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Fructooligosaccharides in honey and effects of honey on growth of *Bifidobacterium longum* BB 536

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Abstract: This research was carried out to determine the fructooligosaccharides content in local honey samples, namely the wild Malaysian Tualang honey and common wild honey obtained from Tapah, Perak and a commercial Tualang honey. Local wild honeys were found to contain a higher concentration of fructooligosaccharides (FOS) compared to the commercial Tualang honey. The FOS quantified from local wild honeys was inulobiose, kestose and nystose. Nystoses were found at a very low amount in the commercial Tualang honey. The effects of honey on the growth of Bifidobacterium longum BB 536 were investigated. Both wild and commercial honey samples including FOS standard were found to support the growth of B. longum. The pH value of the skim milk + honey inoculated with the probiotic strain decreases as expected. Addition of honey was found to support the growth of B. longum BB 536.

Keywords: Fructooligosaccharides, honey, probiotic, *Bifidobacterium longum*

Introduction

Honey was one of the man's earliest foods, as honeybees were producing it long before man appeared on earth. Honey remains the only sweetener that can be stored and used precisely as it is produced by nature (Doner, 2000). Honey is primarily a carbohydrate material, where fructose and glucose account for over 85% of its solids (NHB, 1996). Another 10% of honey solids include at least 25 other more complex sugars ranging from di- to oligosaccharides (Doner, 2000). This group of oligosaccharides are of interest because, they are neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract, and may beneficially affect the health of the consumer by selectively stimulating the growth or and/or activity of desirable bacteria in the colon (Ustunol, 2000; Cummings *et al*., 2001).

Fructooligosaccharides (FOS) are short-chain sugars that occur naturally and have dietary benefits for humans. They are widely distributed in nature and are a natural part of the human diet (Hogarth *et al*., 2000). Non-digestible oligosaccharides also occur naturally in foods such as fruits, vegetables, milk and honey (Tannock, 1999). The ability of honey to enhance the growth and activity of bifidobacteria in milk and intestinal bifidobacteria *in vitro* were reported and suggested that this may be due to the unique carbohydrate composition and complex mixture of oligosaccharides present in honey (Chick *et al*., 2001; Ustunol and Gandhi, 2001; Kajiwara *et al*., 2002). *Bifidobacteria* spp. including *Bifidobacterium longum* are probiotics; living organisms that affect the host in a beneficial manner (Montrose and Floch, 2005).

Materials and Methods

Two types of wild honey known as wild *Tualang* and common wild honey were obtained from Tapah, Perak. A local commercial *Tualang* honey was obtained from a supermarket. All chemicals and reagents used were of analytical grade.

Pre-treatment of honey

The removal of monosaccharrides and disaccharides and the recovery of

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fructooligosaccharides (FOS) from honey were according to Morales et al. (2006). A 0.5 g of honey were mixed with 3 g of activated charcoal and were dissolved in ethanol:water (90:10, v/v) solution. The mixture was stirred for 30 min. at room temperature, filtered through Whatman paper 1 and then washed with 25 ml of ethanol:water (90:10, v/v). The FOS absorbed by the activated charcoal were dissolved using a water:ethanol (50:50, v/v) solution and then filtered. The filtrate was concentrated at 30°C using rotary evaporator. The extract was then filtered through 0.22 µm membrane filter for HPLC analysis.

Determination of fructooligosaccharides

The determination of fructooligosaccharides was carried out according to Da Costa Leite *et al*. (2001). Beneo P95 (fructooligosaccharides) was used as the FOS standard. Analyses were carried out in a Waters 717 Plus Autosampler HPLC system and an evaporative light-scattering detector (ELSD). Separation of fructooligosaccharides was on a Water Spherisorb 5µm NH_2 (250 x 4.6 mm) column using acetonitrile:water (85:15, v/v) as mobile phase and flow rate of 1 ml/min. Quantification was obtained by peak height comparison with the standard FOS. Statistical analysis of data was performed by one way analysis of variance using SPSS 11.0.

Determination of Bifidobacterium longum *BB 536 growth*

A 12% (w/v) of reconstituted skim milk was prepared and mixed with 5% each of honey and FOS standard. Skimmed milk without honey served as the control. Both sample and control were pasteurized at 70°C for 15 minutes in a water bath and then cooled to 37°C before inoculation (Chick *et al*., 2001). An initial 5% (10^5 CFU/ml) inoculum of *B. longum* BB 536 was inoculated into the milk and incubated anaerobically at 37°C for 24 hr. Appropriate dilutions of the fermented milk was then plated on MRS agar (Oxoid) and incubated at 37°C for 48 hr anaerobically to determine the number of colonies. Anaerobic condition was generated by Gas Generating Kit CO_2 BR39 (Oxoid, England). pH was taken after 0 and 24 hour of incubation using a calibrated pH meter (HANNA, USA). The *B. longum* BB 536 strain was a gift from Universiti Putra Malaysia and was stored at 4°C on slants of MRS agar before inoculation.

Results and Discussion

Fructooligosaccharides in honey

The Beneo P95 (fructooligosaccharides) standard was analyzed using HPLC to determine the FOS peaks. The following figure shows the chromatographic profile of the Beneo P95 (fructooligosaccharides) standard (Figure 1) and a chromatogram of the FOS in the common wild honey (Figure 2).

The fructooligosaccharides identified in the honey samples based on retention time were inulobiose (F2), kestose (DP3) and nystose (DP4). The FOS contents are shown in Table 1. Fructofuranosil-nystose (DP5) was not detected in all the honey samples. Ruiz-Matute *et al*. (2007) were among the first researchers to identify the presence of inulobiose (0.93 – 6.14 mg/g) in honey. This FOS was also detected in the wild *Tualang* honey at 0.018 mg/g. Inulobiose may be formed in honey via the β-transfructosylation reaction from invertase which are present in pollens (Ruiz-Matute *et al*., 2007). Kestose were determined in the common wild honey at 0.023 mg/g. The formation of kestose in honey is similar to inulobiose. It could also be due to the trans-D-fructosylation activity on sucrose by α-glucosidase enzyme secreted by honey bees or even yeasts (Siddiqui, 1970). Doner (1991) reported that if 1-kestose was found in honey, it could be caused by the presence of yeasts.

Doner (2000) stated that the formations of more than 25 types of oligosaccharides in honey from the three main nectar sugars (glucose, fructose and sucrose) are very complex. The catalysis activities caused by enzymes are believed to be responsible for the formation of these oligosaccharides. The enzymes in honey are secreted by honey bees to assist in the ripening process of nectar to honey.

Effect of honey on Bifidobacterium longum *BB 536*

The wild and commercial honeys were found to support the growth of *B. longum* in skimmed milk as shown in Table 2. The addition of 5% honey to the skimmed milk did not show any inhibitory effect on the growth of the probiotic strain. The growth of *B. longum* in skimmed milk mixed with wild *Tualang* and common wild honey shows a significant increase from 5.5 to 7.0 $\log_{10}$ CFU/ml cycle and 5.4 to 7.1 $\log_{10}$ CFU/ml cycle respectively after 24 hr incubation. Meanwhile the probiotic inoculated in skimmed milk with commercial *Tualang* honey increased more than 3.0 $\log_{10}$ CFU/ml cycle ($p<0.05$). It is also noted that wild honeys may contain antibacterial components. Čurda and Plocková (1995) suggested that honey obtained from different floral sources shows inhibitory effects on the growth of lactic acid

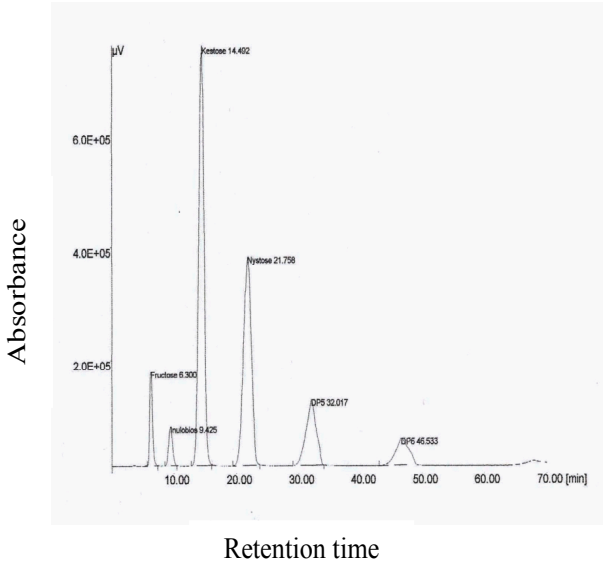

Figure 1. Chromatographic profile of Beneo P95 (fructooligosaccharides) standard obtained by HPLC-ELSD under elution conditions

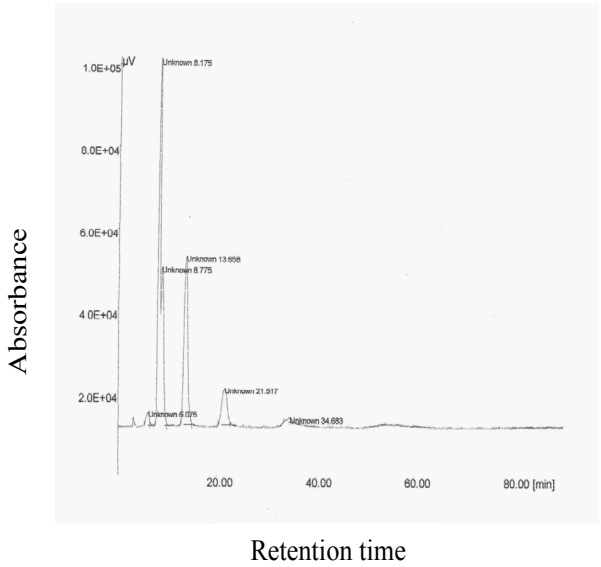

Figure 2. Chromatographic profile of fructooligosaccharides in common wild honey

Table 1. Fructooligosaccharides content in honeys (mg/g)

FOS	Wild *Tualang*	Common wild honey	Commercial *Tualang*
Inulobiose (DP2)	0.018	ND	ND
Kestose (DP3)	ND	0.023	ND
Nystose (DP4)	0.004	0.035	0.001
Total fructans	0.022	0.058	0.001

ND: Not detected

Table 2. Growth of *Bifidobacterium longum* BB 536 in skimmed milk with honey and FOS (log_{10} CFU/ml) at 37°C

Skimmed milk with honey/ FOS	0 hr	24 hr
Wild *Tualang*	$5.5^{b} \pm 0.02$	$7.0^{c} \pm 0.01$
Common wild honey	$5.4^{c} \pm 0.03$	$7.1^{c} \pm 0.02$
Commercial *Tualang*	$5.3^{d} \pm 0.04$	$8.7^{b} \pm 0.05$
Fructooligosaccharides	$5.7^{a} \pm 0.01$	$8.8^{a} \pm 0.01$
Control (skimmed milk only)	$5.8^{a} \pm 0.07$	$7.0^{c} \pm 0.14$

a-d Means in the same column with different letters are significantly different at $p < 0.05$.

bacteria. Some of the inhibitory effects could be due to the high sugar content which reduces the A_w for microbial growth and the presence of organic acids and hydrogen peroxide (Mundo *et al*., 2004).

Even though the total fructans were found to be higher in both the wild honeys, the commercial honey supported the probiotic growth very well. This may indicate the presence of other oligosaccharides in the honey which were not determined in this study that have prebiotic activity. Weston and Brocklebank (1999) also reported that New Zealand honey contains certain oligosaccharides like isomaltose and melezitose.

The pH value of the skimmed milk inoculated with *B. longum*, decreased after 24hr incubation (Table 3). The fermented milk with honey showed a significant pH decrease compared to the control. The pH of the fermented milk with *Tualang* honey and with common wild honey decreased from 6.3 to 4.6 and 6.5 to 4.6 respectively within 24hr of incubation. This is because bifidobacteria produce 3 mol acetic acid and 2 mol lactic acid for each two mol glucose (Scardovi, 1986). The production of these two organic acids and a lower pH value in the colon are among the probiotic factors contributing to the well-being of the host (Crittenden, 1999).

Table 3. pH value of skimmed milk with honey/ FOS inoculated with *B. longum*

Skimmed milk with honey/FOS	0 hr	24 hr
Wild Tualang	6.3c ± 0.01	4.6c ± 0.01
Common wild honey	6.5a ± 0.01	4.6c ± 0.00
Commercial Tualang	6.5a ± 0.01	4.3d ± 0.01
Fructooligosaccharides	6.5a ± 0.01	5.2a ± 0.01
Control	6.4b ± 0.01	5.2b ± 0.01

a-d Means in the same column with different letters are significantly different at $p < 0.05$.

Conclusions

FOS was detected in the local honey but in low amounts ranging from 0.001 to 0.035 mg/g. Results showed that the local honey supported the growth of *B. longum* BB 536 and may have the potential as another prebiotic source.

Acknowledgements

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