# THE BIFIDOGENIC EFFECT OF *AGAVE FEROX* C. KOCH LEAF JUICE*

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The juice of agave leaf (*Agave ferox* C. Koch) stimulated *in vitro* the growth of 17 strains of bifidobacteria. The utilization of oligofructans, glucose, fructose and total saccharides was determined by enzymatic and refractometrical methods. Juice of agave leaf was important source of carbon and energy for beneficial saccharolytical bifidobacteria tested. The agave leaf acts as a prebiotic within the colonic microflora, increasing numbers of bifidobacteria, which can metabolise it to produce short-chain fatty acids. This bifidogenic effect can initiate a number of gastrointestinal and systemic health benefits that may be associated to improved immune system and reduced the incidence of diseases.

agave leaf juice; *Agave;* bifidogenic activity; bifidobacteria; prebiotic

## INTRODUCTION

The plant family Agaveaceae comprises over 200 known species which are chiefly tropical and xerophytic. Juices from the stem of some species are used for making some spirits, e.g. tequila (*Agave tequilana* A. Weber) and pulque (*Agave americana* L.). Components with biological activities; chlorogenin hexasaccharide with cytotoxic and cell cycle inhibitory activities (Ohtsuki et al., 2004) or steroidal sapogenins with anti-inflammatory activity (Peana et al., 1997) were isolated from leaves of some agave species. It is also known that agave leaves contain soluble sugars such as sucrose, glucose, fructose and mainly oligofructans with β (2→1) and β (2→6) linkages, especially neokestose, kestose and nystose (Wang, Nobel, 1998; Lopéz et al., 2003; Mancilla-Margalli, Lopéz, 2006).

Fructooligosaccharides are non-digestible components, which are preferably utilized by bifidobacteria in the large intestine. Bifidobacteria constitute a major part of the human intestinal microflora and have proved considerable health promoting benefits to the host. Fructooligosaccharides are thereby widely used as prebiotics, which are defined as a non-digestible food components selectively promoting growth and/or activity one or more strains of probiotic microorganisms in the large intestine (Manning, Gibson, 2004).

The aims of this study were to analyze sugar contents and to evaluate bifidogenic effect of *Agave ferox* C. Koch leaf juice.

## MATERIALS AND METHODS

### Plant material

*Agave ferox* C. Koch leaves were taken from The Botanical Gardens of Charles University in Prague after blooming. Tested juice was removed from the middle part of the leaves. Cover green layer of agave leaves was cut off and the middle part was mixed. The agave leaf juice was obtained after pressing and filtration.

### Cultures of bifidobacteria

Sixteen strains of 7 species of human bifidobacteria and one strain isolated from milk product were tested. The list of strains tested is shown in Table 1. Wild strains were isolated using MTPY agar (Rada, Petr, 2000) and identified according to Vlková et al. (2005).

### Growth medium with agave leaf juice

The growth medium (pH = 7.3 ± 0.1) contained tryptone (10 $g.l^{-1}$), nutrient broth No. 2 (10 g), yeast extract (5 g), $NaHCO_3$ (3 g), $K_2HPO_4$ (1 g), L-cysteine hydrochloride (0.5 g), tween 80 (1 ml), distilled water (700 ml) and agave leaf juice (300 ml). Growth medium with agave leaf juice was sterilized (120 °C, 15 min) before it was used for testing.

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Table 1. The growth of bifidobacteria in medium with agave leaf juice expressed as optical density of 48h-cultures

| Strain | Origin | Agave leaf juice | Wilkins-Chalgren broth[a] |
|---|---|---|---|
| *B. adolescentis* 1 | CCUG 18 363 | 2.08 ± 0.00* | 1.08 ± 0.14* |
| *B. adolescentis* 2 | adult faeces | 2.14 ± 0.00* | 1.10 ± 0.01* |
| *B. adolescentis* 3 | adult faeces | 2.16 ± 0,02* | 1.10 ± 0.3* |
| *B. animalis* 1 | CCUG 24 606 | 1.43 ± 0.00* | 0.80 ± 0.06* |
| *B. animalis* 2 | MILCOM 93 | 1.72 ± 0.01* | 1.23 ± 0.01* |
| *B. bifidum* 1 | ATCC 29 521 | 1.33 ± 0.04* | 0.26 ± 0.00* |
| *B. bifidum* 2 | CCM 3762 | 1.71 ± 0.02* | 1.18 ± 0.02* |
| *B. bifidum* 3 | adult faeces | 1.24 ± 0.05* | 0.40 ± 0.1* |
| *B. bifidum* 4 | adult faeces | 1.00 ± 0.00* | 0.42 ± 0.17* |
| *B. bifidum* 5 | infant faeces | 1.39 ± 0.05* | 0.45 ± 0.04* |
| *B. breve* 1 | ATCC 15 700 | 2.17 ± 0.01* | 1.10 ± 0.01* |
| *B. breve* 2 | infant faeces | 1.89 ± 0.00* | 0.81 ± 0.01* |
| *B. catenulatum* | CCUG 18 366 | 2.06 ± 0.02* | 1.19 ± 0.00* |
| *B. infantis* | ATCC 17 930 | 2.18 ± 0.00* | 1.22 ± 0.01* |
| *B. longum* 1 | ATCC 15 707 | 2.18 ± 0.01* | 1.03 ± 0.00* |
| *B. longum* 2 | infant faeces | 1.66 ± 0.01* | 0.30 ± 0.02* |
| *B. pseudolongum* | fermented milk product | 1.72 ± 0.02* | 1.16 ± 0.00* |

ATCC – American Type Culture Collection; CCM – Czech Collection of Microorganisms; CCUG – Culture Collection of University Göteborg; CCDM – Culture Collection of Dairy Microorganisms (Laktoflora, Czech Republic)

Values present optical density at 620 nm.$1^{-1}$ cm light path. All values are mean from triplicate determination ± S.D.

[a]control; *significant differences ($P < 0.01$) were evaluated by the *t*-test (STATISTICA 10 CZ program)

Table 2. Concentrations of saccharides in agave medium before and after cultivation with bifidobacteria

| Sample | Total soluble saccharides (g.$l^{-1}$) | Oligofructans (g.$l^{-1}$) | Glucose + fructose (g.$l^{-1}$) | Glucose (g./$l^{-1}$) |
|---|---|---|---|---|
| Agave medium | 52.0 | 3.05 | 4.65 | 1.95 |
| *B. adolescentis* 1 | 4.5 | 0.71 | 0.65 | 1.95 |
| *B. adolescentis* 2 | 5.0 | 1.12 | 3.99 | 1.95 |
| *B. adolescentis* 3 | 4.0 | 1.73 | 4.16 | 1.95 |
| *B. animalis* 1 | 3.5 | < 0.10 | < 0.10 | 1.95 |
| *B. animalis* 2 | 3.5 | 0.41 | 1.20 | 1.95 |
| *B. bifidum* 1 | 13.0 | < 0.10 | 4.26 | 1.35 |
| *B. bifidum* 2 | 5.5 | 0.61 | 2.51 | 1.95 |
| *B. bifidum* 3 | 12.5 | < 0.10 | 5.04 | 0.95 |
| *B. bifidum* 4 | 3.5 | 0.51 | 4.22 | 1.31 |
| *B. bifidum* 5 | 11.5 | < 0.10 | 4.92 | 1.10 |
| *B. breve* 1 | 6.5 | 1.53 | 3.94 | 1.95 |
| *B. breve* 2 | 6.0 | 0.51 | 4.19 | 1.95 |
| *B. catenulatum* | 3.5 | < 0.10 | 4.30 | 1.95 |
| *B. infantis* | 8.0 | 0.92 | 4.65 | 1.95 |
| *B. longum* 1 | 6.5 | 0.71 | 4.65 | 1.95 |
| *B. longum* 2 | 5.0 | 0.31 | 0.98 | 1.95 |
| *B. pseudolongum* | 3.0 | 1.02 | 0.76 | 1.95 |

All values are means from triplicate determination

#### Sugar content analyses

Overnight cultures of bifidobacteria grown in Wilkins-Chalgren broth were inoculated into the hermetically sealed Hungate tubes containing anaerobic growth medium (9 ml). All cultures were incubated at 37 °C for 48 h under anaerobic conditions. Then, the optical density at 620 nm $cm^{-1}$ light path was determined by Densi-La-Meter (Lachema, Czech Republic); Wilkins-Chalgren broth was used as a control medium. Contents of carbohydrates were determined before and after incubation in all bifidobacterial cultures. The following methods were used: phenol-sulfuric acid method (Dubois et al., 1951) for total saccharides, fructan assay procedure kit (Megazyme, Ireland) for oligofructans and reflectoquant tests (Merk, Germany) for fructose and glucose.

## RESULTS AND DISCUSSION

All bifidobacterial strains were able to grow in the medium with agave leaf juice (Table 1). In addition, all strains grew significantly better in this medium compared to control medium (Wilkins-Chalgren anaerobe broth). Bifidobacteria utilized oligofructans of agave leaves in the range from 0% to 57%, 19% on average; fructose in the range from 0% to 100%, 69% in average and free glucose in the range from 49% to 100%, 91% in average (Table 2). As follows from our previous results (Trojanová et al., 2004), oligofructans from dandelion root infusion were important source of carbon and energy for bifidobacteria tested. Although the contents of monosaccharides (40.9 $g.l^{-1}$) in agave leaf juice is approximately three times higher than in dandelion root infusion, the average utilization of oligofructans by bifidobacteria was similar for both media (21% and 19% for dandelion and agave medium, respectively). This fact suggests that bifidobacteria utilize oligofructans in preference to monosaccharides.

Bifidobacteria represent the main bacterial genus (together with lactobacilli) commonly used as animal and human probiotics. Because they belong to the group of saccharolytic bacteria, their growth and activity are supported by free glucose, fructose and in the contrast to lactobacilli by fructooligosaccharides. As it is shown in this paper, agave leaf juice contains relatively high quantity of glucose, fructose and oligofructans, which are utilizable by bifidobacteria. Non-digestible oligofructans presented in agave leaf juice should also be used as prebiotics for humans and animals.

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**Bifidogenní účinek výlisku z listů *Agave ferox* C. Koch**

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Cílem této studie bylo zjistit možný růst různých kmenů bifidobakterií v médiu obsahující výlisek z listů *Agave ferox* C. Koch, resp. prebiotický (bifidogenní) účinek. V případě růstu bifidobakterií také stanovit množství sacharidů ve výlisku, především oligofruktanů, s prebiotickým účinkem. Bylo zjištěno, že kmeny bifidobakterií využívají jako zdroj energie celé spektrum sacharidů obsažených ve výliscích *Agave ferox* včetně oligofruktanů, o nichž je známo, že mají probiotický účinek. Tudíž listy agáve mohou být zdrojem využitelných prebiotik.

šťáva z listu agáve; bifidogenní aktivita; bifidobakterie; prebiotikum

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