifuged and analyzed immediately after extraction. Sample extraction was optimized by using 0.2 M PCA acid, which was obtained from a commercially available ELISA kit for serotonin extraction in biological samples (Immunotech, Marseille, France). Stock solutions of the standard analytes, at a concentration of 500 mg/mL, were prepared using 0.2 M PCA solution. Aliquoted stock solutions were then stored at −30 °C. Extracted samples and standard aliquots remained stable for at least 6 months. Standard working solutions were prepared using Milli-Q® water prior to each use.

2.3. Fluorescence-detection HPLC analysis of serotonin, tryptophan, and tryptamine

A 40.0-μL aliquot of each sample was diluted (10 ×) with the initial mobile phase (10 mM $HCOONH_4$, pH 3.4). Separations were performed using the following gradient profiles: 0–5 min, 10 mM $HCOONH_4$, pH 3.4; 5–15 min, linear gradient of 0–25% acetonitrile in 10 mM $HCOONH_4$, pH 3.4, in an Atlantis C18 column (4.6 × 50 mm, 5 μm, Waters, Milford, MA, USA) at 30 °C with a flow rate of 1.0 mL/min. The total HPLC run time for each sample was 32 min, and the injection volume was 10 μL. Fluorescence was detected at excitation and emission wavelengths of 300 nm and 355 nm, respectively. Peak identifications were made by comparing the retention times. The retention times under the established conditions were 7.75 ± 0.02 min, 9.23 ± 0.07 min, 10.93 ± 0.04 min, and 12.10 ± 0.05 min for 5-hydroxytryptophan, serotonin, tryptophan, and tryptamine respectively (Fig. 1). The resolution of the chromatogram was more than 1, and the peak symmetry was close to 1 during standard analyte analysis.

2.4. Validation of the method

The performance of the assay was evaluated using several figures of merit: limits of detection (LOD) and quantification (LOQ), linearity, linear range, inter-day repeatability, and recovery percentages. LOD is the lowest analyte concentration that can be reliably distinguished from background noise. LOQ was determined from the LOD as follows: LOQ=(10 × LOD)/3 (FDA, 1994). The LOD for serotonin, tryptophan, and tryptamine were 0.00156, 0.1, and 0.0075, respectively, while the LOQ was 0.0052, 0.34, and 0.025 μg/mL, respectively. The analytical curves were constructed by calculating the regression line, and the linearity was defined by the coefficient of correlation (R^2). All analytical curves were linear within the studied range, with an R^2 value of > 0.99. The linear ranges of the calibration curves were 0.0625–8.0 for 5-HW, 0.125–4.0 for serotonin, 0.25–32.0 for tryptophan, and 0.5–16.0 μg/mL for tryptamine.

Inter-day variations were determined by performing HPLC using a freshly prepared standard mixture containing 4 μg/mL of each component repeatedly for three days. Using this analytical procedure, the relative standard deviation was found to be less than 2%. The extraction efficiency was calculated by adding known amounts of 5-HW (0.5, 1.0, and 10 mg/mL) to 1.0 g of tomato extract. The peak heights of 5-HW from spiked tomato samples were compared with those obtained after direct injection of 0.5, 1.0, and 10 μg/mL 5-HW solutions. The recovery percentages of serotonin, tryptophan, tryptamine, and 5-HW were 92.13%, 101.61%, 98.26%, and 100.37%, respectively, indicating that the HPLC – fluorescence detection method was sufficiently precise, accurate, and sensitive for simultaneous quantification. Sample concentrations were calculated by comparing the peak area of the sample with those of the standards and dilution factors using the initial mobile phase and PCA.

3. Results and discussion

3.1 Analysis of serotonin, tryptophan, and tryptamine levels in fruits

Serotonin levels in the fruits tested ranged from 0.05–9.52 μg/g of fresh weight (Table 1). The highest serotonin levels were detected in kiwi and banana. Pineapple also contained relatively high levels of serotonin. However, the serotonin levels detected in pineapple and banana in the current study were lower than the levels reported in a previous study (Feldman and Lee, 1985). The distribution of serotonin in fruits and plant tissues is not uniform, and the levels are known to increase as the fruits ripen in many species, including tomato, although the inverse is true for pineapple (Ramakrishna, Giridhar & Ravishankar, 2011). Although kiwi

Table 1
Serotonin, tryptophan, and tryptamine levels in fruits.

Fruit name:	Serotonin	Tryptophan	Tryptamine
Kiwi (*Actinidia deliciosa*)	9.52 ± 0.62	3.32 ± 0.13	6.38 ± 1.24
Banana (*Musa acuminata*)	9.48 ± 0.09	26.15 ± 0.37	0.959 ± 0.28
Pineapple *(Ananas comosus)*	9.11 ± 0.13	19.83 ± 4.08	1.24 ± 0.15
Avocado (*Persea americana*)	5.37 ± 0.41	16.27 ± 3.49	1.70 ± 0.52
Mikan (*Citrus unshiu*)	2.14 ± 0.08	9.18 ± 0.72	1.61 ± 0.42
Grapefruit (*Citrus paradisi*)	0.97 ± 0.42	7.07 ± 1.36	1.33 ± 1.00
Peach (*Prunus persica*)	0.22 ± 0.17	7.91 ± 4.04	3.75 ± 0.35
Grape (Purple) (*Vitis vinifera*)	0.18 ± 0.03	5.04 ± 2.34	ND
Cherry (*Prunus avium)*	0.17 ± 0.06	7.85 ± 2.39	0.67 ± 0.06
Apple *(Malus pumila)*	0.15 ± 0.03	2.44 ± 1.27	0.84 ± 0.40
Kaki (*Diospyros kaki [Thunb.]*)	0.11 ± 0.05	3.72 ± 0.81	0.08 ± 0.01
Pears (*Pyrus nivalis Jacq.*)	0.07 ± 0.03	2.67 ± 0.22	0.32 ± 0.08
Strawberry (*Fragaria ananassa*)	0.05 ± 0.003	19.58 ± 0.22	ND
Watermelon *(Citrullus lanatus)*	0.06 ± 0.02	30.77 ± 4.49	0.74 ± 0.16
Grape (Green) (*Vitis vinifera*)	ND	25.36 ± 0.47	ND

Values are shown as the mean ± SD μg/g of fresh weight. Levels were measured in triplicate. ND; not determined.