Antioxidant Capacity of Honeys from Various Floral Sources Based on the Determination of Oxygen Radical Absorbance Capacity and Inhibition of in Vitro Lipoprotein Oxidation in Human Serum Samples

NELE GHELDOF AND NICKI J. ENGESETH*

Department of Food Science and Human Nutrition, University of Illinois, Urbana, Illinois

Honeys from seven different floral sources were analyzed for in vitro antioxidant capacity and total phenolic content. Antioxidant capacity was measured by the oxygen radical absorbance capacity (ORAC) assay and by monitoring the formation of conjugated dienes as an index of the inhibition of copper-catalyzed serum lipoprotein oxidation. ORAC values ranged from 3.1 to 16.3 μmol Trolox equivalent/g honey. The darkest colored honeys, such as buckwheat honey, had the highest ORAC values. A linear correlation was observed between phenolic content and ORAC activity of the investigated honeys ($p < 0.0001$, $R^2 = 0.9497$). The relationship between the ORAC activity and inhibition of lipoprotein oxidation by the honeys yielded a correlation coefficient of 0.6653 ($p = 0.0136$). This work shows that honey may be used as a healthy alternative to sugar in many products and thereby serve as a source of dietary antioxidants.

KEYWORDS: Honey; antioxidants; lipoprotein oxidation; phenolics

INTRODUCTION

Oxidative stress, defined as an imbalance between free radical production and antioxidant defense system favoring oxidation, plays a significant role in the development of many chronic diseases. In addition, oxidative reactions are among the most deleterious reactions in food products. This has led to considerable interest in both health-related effects of diets high in antioxidants and the functional role of antioxidants in food systems. Because of concerns about the safety of synthetic antioxidants (*1*, *2*), a great number of studies have focused on the beneficial effects of natural sources of antioxidants. Many epidemiological studies have shown an inverse relationship between vegetable and fruit intake and risk for chronic disorders, such as cancer and cardiovascular disease (*3*, *4*). This inverse correlation is generally attributed to the high level of antioxidant vitamins or provitamins (*5*) and polyphenols (*6*) contained in fruits and vegetables, but also present in a variety of other plant foods (cereals, legumes, and nuts) and beverages (such as wine, tea, beer, and cocoa). Indeed, these dietary antioxidants have been found to significantly strengthen the endogenous antioxidant defense system, and hence decrease the adverse effects of reactive oxygen species on normal physiological functioning in humans (*7*).

All over the world, honey is not only valued as a flavorful sweetener, but is also considered a part of traditional folk medicine. During the past decade, the use of honey as a therapeutic substance has been reevaluated in a more scientific setting. Studies have shown that honey has both antibacterial (*8*) and antiinflammatory properties (*9*), useful in stimulation of wound and burn healing (*10*) and treatment of gastric ulcers and gastritis (*11*). Additionally, honey has been found to have significant antioxidant activity, as demonstrated by a spectrophotometric method (*12*) and by chemiluminescence (*11*). Honeys from various floral sources exhibit a wide range of antioxidant activities, and a linear correlation with honey color has been observed (*12*). Honey, as a source of antioxidants, has been proven to be effective against deteriorative oxidation reactions in foods, such as lipid oxidation (*13*, *14*) and enzymatic browning of fruits and vegetables (*15*−*17*). In general, honey has been reported to have a rich polyphenolic profile (*18*, *19*). Many of the honey flavonoids and phenolic acids are known to have antioxidant activity.

Considering the previous studies on the antioxidant activity of honey, it is important to further elucidate the antioxidant behavior of honey in well-established and sensitive antioxidant assays that are more biologically relevant. The objective of this study was to investigate the potential of honey to reduce oxidative reactions in vitro and to compare its effectiveness to that of a sugar analogue. The oxygen radical absorbance capacity (ORAC) assay, developed by Cao et al. (*20*), has been deemed valuable in assessing the antioxidant capacity of a variety of biological samples, from pure compounds to complex matrixes (*21*). The ORAC values of honeys from various floral sources against peroxyl radicals were determined in comparison to that of a sugar analogue. The antioxidant ability of honey and its sugar analogue was also examined by monitoring the extent of

* Corresponding author. Address: 259 ERML, 1201 W. Gregory Drive, Urbana, IL 61801. Phone: (217) 244-6788. Fax: (217) 244-7877. E-mail: engeseth@uiuc.edu.

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Cu^{2+}-induced lipoprotein oxidation in human serum samples based on the production of conjugated dienes. In addition, we investigated whether the variation in antioxidant content among the honeys is correlated significantly with their phenolic content.

MATERIALS AND METHODS

Chemicals and Honey Samples. β-phycoerythrin (β-PE) from *Porphyridium cruentum* was obtained from Sigma Chemical (St. Louis, MO). AAPH (2,2′-azobis(2-amidinopropane) dihydrochloride) was purchased from Wako Chemicals, Inc. (Richmond, VA), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was from Fisher Scientific (Pittsburgh, PA). All other chemicals were obtained from Sigma Chemical.

Commercial honeys from the following floral sources were obtained from Moonshine Trading Co. (Winters, CA): fireweed (*Epilobium angustifolium*), tupelo (*Nyssa aquatica*), and Hawaiian Christmas berry (*Schinus terebinthifolius*). Clover (*Melilotus spp.*) honey was purchased on two separate occasions from Sue Bee (Sioux City, Iowa), acacia (*Robinia pseudoacacia*) honey was purchased from Langnese Honig KG (Bargteheide, Germany), and NY buckwheat (*Fagopyrum esculentum*) honey was from Wixson Honey Co. (Dundee, NY). IL buckwheat honey and two soybean (*Glycine max*) honey samples (extracted in 1996 and 2000, respectively) were locally obtained from the University of Illinois Beekeeping Facility. Buckwheat honey samples from four additional geographical regions were purchased: from Dutch Gold (Lancaster, PA), Pot o' Gold Honey Co. (Hemingway, SC), Deer Creek Honey Farms (London, OH), and Hilyard & Hilquist (Stanislaus, CA). All of these honeys are vended as "monofloral", meaning that the honey must derive at least 51% of the constituent nectar or 45% of contaminant pollen from a single floral source (*22*). Thus, honeys collected may contain nectars from more than one source, but the nominate floral type predominates. Samples were kept at 4 °C until assayed. A sugar analogue was made consisting of 40% fructose, 30% glucose, 10% maltose, and 20% water.

ORAC Assay. The ORAC assay was based on the procedure described by Cao et al. (*20*). Free radicals are produced by AAPH and the fluorescent indicator protein β-PE is subsequently oxidized. All reagents were prepared in 75 mmol/L phosphate buffer, pH 7.0, and Trolox (0−4 μM) was used as standard. The honey samples were suitably diluted in the phosphate buffer. Quercetin dihydrate (1 μM) (positive control) was dissolved in methanol first and then diluted with buffer (1:249, v/v). When methanol was used in the control sample, methanol was also used in the blank and standard, without having an effect on the 1:1 relationship between Trolox and ORAC value. Reaction mixtures consisted of 1000 μL of β-PE (0.92 nmol/L, preincubated for 15 min at 37 °C), 60 μL of test compound, 40 μL of 75 mmol/L phosphate buffer (pH 7.0), and 100 μL of AAPH (500 mM). Once the AAPH was added, the plate was shaken automatically for 3 s, and fluorescence was measured every 2 min for 70 min with emission and excitation wavelengths of 565 and 540 nm, respectively, using a microplate fluorescence reader FL600 (BioTek, Inc., VT) that was maintained at 37 °C. The ORAC values were calculated according to Cao et al. (*20*) and expressed as μmol Trolox equivalent (TE)/g. Analysis of variance with post-hoc comparisons according to Tukey was performed to compare the ORAC values of the different honeys using SAS Software (SAS Institute Inc, Cary, NC, version 8, 1999).

Lipoprotein Oxidation in Serum. The effect of honeys from various floral sources on copper-catalyzed lipoprotein oxidation in human blood serum was assessed by the method described by Hodgson et al. (*23*). This method provides an indication of diene formation in lipoprotein fatty acids present in serum exposed to Cu^{2+}, assessed by measuring the change in absorbance at 234 nm every 10 min for 4 h at 37 °C. Blood samples were obtained from five healthy volunteers aged 26−40 years. A fasting blood sample from each volunteer was drawn from the antecubital fossa vein into evacuated glass tubes, and the serum was separated using standard procedures. Serum samples were mixed, aliquoted, flushed with nitrogen, and frozen at −80 °C until assayed. Honey samples were suitably diluted (0.2−5 g/L final concentration) and tested in triplicate. Control experiments consisted of identical assay conditions but without the added honey. The variables used to describe the differences between oxidation curves were area under the oxidation curve (AUC), lag time to lipoprotein diene formation, and slope of the propagation phase of the oxidation curve. Lag time was defined as the intercept between the tangent of the absorbance curve during the propagation phase and the baseline (*24*). To determine whether the different concentrations of honey significantly inhibited lipoprotein oxidation, we compared the control to the honey experiments by paired *t*-tests. From the dose−response curves, the concentration of honey that produced a 50% reduction in the area-under-the-curve (IC_{50}) was calculated and used as an index of protection against lipoprotein oxidation.
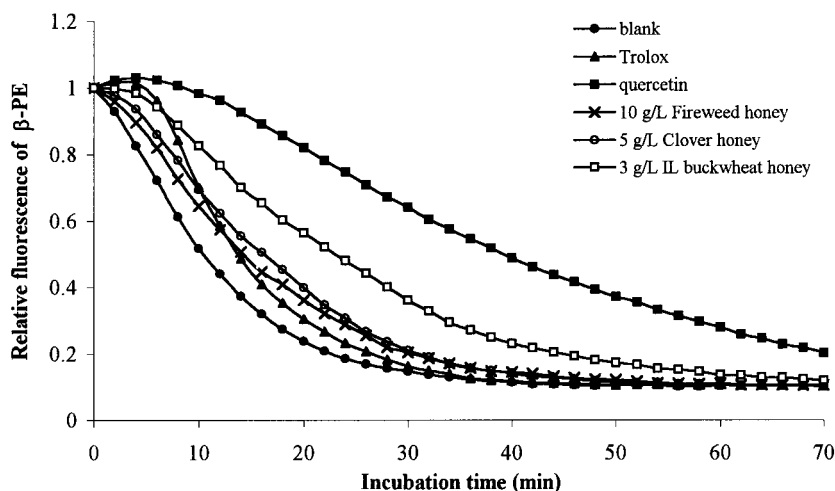
Total Phenolic Analysis. Total phenolic content was determined by a modified procedure of the Folin-Ciocalteau method described by Bonvehi & Coll (*25*). Because reducing sugars, present in large amounts in honey, have been found to interfere with the original Folin-Ciocalteau method (*26*), a blank was prepared by stirring 5 mL of the honey extract with 0.1 g of insoluble polyvinylpolypyrrolidone (PVPP). The quantity of polyphenolics was estimated at 765 nm in relation to a gallic acid standard curve. Additionally, recovery of phenolics was calculated by testing a sugar analogue consisting of 38.4% fructose, 30.3% glucose, and 500 μg/g gallic acid. Linear correlations between total phenolic content and ORAC activity were calculated using SAS Software.

RESULTS

Quenching curves of β-PE fluorescence illustrate the peroxyl radical absorbance ability of selected honeys and quercetin (positive control) as compared to that of the standard Trolox (**Figure 1**). Quercetin (1 μM) was 3.39 ± 0.23 times as potent as 1 μM Trolox, which is consistent with the study by Cao et al. (*27*) who found quercetin to be 3.297 times as powerful as Trolox in the ORAC assay. The ORAC values and total phenolic contents of honeys and the sugar analogue are summarized in **Table 1**. The ORAC values of honey ranged from 3 to 17 μmol TE/g, and were higher than the ORAC value of the sugar analogue ($p < 0.0001$). The ORAC activity of buckwheat honey samples was dependent on the geographical region of the samples: Illinois buckwheat honey had a 2.3-fold higher ORAC activity than California buckwheat honey. All other buckwheat honeys samples had similar ORAC activities, averaging 9.5 μmol TE/g. Hence, only two of the six buckwheat honey samples (IL and NY buckwheat honey) were selected for total phenolic analysis and for the lipoprotein oxidation assay. When comparing ORAC analyses of different batches of clover honey and soybean honey purchased at different time points, no significant differences were observed. Therefore, only one sample of each of the respective honeys was investigated for total phenolic content and in the lipoprotein assay (clover honey purchased in July 2000 and soybean honey extracted in 1996). Total phenolic content (**Table 1**) ranged from 45 to 800 mg/kg gallic acid equivalent, depending on the floral source of the honey. The correlation between phenolic content and ORAC activity was significant ($p < 0.0001$) and total phenolics accounted for over 94% of the variance in ORAC activity ($R^2 = 0.9497$).

Time plots of conjugated diene formation from human serum lipoproteins in the absence or presence of honeys at specified concentrations are shown in **Figure 2**. Significant differences in antioxidant capacity between honeys from different floral sources were found. However, Hawaiian Christmas berry (HCB) honey was markedly less potent in the lipoprotein oxidation assay relative to the ORAC assay. The area-under-the-curve (AUC), lag time, and slope for different honey and sugar test concentrations relative to the control are presented in **Table 2**. AUC was used to compare differences in the overall oxidation curves without focusing on particular aspects. The sugar analogue provided protection against oxidation only when very high concentrations were used. From **Table 2**, it is clear that

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Figure 1. Kinetics of β-PE quenching curves in the ORAC assay with 1 μM Trolox, 1 μM quercetin, and fireweed, clover, and IL buckwheat honey at specified concentrations.

Table 1. Antioxidant Activity (ORAC) and Total Phenolic Content of Honeys from Various Floral Sources and of the Sugar Analogue[a]

floral source	supplier	ORAC (μmol TE/g)	total phenolics[b] (mg/kg)
IL buckwheat	local beekeeper	16.95 ± 0.76[a]	796 ± 32[a]
buckwheat	Dutch Gold	9.81 ± 0.34[b]	n.d.
NY buckwheat	Wixson Honey	9.75 ± 0.48[b]	456 ± 55[b]
soy (2000)	local beekeeper	9.49 ± 0.29[bc]	n.d.
buckwheat	Deer Creek Honey	9.34 ± 0.57[bc]	n.d.
buckwheat	Pot o'Gold Honey	9.17 ± 0.63[bc]	n.d.
Hawaiian Christmas berry	Moonshine Trading	8.87 ± 0.33[bc]	250 ± 26[c]
soy (1996)	local beekeeper	8.34 ± 0.51[bc]	269 ± 22[c]
buckwheat	Hilyard & Hilquist	7.47 ± 0.27[cd]	n.d.
clover (Jan 2000)	Sue Bee	6.53 ± 0.70[d]	n.d.
tupelo	Moonshine Trading	6.48 ± 0.37[d]	183 ± 9[d]
clover (July 2000)	Sue Bee	6.05 ± 1.00[d]	128 ± 11[d]
fireweed	Moonshine Trading	3.09 ± 0.27[e]	62 ± 6[e]
acacia	Langnese Honig	3.00 ± 0.16[e]	46 ± 2[e]
sugar analogue		1.00 ± 0.16[f]	n.d.
ANOVA *p*		< 0.0001	< 0.0001

[a] Data expressed as means ± SEM. Means within a column sharing the same letter are not significantly different by Tukey (*p* < 0.05). [b] Data expressed as milligrams of gallic acid equivalent (GAE) per kg. n.d.= not determined.

the inhibitory effects of honey on copper-induced lipoprotein oxidation were dose dependent. In the appropriate concentration range, an inverse correlation between concentration and relative area-under-the-curve could be established for all honeys ($R^2 \geq$ 0.92) and the IC$_{50}$ value was calculated (**Table 2**). Linear regression analysis revealed a correlation between the IC$_{50}$ values and the ORAC values of 0.6653 (*p* = 0.0136).

DISCUSSION

Honey contains a number of compounds known to act as antioxidants, including polyphenolics, organic acids, vitamins (ascorbic acid), and enzymes (glucose oxidase and catalase) (*28, 29*). Here, we show for the first time a significant correlation between total phenolic content and ORAC activity of the honeys (*p* < 0.0001, R^2 = 0.9497). ORAC values of honey (3–17 μmol TE/g) were in the same range as ORAC values of many fruits and vegetables (0.5-19 μmol TE/g fresh weight) (*30, 31*). These results indicate that honey is comparable to fruits and vegetables in antioxidant capacity on a fresh weight basis. Although honey is not consumed in quantities equivalent in mass to that of most fruits and vegetables, it may be used as a healthy alternative to

sugar in many products and thereby serve as a supplementary source of dietary antioxidants.

There was, however, a large variation in ORAC activity among the investigated honeys. One potential source of variation in antioxidant capacity is the fact that these honey samples were obtained from different beekeepers in various geographical regions and/or at different times. Even within honeys from a particular floral source, the composition can vary depending on climate and environmental stress factors, such as humidity, temperature, and soil composition (*32*). In addition, processing, handling, and storage of honey may dictate part of its composition. All of these attributes may therefore affect the antioxidant capacity of honey. To further investigate the potential impact of geographical origin, beekeeper's handling, and time on the variation in antioxidant capacity, buckwheat honey from six different suppliers and clover honey and soy honey purchased at two different time points were examined for their ORAC activity. Buckwheat honey samples had similar ORAC values, except for the Illinois buckwheat honey, which had a significantly higher ORAC value (**Table 1**). This result is consistent with the study by Frankel et al. (*12*), who demonstrated that the antioxidant activity of IL buckwheat honey was approximately three times higher than the antioxidant content of CA buckwheat honey. When comparing ORAC analyses of different batches of clover honey and soybean honey, no significant differences were observed. However, the most recent soybean honey (extracted in 2000) had a slightly higher ORAC activity than soybean honey from 1996. Hence, floral source appears to be the primary reason for the large variation in antioxidant activity, except for Illinois buckwheat honey.

In addition, Frankel et al. (*12*) demonstrated a significant correlation between color and antioxidant capacity of honey as measured by a spectrophotometric DPPH assay (*p* < 0.00001), with darker colored honeys having a higher antioxidant content. Visual observation revealed a similar association between ORAC activity and color of the investigated honeys. Variation in antioxidant content among the different honeys is, however, much smaller in our study than that reported by Frankel and co-workers. The ORAC values differ approximately 5.5-fold between the highest and lowest value in contrast to the 20-fold variation in antioxidant content as measured by the DPPH assay. The DPPH assay is a spectrophotometric method based on the reduction in absorbance at 517 nm when a stable free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), reacts with an antioxidant (*33*). A variety of methods are available for assessing the
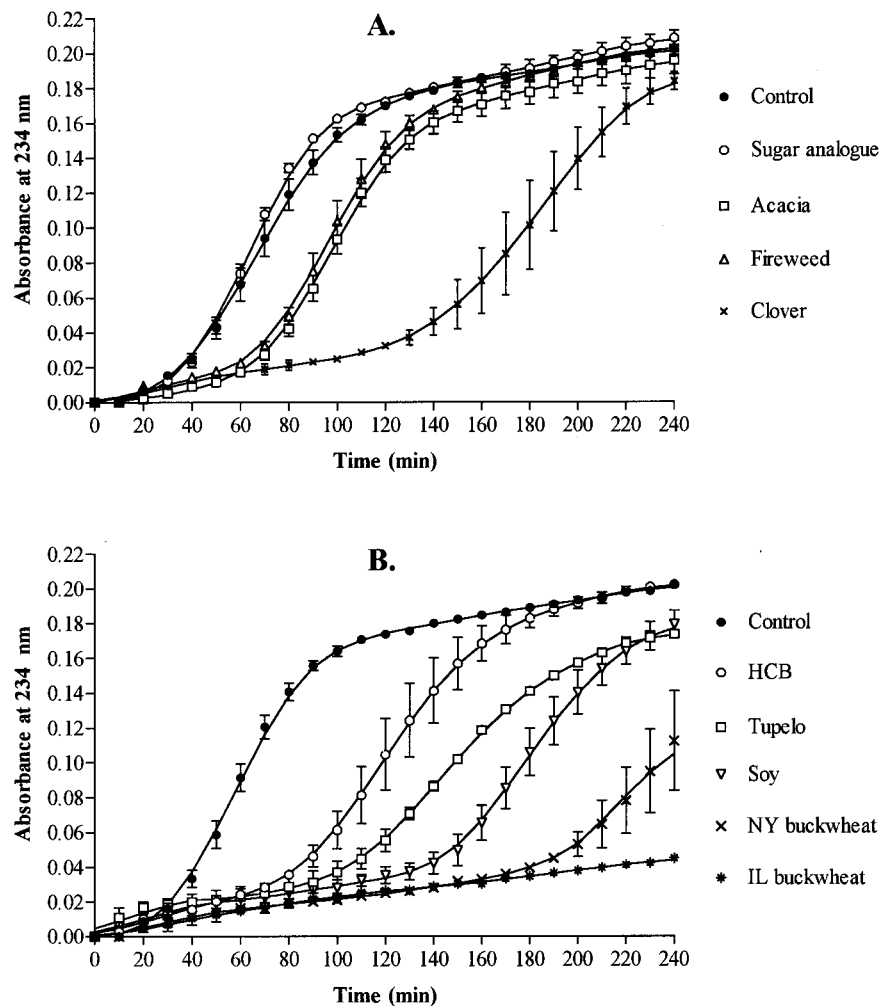
Figure 2. Time plots for conjugated diene formation from human serum lipoproteins in the absence (control) or presence of honeys from various floral sources. (A) Light colored honeys at 2 g/L final concentration, (B) darker colored honeys at 1 g/L final concentration. Data shown as means ± SEM ($n = 3$).

antioxidant capacity of a substance. Given the complexity of oxidative reactions, no single antioxidant test, however, can possibly measure all oxidative events in all matrixes. In addition, the mechanism of antioxidant activity in one system may or may not predict activity in other systems (*34*). Therefore, more reliability can be obtained when a combination of tests is used. The ORAC assay is a relatively simple but sensitive and reliable method. The uniqueness of this method is that it takes a free radical reaction to completion and uses an area-under-the-curve (AUC) technique for quantifying antioxidant capacity, thus combining both inhibition time and inhibition degree of free radical action by antioxidants into a single quantity (*35*). In addition, spectrofluorometric assays, such as the ORAC assay, have been reported to be 100−1000 times more sensitive than spectrophotometric techniques (*36*).

Because ORAC values of honey were demonstrated to be in the same range of ORAC values of many fruits and vegetables, it was of interest to assay the effect of honey in another oxidation system that is more indicative of the potential of a substance to serve as a dietary antioxidant. The effect of honeys from various floral sources on copper-catalyzed lipoprotein oxidation in human blood serum (*23*) was investigated for this purpose. All honeys reported in this paper inhibited lipoprotein oxidation more effectively than the sugar analogue (**Figure 2**). A significant correlation was found between the ORAC activity and inhibition of lipoprotein oxidizability ($p = 0.0136$).

However, the relative antioxidant activity of honeys determined by this assay was slightly different from that found via the ORAC assay. In the ORAC assay, Hawaiian Christmas berry (HCB) honey was significantly more active than lighter colored honeys, such as those from soybean and tupelo, whereas the latter honeys were more active in the lipoprotein oxidation assay than HCB honey. The ORAC assay and lipoprotein oxidation assay differ in the way oxidation is initiated. In the ORAC assay, oxidation is induced by a free radical generator, whereas in the lipoprotein oxidation assay oxidation is initiated by addition of copper. HPLC analysis of the honey phenolics (*29*) revealed that HCB honey has a distinctively different phenolic profile as compared to the other investigated honeys, lacking some of the main honey flavonoids and containing more phenolic acids and/or esters. Flavonoids are known to inhibit lipid oxidation through both metal chelating and free radical scavenging mechanisms, whereas phenolic acids act as antioxidants by free radical trapping mechanisms (*37*). The absence of flavonoids in HCB honey as opposed to other investigated honeys might result in less metal chelating activities and, hence, might explain the lower protection of HCB honey in the lipoprotein oxidation assay.

Oxidative modification of lipoproteins is considered a pivotal step in the pathogenesis of atherosclerosis (*38*). Assessment of in vitro lipoprotein oxidizability in the presence of honey can provide useful initial evidence of the potential biological

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Table 2. IC$_{50}$[a] Values and Area under the Oxidation Curve (AUC), Lag Time, and Slope for Each Honey and the Sugar Analogue at Selected Test Concentrations Relative to those of the Control[b]

floral source and concentration (g/L)	IC$_{50}$ (g/L)	relative AUC[c]	relative lag time	relative slope
IL buckwheat	0.62 ± 0.06[a]			
1		0.188 ± 0.005*	n.d.[d]	n.d.
0.5		0.559 ± 0.089*	3.784 ± 0.464*	0.827 ± 0.120
0.2		0.901 ± 0.069	1.750 ± 0.270*	0.894 ± 0.043*
NY buckwheat	0.74 ± 0.07[a]			
1		0.281 ± 0.071*	n.d.	n.d.
0.5		0.741 ± 0.072*	2.605 ± 0.366*	0.920 ± 0.012*
0.2		0.888 ± 0.059*	1.612 ± 0.082*	0.880 ± 0.208
soy	1.01 ± 0.10[b]			
1		0.461 ± 0.056*	4.406 ± 0.682*	0.775 ± 0.061*
0.5		0.889 ± 0.018*	1.997 ± 0.251 *	0.933 ± 0.030*
0.2		0.913 ± 0.062	1.301 ± 0.229	1.015 ± 0.126
tupelo	1.29 ± 0.08[c]			
2		0.199 ± 0.042*	n.d.	n.d.
1		0.588 ± 0.046*	2.353 ± 0.542*	0.610 ± 0.085*
0.5		0.877 ± 0.087	1.445 ± 0.127*	0.837 ± 0.047*
Hawaiian Christmas berry	1.43 ± 0.02[c]			
2		0.213 ± 0.045*	n.d.	n.d.
1		0.768 ± 0.049*	2.341 ± 0.089*	0.967 ± 0.059
0.5		0.930 ± 0.012*	1.478 ± 0.159*	0.971 ± 0.010
clover	1.85 ± 0.20[d]			
2		0.445 ± 0.072*	4.448 ± 0.781*	0.789 ± 0.102*
1		0.787 ± 0.040*	1.984 ± 0.233*	0.777 ± 0.070*
0.5		0.956 ± 0.041	1.402 ± 0.094*	1.012 ± 0.041
acacia	3.33 ± 0.18[e]			
5		0.158 ± 0.033*	n.d.	n.d.
2		0.810 ± 0.055*	2.016 ± 0.082*	0.965 ± 0.058
1		0.964 ± 0.046	1.359 ± 0.139*	1.042 ± 0.038
fireweed	3.57 ± 0.16[f]			
5		0.188 ± 0.070*	n.d.	n.d.
2		0.852 ± 0.011*	2.076 ± 0.175*	1.032 ± 0.010
1		1.043 ± 0.100	1.197 ± 0.152	1.209 ± 0.143
sugar analogue	7.52 ± 0.06[g]			
5		0.770 ± 0.005*	2.871 ± 0.340*	0.995 ± 0.052
2		1.025 ± 0.016	1.106 ± 0.152	1.132 ± 0.104
ANOVA p	< 0.0001			

[a] IC$_{50}$ is the sample concentration that produces a 50% reduction in AUC. Means sharing the same letter are not significantly different by Tukey ($p < 0.05$). [b] The control consists of identical assay conditions but without the added honey and has a relative value of 1. Results are presented as means ($n=3$) ± SD. [c] * Significant differences between control and sample. Paired *t*-test ($p < 0.05$). [d] n.d.= not determined.

antioxidant effect of honey. Although conjugated diene formation is most commonly monitored in isolated low-density lipoprotein (LDL) particles (*39, 40*), we chose to analyze serum lipoprotein oxidation for two reasons. First, it has been suggested that LDL oxidation is most likely initiated from the aqueous environment surrounding the lipoprotein particle, and thus water-soluble antioxidants serve to prevent the initiation of lipid peroxidation (*41*). Since the serum lipoprotein method takes the water-soluble antioxidants into account, this method may resemble in vivo conditions more closely than prior isolation of LDL from serum (*23*). Second, the serum lipoprotein assay is less complicated because it does not require prior isolation of the LDL particles. LDL is very susceptible to oxidation once it is removed from its natural plasma environment, and this is a major potential source of experimental artifacts (*41*). It must, however, be noted that serum is diluted (1:150) in the assay, and hence the relative antioxidant capacities may be distorted because of this dilution (*42*). In addition, honey is diluted (1: 200 to 1:5000 (w/v)) in the final reaction volume. It is questionable whether these concentrations can be reached in the plasma. This will largely depend on the absorption, metabolism, and bioavailability of the honey components. Conclusions about the efficacy of honey as an antioxidant in the human body cannot be reached if its antioxidant action is not assessed as part of human studies. Nonetheless, this study demonstrates that incorporation of honey in the human diet or as a food ingredient may potentially impart significant health

benefits to consumers in addition to stabilizing food products. Sugar consumption in the United States in 1997 was estimated at 154 pounds of sugar/person/year (www.usda.gov/cnpp). Honey represents only a very small component of the traditional US diet (∼1 pound/person/year). Demonstration of the ability of honey to serve as a natural source of antioxidants may provide good evidence to support the substitution of honey for some of the "empty calories" being consumed as refined sugar.

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