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Composition of Buckwheat Honey

Kris Corey[1], Anwar A. Hamama[1], Haiwen Li[1], Rafat A. Siddiqui[1], Chyer Kim[1] & Harbans L. Bhardwaj[1]

[1] Agricultural Research Station, Virginia State University, Petersburg, VA, USA

Correspondence: Harbans L. Bhardwaj, Agricultural Research Station, Virginia State University, Petersburg, Virginia 23806, USA. Tel: 1-804-524-6723. Fax: 1-804-524-5950. E-mail: hbhardwaj@vsu.edu

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Abstract

Buckwheat has been grown in Virginia since late 1700s; however, today the crop is almost non-existent in Virginia. Since buckwheat flowers profusely in a few weeks after planting, it has potential to support honeybees but there is a lack of information about quality of buckwheat honey produced in Virginia. Our objective was to characterize composition of honey produced by honeybees foraging on buckwheat (Buckwheat honey), compared to that produced by honeybees foraging on wild plants (Wild plant honey). Buckwheat honey differed in composition, antioxidant concentrations, and microbial activities from wild plant honey. Concentrations of fructose, glucose, and melezitos in buckwheat honey were quantitatively lower than that in wild plant honey whereas concentrations of sucrose and maltose exhibited an opposite trend—concentration of maltose being statistical significant. Fructose was the dominant sugar (42 and 52 percent in buckwheat honey and wild plant honey, respectively). Buckwheat honey had significant higher concentrations of K and Cu in comparison to wild plant honey (0.17 and 0.04 percent, and 5.0 and 3.33 ppm, respectively). Concentrations of Trolox and TPC were significantly higher in buckwheat honey than wild plant honey (1.01 and 0.32, and 0.39 and 0.17, respectively). Both types of honeys exhibited anti-microbial activity against gram-positive and gram-negative bacteria. The buckwheat honey was darker in color than the honey from wild plants. We concluded that production of buckwheat as a grain or cover crop can also support honeybees and buckwheat honey might be superior to wild plant honey.

Keywords: *Fagopyrum esculentum*, sugars, minerals, antioxidants, bacterial inhibition

1. Introduction

Buckwheat (*Fagopyrum esculentum* Moench) is considered a pseudo-cereal. Buckwheat has a reputation for growing on poor soils and thrives on a wide variety of soils. Even small amounts of fertilizer are known to considerably enhance buckwheat performance in low fertility soils. Buckwheat can be used as a forage crop, cover crop, grain crop for animal feed and human food, honeybee crop, and a smother crop for weed suppression. Buckwheat is a short-duration crop and has great potential when "normal" crops fail. It improves the soil and suppresses weeds; needs very little attention during the growing season; makes a great rotation crop; often grows well on low-fertility land; is a high-yield crop; and can be planted as late as mid-July in many areas and is fast growing—70 days from planting to harvest. Fertilizer needs of buckwheat are limited producing savings in labor, fuel and chemical inputs.

Buckwheat has been grown in Virginia since late 1700s [Thomas Jefferson's letter (Jefferson, 1794) to George Washington dated May 14, 1794 indicated his interest in buckwheat as a rotation crop]. However, today the crop is almost non-existent in Virginia. We consider buckwheat to be a high-value alternative crop for Virginia and are interested in developing it as a summer forage and food crop, as a crop to support honeybees, as a weed smothering crop in rotations both in traditional and organic systems, and as a feedstock (both plant biomass and seed) for biofuel production.

We got interested in buckwheat when we observed considerable and quick growth of buckwheat at Randolph Farm of Virginia State University during summer of 2014. We planted ten acres of buckwheat on August 12, 2014 as a cover crop following harvest of winter canola and chickpea crops and observed that buckwheat grew extremely well. We obtained 9547, 8807, and 8615 pounds/acre fresh weight biomass 38, 31, and 24 days after planting, respectively. This crop was loaded with foraging honeybees upon flower initiation for about 10-15 days. This observation led us to undertake the present study to compare quality of honey produced by honeybees

foraging on buckwheat (Buckwheat honey) to that produced when honeybees foraged on wild plant types (Wild plant honey).

Even though, differences in honey from different foraging sources are known (Alves et al., 2013; Da Silva et al., 2016; Bayram et al., 2021), information about quality of buckwheat honey from Virginia s unavailable. Therefore, purpose of this study was to characterize the quality of buckwheat honey and compare it to wild plant honey.

2. Materials and Methods

2.1 Plant Material

The plant material for this study consisted of buckwheat variety ManCan. The seeds of buckwheat were purchased from Heretick Feed and Seed, Petersburg, VA.

2.2 Production and Sampling

Buckwheat was planted three times from May to June during 2015 (about 3 to 4 weeks apart) to provide continuing flowers for honeybees at the farms of cooperating beekeepers in Virginia. Plot size at each location was about 0.5 acre. Seeding rate was about 50 pounds/acre. These plantings were done at three locations in Virginia during 2015 [Gordonsville, (38°8′5″N, 78°11′13″W), Glenn Allen, 37°39′36″N; 77°29′8″W), and Nokesville 38°41′54″N; 77°34′25″W)]. Participating beekeepers placed several beehives in their buckwheat fields, collected honey in the fall season (October-November) from these locations, and provided these samples for analysis. They also provided honey samples from their beehives placed around their traditional locations around wild plants, trees, and other flora.

2.3 Analysis of Honey Samples and Data Collection

Six honey samples (Three of buckwheat and three of wild plant honey) were analyzed for concentrations of sugars and various minerals resulting in comparisons of buckwheat and wild plant honeys. Additionally, honey samples were also analyzed for anti-oxidants and their potential as anti-microbial agents.

Concentrations of various macro- and micro-nutrients were determined, using AOAC methods (AOAC, 2016) by Waypoint Analytical Laboratory (Richmond, Virginia, USA). Sugars were extracted from honey samples (1 g) and analyzed by HPLC following the methods optimized by Johansen et al. (1996). Sugars in the extracts were identified by comparing their retention times with standard sugars. For quantification, trehalose was used as internal standard and the sugar concentration was expressed as g/100 g meal (Bhardwaj & Hamama, 2016).

Anti-oxidants analyses were conducted in the Food Science Laboratory of Agricultural Research Station of Virginia State University according to the protocols described by Haiwen et al. (2011). All samples were stored in the dark under nitrogen until analyzed. Three measures of antioxidant activity were used: Oxygen-Radicals Scavenging Activity (ABTS assay), Antioxidation Activity (DPPH assay), and Total Phenolic Content (TPC). These analyses used 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), Folin-Ciocalteu reagent, gallic acid, and 6-hydroxy-2,5,7, 8-tetramethylchroman-2-carboxylic acid (Trolox) which were purchased from Sigma-Aldrich (St. Louis, MO). Honey samples were extracted with 80% methanol at 1 g/25 ml. The extracted samples were evaluated for the DPPH and ABTS+ scavenging activities, and Total Phenolic Content (TPC).

The DPPH Scavenging Capacity was measured using a high throughput assay. A SpectraMax M2 96-well Microplate Readers (Molecular Devices, Sunnyvale, CA) was used for assay determination. The reaction mixtures contained 100 µL 0.2 mM DPPH and 100 µL standards, control, blank, or sample. Absorbance readings were determined at 510 nm. Percent of control was calculated as the percent of effectively reduced DPPH amount comparing with the original DPPH reading following 30 min of reaction. Measurements were conducted in triplicate. For measuring ABTS+ scavenging capacity, ABTS+ was generated by oxidizing a 5 mM aqueous solution of ABTS with manganese dioxide for overnight at ambient temperature. The final reaction mixture contained 80 µL of extract, standard or 80% methanol for control, and 1.0 mL ABTS+ solution with an absorbance of 0.7 at 734 nm. The absorbance at 734 nm was measured after a reaction time of 30 sec. Trolox equivalents were calculated using a standard curve and expressed in mM Trolox equivalents/g sample. TPC was determined using Folin and Ciocalteu's (FC) reagent following a previously described method (Haiwen et al., 2011). The final reaction mixture contained 125 µL FC reagent, , 125 µL sample extracts or standard, and 0.5 mL H_2O, 1 mL 20% Na_2CO_3. Absorbance was measured at 765 nm following 2 hours of reaction at ambient temperature. Gallic acid was used as the standard. Measurements were taken in triplicate.

We also determined antimicrobial potential of buckwheat and wild plant honeys. Two gram-positive (*Listeria monocytogenes* ATCC 19115 and *Staphylococcus aureus* ATCC 29213) and two gram-negative (*Escherichia coli* O157:H7 ATCC 35150 and *Salmonella typhimurium* ATCC 14028) bacteria were used as test pathogenic microorganisms. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each honey sample were determined using a modified broth micro-dilution method as described by Kim et al. (2021).

Two hundred microliters of 50% honey prepared in MHB were transferred into the first well of a 96-well sterile plate. Serial twofold dilutions were made into consecutive wells. Into each well, 5 µl of the bacterial inoculum (grown on MHB for 18 h at 36°C) was added to achieve concentrations of honey ranging from 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.20, and 0.1 % (w/v). Growth control wells with each bacterial inoculum with no honey in MHB (growth control) and sterility control wells with MHB only (background control) were included in the plate. Growth of the bacteria was assessed by measuring optical density after 24 h of incubation at 36°C using a microplate reader (Spectra Max 340PC, Molecular Devices, San Jose CA) at 620 nm. After culturing the test organisms separately as described above in MHB containing various concentrations of the honey, the samples were spread-plated on SMA to assay for the bacteriostatic (inhibitory) and bactericidal effect. The lowest concentration of honey, which showed approximately same or less level of microbial populations as the inoculated level, was regarded as MIC while the lowest concentration of honey, which showed no bacterial growth on SMA after the incubation period, was regarded as MBC.

2.4 Statistical Analysis

All data were analyzed using SAS (2014) using 5% level of significance.

3. Results and Discussion

Honey is a natural food, mainly composed of sugars and other constituents such as enzymes, amino acids, organic acids, carotenoids, vitamins, minerals, and aromatic substances. It is rich in flavonoids and phenolic acids that exhibit a wide range of biological effects and act as natural antioxidants (Alqarni et al., 2012). The composition, color, aroma and flavor of honey depend mainly on the flowers, geographical regions, climate and honeybee species involved in its production, and are also affected by weather conditions, processing, manipulation, packaging and storage time (Escuredo et al., 2014; Tornuk et al., 2013).

Honey in our studies was affected by forage source (Table 1). Concentrations of fructose, glucose, and melezitos in buckwheat honey were quantitatively lower than those in wild plant honey whereas concentrations of sucrose and maltose exhibited an opposite trend - concentration of maltose being statistical significant. We suspect that sugar concentrations in buckwheat honey will exhibit statistical significance if data from more samples spanning times and locations were available. Fructose concentrations in honey from both foraging sources in our studies was the predominant carbohydrate (42 percent in buckwheat honey and 52 percent in wild plant honey). Glucose concentrations of honey in our study were lower than expected and varied from 1.72 to 1.96 percent. In general, honey samples contain approximately 2:1 ratio of fructose and glucose (Tornuk et al., 2013). Based on the total fructose and glucose concentrations in honey in our study, both buckwheat and wild plant honey met the European Codex Honey standards of less than 60 percent. Similarly, sucrose in both buckwheat and wild plant honey was less than 5 percent and met the European Codex Honey standards of less than 5 percent (Tornuk et al., 2013).

Table 1. Sugars and minerals in buckwheat and wild plant honey from three locations in Virginia during 2015

Component	Foraging crop	
	Buckwheat	Wild plants
Fructose (%)	41.69	51.57
Glucose (%)	1.72	1.96
Sucrose (%)	1.26	1.19
Maltose (%)	0.26	0.18*
Melezitos (%)	0.54	0.69
Sulfur (%)	0.02	0.01
Phosphorus (%)	0.02	0.01
Potassium (%)	0.17	0.04**
Magnesium (%)	0.01	0.01
Calcium (%)	0.05	0.02
Boron (ppm)	6.67	4.67
Zinc (ppm)	1.33	1.00
Manganese (ppm)	11.33	9.33
Iron (ppm)	1.00	1.00
Copper (ppm)	5.00	3.33*
Aluminum (ppm)	8.00	1.67

Note. *, **, ***: Means were statistically different at 10, 5, and 1 percent level, respectively.

Buckwheat honey in our study (Table 1) had significantly higher concentrations of K and Cu (0.17 percent and 5.0 ppm for buckwheat honey and 0.04 percent and 3.33 ppm for wild plant honey, respectively). These values compare well with those reported by Alqarni et al. (2012) where the investigators had compared 23 honey samples from seven countries. We did not study composition of nectar produced by buckwheat or the wild plants but it seems it seems that properties of nectar produced by the foraging plant determines the quality of honey.

In addition its' use as a sugar substitute, honey has an additional advantage of being a good source of antioxidant compounds (Ferreira et al., 2009). It is well known that both botanical and geographical origin of the honey influences its antioxidant activity because multiple factors, such as climatic conditions, soil composition, flora diversity and its contribution to the chemical composition of nectar are involved. In our study, buckwheat honey was observed to contain significantly higher concentrations of Trolox and TPC. The concentrations of these constituents were 1.01 and 0.32 in buckwheat honey whereas corresponding values for wild plant honey were 0.39 and 0.17, respectively (Table 2). Our results demonstrate that buckwheat honey is desirable over the wild plant honey for health nutrition aspects related to protection of human cells against free radicals, which may play a role in heart disease, cancer and other diseases (Kurutas, 2016). As a natural source of antioxidants, buckwheat honey may have a role in human health.

Table 2. Comparison of anti-oxidants in buckwheat and wild plant honey from three locations in Virginia during 2015

Foraging crop	ORAC*	DPPH*	TPC*
Buckwheat	1.01a**	78.1b	0.32a
Wild plants	0.39b	89.5a	0.17b

Note. * ORAC is the oxygen radical absorbance capacity measured as μmol Trolox equivalents/g (TE); DPPH scavenging capacity measured as ED_{50} 10 min mg equivalent/ml; TPC is total phenolic content measured as mg gallic acid equivalent/g (GAE mg/g).

**Means followed by different letters were statistically different according to Fisher's Protected Least Significant Difference at 5% level.

In our study, there was a complete lack of bacteria in all honey samples (Results not presented). We observed low value of water activity (~0.6) indicating that no bacteria can survive in our honey samples. Microbial inhibition zone study revealed that the samples have antimicrobial efficacy. Preliminary results indicated that

buckwheat honey produced in Gordonsville was the most effective in inhibiting and killing two gram-positive (*Listeria monocytogenes* ATCC 19115 and *Staphylococcus aureus* ATCC 29213) and two gram-negative (*Escherichia coli* O157:H7 ATCC 35150 and *Salmonella typhimurium* ATCC 14028) bacteria whereas both buckwheat and wild plant honey samples from Glen Allen were ineffective against these pathogens. It is well known that composition of honey is variable and depends upon floral sources but external factors such as seasonal and environmental factors also play a role. Our preliminary results are in agreement with those of Alvarez-Suarez et al. (2010) when they reported existence of differences in abilities of honey from different sources to inhibit bacteria. They studied antimicrobial activities of several honey samples against gram-positive and gram-negative bacteria and observed that dark honeys have a higher antimicrobial activity as compared to clear honeys.

In our study, buckwheat honey was darker in color than wild plant honey. An anecdotal observation indicated that buckwheat honey might be more palatable for human taste as compared to the wild plant honey. In a field crop demonstration that included food, we provided two honey samples—one each from our study. Several attendees indicated that buckwheat honey had a "more" buttery taste as compared to the wild plant honey.

4. Conclusion

This study indicated that quality of honey depends upon foraging source. Concentration of maltose, K, and Cu were significantly higher in buckwheat honey as compared to wild plant honey. Buckwheat honey also contained higher concentrations of antioxidants. It was concluded that production of buckwheat as a grain or cover crop can also help honeybees.

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Disclaimer

This study was conducted as a student-training project for the first author (KC). Use of any trade names or vendors does not imply approval to the exclusion of other products or vendors that may also be suitable.

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