



Extraction, degree of polymerization determination and prebiotic effect evaluation of inulin from *Jerusalem artichoke*

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ABSTRACT

The tubers of *Jerusalem artichoke* are rich of inulin, which makes the plant one of primary inulin resources in China. The aim of this study was to extract inulin from tubers and test the degree of polymerization (DP) 10 days before flowering to 80 days after flowering. The DP of inulin reaches a maximum of 19 at 50 days after flowering. The variation tendencies of inulin content and DP were almost the same, which increase rapidly at the beginning and then decrease gradually at a lower speed. Meanwhile, the effects of inulin on probiotics in yogurt have been evaluated. It indicated that inulin with low DP has higher activities. Experimental data improve the understanding of status change of inulin in whole growth of *Jerusalem artichoke* tubers in Northeastern China and are instructive to get inulin with different properties.

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1. Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) is a native plant of North America, which belongs to sunflower family. Unlike grain crops, *Jerusalem artichoke* can grow well in barren land and it is resistant to frost and drought. Furthermore, it does not compete with grain crops for arable land and could produce high yield of edible tubers (Li, Li, Wang, Du, & Qin, 2013). The edible tubers of *Jerusalem artichoke* store excess energy in the form of fructose polymer inulin, which makes it one of primary inulin resources. To date, *Jerusalem artichoke* is mainly cultivated in North America, Northern Europe, China, Korea, Australia, and New Zealand, and its tubers have already become increasingly popular in European cooking (Bach, Jensen, Clausen, Bertram, & Edelenbos, 2013).

Inulin is a polysaccharide linked by β (2 $\rightarrow$ 1) linkages of D-fructose with the end of glucose residue (Causey, Feirtag, Gahaher, Tuqland, & Slavin, 2000). Because of this special β -linkage configuration between fructose monomers, inulin-type fructans cannot

be degraded by human digestive system so that it does not affect blood sugar level (Bach et al., 2013; Causey et al., 2000; Li et al., 2013). It is scientifically substantiated that inulin-type fructans is a prebiotic based on results of a large number of animal studies and human nutrition intervention trials (Biedrzycka & Bielecka, 2004; Ramnani et al., 2010). The concept of prebiotics is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health (Roberfroid, 2007). β (2 $\rightarrow$ 1)-fructan is currently considered as the only dietary non-digestible oligosaccharide to fulfill all the criteria for prebiotic classification (Biedrzycka & Bielecka, 2004; Roberfroid, 2007).

Inulin has a unique range of molecular weight with the degree of polymerization (DP) varying from 2 to 100. The length, composition, and polydispersity of inulin depend on plant species, harvesting time, and extraction and post-extraction processes (Ronkart et al., 2007). Different inulin-type fructans have probably different efficacies (in terms of effective daily dose), and the most active product is oligofructose-enriched inulin (Roberfroid, 2005). The range of DP has a significant influence on the economic value and industrial utility of inulin and *Jerusalem artichoke*. The properties of inulin have attracted a growing interest in its use as a healthy food ingredient, alternative low-calorie sweeteners, dietary fiber, and a fat substitute.

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In this paper, we extracted inulin from *Jerusalem artichoke* tubers from 10 days before flowering to 80 days after flowering, which was the whole growth process of *Jerusalem artichoke* tubers. Meanwhile, high performance gel filtration chromatography (HPGFC or GFC) method was employed to calculate the average DP of extracted inulin, so that we could observe the molecular weight change of inulin in whole vegetation process. Furthermore, we also tested the effects of extracted inulin on the probiotics in yogurt to find out the correlation between DP and activities. This study may have directive significance to get high yield of inulin with different DP and higher activities from *Jerusalem artichoke* in Northeastern China.

2. Materials and methods

2.1. Materials

2.1.1. Jerusalem artichoke tubers

Jerusalem artichokes (*Helianthus tuberosus* L.) were planted in late May, when temperature was above 20 °C, at *Jerusalem artichokes* cultivating demonstration base in Dongsui Village, Shandong Province, China. The sampling site is located in the Yellow River delta (N37.64°, E118.87°). The climate is damp and hot in summer while dry and cold in winter, and soil is sort of soft and saline-alkali there (Zhao, Li, Xu, & Wang, 1999). Initial flowering began in early September which is about 15 weeks after planting, and the florescence lasts about one month. The tubers of *Jerusalem artichokes* were harvested every 10 days since August 30th and 10 times totally for different maturities which are from 10 days before blossom to a month after that. All tubers were processed immediately on the harvested dates.

2.1.2. Chemicals and instruments

Calcium hydroxide, phosphoric acid, 30% hydrogen peroxide, phenol, sulfuric acid, and 3,5-dinitrosalicylic acid are analytical grade and supplied by Sinopharm Chemical Reagent Co. Ltd (Beijing, China). Deionized water (18.2 MΩ resistivity) was obtained from a Milli-Q Element water purification system (Millipore, Bedford, MA). Dextran standard substances (Mw 1000, 5000, 12,000, 25,000, and 50,000) were purchased from Sigma–Aldrich.

HPLC applied in this study is composed of a quaternary pump, a manual injector, a column oven, and a differential refraction detector (RID) (Agilent 1260 HPLC, USA). A UV–Vis spectrophotometer (T-6, Persee, China) and a pH meter (FE20/EL20, Mettler Toledo, Switzerland) were used in spectrophotometric in pH measurement respectively.

2.2. Inulin extraction

500 g tubers were taken randomly from each batch and chopped into small pieces. Inulin was extracted in hot distilled water according to Gao's methods with minor modification (Gao, Peng, & Xu, 2009). Firstly, the samples were boiled for 5 min to eliminate

enzymes. Then they were stoved at 60 °C for 7 h and smashed with a grinder. The sample power was boiled in deionized water at 90 °C for 40 min twice and filtered. Ca(OH)₂ was added to the filtrate until pH reached 11 to remove the protein, and H₃PO₄ was added until pH was 8 to remove the redundant Ca(OH)₂. Then 30% H₂O₂ (v/v 3%) were used to bleach the solution. Finally, the inulin powder was collected by precipitation with excess ethanol and freeze dried at −40 °C.

2.3. Inulin content assay

Phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) was adopted to assay the total sugar content and the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959) was adopted to assay the reducing sugar content. Inulin content in the extract was the difference between the two. Inulin content in fresh tubers was calculated by the following formula: $w_{\text{in fresh tubers}} = \frac{w_{\text{in extract}}}{w_{\text{fresh tubers}}} \times 100\%$.

2.4. DP determination

The degree of polymerization (DP) of inulin extracts was analyzed by Agilent 1260 HPLC. A Shodex GPC OHPak SB-804 column (7.9 mm × 300 mm) was used with ultrapure water as the mobile phase at a flow rate of 0.8 mL/min. The column temperature was set to 30 °C and injection volume was 20 μL (Zhang, Zhang, Shi, Shi, & Zhang, 2009).

Dextran standard substances were dispersed in ultrapure water at a concentration of 10 mg/mL. The samples were determined at the conditions described above. The standard curve was drawn with the retention time (RT) of Dextran as the abscissa, and the logarithms of molecular weight (log Mw) as the ordinate.

All the inulin samples extracted were also dispersed in ultrapure water at a concentration of 10 mg/mL. These samples were determined at the same chromatographic conditions. The DP was calculated by PL Cirrus for GFC software.

2.5. Assessment of the prebiotic effect of extracted inulin

2.5.1. Growth curve of probiotics determination

Probiotics in yogurt, the mixture of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium lactis*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactococcus lactis* subsp. *cremoris*, and *L. lactis* subsp. *lactis*, were grown under anaerobic conditions at 37 °C in de man Rogosa Sharpe (MRS) broth. The formula of MRS broth is shown in Table 1 (Moreno-Vilet et al., 2014). Growth of mixed strains was monitored every few hours by measuring the optical density (OD) of the cultures at 622 nm and pH (Su, Henriksson, & Mitchell, 2007).

Table 1
The formula of MRS broth.

Substance name	Concentration (g/L)	Manufacturer
Casein peptone	10	Beijing Aoboxing Bio-tech Co., Ltd., China
Meat extract	10	Beijing Aoboxing Bio-tech Co., Ltd., China
Yeast extract	5	Beijing Aoboxing Bio-tech Co., Ltd., China
Tween 80	1	Tianjin Rgent Chemicals Co., Ltd., China
Ammonium citrate	2	Tianjin Rgent Chemicals Co., Ltd., China
Sodium acetate	5	Sinopharm Chemical Reagent Co., Ltd., China
Dipotassium hydrogen phosphate	2	Sinopharm Chemical Reagent Co., Ltd., China
Magnesium sulfate	0.2	Sinopharm Chemical Reagent Co., Ltd., China
Manganese sulfate	0.05	Sinopharm Chemical Reagent Co., Ltd., China
Glucose	5	Sinopharm Chemical Reagent Co., Ltd., China

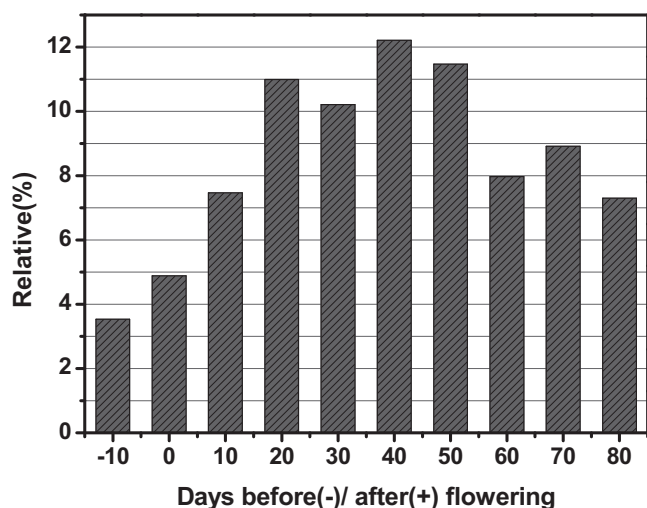


Fig. 1. Inulin content in fresh tubers of *Jerusalem artichoke* from different harvest time.

2.5.2. Prebiotic effect of inulin determination

The response as prebiotic was evaluated using a culture media with the same composition of MRS broth but replacing the carbohydrate source for inulin extracted. This bacterial growth was also evaluated in the prepared MRS broth without carbohydrate source, which was used as control. The carbohydrate concentration in all the assays was 5 g/L. The activated probiotics was inoculated with 1% (v/v) and incubated at 37 °C for 32 h, when the growth of mixed strains was in the stationary phase. The OD and pH were measured to evaluate the growth of probiotics. All assays were performed in duplicate.

2.6. Data analysis

For each experiment, the data was analyzed using the Excel statistical package. The OD readings and standard deviations were calculated from duplicate samples from two separate experiments.

3. Results and discussion

3.1. Determination of the content and DP of inulin

Fig. 1 shows inulin content in fresh tubers at different growth phases of *Jerusalem artichoke*. Inulin content in fresh tubers was calculated by inulin content in extract divided by the weight of fresh tubers. It increases from 3.5% at 10 days before flowering to maximum value 12.21% at 40 days after flowering. After that, percent of inulin begins to decrease gradually and reached 7.3% at the last time point. Compared with previous reports (Frese, Dambroth, & Bramm, 1991; Liu et al., 2014; Yang et al., 2010), the yield of inulin extracted from *Jerusalem artichoke* was greater than or equal to the yield in other species (i.e., chicory, burdock, *Morinda officinalis*). This indicates that *Jerusalem artichoke* could be a resource to produce inulin in China. In addition, it is known that the sugars formed in *Jerusalem artichoke* are polyfructosans of inulin type, the polymerization of which varies during the harvest time (Chabbert, Braun, Guiraud, Arnoux, & Galzy, 1983; Ronkart et al., 2007). The development of DP in each period is presented in Fig. 2. The DP of inulin shows about the same variation tendency with inulin content. It increased fast at the beginning and then decreased gradually at a lower speed, but the final DP of inulin is higher than the original DP. It is 5 at the beginning and jumps to 10 only 10 days later. Then the growth trend increased gradually to the maximum of 19 during 50 days. The time when the DP of inulin reaches the maximum

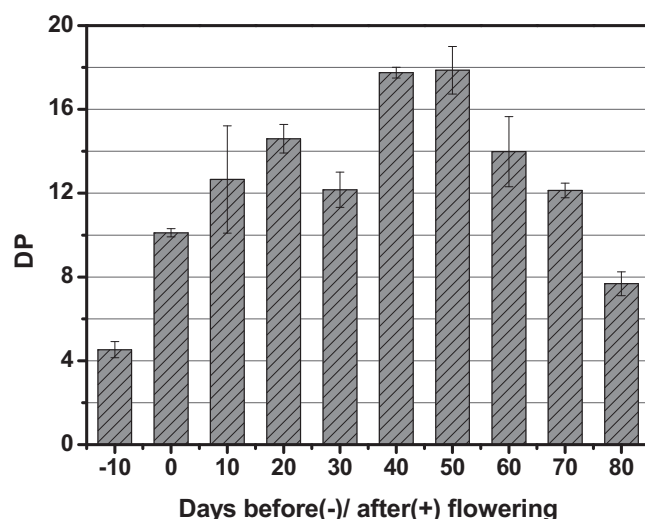


Fig. 2. Change of DP of inulin in the whole growth of *Jerusalem artichoke*.

is about 50 days after flowering. In the end, the DP dropped to 8. Obviously, both changing trends are similar to each other.

The following theory may explain the changes of inulin's content and DP. At the beginning, the low DP of inulin should attribute to high fructo-oligosaccharide content in tubers. After flowering, *Jerusalem artichoke* tubers start translocating photosynthetic assimilates from stems to tubers, which causes the inulin content increases (Meijer, Mathijssen, & Borm, 1993; Zubr & Pedersen, 1993). At the same time, one kind of polysaccharase might start to use one fructo-oligosaccharide as a key intermediate to synthesize high-molecular-weight inulin and consequently the DP of inulin increases (Edelman & Jeeoord, 1968). As leaves and stems dry up, the content and DP of inulin start decreasing slowly after reaching maximum. The decrease would be due to a progressive depolymerization by a certain enzyme known as inulinase. The inulinase activity would increase as the tubers grow older and it could cause the high-molecular-weight sugars to break down into fructose which is utilized by the plant, and sugars would be less polymerized as the season wore on (Limami & Fiala, 1993; Schorr-Galindo & Guiraud, 1997).

3.2. Effects of inulin extracted on the probiotics in the yogurt

First of all, we determined the growth of probiotics in yogurt to decide incubation time to test prebiotic effect. Fig. 3 indicates the

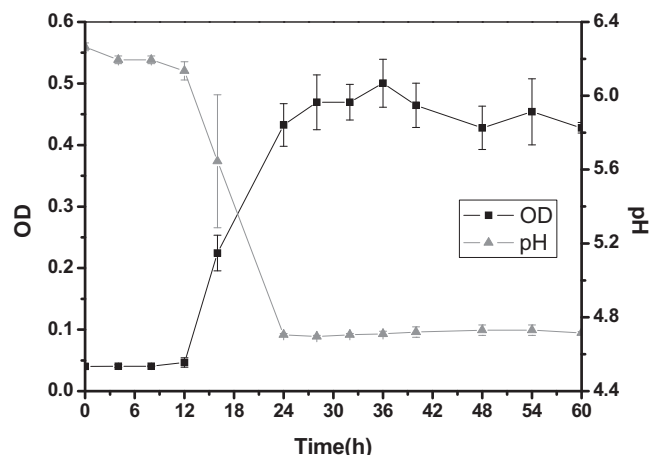


Fig. 3. Growth curve of probiotics in the yogurt.

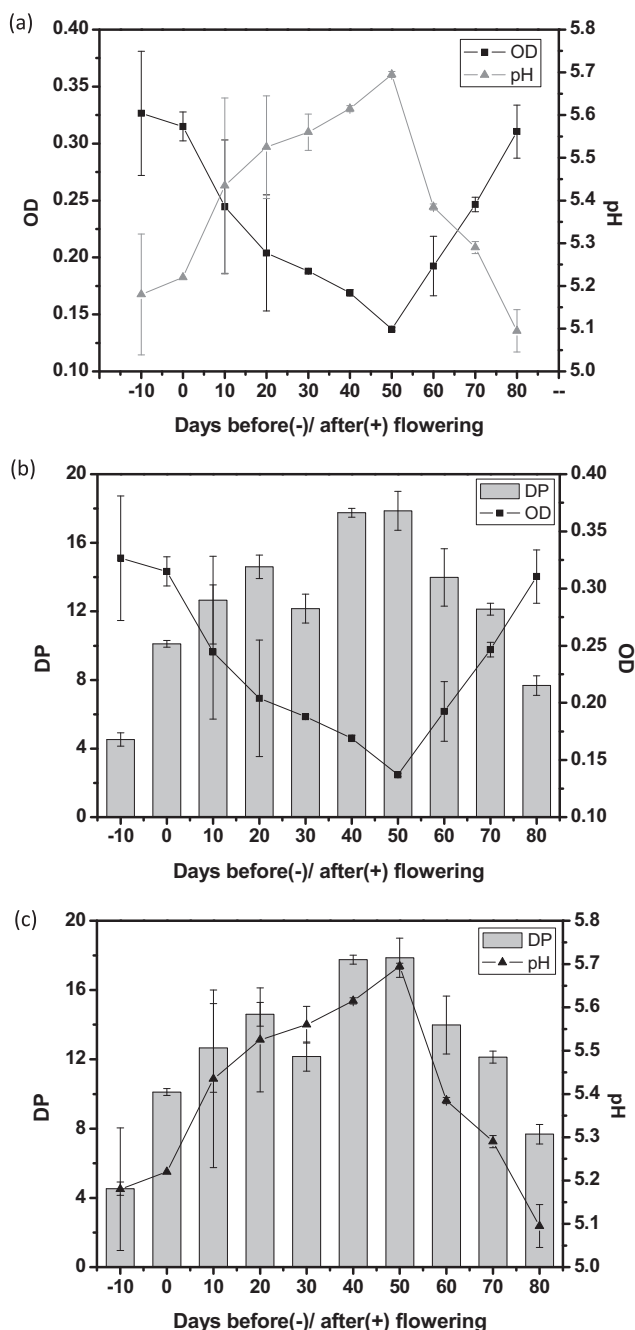


Fig. 4. (a) OD and pH of culture mediums fermented 32 h later with inulin extracted from different harvest time of *Jerusalem artichoke* as carbon sources. (b) Prebiotic effect of inulin DP on probiotics in the yogurt. (c) Effect of Inulin DP on pH of culture mediums.

changes of OD and pH during the growth of probiotics. Since inulin will decompose into some kinds of acids in the growth process of probiotics and the pH of cultures will decrease, this makes pH as an important indicator to characterize the growth phases of strains. As can be seen, lag phase lasts 12 h. Then, the strains grow very fast, which suggests that they are in logarithmic phase. Stationary phase begins at 24 h, and decline phase starts at 40 h. According to the growth curve, 32 h after culture fermentation could be an appropriate time to measure the effect of prebiotics.

Fig. 4(a) reveals OD and pH of culture mediums fermented 32 h with inulin extracted from different harvest time of *Jerusalem artichoke* as carbon sources. A larger OD value means the better growth of probiotics and the more effective utilization of inulin. This

process could produce more acid and the pH would decrease. It is inferred that OD and pH are negative correlation, which can be proved from Fig. 4(a). Fig. 4(b) shows the correlation between the OD and DP. It can be seen that the OD of culture medium is larger with lower DP of inulin as carbon source, which means the inulin with lower DP has a better prebiotic effect. The figure suggests that there is also a negative correlation between DP and OD. Regarding to inference, it should be positive correlation between pH and DP, which is just proved by Fig. 4(c). This indicates that inulin with lower DP can be better used by probiotics. In summary, inulin with lower DP is more active and shows higher degree of utilization. This is a significant guidance for the utilization of inulin in industrial production.

According to these data, we can harvest the tubers at different times to get inulin with different average DP and to improve the utilization of inulin for different purposes. The tubers of *Jerusalem artichoke* can be harvested at the beginning of flowering or after the drying up of leaves and stems to get inulin with low DP which is reported to be the most active product. Nevertheless, the yield of inulin should also take into account. In short, with comprehensive consideration of inulin content and DP, 50 days or even later after flowering may be supposed to be the optimum maturity in Northeastern China.

4. Conclusions

In this paper, we observed changes of inulin content and DP from 10 days before *Jerusalem artichoke* blossomed and 80 days after blossomed. The variation tendencies are almost the same, which increase rapidly at the beginning and then decrease gradually at a lower speed. The maximum of inulin content could reach 12.21% and the maximum of inulin DP could reach 19. Both data reach peak at the similar time which is 40 or 50 days after blossom. It indicates that these changes are related to the growth status of *Jerusalem artichoke* tubers. Experimental data supplied us with deeper knowledge of inulin content and DP changes in whole growth process of *Jerusalem artichoke* tubers. Meanwhile, the effects of the inulin on probiotics in the yogurt have also been evaluated systematically. The result shows that inulin with lower DP has better activities, which could be a theoretical basis for further high-value utilization of inulin. These findings mentioned above may be instructive to get inulin with different properties from *Jerusalem artichoke* in Northeastern China. Further study will be carried out in the near future.

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References

- Bach, V., Jensen, S., Clausen, M. R., Bertram, H. C., & Edelenbos, M. (2013). Enzymatic browning and after-cooking darkening of *Jerusalem artichoke* tubers (*Helianthus tuberosus* L.). *Food Chemistry*, 141, 1445–1450.
- Biedrzycka, E., & Bielecka, M. (2004). Prebiotic effectiveness of fructans of different degrees of polymerization. *Trends in Food Science & Technology*, 15, 170–175.
- Causey, J. L., Feirtag, J. M., Gallaher, D. D., Tuqland, B. C., & Slavin, J. L. (2000). Effects of dietary inulin on serum lipids, blood glucose and the gastrointestinal environment in hypercholesterolemic men. *Nutrition Research*, 20, 191–201.
- Chabbert, N., Braun, P., Guiraud, J. P., Amoux, M., & Galzy, P. (1983). Productivity and fermentability of *Jerusalem artichoke* according to harvesting date. *Biomass*, 3(3), 209–224.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination sugars and related substances. *Analytical Chemistry*, 28(3), 350–356.

- Edelman, J., & Jeeoord, T. G. (1968). The mechanism of fructosan metabolism in higher plants as exemplified in *Helianthus Tuberosus*. *New Phytologist*, 67, 517–531.
- Frese, L., Dambroth, M., & Bramm, A. (1991). Breeding potential of root chicory (*Cichorium intybus* L. var. *sativum*). *Plant Breeding*, 106(2), 107–113.
- Gao, J., Peng, B., & Xu, H. (2009). Study on extraction and separation of inulin from *Jerusalem artichoke*. *Journal of Anhui Agricultural Sciences*, 37(1), 184–185, 214.
- Li, L. L., Li, L., Wang, Y. P., Du, Y. G., & Qin, S. (2013). Biorefinery products from the inulin-containing crop *Jerusalem artichoke*. *Biotechnology Letters*, 35(4), 471–477.
- Limami, A., & Fiala, V. (1993). Fructan polymerization and depolymerization during the growth of chicory (*Chicorium intybus* L.) plants. *Studies in Plant Science*, 3, 191–198.
- Liu, W., Wang, J. J., Zhang, Z. Z., Xu, J. N., Xie, Z. H., Slavin, M., et al. (2014). In vitro and in vivo antioxidant activity of a fructan from the roots of *Arctium lappa* L. *International Journal of Biological Macromolecules*, 65, 446–453.
- Meijer, W. J. M., Mathijssen, E. W. J. M., & Borm, G. E. L. (1993). Crop characteristics and inulin production of *Jerusalem artichoke* and chicory. *Studies in Plant Science*, 3, 29–38.
- Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31, 426–428.
- Moreno-Vilet, L., Garcia-Hernandez, M. H., Delgado-Portales, R. E., Corral-Fernandez, N. E., Cortez-Espinosa, N., Ruiz-Cabrera, M. A., et al. (2014). In vitro assessment of agave fructans (*Agave salmiana*) as prebiotics and immune system activators. *International Journal of Biological Macromolecules*, 63, 181–187.
- Ramrani, P., Gaudier, E., Bingham, M., van Bruggen, P., Tuohy, K. M., & Gibson, G. R. (2010). Prebiotic effect of fruit and vegetable shots containing *Jerusalem artichoke* inulin: A human intervention study. *British Journal of Nutrition*, 104(2), 233–240.
- Roberfroid, M. (2007). Prebiotics: The concept revisited. *Journal of Nutrition*, 137(3), S830–S837.
- Roberfroid, M. B. (2005). Introducing inulin-type fructans. *British Journal of Nutrition*, 93(Suppl. 1), S13–S25.
- Ronkart, S. N., Blecker, C. S., Fourmanoir, H., Fougny, C., Deroanne, C., Herck, J. V., et al. (2007). Isolation and identification of inulooligosaccharides resulting from inulin hydrolysis. *Analytica Chimica Acta*, 604(1), 81–87.
- Schorr-Galindo, S., & Guiraud, J. P. (1997). Sugar potential of different *Jerusalem artichoke* cultivars according to harvest. *Bioresource Technology*, 60, 15–20.
- Su, P., Henriksson, A., & Mitchell, H. (2007). Selected prebiotics support the growth of probiotic mono-cultures in vitro. *Anaerobe*, 13, 134–139.
- Yang, Z. M., Yi, Y. T., Gao, C. C., Hou, D. K., Hu, J., & Zhao, M. Y. (2010). Isolation of inulin-type oligosaccharides from Chinese traditional medicine: *Morinda officinalis* How and their characterization using ESI-MS/MS. *Journal of Separation Science*, 33, 120–125.
- Zhang, Z. S., Zhang, J. P., Shi, S., Shi, J. N., & Zhang, J. (2009). HPGPC determination of relative molecular mass of inulin. *Food Science*, 30(12), 219–221.
- Zhao, G. X., Li, Y. H., Xu, C. D., & Wang, A. L. (1999). Relations and sustainable development of soil–human–environment in Kenli county. *Soil and Environmental Science*, 8(4), 250–253.
- Zubr, J., & Pedersen, H. S. (1993). Characteristics of growth and development of different *Jerusalem artichoke* cultivars. *Studies in Plant Science*, 3, 11–19.