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Nutrients and natural toxic substances in commonly consumed Jerusalem artichoke (*Helianthus tuberosus* L.) tuber

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ABSTRACT

This study determined nutrients, chemical contaminants, (insecticide residues and heavy metals), and natural toxic substances (nitrate, nitrite, cyanide, oxalate, phytate, and trypsin inhibitor) in tubers of Jerusalem artichokes—Kaentawan in the Thai language—grown in four major provinces in Thailand. They were purchased, prepared, homogenized, and freeze-dried for further analysis using standard methods. All Kaentawan samples contained considerable amounts of fructans and dietary fiber (15.4±0.2 g and 3.2±0.8 g/100 g fresh weight [FW], respectively), as well as potassium and iron (339±61 and 0.32±0.05 mg/100 g FW, respectively). All samples had very low amounts of insecticide residues (37 compounds), cyanide, and trypsin inhibitor, as well as Pb, Cd, nitrate, and nitrite (0.82±0.09, 0.10±0.02, 1.9–17.5, and 0.01–0.24 mg kg^{-1} FW, respectively), in addition to oxalate and phytate (14±9 and 0.17±0.02 mg/100 g FW, respectively). This study's data can be used for food composition databases and for safety consumption.

1. Introduction

Kaentawan, or Jerusalem artichoke (*Helianthus tuberosus* L.), is known as an easy growing crop plant (Bach, Clausen, & Edelenbos, 2015; Kays & Nottingham, 2008). Kaentawan tuber has been reported as a rich source of inulin and fructo-oligosaccharide (FOS). Many studies have shown that inulin-type fructans have beneficial effects in terms of dietary fiber and prebiotics. Since Kaentawan has a low caloric value, is fat-free, and has a high mineral content (Kays & Nottingham, 2008), it can be developed and applied as a functional food. Generally, this tuber, though a good source of nutrients, can also contain toxic substances in terms of either chemical contaminants (i.e., insecticide residues or heavy metals) or natural toxins (i.e., nitrate, nitrite, cyanide, phytate, oxalate, and trypsin inhibitor) (Jaworska, 2005; Kays & Nottingham, 2008).

For chemical contaminants, insecticides contain a variety of toxic substances. Most organochlorine (OC) insecticides have been banned, since they can persist in the environment. Carbamate (CRB) and organophosphorus (OP) can replace OC which is most widely used for crop plants (Bai, Zhou, & Wang, 2006). Pyrethroid (PYR) is another choice and has naturally synthetic analogues. PYR insecticides have greater photostability and relatively low toxicity compared to OC, OP, and CRB insecticides (Ware & Whitacre, 2004). Heavy metal contents can contaminate the environment before plants are grown, such as in water and soil, (McGrath, Zhao, & Lombi, 2001). For example, cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg) may be found at certain levels in plants (Onianwa, Adetola, Iwegbue, Ojo, & Tella, 1999).

For natural toxins, nitrate and nitrite are naturally present in a wide range of vegetables. They accumulate in the stem, leaves, and roots of plants and may relate to high levels of ammonia or nitrates in the soil (Nicholson, 2007). Oxalate is widely found in plants in a water soluble form (sodium, potassium, and ammonium) and an insoluble (calcium) form, which may be present at different levels in different plant parts (Holloway, Argall, Jealous, Lee, & Bradbury, 1989; Judprasong, Charoenkiatkul, Sungpuag, Vasanachitt, & Nakjamanong, 2006). Oxalate can bind strongly with calcium, iron, and magnesium (Noonan & Savage, 1999), which can have adverse health effects (Jaworska, 2005). A diet high in oxalates can cause excessive urinary excretion of oxalate (hyperoxaluria) and can increase the risk of developing kidney stones (Nguy n & Savage, 2013). Trypsin is a serine protease that is produced in the pancreas as zymogen trypsinogen and stored in active form (Horton, Moram, Acrimgeour, Perry, & Rawn, 2006). Trypsin inhibitor (TI) inhibits

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the activities of protein digesting enzymes in the digestive tract and reduces the body's capability to use protein in foods (Srinivasan, Giri, Harsullkar, Gatehouse, & Gupta, 2005).

Currently, there is limited, to no, information available on nutrients, chemical contaminants (insecticide residues and heavy metals), and natural toxic substances (nitrate, nitrite, cyanide, oxalate, phytate, and trypsin inhibitor) in Kaentawan tubers. Consequently, these components should be investigated to ensure healthy and safe consumption.

2. Materials and methods

2.1. Samples and sample preparation

Kaentawan tubers were purchased from four major growing areas in Thailand (Nakhon Pathom, Khonkaen, Nakhon Ratchasima, and Phetchabun provinces) during April to May 2014. The tubers were transported to the laboratory at Institute of Nutrition, Mahidol University. Kaentawan tubers from each source were classified to two groups, namely, fresh Kaentawan without skin and boiled Kaentawan without skin. Each sample was peeled, weighed edible part, and washed with deionized water (DI water). Each sample was homogenized using a food processor (Mara®, Thailand) and separated into two portions as fresh sample and dried sample by freeze drying (lyophilization). The prepared samples were then stored at −20 °C until analysis.

2.2. Reagents and standards

Deionized water was obtained by means of a Millipore water purification system with resistivity 18.2 MΩ cm^{-1} (Millipore RiOs-DITM134, Bedford, MA, USA). Nitric acid, perchloric acid, sulfuric acid, hydrochloric acid, acetic acid, cadmium standard, lead standard, silver nitrate, potassium cyanide, potassium hydroxide, sodium hydroxide, sodium chloride, oxalic acid dehydrate (99.5–102.0%), phytic acid, sodium salt hydrate, hexane, and other chemicals were obtained from MERCK (Germany). Acetone, dichloromethane, ethyl acetate, 2, 2, 4 – trimethylpentane (Iso–octane), silica gel (60–200 mesh), and sodium sulfate anhydrous (12–60 mesh) were analytical grade from Sigma-Aldrich®, USA. All other chemicals and solvents used were of analytical and HPLC grade.

2.3. Nutrient determination

Nutrient analyses (proximate compositions and minerals) were conducted using standard AOAC methods (AOAC, 2012). All samples were analyzed by the accredited ISO 17025:2005 laboratory at the Institute of Nutrition, Mahidol University, Thailand. The results of all measurements were presented as mean ± standard deviation in fresh weight of the edible portion.

2.3.1. Proximate composition

Total nitrogen was determined using the Kjeldahl method following AOAC method number 981.10 (AOAC, 2012) and calculated into protein content using 6.25 as the specific (Jones) factor. Moisture was analyzed using a drying method at 100 ± 2 °C until a constant weight, following AOAC method no. 952.45 (AOAC, 2012). Crude fat was determined by acid digestion prior to continuous extraction using petroleum ether in Soxtec system following AOAC method no. 945.16 (AOAC, 2012). Ash was analyzed by incinerating all organic matter at 550 ± 5 °C in accordance with AOAC method no. 945.46 (AOAC, 2012). Carbohydrate was calculated using the formula 100-moisture-protein-fat-ash; energy was calculated by Atwater factor (4 for protein and carbohydrate and 9 for total fat). Total dietary fiber was determined using enzymatic gravimetric method following AOAC method no. 985.29 (AOAC, 2012).

2.3.2. Fructans

Fructans (inulin and fructooligosaccharides) were analyzed following the AOAC method no. 997.08 (AOAC, 2012) by extracting with hot water and hydrolysing by inulinase (Sigma–Aldrich®, USA, I2017 inulinase from Aspergillus niger, CAS Number 9025-67-6 which has enzyme activity of 1740 inu/g). Both hot water extracted and enzyme hydrolysed fractions were derivatized into volatile oxime-trimethylsilyl derivatives. Each individual sugar, both before and after enzyme hydrolysis, was then determined by high temperature gas chromatography (Joye & Hoebregs, 2000; Judprasong, Tanjor, Puwastien, & Sungpuag, 2011) and the fructans in each sample were calculated.

2.3.3. Minerals

The acid solution dissolved from ash residue after incineration at 550 °C for 2 h was used for calcium, sodium, and potassium content analyses by flame atomic absorption spectrophotometer (AAS) following method no. 975.03 (AOAC, 2012). The acid solution was also determined for phosphorus by the gravimetric method following AOAC no, 920.55 (AOAC, 2012). Acid digestion in a closed Teflon vessel was employed for determining magnesium, iron, copper, and zinc using an inductively coupled plasma optical emission spectrophotometer (ICP-OES), method no. 984.27 (AOAC, 2012).

2.4. Toxic substances determination

2.4.1. Insecticide residue

Each sample (about 20 g) was weighed into an Erlenmeyer flask containing 20 g of DI water. Fifty mL acetone, 50 mL dichloromethane, and a little sodium chloride were added to the mixture. The extraction was blended and mixed with ultra-turrax at high speed for 3 min. The organic phase was transferred into a beaker and dried with Na_2SO_4 for 10 min. For the organic phase, 50 mL was taken and reduced in volume to 3–5 mL with rotary evaporator. Thereafter, it was adjusted to the volume of 5 mL with ethyl acetate and re-concentrated (repeat the evaporation twice with ethyl acetate to make sure the complete removal of acetone). The solution was injected into gas liquid chromatography, GLC (Agilent 6890 N, USA), for organophosphorus analysis. Another 2 mL of solution was cleaned up by silica gel and then eluted with hexane:dichrolomethane (4:1) and injected into GLC (Agilent 6890 N, USA) for organochlorine and pyrethroids analyses. The solution was dissolved into methanol:water and injected into high performance liquid chromatography, HPLC (Agilent 1100 Series, USA), for carbamate analysis.

2.4.2. Heavy metals

Each sample (0.5 g) was extracted with 5 mL of 65% HNO_3 and 1 mL $HClO_4$ into a Teflon jar. The extraction was digested at 100 °C for 9 h in a hot air oven (Memmert, USA) and then left to cool under the fume hood. The solution was transferred to a 25 mL volumetric flask and diluted with DI water to the mark. Heavy metals (Pb and Cd) were determined using Inductively Couple Plasma-Optical Emission Spectrometer (ICP-OES, PerkinElmer®, Optima 4300, USA) with WinLab32 software against standard addition methods.

2.4.3. Nitrite and nitrate

Nitrate and nitrite content in all study samples were determined by the cadmium column method (AOAC, 2012). Each sample (5 g) was extracted with 5 mL saturated borax solution and 100 mL hot water, mixed thoroughly, heated in a boiling water bath at 100 °C for 20 min, and then shaken repeatedly. The solution was