

Characterization of transfructosylating activity enzyme from tubers of tropical Jerusalem artichoke (*Helianthus tuberosus* L.) for production of fructooligosaccharides

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Abstract

A crude extract from tubers of tropical Jerusalem artichoke (*Helianthus tuberosus* L.) with high transfructosylating activity was purified by two chromatographic steps (anion exchange and affinity chromatography). Gel filtration under native conditions revealed that the purified enzyme had molecular mass approximately 75 kDa whereas SDS-PAGE indicated it was dimeric protein with molecular mass approximately 66 and 25 kDa. The optimal pH and temperature of purified enzyme were 5.4 and 35°C, respectively. The activity of enzyme was enhanced by pyridoxal-HCl, while KI had an effect to inhibit its activity. Using sucrose as substrate, the K_m values for transfructosylating activity were rather high. This enzyme might be sucrose: sucrose fructosyltransferase (SST) because it can use sucrose as the smallest donor substrate in synthesis of fructooligosaccharides (FOS) with degree of polymerization (DP) less than 5 as major products. When 0.26 U of purified enzyme was incubated with 0.46 M sucrose as substrate at pH 5.4 and 35°C for 144 h, about 54.46% short chain fructooligosaccharides (scFOS, DP<5) was produced. Additionally, the scFOS showed growth promoting to *Bifidobacterium* sp. indicated it has prebiotic property by supporting the growth of probiotics. It might be an alternative means for biosynthesis of scFOS by SST from plant for applications in functional foods and nutraceuticals industry.

Keywords

Tropical Jerusalem
 artichoke
Helianthus tuberosus L.
 Transfructosylating activity
 Sucrose: sucrose
 fructosyltransferase
 Short chain
 fructooligosaccharides

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Introduction

Fructooligosaccharides (FOS) are fructose polymers with a degree of polymerization (DP) in range of 2-10. According to their health promoting properties, they are classified as a prebiotic and widely added in various food products. FOS was not digested in the human small intestine, but fermented in colon where the growth of probiotics such as *Lactobacillus* and *Bifidobacteria* were stimulated (Robertfroid *et al.*, 1993). FOS is a member of a fructan group and highly stored in underground organs of many *Asteraceae* species (Vijn and Smeekens, 1999). Among them, Jerusalem artichoke (*Helianthus tuberosus* L.), domesticated plant in temperate regions, accumulated high contents of FOS with average DP of 6-10 (16% dry weight). Inulin and FOS from Jerusalem artichoke are currently used as functional food ingredient in food industry, feed industry and also considerable for biorefinery industry (Long *et al.*, 2015; Tiengtam *et al.*, 2015; Yang *et al.*, 2015). They are synthesized by the catalytic action of

enzymes with transfructosylating activity in vacuole of plant cells. It is classified as fructosyltransferase (FTase, E.C.2.4.1.9). FTase catalyzes the transfer of fructosyl group to sucrose molecule or FOS (Antosova and Polakovic, 2001). Edelman and Jefford proposed model of long chain FOS (lcFOS) synthesis in plant by concerted action of two FTases, sucrose: sucrose fructosyltransferase (SST, E.C.2.4.1.99) initiated reaction by catalyzing transfer of a fructosyl residue from sucrose to another sucrose molecule, resulting in the formation of 1-kestose (GF2) and fructan: fructan fructosyltransferase (FFT, E.C.2.4.1.100) functioned to elongate the fructose chains in afterward (Edelman and Jefford, 1968). This model was approved by Koops and Jonker (Koops and Jonker, 1994; 1996). It was led to the alternative means for FOS synthesis with expected degree of polymerization under *in vitro* condition which was controllable. Due to the quality and quantity of FOS derived from plants were varied upon plant species, stages, cultivation conditions, harvest time and extraction methods, therefore the *in vitro* enzymatic synthesis of FOS

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should be considered. Temperate Jerusalem artichoke was imported and improved to grow in Thailand where located in tropical region (Judprasong *et al.*, 2011). Most grown varieties still accumulated high contents of FOS in range of 20.8-23.3 g/100g dry weight (Judprasong *et al.*, 2011; Tanjor *et al.*, 2012). This might be considered to either use as FOS or FTases source for further applications. Many studies have purified and characterized enzymes in group of FTases from Jerusalem artichoke; however, all of those were obtained from plants which grown in temperate regions and purified by many purification steps that was not suitable for FOS synthesis in up scaling (Praznik *et al.*, 1990; Lüscher *et al.*, 1993; Koops and Jonker, 1994; 1996). FTases from tubers of tropical Jerusalem artichoke which was improved and grown as potential FOS source for supplying food sections in Thailand has not so far purified and characterized, hence, this present work has aimed to purify and characterize FTases from tubers of tropical Jerusalem artichoke variety grown in Thailand by using two chromatographic steps. In addition, FOS produced by action of purified enzyme and evaluation of their prebiotic property was also conducted for further application in food industry. This investigation might be beneficial for alternative FOS production under in vitro conditions.

Materials and Methods

Plants and chemicals

Tropical Jerusalem artichoke (*Helianthus tuberosus* L. var no.2) tubers were supplied by Research Institute, Petchaboon province, Thailand. All chemicals used were obtained from the Sigma-Aldrich Company, Singapore otherwise specified.

Preparation of tropical Jerusalem artichoke plants

Plants were grown in green house of Department of Biotechnology, Faculty of Engineering and Industrial Technology, Silpakorn University, Nakhonpathom province, Thailand. The sprouts were germinated from tuber bud in pot containing sandy loam soil. Plants were watered thrice a week. The plant samples were harvested at designed times and stored at -80°C until analysis.

Sugar extraction and analysis

The sugar was extracted from roots or tubers of tropical Jerusalem artichoke. Samples were homogenized in 80% ethanol with ratio of 1:2 (w/v). The homogenate was incubated for 10 min at 95°C and filtered through filter paper (3M). The residue was extracted three times. The extracts were concentrated

with evaporator. Total sugars and reducing sugars were determined by phenol sulfuric method (Dubois *et al.*, 1956) and DNS method (Miller, 1959), respectively. Glucose was used as standard for total sugar and reducing sugar analysis. The FOS contents were estimated as non-reducing sugar which derived from total sugar subtracted by reducing sugar.

Crude protein extraction

The crude protein was extracted from the frozen tubers (-80°C) following the method described for SST (Praznik *et al.*, 1990) with some modifications. Plant samples were chopped into small pieces and homogenized by kitchen blender. The enzyme extraction buffer (0.007 M potassium phosphate buffer (pH 7.0) containing 0.5% Triton X-100 and 0.5% cysteine) was added in ratio of 1:1 (w/v). The extraction was incubated for 16 h at 4°C. The slurry was filtered through cheese cloth and centrifuged at 10,000×g for 10 min. The proteins were concentrated by Amicon Ultra-4 (10K) centrifugal filter unit.

Enzyme purification

The crude enzyme solution was applied to an anion exchange column (Q Sepharose® Fast Flow, XK26, Amersham Pharmacia, Sweden) equilibrated with 50 mM potassium phosphate buffer (pH 6.5). The non-adsorbed proteins were eluted with the starting buffer, and the adsorbed proteins and enzymes were eluted with a linear gradient of NaCl (0 to 1 M) in the same buffer at a flow rate of 1 ml min⁻¹. The proteins were concentrated by Amicon Ultra-4 (10K) centrifugal filter unit. The concentrated enzymes with transfructosylating activity were further purified by applying to an affinity chromatography column (Con A® Fast Flow, XK13, Amersham Pharmacia, Sweden) equilibrated with 50 mM potassium phosphate buffer (pH 6.5) containing 0.5 M NaCl, 1 mM MnCl₂, and 1 mM CaCl₂. The non-adsorbed proteins were eluted with the starting buffer, and the adsorbed proteins and enzymes were eluted (0-100%) with 50 mM potassium phosphate buffer (pH 6.5) containing 50 mM NaCl, 1 mM MnCl₂, 1 mM CaCl₂, and α -methylmannopyranoside (C₇H₁₄O₆) at a flow rate of 1 ml min⁻¹. The fractions containing transfructosylating activity were pooled and concentrated by Amicon Ultra-4 (10K) centrifugal filter unit. The protein content from each step of purification was determined by the Bradford (Bradford, 1976) using bovine serum albumin (BSA) as a standard.

Enzyme assay

Transfructosylating activity was determined

at 34°C for 24 h. The enzyme reaction mixture composed of 100 µl crude or purified enzyme, 400 µl of 0.1 M potassium phosphate buffer (pH 5.4) containing 0.02% sodium azide and 0.46 M sucrose as substrate. The reaction was stopped by heating at 95°C for 2 min and centrifuged at 10,000×g for 5 min. Reducing sugar (R) released at the end of reaction was determined with the Somogyi-Nelson method (Nelson, 1994) and glucose (G) released was analyzed by glucose oxidase peroxidase method. To calculate the amount of fructose transferring (F⁷), two times of glucose content was subtracted by reducing sugar content (2G-R). One unit of transfructosylating activity was defined as the amount of enzyme transferring a fructosyl group from sucrose donor to sucrose acceptor that produces 1 µmole of transferred fructosyl in 1 min under the conditions described above.

Estimation of protein molecular mass

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine protein purity and the molecular mass of the enzymes under the denaturing condition, with 12% acrylamide gel. The proteins were stained with Coomassie blue. A mixture of known molecular weight proteins (Vivantis) was used as molecular mass markers. Electrophoresis was performed at 30 mA for 3–4 h. In addition, protein purity and molecular mass under non-denaturing condition was also investigated by gel filtration chromatography. The protein was applied to Sephacryl S-200 column, XK13 equilibrated with 50 mM potassium phosphate buffer (pH 6.5) at a flow rate of 1 ml min⁻¹. The volume of buffer to elute protein containing transfructosylating activity compared to the calibration curve of molecular mass standard and elution volume was used to estimate molecular mass. Lysozyme (MW 14600), trypsin (MW 23800), lipase (MW 45000), BSA (MW 66000), and amyloglucosidase (MW 97000) were used as molecular mass standards.

Characterization of enzyme activity

The activity and stability of the enzyme were measured at various pH values from 3.0 to 10.0 using 0.1 M citrate phosphate (pH 3.0-6.0), 0.1 M potassium phosphate (pH 6.5-7.5), 0.1 M Tris (pH 7.5-9.0), and glycine NaOH buffer (pH 9.0-10.0). The enzyme solution was maintained for 48 h and the residual activity was measured using standard assay conditions. The effect of temperature on the maximum reaction rate was studied from 4 to 70°C at optimum pH. Thermo-stability was determined by incubating the enzyme at temperatures from 4

to 70°C for 48 h. The residual enzyme activity was measured using standard assay conditions. The effect of various metal ions (K⁺, Ca²⁺, Cu²⁺, Mg²⁺, Mn²⁺, Na⁺, Li⁺) and reagents (SDS, EDTA, pyridoxal HCl, mercaptoethanol, PMSF, isopropanol) on transfructosylating activity was studied by the determination of the enzyme activity in a solution consisting 0.1 M potassium phosphate buffer (pH 5.4) containing 0.02% sodium azide and 0.46 M sucrose.

Determination of kinetics parameters

The initial reaction rate was determined for transfructosylating activity under its optimum conditions (pH 5.4, 35°C), at various sucrose concentrations (0.26-1.00 M sucrose). For each condition, five samples were collected within 30 min of reaction, at a constant time interval. 1-Kestose (GF2) produced at the end of reaction was determined by HPLC with refractive index detection using Rezek RNM carbohydrate column, equilibrated and eluted with deionized distilled water at flow rate 1 ml min⁻¹, 45°C. One unit of transfructosylating activity was defined as the amount of enzyme transferring a fructosyl group from sucrose donor to sucrose acceptor that produces 1 µmole of 1-kestose in 1 min under the conditions described above.

FOS production by purified enzyme

From our previous experiments, the production of 1-kestose (GF2) with the highest amount compared with others short chain FOS (scFOS) was obtained within 144 h of partial purified enzyme incubation (Ngampanya et al., 2012) therefore incubation time for FOS production in this study was 144 h. The enzyme reaction mixture composed of 2 ml purified enzyme (0.26 U), 0.1 M potassium phosphate buffer (pH 5.4) containing 0.02% sodium azide and 0.46 M sucrose as substrate. The reaction was incubated for 144 h at 35°C. The enzyme reactions were stopped by heating at 95°C for 2 min and centrifuged at 10,000 g for 5 min. The products from enzyme reaction mixtures were analyzed by HPLC.

Determination on growth promoting to probiotics

The scFOS produced by the purified enzyme and reference sugars (commercial chicory inulin, sucrose, glucose, and fructose) were used as carbon source for determination on growth promoting of *Bifidobacteria* sp. *Bifidobacteria* sp. was inoculated in basal MRS broth plus L-cysteine added by the scFOS or reference sugars with a same final concentration of 2% (w/v). The cultures were incubated at 37°C for 48 h under anaerobic conditions in anaerobic jar. Sample was taken at every 3 h interval for bacterial enumeration

Table 1. The non-reducing sugar content and transfructosylating activity of crude extract from roots and tubers of tropical Jerusalem artichoke

Plant age (days)	Non-reducing sugar content (mg/g Fw)*		Transfructosylating activity (U/ml enzyme)*	
	Roots	Tubers	Roots	Tubers
60	17.181±1.076	-	-	-
90	31.955±1.932	-	0.026±0.013	-
105	41.851±1.791	113.025±5.058	0.012±0.003	0.103±0.002
120	42.039±0.387	112.885±2.274	0.005±0.005	0.098±0.008
135	25.195±0.798	90.061±2.784	0.001±0.001	0.086±0.004
150	31.654±0.761	59.762±2.053	-	0.069±0.021
180	-	84.554±0.887	-	0.033±0.004

* Mean ± S.D. (n=3)

- not determine

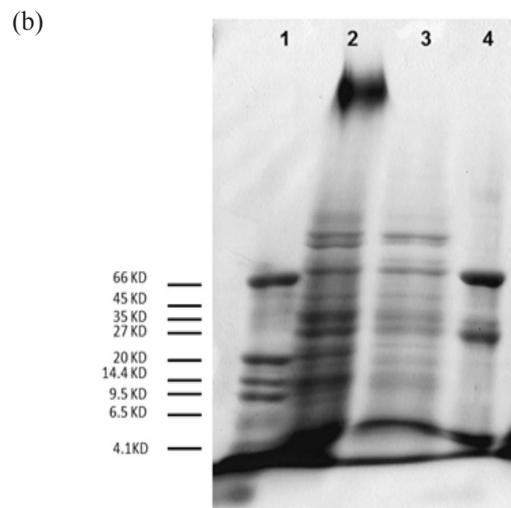
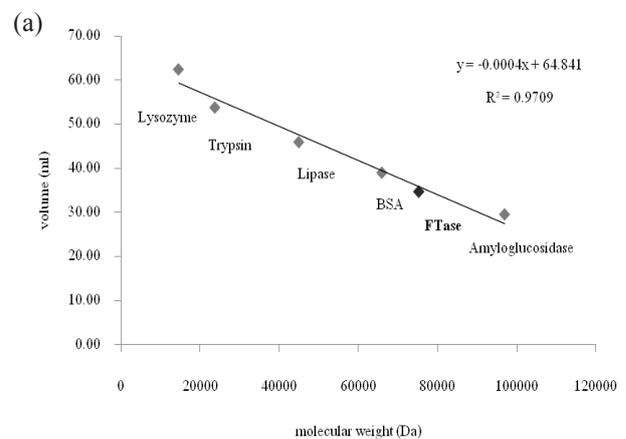
by serial dilution and plate counts and measuring the absorbance at 600 nm. The pH, reducing sugar and total sugar concentration of the cell-free supernatant from cultures was also determined by pH meter, DNS method (Miller, 1959) and the phenol-sulfuric acid method (Dubois *et al.*, 1956) respectively.

Results and Discussion

The correlation of FOS content and transfructosylating activity during developing stage of tropical Jerusalem artichoke was reported. The accumulation of FOS content determined as non-reducing sugars from roots to tubers was detected during rapid development in tuber filling stage (105-120 days after cultivation) as shown in Table 1. The high content of non-reducing sugar in 105-120 days old tubers was also correlated with transfructosylating activity of crude extract. Hence, the tubers of tropical Jerusalem artichoke might be a candidate source of transfructosylating activity enzymes.

Purification and characterization of enzyme

As shown in Table 2, after crude extract was purified by anion exchange chromatography (Q Sepharose column) and affinity chromatography (ConA Sepharose column), the purified enzyme had specific activity of 5.39 and 44.40 U/mg proteins respectively. The purification fold was increased from 5.26 to 43.28 when crude enzymes were applied to Q Sepharose and ConA Sepharose column, respectively. It indicates those tubers were suitable for use as a candidate source of FTases production. Considering to molecular weight of the purified enzyme as shown in Figure 1a, the molecular weight of native form of the purified enzyme in present study was 75.3 kDa. Two bands of proteins with molecular mass approximately 66 and 25 kDa were appeared



Lane 1: Proteins molecular weight standard marker (Vivantis)
 Lane 2: Crude enzymes
 Lane 3: The purified enzyme after Q sepharose purification
 Lane 4: The purified enzyme after ConA sepharose purification

Figure 1. Molecular weight estimation of the purified enzyme by gel filtration chromatography (a) and SDS-PAGE (b)

Table 2. Purification of transfructosylating activity enzyme from 105-120 days old tuber of tropical Jerusalem artichoke

Purification steps	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification (fold)	Yield (%)
Crude extract	38.11	37.14	1.03	1.00	100.00
Q sepharose + ultrafiltration	9.82	1.82	5.39	5.26	25.77
ConA sepharose + ultrafiltration	6.50	0.15	44.40	43.28	17.06

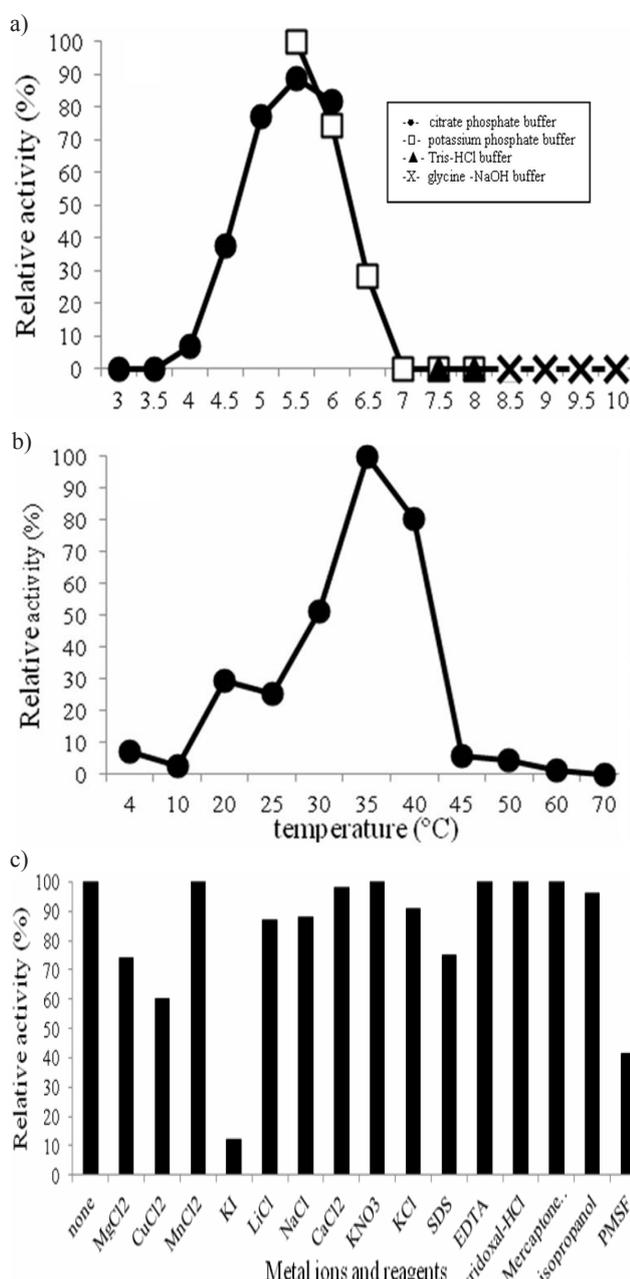


Figure 2. Effect of pH (a), temperatures (b) and ions (c) on enzyme activity

under denaturing conditions as can be seen in Figure 1b. It suggested that the purified enzyme was dimeric protein. Plant FTases are glycoproteins containing 20-50% of carbohydrates in the mass of their molecules. Generally, molecular weight of plant FTases are in range of 60-85 kDa. They are formed from two different subunits having molecular weight about 50-60 and 22-27 kDa (Antosova and Polakovic, 2001). The molecular mass of SST in native form from tubers of temperate Jerusalem artichoke in previous reports were 67 (Praznik *et al.*, 1990), 67 (Koops and Jonker, 1996), and 59 kDa (Luscher *et al.*, 1996). In addition, all SST previously reported also composed of two subunits with molecular mass about 55 and 27 (Koops and Jonker, 1996) 59 and 26 kDa (Luscher *et al.*, 1996). It is possible that the purified enzyme in this study is SST. This is because it consisted of 2 subunits whereas FFT had been reported that it had 5 subunits (Luscher *et al.*, 1993; Koops and Jonker, 1994). In addition, SST could synthesis only short chain FOS (scFOS, DP≤5) and use sucrose as substrate donor meanwhile FFT could synthesis FOS with higher DP and use 1-kestose and higher DP as donor substrate. Considering to the method of purification (anion exchange and affinity chromatography), the specific activity of SST from tubers of tropical Jerusalem artichoke (Table 2) was higher than those of SST from temperate Jerusalem artichoke tubers (Praznik *et al.*, 1990; Koops and Jonker, 1996). It may due to the effects of plant varieties, developmental stage (age of tuber), geography and cultivation conditions.

The pH ranges used in this study (3.0-10.0) affected to the transfructosylating activity of the enzyme. As it can be seen in Figure 2a, the optimal pH for the enzyme was in range of 5-6 and the highest activity of enzyme was reached at pH 5.4. The residual activity of enzyme was found to be higher than 70% at pH of 4.5-6.5. The SST showed

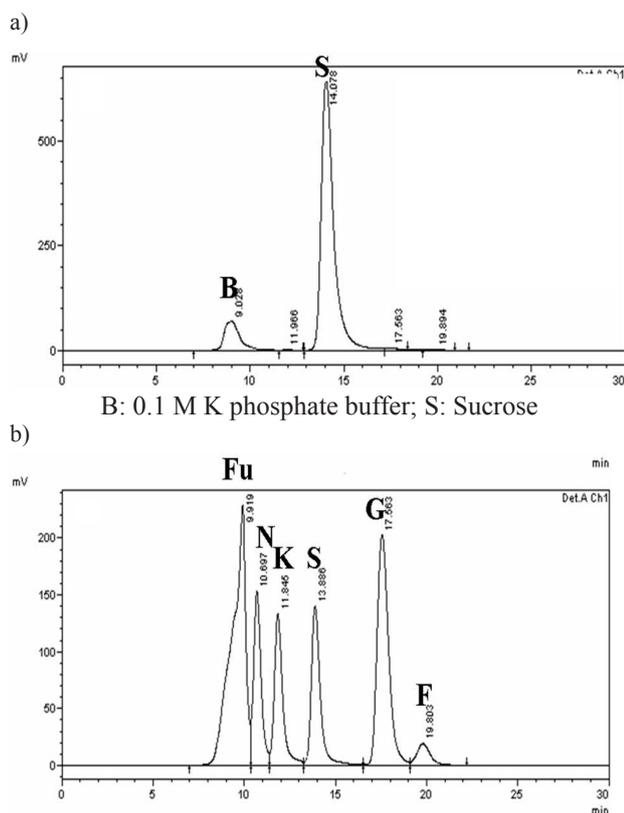


Figure 3. FOS production by SST from 105-120 days old tubers of tropical Jerusalem artichoke at 0 (a) and 144 h (b)

F: Fructose; G: Glucose; S: Sucrose; K: 1-kestose (GF₂); N: nystose (GF₃); Fu: 1- β -fructofuranosylnystose (GF₄)

optimum activity at pH 5.4 when 0.46 M sucrose was used as substrate. In case of SST isolated from tubers of *Helianthus tuberosus* Colombia, it had optimal pH in range of 3.5-5.0 (Koops and Jonker, 1996). The similar range of optimal pH for SST of both tropical and temperate Jerusalem artichoke indicated that it was mild acidic enzyme.

The optimum temperature for enzyme activity was 35°C as shown in Figure 2b. When enzyme was incubated at temperatures in range of 4 to 70°C for 48 h, the residual enzyme activity of higher than 80% was detected at temperatures lower than 35°C. The enzyme was not stable at 40 to 70°C. The SST in this study showed the highest activity at 35°C however it had been reported that this enzyme had the highest activity at lower temperature (20-25°C) (Koops and Jonker, 1996). It might be explained in term of different cultivated locations, the adaptation of Jerusalem artichoke grown in tropical regions including Thailand for survival under elevated temperature. Therefore, the plant had to change in their physiology and metabolism involved enzymes in the pathway. High contents of FOS had been reported in various varieties of Jerusalem artichoke grown in Thailand (Judprasong *et al.*, 2011; Tanjor *et al.*, 2012). They suggested that the enzymes involved

in FOS synthesis had slightly different properties among each variety. In addition, the SST was not stable at higher temperature after it was extracted from tubers.

As shown in Figure 2c, the activity of enzyme was slightly increased in reaction mixtures containing MnCl₂ (104.33%) and KNO₃ (101.87%) whereas MgCl₂, CuCl₂, KI, NaCl, CaCl₂ and KCl inhibited enzyme activity. In agreement with the inhibitory effects of Cu²⁺ and Ca²⁺ on activity of SST of *Helianthus tuberosus* Colombia (Koops and Jonker, 1996) and *Allium cepa* (Shiomi *et al.*, 1985). The activity of SST detected in this study was also inhibited by various divalent metal ions such as Cu²⁺ and Ca²⁺. In addition, enzyme activity was enhanced by EDTA (102.95%), pyridoxine-HCl (115.34%), and mercaptoethanol (105.84%). The similar effect of pyridoxyl-HCl to increase SST activity had been also reported in *Helianthus tuberosus* Colombia (Koops and Jonker, 1996). Sodium dodecyl sulfate (SDS), isopropanol and PMSF inhibited activity of the enzyme. In general, the SST from tubers of Jerusalem artichoke grown in tropical region and in temperate region had similar properties except for the optimal temperature.

The kinetics parameters for transfructosylating activity of the enzyme were determined using the Michaelis-Menten model. The K_m and V_{max} of the enzyme were 0.372 M and 1.218 $\mu\text{mol}\cdot\text{ml}^{-1}\cdot\text{h}^{-1}$, respectively. This was higher than the K_m (0.042 M) of SST purified from dormant tubers of Jerusalem artichoke by using the same two chromatographic steps (Praznik *et al.*, 1990). The K_m value of SST purified from dormant tubers reported by Praznik and co-worker was about one-tenth compared to it was reported by Scott and co-worker (Scott *et al.*, 1966). Praznik suggested that it might be due to enzyme was prepared by different methods. The different in developmental stage (age of tubers) may affect to enzyme properties as well. The enzyme purified from dormant tubers may have an SST isoform that is not active. In case of SST purified by six steps to 655-fold, the rate of GF₂ formation from sucrose is barely saturable and K_m could not be calculated (Koops and Jonkers, 1996). The rate of GF₂ formation versus sucrose concentration curves of reaction are often interpreted as first-order Michaelis-Menten equations with K_m values usually higher than 0.1 M (Mahler and Cordes, 1971). It indicated the requirement of high substrate (sucrose) concentrations for FOS synthesis. In fructan accumulating plant, such as Jerusalem artichoke and chicory, they imported sucrose resulting from photosynthesis into vacuole where FOS synthesized enzymes located. The high accumulation

of imported sucrose in vacuole was then converted to FOS by using rather transfructosylating activity than hydrolysis activity of FTase in order to make them survive under cold and drought conditions (Vijn and Smeekens, 1999). In addition, FTase has a little affinity towards water as acceptor which means that the hydrolase activity of FTase is very low (Antosova and Polakovic, 2001). This may explain why the SST required high sucrose concentration as substrate.

FOS production by the purified SST

According to 1-kestose (GF_2) was synthesized in the highest amount compared with others short chain FOS within 144 h incubation in our previous report (Ngampanya et al., 2012), therefore the production of FOS by using SST was incubated for 144 h. Short chain FOS consisted of 1-kestose (GF_2), nystose (GF_3) and 1- β -fructofuranosylnystose (GF_4) were synthesized by action of the purified SST within 144 h of incubation in 0.1 M potassium phosphate buffer (pH 5.4) containing 0.02% sodium azide and 0.46 M sucrose as substrate (Figure 3). About 75% of substrate was used during enzymatic synthesis reaction. SST catalyzes the irreversible transfer of a fructosyl unit (F) from a donor molecule (sucrose, GF) to an acceptor molecule of sucrose when 1-kestose (GF_2) and glucose (G) are formed (Antosova and Polakovic, 2001). The occurrence of nystose (GF_3) and 1- β -fructofuranosylnystose (GF_4) might be a result of self-transfructosylations with GF_2 and GF_3 as substrates yielding GF_3 and GF_4 . This was also found in the FOS synthesis by SST purified from tubers of *Helianthus tuberosus* Colombia (Koops and Jonkers, 1996). For synthesis of long chain FOS (up to DP 20), FFT need to be involved using 1-kestose as donor substrate (Luscher et al., 1993; 1996). These synthesized FOS with short chains could be used as low calorie sweetening agents or prebiotics in food products. According to residual substrate (sucrose) still remained in the scFOS mixture about 25%, ratio of enzyme and substrate (sucrose) should be further investigated in order to obtain high purity of scFOS. Although commercial production of FOS is performed by using β -fructofuranosidase (FFase) from bacteria (*Aureobasidium pullulans*) and fungi (*Aspergillus niger*) with about 60% yield (Yoshikawa et al., 2008). However, the main component of FOS in commercial production is 1-kestose whereas FOS produced by SST from plant (Jerusalem artichoke) had comparable yield (approximately 55%). Interestingly, the scFOS has mixed DP with very high in DP5. So that molecular weight distribution and oligosaccharides profile of commercial FOS and the scFOS produced in this study are different in term

of prebiotic property. To bring plant SST for up-scale or commercial production, several approaches could be considered. The enzyme is collectible from waste during processing of tubers of Jerusalem artichoke. The enzyme can be sequenced and mimicked for production in bioreactor similar to current industrial production of FOS. Plant tissue culture is being invested in our research group to produce callus accumulated very high content of scFOS.

Growth promoting to probiotics

The growth of *Bifidobacteria* sp. was higher when glucose and fructose were used as carbon source while the scFOS and sucrose showed similar effects to promote growth of probiotic. Additionally, commercial FOS from chicory root had lower effect to stimulate growth of probiotic. The preferable of bacteria to metabolize sugar in a reduced form supported the high growth rate of *Bifidobacteria* sp. when glucose and fructose was used as sole carbon. Considering to the same concentration of total sugar in MRS broth L-cysteine and the lower level of reducing sugar in medium supplemented the scFOS compared to those media containing glucose and fructose, it may suggest that the scFOS had an effect to stimulate the growth of *Bifidobacteria* sp. From the results indicated that the scFOS produced in this study by SST from tropical Jerusalem artichoke had prebiotic property that can be used as functional food ingredient.

Conclusions

A crude extract from tubers of tropical Jerusalem artichoke (*Helianthus tuberosus* L.) was purified by anion exchange and affinity chromatography. Gel filtration under native conditions predicted that the purified enzyme had molecular mass approximately 75 kDa whereas SDS-PAGE indicated it was dimeric protein with molecular mass approximately 66 and 25 kDa. The optimal pH and temperature of purified enzyme were 5.4 and 35°C, respectively. The activity of enzyme was enhanced by pyridoxal-HCl, while KI had an effect to inhibit its activity. Using sucrose as substrate, the K_m and V_{max} values for transfructosylating activity were 0.372 M and 1.218 $\mu\text{mol}\cdot\text{ml}^{-1}\cdot\text{h}^{-1}$, respectively. This enzyme suggested to be sucrose: sucrose fructosyltransferase (SST) because it can use sucrose as donor substrate in synthesis of fructooligosaccharides (FOS). Moreover, it can synthesize mostly of short chain FOS with $DP \leq 5$. About 54.46% scFOS ($DP \leq 5$) was produced when 0.26 U of SST was incubated with 0.46 M sucrose as substrate at pH 5.4 and 35°C for

144 h. In addition, the scFOS was also shown on growth promotion of *Bifidobacterium* sp. indicated it has prebiotic property by supporting the growth of probiotics.

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References

- Antosova, M. and Polakovic, M. 2001. Fructosyltransferases: the enzymes catalyzing production of fructooligosaccharides. *Chemical Papers* 55(6): 350–358.
- Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956. Calorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28: 350–356.
- Edelman, J. and Jefford, T. G. 1968. The mechanism of fructosan metabolism in higher plants as exemplified in *Helianthus tuberosus*. *New Phytologist* 67: 517-531.
- Judprasong, K., Tanjor, S., Puwastien, P. and Sungpuang, P. 2011. Investigation of Thai plants for potential sources of inulin-type fructans. *Journal of Food Composition and Analysis* 24: 642-649.
- Koops, A. J. and Jonker, H. H. 1994. Purification and characterization of the enzymes of fructan biosynthesis in tubers of *Helianthus tuberosus* 'Colombia'. *Journal of Experimental Botany* 45: 1623-1631.
- Koops, A. J. and Jonker, H. H. 1996. Purification and characterization of the enzymes of fructan biosynthesis in tubers of *Helianthus tuberosus* Colombia. II. Purification of sucrose:sucrose 1-fructosyltransferase and reconstitution of fructan synthesis in vitro with purified sucrose:sucrose 1-fructosyltransferase and fructan:fructan 1-fructosyltransferase. *Plant Physiology* 110: 1167-1175.
- Long, H. X., Shao, H. B., Liu, L., Liu, L. P. and Liu, Z. P. 2015. Jerusalem artichoke: a sustainable biomass feedstock for biorefinery. *Renewable and Sustainable Energy Reviews* 54: 1382–1388.
- Luscher, M., Frehner, M. and Nosberger, J. 1993. Purification and characterization of fructan: fructan fructosyltransferase from Jerusalem artichoke (*Helianthus tuberosus* L.). *New Phytologist* 123: 717-724.
- Luscher, M., Erdin, C., Sprenger, N., Hochstrasser, U., Boller, T. and Wiemken, A. 1996. Inulin synthesis by a combination of purified fructosyltransferases from tubers of *Helianthus tuberosus*. *FEBS Letters* 385: 39-42.
- Mahler, H. R. and Cordes, E. H. 1971. *Biological chemistry*. 2nd ed. London: Harper & Row.
- Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* 31: 420-428.
- Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *The Journal of Biological Chemistry* 153: 375-380.
- Ngampanya, B., Keayarsa, S., Ngermmeesri, K., Prakobtran, P. and Wichienchot, S. 2012. Production of fructo-oligosaccharides by partial purified fructosyltransferase from variety of Jerusalem artichoke grown in Thailand. *Proceedings of the 7th International Symposium of the Protein Society of Thailand*, p 172-177. Bangkok: the Protein Society of Thailand.
- Praznik, W., Beck, R. H. F. and Spies, T. H. 1990. Isolation and characterisation of sucrose:sucrose 1F-β-D-fructosyltransferase from tubers of *Helianthus tuberosus* L. *Agricultural Biology and Chemistry* 54(9): 2429-2431.
- Roberfroid, M. B. 1993. Dietary fiber, inulin, and oligofructose: a review comparing their physiological effects. *Critical Reviews in Food Science and Nutrition* 33(2): 103-148.
- Robertfroid, M. B., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R. A. and Rowland, I. 2010. Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition* 104: S1–S63.
- Scott, R. W., Jefford, T. G. and Edelman, J. 1966. Sucrose fructosyltransferase from higher plant tissues. *Biochemical Journal* 100: 23-24.
- Shiomi, N., Kido, H. and Kiriyama, S. 1985. Purification and properties of sucrose: sucrose 1F-β-D-fructosyltransferase in onion seeds. *Phytochemistry* 24: 695-698.
- Tanjor, S., Judprasong, K., Chaito, C. and Jogloy, S. 2012. Inulin and fructooligosaccharides in different varieties of Jerusalem artichoke (*Helianthus tuberosus* L.). *KKU Research Journal* 17(11): 25-34.
- Tiengtam, N., Khempaka, S., Paengkoum, P. and Boonanutanasarn, S. 2015. Effects of inulin and Jerusalem artichoke (*Helianthus tuberosus*) as prebiotic ingredients in the diet of juvenile Nile tilapia (*Oreochromis niloticus*). *Animal Feed Science and Technology* 207: 120–129.
- Vijn, I. and Smeekens, S. 1999. Fructan: more than a reserve carbohydrate? *Plant Physiology* 120: 351–359.
- Yang, L., He, Q. S., Corscadden, K. and Udenigwe, C. C. 2015. The prospects of Jerusalem artichoke in functional food ingredients and bioenergy production. *Biotechnology Reports* 5: 77–88.
- Yoshikawa, J., Amachi, S., Shinoyama, H. and Fujii, T. 2008. Production of fructooligosaccharides by crude enzyme preparations of β-fructofuranosidase from *Aureobasidium pullulans*. *Biotechnology Letters* 30: 535–53.