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Effect of dietary fibres present in traditional Japanese vegetables on carbohydrate-digesting enzyme activity

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ABSTRACT

Introduction: Dietary fibre intake is widely recommended as a nutritional therapy to alleviate hyperglycaemic and diabetic conditions. Inulin is a heteropolysaccharide mainly found in burdock. It is known that inulin intake reduces postprandial glucose level; however, details regarding the mechanism of this action remain unclear. In this study, we investigated the effect of burdock and inulin on glucose metabolism. **Methods:** Burdock and inulin were added to a solution containing maltose and α-glucosidase. Glucose produced from digested maltose was measured using mutarotase-glucose oxidase assay as the activity of α-glucosidase. We employed Ostwald viscometer to measure viscosity of inulin solution. **Results:** Addition of 0.1-1.0 g raw burdock in 6 mL incubation solution significantly decreased activity of α-glucosidase in an amount-dependent manner. Boiled burdock and its supernatant liquid sample also significantly decreased activity of this enzyme, although degree of suppression in the boiled sample was less than in raw burdock. Addition of inulin, in the range of 0.67 to 6.70%, significantly decreased activity of α-glucosidase. Viscosity of inulin solution depended on its concentration from 0 to 6.70% and the relationship showed an almost direct proportion. α-glucosidase activity significantly decreased in the range of 1.03 to 1.40 in relative viscosity of inulin solution. **Conclusion:** These results suggested that inulin has a suppressive effect on α-glucosidase activity and that high viscosity of inulin solution may inhibit reaction of digestive enzyme.

Keywords: α-glucosidase activity, burdock, dietary fibre, inulin, liquid viscosity

INTRODUCTION

Various medical and nutritional therapies to treat glucose metabolism disorders, particularly hyperglycaemia and diabetes, have been developed worldwide. As a nutritional therapy for these disorders, dietary fibre intake suppresses postprandial increments in plasma glucose levels. Moreover, the intake of vegetables containing abundant dietary fibre before carbohydrate intake attenuates postprandial glycaemia in healthy participants and patients with type 2 diabetes after carbohydrate intake (Sun *et al.*, 2020; Imai *et al.*, 2011).

Dietary fibres, such as cellulose, pectin, guar, and inulin, improve glucose metabolism (Jenkins *et al.*, 1978; Johnson & Gee, 1981; Kaur & Gupta, 2002). Cellulose is a structural component of the plant cell wall and a homopolysaccharide consisting of

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a linear chain of several hundreds to thousands of β1–4 glycosidic-bound D-glucose molecules. Pectin is a heteropolysaccharide found in apples and citrus fruits; its main component is galacturonic acid. Guar, also known as guar gum, is a galactomannan polysaccharide found in guar beans. These polysaccharides attenuate the rapid increase in postprandial plasma glucose levels by decreasing glucose absorption in the small intestine (Johnson *et al.*, 1981; Kay, 1982; Flourie *et al.*, 1984). The presence of dietary fibres in the intestine increases luminal fluid viscosity and thickening, thereby delaying glucose absorption from the luminal to the serosal side. Inulin is a water-soluble dietary fibre and the main heteropolysaccharide found in burdock, Jerusalem artichokes, and chicory. Inulin mainly comprises fructose, fructans, and terminal glucose units. Inulin intake reduces postprandial glycaemia over a long period, such as 1–2 months (Kaur *et al.*, 2002; Gargari *et al.,* 2013). Shao *et al.* (2020) found that inulin attenuates hyperglycaemia in diabetic mice by improving the intestinal microbiota.

Burdock (Arctium) is routinely used as a dietary fibre-rich food and traditional medicine in Asian countries, including Japan. Although burdock extract has anti-hyperglycaemic effects (Mondal & Eun, 2022), the detailed mechanism underlying the effect of direct burdock intake on glucose metabolism is unclear. Burdock contains various bioactive substances, such as polyphenols and inulin (Mondal & Eun, 2022); inulin is the main heteropolysaccharide present in burdock (Moro & Clerici, 2021). It has been reported that flavonoids in burdock leaf suppress α-glucosidase activity (Cui, Zeng & Zhang, 2022). However, the mechanism by which inulin intake affects glucose metabolism remains unclear. To understand these mechanisms, we hypothesised that burdock and inulin intakes influence the activity of carbohydrate-digesting enzymes. In this study, we investigated the effects of burdock and inulin on maltose-digesting α-glucosidase activity.

METHODOLOGY

Materials

Burdocks used for this research were domestically cultivated in Aomori Prefecture, Japan, and purchased in September 2023. They were planted in May 2023 and harvested in the beginning of September 2023. α-glucosidase (a maltose-digesting enzyme from yeast) was purchased from ORIENTAL YEAST, Ltd. (Tokyo, Japan). Pure inulin powder was purchased from NACALAI TESQUE Inc. (Kyoto, Japan).

Preparation of burdock

Raw burdock (RB) was smashed using a grater and a mortar-pestle set before use. Boiled burdock (BB) was prepared by adding 2 mL water to 1 g RB and boiled at 100 °C for 10 minutes. After boiling the sample, the filtered solution was collected as the supernatant liquid (SL) using a funnel with filter paper.

Measurement of α-glucosidase activity

α-glucosidase (0.2 mg/mL) was dissolved in 66.7 mM phosphate buffer solution (pH 7.0, 66.7 mM $NaH_2PO_4 \cdot 2H_2O$, 66.7mM $Na_2HPO_4 \cdot 12H_2O$ = 2:3). To evaluate the effect of burdock on α-glucosidase activity, reaction solutions containing 3 mL phosphate buffer solution, 3 mL 80 mM maltose solution, and 0.1-1.0 g BB, RB, or SL, respectively, were incubated at 37°C for 20 minutes. In control, the reaction solution contained no burdock sample. Then, all reaction solutions were heated at 100°C for 3 minutes to deactivate α-glucosidase. The glucose concentrations from digested maltose were then estimated using

Table 1. The effect of raw and boiled burdock on α-glucosidase activity

Type of burdock	*α-glucosidase activity (μg/mL/h)*		
	0.1g	*0.5g*	*1.0g*
Without burdock	599.6±18.0	583.6±18.3	576.2±10.6
Raw burdock	489.2±5.8*	356.6±59.4*	258.2±77.1*
Boiled burdock	561.1±1.9*†	460.0±5.2*†	389.9±19.2*†

Raw and boiled burdock were incubated with α-glucosidase and maltose at 37°C for 20 minutes.
Data expressed as mean±*SE*.
*p<0.05, compared to the control; †p<0.05, compared to the group of raw burdocks

the mutarotase-glucose oxidase assay (Glucose CII-Test, Wako, Osaka, Japan) from the absorbance at 505 nm. The amount of glucose (μg/mL/h) produced from maltose was considered the activity of α-glucosidase.

To evaluate the effect of inulin on α-glucosidase activity, the reaction solution containing 1 mL phosphate buffer solution, 1 mL 80 mM maltose solution, and 0.9 mL inulin solution was incubated at 37°C for 20 minutes. The reaction solution for control contained no inulin.

Determination of inulin solution viscosity

Ostwald viscometer was used to determine the viscosity of each inulin solution. Viscosity was calculated as relative to that of water at 37°C. The Ostwald viscometer has a capillary with a 0.5 mm inner diameter. To calculate the relative viscosity of the solution, the time (seconds) required for it to flow through the capillary was measured. Solution density (g/cm^3) was determined using a specific gravity bottle (Gay–Lussac type). Relative inulin solution viscosity was determined as:

Relative inulin solution viscosity
= [inulin solution density (g/cm^3) × t_i] / [water density (g/cm^3) × t_w]

with t_i and t_w representing flowdown time (seconds) of inulin solution and water, respectively, in the capillary of the viscometer.

Statistics

Data were expressed as mean ± standard error (*SE*). All experiments were repeated four times as independent trials. Statistical comparisons between two means were performed using paired Student's *t*-test. More than three mean values were compared using analysis of variance (ANOVA), followed by the Bonferroni–Dunn post-hoc test using StatView software (SAS Institute, Cary, NC, USA). P<0.05 was considered statistically significant.

RESULTS

First, the effect of RB on α-glucosidase activity was determined. In this study, α-glucosidase isolated from yeast cells was used because it is widely used as an intestinal α-glucosidase substitute (Tan *et al.*, 2013; Ardiansah *et al.*, 2023). Table 1 shows the effects of each burdock sample on α-glucosidase activity. Adding 0.1, 0.5, and 1.0 g RB significantly decreased α-glucosidase activity in an amount-dependent manner, with suppressive ratios of 18.4, 38.9, and 55.2%, respectively. BB also significantly decreased α-glucosidase activity in an amount-dependent manner; however, the degrees of suppression were lower than that of RB, with suppressive ratios of 6.4, 21.2, and 32.3% for 0.1, 0.5,

Table 2. The effect of burdock supernatant liquid on α-glucosidase activity

Type of burdock	*α-glucosidase activity (μg/mL/h)*		
	0.1g†	*0.5g*†	*1.0g*†
Without burdock	577.5±3.3	572.5±14.7	566.4±13.9
SL of burdock	481.0±23.7*	318.2±5.7*	337.0±14.7*

SL: Supernatant liquid. Supernatant liquid was incubated with α-glucosidase and maltose at 37°C for 20 minutes.
†Amount of raw burdock for preparing the supernatant sample, respectively.
Data expressed as mean±SE; *p<0.05, compared to the control.

and 1.0 g BB, respectively. Moreover, SL obtained from 0.1, 0.5, and 1.0 g RB significantly decreased α-glucosidase activity, and the suppressive ratios were 16.7, 44.4, and 40.5%, respectively (see Table 2). These results suggest that by boiling the burdock, its effective components were leached out of the cells and tissues, resulting in a SL with suppressive effects on α-glucosidase activity.

Second, the effect of inulin, the main heteropolysaccharide in burdock, on α-glucosidase activity and relative inulin solution viscosity was determined. The addition of 0.67–6.70% inulin significantly decreased α-glucosidase activity (see Figure 1). In the 6.70% inulin solution, the suppressive ratio to non-inulin solution (control bar) was 17%. The relative viscosity to non-inulin solution of 0–6.70% inulin solution was almost directly proportionally concentration dependent (R^2=0.9927; see Figure 2). Figure 3 shows the relationship between inulin solution

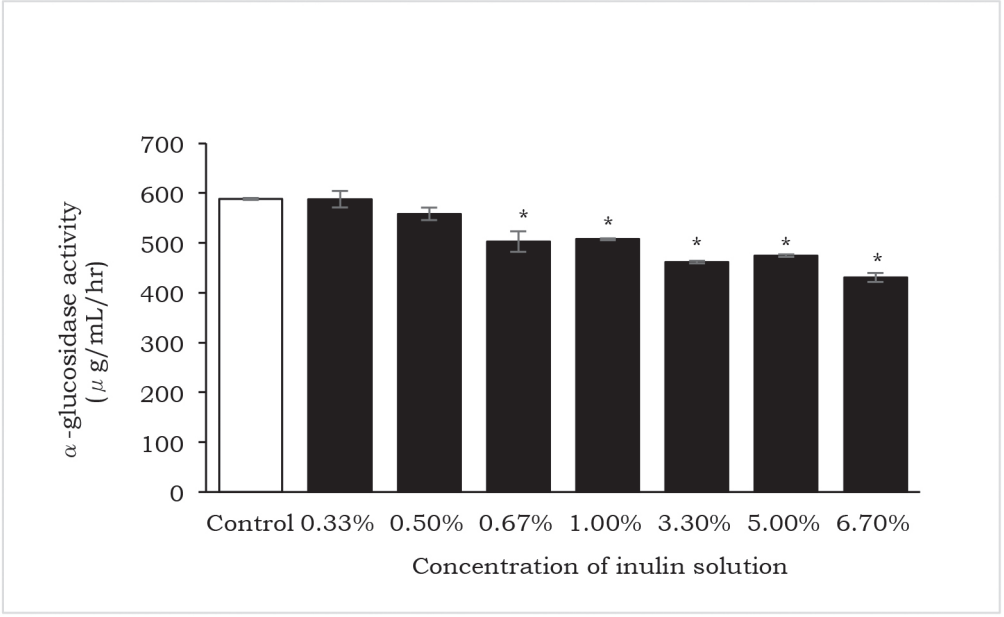

Figure 1. Effect of inulin on α-glucosidase activity
Inulin (0.33–6.70%) were added to each reaction solution and incubated with α-glucosidase and maltose at 37°C for 20 minutes. Control indicates activity in the absence of inulin.
Data expressed as mean±*SE*; *p<0.05, compared to the control.

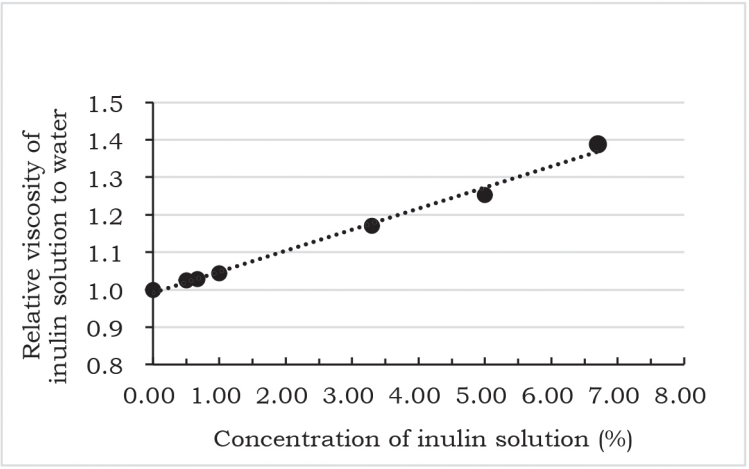

Figure 2. Relationship between inulin solution viscosity and concentration. Inulin viscosity was determined relative to that of water (vertical axis, 1.0)

viscosity and α-glucosidase activity. Each value in the bracket showed the concentration of inulin corresponding to its relative viscosity. The enzyme activity significantly decreased sharply in the relative viscosity of 1.03 (equivalent to 0.67% inulin solution) and then decreased gradually at 1.40 relative viscosity. These results suggest that the effect of inulin on α-glucosidase activity depends on the viscosity of its solution.

DISCUSSION

This study showed that the addition of burdock and inulin decreased α-glucosidase activity. In this study, RB tissues and cells were physically

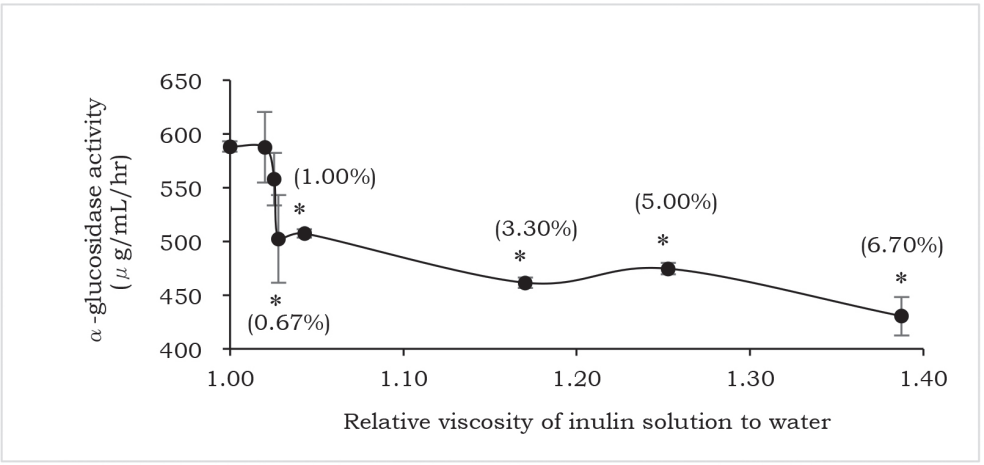

Figure 3. Relationship between inulin solution viscosity and α-glucosidase activity
Inulin viscosity was determined relative to that of water (1.00 on the horizontal axis). Each value in the bracket shows the concentration of inulin corresponding to its relative viscosity. Measurement of α-glucosidase activity was performed under the same condition as Figure 1. *p<0.05 compared to α-glucosidase activity in the absence of inulin (1.00 in horizontal axis).

destroyed, causing inulin to probably leach into the reaction solution, consequently affecting α-glucosidase activity. BB also decreased α-glucosidase activity, although its effect was weaker than that of RB. Moreover, SL obtained by boiling burdock also suppressed α-glucosidase activity. Zhang *et al.* (2022) reported in their study that inulin was purified from Jerusalem artichoke using high-temperature water. Similarly, our results suggest that inulin was partially removed from RB tissues by high-temperature liquid, resulting in BB having a less suppressive effect on α-glucosidase activity.

This study also reported that inulin suppressed α-glucosidase activity and the viscosity of 0–6.70% inulin solution was concentration dependent. Moreover, the high-viscosity inulin solution suppressed α-glucosidase activity (Figure 3), which is similar to the results of previous studies that showed the suppressive effect of high-viscosity liquid on enzyme activity (Uribe & Sampedro, 2003; Barbier & Campbell, 2005). Our present results suggest the possibility that the enzyme–substrate reaction between maltose and α-glucosidase may be physically suppressed by high-viscosity inulin solution. To clarify this possibility, further study using positive control substances is needed. One candidate substance is glycerol. It is a simple triol compound with low molecular weight, and its solution has high viscosity. The effect of this positive control substance on α-glucosidase activity should be investigated in the same inulin viscosity as in this study. However, we cannot exclude the possibility that inulin directly suppresses α-glucosidase activity without depending on inulin viscosity. The molecular interaction between inulin and enzyme protein is unknown. Further study will be needed to determine this.

Although the direct relationship between inulin solution viscosity and α-glucosidase activity was not examined, interestingly, 6.70% inulin solution suppressed α-glucosidase activity by 17%, which was lower than that by burdock (Figures 1 and 3). These results suggest that other components also suppress α-glucosidase activity. Burdock contains various bioactive substances, such as polyphenols, in addition to inulin (Mondal & Eun, 2022). It has been reported that burdock leaf flavonoids, as well as ethanol and hexane burdock extracts, also inhibit α-glucosidase activity (Cui, Zeng & Zhang, 2022). Therefore, we consider the possibility that inulin and other bioactive compounds simultaneously suppress α-glucosidase activity. Previous studies reported that raw burdock contains approximately 10.00% inulin, while burdock dry powder contains approximately 50.00% inulin (Kato *et al.*, 1993; Watanabe *et al.*, 2020). Although inulin had a suppressive effect on α-glucosidase activity in this study, the concentration of inulin in the reaction solutions with burdock samples was not determined. To compare the suppressive effects of inulin and burdock in our present study, it will be important to measure inulin concentration in the reaction solutions with burdock. In addition, cellulose is a major component of the plant cell walls, including vegetables, and is a type of insoluble dietary fibre. Because burdocks contain cellulose like other plants, further study will be needed to determine cellulose concentration in burdock-containing solutions and its direct effect on α-glucosidase activity.

Several seasonings, such as salt and soy sauce, are generally used to cook burdocks. We did not examine the effect of NaCl concentration on burdock-induced α-glucosidase activity suppression. Turner, Tomlinson & Caldwell (1980)

found that salts, such as sodium chloride (NaCl), potassium chloride (KCI), and potassium phosphate, reduced the inhibitory effect of phosphoenolpyruvate on carrot phosphofructokinase. Accordingly, further studies should aim to elucidate the relationship between solution concentration (or osmolality) and burdock-induced α-glucosidase activity suppression. In addition, the temperature of a frying pan reaches 150–200°C while cooking on a stovetop, which is substantially higher than that of boiled water. This may change the structure and characteristics of the bioactive components in burdock. Therefore, further studies should focus on determining the influence of very high temperature on the suppressive effects of burdock and inulin on α-glucosidase activity.

With regards to dietary and health implications of our present study, we consider burdock as a food material candidate contributing to ameliorate and prevent hyperglycaemia and diabetes. Recently, purified inulin is used as a dietary supplement. In addition, it has been reported that other purified physiological substances from burdock had improving effects on glucose metabolism (Mondal & Eun, 2022).

The novelty of our present study is that inulin was demonstrated as a key dietary fibre in regulating carbohydrate-digesting enzyme activity in the intestine and that direct intake of burdock is a useful method to regulate intestinal enzyme activity. However, this study also has limitations. The effects of burdock and inulin on digestion and absorption of saccharides in the human or animal intestine were not determined. This determination is important to understand more physiologically the effects of burdock and inulin on the improvement of glucose metabolic problems in the human body.

CONCLUSION

In conclusion, our results showed that burdock and inulin suppressed α-glucosidase activity. The high viscosity of the inulin solution may also inhibit the reaction between the digestive enzyme and its substrate. Therefore, burdock may contribute to intestinal glucose absorption suppression and improvements in glucose metabolic diseases.

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Authors' contributions

Tsuchiya Y, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript, and reviewed the manuscript; Maeda A, conducted the study, data analysis and interpretation, assisted in drafting of the manuscript, reviewed the manuscript.

Conflict of interest

No conflicts of interest to be declared.

References

Ardiansah B, Rohman N, Nasution MAF, Tanimoto H, Cahyana AH, Fadlan A & Ariyanid T (2023). Synthesis, α-glucosidase inhibitory activity and molecular docking study of chalcone derivatives bearing a 1H-1,2,3-triazole unit. *Chem Pharm Bull* 71:342–348.

Barbier GG & Campbell WH (2005). Viscosity effects on eukaryotic nitrate reductase activity. *J Biol Chem* 280:26049–26054.

Cui J, Zeng S & Zhang C (2022). Anti-hyperglycaemic effects of burdock (Arctium lappa L.) leaf flavonoids through inhibiting α-amylase and α-glucosidase. *Int J Food Sci Technol* 57:541-551.

Flourie B, Vidon N, Florent CH & Bernier JJ (1984). Effect of pectin on jejunal glucose absorption and unstirred layer thickness in normal man. *Gut* 25:936-941.

Gargari BP, Dehghan P, Aliasgharzadeh A & Jafar-Abadi M (2013). Effects of high performance inulin supplementation on glycaemic control and antioxidant status in women with type 2 diabetes. *A. Diabetes Metab J* 37:140-148.

Imai S, Matsuda M, Hasegawa G, Fukui M, Obayashi H, Ozasa N & Kajiyama S (2011). A simple meal plan of eating vegetables before carbohydrate' was more effective for achieving glycaemic control than an exchange-based meal plan in Japanese patients with type 2 diabetes. *Asia Pac J Clin Nutr* 20:161-168.

Jenkins DJ, Wolever TM, Leeds AR, Gassull MA, Haisman P, Dilawari J, Goff DV, Metz GL & Alberti KG (1978). Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. *BMJ* 1:1392-1394.

Johnson IT & Gee JM (1981). Effect of gel-forming gums on the intestinal unstirred layer and sugar transport in vitro. *Gut* 22:398-403.

Kato Y, Fujita M & Asari U (1993). Changes in inulin of edible burdock during storage and heating. *Bulletin of Faculty of Education, Hirosaki University* 69:131-135.

Kaur N & Gupta AK (2002). Applications of inulin and oligofructose in health and nutrition. *J Biosci* 27:703-714.

Kay RM (1982). Dietary fibre. *J Lipid Res* 23:221-242.

Mondal SC & Eun JB (2022). Mechanistic insights on burdock (Arctium lappa L.) extract effects on diabetes mellitus. *Food Sci Biotechnol* 31:999–1008.

Moro TMA & Clerici MTPS (2021). Burdock (Arctium lappa L) roots as a source of inulin-type fructans and other bioactive compounds: Current knowledge and future perspectives for food and non-food applications. *Food Res Int* 141:109889.

Shao T, Yu Q, Zhu T, Liu A, Gao X, Long X & Liu Z (2020). Inulin from Jerusalem artichoke tubers alleviates hyperglycaemia in high-fat-diet-induced diabetes mice through the intestinal microflora improvement. *Br J Nutr* 123:308-318.

Sun L, Goh HJ, Govindharajulu P, Leow MK & Henry CJ (2020). Postprandial glucose, insulin and incretin responses differ by test meal macronutrient ingestion sequence (PATTERN study). *Clin Nutr* 39:950-957.

Tan C, Wang Q, Luo C, Chen S, Li Q & Li P (2013). Yeast α-Glucosidase Inhibitory Phenolic Compounds Isolated from Gynura medica Leaf. *Int J Mol Sci* 14:2551-2558.

Turner JF, Tomlinson JD & Caldwell RA (1980). Effect of salts on the activity of carrot phosphofructokinase. *Plant Physiol* 66:973-977.

Uribe S & Sampedro JG (2003). Measuring Solution Viscosity and its Effect on Enzyme Activity. *Biol Proced Online* 5:108-115.

Watanabe A, Sasaki H, Miyakawa H, Nakayama Y, Lyu Y & Shibata S (2020). Effect of Dose and Timing of Burdock (Arctium lappa) Root Intake on Intestinal Microbiota of Mice. *Microorganisms* 8:220

Zhang X, Zhu X, Shi X, Hou Y & Yi Y (2022). Extraction and purification of inulin from jerusalem artichoke with response surface method and ion exchange resins. *ACS Omega* 7:12048-12055.