Honeys from Different Floral Sources as Inhibitors of Enzymatic Browning in Fruit and Vegetable Homogenates

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Honeys from different floral sources were evaluated for their antioxidant content and for their ability to inhibit enzymatic browning in fruits and vegetables. Antioxidant contents of honeys vary widely from different floral sources, as do their abilities to protect against enzymatic browning. Polyphenol oxidase (PPO) activity was reduced over a range of ~2−45% in fruit and vegetable homogenates, corresponding to a reduction in browning index by 2.5−12 units. Soy honey was particularly effective when compared to clover honey, which had a similar antioxidant content. When compared to commercial inhibitors of browning, honeys were less effective; however, in combination they added to the effectiveness of metabisulfite and ascorbic acid. Honey has great potential to be used as a natural source of antioxidants to reduce the negative effects of PPO browning in fruit and vegetable processing.

Keywords: *Honey; enzymatic browning; polyphenol oxidase; fruits and vegetables*

INTRODUCTION

Enzymatic browning in foods has a major impact on food quality. Polyphenol oxidase (PPO) action is useful in the production of black tea and raisins, imparting characteristic pleasant colors and aromas. However, PPO enzymatic browning also represents an adverse reaction during the processing of fruit juice and also in fresh fruits and vegetables, often associated with undesirable brown colors, off-flavors, and negative effects on the nutritional value. Prevention of undesirable browning reactions, catalyzed by PPO, has traditionally been accomplished by various chemicals, including ascorbic acid, citric acid, and sulfites. Sulfites are among the most effective browning inhibitors, yet are limited by the fact that a significant proportion of the population is sensitive to sulfites, leading to potentially fatal health problems such as severe asthmatic or anaphylactic-like reactions (Taylor et al., 1986; Sapers, 1993). Natural alternatives to these costly and potentially toxic inhibitors would be desirable.

Honey has been used since ancient times and has gained appreciation as the only concentrated form of sugar available worldwide (FAO, 1996). Traditionally, its use in food has been as a sweetening agent. However, several aspects of its use indicate that it also functions as a food preservative. Honeys contain a number of components known to act as preservatives; these include α-tocopherol, ascorbic acid, flavonoids, and other phenolics and enzymes such as glucose oxidase, catalase, and peroxidase (Ferreres et al., 1993; Ioyrish, 1974; Crane, 1975). Many of these substances owe their preservative properties to their antioxidant activity. Honey has been investigated as an alternative to the use of sulfites for browning control in the processing of light raisins (McLellan et al., 1995) and in grape juice processing (Lee, 1996). The efficacy of honey at reducing PPO activity in apple slices, in grape juice, and in model systems has also been investigated (Oszmianski and Lee, 1990).

Previous investigations into the positive effects of honey at reducing PPO activity and enzymatic browning have dealt primarily with honeys from single floral sources. The antioxidant content of honey varies widely depending on the floral source and is strongly correlated with the color of honey (Frankel et al., 1998). Because of the dramatic differences in antioxidant content of different honeys, we sought to determine the effectiveness of honeys from different floral sources at inhibition of enzymatic browning and PPO activity in fruit and vegetable homogenates.

MATERIALS AND METHODS

Honeys from the various floral sources were obtained from Moonshine Trading Co. (Winters, CA). Those tested included honeys from the following flower sources: acacia (*Robinia pseudoacacia*), fireweed (*Epilobium angustifolium*), soybean (*Glycine max*), clover (*Melilotus* spp.), tupelo (*Nyssa aquatica*), Hawaiian Christmas berry (*Schinus terebinthifloiius*), and buckwheat (*Fagopyrum esculentum*). Flower sources of honey are typically designated on the basis of having at least 51% of constituent nectar or 45% of contaminant pollen from a single floral source (Yoirish, 1977; Louveaux et al., 1978). Thus, the honeys collected may contain nectars from more than one source, but the nominate floral type predominates. All chemicals/reagents were obtained from Sigma Chemical (St. Louis, MO).

Overall Design. Honeys from different floral sources were selected over a wide range of antioxidant contents to evaluate their effectiveness at reducing enzymatic browning in fresh fruit and vegetable homogenates. Honeys were tested in homogenates for their ability to reduce browning index versus control samples (heat-inactivated PPO). Also tested were the

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10.1021/jf000373j CCC: $19.00
Published on Web 09/07/2000

effects of honey on PPO enzyme activity. After it was established that these honeys would be effective, they were then compared to commercial browning inhibitors. All assays were conducted in triplicate.

Measurement of Antioxidant Content. Honeys from different floral sources were evaluated for antioxidant content by using a spectrophotometric assay (Glavind, 1963) based on the reduction in absorbance at 517 nm when a stable free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), reacts with an antioxidant. Honey was dissolved in warm water (0.05−0.2 g/mL), and 0.75 mL of this was mixed with 1.5 mL of a 0.09 mg/mL solution of DPPH in methanol. After the mixture had sat at room temperature for 5 min, 2 mL of xylene was added, and the mixture was mixed and then allowed to separate. The xylene layer was centrifuged (3000 rpm, 2 min), and the water-soluble antioxidant content was determined spectrophotometrically (Spectronic Genesys 2 spectrophotometer, Spectronic Instruments, Rochester, NY; 517 nm) using ascorbic acid (0−0.04 mg/mL) for the standard curve.

Determination of the Efficacy of Honeys in Inhibiting Browning. A spectrophotometric assay was used to determine the browning index of treated and untreated fruits and vegetables following a modification of the method presented by Omidiji and Okpuzor (1996). Browning index is represented in browning units, where 1 browning unit is the equivalent of a difference of 0.01 absorbance unit from control per gram homogenate. Fresh homogenates of Red Delicious apples, d'Anjou pears, Idaho baking potatoes and sweet potatoes were assayed. A 1-h incubation period with and without honey was conducted; honey concentration varied from 0.5 to 4% of total homogenate. Controls included heat treatment of homogenates (placement in a boiling water bath for 10 min) to inactivate PPO. Following incubation, an aliquot of each homogenate was extracted with aqueous methanol. The extract was centrifuged (10000*g*, 5 min), and optical density was determined at 420 nm against an aqueous methanol blank. Data are represented as the change in browning units; thus, those samples with the highest numbers indicate the greatest inhibition of browning. Treatment means were compared by ANOVA with post-hoc comparisons made according to Tukey (using SPSS software, SPSS Inc., Chicago, IL).

Determination of the Efficacy of Honeys in Inhibiting PPO. PPO assays were conducted following a modified method of Omidiji and Okpuzor (1996). PPO was extracted from fruits and vegetables by homogenizing freshly peeled tissue in 0.05 M sodium phosphate buffer, pH 7, and centrifuging (12100*g*, 12 min at 4 °C). Crude enzyme extract (0.1 mL) was incubated with 2 mL of catechol (12.5 mM in 0.2 M phosphate buffer) and 0.1 mL of 1 M phosphate buffer (total reaction mixture = 2.2 mL). Changes in absorbance at 420 nm were monitored for 90 s in a spectrophotometer equipped with an automatic cell changer (Spectronic Genesys 2 spectrophotometer, Spectronic Instruments). Alterations in homogenate concentration were made on the basis of achieving a linear response. Honey concentrations were tested over a range of 0.045−0.27% of the total reaction mixture. Polyvinylpolypyrrolidone (PVPP) was also added to tie up phenolic substances in homogenates (Smith and Montgomery, 1985). This served to eliminate browning of extracts prior to addition of catechol (substrate) and allow more accurate monitoring of enzymatic activity.

Comparison of Honeys to Commercial Inhibitors of Enzymatic Browning. The above assays for browning and PPO inhibition were also conducted in a comparison of honeys and commercial inhibitors. Ascorbic acid (AA) and sodium metabisulfite (MBS) were tested at 0.1% of total homogenate. To determine whether addition of honey to antioxidant-supplemented food enhanced inhibition of browning, we compared antioxidant alone to antioxidant plus honey (1.0% of total homogenate) treatments by paired *t*-tests.

RESULTS AND DISCUSSION

Antioxidant Content of Honeys from Different Floral Sources. Antioxidant contents of the various honeys ranged from 2.5×10^{-4} to 59.2×10^{-4} μequiv (Table 1). The darkest honey, buckwheat, had the highest antioxidant content; acacia, the lightest in color, had the lowest antioxidant content. These data followed the same trends as those presented by Frankel et al. (1998), who demonstrated that honeys with darker colors exhibited higher antioxidant contents ($p < 0.00001$). Honey color accounted for >60% of variance in antioxidant capacity for honeys examined ($R^2 = 0.634$). Although the antioxidant capacity of buckwheat honey is noted here for comparison, it was impossible to use this honey in assays to determine the effectiveness of honey at reducing oxidative browning due to its dark brown color.

Table 1. Antioxidant Content of Honeys Utilized

honey type	antioxidant content[a] (10^{-4} μequiv)
buckwheat	59.2 ± 1.0
Hawaiian Christmas berry	20.0 ± 1.3
tupelo	17.4 ± 0.7
sweet clover	6.7 ± 0.6
soybean	5.9 ± 1.0
fireweed	5.4 ± 0.2
acacia	2.5 ± 0.1

[a] Values represent means of at least three replicates ± SE.

Efficacy of Honeys at Inhibition of Enzymatic Browning. Honeys from different floral sources exhibited various degrees of alteration in the browning index of fruit and vegetable homogenates (Table 2). The values represent changes in browning as compared to controls; thus, the higher values indicate greatest effectiveness at inhibition of browning. Effectiveness of honeys at reducing browning followed the trend of antioxidant content, with the exception of soy honey. Soy honey (5.9×10^{-4} μequiv) was slightly lower than clover honey (6.70×10^{-4} μequiv) in antioxidant content, yet was almost as effective as Hawaiian Christmas berry honey, with an antioxidant content of 20×10^{-4} μequiv, at reducing browning. This is not the first report of the use of honeys for enhancement of fruit and vegetable products; however, our research demonstrates the effectiveness of honeys from different floral sources with different antioxidant contents. Honeys have previously been successfully used in the clarification of apple juice (Lee and Kime, 1984). This property was thought to be due to protein components of honey forming a complex with polyphenolic tannins. Further studies showed that, although this may be a property of a protein component of honey (Lee et al., 1990), the clarification rate did not correlate well with any particular phenolic group of the juices (Wakayama and Lee, 1987). Honey has also been used for the prevention of browning in apple slices (Oszmianski and Lee, 1990), in grape juice (Oszmianski and Lee, 1990; Lee, 1996), and in grapes used for light raisin production (McLellan et al., 1995). Apple slices in honey solution browned less than those in a sucrose solution of comparable sugar content (Oszmianski and Lee, 1990), indicating that some component other than sugar was providing protection against enzymatic browning.

Efficacy of Honeys in Inhibiting PPO. Trends in alterations of PPO activity upon introduction of various honeys to fruit and vegetable homogenates were similar to those of alteration of browning index (Table 3). This was expected as a close correlation of browning with PPO activity was previously demonstrated (Lee et al., 1990). Again, soy honey proved to be quite effective in

Table 2. Alterations in Browning Index for Fruits and Vegetables in the Presence of Honeys from Different Floral Sources

	change in browning index[a] (mean ± SE)			
honey type	Idaho baking potato	sweet potato	Red Delicious apple	d'Anjou pear
Hawaiian Christmas berry	12.07 ± 0.12[a]	11.73 ± 0.09[a]	8.53 ± 0.20[a]	8.57 ± 0.12[a]
tupelo	11.43 ± 0.18[b]	11.10 ± 0.12[b]	8.38 ± 0.15[a]	8.30 ± 0.12[a]
sweet clover	5.40 ± 0.10[c]	4.97 ± 0.12[c]	3.12 ± 0.33[b]	3.33 ± 0.09[b]
soybean	10.87 ± 0.12[d]	10.07 ± 0.09[d]	8.15 ± 0.33[a]	8.07 ± 0.12[a]
fireweed	4.10 ± 0.10[e]	4.03 ± 0.03[e]	2.74 ± 0.13[b]	2.83 ± 0.18[b]
acacia	3.17 ± 0.09[f]	3.00 ± 0.06[f]	2.67 ± 0.08[b]	2.57 ± 0.09[b]
ANOVA *p*	<0.001	<0.001	<0.001	<0.001

[a] One browning unit is the equivalent of a difference of 0.01 absorbance unit from control per gram of homogenate. Means with the same letters are not significantly different from one another by Tukey HSD ($p < 0.05$).

Table 3. Alterations in PPO Activity for Fruits and Vegetables in the Presence of Honeys from Different Floral Sources (PVPP Method)[a]

	change in PPO activity (mean ± SE)			
honey type	Idaho baking potato	sweet potato	Red Delicious apple	d'Anjou pear
Hawaiian Christmas berry	45.7 ± 2.0[a]	42.1 ± 1.2	37.6 ± 1.3	45.6 ± 2.2
tupelo	28.0 ± 8.9[ac]	36.0 ± 0.8	31.0 ± 1.5	39.7 ± 0.8[a]
sweet clover	21.7 ± 0.8[bcd]	19.0 ± 1.1	9.3 ± 0.2[a]	27.2 ± 0.6
soybean	39.3 ± 0.5[ad]	27.9 ± 1.2	20.1 ± 0.4	34.6 ± 0.8[a]
fireweed	14.1 ± 1.6[bc]	11.9 ± 0.4[a]	7.2 ± 0.3[a]	15.1 ± 0.8[b]
acacia	9.7 ± 0.9[b]	7.9 ± 0.3[a]	2.1 ± 0.3	11.8 ± 1.0[b]
ANOVA *p*	<0.001	<0.001	<0.001	<0.001

[a] Means sharing the same letter are not significantly different by Tukey HSD ($p < 0.005$).

Table 4. Reduction of Browning by Commercial Inhibitor [Metabisulfite (MBS)] in the Presence and Absence of Honeys

	change in browning index[a] (mean ± SE)							
	Idaho baking potato		sweet potato		Red Delicious apple		d'Anjou pear	
honey type	MBS	MBS + honey	MBS	MBS + honey	MBS	MBS + honey	MBS	MBS + honey
Hawaiian Christmas berry	39.37 ± 0.07	44.07 ± 0.09*	31.37 ± 0.09	35.47 ± 0.19*	34.96 ± 0.26	38.77 ± 0.27*	33.70 ± 0.18	37.73 ± 0.20*
tupelo	39.43 ± 0.12	43.70 ± 0.06*	31.33 ± 0.13	34.87 ± 0.09*	34.88 ± 0.33	38.62 ± 0.23*	33.67 ± 0.21	37.30 ± 0.12*
sweet clover	39.30 ± 0.06	41.07 ± 0.12*	31.07 ± 0.22	32.10 ± 0.25*	35.26 ± 0.40	35.72 ± 0.33	33.40 ± 0.16	34.33 ± 0.24
soybean	39.23 ± 0.09	43.17 ± 0.09*	31.13 ± 0.09	34.77 ± 0.13	34.09 ± 0.27	37.33 ± 0.20*	33.53 ± 0.12	36.67 ± 0.23*
fireweed	39.40 ± 0.06	40.40 ± 0.06*	31.23 ± 0.03	32.07 ± 0.13*	34.58 ± 0.15	34.96 ± 0.13*	33.10 ± 0.18	33.57 ± 0.15
acacia	39.30 ± 0.15	39.97 ± 0.24*	31.30 ± 0.15	31.80 ± 0.17*	34.88 ± 0.20	35.11 ± 0.21*	33.57 ± 0.21	33.80 ± 0.21*

[a] One browning unit is the equivalent of difference of 0.01 absorbance unit from control per gram homogenate. Asterisks indicate significant differences between MBS alone and MBS + honey. Paired *t*-test ($p < 0.05$).

Table 5. Reduction of Browning by Commercial Inhibitor [Ascorbic Acid (AA)] in the Presence and Absence of Honeys

	change in browning index[a] (mean ± SE)							
	Idaho baking potato		sweet potato		Red Delicious apple		d'Anjou pear	
honey type	AA	AA + honey	AA	AA + honey	AA	AA + honey	AA	AA + honey
Hawaiian Christmas berry	20.63 ± 0.09	25.13 ± 0.11*	25.47 ± 0.09	29.57 ± 0.09*	21.10 ± 0.20	24.98 ± 0.20*	27.57 ± 0.15	31.80 ± 0.10*
tupelo	20.40 ± 0.11	24.57 ± 0.13*	25.57 ± 0.09	29.10 ± 0.16*	21.40 ± 0.15	24.83 ± 0.08*	27.67 ± 0.15	31.43 ± 0.44*
sweet clover	20.57 ± 0.24	22.43 ± 0.18*	25.33 ± 0.12	26.23 ± 0.15*	20.79 ± 0.23	21.40 ± 0.33*	27.47 ± 0.24	28.20 ± 0.27*
soybean	20.40 ± 0.15	24.30 ± 0.06*	25.53 ± 0.09	28.87 ± 0.13*	20.35 ± 0.11	23.85 ± 0.14*	27.63 ± 0.27	30.50 ± 0.29*
fireweed	20.47 ± 0.09	21.57 ± 0.09*	25.43 ± 0.09	26.13 ± 0.16*	20.72 ± 0.20	21.02 ± 0.26*	27.43 ± 0.21	27.80 ± 0.15
acacia	20.27 ± 0.10	20.97 ± 0.04*	25.37 ± 0.09	25.90 ± 0.12*	20.79 ± 0.26	21.02 ± 0.23*	27.43 ± 0.18	27.60 ± 0.19*

[a] One browning unit is the equivalent of a difference of 0.01 absorbance unit from control per gram homogenate. Asterisks indicate significant differences between AA alone and AA + honey. Paired *t*-test ($p < 0.05$).

comparison to clover honey, which was similar in antioxidant content. It is speculated that soybean honey has unique antioxidant compounds that impart greater protection in these specific assays, allowing for higher protection. It is our hypothesis that the floral sources protect differently in various oxidative reactions depending on some unique distribution of antioxidants. Also, it is possible that there are components acting as antibrowning agents and as inhibitors of PPO activity, which may not be measured using the DPPH antioxidant assay. A thorough investigation of antioxidant components of the various honeys is being conducted.

Comparison of Honeys to Commercial Inhibitors of Enzymatic Browning. MBS (Table 4) and AA (Table 5), tested at 0.1% of total homogenate, consistently reduced browning (as evidenced by browning index) (Tables 4 and 5). For AA the change in browning index is from 20 to 30 units (1 browning unit represents a change in absorbance of 0.01). For MBS the change is even greater, indicating a strong inhibition of browning.

Also tested was the effect of these potent inhibitors in combination with honey. The literature is suggestive of the fact that browning inhibitors may act synergistically (Kim et al., 1995; Montoya et al., 1997). We found that the combination of honey with AA or MBS was not synergistic, nor exactly additive. Addition of honey improved browning by AA and MBS to a level intermediate between that of AA (or MBS) alone and that of the added sum of AA (or MBS) plus honey.

Results of our study suggest that it may be possible to select honey varieties for use as natural antioxidants in fruit and vegetable preparation. In addition to enhancing flavor, honey may also contribute to improving appearance and preserving nutritive value.

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Received for review March 23, 2000. Revised manuscript received July 19, 2000. Accepted July 24, 2000. This work was supported by a grant from the National Honey Board.

JF000373J