Functional characterization of oligosaccharides purified from *Asparagus officinalis* peel

XIAOHONG KOU – CUIYU MAO – BING XIE – XINGYUAN LI – ZHAOHUI XUE – ZHI ZHANG

Summary
In this work, oligosaccharides were subjected to functional identification after purification by Sephadex G-25 gel filtration column (Pharmacia, Jersey City, New Jersey, USA). Physico-chemical characteristics, molecular weight and functional groups were also studied. The molecular weight of the purified asparagus oligosaccharides was estimated to be 569 Da by gel permeation chromatography analysis. Infrared spectral analysis identified different functional groups on the asparagus oligosaccharides, laying a theoretical foundation to determine additional functions of asparagus oligosaccharides in future work. By in vitro tests, we determined that asparagus oligosaccharides were able to remove hydroxyl and superoxide radicals with a dose-effect relationship. Asparagus oligosaccharides promoted proliferation of bifidobacteria, *Lactobacillus lactis* and *Streptococcus lactis*. The growth of *Escherichia coli* in the asparagus oligosaccharides media was significantly weaker compared to the glucose media, indicating that *E. coli* preferred glucose as the source of carbon over oligosaccharides. Our research showed that asparagus oligosaccharides are ideal candidates as antioxidants and prebiotics in the food industry. These results provide a valuable information to expand the comprehensive utilization of asparagus, further developing the edible and medicinal value of asparagus oligosaccharides in the health-promoting food and feed industries.

Keywords
asparagus; oligosaccharides; purification; antioxidants; prebiotics

Asparagus is an economically important vegetable that is cultivated in temperate zones all over the world [1]. Asparagus is mainly produced in China, the United States, Japan, Spain and France. The global asparagus cultivation area is 218 000 ha with approx. 66 000 ha in China [2]. Asparagus contains various significant compounds and essential nutrients, including dietary fibre [3], oligosaccharides [4], amino acid derivatives [5], lignans [6], vitamins and minerals [7]. Asparagus also contains flavonoids (mainly rutin) and phenolic compounds, which possess strong antioxidant properties [8]. At present, asparagus is marketed as a new and special vegetable, and is primarily exported to Japan, Europe and other countries, but some of the exported asparagus is wasted in the international market [9].

Oligosaccharides are obtained by extraction from natural sources or by chemical or enzymatic synthesis [10–12]. KAMAT et al. reported that asparagus extract could significantly enhance bowel peristalsis and reduce the occurrence of ulcers [13]. Asparagus is commonly extracted by a hot water extraction method [14]. It has been shown to repair gamma-rays damage of mitochondria in the mouse liver due to its antioxidant ability, which has been studied by NWAFOR et al. [1]. GAUTAM et al. discovered that injecting immune acces-

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sory factors that were extracted from asparagus root can significantly improve the density of the whooping cough antibody, reducing the incidence of the disease and the mortality rate of mice [15]. The immuno-stimulating, antioxidant, antiangiogenic and antithrombotic activities of plant or algae poly/oligosaccharide molecules are induced by linkages between a glycan sequence and receptors on cell membranes [16]. Over the past few years, many researchers have suggested that dietary deficiencies can lead to disease states and that some diseases can be avoided through the adequate intake of prebiotics, such as fructooligosaccharides [12]. Since the late 1990s and the birth of the prebiotic concept, many scientists have studied the health-promoting properties of prebiotic compounds, which has resulted in a number of scientific publications describing their relation to human health [17].

Asparagus, in addition to its nutritional value, is beneficial to human health [18]. Some studies suggest that oligosaccharides can be viewed as the key component of prebiotics in human milk [19]. Consumption of human milk oligosaccharides, for example, is known to improve immune function, prevent adhesion of pathogens to intestinal epithelial tissues, increase absorption of minerals and improve glucose homeostasis. The research on oligosaccharides from plant food is commonly reported for fruits [20], but less for vegetables, in particular asparagus. The function of polysaccharides and flavonoids has been intensely studied in asparagus. Plant polysaccharides have high free radical scavenging and antimicrobial activities, making them ideal candidates for antioxidants and prebiotics [21]. However, the functions of asparagus oligosaccharides remain unknown. In our previous works, we mainly studied the extraction and removal of proteins from asparagus oligosaccharides, indicating that oligosaccharides mainly exist in the asparagus peel, which is usually discarded, thereby causing unnecessary waste [22]. Furthermore, oligosaccharides and their conjugates (glycoproteins and glycolipids) are important signalling compounds in vivo [23]. Therefore, research on asparagus oligosaccharide is of great significance.

In the present study, oligosaccharides were subjected to functional identification after purification by Sephadex G-25 gel filtration column (Pharmacia, Jersey City, New Jersey, USA). The study demonstrated that asparagus oligosaccharides are an excellent source of antioxidants and prebiotics. This study may be useful to the food industry regarding the comprehensive utilization of asparagus oligosaccharides as antioxidants and prebiotics for human health. Physico-chemical characteristics, the molecular weight and different functional groups were also studied, laying a theoretical foundation to determine additional functions of asparagus oligosaccharides in future work.

MATERIALS AND METHODS

Materials

Green asparagus Apollo F1 generation cultivars (Agricultural Science and Technology enterprises, Hebei, China) was used. Asparagus was subjected to hot water extraction and proteins removal using techniques from our previous study [22].

Purification and physico-chemical characterization of asparagus oligosaccharides

Asparagus oligosaccharides were purified by Sephadex G-25 gel filtration column (Pharmacia, Jersey City, New Jersey, USA). Sephadex G-25 gel filtration column (15 mm × 400 mm) was eluted with distilled water at a flow rate of 0.2 $ml \cdot min^{-1}$. Each fraction of 1 ml of eluate was collected. All collected fractions were characterized by the phenol-sulphuric acid method [24]. Absorbance was measured at 490 nm using ultraviolet-visible spectrophotometer Cary 8454 (Agilent, Santa Clara, California, USA).

Glucose (Sheng Baihao, Tianjin, China) standards were prepared in a series of concentrations to construct a standard curve for the asparagus oligosaccharide quantification. The chromatography profile was plotted using Microsoft Excel 2003 (Microsoft, Redmond, Washington, USA). The peak with the highest oligosaccharide content was collected and then freeze-dried.

Physico-chemical characterization included state, colour, smell, solubility in water, solubility in organic solvents, hydroscopicity, Molisch reaction, ultraviolet spectrum detection, Biuret reaction [25] and iodine-potassium iodide reaction.

Gel permeation chromatography and infrared spectra analysis

The molecular weight of purified asparagus oligosaccharides was determined by gel permeation chromatography (GPC) analysis using HP1100 (Agilent) [26]. The molecular weight was calculated by comparison to a calibration curve constructed using dextran standards (Sheng Baihao) of different molecular weights. Further structural characterization were performed using infrared spectrometer Cary 680 (Agilent). Pellets

for infrared analysis were prepared using 1 mg of dried oligosaccharide sample and 100 mg of potassium bromide, and spectral data were collected from 4000 cm^{-1} to 400 cm^{-1} [27].

Determination of hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of asparagus oligosaccharides was analysed using the method developed by COURTOIS [28]. Solutions of varying oligosaccharide concentrations, 1, 5, 10, 20 and 30 $mg \cdot ml^{-1}$, were added to tubes containing a phenanthroline–ferric ion– H_2O_2 system. The solutions were subsequently incubated in a water bath (37 °C) for 1 h. The absorbance value at 510 nm was measured in triplicate for each experiment.

The hydroxyl radical scavenging rate (RSR_{OH}) was calculated according to the following formula and expressed in percent:

$$RSR_{OH} = \frac{A_S - A_1}{A_2 - A_1} \times 100 \quad (1)$$

where A_S is the absorbance of the sample, A_1 is the absorbance of H_2O_2 in solutions without the samples, and A_2 is the absorbance of the sample without H_2O_2 present in the solution.

Determination of superoxide anion radical scavenging activity

The superoxide anion radical scavenging activity of asparagus oligosaccharides was analysed using the medthod developed by Chen et al. [29]. Oligosaccharide solutions with concentrations of 1, 5, 10, 20 and 30 $mg \cdot ml^{-1}$ were added to tubes and incubated in a water bath at 25 °C for 20 min. The absorbance value at 325 nm was measured every 30 s for 3 min, and each experiment was carried out in triplicate.

The superoxide anion radical scavenging rate (RSR_{O2}) was calculated according to the following formula and expressed in percent:

$$RSR_{O2} = \frac{A_C - A_S}{A_C} \times 100 \quad (2)$$

where A_C is the absorbance of the control, A_S is the absorbance of the sample.

Effect on proliferation of intestinal bifidobacteria and *Escherichia coli*

Our laboratory separated bifidobacteria and *E. coli*. Three different media were used for the tests: general anaerobic medium (GAM) liquid medium (Cat. No. HB8518, Sheng Baihao) with asparagus oligosaccharides (10 $mg \cdot ml^{-1}$, 20 $mg \cdot ml^{-1}$, 30 $mg \cdot ml^{-1}$), GAM liquid medium with added glucose (20 $mg \cdot ml^{-1}$), and GAM liquid medium without additional saccharides (control group). Fifty microliters of 10^{10} $CFU \cdot l^{-1}$ bifidobacteria and *E. coli* suspensions were added to these media, respectively, the inoculum concentration of the bacterial suspension being 10^8 $CFU \cdot l^{-1}$. The absorbance value at 550 nm (A_{550}) and pH were determined after the cultures were incubated for 0 h, 12 h and 24 h, each experiment being performed in triplicate.

Effect on proliferation of *Lactobacillus lactis* and *Streptococcus lactis*

Our laboratory separated *Lb. lactis* and *S. lactis*. Fifty microliters of 10^{10} $CFU \cdot l^{-1}$ *Lb. lactis* and *S. lactis* suspensions were added to DeMan-Rogosa-Sharpe (MRS) medium (Sheng Baihao) and ammonium paratungstate (APT) broth (Cat. No. HB0393-1, Sheng Baihao), respectively, the final concentration of the bacterial suspension being 10^8 $CFU \cdot l^{-1}$. The media without added asparagus oligosaccharides were used as the control group, and the medium with 20 $mg \cdot ml^{-1}$ asparagus oligosaccharides added was used as the experimental group. All media were cultured at 37 °C in an incubator. Aliquots of 0.1 ml of the bacterial suspension were removed from each experimental group at 0 h, 24 h and 48 h and subsequently diluted for plate counting, with each experiment performed in triplicate. The results are presented in the logarithmic format.

Statistical analyses

All treatments were run with 3 replicates and are presented as mean ± standard deviation of each experiment. The significant differences ($p < 0.05$) between the mean values were tested by analysis of variance (ANOVA) and Dunnett's T3 test using IBM SPSS Statistics 20 (SPSS, Chicago, Illinois, USA) software package.

RESULTS AND DISCUSSION

Purification and physico-chemical characterization of asparagus oligosaccharides

Fig. 1 shows the gel filtration chromatogram of the eluted asparagus oligosaccharides. Two major peaks were observed in the chromatogram. The first peak accounted for 95.6% of the total asparagus oligosaccharides collected. The second peak represented an oligosaccharide fraction that accounted for 3.4% of the total eluted product. The first peak was collected and chosen for further functional characterization (Fig. 2). Physico-

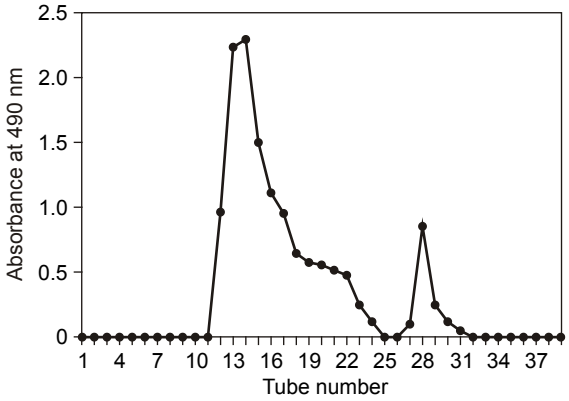

Fig. 1. Gel filtration chromatogram of asparagus oligosaccharides using a Sephadex G-25 gel column.

Fig. 2. Purified asparagus oligosaccharides.

chemical characterization suggested that the oligosaccharides purified by the Sephadex G-25 gel filtration column did not contain starch, protein, nucleic acids or other impurities (Tab. 1).

Molecular weight and functional groups of asparagus oligosaccharides

Pectic oligosaccharides have been shown to induce apoptosis in human colonic adenocarcinoma cells. They are extremely diverse in their structures and rich in galacturonic acid. The complex structures are important with regard to functional properties such as anti-cancer activities and other bioactive properties [30]. In our present study, GPC analysis was employed to determine the molecular weight of the collected asparagus oligosaccharides. Based on the equation from the standard curve developed using different dextran standards, and the retention time of the asparagus oligosaccharides, the molecular weight of the purified asparagus oligosaccharides was estimated to be 569 Da. The infrared spectrum of the purified asparagus oligosaccharides is displayed in Fig. 3. The spectrum suggests the identity of the functional groups on the asparagus oligosaccharides. A wide absorption peak was observed at 3367 cm^{-1}, identified as the O–H stretching vibration, which indicates the presence of inter- and intramolecular hydrogen bonds in the oligosaccharides. An absorption peak at 2936 cm^{-1} was assigned to C–H stretching vibrations, an absorption peak at 1743 cm^{-1} was assigned to C=O stretching vibrations, and an absorption peak at 1621 cm^{-1} indicated the presence of crystallized water and C=O stretching vibration. Based on the observed peaks, we can conclude the presence of carboxyl group and uronic acid. Absorption peaks at 1400–1200 cm^{-1} were associated with vibrations

Tab. 1. Physico-chemical characteristic of asparagus oligosaccharides.

Item	Characteristic
State	Loose, powder
Colour	White
Smell	None
Solubility in water	Soluble
Solubility in organic solvents	Insoluble
Hydroscopicity	Weak
Molisch reaction	Positive
Ultraviolet spectrum detection	No absorption peaks at 260–280 nm
Biuret reaction	Negative
Iodine-potassium iodide reaction	Negative

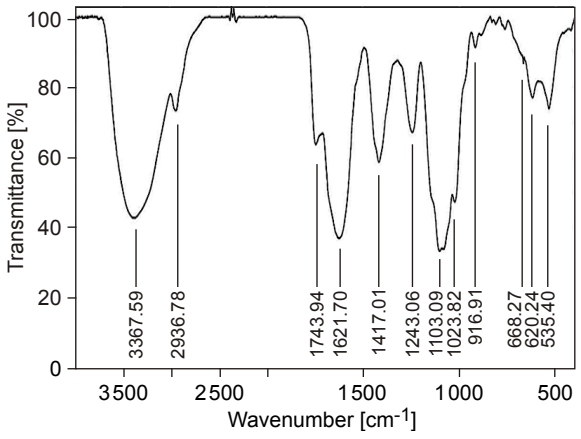

Fig. 3. Infrared spectrum of asparagus oligosaccharides.

of the C–H bond. Absorption peaks at 1 103 cm^{-1} and 1 023 cm^{-1} suggest that the saccharide rings in asparagus oligosaccharides belong to the pyran class. An absorption peak at 916 cm^{-1} was assigned to the asymmetric stretching vibration of the pyran ring. The monosaccharide residues of fructooligosaccharides in asparagus roots exist in the forms of the pyran rings and furan rings [31]. Human milk oligosaccharides contain various glycosidic bonds. This structural complexity of oligosaccharides appears to be the key to their biological activity [32]. Some neutral or acidic oligosaccharides with substituents may be active, and the presence of carboxyl group and uronic acid has previously been established in oligosaccharides. Therefore, analysis of the molecular structure of oligosaccharides is essential to understand the structure-function relationship in food science [33].

Antioxidant capacity of asparagus oligosaccharides

Asparagus oligosaccharides are efficient at scavenging hydroxyl radicals (Fig. 4A), and a dose-response relationship was observed. The scavenging activity was greater than 50% when 20 $mg \cdot ml^{-1}$ asparagus oligosaccharide was added. Additionally, mannitol is an effective hydroxyl radical scavenger [34]. Our results indicated that the scavenging activity of asparagus oligosaccharides for hydroxyl radical was greater than that of mannitol at the same concentration ($p < 0.05$). Asparagus oligosaccharide (10 $mg \cdot ml^{-1}$) and mannitol (30 $mg \cdot ml^{-1}$) have similar hydroxyl radical scavenging activities. Hydroxyl radicals were generated by lipid peroxidation [35]. Oligosaccharide has reductive hemiacetal hydroxyl, which can capture reactive oxygen, shorten the length of the lipid peroxidation chain and slow down lipid peroxidation. In addition, hydrogen atoms on the hydrocarbon chains of asparagus oligosaccharides can bind to hydroxyl radical to form water and free radical carbons [36].

The carbon free radicals further participate in oxidation reactions to form peroxy radicals and finally decompose to harmless products. This reaction pathway was also reported by Hernandez-Marin and Martínez in a theoretical analysis of the free radical scavenging properties of selected carbohydrates, which suggested a hydrogen atom transfer from the investigated hydrocarbons to the hydroxyl radicals [37]. The primary polysaccharide of asparagus, which has a molecular weight 5.75×10^4 Da, has a significant function in scavenging hydroxyl radicals. Using same concentrations, the hydroxyl radical scavenging ability of asparagus oligosaccharides was weaker compared to that of asparagus polysaccharides [38], suggesting that asparagus oligosaccharides have some oxidative resistance, but they are not the primary antioxidant in asparagus.

Fig. 4B suggests that the clearance rate of the superoxide anion increased with an increase in the concentration of asparagus oligosaccharides ($p < 0.05$). Asparagus oligosaccharides can restrain the pyrogallol autoxidation and clear superoxide anions produced via pyrogallol autoxidation. Once superoxide anions react with carbonyls, they can damage DNA and hinder physical functions. Asparagus oligosaccharides

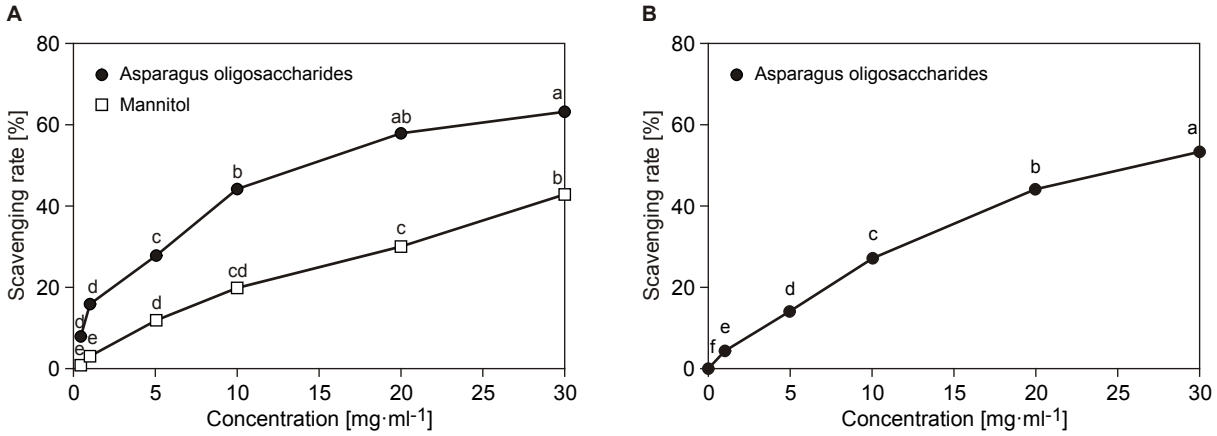

Fig. 4. Effects of asparagus oligosaccharides on scavenging activity of hydroxyl radical and superoxide anion radical.

A – Hydroxyl radical scavenging rate, B – Superoxide anion radical scavenging rate.
Data are mean ± standard deviation of three independent experiments. Significant differences ($p = 0.05$) between means are indicated by different letters.

can remove superoxide anion with a dose-effect relationship by participating in an oxidation reaction with the superoxide anion and preventing pyrogallol autoxidation. Our study showed that the maximum inhibition rate was 53.3% when adding 30 mg·ml^{-1} asparagus oligosaccharides. Therefore, asparagus oligosaccharides can be used as a natural free radical scavenger. There is a growing interest in the substitution of drugs and medicines by natural compounds from plant food for the prevention of diseases and the enhancement of general human health. Apple pectic oligosaccharides exhibit scavenging activity for superoxide anion and hydroxyl radicals [39].

Our study shows that the oligosaccharides obtained from asparagus are suitable for use as a functional ingredient. Based on the antioxidant study, we found that asparagus oligosaccharides also have a relatively high free radical scavenging capacity, making them ideal candidates for use as antioxidants. It could be proposed that the regular use of asparagus oligosaccharides may help in the prevention of several diseases related to oxidative damage.

Fan et al. [40] investigated the relation between total antioxidant activity and total phenolics extracted from the residues of asparagus. Antioxidant activity evaluation indicated that phenolics were dominant bioactive compounds. Rodríguez et al. [41] studied the correlation between antioxidant activity and the content of total phenolics extracted from green asparagus. Results obtained for antiradical capacity and reducing power were very similar, and a high correlation with phenolics was found. Asparagus inhibits lipid primary oxidation, but no correlation between the inhibition percentage and phenolics was observed. Bonoli et al. [42] reported that phenolic compounds have strong in vitro and in vivo antioxidant activities associated with their ability to scavenge free radicals, break radical chain reactions, and chelate metals. 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity was significantly correlated with total phenolics content. Decreasing of phenolics content was shown to be a response to the senescence of asparagus [43]. Phenolic antioxidants were reported to be able to quench oxygen-derived free radicals as well as substrate-derived free radicals by donating a hydrogen atom or an electron to the free radical. For instance, phenolic extracts of plant materials were shown to neutralize free radicals in various model systems [44]. Asparagus was divided into three sections: apical, middle and basal. In the fresh asparagus, content of uronic acid is higher in the apical and basal sections and slightly lower in the middle one [45]. However, we noticed that at present studies, as far as we know, there are very few works on percentage of uronic acid and investigating the relationship between uronic acid and antioxidant activity. This question is still very meaningful to be further studied and discussed. We will conduct the deep research of ascertaining the percentage of uronic acid or bound phenolics to better explain the antioxidant activity and study the specific structure-antioxidant activity relationship of asparagus oligosaccharides in our further studies.

Effect of asparagus oligosaccharides on bacteria

It is widely accepted that plant polysaccharides have high antimicrobial activity, which make them ideal candidates for prebiotics [21]. However, the functions of plant oligosaccharides are barely understood. In this study, we examined the effects of asparagus oligosaccharides on bifidobacteria, *E. coli*, *Lb. lactis* and *S. lactis* to further analyse the properties of asparagus oligosaccharides.

Bifidobacteria dominate the intestinal microbiota of many mammals, especially during the milk-feeding period, as they are supported by components with the mother's milk, and their presence in high numbers is associated with good health [46–48]. Our results indicated that the absorbance in media containing 20 mg·ml^{-1} asparagus oligosaccharides was higher compared to media with 20 mg·ml^{-1} glucose added and control media ($p < 0.05$) (Fig. 5A). The pH experiments exhibited the opposite trend (Fig. 5B). These data suggested that asparagus oligosaccharides could effectively promote the growth of bifidobacteria. Commercially available fructooligosaccharides are typical examples of prebiotics for bifidobacteria. The selective increase in the number of bifidobacteria in the human and animal large intestine due to the ingestion of fructooligosaccharides confers several benefits to their host, such as the competitive exclusion of intestinal pathogens, a reduction in serum cholesterol, an increase in calcium and magnesium absorption, the prevention of colon cancer and the production of B vitamins [49, 50].

Fig. 6A suggests that bifidobacteria grow rapidly in all types of GAM liquid media equipped with different doses of asparagus oligosaccharides. A_{550} increased with the increase of the dose and peaked in the high-dose group ($p < 0.05$). The pH of the three dose groups exhibited a negative trend, and the high dose group decreased more rapidly compared to the other groups ($p < 0.05$) (Fig. 6B). These results demonstrated that the growth of bifidobacteria accelerated with the increase in concentration of asparagus

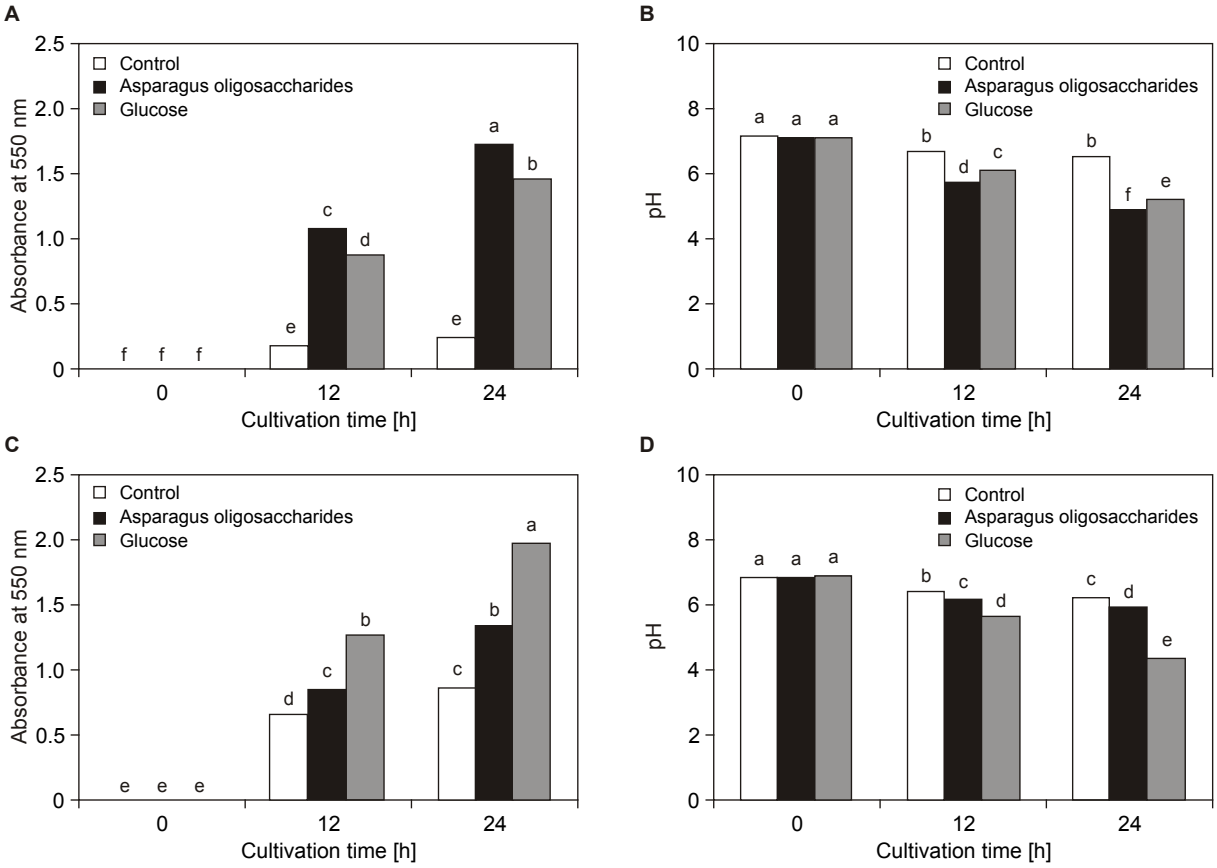

Fig. 5. Effect of asparagus oligosaccharides and glucose in GAM medium on bifidobacteria and *E. coli*.

A – absorbance of medium inoculated with bifidobacteria, B – pH of medium inoculated with bifidobacteria, C – absorbance of medium inoculated with *E. coli*, D – pH of medium inoculated with *E. coli.*
Concentration of asparagus oligosaccharides and glucose in media was 20 mg·ml^{-1}. Data are mean ± standard deviation of three independent experiments. Significant differences ($p = 0.05$) between means are indicated by different letters.

oligosaccharides. Carbohydrates can be fermented by intestinal microorganisms and produce a variety of volatile fatty acids, including formic acid, acetic acid, propionic acid, butyric acid and large amounts of lactic acid [51, 52]. The pH value was significantly decreased as observed during growth of bifidobacteria on culture with oligosaccharides. We deduce that adding oligosaccharides may increase volatile fatty acid concentration, and thus decrease pH. The use of fructooligosaccharides is mediated by bacterial hydrolases of the colon, so that the bacteria produce glycolytic enzymes that hydrolyse oligosaccharides to mono- or disaccharides. These are transported to the interior of the cell, where they are metabolized to short chain volatile fatty acids, CO_2 and hydrogen [53, 54]. These fatty acids, particularly acetate, propionate and butyrate, are the main end products of bacterial fermentation reactions that acidify the colon [55]. This decrease in pH favours the development of bacteria such as bifidobacteria [56]. Adding oligosaccharides to feed can increase the content of volatile fatty acids and decrease the pH value in chicken and rat [57–59].

For *E. coli,* the absorbance of cultures in media with asparagus oligosaccharides was higher compared to that of the control media, and the trend of the pH experiments was negative (Fig. 5C and Fig. 5D), indicating that asparagus oligosaccharides can be used by *E. coli* as carbon sources if no other sources are available. Compared to the media with glucose, the absorbance of the asparagus oligosaccharides media was lower and the pH value was higher ($p < 0.05$), suggesting that the growth of *E. coli* in the asparagus oligosaccharides media was significantly weaker compared to the glucose media. We conclude that *E. coli* preferred glucose as the source of carbon over oligosaccharides. In addition, *E. coli* could grow in GAM liquid media with three different doses of asparagus oligosaccharides added (Fig. 6C and Fig. 6D). A_{550} of the media in the

high-dose group was greater compared to that of the other groups after 24 h. During that 24 h period, pH of the three dose groups displayed a downward trend. The pH value of the high-dose group decreased more rapidly, while the low-dose group decreased more slowly. *E. coli* is a potentially pathogenic organism [60]. Blocking microbial lectins from binding to host cells is a possible antibacterial mechanism, which has been reported at anti-adhesive oligosaccharides [61]. Binding of enteropathogenic *E. coli* O127 to the surface of Caco-2 epithelial cells was found to be inhibited by β-galactooligosaccharides [62].

Lb. lactis and *S. lactis* can grow in MRS medium. After adding asparagus oligosaccharides to the nutrient-rich MRS medium, the proliferation rates of *Lb. lactis* and *S. lactis* were higher compared to the control group (Fig. 7). As shown in Fig. 8, amounts of *Lb. lactis* and *S. lactis* increased in the control group during the first 24 h and exhibited a downward trend from 24 h to 48 h. *Lb. lactis* and *S. lactis* in the asparagus oligosaccharides group proliferated throughout the experiment. The amounts of *Lb. lactis* and *S. lactis* in the asparagus oligosaccharides group were higher compared to the control group at 24 h and 48 h ($p < 0.05$). This study suggests that oligosaccharides can promote the proliferation rates of *Lb. lactis* and *S. lactis* effectively. The known health-promoting effects of dairy products, especially sour milk and yoghurt, stem from viable probiotic lactic acid bacteria that have scientifically proven health effects and safety. Some lactic acid bacteria are exploited for their role as producers of peptides with antimicrobial activity [63]. The effect of combining the actions of oligosaccharides and lactic acid bacteria lays a theoretical foundation for prebiotic products.

A prebiotic can be defined as a non-digestible food ingredient that has beneficial effects

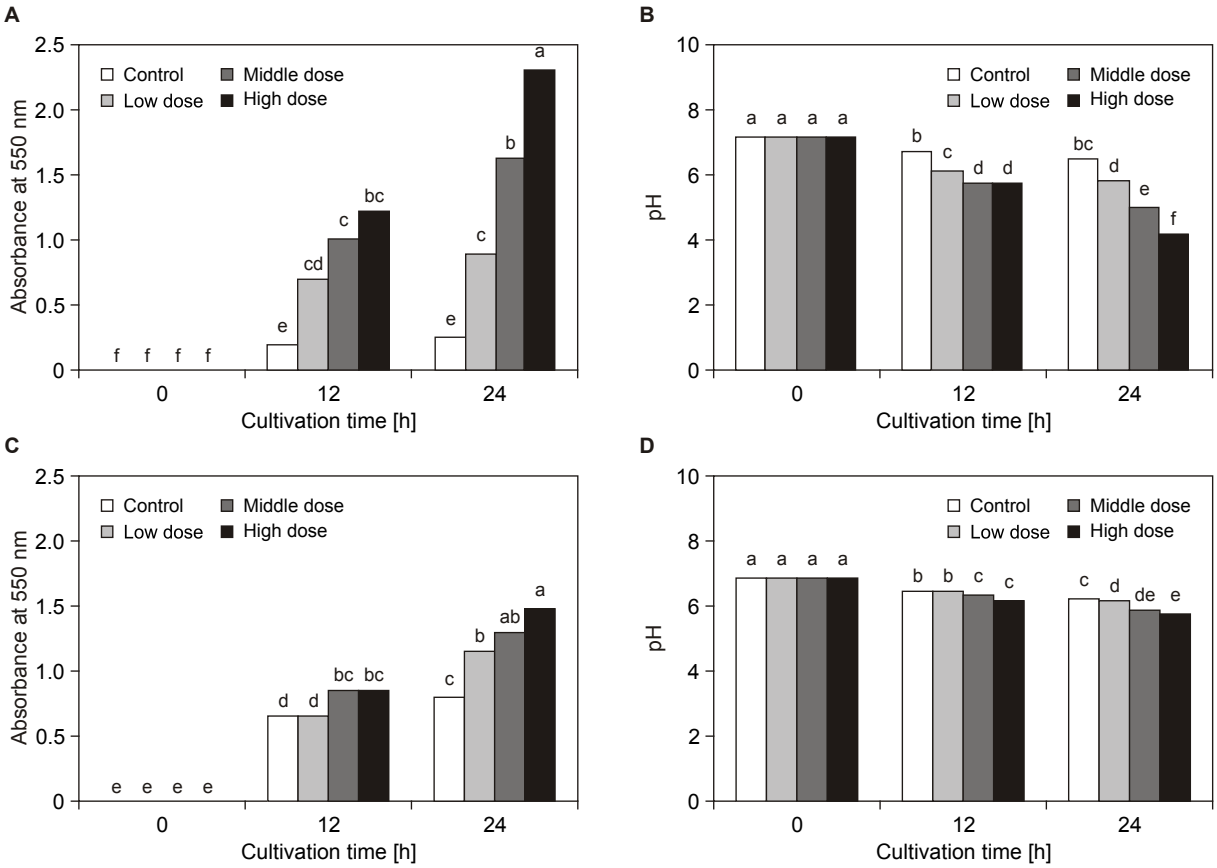

Fig. 6. Effect of different doses of asparagus oligosaccharides in GAM medium on bifidobacteria and *E. coli*.

A – absorbance of medium inoculated with bifidobacteria, B – pH of medium inoculated with bifidobacteria, C – absorbance of medium inoculated with *E. coli*, D – pH of medium inoculated with *E. coli.*
Concentration of asparagus oligosaccharides: low dose – 10 mg·ml^{-1}, middle dose – 20 mg·ml^{-1}, high dose – 30 mg·ml^{-1}.
Data are mean ± standard deviation of three independent experiments. Significant differences ($p = 0.05$) between means are indicated by different letters.

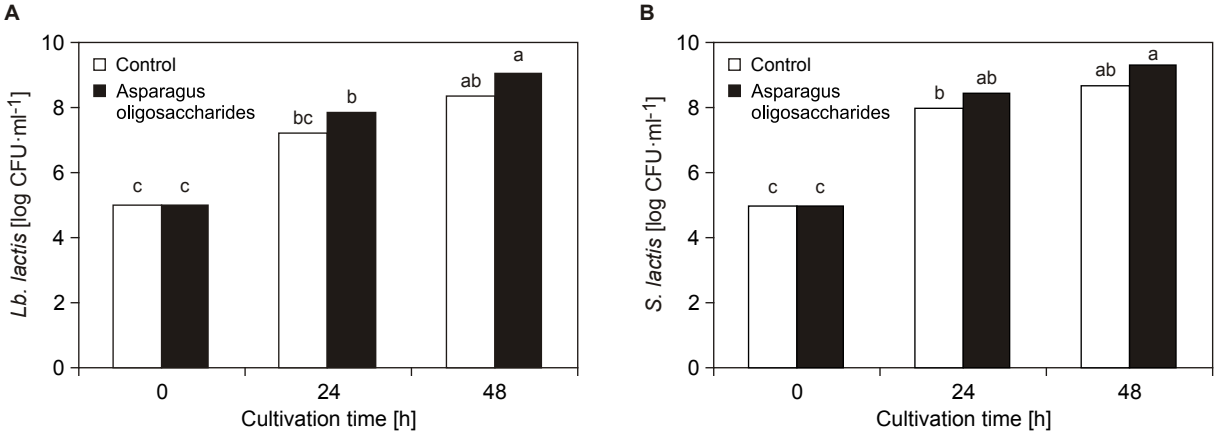

Fig. 7. Effect of asparagus oligosaccharides in MRS medium on *Lactobacillus lactis* and *Streptococcus lactis*.

A – medium inoculated with *Lb. lactis*, B – medium inoculated with *S. lactis*.
Data are mean ± standard deviation of three independent experiments. Significant (p = 0.05) differences between means are indicated by different letters.

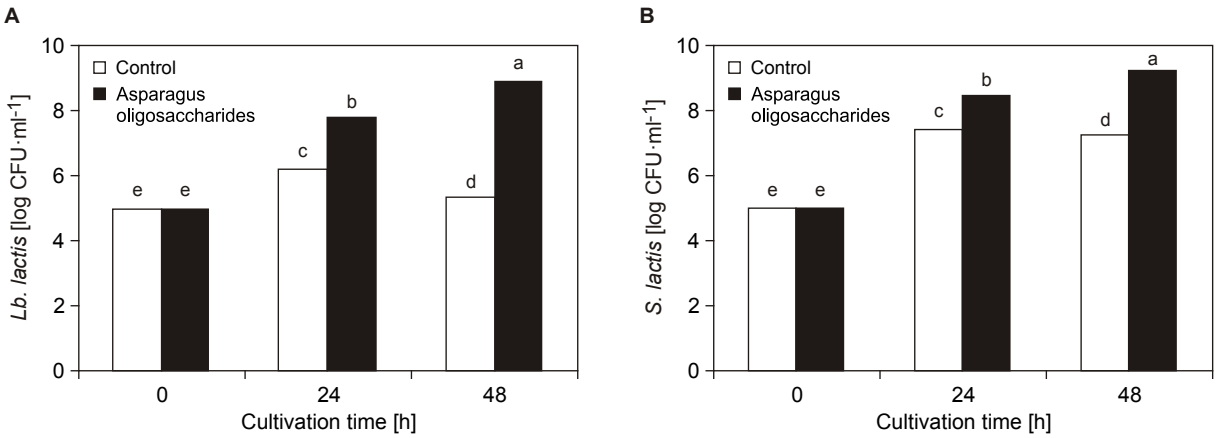

Fig. 8. Effects of asparagus oligosaccharides in APT broth on *Lactobacillus lactis* and *Streptococcus lactis*.

A – medium inoculated with *Lb. lactis*, B – medium inoculated with *S. lactis*.
Data are mean ± standard deviation of three independent experiments. Significant (p = 0.05) differences between means are indicated by different letters.

on the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving the host's health. GALEOTTI et al. [64] reported that human milk oligosaccharides have many biological and protective functions. Based on these previous findings and the results of this study, it could be concluded that asparagus oligosaccharides are functional components, which can be used as ideal candidates for prebiotics to provide effective help for improving intestinal tract dysfunction, promote intestinal peristalsis and enhance the human immune system.

CONCLUSIONS

Asparagus is rich in nutritional and functional compounds, and therefore analysing the function of asparagus oligosaccharides more thoroughly can theoretically increase the use of asparagus. In this study, asparagus oligosaccharides were subjected to characterization after purification by a Sephadex G-25 gel filtration column. The study results show that asparagus oligosaccharides are an excellent source of antioxidants and prebiotics. This could be profitable for the food industry regarding the comprehensive use of asparagus

oligosaccharides as antioxidants and prebiotics for human health. The physico-chemical characteristics, molecular weight and different functional groups were also studied, laying a theoretical foundation to determine additional functions of asparagus oligosaccharides in future work.

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REFERENCES

1. Nwafor, P. A. – Okwuasaba, F. K. – Binda, L. G.: Antidiarrhoeal and antiulcerogenic affects of methanolic extract of *Asparagus pubescens* root in rats. Journal of Ethnopharmacology, *72*, 2000, pp. 421–427. DOI: 10.1016/S0378-8741(00)00261-0.
2. Ahemad, M. – Khan, M. S.: Comparative toxicity of selected insecticides to pea plants and growth promotion in response to insecticide-tolerant and plant growth promoting *Rhizobium leguminosarum*. Crop Protection, *29*, 2010, pp. 325–329. DOI: 10.1016/j.cropro.2010.01.005.
3. Sun, Z. – Huang, X. – Kong, L.: A new steroidal saponin from the dried stems of *Asparagus officinalis* L. Fitoterapia, *81*, 2010, pp. 210–213. DOI: 10.1016/j.fitote.2009.09.002.
4. Fukushi, E. – Onodera, S. –Yamamori, A. – Shiomi, N. – Kawabata, J.: NMR analysis of tri- and tetrasaccharides from asparagus. Magnetic Resonance in Chemistry, *38*, 2000, pp. 1005–1011. DOI: 10.1002/1097-458X(200012)38:12<1005::AID-MRC772>3.0.CO;2-Q.
5. Kasai, T. – Sakamura, S.: *N*-carboxymethyl-L-serine, a new acidic amino acid from asparagus (*Asparagus officinalis*) shoots. Agricultural and Biological Chemistry, *45*, 1981, pp. 1483–1485. DOI: 10.1080/00021369.1981.10864709.
6. Thompson, L. U. – Robb, P. – Serraino, M. – Cheung, F.: Mammalian lignan production from various foods. Nutrition and Cancer, *16*, 1991, pp. 43–52. DOI: 10.1080/01635589109514139.
7. Kim, B. Y. – Cui, Z. G. – Lee, S. R. – Kim, S. J. – Kang, H. K. – Lee, Y. K. – Park, D. B.: Effects of *Asparagus officinalis* extracts on liver cell toxicity and ethanol metabolism. Journal of Food Science, *74*, 2009, pp. H204–H208. DOI: 10.1111/j.1750-3841.2009.01263.x.
8. Makris, D. P. – Rossiter, J. T.: Domestic processing of onion bulbs (*Allium cepa*) and asparagus spears (*Asparagus officinalis*): Effect of flavonol content and antioxidant status. Journal of Agricultural and Food Chemistry, *49*, 2001, pp. 3216–3222. DOI: 10.1021/jf001497z.
9. Villanueva, M. J. – Tenorio, M. D. – Sagardoy, M.: Physical, chemical, histological and microbiological changes in fresh green asparagus (*Asparagus officinalis* L.) stored in modified atmosphere packaging. Food Chemistry, *91*, 2005, pp. 609–619. DOI: 10.1016/j.foodchem.2004.06.030.
10. Mussatto, S. I. – Mancilha, I. M.: Non-digestible oligosaccharides: A review. Carbohydrate Polymers, *68*, 2007, pp. 587–597. DOI: 10.1016/j.carbpol.2006.12.011.
11. Jovanovic-Malinovska, R. – Fernandes, P. – Winkelhausen, E. – Fonseca, L.: Galactooligosaccharides synthesis from lactose and whey by immobilized β-galactosidase in PVA. Applied Biochemistry and Biotechnology, *168*, 2012, pp. 1197–1211. DOI: 10.1007/s12010-012-9850-1.
12. Dominguez, A. L. – Rodrigues, L. R. – Lima, N. M. – Teixeira, J. A.: An overview of the recent developments on fructooligosaccharide production and applications. Food and Bioprocess Technology, *7,* 2014, pp. 324–337. DOI: 10.1007/s11947-013-1221-6.
13. Kamat, J. P. – Bolour, K. K. – Devasagayam, T. P. A. – Venkatachalam, S. R.: Antioxidant properties of *Asparagus racemosus* against damage induced by γ-radiation in rat liver mitochondria. Journal of Ethnopharmacology, *71*, 2000, pp. 425–435. DOI: http://dx.doi.org/10.1016/S0378-8741(00)00176-8.
14. Singh, R. S. – Dhaliwal, R. – Puri, M.: Production of high fructose syrup from *Asparagus* inulin using immobilized exoinulinase from *Kluyveromyces marxianus* YS-1. Journal of Industrial Microbiology and Biotechnology, *34*, 2007, pp. 649–655. DOI: 10.1007/s10295-007-0237-1.
15. Gautam, M. – Diwanay, S. – Gairola, S. – Shinde, Y. – Patki, P. – Patwardhan, B.: Immunoadjuvant potential of *Asparagus racemosus* aqueous extract in experimental system. Journal of Ethnopharmacology, *91,* 2004, pp. 251–255. DOI: 10.1016/j.jep.2003.12.023.
16. Becker, C. F. – Guimarães, J. A. – Mourão, P. A. S. – Verli, H.: Conformation of sulfated galactan and sulfated fucan in aqueous solutions: Implications to their anticoagulant activities. Journal of Molecular Graphics and Modelling, *26*, 2007, pp. 391–399. DOI: 10.1016/j.jmgm.2007.01.008.
17. Peshev, D. – Van den Ende, W.: Fructans: prebiotics and immunomodulators. Journal of Functional Foods, *8*, 2014, pp. 348–357. DOI: 10.1016/j.jff.2014.04.005.
18. Jovanovic-Malinovska, R. – Kuzmanova, S. – Winkelhausen, E.: Application of ultrasound for enhanced extraction of prebiotic oligosaccharides from selected fruits and vegetables. Ultrasonics Sonochemistry, *22*, 2015, pp. 446–453. DOI: 10.1016/j.ultsonch.2014.07.016.
19. Laitinen, K. – Poussa, T. – Isolauri, E.: Probiotics and dietary counselling contribute to glucose regulation during and after pregnancy: a randomised controlled trial. British Journal of Nutrition, *101*, 2009, pp. 1679–1687. DOI: 10.1017/S0007114508111461.
20. Benkeblia, N. – Lopez, M. G.: Saccharides and fructooligosaccharides composition of green and ripe

Averrhoa carambola, *Blighia sapida* and *Spondias dulcis* fruits. Food Chemistry, *176*, 2015, pp. 314–318. DOI: 10.1016/j.foodchem.2014.12.080.
21. Xie, J. H. – Liu, X. – Shen, M. Y. – Nie, S. P. – Zhang, H. – Li, C. – Gong, D. M. – Xie, M. Y.: Purification, physicochemical characterisation and anticancer activity activity of a polysaccharide from *Cyclocarya paliurus* leaves. Food Chemistry, *136*, 201, pp. 1453–1460. DOI: 10.1016/j.foodchem.2012.09.078.
22. Li, X. Y. – Xie, B. – Guo, W. L. – Guo, R. Z. – Kou, X. H.: Extraction and purification of *Asparagus* oligosaccharides. Advanced Materials Research, *550–553,* 2012, pp. 1886–1892. DOI: 10.4028/www.scientific.net/AMR.550-553.1886.
23. Seeberger, P. H. – Werz, D. B.: Synthesis and medical applications of oligosaccharides. Nature, *446*, 2007, pp. 1046–1051. DOI: 10.1038/nature05819.
24. Dubois, M. – Gilles, K. A. – Hamilton, J. K. – Rebers, P. A. – Smith, F.: Colorimetric method for determination of sugar and related substances. Analytical Chemistry, *28*, 1956, pp. 350–356. DOI: 10.1021/ac60111a017.
25. Goshev, I. – Nedkov, P.: Extending the range of application of the biuretreaction: Quantitative determination of insoluble proteins. Analytical Biochemistry, *95*, 1979, pp. 340–343. DOI: 10.1016/0003-2697(79)90736-X.
26. Van Langenhove. A. – Reinhold, V. N.: Determination of polysaccharide linkage and branching by reductive depolymerization. Gas-liquid chromatography and gas-liquid chromatography-mass spectrometry reference data. Carbohydrate Research, *143*, 1985, pp. 1–20. DOI: 10.1016/S0008-6215(00)90691-8.
27. Agarwal, R. – Diwanay, S. – Patki, P. – Patwardhan, B.: Studies on immunomodulatory activity of *Withania somnifera* (Ashwagandha) extracts in experimental immune inflammation. Journal of Ethnopharmacology, *67*, 1999, pp. 27–35. DOI: 10.1016/S0378-8741(99)00065-3.
28. Courtois, J.: Oligosaccharides from land plants and algae: production and applications in therapeutics and biotechnology. Current Opinion in Microbiology, *12*, 2009, pp. 261–273. DOI: 10.1016/j.mib.2009.04.007.
29. Xu, C. – Liu, S. – Liu, Z. – Song, F. – Liu, S.: Superoxide generated by pyrogallol reduces highly water-soluble tetrazolium salt to produce a soluble formazan: A simple assay for measuring superoxide anion radical scavenging activities of biological and abiological samples. Analytica Chimica Acta, *793*, 2013, pp. 53–60. DOI: 10.1016/j.aca.2013.07.027.
30. Willats, W. G. T. – Knox, J. P. – Mikkelsen, J. D.: Pectin: new insights into an old polymer are starting to gel. Trends in Food Science and Technology, *17*, 2006, pp. 97–104. DOI: 10.1016/j.tifs.2005.10.008.
31. Shiomi, N. – Yamada, J. – Izawa, M.: Isolation and identification of fructooligosaccharides in roots of asparagus (*Asparagus officinalis* L.). Agricultural and Biological Chemistry, *40*, 1976, pp. 567–575. DOI: 10.1080/00021369.1976.10862085.
32. Strum, J. S. – Aldredge, D. – Barile, D. – Lebrilla, C. B.: Coupling flash liquid chromatography with mass spectrometry for enrichment and isolation of milk oligosaccharides for functional studies. Analytical Biochemistry, *424*, 2012, pp. 87–96. DOI: 10.1016/j.ab.2012.02.012.
33. Li, S.-P. – Wu, D.-T. – Lv, G.-P. – Zhao, J.: Carbohydrates analysis in herbal glycomics. Trends in Analytical Chemistry, *52*, 2013, pp. 155–169. DOI: 10.1016/j.trac.2013.05.020.
34. Silvestre, C. I. C. – Santos, J. L. M. – Lima, J. L. F. C. – Zagatto, E. A. G.: Single reaction interface flow system for chemiluminescent monitoring of mannitol based on its hydroxyl radical scavenger activity. Talanta, *77*, 2008, pp. 518–521. DOI: 10.1016/j.talanta.2008.03.021.
35. Smirnoff, N. – Cumbes, Q. J.: Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry, *28*, 1989, pp. 1057–1060. DOI: 10.1016/0031-9422(89)80182-7.
36. Yuan, X. – Wang, J. – Yao,H. – Chen, F.: Free radical-scavenging capacity and inhibitory activity on rat erythrocyte hemolysis of feruloyl oligosaccharides from wheat bran insoluble dietary fiber. LWT - Food Science and Technology, *38*, 2005, pp. 877–883. DOI: 10.1016/j.lwt.2004.09.012.
37. Hernandez-Marin, E. – Martínez, A.: Carbohydrates and their free radical scavenging capability: A theoretical study. Journal of Physical Chemistry B, *116*, 2012, pp. 9668–9675. DOI: 10.1021/jp304814r.
38. Zhao, Q. – Xie, B. – Yan, J. – Zhao, F. – Xiao, J. – Yao, L. – Zhao, B. – Huang, Y.: *In vitro* antioxidant and antitumor activities of polysaccharides extracted from *Asparagus officinalis*. Carbohydrate Polymers, *87*, 2012, pp. 392–396. DOI: 10.1016/j.carbpol.2011.07.068.
39. Fuentes-Alventosa, J. M. – Jaramillo-Carmona, S. – Rodríguez-Gutiérrez, G. – Guillén-Bejarano, R. – Jiménez-Araujo, A. – Fernández-Bolaños, J. – Rodríguez-Arcos, R.: Preparation of bioactive extracts from asparagus by-product. Food and Bioproducts Processing, *91*, 2013, pp. 74–82. DOI: 10.1016/j.fbp.2012.12.004.
40. Fan, R. – Yuan, F. – Wang, N. – Gao, Y. – Huang, Y.: Extraction and analysis of antioxidant compounds from the residues of *Asparagus officinalis* L. Journal of Food Science and Technology, *52*, 2015, pp. 2690–2700. DOI: 10.1007/s13197-014-1360-4.
41. Rodríguez, R. – Jaramillo, S. – Rodríguez, G. – Espejo, J. A. – Guillén, R. – Fernández-Bolaños, J. – Heredia, A. – Jiménez, A.: Antioxidant activity of ethanolic extracts from several asparagus cultivars. Journal of Agricultural and Food Chemistry, *53*, 2005, pp. 5212–5217. DOI: 10.1021/jf050338i.
42. Bonoli, M. – Verardo, V. – Marconi, E. – Caboni, M. F.: Antioxidant phenols in barley (*Hordeum vulgare* L.) flour: comparative spectrophotometric study among extraction methods of free and bound phenolic compounds. Journal of Agricultural and Food Chemistry, *52*, 2004, pp. 5195–5200. DOI: 10.1021/jf040075c.
43. Wei, Y. – Liu, Z. – Su, Y. – Liu,D. – Ye, X.: Effect of salicylic acid treatment on postharvest quality, antioxidant activities, and free polyamines of asparagus. Journal of Food Science, *76*, 2011, pp. S126–S132.

DOI: 10.1111/j.1750-3841.2010.01987.x.
44. Wettasinghe, M. – Shahidi, F.: Antioxidant and free radical-scavenging properties of ethanolic extracts of deffated borage (*Borago officinalis* L.) seeds. Food Chemistry, *67*, 1999, pp. 399–414. DOI: 10.1016/S0308-8146(99)00137-5.
45. Rodríguez, R. – Jiménez, A. – Guillén, R. – Heredia, A. – Fernández-Bolaños, J.: Postharvest changes in white asparagus cell wall during refrigerated storage. Journal of Agricultural and Food Chemistry, *47,* 1999, pp. 3551–3557. DOI: 10.1021/jf981295r.
46. Chiu, Y.-H. – Tsai, J.-J. – Lin, S.-L. – Chotirosvakin, C. – Lin, M.-Y.: Characterisation of bifidobacteria with immunomodulatory properties isolated from human breast milk. Journal of Functional Foods, *7*, 2014, pp. 700–708. DOI: 10.1016/j.jff.2013.12.015.
47. Russell, D. A. – Ross, R. P. – Fitzgerald, G. F. – Stanton, C.: Metabolic activities and probiotic potential of bifidobacteria. International Journal of Food Microbiology, *149*, 2011, pp. 88–105. DOI: 10.1016/j.ijfoodmicro.2011.06.003.
48. Sánchez, B. – Ruiz, L. – Gueimonde, M. – Ruas-Madiedo, P. – Margolles, A.: Adaptation of bifidobacteria to the gastrointestinal tract and functional consequences. Pharmacological Research, *69,* 2013, pp. 127–136. DOI: 10.1016/j.phrs.2012.11.004.
49. Ritsema, T. – Smeekens, S. C. M.: Fructans: beneficial for plants and humans. Current Opinion in Plant Biology, *6*, 2003, pp. 223–230. DOI: 10.1016/S1369-5266(03)00034-7.
50. Van der Meulen, R. – Avonts, L. – De Vuyst, L.: Short fractions of oligofructose are preferentially metabolized by *Bifidobacterium aaninalis* DN-173010. Applied and Environmental Microbiology, *70*, 2004, pp. 1923–1930. DOI: 10.1128/AEM.70.4.1923-1930.2004.
51. Imoto, S. – Namioka, S.: VFA production in pig large intestine. Journal of Animal Science. *47,* 1978, pp. 467–478. DOI: 10.2527/jas1978.472467x.
52. Canh, T. T. – Sutton, A. L. – Aarnink, A. J. – Verstegen, M. W. – Schrama, J. W. – Bakker, G. C.: Dietary carbohydrates alter the fecal composition and pH and the ammonia emission from slurry of growing pigs. Journal of Animal Science, *76*, 1998, pp. 1887–1895. DOI: 10.2527/1998.7671887x.
53. Sabater-Molina, M. – Larqué, E. – Torrella, F. – Zamora, S.: Dietary fructooligosaccharides and potential benefits on health. Journal of Physiology and Biochemistry, 6*5*, 2009, pp. 315–328. DOI: 10.1007/BF03180584.
54. Salminen, S. – Bouley, C. – Boutron, M. C. – Cummings, J. H. – Franck, A. – Gibson, G. R. – Isolauri,E.–Moreau,M.-C.–Roberfroid,M.–Rowland,I.: Functional food science and gastrointestinal physiology and function. British Journal of Nutrition, *80*, 1998, pp. S147–S171. DOI: 10.1079/BJN19980108.
55. Swennen, K. – Courtin, C. M. – Delcour, J. A.: Non-digestible oligosaccharides with prebiotic properties. Critical Reviews in Food Science and Nutrition, *46*, 2006, pp. 459–471. DOI: 10.1080/10408390500215746.
56. Blaut, M.: Relationship of prebiotics and food to intestinal microflora. European Journal of Nutrition, *41*, 2002, pp. 11–16. DOI: 10.1007/s00394-002-1102-7.
57. Hidaka, H. – Eida, T. – Takizawa, T. – Tokunaga, T. – Tashiro, Y.: Effect of fructooligosaccharides on intestinal flora and health. Bifidobacteria and Microflora, *5*, 1986, pp. 37–50. DOI: 10.12938/bifidus1982.5.1_37.
58. Morishita, Y. – Fuller, R. – Coates, M. E.: Influence of dietary lactose on the gut flora of chicks. British Poultry Science, *23*, 1982, pp. 349–359. DOI: 10.1080/00071688208447968.
59. Tellez, G. – Dean, C. E. – Corrier, D. E. – Deloach, J. R. – Jaeger, L. – Hargis, B. M.: Effect of dietary lactose on cecal morphology, pH, organic acids, and *Salmonella enteritidis* organ invasion in Leghorn chicks. Poultry Science, *72*, 1993, pp. 636–642. DOI: 10.3382/ps.0720636.
60. Rufián-Henares, J. A. – Morales, F. J.: Microtiter plate-based assay for screening antimicrobial activity of melanoidins against *E. coli* and *S. aureus*. Food Chemistry, *111*, 2008, pp. 1069–1074. DOI: 10.1016/j.foodchem.2008.05.027.
61. Ryu, S.-I. – Lee, S.-B.: Synthesis of nucleotide sugars and α-galacto-oligosaccharides by recombinant *Escherichia coli* cells with trehalose substrate. Enzyme and Microbial Technology, *53*, 2013, pp. 359–363. DOI: 10.1016/j.enzmictec.2013.07.009.
62. Quintero, M. – Maldonado, M. – Perez-Munoz, M. E. – Jimenez, R. – Fangman, T. – Rupnow, J. – Wittke, A. – Russell, M. – Hutkins, R.: Adherence inhibition of *Cronobacter sakazakii* to intestinal epithelial cells by prebiotic oligosaccharides. Current Microbiology, *62*, 2011, pp. 1448–1454. DOI: 10.1007/s00284-011-9882-8.
63. de Vos, W. M.: Gene expression systems for lactic acid bacteria. Current Opinion in Microbiology, *2*, 1999, pp. 289–295. DOI: 10.1016/S1369-5274(99)80050-2.
64. Galeotti, F. – Coppa, G. V. – Zampini, L. – Maccari, F. – Galeazzi, T. – Padella, L. – Santoro, L. – Gabrielli, O. – Volpi, N.: Capillary electrophoresis separation of human milk neutral and acidic oligosaccharides derivatized with 2-aminoacridone. Electrophoresis, *35*, 2014, pp. 811–818. DOI: 10.1002/elps.201300490.

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