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Fatty acids, minerals, phenolics and vitamins in the seeds of *Inocarpus fagifer*, a Pacific Island underutilized legume

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Summary

Recently, Pacific nations have faced to alarming increase in prevalence of noncommunicable diseases connected with consumption of non-traditional processed food. It is believed that re-introduction of native diet may mitigate these negative trends. One of the traditional staple food of Pacific region are seeds of underutilized leguminous tree *Inocarpus fagifer*. Nevertheless, information on their chemical composition and nutritional properties are missing. Therefore we decided to analyze this crop for the presence of fatty acids, minerals, phenolics and vitamins. Performed analyses revealed a slightly predominating portion of unsaturated (e.g. 18:2 *n*-6; 18:1 *n*-9 and 18:3α *n*-3) over saturated (e.g. C18 and C16) fatty acids. Considering minerals, the substantial concentrations of copper, magnesium, manganese, potassium and zinc (19.32; 1823.21; 8.44; 23308.41 and 77.99 mg kg^{-1} of dry matter respectively) were recorded. Ferulic and coumaric acids were the most abundant phenolics (3.23 and 1.48 mg kg^{-1} of dry matter respectively), whereas flavonoids, isoflavonoids and coumestrol were also present. Regarding vitamins, niacin and riboflavin were found in respective concentrations 131.80 and 4.47 mg kg^{-1} of dry matter. Our findings suggest *I. fagifer* seeds as a prospective food source of several health-beneficial constituents which might contribute to the well-being of Pacific islanders.

Introduction

Globalization of processed, carbohydrate rich diets with lack of micronutrients leads to a higher vulnerability to overweight and non-communicable diseases (NCDs) not only in the developed world but also in low- and middle- income countries where almost three quarters of the world's NCD deaths currently occur. Despite the remote location, Pacific islanders have also been facing severe changes in dietary habits, whereas the current prevalence of raised blood glucose and obesity is dramatically increasing in certain regions. For example in Samoa, the levels of both above mentioned factors are more than two-fold higher than the global average (Curtis, 2014; WHO, 2014; WHO, 2002; Win Tin et al., 2015). Since many underutilized crops are being recognized for their dietetic value due to the presence of specific health-beneficial constituents, their re-introduction to the diet has a potential to reverse the negative impact of recently occurring trends in the nutrition and health of the local populations (Ebert, 2014; Gibson et al., 2015; Thies, 2000). Nevertheless, before any recommendations can be made on consumption of novel plant foods, it is necessary to assess their dietetic and toxicological properties by detailed analysis (Nasi et al., 2009).

Edible seeds of leguminous plants are considered as a valuable source of nutrition and functional compounds. Besides the basic nutritional proteins and oligosaccharides they also provide other essential factors such as unsaturated omega-3 and omega-6 fatty acids, minerals and vitamins, which play an irreplaceable role in the human metabolism and health (Combs, 2001; Schieber et al., 2001; Yildiz, 2010). Characteristic secondary metabolites of legumes are isoflavonoids (Veitch, 2013), which exert various biological effects. Some of them possess an ability to bind to the estrogen receptor (Andres et al., 2015), while it might lead to e.g. relief of menopausal symptoms, inhibition of intestinal glucose-uptake (Vedavanam et al., 1999) or prevention of steroid hormone-dependent cancers (Perabo et al., 2008). Other biologically active phenolic derivatives found in leguminous plants are flavonoids and phenolic acids (Dewick, 2009). Their consumption is connected with a lower risk of oxidative-stress related diseases due to their antioxidant potential (Gordon, 1996).

Inocarpus fagifer (Parkinson) Fosberg, known as Tahitian or Polynesian chestnut, is a medium-sized tree belonging to the Leguminosae family (Lewis et al., 2005) and naturalized in the lowland tropical forests of Melanesia, Micronesia and Polynesia. Its indehiscent pod contains a kidney-shaped seed with an edible kernel which when prepared raw or cooked is considered to be among the most important cash crops and culinary nut species of the Pacific (Lim, 2012), however, as a food, it is practically unknown in the global context. In Samoa, this multipurpose tree called "ifi" has played an important role in the livelihood of local inhabitants and even nowadays its seed is considered a staple food. It has been also consumed in other Pacific regions, where it is traditionally prepared in many different ways, including grilling, roasting, baking, boiling or mashed in pudding. Moreover, significance of this tree also relies

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on the medicinal use of various tree parts and its timber is suitable for light constructions, tool handles, furniture and canoes manufacturing (PAUKU, 2006; WHISTLER, 2002). For above mentioned reasons, *I. fagifer* was designated as one of the priority crops for the Pacific region (APAARI, 2011).

Despite the well recorded traditional food use of *I. fagifer* in many Pacific island nations, the plant has received little attention from research and extension services and the data on chemical composition and nutritional properties of this species are totally missing. Therefore we decided to determine the presence of certain basic nutritional components of its seeds collected in Samoa.

Materials and methods

Plant material

The fruits of *Inocarpus fagifer w*ere collected from a wild population of trees (13°30.743´ S, 172°47.828´ W) growing in the coastal area near to the village of Falealupo (Vaisigano district) located at Savai'i island of the Independent State of Samoa in August 2014. During randomized collection, 30 fruits were gathered from three different trees (ten fruits from each tree). The plant was authenticated by Prof. Ladislav Kokoska and voucher specimen (No. 03015KBFR) is deposited in the herbarium of the Department of Botany and Plant Physiology of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague (Czech Republic).

Sample preparation and dry matter determination

A fleshy mesocarp and testa, which are inedible for humans, were removed and hulled seeds were lyophilized in the Free-Zone 1 freeze dry system (Labconco, Kansas city, MO, USA). Immediately after lyophilization the seeds were pulverized using an IKA A11 analytical mill (IKA Werke GmbH & Co. KG, Staufen, Germany). Homogenized material was stored under -20 °C until analysis. The residual moisture of lyophilized samples was determined gravimetrically at 130 °C for 60 min by the Scaltec SMO 01 analyzer (Scaltec Instruments, Gottingen, Germany) according to the Official Methods of Analysis of AOAC INTERNATIONAL (2012).

Chemicals and reagents

Bacto Niacin Assay Medium was purchased from Becton, Dickson and Company (Franklin Lakes, NJ, USA), coumestrol, daidzein, daidzin, 7,4-dimethoxyisoflavone, formononetin, genistein, genistin, glycitein, glycitin, kaempferol, liquiritigenin, luteolin, naringenin, naringenin-7-*O*-glucoside, prunetin, rutin, sissotrin from Indofine (Hillsborough, NJ, USA), chloroform, nitric acid, sodium carbonate, sodium hydroxide, sodium sulfate from Lach-Ner (Neratovice, Czech Republic), ethanol, Folin-Ciocalteu reagent, formic acid, gallic acid, methanol from Merck (Darmstadt, Germany), acetic acid, dichlormethan, hydrogen peroxide, *n*-hexane, sodium acetate, sulfuric acid, potassium permanganate from Penta (Prague, Czech Republic), tectoridin from PhytoLab (Vestenbergsgreut, Germany), apigenin-7-*O*-glucoside, boron trifluoride-methanol solution, bromothymol blue indicator, caffeic acid, *p*-coumaric acid, (-)-epicatechin, ferulic acid, gallic acid, isoquercitrin, nicotinic acid, salicylic acid, sinapic acid and takadiastase (EC 3.2.1.1) from *Aspergillus oryzae* from Sigma-Aldrich (Saint Luis, MO, USA), standard 37 component FAME mixture was obtained from Supelco (Bellefonte, PA, USA).

Minerals analysis

Determination of minerals was carried out after acid digestion of samples performed according to the method previously described by VANEK et al. (2010). Initially, a 0.5 g of sample was kept overnight at laboratory temperature in a closed, but not sealed, Teflon vessel (Savillex, Eden Prairie, MN, USA) with 10 mL of 65% nitric acid. Then the Teflon vessel was sealed and the mixture was heated at 120 °C on a hot plate for 2 hours. The digested solution was then quantitatively transferred to the 50 mL volumetric flask and filled up to the mark with deionized water. The solution was filtered through 0.45 μm Nylon disk filters (Cronus, Gloucester, United Kingdom) prior to analysis. An inductively coupled plasma optical emission spectrometer DUO iCap 7000 (Thermo Scientific, Waltham, MA, USA) operating at 61.15 kW with respective nebulizer and auxiliary gas flow rates of 0.5 and 1 L/min was used for contents determination of selected elements, which were monitored at the following spectral lines (wavelength in nm): boron (B, 208.959), calcium (Ca, 317.933), copper (Cu, 224.700), iron (Fe, 238.204), magnesium (Mg, 279.079), manganese (Mn, 257.610), phosphorus (P, 177.495), potassium (K, 766.490), sodium (Na, 589.592), sulfur (S, 180.731), and zinc (Zn, 202.548). Quality of digestion and analysis were controlled using blanks and the standard reference materials (NIST SRM 1575a Pine Needles and NCS DC 73351 Tea).

Fatty acids analysis

The oil was obtained from the sample (about 3 g) by multiple extraction with 180 mL *n*-hexane:dichlormethan 80:20 v/v in a Soxhlet apparatus for 6 h. The fat was recovered under vacuum and dried over anhydrous sodium sulfate and transferred to a capped bottle and stored at 4 °C until used for analysis.

Derivatization of fatty acids was based on the base-catalyzed reaction using sodium hydroxide-methanol as a reagent with boron trifluoride as a catalyst. Fatty acid methyl esters (FAMEs) were then extracted to hexane. FAMEs were analyzed by gas-liquid chromatography using a SP-2560 fused silica capillary column (100 m × 0.25 mm i.d., 0.2 μm film thickness) from Supelco (Bellefonte, PA, USA) in Agilent 6890 gas chromatograph (Agilent, Palo Alto, CA, USA) equipped with a flame ionization detector. The oven temperature was 175 °C for 30 min, then it was increased by 1 °C min^{-1} to 210 °C and this temperature was maintained for 140 min. Detector and injection port temperatures were 220 °C and the nitrogen carrier gas flow was 1 mL min^{-1}. For the identification of FAMEs, standard FAME mixtures were analyzed. To confirm the identification of some FAMEs, GC/MS analysis was carried out in the GC/MSD system Agilent 5975 (Agilent, Palo Alto, CA) with the same column and temperature conditions as above, except for the helium flow, which was 0.6 mL/min and the detector temperature was 250 °C. GC-FID chromatogram of fatty acids composition in *I. fagifer* seed extract is available in supplementary information (Fig. S1).

UHPLC-MS/MS analysis of phenolic compounds

Prior to analysis, the plant material (2 g) was extracted in a Soxhlet-like fex IKA 50 extractor (IKA Werke GmbH & Co. KG, Staufen, Germany) in 70% ethanol in 1/20 (w/v) proportion during three 7-min cycles at 130 °C followed by cooling to 50 °C. The extracts were evaporated on a rotary evaporator, dissolved in 5 mL of 40% methanol and filtered through a Teflon (PTFE) syringe filter (0.45 μm) (Labicom, Olomouc, Czech Republic). Standard stock solutions of coumestrol, daidzein, daidzin, 7,4-dimethoxyisoflavone, formononetin, genistein, genistin, glycitein, glycitin, kaempferol, liquiritigenin, luteolin, naringenin, naringenin-7-*O*-glucoside, prunetin, rutin, sissotrin, tectoridin, apigenin-7-*O*-glucoside, caffeic acid, *p*-coumaric acid, (-)-epicatechin, ferulic acid, gallic acid, isoquercitrin, salicylic acid and sinapic acid were prepared in methanol at a concentration of 1 mg mL^{-1} and were subsequently diluted in 40% (v/v) methanol-water solution at concentrations ranging from 0.1 to 1000 ng mL^{-1}.

UHPLC-MS/MS analysis of polyphenolic compounds was carried out using an Agilent 1290 Infinity instrument (Agilent, Santa Clara, CA, USA) equipped with a binary pump (G4220B), an autosampler (G4226A), an autosampler thermostat (G1330B) and a column compartment thermostat (G1316C), coupled to an Agilent triple quadrupole mass spectrometer (6460A) with a Jet Stream ESI ion source. A Kinetex PFP column (2.6 μm, 100A, 150 × 3.00 mm) from Phenomenex (Torrance, CA, USA) was used for the chromatographic separation of the extracts. Column temperature was set at 35 °C and the injection volume was 3 μL. 10 mM formic acid (eluent A) and methanol (eluent B) were used as mobile phases for a gradient elution. The linear gradient solvent system started in 40 % of eluent B and increased to 100 % of eluent B in 10 min. This was maintained for 4 min and in 1 min the conditions returned to the initial ratio, which was maintained for a further 4 min. The flow rate was set at 0.3 mL min^{-1}. The MS/MS apparatus was operating in positive and negative mode in the same analysis. The applied conditions of Jet Stream Ion Source were: drying gas temperature 290 °C; drying gas flow 4 L min^{-1}; sheath gas temperature 380 °C; sheath gas flow 10 L min^{-1}; nebulizer pressure 35 psi; capillary voltage was set at 3500 and 5000 V in positive and negative acquisitions, respectively. Multiple reaction monitoring (MRM) mode was used for the detection. Except for *p*-coumaric acid, the two most abundant transitions per analyte were used. The transitions and specific MS/MS parameters used for each compound are available in supplementary information (Tab. S1). An Agilent Mass Hunter (Agilent, Santa Clara, CA, USA) was used for data acquisition and quantification of samples. Extracted ion chromatograms of phenolic compounds detected in *I. fagifer* seed extract in MRM mode can be found also in supplementary information (Fig. S2).

Total phenolic content

Total phenolic content was measured using slightly modified method previously developed by Singleton et al. (1998). Firstly, each sample in volume of 100 μL was added to a 96-well microtiter plate. Thereafter, 25 μL of pure Folin-Ciocalteu reagent was added. The plate was inserted in an orbital shaker at 400 rpm for 5 min. Reaction was started by adding 75 μL of 12 % sodium carbonate. The mixture was kept in dark at 37 °C for 1 h. Absorbance was measured at 760 nm by Infinite M200 PRO (Tecan, Grödig, Austria). Results were expressed as gallic acid equivalents.

Vitamin B_2 and B_3 content

The content of vitamin B2 (riboflavin) was evaluated by fluorimetric method after its conversion to lumiflavin according to the Czech technical standard method CSN 560054 (1972). The riboflavin was extracted after hydrolysis with 0.05M sulfuric acid at 100 °C for 30 min followed by dephosphorylation using an enzymatic treatment (Takadiastase from *Aspergillus oryzae*) at 37 °C for 16 h after pH adjustment to 4.5 by 2.5M sodium acetate solution. The sample was filtrated and the aliquot of the filtrate was shaken with chloroform. Concentrated sodium hydroxide solution (7M) was added to the clear aqueous layer; the solution was then illuminated with a 40 W fluorescent tube and riboflavin in the sample converted to lumiflavin. The solution was acidified with a few drops of acetic acid and interfering substances of reaction were inactivated by oxidation with 4 % potassium permanganate solution. After 1 minute of exposure the decolorization of the solution by 3 % of hydrogen peroxide was performed. Lumiflavin was then extracted by chloroform and measured fluorimetrically (excitation 435 nm, emission 525 nm) at Spekol 11 (Carl Zeiss, Oberkochen, Germany).

The microbiological determinations of vitamins B3 (niacin) was performed according to the Czech technical standards CSN 56 0051 (1987). The sample was hydrolyzed with 0.5M sulfuric acid at 121 °C for 60 min and then cooled to room temperature. The pH of the sample was adjusted to 4.5, filtered and diluted to a concentration suitable for the assay calibration range. For determination of vitamin B3, pH was adjusted by 1M sodium hydroxide solution to 6.8. The diluted filtrate, appropriate medium (Bacto Niacin Assay Medium), standard solution of nicotinic acid and deionized water were pipetted into assay tubes. Tubes were sterilized by autoclaving (116 °C, 15 min) and after cooling to room temperature inoculated with *Lactobacillus plantarum* ATCC 8014 (The Czech National Collection of Type Cultures, The National Institute of Public Health, Prague, Czech Republic). After 72 hours of incubation at 30 °C, the content of B3 was determined by titration with 0.1M sodium hydroxide and bromothymol blue indicator. The growth response of the tested microorganism was compared quantitatively to that of known standard solutions.

Statistical analysis and calculations

All analytical experiments were performed in triplicate and results are expressed as mean and standard deviation. For comparison of our results with contents of minerals and vitamins reported by the USDA (2014), the conversion of nutrient values (NV) from fresh weight (FW) to dry matter (DM) basis was performed according to the formula NV in g kg^{-1} of DM = (100 × NV in g kg^{-1} of FW)/(100 – water content in g kg^{-1} of FW) as recommended in FAO/INFOODS guidelines (2012).

Results and discussion

A complex set of analysis has revealed substantial contents of several minerals together with the presence of various fatty acids, phenolic compounds and B vitamins. The complete results are shown in the Tab. 1-3.

The determined oil content of *I. fagifer* seed (2.2 ± 0.2 % w/w) correlates with oil content of other legumes such as peas, lentils and beans (USDA, 2015). Within the oil fraction, overall 14 unsaturated fatty acids (UFAs) and 21 saturated fatty acids (SFAs) were detected while the quantity of each compound was expressed as a relative percentage (Tab. 1). The most abundant UFAs determined in our study were linoleic (18:2 *n*-6; represented by 26.72 %), followed by oleic (18:1 *n*-9; 18.49 %); α-linolenic (18:3α *n*-3; 4.42 %) and vaccenic acid (18:1 *n*-7; 1.27 %). The rest of UFAs were represented by less than 1 %. Among SFAs, palmitic (C16; 22.73 %), stearic (C18; 7.35 %), myristic (C14; 4.41 %) and lignoceric (C24; 4.20 %) acids were the most predominant. Behenic (C22), arachidonic (C20) and cerotic acid (C26) were detected in respective amounts of 1.70, 1.33 and 1.17 %. Remaining SFAs were found in lower concentration than 1 %.

The complete results of fatty acids (FAs) analysis showed that UFAs were slightly predominating (53.57 %) over SFAs (46.22 %). The overall profile also demonstrated a greater portion of polyunsaturated FAs (31.14 %) over monounsaturated FAs (22.06 %). Recent nutritional studies are focusing on the omega-6/omega-3 polyunsaturated FAs proportion as one of the keystones to prevent health complications caused by an unhealthy diet. It is proposed that their high ratio (1:15-25) occurring in the western diet is connected with the development of NCDs (Simopoulos, 2012). In the case of *I. fagifer* seed, the results showed a relatively desirable proportion of omega-6/omega-3 FAs (1:6.05) whereas this ratio is comparable to those of other legumes such as soybean (Rusnikova et al., 2013). These findings may be interpreted as indicating the potential of this traditional crop to be an alternative to current diets with an undesirable UFAs ratio.

The results of mineral elements analysis (Tab. 2) showed that calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P) and

Tab. 1: Fatty acids composition of *Inocarpus fagifer* seed oil

Fatty acid shorthand designation	Content (%)	SD[a]
Saturated fatty acids (SFA)		
C 6:0	0.15	0.02
C 8:0	0.15	0.02
C 10:0	0.10	0.02
C 12:0	0.91	0.07
C 14:0	4.41	0.27
C 15:0	0.22	0.02
C 16:0	22.73	0.57
C 17:0	0.23	0.02
C 18:0	7.35	0.26
C 19:0	0.16	0.02
C 20:0	1.33	0.11
C 21:0	0.07	0.02
C 22:0	1.70	0.14
C 23:0	0.49	0.06
C 24:0	4.20	0.38
C 25:0	0.43	0.06
C 26:0	1.17	0.12
C 27:0	0.09	0.02
C 28:0	0.20	0.04
C 29:0	0.05	0.02
C 30:0	0.08	0.02
Total SFA	43.22	
Unsaturated acid (UFA)		
C14:1 (*cis* 9)	tr.[b]	
C16:1 (*cis* 7)	0.29	0.03
C16:1 (*cis* 9)	0.88	0.07
C16:1 (*cis* 11)	0.60	0.05
C17:1 (*cis* 9)	0.03	n.a.[c]
C18:1 (*trans* isomers)	tr.	
C18:1 (*cis* 9)	18.49	0.46
C18:1 (*cis* 11)	1.27	0.10
C18:1 (*cis* isomers)	tr.	
C18:2 (*cis trans* isomers)	0.19	n.a.
C18:2 (*cis* 9, *cis* 12, *n*-6)	26.72	0.67
C18:3 (*cis trans* isomers)	0.18	n.a.
C18:3 (*cis* 9, *cis*12, *cis*15, *n*-3)	4.42	0.27
C20:1 (*cis* 11)	0.50	0.05
Total UFA	53.57	

[a]standard deviation (n=3), [b]traces (below 0.01 %), [c]not applicable

sulfur (S) were the most abundant entities detected in the *I. fagifer* seeds; while boron (B), copper (Cu), iron (Fe), manganese (Mn), sodium (Na) and zinc (Zn) were found in lower concentrations. K was present in the highest amount of 23.31 g kg^{-1}, exceeding those of soybean and peanut (19.65 and 7.54 g kg^{-1} respectively) (USDA, 2015). According to the minimum recommended dietary allowances (RDA; 1.6-2 g day^{-1} per healthy adult) this study indicates

Tab. 2: Minerals and vitamins content of *Inocarpus fagifer* seed

Minerals	Content (mg kg^{-1} dry matter)	SD[a]
boron	37.77	19.45
calcium	1536.39	151.27
copper	19.32	10.11
iron	44.84	42.30
magnesium	1823.21	86.32
manganese	8.44	0.69
phosphorus	3904.02	34.48
potassium	23308.41	570.80
sodium	348.35	98.01
sulfur	2179.05	41.31
zinc	77.99	7.01
Vitamins		
niacin	131.80	2.67
riboflavin	4.47	1.62

[a]standard deviation (n=3)

I. fagifer as a valuable dietary source of this element (NRC, 1989a). Considering the importance of K in the human diet, this finding could boost its availability and intake for people from Pacific island nations. Relatively high Na/K ratio (1:67) with low Na concentration (348.35 mg kg^{-1}) also suggests a possible beneficial effect of seed consumption in persons suffering from hypertension (NRC, 1989b; Perez and Chang, 2014). Taking into the account the RDA of other micronutrients, the notable concentrations of Cu (19.32 mg kg^{-1}), Mg (1823.21 mg kg^{-1}), Mn (8.44 mg kg^{-1}) and Zn (77.99 mg kg^{-1}) were detected. These results indicate that 100 g of the dry matter edible portion of *I. fagifer* seed contains approximately half of the RDA of mentioned micro-elements (Cu = 1.5-3 mg day^{-1}; Mg = 200-350 mg day^{-1}; Mn = 2-5 mg day^{-1}; Zn = 11-15 mg day^{-1} per healthy adult) (NRC, 1989a) whereas analyzed material has higher content of Zn than soybean (53.47 mg kg^{-1}) (USDA, 2015).

In order to evaluate the presence of phenolic compounds in *I. fagifer* seed, the total phenolic content (TPC) was determined (63.75 ± 4.62 mg of gallic acid equivalents kg^{-1} of dry matter). Furthermore, 27 phenolic entities with a total sum 6.81 mg kg^{-1} were detected by detailed analysis (Tab. 3). The most abundant phenolic compounds found in the seed of *I. fagifer* were hydroxycinnamic acids, namely ferulic and coumaric, in respective amounts of 3.23 and 1.48 mg kg^{-1} while other phenolic acids were determined in much lower concentrations. The analysis also revealed the presence of isoflavonoids. From this class of compounds, the most abundant were glycitein, its glycoside glycitin and formononetin (568.64; 71.28 and 179.73 µg kg^{-1} respectively). Daidzein and genistein were identified along with their glycosides daidzin and genistin in concentrations ranging from 2.46 to 14.48 µg kg^{-1}. It is known that quantities of isoflavonoids may vary in underutilized legumes significantly (Leuner et al., 2013). Although the amounts of isoflavonoids detected in *I. fagifer* seeds are rather low, the presence of this metabolic pathway within this species is in agreement with authors studying other members of the Dalbergieae tribe (Hegnauer and Hegnauer, 2001). Coumestrol was the only coumestan found (530.06 µg kg^{-1}). Among flavonoids, the highest concentration was recorded in the case of liquiritigenin and rutin (152.92 and 123.68 µg kg^{-1}respectively).

The determined TPC of *I. fagifer* seed was by several orders of magnitude lower in the comparison with other pulses e.g. soybean and peanut (Djordjevic et al., 2011). Although it is obvious that

Tab. 3: Phenolic compounds content of *Inocarpus fagifer* seed

Phenolics acids	Content (μg kg^{-1} dry matter)	SD[a]
Caffeic acid	97.89	1.52
p-Coumaric acid	1478.47	15.11
Ferulic acid	3229.08	32.90
Gallic acid	tr.[b]	
Salicylic acid	88.75	0.90
Sinapinic acid	65.80	0.29
Isoflavonoids		
Daidzein	13.87	0.07
Daidzin	2.46	0.03
7,4´-dimethoxyisoflavon	64.99	0.49
Formononetin	179.73	1.58
Genistein	13.53	0.16
Genistin	14.48	0.11
Glycitein	568.64	7.84
Glycitin	71.28	0.89
Prunetin	7.78	0.05
Sissotrin	3.31	0.05
Tectoridin	15.11	0.10
Flavonoids		
Apigenin-7-glu	tr.	
Epicatechin	tr.	
Isokvercitrin	21.53	0.21
Kaempferol	46.91	0.39
Liquiritigenin	152.92	2.74
Luteolin	7.05	0.05
Naringenin	13.75	0.26
Naringenin-7-glu	tr.	
Rutin	123.68	0.17
Coumestans		
Coumestrol	530.06	8.61

[a]standard deviation (n=3), [b]traces (below 0.01%)

this crop represents a rather weak source of phenolics, as long as it serves as a staple food in certain regions, it would appear that the cumulative amount consumed on a daily bases might contribute to the prevention of oxidative stress-related diseases. Because the determined value of TPC and the sum of quantified phenolics do not match, it seems that a considerable amount of other phenolic entities might be present within the examined material. Possible candidates include condensed tannins which are found in numerous legumes (Coda et al., 2015; Curiel et al., 2015).

Considering the vitamins content (Tab. 2), representatives of group B, niacin and riboflavin were found in respective concentrations of 131.80 and 4.47 mg kg^{-1}. Niacin was present in the seed of *I. fagifer* in a higher concentration than in soybean or peanut (USDA, 2015), while a 100 g dry matter edible portion along with sufficient intake of tryptophan (200 mg) should cover daily recommended minimum consumption (NRC, 1989a). Administration of niacin increases adiponectin secretion and may therefore contribute to the reduction of obesity via lipid-lowering effect (Westphal et al., 2010). In the case of riboflavin, a 100 g dry matter edible portion represents ca. 1/3 of re-commended minimum intake (1.2 mg day^{-1} per healthy adult) (NRC, 1989a).

Conclusion

This study, to our best knowledge, is a pioneering investigation on the chemical composition of *I. fagifer* seed highlighting the presence of minerals, fatty acids, phenolics and B vitamins. Of great significance is the presence of micronutrients such as Cu, Mg, Mn and Zn in substantial amounts. These micronutrients together with K are essential to the human metabolism. Appropriate to the human diet seem to be also concentrations of niacin and riboflavin together with determined ratios of sodium/potassium and omega-6/omega-3 UFAs, indicating its potential as a valuable dietary source. Considering the obtained results, it seems that this crop possesses several health-beneficial attributes and therefore might potentially play a positive role in the lowering of prevalence of obesity and NCDs mainly in Pacific island nations where it is traditionally consumed as a staple food. However, further studies on chemical composition, biological activities and toxicology of this underutilized crop which has received little attention from researches in the past, are necessary to verify its potential as a healthy food.

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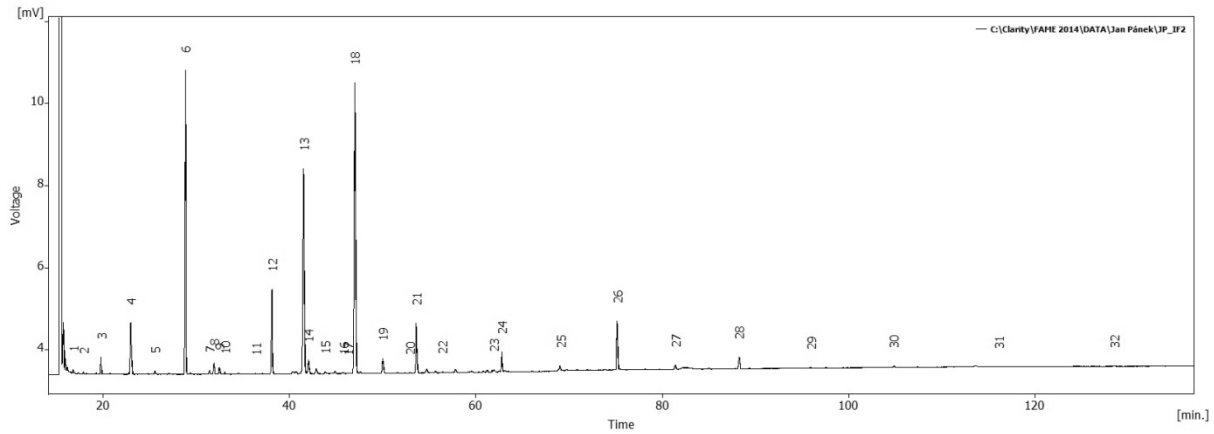

Fig. S1: GC-FID chromatogram of *Inocarpus fagifer* seed extract. Peak identification: 1 = caprylic acid, 2 = capric acid, 3 = lauric acid, 4 = myristic, 5 = pentadecylic acid, 6 = palmitic acid, 7 = hexadecenoic acid (*cis* 7), 8 = hexadecenoic acid (*cis* 9), 9 = hexadecenoic acid (*cis* 11), 10 = heptadecanoic acid, 11 = heptadecenoic acid (*cis* 9), 12 = stearic acid, 13 = oleic acid (*cis* 9), 14 = octadecenoic acid (*cis* 11), 15 = nonadecanoic acid, 16 + 17 = octadecadienoic acid (*cis trans* isomers), 18 = linoleic acid, 19 = arachidic acid, 20 = eicosenoic acid (*cis* 11), 21 = α-linolenic, 22 = heneicosanoic acid, 23 = unidentified fatty acid, 24 = behenic acid, 25 = tricosanoic acid, 26 = lignoceric acid, 27 = pentacosanoic acid, 28 = cerotic acid, 29 = heptacosanoic acid, 30 = octacosanoic acid, 31 = nonacosanoic acid, 32 = triacontanoic acid.

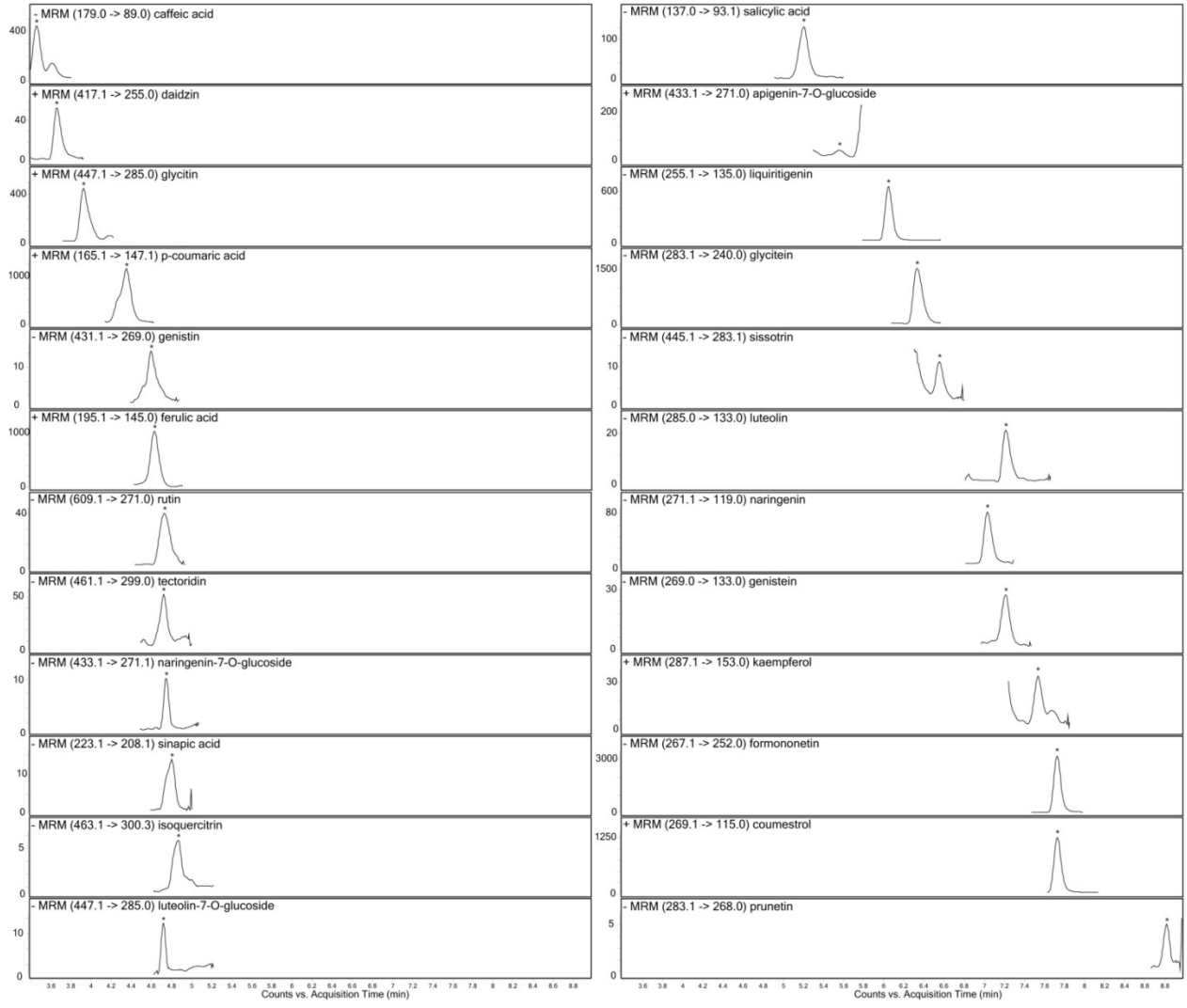

Fig. S2: Extracted ion chromatograms of phenolic compounds detected in *Inocarpus fagifer* seed extract.

Tab. S1: The transitions and specific MS/MS parameters used for analysis of phenolic compounds

	ionisation mode	retention time (min)[a]	fragmentor (V)	precursor ion (m/z)	product ion (m/z)				LOD (ng/mL)[c]	LOQ (ng/mL)[d]
					quantification transition	CE (eV)[b]	confirmation transition	CE (eV)[b]		
7.4´-dimethoxyisoflavone	ESI +	9.05 (0.5)	117	283.10	171.3	42	176.0	18	0.4	1.4
apigenin-7-*O*-glucoside	ESI +	5.54 (0.5)	109	433.12	271.0	13	153.0	60	0.2	0.8
caffeic acid	ESI -	3.60 (0.4)	81	179.00	89.0	30	135.1	13	2.3	7.7
chlorogenic acid	ESI -	3.01 (1.0)	81	353.09	191.1	9			0.6	1.9
p-coumaric acid	ESI +	4.37 (0.5)	60	165.05	147.1	9			0.9	3.0
coumestrol	ESI +	7.88 (0.5)	121	269.05	115.0	53	157.0	33	0.5	1.6
daidzein	ESI -	6.01 (0.5)	126	253.05	132.1	42	91.0	34	0.1	0.3
daidzin	ESI +	3.66 (0.5)	85	417.12	255	14	152.0	78	0.1	0.2
epicatechin	ESI -	3.10 (0.5)	111	289.07	109	13	245.1	5	0.7	2.4
ferulic acid	ESI +	4.65 (0.5)	63	195.07	145.0	13	177.0	5	1.0	3.2
gallic acid	ESI -	2.43 (0.6)	75	169.01	124.9	9	169.0	5	0.7	2.2
genistein	ESI -	7.21 (0.5)	123	269.04	133.0	30	63.1	34	0.1	0.4
genistin	ESI -	4.62 (0.5)	162	431.10	269.0	10	239.1	46	0.1	0.4
glycitein	ESI -	6.32 (0.5)	99	283.06	240.0	18	268.0	10	0.1	0.2
glycitin	ESI +	3.96 (0.5)	82	447.13	285.0	14	270.0	46	0.1	0.1
isoquercitrin	ESI -	4.87 (0.5)	150	463.09	300.3	18	271.0	42	0.4	1.2
kaempferol	EIS +	7.55 (0.6)	161	287.06	153.0	41	69.1	53	1.1	3.6
liquiritigenin	ESI -	6.06 (0.5)	90	255.06	135.0	9	119.1	21	0.1	0.3
luteolin	ESI -	7.23 (0.8)	128	285.04	133.0	33	151.0	20	0.4	1.4
naringenin	ESI -	7.05 (0.5)	93	271.06	119.0	21	151.0	9	0.1	0.1
naringenin-7-*O*-glucoside	ESI -	4.76 (0.5)	117	433.11	271.1	10	119.0	50	0.1	0.4
prunetin	ESI -	8.82 (0.3)	111	283.06	268.0	13	239.0	25	0.3	0.9
rutin	ESI -	4.69 (0.5)	163	609.14	271.0	61	300.0	65	0.3	0.9
salicylic acid	ESI -	5.22 (0.6)	72	137.02	93.1	13	65.1	29	0.5	1.8
sinapic acid	ESI -	4.80 (0.4)	81	223.06	208.1	9	149.0	17	0.1	0.2
sissotrin	ESI -	6.55 (0.5)	162	445.11	283.1	10	268.0	30	0.1	0.3
tectoridin	ESI -	4.74 (0.5)	138	461.11	299.0	13	283.0	29	0.1	0.4

[a]Retention time window (min) is given in brackets, [b]Collision energy, [c]Limits of detection (signal-to-noise ratio of 3), [d]Limits of quantification (signal-to-noise ratio of 10)