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Flavonoids of Buckwheat Honeys

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ABSTRACT

Because of the health benefits of dietary flavonoids, two buckwheat honeys were compare with six other types of honey (including alfalfa, clover, prairie flower, sunflower, wildflower and regular/unspecified) for total flavonoid content by a spectrophotometric assay and flavonoid profiles by HPLC. The flavonoid composition of all honey samples was analysed by HPLC, combined HPLC-MS and low-energy collision induced dissociation (CID) MS-MS. Negative-mode atmospheric pressure chemical ionization (APCI) MS and MS-MS were utilized to provide molecular mass information and product-ion spectra of the glycosyl compounds. Product-ion spectra of the aglycone verified the identity by comparison with the product-ion spectra of the standards. The major flavonoids in buckwheat honey samples were chrysin, galangin, kaempferol, naringenin, pinocembrin and quercetin. Kaempferol appeared in higher concentration in buckwheat honeys. The total flavonoid content of buckwheat honeys was similar to that of red wine and ranged from 16-18 μg flavonoids/g honey.

Keywords: Buckwheat, flavonoids, honey.

INTRODUCTION

Canadian pure honey is renowned world wide for its quality and flavour. The average annual Canadian honey production during the five-year period 1994-1998 was 33,569 tonnes. In 1998, the total value of Canadian honey reached $93.5 million. Alberta and Saskatchewan virtually tied for the highest producing province in 1999, with each accounting for 26% of Canadian production, followed by Manitoba with 22%, Ontario with 14% and Quebec with 5%. Canada is currently the fifth largest honey producer in the world with 7.4% of world production in 1999. China, USA, Argentina and Mexico are the top four prodcuing countries. Canadian honey exports reached 14,715 tonnes at a value of $30.9 million in 1999. During the period 1994-1998, exports averaged about 31% of Canadian production while imports averaged about 15% of production (Statistics Canada, http://www.statcan.ca/english/freepub/23-221-XIB/free.htm).

Depending on the nectar source (the blossoms), honey varies widely in colour, viscosity and chemical composition (sugar constituents, water, ash, nitrogen and metal). There are many kinds of honey in Canada, originating from such diverse sources as clover, alfalfa, canola, sunflower, wild flower and buckwheat. It is estimated that buckwheat honey represents about 1% of total Canadian honey production. Since fruits and vegetables are an especially rich

source of flavonoids, we hypothesized that honey, which originates from plant nectar, might also contain disease-fighting flavonoids. The aim of this study was to evluate the profiles and total content of flavonoids from seven types of Canadian honey.

MATERIALS AND METHODS

Materials :

Eight honey samples were collected from commercial stores in Saskatoon in 2000.

Reagents :

Flavonoid standards, chrysin (5,7- dihydroxyflavone, Mw 254), galangin (3, 5, 7-trihydroxyflavone, Mw 270), isoquercitrin (3, 3'. 4', 5, 7 - pentahydroxyflavone-3-glucoside, Mw 464), kaempferol (3, 4', 5, 7-tetrahydroxyflavone, Mw 286), naringenin (4', 5, 7-trihydroxyflavanone, Mw 272), pinocembrin (5, 7-dihydroxyflavanone, Mw 256), quercetin (3, 3', 4', 5, 7-pentahydroxyflavone, Mw 302), rutin (3, 3',4',5, 7'-pentahydroxyflavone-3-rutinoside, Mw 610), and tectochrysin (5-hydroxy-7-methoxyflavone, Mw 268) were purchased from Indofine Chemical Company Inc. (Somerville, NJ, USA). Apigenin (4', 5, 7-trihydroxyflavone, Mw 270) and luteolin (3', 4', 5, 7-tetrahydrixyflavone, Mw 286) were bought concentration of approximately 0.5 g/l and kept protected from light at- 20 C for up to 3 months. Working solutions were made up each day by diluting standard stock solution to 0.025-0.25 g/l with methanol. HPLC-grade solvents were supplied by Ficher Scientific Ltd. (Nepean, ON, Canada).

Sample extraction (column chromatography):

Honey samples (10 g each) were mixed with one part of water until completely fluid, and the fluid samples were filtered through a glass fiber (GF/A) Whatman filter paper to remove solid particles. The filtrate was then loaded onto a prepared Oasis HLB 3-cc/60 mg SPE cartridge (Waters Inc.) which was preconditioned with 1 ml of methanol and 1ml of purified water. The cartridge was then washed with 20 ml of purified water followed by 20 ml of 15% methanol. The whole phenolic fraction was then eluted with 2ml of 100% methanol and taken to dryness at 40°C under reduced pressure. The residue was redissolved in 3 ml of water and extracted with diethyl ether (5 ml X 3). The ether extracts were combined, concentrated at 40°C under reduced pressure and redissolved in 1 ml of methanol for HPLC analysis.

HPLC Analysis of honey flavonoids:

This was carried out on a reversed-phase Waters Symmetry C18 column (3 x 150 mm, 5μm) using water + 0.05% trifluoroacetic acid (Solvent A) and acetonitrile + 0.05% trifluoroacetic acid (Solvent B) at a flow rate of 0.4ml/min. The Waters Alliance HPLC consisted of a 2690 Separations Module and a 996 photodiode array (PDA) detector (Waters Inc.). UV spectra were recorded from 200 to 400 nm. The elution gradient is shown in Table 1.

Flavonoid identification:

The different flavonoids were identified by chromatographic comparisons with authentic standards, their UV spectra and HPLC-MS-MS. LC-MS analysis was performed on a Quattro LC triple quadrupole instrument (Micromass, Manchester, UK) with a Z-spray API source using an APCI probe. Chromatographic separation was undertaken on a 2690 Separations

Module (Waters Inc.) equipped with a 996 PDA detector (Waters Inc.). Instruments were controlled by and all data collected and analyzed using MassLynx software instrument was set to scan from 120 to 1000 mass units. Selected Ion Recording (SIR) data were collected using the molecular weights of the authentic standards The probe and source parameters were: source temperature 80°C, probe temperature 450°C, cone voltage 30 V, corona discharge 2.7 kV, analyser vacuum 5.1x 10^{-6} torr and gas cell 2.0 x 10^{-5} torr. When the instrument was operated in the MS-MS mode the following parameters were changed : source temperature 120 °C, probe temperature 400°C, analyser vacuum 1.8 x 10^{-5} torr and gas cell 7.6 x 10^{-4} torr.
Both positive and negative product ions were analysed for all pseudomolecular (M+H, M-H) ions generated in the source. The gas pressure in the collision cell and the collision voltage applied to the collision cell were varied in order to achieve fragmentation of each pseudomolecular ion.

Flavonoid quantification:

The flavonoids were quantified by absorbance of their corresponding peaks in the chromatograms. Pinocembrin and naringenin were detected at 290 nm, whereas other flavonoids were detected at 340nm.

Table 1. HPLC Gradient

Time(minutes)	Water + 0.05% TFA (% volume)	Acetonitrile + 0.05% TFA (% volume)
0	90	10
2	75	25
12	75	25
20	65	35
30	65	35
50	50	50
52	30	70
58	90	10
60	90	10

Total flavonoids:

Determination of total flavonoids was carried out using a simple spectrophotometric assay as described by Hairi *et al* (1991). Briefly, a honey sample (10 g) was extracted with 80% methanol (25 ml) with a magnetic stirrer for 2 hour at 70°C. The extract was filtered through a Whatman #5 filter paper. The filtrate (1ml) was diluted with 3 ml of deionized water. An aliquot (2ml) of diluted extract was mixed with 100µl of 1% 2-aminoethl-diphenylborate methanolic solution. The absorbance was determined spectrophotometrically at 404 nm, and compared to that of a standard rutin.

RESULTS AND DISCUSSION

CID-MS-MS analysis was used to identify the aglycone by comparison with product-ion spectra of authentic standards. Methoxylated flavonoids exhibit specific fragmentation due to loss of · CH_3 radicals from the deprotonated aglycone ions that add to the usefulness of the method for identifying unknown flavonoids. Negative-mode APCI mass spectrometry is demonstrated to be an excellent alternative to the commonly used positive mode operation.

Canadian honey contains low to medium levels of dietary flavonoids, ranging from 2-18 μg per g of honey (Table 2). This is in agreement with Spanish honey which ranged from 5-20 μg per g of honey (Ferreres *et al.* 1994). This is similar to the levels of total flavonoids in red wine, 4-16 μg per ml of wine (Hertog *et al.* 1993). Honey from alfalfa, buckwheat, clover, sunflower and wildflower seems to have higher flavonoid content. The major flavonoids in these honey samples were naringenin, pinocembrin, kaempferol, galangin, chrysin, quercetin and tectochrysin (Table 2). Each honey tends to have a distinctive flavonoid profile. Pinocembrin and chrysin were found in all honey samples. Kaempferol appeared in higher concentration in buckwheat honeys.

Table 2. Concentrations (μg/g) of selected and total flavonoids in various honey samples

Honey Type	Total Flavonoids (Spectrophotometric)	Selected Flavonoids (by HPLS)						
		C	G	K	N	P	Q	T
Alfalfa	15	0.65	0.91	---	4.10	3.91	---	---
Buckwheat I	16	0.86	1.05	3.27	4.40	3.90	---	---
Buckwheat II	18	0.22	0.18	2.88	---	1.06	0.97	---
Clover	18	1.12	1.70	---	6.76	6.15	---	---
Prairie Flower	2	0.02	0.38	---	1.62	2.12	---	0.07
Sunflower	18	0.82	0.62	---	3.05	3.42	---	0.15
Unspecified	5	0.21	---	1.48	---	0.79	0.37	---
Wildflower	18	0.91	1.41	---	6.76	5.09	---	---

C=chrysin, G=galangin, K=kaempferol, N=naringenin, P=pinocembrin, Q=quercetin, T=tectochrysin, (-)=flavonoids below limit of quantitation.

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