Supplemental Material

Quantification and Comparison of Nutritional Components in Oni Walnut (*Juglans ailanthifolia* Carr.), Hime Walnut (*Juglans subcordiformis* Dode.), and Cultivars

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Measurement of the carbohydrates

1. Reagents

For analyses of the carbohydrates in four walnut species, all reagents employed were of HPLC (High performance Liquid Chromatography) grade or higher. These reagents were purchased from the Fujifilm Wako Pure Chemical Corp. (Osaka, Japan).

2. Methods

For the analysis of carbohydrates in walnuts, the following defatting process was first performed. 0.5 g of crushed walnut was placed in a centrifuge tube with a lid (50 mL). To this was added 20 mL of hexane. The mixture was shaken with a shaker (room temperature, 1500 rpm, 10 min) and centrifuged (4 °C, 850 g, 10 min). The upper layer of hexane was removed, and 20 mL of hexane was added again. This procedure was repeated twice. Obtained defatted residue was dried in a desiccator.

Measurement of carbohydrate was carried out using a slight modification of the method of Cichonska et al [1]. The extraction of carbohydrates was performed as follows. 10 mL of 50 % ethanol was added to the defatted residue obtained. The mixture was shaken with a shaker (room temperature, 1500 rpm, 10 min) and centrifuged (4°C, 850 g, 10 min). The upper layer was transferred to a 20 mL volumetric measuring flask and 8 mL of 50 % ethanol was added again. The same procedure was then performed, and the extract was made up to 20 mL. Obtained extract was filtered through disposable syringe filter with φ0.45 μm (GL Chromatodisc, GL Sciences Inc., Tokyo, Japan). The filtered extract was subjected to solid phase extraction (Oasis HLB, Waters, MA, USA) and ultrafiltration (Amicon Ultra 3k, Merck KGaA, Darmstadt, Germany) to make the working solution. The working solution was measured by liquid chromatography coupled with evaporative light scattering detection (HPLC-ELSD, SofTA 300S ELSD, Teledyne Technologies Inc., CA, USA). Analytical conditions for HPLC-ELSD were set as follows. Column: COSMOSIL Sugar-D (φ4.6 x 250 mm, Nacalai Tesque Inc., Kyoto, Japan); mobile phase: water and acetonitrile; flow rate: 1.0 mL/min; column oven temperature: 30°C; injection volume: 20 μL. Under these analytical conditions, three carbohydrates (Glucose, Fructose, and Sucrose) were measured. Data (n=1) were collected triplicate.

3. Results

The quantitative results showed that glucose and fructose were below the detection limits and that only sucrose was detected in all four walnut species.

Table S1 Carbohydrate contents of walnut kernels.

Constituent	Walnuts			
	English walnut	**Shinano walnut**	**Oni walnut**	**Hime walnut**
Sucrose (g/100 g wet wt)	1.52	1.29	1.06	0.99

References

1. Cichońska, P.; Ziębicka, A.; Ziarno, M. Properties of rice-based beverages fermented with lactic acid bacteria and propionibacterium. *Molecules* **2022**, 27, 2558.