

ผลของการบริโภคแก่นตะวันแบบสดและแบบอบแห้งต่อปริมาณ *Bifidobacterium* spp. ในอาสาสมัครชาวไทย

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บทคัดย่อ

ปัจจุบันมีการนำเข้าแก่นตะวัน (*Helianthus tuberosus* L.) เพื่อเพาะปลูกในประเทศไทยและส่งเสริมให้เป็นอาหารสุขภาพเนื่องจากมีอินูลิน (inulin) เป็นส่วนประกอบในปริมาณสูง อย่างไรก็ตามยังไม่มีข้อมูลสำหรับปริมาณแก่นตะวันแบบสดและแบบอบแห้งต่อการบริโภคในแต่ละวัน งานวิจัยนี้จึงได้ศึกษาผลของการบริโภคแก่นตะวันแบบสดและแบบอบแห้งต่อปริมาณของ *Bifidobacterium* spp. ในลำไส้ โดยแบ่งอาสาสมัครเป็น 2 กลุ่ม กลุ่มแรก 77 คนบริโภคแก่นตะวันแบบสดปริมาณ 150 กรัมต่อวัน (ประกอบด้วยอินูลิน 29.1 กรัม) และกลุ่มที่สอง 55 คนบริโภคแบบอบแห้งปริมาณ 32 กรัมต่อวัน (เตรียมจากแก่นตะวันสด 150 กรัม) เป็นเวลา 4 สัปดาห์ เก็บตัวอย่างอุจจาระของอาสาสมัครก่อนและหลังบริโภคแก่นตะวันครบ 4 สัปดาห์ อาสาสมัครต้องบันทึกการบริโภคแก่นตะวันทุกวัน เมื่อสิ้นสุดการทดลองมีอาสาสมัครจำนวน 46 คนและ 30 คนที่บริโภคแก่นตะวันแบบสดและแบบอบแห้งทุกวันครบ 4 สัปดาห์ตามลำดับ ผลการวิเคราะห์ปริมาณของ *Bifidobacterium* spp. ในอุจจาระของอาสาสมัครด้วยเทคนิค real-time polymerase chain reaction (PCR) ซึ่งมียีนเป้าหมายคือยีน xylulose-5-phosphate/fructose-6-phosphate phosphoketolase (*xfp*) พบว่าหลังบริโภคแก่นตะวันทั้งแบบสดและแบบอบแห้งอาสาสมัครที่มีปริมาณของ *Bifidobacterium* spp. ในอุจจาระเพิ่มขึ้นมีจำนวนมากกว่าอาสาสมัครที่มีปริมาณเชื้อลดลงอย่างมีนัยสำคัญทางสถิติ ($P < 0.001$) และพบว่าปริมาณของ *Bifidobacterium* spp. ในอุจจาระของอาสาสมัครทั้งสองกลุ่มหลังการบริโภคสูงกว่าก่อนบริโภคอย่างมีนัยสำคัญทางสถิติ ($P < 0.001$) นอกจากนี้ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของปริมาณเชื้อระหว่างกลุ่มบริโภคแบบสดและแบบอบแห้ง ($P = 0.314$) การศึกษาครั้งนี้แสดงผลของแก่นตะวันต่อการเพิ่มจำนวนของ *Bifidobacterium* spp. ในลำไส้ซึ่งเป็นข้อมูลที่สนับสนุนการบริโภคแก่นตะวันเป็นอาหารสุขภาพอีกทางเลือกหนึ่ง

คำสำคัญ: *Bifidobacterium*, อินูลิน, อาหารสุขภาพ, แบคทีเรียที่ดีต่อสุขภาพ

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Bifidogenic effect from consumption of fresh and oven-dried Jerusalem artichoke (*Helianthus tuberosus* L.) in Thai subjects

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Abstract

Recently, Jerusalem artichoke (*Helianthus tuberosus* L.) has been introduced to Thailand and promoted as a potential health food because of its high inulin content. However, the effect of daily intake of fresh (FJA) or oven-dried (OJA) Jerusalem artichoke on intestinal *Bifidobacterium* has not yet been investigated. To evaluate the bifidogenic effect of FJA and OJA intake, 77 volunteers received 150 g/d of FJA (containing 29.1 g of inulin) and 55 volunteers consumed 32 g/d of OJA (prepared from 150 g fresh weight) for 4 wk. Fresh stool samples were collected on days 0 and 28. During the experimental period, a daily intake of FJA or OJA was recorded. At the end of the study, 46 and 30 volunteers completely consumed the FJA and OJA daily for 4 wk, respectively. Quantitative analysis of fecal *Bifidobacterium* by a real-time polymerase chain reaction (PCR) technique targeting a xylulose-5-phosphate/fructose-6-phosphate phosphoketolase gene (*xfp*) revealed that the numbers of volunteers with increased fecal *Bifidobacterium* were significantly higher than those with decreased levels in both experimental groups ($P < 0.001$). In addition, the total numbers of fecal *Bifidobacterium* after both interventions were significantly increased ($P < 0.001$). Moreover, there was no significant difference in the changes of fecal *Bifidobacterium* numbers between FJA and OJA dietary groups ($P = 0.314$). In conclusion, the consumption of both FJA and OJA was effective in increasing intestinal *Bifidobacterium*, indicating that Jerusalem artichoke could be promoted as an alternative health food.

Keywords: *Bifidobacterium*, Inulin, Prebiotics, Probiotics

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Introduction

Inulin, a non-digestible food ingredient, is a polydisperse fructan with β (2 $\rightarrow$ 1) fructosyl-fructose glycosidic linkages with either fructose or glucose starting unit with a degree of polymerization between 2 and 60. It is found in a wide variety of plants such as chicory root, Jerusalem artichoke, garlic, asparagus, onion, wheat and banana. Because of its unique structure and physiological properties, inulin resists to digestive enzymes from saliva and small intestine. This allows inulin to arrive large intestine where it selectively stimulates growth of beneficial gut microflora, particularly *Bifidobacterium*⁽¹⁾. This organism plays a crucial role in the host well-being and health by various mechanisms such as maintenance of gastrointestinal tract normal state, provision of nutrients, alleviation of constipation, production of bacteriocins against pathogenic bacteria and stimulation of immune system⁽²⁾. Several studies have shown enhancing effect of inulin supplement on the amounts of intestinal *Bifidobacterium* by using various doses ranging from 2.5 to 40 g/d for 14 to 64 days^(1,3-9).

Jerusalem artichoke (*Helianthus tuberosus* L.) is a member of family *Asteraceae* and a native plant in the central United States and Canada, where it was cultivated by the American-Indians during the 15th century. It was introduced to Europe in the 17th century and is now grown in a wide cross section of countries including Europe and Asia. More recently, Jerusalem artichoke has been introduced to Thailand and a breeding program was initiated since then⁽¹⁰⁾. Judprasong et al.⁽¹¹⁾ reported high levels of inulin (19.4 $\pm$ 1.04 g/100 g fresh weight) in Jerusalem artichoke variety Kaentawan from Thailand. In 2009, Kanungskasem et al.⁽¹²⁾ reported the prevalence of inadequate fruit and vegetable consumption of more than 84% in men and more than 79 % in women from Thailand. This may cause a health problem among Thai people. In addition, the World Health Organization (WHO)

recommends a minimum daily intake of 400 g (or five servings) of fruit and vegetables to prevent chronic diseases such as heart diseases, cancer, diabetes and obesity. The fresh Jerusalem artichoke (FJA) can be taken as a side dish with salty or spicy foods such as chili paste, a common household Thai food. Therefore, Jerusalem artichoke could be an alternative health food in Thailand as well as other Asian countries. However, a recommended daily intake of the FJA for increasing intestinal *Bifidobacterium* has not yet been established. Therefore, this study focused on the bifidogenic effect of consuming the FJA. In addition, oven-dried Jerusalem artichoke (OJA) intake was also carried out in order to test whether it still increased intestinal *Bifidobacterium* after preserved in this form.

Materials and Methods

Subjects and experimental design

The experimental protocol was approved by the Ethics Committee of Khon Kaen University (Project ID: HE522016). The study was carried out according to the guidelines of Declaration of Helsinki. Informed consent was obtained from each volunteer after the experimental procedures had been completely explained. One hundred and thirty-two subjects, 97 females and 35 males, 18-67 years old, who had undergone no antibiotic treatment prior to the beginning of the study at least 1 wk, were participated and divided into two groups; 77 volunteers ingesting 150 g/d of FJA and 55 volunteers consuming 32 g/d of OJA for 4 wk. They were physically examined by a clinician prior to the starting of the study and instructed to maintain their dietary habits and physical activity patterns and to avoid taking antibiotics and probiotic products during the study period. Age, sex, weight and height of each volunteer were recorded. During the intervention period, the volunteers recorded their daily intake such as Jerusalem artichoke, probiotics and antibiotics.

Jerusalem artichoke preparation

Tubers of Jerusalem artichoke (variety Kaentawan obtained from Khon Kaen University, Thailand) were harvested after growing period of 120 days. They were peeled, washed and weighed at 150 g for daily intake. The OJA was prepared from 150 g fresh weight, all of which was chopped, oven-dried at 60°C for 8 h and crushed into small pieces for daily intake.

Sample collections

Freshly voided fecal samples were obtained from each volunteer on days 0 and 28. They were transported in a cooling box and delivered to the laboratory within 24 h. The fecal consistency was recorded as watery, loose, soft, formed and hard. The feces were weighed at 200 to 220 mg and then frozen at -20°C. The exact amount of each fecal sample was recorded.

DNA extraction from stool samples and reference strain

DNA from either stool samples or *Bifidobacterium breve* ATCC 15700 (MicroBioLogic, Minnesota, USA), a reference strain, was extracted by QIAamp DNA stool mini kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions and then stored at -20°C until analysis.

Quantitative analysis of fecal Bifidobacterium

A plasmid containing an *xfp* was constructed for generating a standard curve for quantitative analysis of *Bifidobacterium* by the real-time PCR. A 232 bp DNA fragment of the *xfp* from *B. breve* ATCC 15700 DNA was amplified using primers by the method of Cleusix et al.⁽¹³⁾. The DNA fragments were then purified by Wizard® PCR Preps DNA Purification System (Promega, Madison, USA), cloned into pGEM-T Easy vector (Promega) and transformed into *E. coli* JM109 (Promega). The plasmid DNA concentration was measured by NanoVue spectrophotometer (GE Healthcare, New Jersey, USA). Mass of one plasmid DNA copy was calculated as described by Loilome et al.⁽¹⁴⁾. The SYBR Green assay targeting the *xfp* was carried out

for quantitative analysis of fecal *Bifidobacterium* using Light Cycler 480 SYBRGreen Mastermix and LightCycler 480II instrument (Roche Diagnostic, Mannheim, Germany). Each fecal DNA sample was performed in triplicate and each run included the plasmid DNA as a control. The copy numbers of the *xfp* was calculated from the threshold cycle (C_t) values using the constructed standard curve and expressed as the log no. of cells/g of feces.

Data analysis

Statistical analysis was performed by the software package SPSS for Windows (version 19.0; SPSS, Inc. Armonk, NY, USA). The proportion of volunteers with increased fecal *Bifidobacterium* after the intervention was compared to that with decreased levels by a Chi-square test. All values were tested for normal distribution by a Kolmogorov-Smirnov test. Differences in the amounts of fecal *Bifidobacterium* between before and after the intervention were tested by a paired *t*-test, whereas those between FJA and OJA dietary groups were tested by a *t*-test. The *P* value of < 0.05 was considered significantly different.

Results

After the intervention period, 46 of 77 and 30 of 55 volunteers completely consumed the FJA and OJA daily for 4 wk, respectively. In addition, 22 of 77 and 18 of 55 volunteers ingested the FJA and OJA for approximately 3 wk respectively during the 4-wk intervention period. They were also included in order to compare with the volunteers that completed the study. The remaining volunteers who took antibiotics and/or probiotic products during the intervention period (9 subjects), who consumed the FJA or OJA less than 3 wk (4 subjects) or who did not submit the intake daily diary (3 subjects), were excluded from this study. The baseline characteristics of the volunteers are shown in **Table 1**. There was no significant difference between FJA and OJA intake groups in term of age and body mass index (BMI).

Table 1 Baseline characteristics of volunteers consuming Jerusalem artichoke

Characteristics	Volunteers consuming Jerusalem artichoke			
	Fresh		Oven-dried	
	3 wk (n=22)	4 wk (n=46)	3 wk (n=18)	4 wk (n=30)
Age (years)				
range	25-55	20-67	18-51	24-56
(means $\pm$ SD)	(37.73 $\pm$ 9.85)	(41.6 $\pm$ 10.52)	(35.89 $\pm$ 10.99)	(41.57 $\pm$ 10.39)
Body mass index (kg/m²)				
range	18.12-33.02	18.65-42.19	19.53-31.08	18.41-38.37
(means $\pm$ SD)	(23.88 $\pm$ 3.88)	(25.85 $\pm$ 5.56)	(24.44 $\pm$ 3.11)	(24.25 $\pm$ 4.01)
Male/female	6/16	12/34	3/15	9/21

The sensitivity and linearity for detection of *Bifidobacterium* by the SYBR Green assays ranged from 1.6×10^3 to 1.6×10^{10} cells/mL. The total numbers of fecal *Bifidobacterium* were presented as means $\pm$ SD of log no. of cells/g of feces. Among the volunteers consuming the FJA and OJA for 4 wk, 36 of the 46 volunteers (78.3 %) and 25 of the 30 volunteers (83.3 %) had increased fecal *Bifidobacterium* at the end of the study respectively (Table 2). The proportions

of volunteers with increased fecal *Bifidobacterium* were significantly higher than those with decreased or unchanged levels for both intervention groups ($P < 0.001$). A significant increase in the total numbers of fecal *Bifidobacterium* was also found in both groups ($P < 0.001$) (Table 3). In addition, the changes of fecal *Bifidobacterium* numbers after consuming the FJA and the OJA were not significantly different (Table 3).

Table 2 Changes of the amounts of fecal *Bifidobacterium* in volunteers after consuming Jerusalem artichoke

No. of volunteers with changes of the amounts of fecal <i>Bifidobacterium</i> (%)										
Groups	Fresh					Oven-dried				
	n	Increase	Decrease	No change	P value ^a	n	Increase	Decrease	No change	P value ^a
3 wk	22	17 (77.3)	5 (22.7)	0 (0)	0.011	18	13 (72.2)	5 (27.8)	0 (0)	0.059
4 wk	46	36 (78.3)	9 (19.5)	1 (2.2)	< 0.001	30	25 (83.3)	4 (13.3)	1 (3.3)	< 0.001

^aChi-square test

Table 3 Total numbers of fecal *Bifidobacterium* in volunteers before and after consuming Jerusalem artichoke

Groups	Means±SD of log no. of cells/g of feces										Fresh vs. Oven-dried
	Fresh					Oven-dried					
	n	Before	After	Change	<i>P</i> value ^a	n	Before	After	Change	<i>P</i> value ^a	
3 wk	22	7.63±1.08	8.19±1.15	0.56±0.98	0.015	18	6.48±1.43	6.95±1.60	0.46±1.25	0.134	0.797
4 wk	46	7.17±1.36	8.07±1.09	0.90±1.05	< 0.001	30	7.11±0.88	8.26±1.16	1.15±1.08	< 0.001	0.314

^apaired *t*-test, ^b*t*-test

Regarding to the volunteers receiving the FJA and OJA for 3 wk, 17 of 22 (77.3 %) and 13 of 18 volunteers (72.2 %), respectively had increased fecal *Bifidobacterium* (Table 2). A significant difference between the proportion of volunteers with increased fecal *Bifidobacterium* and that with decreased and unchanged levels was found in the FJA dietary group only ($P = 0.011$). Similarly, there was a significant increase in the amounts of fecal *Bifidobacterium* in the FJA dietary group only ($P = 0.015$) (Table 3). The amounts of fecal *Bifidobacterium* in both 3-wk intake groups were increased in lower levels than those in the 4-wk intake groups. However, there was no significant difference between the changes of fecal *Bifidobacterium* numbers after 3-wk and 4-wk consumption ($P = 0.283$ and 0.056 for FJA and OJA intake groups, respectively).

In the present study, 4 of 22 (18.2 %), 4 of 18 (22.2 %), 10 of 46 (21.7 %) and 7 of 30 (23.3 %) volunteers from FJA and OJA dietary groups for the respect 3 wk and 4 wk had fecal consistency changing from formed stool to soft stool.

Discussion

The recommended daily intake of inulin ranged from 2.5 to 40 g/d for 14 to 64 days^(1,3-9). Judprasong et al.⁽¹¹⁾ found that Jerusalem artichoke or Kaentawan from Thailand contained 19.4 g of inulin/100 g fresh weight. We therefore assigned volunteers to consume either 150 g/d of the FJA or 32 g/d of the OJA,

which was equal to inulin dose of 29.1 g/d for 4 wk. In the present study, we focused on *Bifidobacterium* because this organism was the main target of prebiotics like inulin^(1,3-5,8). In addition, the real-time PCR technique was used for quantitative analysis of fecal *Bifidobacterium* because it is rapid and provides high sensitivity and specificity. The target genes of *Bifidobacterium* used for the real-time PCR were either multi-copy genes like 16S rRNA (one to five copies in bifidobacterial chromosome) or single copy genes such as the *xfp*^(12,15). The SYBRGreen assay targeting single copy of the *xfp* was then chosen to determine the amounts of fecal *Bifidobacterium*.

The present study showed the significant increase of fecal *Bifidobacterium* after consumption of 150 g/d of FJA and 32 g/d of OJA for 4 wk ($P < 0.001$), similar to previous reports^(5,7). The FJA is suitably eaten as vegetable and could be stored at least 1 wk at 4°C. The OJA is tasty such as when taking with milk or yogurt and could be kept at least 2 wk at room temperature. Kleesen et al.⁽⁴⁾ found that the addition of inulin in bakery products still stimulated the growth of *Bifidobacterium*. Therefore, the OJA may be used as a supplement in various foods or cooking. In this study, the decrease or no change in fecal *Bifidobacterium* after both interventions was also observed in 25 of 116 volunteers (21.6 %) (Table 2). Moubareck et al.⁽¹⁶⁾ reported that *Bifidobacterium* was susceptible to several antimicrobials including amoxicillin, which is commonly used for treatment of bacterial infections

such as upper respiratory tract infections. Thus, it might be possible that these volunteers may take antibiotics during the intervention period but did not note it.

It is noteworthy that the significant increase in fecal *Bifidobacterium* was also observed after the FJA consumption for 3 wk ($P = 0.015$). However, there was no significant difference in the OJA dietary group. In this study, the amounts of fecal *Bifidobacterium* from volunteers who completed the intervention were increased in higher levels than those of the 3-wk consumption. Bouhnik et al.⁽³⁾ and Ramirez-Farias et al.⁽¹⁷⁾ found that the amounts of *Bifidobacterium* tended to reduce and return to their baseline levels at 14 days or 21 days after the end of consumption. Therefore, Jerusalem artichoke should be taken continuously to maintain the amounts of intestinal *Bifidobacterium*. Approximately 20% of volunteers receiving the FJA or OJA had softened stool after the intervention period, consistent with the previous study⁽⁹⁾. Bouhnik et al.⁽³⁾ and Marteau et al.⁽⁸⁾ reported the digestive symptoms such as flatus, bloating, borborygmi and abdominal pain during inulin consumption. Although we did not assign volunteers to record their bowel symptoms, some of them had complained about these digestive symptoms including flatulence, borborygmi and bloating after Jerusalem artichoke ingestion. Previous studies showed the bifidogenic effect after receiving 15 g/d inulin for 28 to 45 days^(1,8). Therefore, intake of Jerusalem artichoke with lower doses may not only increase intestinal *Bifidobacterium* but also reduce its side effect. In conclusion, this study clearly illustrated the bifidogenic effect of consuming 150 g/d of FJA and 32 g/d of OJA for 4 wk. This is useful information to promote Jerusalem artichoke as a potential health food for Thai and Asian people.

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