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Taraxacum kok-saghyz (TK): compositional analysis of a feedstock for natural rubber and other bioproducts

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ABSTRACT

Natural rubber (NR) is a critical raw material primarily produced in Asia from a single plant species (*Hevea brasiliensis*). This lack of biologic and geographic diversity, coupled with increasing demand, makes it imperative that alternative sources of NR are developed. *Taraxacum kok-saghyz* (TK) is a rubber-producing dandelion being developed as an alternative to the traditional source of NR. In order to improve the value of this crop and make it competitive with other sources of NR, germplasm improvement, crop production optimization, efficient extraction and purification methods and the valorization of TK co-products are needed. In this study, a compositional analysis of field-grown TK roots was conducted in which total solids, ash content, protein, crude fat, fatty acids, carbohydrates, lignin, and total water-, acetone-, and hexane-extract (rubber) were quantified and characterized. Mass closure was greater than 95%. The analysis showed that 5.4% (g/g root dw) of the root consisted of rubber and 1.7% was acetone extractable (mainly sterols). Approximately 60% (g/g dw) of the root was hot water extractable. Soluble sugars (32%) and proteins (10%) were the most abundant water soluble fractions. Inulin (18%) and sucrose (10%) were the most abundant sugars. Insoluble components included cellulose 9% (glucan), hemicellulose 7% (xylan, mannan, arabinan, galactan), lignin 5%, protein 5% and pectin 3%. This compositional analysis provides a baseline which can be used to assess compositional changes induced by altering plant genetics, environmental conditions and cultivation practices. Based on these results, pathways for TK processing are proposed, and its potential as a biorefinery feedstock is evaluated. This analysis indicates that TK has potential as a source of not only NR but other products and/or raw materials of importance including inulin and proteins.

1. Introduction

Natural rubber (NR) is an irreplaceable raw material used to produce a wide variety of products ranging from medical devices to aircraft tires (Puskas et al., 2014). The United States of America has categorized NR as, “a commodity of vital importance to the economy, the defense, and the general well-being of the nation”, and recognized as important the development of a domestic source of NR through the Critical Agricultural Materials Acts of 1984 and 2002 (Mathers and Meier, 2011; Whalen et al., 2013).

The amount, and relative share of NR in rubber products, has increased over the last 35 years. NR accounted for 30% of the total rubber (natural and synthetic) used in the world in 1981 and by 2013 the share had increased to 42% (iHS, 2014). Its consumption in the USA grew approximately 80% between 2000 and 2013 (Statistica, 2016), reaching 12×10^9 kg representing a US$50 billion market (Accenture, 2014). The global market for industrial rubber products had a value of US$115 billion in 2013 and by 2018 it is expected to reach US$158 billion (Karpus-Romain, 2015). Along with the expansion of the market, prices and demand for NR are forecast to rise in response to increases in demand from developing countries, such as China, India and Brazil (Accenture, 2014; van Beilen and Poirier, 2007a,b)

NR is traditionally obtained from one, extremely genetically-narrow source, the Para rubber tree (*Hevea brasiliensis*). This tree is mainly grown in Asian-Pacific countries, where 90% of the NR global production is concentrated (Accenture, 2014). This lack of biological and geographical diversity makes the NR supply highly vulnerable to disruption. Particular factors endangering NR supply are: 1. Possible outbreak of pathogens, including the South American Leaf Blight (SALB) which could rapidly destroy rubber tree plantations if it escapes control measures (van Beilen and Poirier, 2007a); 2. The strict climate requirements for *H. brasiliensis* that limit its cultivation to specific

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tropical regions, and make it vulnerable to climate change (Mooibroek and Cornish, 2000; van Beilen and Poirier, 2007b); 3. The rise in demand for NR from developing countries, such as China, India and Brazil (van Beilen and Poirier, 2007a; b; Whalen et al., 2013); 4. Displacement of rubber-tree plantations by more profitable palm-oil plantations to meet the demands for biofuels (Belcher et al., 2004); 5. Limits to the unsustainable expansion of rubber-tree plantations that destroy natural forest ecosystems (Ahrends et al., 2015; Warren-Thomas et al., 2015). 6. Geopolitical factors that may limit access to, and production of, NR.

Synthetic rubbers can be made from petroleum. These are often used in applications requiring resistance to oil, certain chemicals and oxygen, and they have better aging and weathering characteristics than natural rubber. But synthetic rubber properties are inferior to the properties of NR for many of the most important applications (Puskas et al., 2014). NR has superior resilience, tack, strength, thermal properties, elasticity, abrasion, and impact resistance compared with synthetic rubber which are attributed to its unique molecular structure and high molecular weight (> 1 million g/mol) (Cornish, 2001; Musto et al., 2016). NR consists primarily of linear rubber polymers (*cis-1*,4-polyisoprene) and secondary components (non-rubber constituents) that make it different than synthetic rubber. The non-rubber constituents include proteins and resins (fatty acids, triglycerides, sterols, etc). Some of the non-rubber constituents in *H. brasiliensis*, at least, are linked to the ends of rubber chain and participate actively in the naturally occurring network of natural rubber (Amnuaypornsri et al., 2008). Natural and chemically-induced networks give NR the ability to crystallize upon stretching which is integral to its outstanding mechanical properties (Ikeda et al., 2016).

Taraxacum kok-saghyz (TK) is one of the most promising crops for alternative NR production (Buranov and Elmuradov, 2010). Many countries have investigated the development of TK as a source of NR (Mooibroek and Cornish, 2000). TK was first developed by the Soviet Union (Volis et al., 2009) and introduced into the U.S. during the Emergency Rubber Project in 1942 as a source of natural rubber to replace supplies cut off during WWII (Whaley and Bowen, 1947). However, with hevea plantations being rebuilt in the aftermath of the war, efforts to exploit TK were discontinued because TK-based NR production could not compete economically with hevea NR (Cornish et al., 2016; Kirschner et al., 2013; Whaley and Bowen, 1947). Nonetheless, a number of research groups around the world, including The Program of Excellence in Natural Rubber Alternatives (PENRA) at The Ohio State University (OSU), are currently working to transform TK into a viable and profitable source of NR. Current research has shown that TK can be grown in temperate regions as direct seeded annual crop and so can readily fit into existing farm infrastructure. However, TK is poorly competitive, slow growing, and is rapidly overwhelmed by weeds. TK also needs steady moisture content during germination and establishment. Chemical weed control, greater vigor and size, and higher rubber concentrations are needed to make TK germplasm commercially competitive (Cornish, 2017; Cornish et al., 2013; Cornish et al., 2016).

TK is a dandelion plant, adapted to temperate regions, native to Kazakhstan and Uzbekistan. Its root system has laticifers, long tubular vessels that produce and store rubber particles, similar to those in the rubber tree's bark. However, in contrast to NR from *H. brasiliensis*, that can be obtained by tapping NR-containing trees in the form of latex, NR in fresh TK roots is present in both solid and latex forms (Buranov and Elmuradov, 2010; Cornish et al., 2015). The presence of both rubber forms in TK roots requires processes for the recovery of solid rubber over latex rubber. This is because upon drying, the latex rubber is converted to solid rubber that can be extracted by mechanical, biological, and/or chemical processes (Buranov, 2009; Huang et al., 2015; van Beilen and Poirier, 2007a; Wade and Swiger, 2013). The rubber content in TK roots has been reported to range from 5 to 24% w/w dry root, and molecular characterization showed a M_w of 1.4–1.8 × 10^6, M_w/M_n of 1.8, and gel content of 34% (Buranov and Elmuradov, 2010; Musto et al., 2016). These molecular features of TK rubber are comparable to those for hevea NR, which suggests that the quality of TK rubber is similar to that from hevea rubber. However, rubber concentration (and possibly its molecular characteristics) depend on cultivation method, age of the plant, climate, and growth conditions (Buranov and Elmuradov, 2010). TK roots are also known to contain significant amounts of inulin, proteins, resin, and fatty acids (Buranov and Elmuradov, 2010). However, there have been no studies of the compositional analysis of field grown TK roots.

Developing a process to produce NR from TK roots should be based on producing rubber at high yield without compromising its physical and mechanical properties. Additionally, one of the major challenges to the development of an economic supply of NR from TK roots is the large quantity of byproducts that result from separating and purifying its NR. These include the non-rubber parts of the roots and the leaves which must also be separated and converted into products and/or raw materials of value to different commercial sectors. Thus, any techno-economic analysis of the use of TK as a source of NR should also consider its overall composition. The primary objective of this study was to quantify and characterize these components of TK roots. Analytical methods that have advanced with the recent research emphasis on biomass conversion for biofuels (Sluiter et al., 2013), are used to provide biomass composition data with near-quantitative mass closure.

Additional objectives were to propose process pathways to obtain TK components within a biorefinery and estimate the value of potential products made from TK root components.

2. Experimental section

2.1. Chemical reagents

All chemicals and reference standards were reagent grade and High Pressure Liquid Chromatography (HPLC) grade, respectively, and obtained from commercial vendors

2.2. TK roots

A representative sample was obtained from field grown TK roots, produced from 1.7 million TK seeds which were planted in greenhouses in spring 2013. Seedlings in 3420 total trays were transplanted into seven fields (~3 ha total) in Ohio that summer. The seed germination/establishment rate was 83%. At the end of the autumn 2013, plants were harvested using a potato digger and shovels. Harvested plants were washed with cold water in batches to remove soil. Bins of plants were turned over onto expanded metal greenhouse carts (MET-14 Big Wheel Push Cart, Gothic Archhouses, Mobile, AL) and sprayed with cold tap water using a hose nozzle until most of the soil was visibly washed off. Each cart held 50–100 plants. Washed plants were air-dried for 10 min before rosettes (crowns) were removed using a knife, taking care not to cut into the roots to avoid latex bleed out from the plant. The total harvest was 752 kg of fresh roots. After harvest, roots were dried at 50 °C for 4–7 days in a 765 L forced-air oven (Hoffman Manufacturing, Jefferson, OR), resulting in 188 kg of dry roots. Roots were then chopped and flattened into pieces with a maximum length of 2 cm using a Gran-U-Lizer model 666F (Modern Processing Equipment, Chicago, IL) cutter/roller mill. This resulted in a well-mixed heterogeneous material.

2.3. Sampling

The chopped and flattened dry roots were stored in a tote which, over time, led to segregation and stratification of the material. Therefore, the roots were sampled through a composite sampling procedure (Petersen et al., 2005). During this procedure, the roots were thoroughly mixed before every sampling. A total of 6 sampling increments (partial samples of the tote, that when combined with other sub-

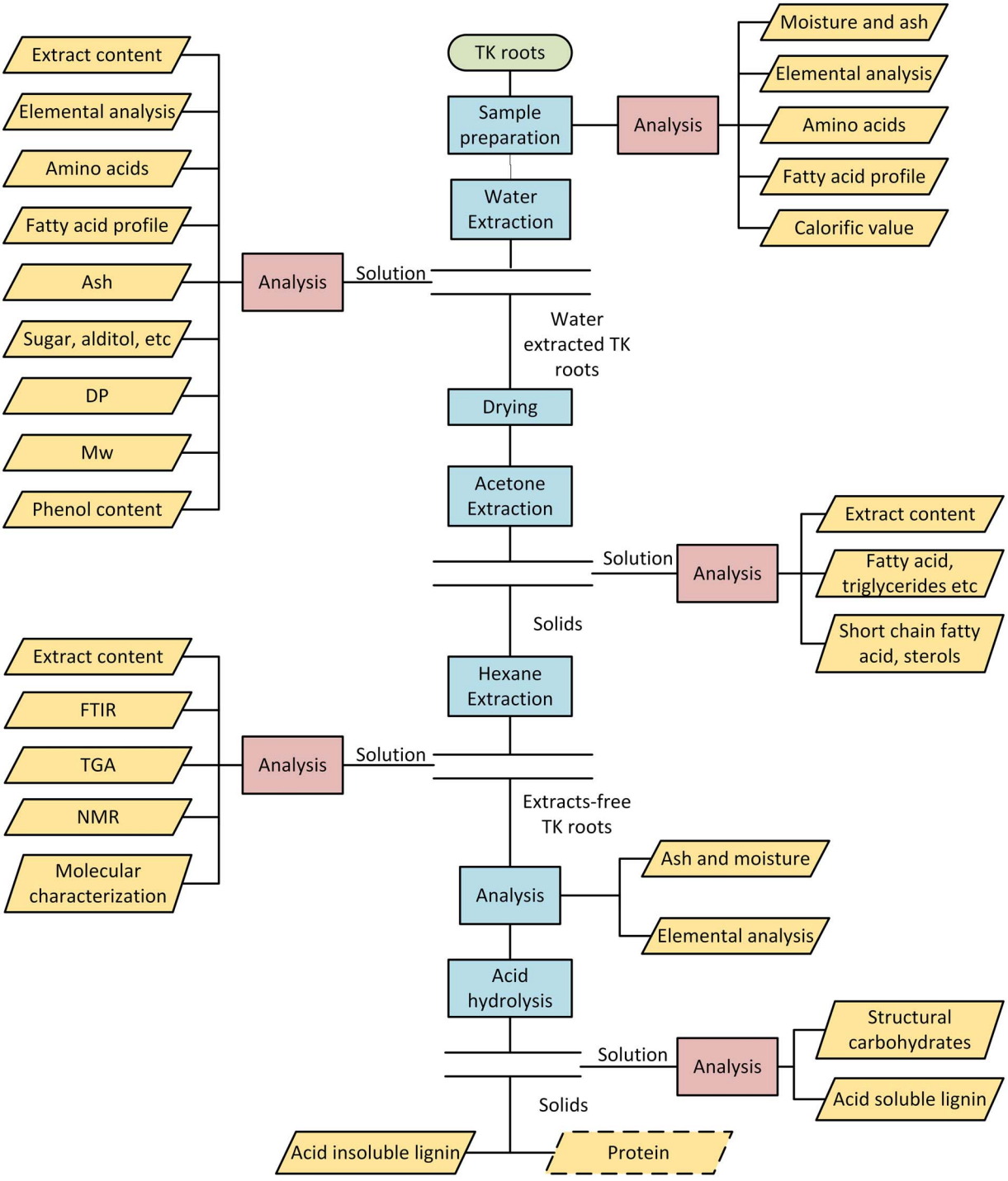

Fig. 1. Flow chart showing the sequence of extractions and analyses used for the compositional analysis of roots of *Taraxacum kok-saghyz* (TK). Dashed line for protein in the acid insoluble fraction indicates that an indirect measure of protein was used, see section 2.17.

samples, provided the final sample) of approximately equal weights from different parts of the tote were taken with a plastic scoop to give a 3 kg representative sample. The representative sample was stored in sealed bags at 4 °C.

2.4. Experimental procedures

Fig. 1 depicts a flow diagram of the experimental procedures followed for the analysis of TK roots. All tests were performed in triplicate unless otherwise indicated.

2.5. Sample preparation

Each subsample taken during this study was dried in a convection oven at 45 °C to constant weight. Three dried subsamples of 10 g were separately ground for 20 s by impact grinding using an IKA A11 basic analytical mill (IKA-Laboratory, Analytical and Processing Technology, Staufen, Germany) and sieved through a sieve N.10 (2 mm sieve). The portion of the sample that passed through the sieve (99.7%) was collected and stored in sealable bags, and refrigerated at 4 °C.

2.6. Moisture and ash content

Moisture and ash content of the ground samples were determined by drying a 1 g sample at 105 °C in a convection oven for 24 h (Sluiter et al., 2008a) and reweighing, then heating to 575 °C for 4 h in air and reweighing (Sluiter et al., 2005).

2.7. Elemental analysis

Elemental analysis of the dried and ground TK root samples was performed according to published methods (Isaac and Johnson, 1985; Jones et al., 1991) using a microwave digestion system (CEM microwave digestion system, CEM Corporation, Matthews, NC) and inductively coupled plasma (ICP) emission spectrometry (Prodigy dual view inductively coupled plasma spectrophotometer, Teledyne Leeman Labs, Hudson, NH). Elemental analysis included determination of: P, K, Ca, Mg, S, Al, B, Cu, Fe, Mn, Mo, Na, and Zn. Total nitrogen and carbon were quantified by a total nitrogen and carbon-combustion method using a Vario Max carbon-nitrogen analyzer (Vario max Carbon-Nitrogen analyzer, Elementar Americas, Mt. laurel, NJ) according to AOAC official methods 990.03 (AOAC, 2002) and 972.43 (AOAC, 2006b), and nitrate-nitrogen was determined potentiometrically (Baker and Smith, 1969).

2.8. Amino acids

The amino acid profile of dried and ground TK root samples was determined according to the AOAC official method 982.30 E(a, b, c) (AOAC, 2006a). Amino acids methionine and cysteine were determined by performic acid oxidation of the ground sample (4 °C overnight) followed by acid hydrolysis in HCl (6 mol/L HCl, 110 °C, 24 h, vacuum-sealed vial). Tryptophan was determined by alkaline hydrolysis using NaOH (4.2 mol/L NaOH, 110 °C, 22 h, vacuum-sealed vial). All other amino acids were determined by acid hydrolysis using HCl (6 mol/L HCl, 110 °C, 24 h, vacuum-sealed vial). Hydrolysates were analyzed using a Hitachi amino acid analyzer L-8900 (Hitachi High-Technologies Corporation, Tokyo, Japan), which uses anion exchange chromatography and ninhydrin detection system.

2.9. Fatty acid profile

Crude fat concentration of dried and ground TK root samples was determined by ether extraction following the AOAC official method 920.39 (AOAC, 2005). Fatty acids were identified and quantified according to AOAC official method 996.06 (AOAC, 2001) using a gas chromatography and flame ionization detection (GC-FID) Agilent 7890A (Agilent Technologies, Palo Alto, CA).

2.10. Calorific value

Calorific value was determined using an automatic adiabatic bomb calorimeter IKA C 2000 basic version 1 using pelletized benzoic acid (IKA C 723) as a standard for calibration (IKA-Laboratory, Analytical and Processing Technology, Staufen, Germany). Approximately 0.4 g of ground TK roots were weighed into a tared stainless steel crucible and the calorific value was measured in the bomb calorimeter using the preconfigured dynamic 25 °C mode.

2.11. Water extraction

200 g of the chopped and flattened root sample was mixed with 1800 mL of DI water and boiled for 20 min. The slurry was filtered through a 0.074 mm sieve (sieve N.200) and the liquid fraction was collected and allowed to cool to room temperature, then volumetrically diluted to 2000 mL. The extraction procedure was repeated 6 times. 10 mL of each extract were combined and used for analysis. The solid fraction was washed 4 times with deionized (DI) water at room temperature and dried at 55 °C for 48 h in a convection oven. The dry, water extracted, TK roots were ground according to the sample preparation method (section 2.5) and stored at 4 °C.

2.12. Analysis of water extract

2.12.1. Water extract solids content

Water extract solids were measured by filtering the extract through a 0.22 µm nylon syringe filter then volumetrically transferring 2 mL of the filtered extract into a plastic weigh boat. The sample was dried at 55 °C for 24 h and the weight of the dried residue was measured. The dried residue was considered the solids content.

2.12.2. Elemental analysis

Elemental analysis of the extract was performed by inductively coupled plasma (ICP) emission spectrometry (Prodigy dual view inductively coupled plasma spectrophotometer, Teledyne Leeman Labs, Hudson, NH) and included the determination of: P, K, Ca, Mg, S, Al, B, Cu, Fe, Mn, Mo, Na, and Zn (APHA et al., 2010a). Total nitrogen and phosphorous, anions NO_3-N, PO_4-P, and NH_3-N were measured by flow injection analysis using a flow injection analyzer (Latchat Quick Chem 8500 flow injection analyzer, Hach Company, Loveland, CO) (APHA et al., 2010c). F, Cl, Br, NO_2-N, SO_4-S were measured by ion chromatography (Dionex Liquid Ion Chromatograph Dionex ICS-1600, Dionex ThermoFisher Scientific, West Palm Beach, FL) (APHA et al., 2010b).

2.12.3. Ash, amino acids and fatty acids profiles

Ash, amino acids and fatty acids (see sections 2.6, 2.8, and 2.9) were determined in both the starting material (TK roots) and in the water extracted TK roots. The amounts of these components in the water extract were calculated by difference.

2.12.4. Sugar, alditol, organic acids and aldehyde measurement

Carbohydrates in the water extract were determined by ion-exclusion high-pressure liquid chromatography (IEC-HPLC). IEC-HPLC analysis was conducted using an Agilent 1200 system with RI detector (Agilent Technologies, Palo Alto, CA), and a Rezex RPM-Monosaccharides Pb + 2 (8%) column (Phenomenex®, Torrance, CA) with a security guard column AJO-4490 (Phenomenex®, Torrance, CA) in series with a micro-guard de-ashing cartridge (Bio Rad®, Hercules, CA). The mobile phase was double deionized (DDI) water at a flow rate of 0.4 mL/min and the injection volume was 10 µL. The column and the guard column were heated to 60 °C. The system was calibrated using HPLC grade standards of inulin, nystose, 1-kestose, sucrose, glucose, and fructose. Linear calibration curves were calculated for each standard using eight calibration points. All had R^2 values greater than 0.99.

Alditol, organic acids and aldehyde content in the water extract were determined using an IEC-HPLC Agilent 1200 system with an RI detector (Agilent Technologies, Palo Alto, CA), a Rezex ROA-Organic Acid H+ (8%) column (Phenomenex®, Torrance, CA), and a security guard column AJO-4492 (Phenomenex®, Torrance, CA) at 80 °C. The mobile phase was 0.005 mol/L H_2SO_4 at a flow rate of 0.6 mL/min and an injection volume of 10 µL. The system was calibrated using HPLC grade standards of galacturonic acid, succinic acid, acetic acid, glycerol, xylitol, ribose, rahmnose, glucose, mannose, galactose, arabinose, xylose, hydroxymethyl furfural (HMF), and furfural. Linear calibration curves were calculated for each standard using eight calibration points. All had R^2 values greater than 0.99.

2.12.5. Degree of polymerization of inulin (DP)

DP and the molecular weight distribution of inulin in the water extract was measured in relation to an inulin standard (I2255) from Sigma Aldrich (Sigma Aldrich, St. Louis, MO). Free sucrose (S_{free}), free glucose (G_{free}) and free fructose (F_{free}) were quantified in water extracts using IEC-HPLC as described in section 2.12.5. To measure sugar

oligomers and inulin, the water extract was hydrolyzed in two ways; (i) using Megazyme fructan kit (Megazyme International Ireland, Bary Business Park, Bary, Co., Wicklow, Ireland); and (ii) sulfuric acid. Total glucose (G_{total}) and total fructose (F_{total}) were measured using IEC-HPLC as described in section 2.12.4.

The fructan assay procedure recommended by Megazyme (Megazyme International Ireland, Bary Business Park, Bary, Co., Wicklow, Ireland) was used for enzymatic hydrolysis (Megazyme, 2014). Acid hydrolysis was adapted from a previously published method (Sluiter et al., 2006). A 3 mL aliquot was taken and diluted with 7 mL of DDI water, and hydrolyzed with 4% sulfuric acid at 80 °C for 2 h. The samples were removed and once they reached room temperature, the pH was adjusted to 5–6 with calcium carbonate.

The glucose (G_f) and fructose (F_f) released from fructans were calculated as follows

$$G_f = G_{total} - G_{free} - 0.53S_{free}$$

$$F_f = F_{total} - F_{free} - 0.53S_{free}$$

The average DP of inulin was calculated by the fructose-to-glucose ratio (number of fructose units per number of glucose units) (Moerman et al., 2004).

DP = number of fructoseunits per number of glucose units

$$DP = \frac{F_f}{G_f} + 1 \text{ glucose unit} + 1$$

2.12.6. Molecular weight distribution

Molecular weight distribution of the components of the water extract was determined using size exclusion chromatography (SEC). SEC analysis was conducted using an Agilent 1200 system with an RI detector (Agilent Technologies, Palo Alto, CA), and a PolySep-SEC GFC-P 200 column (Phenomenex®, Torrance, CA) coupled with a PolySep-GFC-P guard column 83Q-3248-k0 (Phenomenex®, Torrance, CA). The mobile phase was DDI water at a flow rate of 0.8 mL/min, and the injection volume was 15 µL. The column and the guard column were heated up to 55 °C. The system was calibrated using dextran standards (Dextran standards kit, Phenomenex®, Torrance, CA) with molecular weights ranging from 180 to 12,000 Da, since there are no commercial standards for fructans. Therefore, any analysis must be related to dextran. The calibration curve correlation coefficient R^2 was greater than 0.99. The PolySep-SEC GFC-P 2000 column has a linear M_w operation range from 100 to 10,000 Da. Molecules greater than 10,000 Da, such as proteins, were not separated and were carried by the void volume, which was removed during the analysis. The RI signals were exported using ChemStation software (Agilent Technologies, Palo Alto, CA) and processed using R (R Development Core Team, 2010) according to methods previously described to determine molecular weight distribution values (Mori and Barth, 1999).

2.12.7. Total phenol content

Phenol content was determined spectrophotometrically at 760 nm (Agilent 8453 spectrophotometer, Agilent Technologies, Palo Alto, CA) using the Folin-Ciocalteu method (Singleton et al., 1999). Gallic acid was used as a standard.

2.13. Acetone extraction

Water extracted TK roots were prepared according to section 2.5 and extracted with acetone using an accelerated solvent extractor (ASE) (ASE-200, Dionex Corp., now Thermo Fischer Scientific Inc., Waltham, MA) under pressurized conditions. One glass fiber filter of appropriate size was placed in the bottom of a 10 mL ASE extraction cell. 1 g of water extracted roots was weighed in a tared extraction cell. All samples were extracted 3 times by pressurized acetone in order to completely remove the acetone extractable material from the samples. Reagent grade acetone was used. ASE parameters were: N_2 pressure 1500 psi; temperature 40 °C; preheating time 5 min; heat time 5 min (automatic software default); static time 10 min; flush volume 150%; purge time 90 s; static cycles 2.

2.14. Analysis of acetone extract

2.14.1. Acetone extract solids content

After extraction, the solution was poured into a tared aluminum boat and the acetone solvent was allowed to evaporate at room temperature in a fume hood for 6 h. The acetone extract was determined gravimetrically.

2.14.2. Characterization of acetone extract

2.14.2.1. Quantification of free fatty acids, steryl esters, sterols and tryglycerides. Acetone extract was characterized by adapting published methods (Nordic Standardization Programme (NSP), 2008; Örså and Holmbom, 1994). Dihydrocholesterol (ISTD 1), cholesteryl heptadecanoate (ISTD 2), and 1,3-dipalmitoyl-2-oleyl-glycerol (ISTD 3) at concentrations of 0.2 mg/mL were used as internal standards.

1 mL of acetone extract was filtered through 0.45 µm syringe filters into 2 mL vial and mixed with 200 µL of internal standards. This solution was evaporated to dryness under a nitrogen stream at 60 °C using a heating block. 100 µL N,O-Bis(trimethylsilyl)-triflouroacetamide (BSTFA) and 50 µL trimethylchlorosilane (TMCS) were added to the dried extract to redissolve the extracts. The vial was sealed, gently shaken, and warmed at 70 °C for 30 min to completely dissolve and mix the components.

The sample was cooled to room temperature and determination of acetone extract composition was conducted using a GC-FID Agilent 6890 (Agilent Technologies, Palo Alto, CA). Carrier gas used was helium. A sample volume of 1 µL was manually injected in the splitless mode. A 100% dimethylpolysiloxane capillary column by Agilent (reference: Agilent J & W DB-1, 7.5 m x 0.53 mm i.d. x 0.15 µm d.f.) was used. The oven was temperature-programmed from 100 °C in 1.5 min to 340 °C at 12 °C/min. Injector temperature program was 80 °C in 0.5 min to 150 °C at 50 °C/min to 340 °C at 15 °C/min and FID detector temperature was 340 °C. GC-FID signal was analyzed using Chromeleon 6.60 SP2 by Dionex (Dionex Corp., now Thermo Fischer Scientific Inc., Waltham, MA).

Peaks in the chromatogram were integrated and each group was identified and quantified against one of the internal standards as follows: fatty acids and sterols against ISTD 1, steryl-esters against ISTD 2, and triglycerides against ISTD 3 (see supporting information).

2.14.2.2. Analysis of short chain fatty acids and sterols. Short chain fatty acids (C16 to C18) and sterols were further analyzed to identify individual components within these groups. 1 mL of acetone extract was filtered through a 0.45 µm syringe filter into a 2 mL HPLC vial and mixed with 200 µL of internal standards. In this case, standards of isopalmitic acid (C16:0) (ISTD 4) and dihydrocholesterol (ISTD 5), were diluted in ethanol to obtain a concentration of 0.2 mg/mL. The solution of sample and internal standards was evaporated using a nitrogen stream at 60 °C using a heating block. The dried extract (with internal standards) was redissolved in 100 µL dry acetone (acetone dried by addition of sodium sulfate) and 100 µL BSTFA. The vial was sealed, gently shaken, and warmed at 70 °C for 20 min to completely dissolve and mix the components. 10 µL of hexacosane diluted in dichloromethane in a concentration of 2 mg/mL was added as an external standard. The sample was cooled down to room temperature and determination of fatty acid and sterols was conducted using gas chromatography–mass spectroscopy (GC–MS). GC–MS analysis was conducted using a Waters AutoSpec Premier MS (Waters Corporation, Milford, MA) with an integrated GC Agilent 7890 (Agilent

Technologies, Palo Alto, CA); an spli/splitless injector; an autosampler Agilent 7683, CTC-GCPal and CombiPal; and a 5% phenyl polysilphenylene-siloxane capillary column (Reference: SGE Analytical Science BPX5, 30 m x 0.25 mm i.d. x 0.25 µm d.f.). The carrier gas used was helium and the injection volume was 1 µL (automatic injection). The temperature program applied was as follows: 120 °C (1 min) to 300 °C at 10 °C/min. The temperature of the injector during the injection was 160 °C and after the injection was programmed to 260 °C at a rate of 8 °C/min. Compounds were identified by computer comparison of the mass spectra with those in the MassLynx™ V4.1 by Waters (Waters Corporation, Milford, MA).

Small fatty acids and sterols (retention time > 30 min) were quantified by comparing mass spectra against ISTD 5 and ISTD 6, respectively (see supporting information).

2.15. Hexane (Rubber) extraction

Hexane extract was obtained after water and acetone extraction of roots using the ASE. Each sample was extracted 3 times by pressurized hexane in order to completely remove the hexane extractable material. Hexane reagent grade was used and ASE parameters were as follows: N_2 pressure at 1500 psi; temperature 120 °C; preheating time 1 min; heat time 6 min (automatic software default); static time 15 min; flush volume 90%; purge time 60 s; static cycles 2.

2.16. Analysis of hexane extract (rubber)

2.16.1. Hexane extract solids content

After extraction, each solution was poured into a tared aluminum weigh boat and the hexane was allowed to evaporate at room temperature in a fume hood for 6 h. The total amount of hexane extractable material was determined gravimetrically.

2.16.2. Characterization of hexane extract

In order to obtain hexane-soluble material for further characterization, a 12 g sample of water extracted TK roots was prepared from 3 equal parts of 3 separately dried water extracted TK roots. Nine ASE cells were loaded with 1 g of sample and first extracted using acetone and then hexane according to the ASE acetone and hexane methods described in section 2.13 and 2.15, respectively. Each hexane extract was poured into a glass Petri dish and the hexane was evaporated under vacuum at room temperature. Once the extract was dry, the Petri dish was sealed and stored at −20 °C until use. Fourier transform infrared spectroscopy (FTIR) (section 2.16.3) and thermogravimetric (TGA) analyses (section 2.16.4) of the extract was carried out and compared with Hevea natural rubber (SVR-L) purchased from Centrotrade (Chesapeake, VA).

2.16.3. Fourier transform infrared spectroscopy (FTIR) analysis

Approximately 15 mg of dried TK hexane extract was used for FTIR analysis. Fourier transform infrared (FTIR) spectra were recorded using a Spectrum Two™ (Perkin Elmer Inc., Waltham, MA), which was equipped with a universal attenuated total reflectance accessory (UATR). The FTIR/UATR has a diamond crystal which allows direct recording of the spectra from TK NR without sample preparation. The spectra were averaged from 16 scans in the range of 4000 − 450 cm^{-1} with a resolution of 4 cm^{-1}. A background scan of the clean diamond was recorded before scanning the samples.

2.16.4. Thermogravimetric (TGA) analysis

6 mg of dried TK hexane extract was used for thermogravimetric analyses. TGA was carried out at a heating rate of 10 °C/min from 40 to 800 °C under nitrogen atmosphere using a TA Q 50 thermogravimetric analyzer (TA Instruments, New Castle, DE).

2.16.5. Nuclear magnetic resonance (NMR) analysis

Approximately 50 mg of dried hexane extract was solubilized in CD_2Cl_2 and analyzed for one-dimensional ^1H and ^{13}C NMR at 400 MHz using an AVANCEIII-400 spectrometer (Bruker, Rheinstetten, Germany). Data were processed using ACDlabs NMR Processor.

2.16.6. Molecular characterization

The molecular distribution of hexane extracts was determined using gel permeation chromatography (GPC). Each hexane extract was solubilized at a concentration of 1 mg/mL by gently shaking overnight in tetrahydrofuran (THF). The solution was syringe-filtered through a 1.6 µm glass microfiber GF/A filter then injected into an Hewlett-Packard 110 series HPLC (1 mL/min, 35 °C, 100 µL injection volume, THF continuous phase flow rate) with a multi-angle laser light scattering (MALS) detector (DAWN Heleos II, Wyatt Technology, Santa Barbara, CA), a refractive index detector (RI) (Agilent 1260 Infinity, dn/dc = 0.129), and UV detector (HP 110 series @254 nm). The system was equipped with two Agilent PLgel 10 µm Mixed-B 300 × 7.5 mm columns in series. PL gel 10 µm Mixed-B columns have a linear M_w operation range from 500 to 10,000,000 g/mol.

2.17. Measurement of structural carbohydrates and lignin

For the analysis of structural carbohydrates and lignin, roots were first extracted with water, acetone and hexane as described in section 2.11, 2.13, and 2.15, respectively. The extracted TK roots (extract-free TK roots) were subjected to ash, moisture and elemental analysis as previously described in sections 2.6 and 2.7. Protein content was determined by multiplying the nitrogen concentration by the nitrogen-to-protein factor for TK roots (see discussion section 3.2). The extracts-free roots were also hydrolyzed using two-step acid hydrolysis to determine the content of structural carbohydrates and lignin in TK roots (Sluiter et al., 2008b). After acid hydrolysis, the liquid and solid fractions were separated by vacuum filtration through a 15 µm crucible filter.

The liquid fraction was neutralized with calcium carbonate and analyzed by IEC-HPLC using an Agilent 1200 system with an RI detector (Agilent Technologies, Palo Alto, CA) and UV/VIS spectrophotometer SpectraMax Plus 384 at 320 nm (Molecular devices, Sunnyvale, CA) to characterize soluble sugars and byproducts of the hydrolysis, and the acid soluble lignin (ASL), respectively. For IEC-HPLC, the same two column systems described in section 2.12.5 were used.

The solid fraction, or acid insoluble fraction (AIF), was dried at 105 °C for 24 h and the ash content was determined by thermal oxidation at 575 °C. Acid insoluble lignin (AIL) content was quantified assuming that it precipitates along with protein, which was quantified in the extracts-free roots (see above in this section) and assumed to be part of the AIF fraction, and inorganic fraction (ash) after acid hydrolysis. Therefore, AIL was quantified as follows:

$$AIL(\%) = AIF(\%) - ash(\%) - protein(\%)$$

Lignin was then quantified: $L(\%) = AIL(\%) + ASL(\%)$

3. Results

3.1. Moisture and ash content, and elemental analysis

The moisture content of the representative TK root sample after drying was 7.3% (± 0.6%) w/w and the ash content was 11.8% (± 0.6%) w/w dry TK roots. This moisture content was significantly lower than that of the roots immediately after harvesting (section 2.2) due to drying to avoid root deterioration during storing. The high ash content is similar to the value of 10.65% w/w dry TK root reported in a previous study (Zhuo et al., 2015). However previous measurements of the ash content of field grown TK roots in our laboratory (data unpublished) have shown values of 4% ash which are similar to those for other root crops. The high ash value observed in this study may be due

Table 1
Elemental analysis of TK roots.

Concentration (mg/g dry root)	
N	32
C	440
Cl	4.43
P	5.16
K	20.18
Ca	6.65
Mg	2.95
S	2.23
Al	1.27
B	0.02
Cu	0.02
Fe	1.51
Mn	0.05
Mo	0.00
Na	1.22
Zn	0.08

to residual soil on the roots not removed by washing.

The major inorganic elements in the roots were K, Ca, P, and Cl. In general, the elemental analysis showed that the relative concentration of elements was K > Ca > P > Cl > Mg > S > Fe > Al > Na > Zn > Mn > B > Cu > Mo (Table 1).

3.2. Amino acid and fatty acid profiles

Both proteinogenic and non-proteinogenic amino acids were found in TK roots (Table 2A). The concentration of proteinogenic amino acids in the dry roots was 14% w/w, while the non-proteinogenic amino acids were 0.5% w/w. The protein concentration, which was calculated by summing the masses of the proteinogenic amino acids and correcting for water addition during hydrolysis, was 12.4% w/w of the dry roots. The most abundant amino acid (% w/w dry root) was arginine followed by aspartic acid, glutamic acid, and proline (Table 2A).

The proteinogenic amino acid concentrations (Table 2A) and the nitrogen content (Table 1) were used to calculate a nitrogen-to-protein ratio for TK roots (NREL, 2016) to be used in future studies. The average calculated nitrogen to protein ratio was 4.6 with a range from 3.9 to 5.2. This factor can be used to estimate crude protein based on the nitrogen content according to the following formula:

$$\text{Crude protein}(\%) = \text{Nitrogen content}(\%) \times \text{Nitrogen} - \text{to} - \text{protein factor}$$

Fatty acids comprised 5.2% of TK root weight. The fatty acid profile (Table 2B) showed that the most abundant fatty acids were linoleic acid, palmitic acid and linolenic acid. 72% of the total crude fat was identified as fatty acids. The unknown fraction, 28% of the crude fat, may have consisted of polyisoprene (rubber), since it is also partially soluble in ether (Gregg Jr and Macey, 1973).

3.3. Water extract

Water-soluble materials were the major components of the TK root accounting for approximately 60% w/w of the root dry mass (Table 3). Water soluble sugars were the largest water soluble component accounting for 54% of the water extract (32% w/w dry root). The two present at the greatest concentration were inulin and sucrose which comprised 29% and 16% of water extract (17% and 10% w/w dry root), respectively. Monomeric sugars, fructose and glucose, were also present at 3% and 1% of water extract (2% and 0.6% w/w dry root), respectively. Cations and anions were present at concentrations of 3% and 2% of the water extract (2% and 1% w/w dry root), respectively. Potassium and chloride dominated the cation and anions. Nitrite, nitrate, and bromine anions were also found in the extract but their concentrations

Table 2
Amino acid (A) and fatty acid profiles (B) of TK roots.

A) Amino acid profile

Amino acid Proteinogenic amino acids	w/w dry TK root (%)	% of protein
Aspartic Acid	2.57 ± 0.07	17.85 ± 0.10
Threonine	0.40 ± 0.01	2.72 ± 0.11
Serine	0.32 ± 0.01	2.10 ± 0.07
Glutamic Acid	1.93 ± 0.05	13.61 ± 0.09
Proline	1.80 ± 0.06	12.18 ± 0.15
Glycine	0.46 ± 0.00	2.82 ± 0.07
Alanine	0.49 ± 0.01	3.14 ± 0.08
Cysteine	0.16 ± 0.00	1.12 ± 0.02
Valine	0.47 ± 0.01	3.19 ± 0.12
Methionine	0.15 ± 0.02	1.02 ± 0.10
Isoleucine	0.40 ± 0.00	2.74 ± 0.07
Leucine	0.56 ± 0.01	3.90 ± 0.12
Tyrosine	0.24 ± 0.00	1.73 ± 0.03
Phenylalanine	0.36 ± 0.00	2.60 ± 0.07
Lysine	0.60 ± 0.01	4.22 ± 0.09
Histidine	0.33 ± 0.01	2.35 ± 0.02
Arginine	3.07 ± 0.11	22.12 ± 0.40
Tryptophan	0.08 ± 0.01	0.59 ± 0.05
Non-Proteinogenic amino acids		
Taurine	0.20 ± 0.01	
Hydroxyproline	0.10 ± 0.00	
Hydroxylysine	0.14 ± 0.01	
Ornithine	0.04 ± 0.00	

B) Fatty acid profile

Fatty Acid	w/w dry TK root (%)	% total fat
Myristic (14:0)	0.03 ± 0.01	0.67 ± 0.05
Myristoleic (9c-14:1)	0.01 ± 0.00	0.11 ± 0.03
C15:0	0.02 ± 0.00	0.42 ± 0.01
Palmitic (16:0)	0.83 ± 0.16	16.07 ± 0.32
Palmitoleic (9c-16:1)	0.06 ± 0.01	1.10 ± 0.11
Margaric (17:0)	0.03 ± 0.00	0.51 ± 0.12
Stearic (18:0)	0.06 ± 0.01	1.23 ± 0.22
Oleic (9c-18:1)	0.20 ± 0.05	3.77 ± 0.39
Vaccenic (11c-18:1)	0.05 ± 0.01	0.93 ± 0.04
Linoleic (18:2n6)	1.71 ± 0.36	32.90 ± 1.23
Linolenic (18:3n3)	0.51 ± 0.13	9.83 ± 0.88
Arachidic (20:0)	0.07 ± 0.02	1.26 ± 0.09
Gonodic (20:1n9)	0.01 ± 0.01	0.25 ± 0.09
Behenoic (22:0)	0.08 ± 0.02	1.52 ± 0.08
Erucic [22:1n9]	0.00 ± 0.00	0.09 ± 0.03
Lignoceric (24:0)	0.08 ± 0.01	1.58 ± 0.21
Nervonic (24:1n9)	0.02 ± 0.00	0.31 ± 0.03

Amino acids that were not found: lanthionine.
Fatty acids that were not found: 10c-17:1, Elaidic (9t-18:1), Stearidonic (18:4n3), Homo-a-linolenic(20:3n3), Arachidonic [20:4n6], 3n-Arachidonic (20:4n3), EPA (20:5n3), Clupanodonic (22:5n3), DHA (22:6n3).

were low (< 0.1% of the dry root). Phenolic compounds were found at a concentration of 3% of the water extract (2% w/w dry root).

Of the total protein in TK roots 78% was water soluble accounting for 16% of the water extract (10% w/w dry root). Amino acids that predominated in whole roots were also found to predominate in the water extract (Table S1). This shows the high solubility of TK proteins in water. Likewise, 70% of the ash in TK roots was soluble in water (14% of water extract, 8% w/w dry root). In contrast, only about 8% of the crude fat was found in the water extractable fraction (0.6% of water extract, 0.4% w/w dry root). Table S2 of the supporting information summarizes the elemental composition of the water extract of TK roots. K, P, Cl and Ca were the dominant elements in water extract. The composition of water-soluble materials in TK roots was determined with 93% mass closure.

The degree of polymerization of TK inulin was found to be 20. Both methods used for hydrolysis showed similar results for DP. Fig. S1 shows the SEC chromatograms for inulin standard and TK roots water

Table 3
Composition of water extract from TK root.

Water soluble compounds in TK roots		
Compound	w/w dry TK root (%)	w/w dry water extract (%)
Water extract	59.64 ± 2.23	
Ash[a]	8.17 ± 0.99	13.70 ± 1.74
Protein	9.75 ± 0.22	16.35 ± 0.71
Non-proteinogenic amino acids	0.34 ± 0.02	0.57 ± 0.04
Crude fat	0.37 ± 1.00	0.62 ± 1.68
Phenols	1.92 ± 0.00	3.22 ± 0.12
Water soluble cations	2.01 ± 0.06	3.37 ± 0.16
K	1.56 ± 0.06	2.61 ± 0.14
Ca	0.18 ± 0.02	0.30 ± 0.03
Mg	0.14 ± 0.00	0.23 ± 0.01
Na	0.12 ± 0.00	0.20 ± 0.01
Water soluble anions	1.11 ± 0.01	1.86 ± 0.07
PO_4	0.33 ± 0.00	0.55 ± 0.02
NH_3	0.08 ± 0.01	0.13 ± 0.02
SO_4	0.09 ± 0.00	0.15 ± 0.01
F	0.09 ± 0.00	0.15 ± 0.01
Cl	0.46 ± 0.01	0.77 ± 0.03
Water soluble sugars	31.89 ± 0.90	53.47 ± 2.50
Inulin	17.33 ± 0.76	29.06 ± 1.67
Nystose	1.05 ± 0.00	1.76 ± 0.06
1-Kestose	1.39 ± 0.18	2.33 ± 0.31
Sucrose	9.85 ± 0.42	16.51 ± 0.94
Glucose	0.59 ± 0.00	0.99 ± 0.04
Fructose	1.68 ± 0.19	2.82 ± 0.33
Mass closure (%)		**93.16**

extract. The inulin standard had a degree of polymerization ranging from 10 to 60 which corresponds to 1800–10000 g/mol as shown by the unimodal peak around that region (Table 4). However, the water extract showed a wide multi-peak chromatogram ranging from fructan monosaccharides to inulin with DP 60 (Table 4 and Fig. S1).

3.4. Acetone extract

The acetone extractable material accounted for 2% of the TK root dry mass. Five groups of components were identified. The primary group was sterols which represented 1% w/w dry TK roots (57% of the acetone extract), followed by fatty acids C14-C18 (0.2% w/w dry TK roots, 10% of acetone-soluble material), steryl esters (0.2% w/w dry TK roots, 9% of acetone-soluble material), triglycerides (0.2 w/w dry TK roots, 9% of acetone-soluble material), and fatty acids C19-C24 (0.1% w/w dry TK roots, 5% of acetone-soluble material) (Table 5). Unknown components comprised 10% of the acetone extract.

Further analysis of the acetone extract after derivatization of the components by GC–MS is summarized in table S3 and Fig. S3 of the supporting information. Lupeol and β-amyrin were identified as the most abundant sterols in the acetone extract with 0.4% w/w dry TK roots and 0.2% w/w dry TK roots (21% and 11% of the acetone extract), respectively (Table S3). Some other components identified were sitosterol, stigmasterol, and campestarol (0.02%, 0.01%, 0.01% w/w dry TK roots, or 1.1%, 0.9% and 0.5% of the acetone extract, respectively). There were other sterols and short chain fatty acids in the acetone extract that were quantified but not identified (see table S3 and Fig. S3 of the supporting information).

3.5. Hexane (rubber) extract

The hexane extractable material represented 5.4% of the dry TK roots. Hexane solubilizes natural rubber in rubber-bearing plants (Black et al., 1983; Buranov and Elmuradov, 2010; Pearson et al., 2010; Pearson et al., 2013; Salvucci et al., 2009). Thus, most of the hexane extractable material was likely to be rubber. To confirm this, FTIR and

Table 4
SEC analysis of standard inulin and inulin in the TK roots water extract.

	Inulin standard	Water Extract
Degree of Polymerization (DP)	28	20 ± 2
M_n (g/mol)	3537	2410 ± 179
M_w (g/mol)	4341	3950 ± 154
Polydispersity Index (d)	1.23	1.64 ± 0.10

Table 5
Composition of acetone extract.

Acetone extract		
Group	w/w dry TK root (%)	% acetone extract
Acetone extract		1.74 ± 0.11
Fatty acid C14-C18	0.18	10.34
Fatty acid C19-C24	0.08	4.60
Sterols	0.99	56.90
Steryl esters	0.16	9.20
Triglycerides	0.15	8.62

TGA analyses of TK root hexane-soluble material were conducted and compared with samples of *hevea* standard Vietnamese rubber grade L (SVR-L) rubber.

FTIR spectra for hexane-soluble material from TK root (Fig. 2) showed bands characteristic of *cis*-1,4 polyisoprene at 2960, 2925, 2853, 1663, 1447, 1375,and 837 cm^{-1} due to CH_3 stretching, CH_2 stretching, CH_2 stretching, C=C stretching, CH_2 deformation, CH_3 deformation, and C−H out-of-plane bending, respectively. The spectrum was very similar to that of hevea standard rubber. These results confirm that hexane extract of TK roots contained NR.

Nevertheless there were also bands not characteristic of *cis-1*,4-polyisoprene (Fig. 2). These include amine (N−H), and amide I (R_1−**C**=**O**-NH-R_2) and II (C−N, N−H) bands around 3400–3200 cm^{-1}, 1630 cm^{-1} and 1540 cm^{-1}, respectively (Rolere et al., 2015). Amide I and II are specific to peptide bonds and linked to proteins (Rolere et al., 2015). The band around 3400–3200 cm^{-1} corresponds to −O−H, possibly indicating some rubber oxidation (Montha et al., 2016). While all these bands were detected in the hevea NR sample, only a small peak for amide II was observed in TK NR from this study. The absorbance of the amide I band can be correlated with protein content in NR (Rolere et al., 2015) (2015), and, therefore, the absence of amide I band in TK NR indicates that protein was not likely extracted from TK roots with hexane.

Lipids in NR have been associated with ester (R_1−**(C**=**O)**−O−R_2) and carboxyl (R_1−**(C**=**O)**−OH) bands at 1748–1738 cm^{-1} and 1711 cm^{-1}, respectively (Rolere et al., 2015). An ester band was observed in both the hevea and TK NR samples. These results indicate that lipids such as triglycerides, waxes or sterol esters may be linked to macromolecules (Rolere et al., 2015) in hevea and TK NR. The carboxyl band can be associated with a high concentration of free fatty acids (Rolere et al., 2015). The absence of this carboxyl band in TK NR indicates that the preceding acetone extraction removed free fatty acids from the sample.

A comparison of TGA mass loss curves and TGA derivative profiles (DTGA) for TK and hevea NR are shown in Fig. 3. DTGA curves show that pyrolysis of rubber in both samples took place in two steps, the first was at the maximum peak or maximum decomposition temperature of 376 °C, and 374.6 °C for TK and hevea rubber, respectively, and a small shoulder at about 400–465 °C. To compare the two rubber samples based on TGA mass loss curves, three temperature regions were analyzed; ambient to 250 °C, 250–450 °C, and above 450 °C. Up to 250 °C, the weight loss of TK and hevea rubber were 0.4% and 1.6%, respectively. During the second stage, 99.1% and 98.9% of the initial weight were lost from TK and hevea rubber, respectively. At 600 °C, 0.1% and

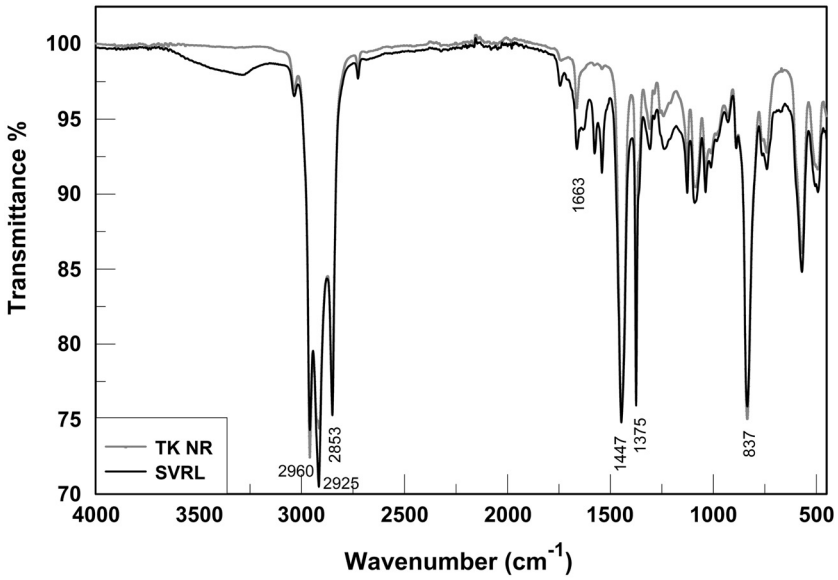

Fig. 2. Fourier transform infrared (FTIR) spectra of rubber extracted from *Taraxacum kok-saghyz* roots and from *Hevea brasiliensis*.

0.06% of the initial weight of TK and hevea rubber still remained. The DTGA and TGA mass loss curves for hevea rubber are similar to those reported previously (Bhowmick et al., 1987; Moreno et al., 2006). The TK NR DTGA and TGA mass loss curves were similar to those of hevea NR after lipid removal (Bhowmick et al., 1987). This indicates that the TK sample had a low lipid/resin content. TGA studies of hevea NR after lipid removal in nitrogen have shown that the degradation of rubber starts around 330 °C, and 400 °C is the temperature at which the degradation rate is maximal (Bhowmick et al., 1987). The mass loss from hevea and TK rubber at temperatures below 330 °C (Fig. 3) is due to the presence of non-rubber organic constituents.

One-dimensional NMR was performed to determine the chemical characteristics of the hexane extractable material. The ^{1}H and ^{13}C NMR of the hexane extract using CD_2Cl_2 as a solvent were consistent with NR (Fig. 4A and B). ^{1}H NMR showed three main peaks located at 5.16, 2.06, and 1.68 ppm which are characteristic of olefinic protons (H−C═C), methylene protons (-CH_2-), and methyl protons (−CH_3−C═C), respectively (fig. 4A). ^{13}C NMR clearly showed five significant peaks at chemical shifts that are consistent with poly *cis*-1,4-isoprene at 135.77, 125.62, 32.77, 27.04, and 23.77 ppm. These are the result of the two ethylenic carbons (−C═C−), two methylenic carbons (−CH_2−), and methyl carbon (−CH_3), respectively. The NMR analyses were in agreement with previously reported analyses of TK NR (Musto et al., 2016).

GPC-RI analysis of the TK root hexane extract showed a unimodal molecular weight distribution with a long tail towards low molecular weight rubber (Fig. S4). Characterization of TK root hexane extract with GPC-MALS gave a M_n 2.42×10^5 g/mol, M_p 5.96×10^5 g/mol, M_w 7.23×10^5 g/mol, M_z 1.72×10^6 g/mol and polydispersity of 2.98. Additionally, a small peak was also identified out of the rubber peak in GPC-RI (Fig. S4) which may indicate the presence of unextracted resin.

3.6. Unextractable components of TK roots

Approximately 33% of the TK root consisted of material that was not extractable by hot water, acetone or hexane (Table 6). This fraction of TK roots was composed primarily of lignocellulosic material, un-

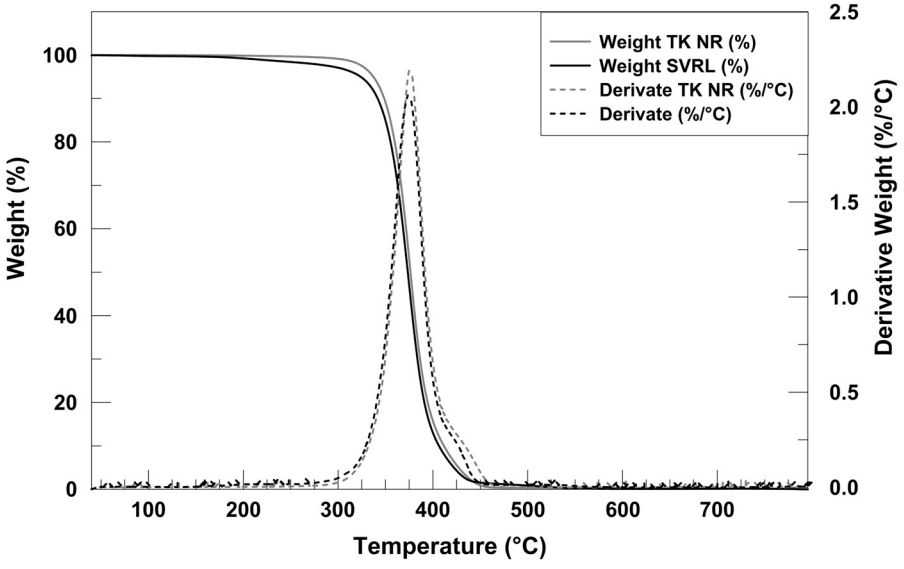

Fig. 3. Thermogravimetric analysis (TGA) and Derivative Thermogravimetric Analysis (DTGA) of natural rubber extracted from *Taraxacum kok-saghyz* roots and from *Hevea brasiliensis*.

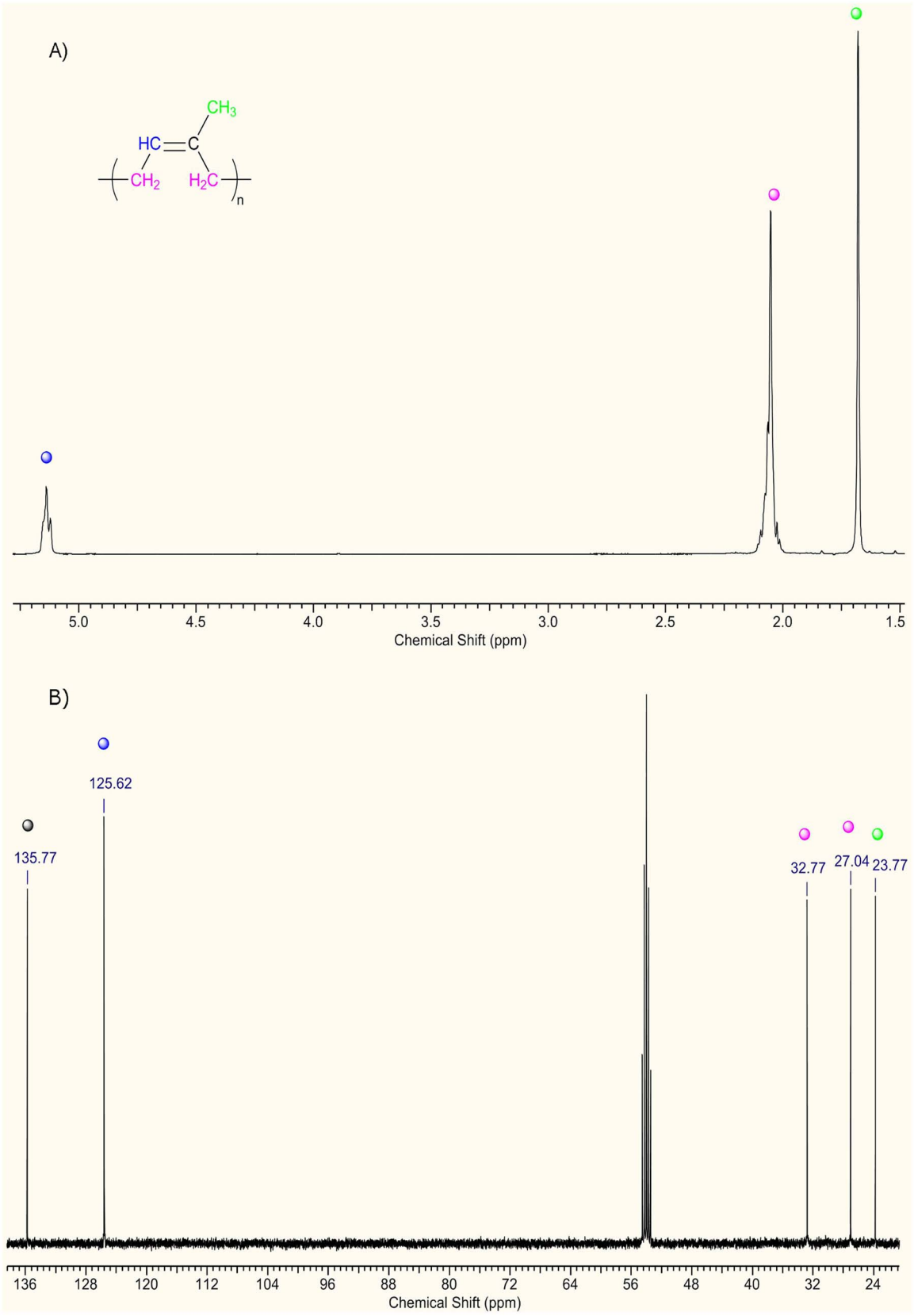

Fig. 4. Nuclear Magnetic Resonance (NMR) analysis of hexane extract from roots of *Taraxacum kok-saghyz*. A) ^{1}H NMR and B) ^{13}C NMR.

extractable proteins, and ash (Table 6). Cellulose was the greatest single component followed by hemicellulose, lignin, non-extracted proteins, pectin, and ash (Table 6). Lignin is normally divided into acid insoluble (AIL) and acid soluble (ASL) fractions. Average AIL and ASL were 4.5% w/w and 0.5% w/w of dry root weight (13.55% and 1.5% w/w dry extracts-free), respectively. The mass closure was 91% of the dry extracts-free TK roots.

Table 6
Composition of the unextractable fraction of TK roots.

Composition of unextractable fraction of TK roots		
Compound	w/w dry TK root (%)	w/w unextractable material (%)
Unextractable Material	**33.21 ± 2.26**	
Lignocellulosic fraction	**24.38 ± 0.76**	**73.41 ± 5.50**
Cellulose	8.98 ± 0.44	27.04 ± 2.27
Glucan	8.98 ± 0.44	27.04 ± 2.27
Hemicellulose	6.89 ± 0.23	20.75 ± 1.57
Xylan	2.16 ± 0.11	6.50 ± 0.55
Galactan	1.47 ± 0.06	4.43 ± 0.35
Arabinan	2.17 ± 0.18	6.53 ± 0.70
Mannan	0.24 ± 0.05	0.72 ± 0.16
Acetate	0.85 ± 0.05	2.56 ± 0.23
Pectin	3.46 ± 0.33	10.42 ± 1.22
Lignin	5.05 ± 0.48	15.21 ± 1.78
non-lignocellulosic fraction	**5.93 ± 0.64**	**17.86 ± 2.28**
Non-extracted protein	4.68 ± 0.37	14.09 ± 1.47
Ash	1.25 ± 0.52	3.76 ± 1.59
Mass closure (%)		**91.27**

3.7. Overall composition of TK roots

Overall mass closure was calculated by summing the masses of water-, acetone-, hexane-soluble materials and the acid soluble (ASF) and acid insoluble (AIF) fractions of the unextractable material of TK roots and dividing this by the original root dry mass (Fig. 5). Mass balance closure was greater than 95%. Water, acetone and hexane extract represented 59.6%, 1.7% and 5.4% of the root dry mass, respectively. Cellulose, hemicellulose, acid soluble lignin and pectin constituted 9.0%, 7.1%, 0.5%, and 3.5% w/w of the root dry mass, respectively. Acid insoluble lignin, protein and ash constituted 4.5%, 4.7%, and 1.2% of the root dry mass, respectively. The total lignin (ASL 0.5% and AIL 4.5%) was 5.0% of the dry TK roots.

Root composition was also calculated based on the total crude protein, ash, crude fat, lignin, and phenol (Table 7). Total carbohydrates were calculated as the sum of saccharides in both water extract (31.9%) and extracts-free TK roots (19.3%). Protein was determined from the proteinogenic amino acids profile of non-extracted TK roots. Ash was measured by thermal oxidation of the whole root. Crude fat was measured based on ether extraction. Lignin was quantified after the acid hydrolysis of the extracts-free TK roots. Moisture content in the representative TK roots after drying was 7.3% and the calorific value of the roots measured as higher heating value (HHV) was 17,012 J/g (Table 7).

4. Discussion

4.1. TK roots compositional analysis

A key economic driver of the development of TK as alternative source of NR is the large quantity of byproducts that result from extracting and purifying its NR. These byproducts can be separated and potentially transformed into bioproducts and/or materials of value to various commercial sectors within a biorefinery concept. This compositional analysis was used to identify and quantify valuable components in the TK roots.

Compositional analyses of various biomass feedstocks have been conducted previously to assess them as potential sources for biofuels (Agblevor et al., 2004; Augustus et al., 2003; Chen et al., 2007; Gao et al., 2013; Hames, 2009; Scurlock et al., 2000; Szczerbowski et al., 2014; Thammasouk et al., 1997), and establish their potential as renewable resources of energy and chemicals (Chow et al., 2008; Kalita and Saikia, 2004).

In this study a compositional analysis of TK roots was conducted to assess its potential value as a biorefinery feedstock. The water-, acetone-, and hexane-soluble as well as the insoluble, or the extracts-free, components in TK roots were identified and quantified. Mass closure of these individual fractions was greater 90% or greater, giving an overall mass closure greater than 95%. Additionally, fatty acids and amino acids profiles, and elemental analysis for TK roots were also generated. A previous study analyzing the chemical composition of TK roots was mainly focused on determining the content of ash, acetone extract, holocellulose, α-cellulose and lignin in TK roots, without including inulin, protein and rubber content (Zhuo et al., 2015). The authors reported similar values for ash (10.65% w/w dry TK root) and acetone extract (1.61% dry TK root). Previous measurements of the ash content of field grown TK roots by our group have found lower ash contents of 4% g ash/g dw which are more similar to other roots crops. Even though the TK roots were washed prior to analysis, it is possible that the high ash contents observed in our, and the previous study by Zhou et al., could be attributed to dirt remaining on the roots. However, cellulose and lignin content were 27% and 12% w/w dry TK roots, respectively. The higher content of cellulose could be because inulin was not extracted and some of its saccharides were quantified as cellulose. In the case of lignin, protein could have interfered with its quantification in the previous study. This analysis did not include further details on monosaccharides and extract composition. Therefore, the present study is the first comprehensive compositional analysis generated for a representative sample of field grown TK roots (to our knowledge), and as such, it represents a reference which will allow differences across germplasm, seasons and cultivation practices to be assessed during the domestication and development of TK as an alternative crop for NR production. In addition, this analysis has highlighted specific components which may be valorized or which must be addressed in the development of efficient extraction and purification processes.

4.2. TK NR

Non-polar solvents such as hexane, cyclohexane, toluene, among others are used to solubilize NR from rubber bearing-plants (Pearson et al., 2010; Pearson et al., 2013; Post et al., 2012). Hexane extraction was conducted immediately after acetone extraction of water extracted TK roots to extract NR in this study. FT-IR, TGA, NMR, and GPC analyses of hexane extract (Figs. 2–4, and S4 respectively) indicated that the extracted rubber contained only a low concentration of non-rubber constituents.

Previous studies have shown that TK natural rubber is very similar to hevea NR. The macro-molecular structure and composition of TK NR has not been studied in the same detail as NR from *Hevea brasiliensis*. However, TK NR is belived to consist of similar long chains of *cis*-1,4-polyisoprene, linked to non-rubber constituents, lipids and proteins, generating a branching or network topology (Toki et al., 2008; Xu et al., 2015). Studies on NR biosynthesis in TK rubber particles − the active structures where rubber biosyntheis take place inside specialized latex-producing parenchyma cells (laticifers)- have shown that this is similar to that in hevea (Schmidt et al., 2010a; Schmidt et al., 2010b). Rubber particles in TK contain rubber with a purity greater than 95% (Schmidt et al., 2010b). Moreover, similar non-rubber components have been reported in both TK and hevea NR (Cornish et al., 2015; Musto et al., 2016). Additionally, TK NR under mechanical strain behaves like hevea NR (Ikeda et al., 2016). Both undergo strain-induced crystallization (SIC) under tension, which has been attributed to the non-rubber constituents (Musto et al., 2016). FTIR, TGA, and ^{1}H and ^{13}C NMR confirmed the predominance of *cis*-1,4-polyisoprene in the hexane extract from TK roots. However, FTIR results also pointed out a lack of proteins and lipid peaks in TK NR which are characteristic of hevea NR. This was confirmed by TGA analysis showing less mass loss from TK NR than from hevea NR at temperatures below 350 °C (Fig. 3). Previous studies

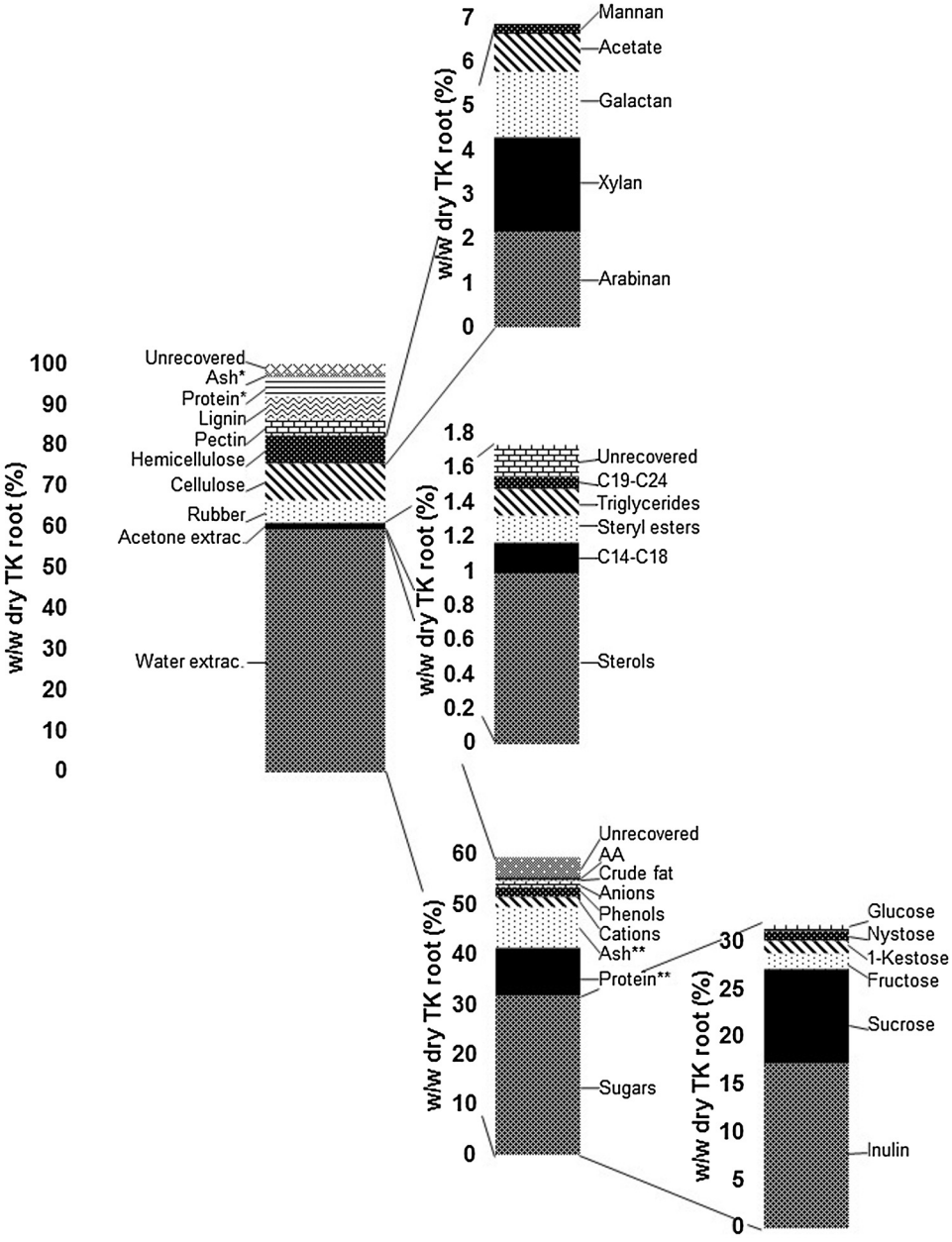

Fig. 5. Composition of *Taraxacum kok-saghyz* (TK) in whole roots and in various extract fractions and in hydrolyzed unextractable root material. *Protein and ash content measured in the unextractable fraction of TK roots. ** Protein and ash content measured in water extracts.

Table 7
Overall composition of whole field grown TK roots.

Component	w/w dry TK root (%)
Moisture	7.31 ± 0.57
Ash[a]	11.78 ± 0.65
Lignin	5.05 ± 0.48
Carbohydrates	51.22 ± 1.08
Protein	12.44 ± 0.26
Crude fat	5.17 ± 0.91
Phenols	1.92 ± 0.00
Unrecovered	5.11 ± 3.95
Calorific value (J/g)	17012 ± 36

[a] Ash value was measured in TK root prior to extraction.

of TK NR have shown M_w 1.4 × 10^6 g/mol (Musto et al., 2016). The lower M_w observed in rubber samples extracted by hexane in the current study likely is a consequence of the high temperatures used by the ASE that may have reduced the M_w of the rubber, since NR is a thermodegradable polymer (Li et al., 2000). The wide peak and left tail in Fig. S4 can be attributed to rubber degradation of NR since this is characteristic of rubber degradation (Enoki et al., 2003). These results demonstrate that the ASE method of extraction, using high temperature acetone and hexane, likely disturbed the network topology of TK NR by removing non-rubber components leading to the scission of *cis*-1,4-polyisoprene.

It should be noted that the superior properties of NR compared with synthetic rubbers are attributed to the non-rubber constituents, mainly proteins and lipid/resins. Some of the non-rubber constituents are linked to the linear chain of rubber and participate actively in the natural occurring network of natural rubber (Amnuaypornsri et al., 2008). This network gives NR a characteristic molecular weight distribution (M_w) and determines the physical and mechanical characteristics of NR (Amnuaypornsri et al., 2008; Baranwal and Stephens, 2001; Sakdapipanich and Rojruthai, 2012). These characteristics are due to the self-reinforcement capacity, which is manifested by strain-induced crystallization (SIC) (Ikeda et al., 2016). That is, NR is an amorphous material in undeformed state, but it is highly crystallizable upon stretching (Ikeda et al., 2016). Thus, any industrial processes used to

isolate proteins, amino acids and/or fatty acids from TK roots as co-products during the extraction of TK NR should avoid not adversely impacting NR mechanical and physical properties.

4.3. Other TK root components of interest to support TK NR economics

The significant amount of water extractable material in TK roots and the high concentration of water-soluble carbohydrates and proteins represents an opportunity for economic valorization of these components. Inulin, the most abundant component of the water extract, is a linear chain of β-2,1-linked D-fructofuranose molecules, containing one terminal glucose residue (Chi et al., 2011). Inulin is widely distributed among plants and serves as a reserve carbohydrate (Apolinário et al., 2014). Inulin is used in a large variety of food and pharmaceutical applications, including as a low calorie sweetener, fat substitute, prebiotic and drug delivery vehicle (Apolinário et al., 2014). The global market for inulin was 2.46×10^8 kg in 2013 and is expected to reach 4×10^8 kg by 2020, with a value of US$2.35 billion. This has resulted in expansion of inulin producing crops, mainly in Europe (Grand View Research, 2016). The plants used most commonly for commercial production of inulin are *Helianthus tuberosus* (Jerusalem artichoke) and *Cichorium intybus* (chicory) (Chi et al., 2011). The concentration of inulin and average DP of Jerusalem artichoke and chicory are 17% w/w dry mass (Johansson et al., 2015) and 6 (Flamm et al., 2001), and 15% w/w dry weight (Amaducci and Pritoni, 1998) and 10–20 (Flamm et al., 2001), respectively. The concentration and DP of TK inulin, 17% w/w dry TK root and 20, respectively, suggest that TK roots could also be a commercially viable source of inulin, especially as a rubber co-product.

Inulin is also receiving attention as a relatively inexpensive and abundant feedstock for the production of biofuels and biochemicals (Chi et al., 2011; Yang et al., 2011). Recent studies indicate the possibility of using inulin as a source of sugars for ethanol, single cell oil for biodiesel production, citric acid, 2,3-butanediol, lactic acid, and sugar alcohols (Chi et al., 2011; Ujor et al., 2015). Hydrolysis of inulin and the dehydration of the resulting fructose and glucose to form 5-hydroxymethylfurfural (5-HMF) is another potential pathway to important renewable fuels and valuable chemicals. Several of its derivatives such as 2,5-dymethylfuran (DMF), 2,5-furfurydiisocyanate, and 5-hydroxymethyl furfurylidenester, are value-added fuels and starting materials in the polymer industry for the preparation of polyamides, and polyurethane (Tong et al., 2010; Yang et al., 2011).

Soluble proteins and the amino acids they contain represent a relatively large fraction of TK roots that could be considered as valuable byproducts. Several previous studies have evaluated co-production of protein and/or amino acids from biomass feedstocks within a biorefinery (Bals and Dale, 2011; Chiesa and Gnansounou, 2011; Dale et al., 2009; Kammes et al., 2011; Lammens et al., 2012; Laser et al., 2009; Scott et al., 2007; Widyarani et al., 2016; YingLai, 2014). Protein can be recovered and used for animal feed and even in the human diet (Chiesa and Gnansounou, 2011; Kammes et al., 2011; Laser et al., 2009), or hydrolyzed and used as a platform for the production of several biochemicals (Lammens et al., 2012; Scott et al., 2007; Widyarani et al., 2016; YingLai, 2014). The most abundant amino acids in TK roots can serve as building blocks for a number of chemicals of interest in the petrochemical, plastic, polymer, cosmetic industries (Lammens et al., 2012; Scott et al., 2007).

In previous studies of water-soluble extracts from plant materials, such as corn stover (Chen et al., 2007) and switchgrass (Chen et al., 2010), complex mixtures of over 30 different compounds were found. The components included sugars, alditols, organic acids, cations, anions and lignin monomers. In this study, two main sugars (fructose and glucose) in addition to fructan oligo- and poly-saccharides were found. Neither alditols nor organic acids were detected in the water extract with the methods used in the present study.

The presence of large amounts of water-soluble material in TK roots poses a challenge during root washing. Root washing is required to remove soil from roots since it would contaminate downstream processing, but inulin and protein could be lost in this step. Use of hot water during washing, and storage in hot places in which there could be contact with water should be avoided. There are several options for root-washing that need to be further investigated to determine the best method for TK root washing. The use of cold water is preferable since inulin is not soluble in such conditions (Berghofer et al., 1993). Some of the methods that can be explored are: drying sieving, rotary drum washing, flotation, use of chemicals to facilitate washing, water/air high pressure cleaner, washing roots in frozen state (Böhm, 1979; Laufenberg and Meuser, 2015).

The unextractable part of TK roots was analyzed following the widely used National Renewable Energy Laboratory (NREL) method for the analysis of structural carbohydrates in biomass (NREL, 2008). The results showed that cellulose and hemicellulose carbohydrates were the dominant components in the extracts-free TK roots, representing 47% of the dry weight (16% w/w dry TK roots). The hemicellulose had a high arabinose content (31% of the hemicellulose, 2% w/w dry TK roots) which is unusual for dicotyledonous plants, but similar to that found in *Gramineae* such as cereal straws (Zhao et al., 2012).

Carbohydrates are a source of fermentable sugars for the production of biofuels and commodity chemicals (Zhao et al., 2012). Given the high concentration of C5 sugars in TK roots, microorganisms capable of fermenting both C6 and C5 sugars would be preferred for the efficient conversion of the TK roots' lignocellulosic fraction to bioproducts.

Acetone has commonly been used to solubilize resins from rubber-bearing plants, such as guayule (*Parthenium argentatum*) (Black et al., 1983; Pearson et al., 2013; Salvucci et al., 2009), sunflower (*Helianthus annuus*) (Pearson et al., 2010), and also TK (Buranov and Elmuradov, 2010). However, the acetone extract is normally quantified gravimetrically but not characterized. The chemicals quantified in our study accounted for 90% of the acetone extractable material.

The fatty acids in TK roots were typical of the saturated and unsaturated C16 and C18 fatty acids commonly found in plants and oil seeds (Jaworski and Cahoon, 2003). Complex mixtures of fatty acids like this are not well-suited for industrial applications, which prefer a mixture that predominantly contains a single fatty acid containing double bounds or specific functional groups. For example, fatty acids with high oxidative stability are preferred for lubricants, while drying oils are manufactured from readily oxidized fatty acids (Jaworski and Cahoon, 2003). However, extraction and isolation of unsaturated fatty acids can potentially lead to chemicals useful in cross-linking applications, such as coatings and resins (Meier et al., 2007).

4.4. Dynamics of TK root composition

In previous research on wild TK roots, rubber and inulin concentrations and the DP of the inulin showed a seasonal dependence (Kreuzberger et al., 2016). Rubber concentrations ranged from 4% to 9%, and inulin concentrations were between 5% to 29% with a DP ranging from 8 to 29 (Kreuzberger et al., 2016). The results obtained in this study were within these ranges. The maximum rubber and inulin concentration, and inulin DP reported previously were obtained in plants 16 months old. Likewise, maximum root biomasses were reported at the same time (Kreuzberger et al., 2016). Comparing the highest rubber and inulin concentration in 7–8 month old TK plants studied by Kreuzberger et al. (2016) to those of a similar age used in this study, reveals that TK plants grown in Ohio contained 35% and 10% more rubber and inulin, respectively. Ohio biennial plants might generate even greater concentrations of rubber and inulin. However, the plants used for this study were transplanted instead of sowed, which could favor their productivity, and climatic conditions could also influence these values.

Based on the previous results (Kreuzberger et al., 2016), it would seem reasonable to conduct compositional analysis on TK roots produced in a biennial crop system, but, this system has yet to be tested

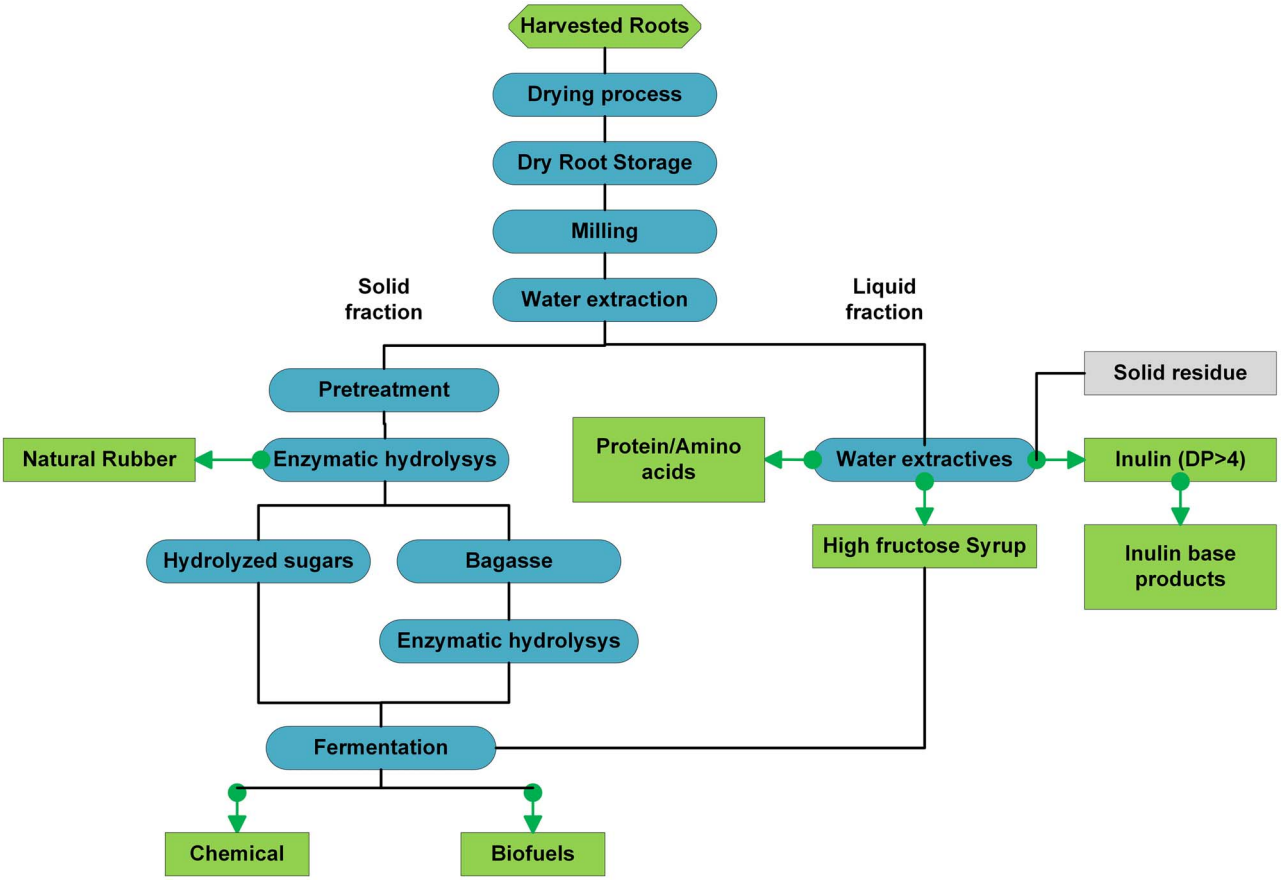

Fig. 6. Proposed process diagram for a *Taraxacum kok-saghyz* root biorefinery.

and its feasibility assessed in Ohio, USA. Overwintered TK plants in Ohio were highly prone to summer die back after spring flowering which would hinder production in a biennial system (Cornish et al., 2016). This research suggests that TK crops in Ohio should be planted as early as possible in the spring, and harvested as late as possible in the autumn to maximize growing days and product yield. This agrees with earlier studies conducted by the US Department of Agriculture who proposed TK planting in spring or summer to obtain better root yield in the first year (Whaley and Bowen, 1947). However, a seed crop would be overwintered as a biennial because seed production in the following spring is much greater than in the first year.

There is still debate about the best agronomic practices for TK production for maximizing rubber yield. Nonetheless, this study shows TK as a potential source of not only NR, but also inulin, C5-C6 sugars, and protein/amino acids, which would valorize TK and turn it into a biorefinery feedstock with economic benefit. Thus, any discussion on TK production should also build on the possibility of obtaining multiple valorized products.

4.5. Proposed biorefinery based on TK roots

Chemical characterization of TK root components through compositional analysis is an important first step in determining the potential for the valorization of TK roots. It provides insights into potential co-products and treatments and technology for processing. Based on the compositional analysis, a scheme for the processing of TK roots for the production of NR and multiple co-products was developed

The first step in the proposed process is water extraction of inulin and fructan oligo- and mono-saccharides, and protein/amino acids from TK roots. NR, a component of specific interest, is associated with the lignocellulosic fraction and can be separated by removing the fraction mechanically (Eskew, 1946) or enzymatically using cellulase and hemicellulase as previously proposed by Wade and Swiger (2013). To liberate NR and release cellulose and hemicellulose, a pretreatment process, such as alkaline and/or acid pretreatment, may be needed. Monosaccharides from the lignocellulosic fraction and those from water extraction could be combined and fermented for chemical and/or biofuels production. Insoluble residues could be incinerated to create a source of heat energy for the biorefinery, or used as a soil-amendment to return carbon and minerals to the land (Adom et al., 2014).

Based on the characterization of TK roots and this scheme (Fig. 6), potential yields of various biobased products were calculated (Table 8). TK field trials conducted at OARDC/OSU Wooster, Ohio showed that when 1.2 million TK plants can be planted per hectare and that the mean dry weight root per plant was approximately 6 g. Using these values, the theoretical yields of TK compounds per hectare were estimated using the equation below, where X represents the concentration of the compound of interest per gram of dry weight root.

$$TK\ root\ product\left(\frac{Kg}{ha}\right) = \frac{1.2 \times 10^6 plant}{ha} \times \frac{6g\ DW\ root}{plant} \times \frac{Xg\ compound\ of\ interest}{g\ DW\ root} \times \frac{1kg}{1000g}$$

For products obtained through the fermentation of sugars (ethanol, 2,3-butandiol, succinic acid, acetone-butanol-ethanol (ABE)), the TK root fermentable sugars were multiplied by theoretical fermentation yields (Adom et al., 2014; Gao et al., 2010; Ujor et al., 2015; Wang et al., 2016) (Table 8).

A highlight of this analysis is its ability to estimate the potential of TK roots as a biorefinery feedstock and estimate yields of potential

Table 8
Potential yields of NR and other bioproducts from TK roots within a biorefinery context.

Potential biobased chemical		Production	Units	Yield	Source
Rubber		389	kg/ha	1.0	Hexane extract
Inulin		1248	kg/ha	1.0	Inulin
Protein		702	kg/ha	1.0	Water soluble protein
	Arginine	202	kg/ha	1.0	Water soluble protein
	Aspartic acid	149	kg/ha	1.0	Water soluble protein
	Glutamic acid	99	kg/ha	1.0	Water soluble protein
	Proline	114	kg/ha	1.0	Water soluble protein
Fructose and glucose		1051	kg/ha	1.0	Water soluble oligo- and mono-saccharides
Cellulose		647	kg/ha	1.0	Lignocellulosic fraction
Hemicellulose		496	kg/ha	1.0	Lignocellulosic fraction
Ethanol		794	kg/ha	0.721[a]	Water soluble sugars (Inulin not included)
Ethanol		944	kg/ha	0.738[a] and 0.721[a]	Cellulose and hemicellulose
Ethanol		612	kg/ha	0.49[b]	Inulin
2,3-Butandiol		600	kg/ha	0.48[c]	Inulin
Succinic acid		782	kg/ha	0.71[a]	Water soluble sugars (Inulin not included)
Acetone butanol ethanol (ABE)		792	kg/ha	0.33[d]	Water soluble sugars

a. (Adom et al., 2014); b. (Wang et al., 2016); c. (Gao et al., 2010); d. (Ujor et al., 2015).

products from TK roots, including rubber, inulin, and protein. There is one commercial source in production, hevea, and two alternative sources of NR currently under development: guayule and TK. To compare the production of rubber from these three sources, the full cycles of plant establishment and production must be considered. For the production of hevea NR, there is a period of seven years for establishment, after which yields range from 500 to 2200 kg dry rubber/ha/yr for up to 25 years (Mak et al., 2008; van Beilen and Poirier, 2007b). In the case of guayule, there is a 1.5–3 year growth period before harvest after which the yield ranges from 300 to 1000 kg dry rubber/ha (Ray et al., 2005). In contrast, TK can be established and harvested in the same year. The potential yield of rubber and inulin from TK obtained in this study was 389 and 1248 kg/ha, respectively (Table 8). Earlier studies of TK rubber production reported rubber yields ranging from of 73–122 kg/ha for two year TK stands (Whaley and Bowen, 1947). Recent investigations using wild TK seeds showed maximum rubber and inulin yields of 62 kg/ha and 209 kg/ha, respectively, for a 16 month old crop (Kreuzberger et al., 2016). The greater yield of rubber estimated in this study is due to increased planting density and improved germplasm. Current breeding and agronomy research is likely to increase rubber yield potential from TK (Cornish et al., 2016), especially as chemical weed control will allow higher planting densities. Studies of 8 month old TK plants grown from wild seeds in small plots showed that the estimated rubber and inulin yields could be 47 kg/ha and 311 kg/ha, respectively (Arias et al., 2016).

The primary commercial source of inulin is chicory (*Cichorium intybus*), a biennial plant with a yield of 5000 kg inulin/ha (De Leenheer, 1994). This is much greater than the potential yield of 1248 kg/ha for TK (Table 8). However, since TK can be harvested annually rather than every other year (as for chicory) the yield of inulin per year from TK is much closer to that of chicory.

Plant protein is another potential product from TK. TK plant protein can be compared with plant protein produced as 'leaf protein concentrate' from alfalfa (*Medicago sativa*). This source has a yield of 876 kg/ha/yr as compared with TK with a yield of 702 kg/ha/y of soluble protein.

While none of the individual TK product yields exceed those of commercial sources, taken together the value of all TK products may generate a value greater than that for other crops. Results presented in Table 8 are for an estimated production level, and actual production levels could be affected by variables such as plant growing conditions, process parameters, and economic, and other factors. Further development of a biorefinery approach for TK roots would benefit from an appropriate techno-economic analysis to assess its feasibility.

5. Conclusions

A compositional analysis of field harvested TK roots was conducted to establish a detailed baseline and to identify components of the roots that may have commercial value. This analysis will be the foundation upon which future work on breeding, germplasm development, seasonal and cultivation practices are built.

Based on the compositional analysis, and current TK field plant density data, yields of products from TK roots within a biorefinery concept were determined. In this scheme, inulin and proteins are water extracted, NR is released after chemical pretreatment and enzymatic hydrolysis of extracted roots and sugars are fermented to chemicals and/or biofuels.

These results indicate that TK could be an economical biorefinery feedstock for the production of NR, inulin, protein, chemicals and biofuels. These results provide valuable data for the development of techno economic models for alternative natural rubber production from TK.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.indcrop.2017.05.043.

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