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Prebiotic properties of licorice root extracts

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Abstract. The study objective is to investigate effect of licorice root extract on growth of probiotic microorganisms. As fructan percentage in licorice roots is 27.8–28.8%, this value is sufficient to enable the raw material suitability as a prebiotic ingredient. The licorice root extract (0.1%, 1% and 10% of medium volume) was added to media. The same media without added extract were considered as controls. Effect of the licorice root extract on growth of probiotic microorganisms was studied in 2 commercial probiotic drug Bifiform (Denmark) and Bifidobacterin forte (Russia). Licorice root extract provides probiotic bacteria an opportunity to tolerate acidity/alkalinity gradient of model media well and to decrease their count slower. In vitro studies showed, what 1% extract provides more favorable conditions for microorganisms that 10% one.

Key words: licorice root, fructans, functional food products, diabetes, intestinal microflora.

INTRODUCTION

It is well known that intestinal microflora indicates functional microorganism status. This microflora produce great number of enzymes (e.g., proteases, lipases, amylases, cellulases, etc.) involved in protein, lipid, carbohydrate, bile acid and cholesterol metabolism, as well as water-electrolyte metabolism. Also, it provides absorption of calcium, iron and vitamin D. So, it plays a key role in consumed food-based power production (Bâckhed et al., 2005).

It should be noted that human intestinal microflora is very unstable system liable to variations. It depends on number of factors such as endogenous (neurological status, food, body temperature, etc.) and exogenous (season, atmospheric oscillations, etc.) ones.

Gastrointestinal diseases have been an increasing global health problem in recent years (Faghfoori et al., 2015). These diseases act directly on the destruction or decline of beneficial gut microbiota as a consequence of physiological and metabolic disturbances induced by a stressful lifestyle, diet modifications, antibiotic consumption and age-related events, i.e., reduction of functionality of the immune system (Biagi et al., 2012). Diets based on prebiotics have been increasingly accepted for improving intestinal health (Gionchetti et al., 2005; Boirivant & Strober, 2007; Hatakka & Saxelin, 2008; Lutgendorf et al., 2008).

Fructans represents a category of natural prebiotic compounds that includes fructose polymers synthesized from sucrose and fructose molecules (Banguela & Hernández, 2006). linked by fructose-fructose glycosidic β-(2 → 1) and β- (2 → 6) bonds and having one terminal glucose unit. Due to the structure and type of linkage-β, fructans are not metabolized by host enzymes in the upper gastrointestinal tract, reaching the lower tract where they become available for the resident microbiota to use as substrates. Therefore, fructans stimulate the proliferation of beneficial bacteria, mainly *Lactobacillus* and *Bifidobacterium*, associated with health-promoting effects and the production of short-chain fatty acids (SCFAs), mainly acetic, propionic, and butyric acids, whose increase antagonizes the growth of some pathogenic bacterial strains and favors mucin production in the colon (Andrade et al., 2019). Licorice root can be one of advantageous sources of probiotic substances, including fructans (Banguela & Hernández, 2006).

Three polysaccharides (i.e., glycyrrhizans UA, UB and UC) were extracted from *Glycyrrhizae uralensis* root, and several polysaccharides (glycyrrhizans GU and GA) were derived from a stolon (i.e., an elongated side shoot) of *Glycyrrhizae glabra var glandulifera*. Structure of glycyrrhizan GA is based on β-1,3-bound galactose residues. α-arabino-β-3,6-galactan (L-arabinose, D-galactose, L-rhamnose, D-galacturonic and D-glucuronic acids – 22:10:1:2:1) is a main structural unit of the glycyrrhizan. Glycyrrhizan UA consists of L-arabinose, D-galacturonic acid, D-galactose and L-rhamnose (molar ratio – 20:3:1:14). β-1,3-bound galactose whose residues contain α-1,5-bound L-arabinose (position 6) in a side chain is a structural unit of glycyrrhizan UA. The glycyrrhizan consists of L-arabinose, L-rhamnose, D-galactose and D-glucose (molar ratio – 12:20:1:10:10). Also, it contains few O-acetyl groups and approximately 10% and 35% of glycyrrhizan UA and UB residues, respectively, peptide residues and D-galacturonic acid as methyl ethers. Glycyrrhizan UC (i.e., a neutral polysaccharide) consists of L-arabinose, D-galactose, L-rhamnose and D-glucose (molar ratio – 10:30:1:27). It may be determined as arabino-3,6-galacto-glucan. Galactose, xylose, arabinose, glucose and mannose were found in polysaccharide hydrolisates. According to several studies presented herein, about 15% of mono- and oligosaccharides were extracted from licorice roots with 82% ethanol. These compounds contained glucose, galactose, mannose, sucrose and glucofructan (Denisova, 2000).

Shen et al. (2015) derived and described a water-soluble polysaccharide GIP1 from *Inflata* licorice roots. Mocanu et al. (2009) obtained a novel probiotic product named ROSALACT®, prepared from pasteurized milk with rosehip and licorice extractusing a mixed culture of probiotic bacteria. *L. plantarum*, was found to be capable of rapidly utilizing liquorice root extract for probiotic cell cultivation. From the results of this study, it can be concluded that the liquorice root extract could be used as a non-dairy raw material for probiotic lactic acid bacteria (Mousavi, Z.E. & Mousavi, M., 2019). In terms of abovementioned data, we can consider the plant as a potential source of substances stimulating growth of probiotic microorganisms.

The study objective is to investigate effect of licorice root extract on growth of probiotic microorganisms.

MATERIALS AND METHODS

Licorice root extraction

At the first stage, extracts were obtained from four samples of licorice root produced by various pharmaceutical companies in Russia (Sample 1–4).

To remove fructose, sucrose and other oligosaccharides in the first stage, extraction was carried out with a 95% solution of ethyl alcohol. Then, water extraction was repeated under the same conditions (80 °C, 150 minutes).

The presence of fructosans in solution was determined using acid hydrolysis by spectrophotometric method. In an acidic environment, these compounds are capable of forming the product of ¾5-hydroxymethylfurfural in the wavelength range from 280 to 380 nm. In literary sources, the greatest formation of this compound is noted at wavelengths of 283 and 285 nm (Ananyina, 2008).

The quantitative determination of the content of fructans (X) in terms of fructose in% was calculated by the formula 1.

$$X = \frac{D \cdot 100 \cdot 25}{298 \cdot m \cdot 1} \quad (1)$$

where X – the content of fructosans, %; D – the optical density of the test solution; 298 – specific absorption rate of the fructose transformation product after acid hydrolysis; m – the mass of the plant sample in the studied extract.

The licorice root extract (0.1%, 1% and 10% of medium volume) was added to model media. The same media without added extract were considered as controls.

Microorganism growth study

Effect of the licorice root extract on growth of probiotic microorganisms was studied in 2 commercial probiotic drugs described in the Table 1.

Table 1. Description of probiotic drugs

S/N	Drug name, country of origin	Microorganism species composition	Bacterial count
1	Bifiform, Denmark	*B. longum*, *E. faecium*	$1 \cdot 10^7$ bacteria/dose
2	Bifidobacterin forte, Russia	*B. bifidum*	$5 \cdot 10^7$ bacteria/dose

Probiotic microorganisms were cultivated in solid media with recommended composition (Likhacheva et al., 1992; Darmov et al., 2011). During the microaerophilic cultivation we applied Anaerobic system Mark III – LE003 (Hi Media Laboratories Pvt. Ltd, Mumbai, India) and Hi Anaero Gas Pacet gas-producing bags. Probiotic microorganism survival rate was tested *in vitro* simulating human digestive conditions.

Model media were prepared on basis of citrate phosphate buffer and enzyme drugs (Acidin-pepsin, Panzinorm forte 20000). After interaction with enzyme drugs along with incubation of model media and bacterial suspensions living microorganism count in probiotic drugs and suspensions was evaluated by inoculation of appropriate 10-fold serial dilutions of test drugs and suspensions in solid media in Petri dishes and colony counting after incubation.

The main point of these tests was to incubate microorganisms of investigated probiotic drugs and licorice root extract in acidic model medium with Acidin-pepsin (pH = 2.3) and alkaline model medium with Panzinorm forte 20000 (pH = 7.2) successively during mean time of mixed meal presence in stomach and intestine,

respectively. Then, we counted survived microorganisms as compared with their initial number. Viable probiotic microorganism count per a drug dose (CFU mL^{-1}) was evaluated initially (0 h), in 4 hours after incubation in acidic model medium with Acidin-pepsin (0.5 mg mL^{-1}) and in 12 hours after incubation in alkaline model medium with Panzinorm forte 20000 (2.5 mg mL^{-1}) (Darmov et al., 2011).

Statistical analysis

All analyses were carried out in triplicate. The results were presented as means ± standard deviation of three replicates of independent experiments. Statistical analysis was performed using the SPSS 17.0, a value of $p < 0.05$ was considered statistically significant.

RESULTS

Oligosaccharides (i.e., fructans and raffinose) are the most important water-soluble prebiotic carbohydrates. See fructans level in licorice root extracts produced by various manufacturers in the Fig. 1.

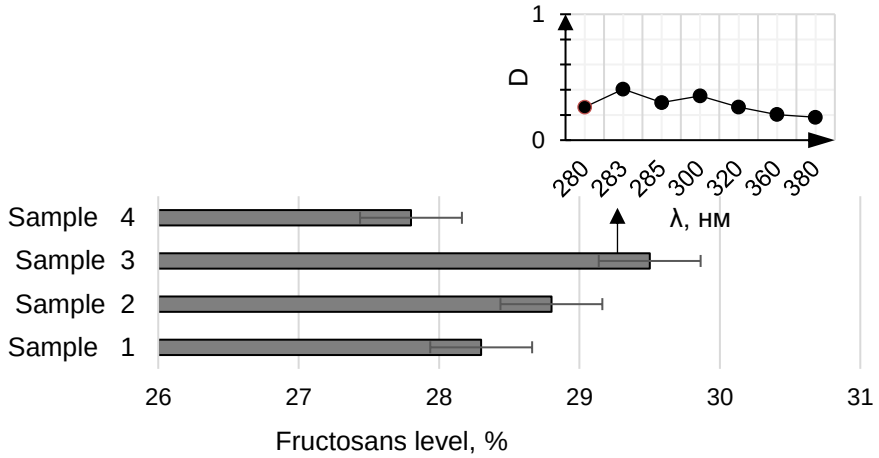

Figure 1. Fructans level in licorice root extracts (equivalent to fructose. Statistically significant differences ($p < 0.05$) between test and reference samples by the Kruskal-Wallis criteria.

As fructan percentage in licorice roots is 27.8–28.8%, this value is sufficient to enable the raw material suitability as a prebiotic ingredient (Isbrucker & Burdock, 2006). On average, the level of fructans in different plants varies from 5 to 30% (Judprasong et al., 2011).

To assess licorice root extract effect on growth of probiotic microorganisms, we simulated human gastrointestinal medium at the next stage of the study.

See results related to the study of licorice root extract effect on probiotic microorganism growth in the Table 2.

The Table 2 demonstrates that all the probiotic microorganisms are exposed to negative effect of factors related to model media simulating human gastrointestinal factors. Bacterial count decreases both in acidic model medium with Acidin-pepsin (4 h) and in alkaline model medium with Panzinorm forte 20000 (i.e., an enzyme drug).

Table 2. Effect of licorice root extract on probiotic microorganism growth

Licorice extract level in medium, %	Bacterial count in a sample during the testin ... hours, CFU mL^{-1} ($X \pm I_{95}$)				
	Bifiform			Bifidobacterin	
	0	4	12	4	12
0.1	$(1.0 \pm 0.1) \cdot 10^7$	$(3.0 \pm 0.2) \cdot 10^4$	$(1.5 \pm 0.2) \cdot 10^3$	$(9.0 \pm 0.5) \cdot 10^5$	$(1.5 \pm 0.2) \cdot 10^3$
1.0	$(1.0 \pm 0.1) \cdot 10^7$	$(1.5 \pm 0.3) \cdot 10^5$	$(5.0 \pm 0.4) \cdot 10^4$	$(6.0 \pm 0.5) \cdot 10^5$	$(2.0 \pm 0.4) \cdot 10^4$
10	$(1.0 \pm 0.1) \cdot 10^7$	$(1.0 \pm 0.4) \cdot 10^5$	$(7.0 \pm 0.2) \cdot 10^3$	$(2.2 \pm 0.4) \cdot 10^5$	$(3.9 \pm 0.4) \cdot 10^3$
control	$(1.0 \pm 0.1) \cdot 10^7$	$(3.0 \pm 0.2) \cdot 10^4$	$(1.5 \pm 0.2) \cdot 10^3$	$(9.0 \pm 0.5) \cdot 10^4$	$(1.5 \pm 0.2) \cdot 10^3$

Licorice root extract provides probiotic bacteria an opportunity to tolerate acidity/alkalinity gradient of model media well and to decrease their count slower. B. Bifidum cells were more sensitive to licorice extracts than B. Longum and E. Faecium cells. This may be due to the differences in growth conditions and adaptation of the strain to the new environment (Mousavi & Mousavi, 2019). The viability of B. Bifidum cells after 4 hours (pH-2,3) is an order of magnitude higher compared to the control sample, even with a minimum concentration of the added extract (0.1%). According to the literature, the optimum condition for growth of Bifidobacterium is at pH 6.5–7.0 (Cronin et al., 2011). This tendency to maintain high viability for bifidobacteria cells is preserved in an alkaline medium after 12 hours.

Along with this, 1% extract provides more favorable conditions for microorganisms that 10% one. The effect can be attributed to glycerritic acid (i.e., a licorice root ingredient) that can inhibit growth of both gram-positive and gram-negative microorganisms (Astafieva, 2013).

CONCLUSION

Nowadays pre- and probiotics are common tools to manage gastrointestinal diseases, immune disorders and allergic diseases.

Licorice root can be one of advantageous sources of probiotic substances, including fructans. Licorice root extract has a sufficient fructan level to provide good resistance of probiotic bacteria to acidity/alkalinity gradient.

In general, 1% licorice root extract can be considered the minimum effective dose in which the higher growth of probiotic microorganism is stimulated. In addition, our results corroborate the prebiotic concept that suggests that fructolygosaccharides stimulate the growth of one or a limited number of bacteria in the colon, improving the health of the host.

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