JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Honey as a Protective Agent against Lipid Oxidation in Ground Turkey

JASON MCKIBBEN† AND NICKI J. ENGESETH*,‡

Anheuser Busch, Santa Monica, California 90403, and Department of Food Science and Human Nutrition, University of Illinois, Urbana, Illinois 61801

Lipid oxidation is a major deteriorative factor in meats. Sources of natural antioxidants that are as effective as commercially available antioxidants are desired. The objective of this research was to investigate honey as an inhibitor of lipid oxidation in ground poultry. The antioxidant content of different varieties of honey was investigated spectrophotometrically and honey's effectiveness in reducing oxidation of ground poultry determined by monitoring thiobarbituric acid reactive substances (TBARS). Buckwheat honey had the highest antioxidant content and acacia honey the lowest. Honeys of different floral sources differed in their protection against lipid oxidation. Buckwheat honey (5%, w/w) reduced TBARS ~70%, whereas acacia honey reduced TBARS ~34% at 3 days of storage at 4 °C. In comparison to butylated hydroxytoluene and tocopherol (0.02% of total fat), honey (at 5% of the weight of the meat) was much more effective at preventing oxidation. Honey has great potential as an antioxidant source and may result in greater acceptability of meat products and prevent negative health implications of oxidized meats.

KEYWORDS: Honey; lipid oxidation; poultry; thiobarbituric acid reactive substances (TBARS)

INTRODUCTION

Lipid oxidation is a major deteriorative factor in meat systems during storage. Lipid hydroperoxides, and their breakdown products, have been implicated in a number of deleterious effects, including off-flavor and off-color development (*1*), possible reaction with certain food components such as amino acids and proteins with concomitant losses of nutritional value and functionality (*2*), and a variety of health-related problems such as heart disease (*3*) and cancer (*1, 4*).

The addition of antioxidants has been used as an effective method for prevention of oxidation in meat systems. There are many synthetic antioxidants that can be used in processed meat products. However, with today's consumer trends away from the addition of synthetic chemicals to foods, there is an interest in the development of natural antioxidants. One solution is the supplementation of the diet of food-producing animals with α-tocopherol (a naturally occurring antioxidant). Numerous studies have documented the effectiveness of tocopherol supplementation in the feed of food-producing animals for the prevention of lipid oxidation in meats (*5*−*10*). However, there is no economic incentive for farmers to supplement in such a manner. Thus, it is more practical to incorporate natural antioxidants at the processing stage.

Honey has been used traditionally as a sweetening agent. Honey has, however, been shown to act in a preservative fashion, including the minimization of oxidative deteriorative reactions in foods (*11*, *12*) and protection against microbial growth (*13*). Honeys may contain a variety of preservative substances such as α-tocopherol, ascorbic acid, catalase, flavonoids and other phenolics, and enzymes such as glucose oxidase, catalase, and peroxidase (*14*−*16*). Many of these substances have antioxidant properties. In this study we investigated the antioxidant potential of honeys from different floral sources. The antioxidant content of honeys from different floral sources varies greatly (*17*). We tested the hypothesis that honeys of differing antioxidant content vary in ability to protect against deteriorative oxidation reactions. The major objective of this research was to evaluate the effectiveness of honey as an inhibitor of lipid oxidation in a meat system and to compare its effectiveness to that of some commonly used antioxidants.

MATERIALS AND METHODS

Materials. 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*), soybean (*Glycine max*), clover (*Melilotus* spp.), 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 (*18*, *19*). Thus, the honeys collected may contain nectars from more than one source, but the nominate floral type predominates.

Fresh lean ground turkey (Turkey Store brand, Jerome Foods, Inc., Barron, WI) was purchased from a local supermarket on day 0 of each analysis. The age of the meat at the time of purchase was unknown.

* Address correspondence to this author at 259 ERML, 1201 W. Gregory Dr., University of Illinois, Urbana, IL 61801 [telephone (217) 244-6788; fax (217) 244-7877; e-mail engeseth@uiuc.edu].
† Anheuser Busch.
‡ University of Illinois.

10.1021/jf010820a CCC: $22.00
Published on Web 12/19/2001

Sell-by dates were indicated, and the package with the latest sell-by date was selected so as to obtain the "freshest" package. There was no indication on the package as to which muscles were included in the ground meat. The percent fat of the samples was 7% (8 g of a 112 g serving, based on raw meat). Dow Corning Antifoam Spray was obtained from Thomas Scientific (Swedesboro, NJ). All other chemicals/reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

Antioxidant Quantitation. Honeys from different floral sources were evaluated for antioxidant content by using a spectrophotometric assay (*20*) based on the loss of absorbance at 517 nm when a stable free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), reacts with an antioxidant. Water-soluble antioxidant content was determined spectrophotometrically using ascorbic acid (0−0.04 mg/mL) for the standard curve.

Assay of Lipid Oxidation in Ground Poultry. Cooked, ground poultry was evaluated for extent of lipid oxidation by measuring thiobarbituric acid reactive substance (TBARS) values following a modified procedure of Tarladgis et al. (*21*). Ground turkey (30 g) was homogenized with an equal weight of water to which butylated hydroxytoluene (BHT) (0.02% of the weight of fat in sample) in propylene glycol was added. Aliquots of homogenate (20 g) were weighed and transferred to distillation flasks. Distillation proceeded in the presence of 87.5 mL of water, 2.5 mL of HCl/H_2O (2:1), and four sprays of antifoam (10% silicone emulsion in deionized water). Fifty milliliters of distillate was collected; 5 mL aliquots were exposed to 5 mL of TBA reagent, placed in a boiling water bath for 35 min, and cooled to room temperature prior to absorbance reading at 532 nm. TBARS values were obtained by multiplication of a predetermined *K* value with the absorbance readings.

Experimental Design. Ground turkey was obtained from a local supermarket on day 0 of analysis. It was immediately prepared into patties of uniform size and weight (140 g; control and honey, or other antioxidant, added by drizzling over the meat and mixing in by hand wearing gloves) and cooked in an electric skillet to an endpoint temperature of 170 °F. TBARS values were determined at days 0, 1, and 3 of storage at 4 °C. A selection of four honeys was made on the basis of their range of antioxidant contents.

Three experiments were completed. First, the effectiveness of soy honey was tested at three levels (1, 5, and 10% of the weight of the meat). The 5% level was chosen for our second experiment, which involved comparison of honeys from four different floral sources. Finally, the effectiveness of honey as a source of antioxidants was also compared to that of BHT and α-tocopherol. It was very difficult to quantitate the amount of antioxidant added through 5% addition of honey. Initially, we tried to quantitate the antioxidant content of honey on the basis of the microequivalents of ascorbic acid and calculated antioxidant content with the molecular weight of ascorbic acid. However, processed honey is very low in ascorbate, and this was believed to be inaccurate. Preliminary data collected in our laboratory show that there is a strong correlation of antioxidant capacity with total phenolic content; thus, we have incorporated this into a new calculation. We used the quantity of total phenolics in soy honey to calculate the percentage of total phenolics added to the meat. This was determined to be ∼0.02% of the weight of the fat. Whether or not these phenolics are all serving as antioxidants is unknown. Because the reduction of TBARS was so dramatic in the case of soy honey (at 5% of total weight of the meat), it was compared to α-tocopherol and BHT (at 0.02% of total fat).

Design and Statistical Analysis. Each treatment [honey (four types), tocopherol, BHT, and a no-antioxidant control] was tested over a 3-day storage period. Our preliminary experiments indicated that this was a sufficient storage period in terms of obtaining adequate absorbance and thus meaningful TBARS values. Other experiments had been designed to collect TBARS over a 10-day period (*10*); we felt this to be unnecessary as our samples had detectable TBARS on day 0 and were sufficiently oxidized over the 3-day period. The goal was to be realistic in terms of an actual storage period that may be encountered in a home setting. Turkey was obtained on day 0 and each treatment was tested in triplicate (i.e., on three different 3-day storage periods). The no-antioxidant control was tested each time for immediate comparison. Duplicate TBARS measurements were made of each sample. Values were averaged and standard errors calculated. Data were analyzed by ANOVA (SAS). Significant differences at $p < 0.05$ were determined by Duncan's multiple-range test.

Table 1. Antioxidant Content of Honeys Utilized[a]

honey	antioxidant content (μequiv × 10^{-4})	honey	antioxidant content (μequiv × 10^{-4})
acacia	2.50 ± 0.1^{a}	clover	6.70 ± 0.6^{b}
soy	5.90 ± 1.0^{b}	buckwheat	59.20 ± 1.0^{c}

[a] Values represent means ± standard errors of three replicates. Means with different superscript letters are significantly different ($p < 0.05$).

Table 2. TBARS Values of Cooked, Ground Turkey after 0, 1, and 3 Days of Storage at 4 °C[a]

sample	day 0	day 1	day 3
control (no honey)	1.22 ± 0.05^{ab}	3.04 ± 0.50^{a}	7.89 ± 0.22^{a}
1% (w/w) soy honey	1.21 ± 0.22^{ab}	2.33 ± 0.10^{ab}	3.66 ± 0.67^{b}
5% (w/w) soy honey	1.08 ± 0.20^{bc}	1.54 ± 0.33^{b}	2.74 ± 0.29^{c}
10% (w/w) soy honey	1.49 ± 0.15^{a}	2.10 ± 0.25^{b}	2.61 ± 0.17^{c}

[a] Values represent means ± standard errors of three replicates. Within columns, means with different superscript letters are significantly different ($p < 0.05$).

RESULTS AND DISCUSSION

Antioxidant Content of Honeys. The antioxidant contents of honeys from different floral sources selected for this study are presented in **Table 1**. Honey types were selected because they represent a wide range of values of antioxidant content. Our sample antioxidant values followed trends similar to those presented by Frankel et al., who found antioxidant content to correlate with the color of honey; that is, those with the darkest colors have the highest antioxidant values ($p < 0.00001$) (*17*). Buckwheat honey had the highest antioxidant content (59.20 × 10^{-4} μequiv), ∼25 times greater than the lowest sample, acacia honey (2.50 × 10^{-4} μequiv). The antioxidant content reported is based on water-soluble components. Further research is being directed into determining the nature and quantity of these antioxidant compounds; preliminary analysis indicates that total phenolic content correlates well with antioxidant capacity (unpublished data).

Evaluation of Effectiveness of Soy Honey at Different Concentrations. The concentration of soy honey at 5% (w/w) was most effective at reducing lipid oxidation (TBARS values) at 1 day of storage at 4 °C (**Table 2**). Although the 5% levels did not provide protection significantly greater than 10% at 1 day of storage, the 5% addition had lower TBARS. At 3 days the protection levels provided by 5 and 10% honey were not significantly different and were better than that provided by 1%. We decided to use 5% because it provided early protection; we also wanted to avoid high amounts of sugar added to the meat. Analysis of our data shows that any of the three concentrations of soy honey tested would be beneficial at reducing lipid oxidation in such samples over a 3-day period. Honey appears to be effective over a fairly wide range of added concentrations.

Prevention of Lipid Oxidation by Honey. The effectiveness of the various honeys at reducing lipid oxidation in cooked, ground turkey is demonstrated after 1 and 3 days of storage at 4 °C (**Table 3**). Day 0 starting TBARS values were detectable. The age of the meat at the time of purchase was unknown, so samples were collected for each treatment on three different starting days. Considerable oxidation occurred over a 3-day period, leading us to monitor oxidation over 3 days. The capacity

Table 3. TBARS Values of Cooked, Ground Turkey after 0, 1, and 3 Days of Storage at 4 °C[a]

honey	day 0	day 1	day 3
control (no honey)	1.16 ± 0.22^{a}	2.45 ± 0.40^{a}	5.57 ± 0.90^{a}
acacia	1.06 ± 0.06^{a}	2.07 ± 0.44^{ab}	3.68 ± 0.46^{b}
clover	1.95 ± 1.17^{b}	2.06 ± 0.62^{ab}	3.02 ± 0.48^{b}
soy	1.08 ± 0.20^{a}	1.54 ± 0.33^{b}	2.74 ± 0.29^{bc}
buckwheat	1.05 ± 0.03^{a}	1.42 ± 0.20^{b}	1.62 ± 0.19^{c}

[a] Values represent means ± standard errors of at least three replicates. Within columns, means with different superscript letters are significantly different ($p < 0.05$).

Table 4. TBARS Values of Cooked, Ground Turkey after 1 and 3 Days of Storage at 4 °C[a]

	day 0	day 1	day 3
control (no antioxidant added)	1.18 ± 0.13^{a}	3.32 ± 0.68^{a}	7.86 ± 1.49^{a}
α-tocopherol	1.03 ± 0.05^{a}	2.77 ± 0.22^{a}	6.60 ± 1.73^{ab}
BHT	1.33 ± 0.38^{a}	2.26 ± 0.85^{a}	4.90 ± 0.43^{b}
soy honey	1.27 ± 0.25^{a}	1.80 ± 0.30^{b}	2.89 ± 0.26^{c}

[a] Values represent means ± standard errors of at least three replicates. Within columns, means with different superscript letters are significantly different ($p < 0.05$).

of the various honeys to reduce TBARS development in turkey samples increased with increasing water-soluble antioxidant capability of the honeys. Buckwheat honey had the highest antioxidant content and also was most effective in preventing lipid oxidation. Acacia, the honey with the lowest antioxidant content, was least effective in protecting against oxidation. Even though clover honey had a slightly higher (but not statistically different) water-soluble antioxidant content than soy honey (**Table 1**), soy honey was equally as effective as clover honey in the prevention of lipid oxidation in meat samples. Initially, we were concerned that, because honey is water-soluble, the antioxidant components may not disperse well among the lipids of the muscle and thus not be in a position where they would provide the most benefit in protection against oxidation. We chose ground turkey partially for this reason. Research on using the honeys as marinades for preparation of intact pieces of meat is planned.

Related research by Dawson and co-workers (*12*, *22*) has also demonstrated the protective effect of honey against lipid oxidation in a turkey roll product. These researchers evaluated the effect of Maillard browning reaction products on lipid oxidation in turkey rolls. Maillard reaction products are known for their potential antioxidant activity (*23*−*29*). The Maillard reaction is facilitated by the addition of honey to the turkey before heating, as the principal reactants are reducing sugars (from the honey) and free amino groups (from the proteins in meat) (*23*). These researchers also isolated/purified Maillard reaction products and added them to turkey rolls. They found the turkey was protected against lipid oxidation by the isolated Maillard products as demonstrated in other meat systems (*26*, *27*). Inhibition of oxidation was greater with honey than with the isolated Maillard reaction products (*12*). It was visually apparent that the Maillard reaction was more extensive in our samples to which honey was added. It is known that Maillard reaction products are capable of acting as antioxidants. We speculate that our samples are indeed protected against lipid oxidation by the enhanced degree of Maillard browning in the presence of honey. However, these Maillard products are not the only antioxidant substances, especially as the sugar compositions of honeys from different floral sources are very similar (*15*). Reducing sugars, fructose and glucose, account for 85−95% of honey carbohydrates, and these are the components that react with amino groups to result in Maillard browning. Differing capacities of honeys from different floral sources to protect against oxidation are thus most likely due to the presence of different antioxidant profiles (aside from Maillard products), depending on the floral source. Additionally, the presence of components other than Maillard products would explain the differences in the Mathew et al. (*22*) study between added honey and added Maillard reaction products. We know the quantities of total phenolics differ among honeys from different floral sources (unpublished data); we are in the process of characterizing them to determine if there are significant differences in phenolic profiles. Having different phenolic profiles may indicate differences in radical scavenging versus metal-chelating abilities.

Comparison of the Antioxidant Effectiveness of Soy Honey with That of Other Antioxidants. Soy honey (5%, w/w) was much more effective at reduction of TBARS over the 3-day storage period at 4 °C than was either α-tocopherol or BHT (**Table 4**). The antioxidant content of the soy honey was first calculated on the basis of the number of microequivalents of ascorbic acid (as determined by the spectrophotometric antioxidant assay, as described above). However, later, total phenolics levels were used to estimate the amount of antioxidant added from the 5% soy honey (at 5% of the weight of the meat) to be comparable to the allowable limit of antioxidant addition (0.02% of the weight of the lipid). We tested both α-tocopherol and BHT at 0.02% of the total fat. At an estimated comparable antioxidant level the soy honey was more protective than either α-tocopherol or BHT. The reason for the differences in antioxidant protection is not known. One reason might be that the phenolic antioxidants α-tocopherol and BHT function in radical scavenging. Honey is a complex system of more than one phenolic component. Honey thus may provide radical-scavenging activities but may also contribute metal chelation activities, leading it to perform differently from α-tocopherol and BHT. Maillard reaction products may also lead to the enhanced level of protection provided by honey versus tocopherol and BHT (due to the presence of reducing sugars in honey as explained above). Additionally, some of the antioxidant substances may act synergistically in honey, contributing to the additional antioxidant effectiveness in this system.

Summary/Conclusions. Honeys of different floral sources (and different antioxidant contents) were effective at reducing lipid oxidation in cooked, ground turkey patties held at 4 °C. Buckwheat honey was the most effective of the honeys tested and acacia the least. Honeys were effective in order of increasing antioxidant content. However, even though clover honey had a slightly higher water-soluble antioxidant content than soy honey, soy was equally effective as clover in the prevention of lipid oxidation in these samples. Honey appears to be a good source of natural antioxidants in addition to its properties of contributing various flavor notes to meats. It has been found to be more effective in our system than either α-tocopherol or BHT.

ACKNOWLEDGMENT

We thank Dr. May Berenbaum for contributions of honey and for reviewing the manuscript. We give special thanks to Jun Guo, who assisted with the TBARS assays, and to Jill Branen, who assisted with the statistical analysis of the data.

LITERATURE CITED

(1) Pearson, A. M.; Gray, J. I.; Wolzak, A. M.; Horenstein, N. A. Safety implications of oxidized lipids in muscle foods. *Food Technol.* **1983**, *37* (7), 121−129.

(2) Matsushita, S. Specific interactions of linoleic acid hydroperoxides and their secondary degraded products with enzyme proteins. *J. Agric. Food Chem.* **1975**, *23*, 150−154.

(3) Yagi, K. Lipid peroxides as agents involved in atherogenesis. In *Lipid Peroxidation in Biological Systems*; Sevanian, A., Ed; AOCS: Champaign, IL, 1988; Chapter 16.

(4) Addis, P. B. Occurrence of lipid oxidation products in foods. *Food Chem. Tox.* **1986**, *24*, 1021−1030.

(5) Ellis, R.; Kimoto, W. I.; Bitman, J.; Edmondson, L. F. Effect of induced high linoleic acid and tocopherol content on the oxidative stability of rendered veal fat. *J. Am. Oil Chem. Soc.* **1974**, *51*, 4−7.

(6) Lin, C. F.; Gray, J. I.; Asghar, A.; Buckley, D. J.; Booren, A. M.; Flegal, C. J. Effects of dietary oils and α-tocopherol supplementation on lipid composition and stability of broiler meat. *J. Food Sci.* **1989**, *54*, 1457−1460.

(7) Engeseth, N. J.; Gray, J. I.; Booren, A. M.; Asghar, A. Improved oxidative stability of veal lipids and cholesterol through dietary vitamin E supplementation. *Meat Sci.* **1993**, *35*, 1−15.

(8) Morrissey, P. A.; Buckley, D. J.; Sheehy, P. J. A.; Monahan, F. J. Vitamin E and meat quality. *Proc. Nutr. Soc.* **1994**, *53* (2), 289−295.

(9) DeWinne, A.; Dirinck, P. Studies on vitamin E and meat quality. 2. Effect of feeding high vitamin E levels on chicken meat quality. *J. Agric. Food Chem.* **1996**, *44*, 1691−1696.

(10) Garber, M. J.; Roeder, R. A.; Davidson, P. M.; Pumfrey, W. M.; Schelling, G. T. Dose−response effects of vitamin E supplementation on growth performance and meat characteristics in beef and dairy steers. *Can. J. Anim. Sci.* **1996**, *76* (1), 63−72.

(11) Chen, L.; Mehta, A.; Berenbaum, M.; Zangerl, A.; Engeseth, N. J. Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. *J. Agric. Food Chem.* **2000**, *48*, 4997−5000.

(12) Antony, S.; Rieck, J. R.; Dawson, P. L. Effect of dry honey on oxidation in turkey breast meat. *Poult. Sci.* **2000**, *78*, 1846−1850.

(13) Willix, D. J.; Molan, P. C.; Harfoot, C. G. A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. *J. Appl. Bacteriol.* **1992**, *73* (5), 338−394.

(14) Ioyrish, N. *Bees and People*; MIR Publishers: Moscow, Russia, 1974.

(15) Crane, E., Ed. *Honey, a Comprehensive Survey*; Crane Russak and Co.: New York, 1975.

(16) Ferreres, F.; García-Viguera, C.; Tomás-Lorente, F.; Tomás-Barberán, F. A. Hesperetin: A marker of the floral origin of citrus honey. *J. Sci. Food Agric.* **1993**, *61*, 121−123.

(17) Frankel, S.; Robinson, G. E.; Berenbaum, M. R. Antioxidant content and correlated characteristics of 14 monofloral honeys. *J. Apic. Res.* **1998**, *37*, 27−31.

(18) Yoirish, N. *Curative Properties of Honey and Bee Venom*; Glide Publications: San Francisco, CA, 1977.

(19) Louveaux, J.; Maurizio, A.; Vorwohl, G. Methods of melissopalynology. *Bee World* **1978**, *59*, 139−157.

(20) Glavind, J. Antioxidants in animal tissue. *Acta Chem. Scand.* **1963**, *17*, 1635−1640.

(21) Tarladgis, B. G.; Watts, B. M.; Younathan, M. T. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. Am. Oil Chem. Soc.* **1960**, *37*, 44−48.

(22) Mathew, S.; Dawson, P.; Han, I. Antioxidative Maillard reaction products formed using honey in a turkey meat model system. *Institute of Food Technologists Annual Meeting Book of Abstracts*; Chicago, IL, 1998; Abstract 46C-9, p 108.

(23) Bailey, M. E.; Um, K. W. Maillard reaction products and lipid oxidation. *ACS Symp. Ser.* **1992**, *No. 500*, 122−139.

(24) Nakamura, S.; Kato, A.; Kobayashi, K. Enhanced antioxidative effect of ovalbumin due to covalent binding of polysaccharides. *J. Agric. Food Chem.* **1992**, *40*, 2033−2037.

(25) Eiserich, J. P.; Shibamoto, T. Antioxidative activity of volatile heterocyclic compounds. *J. Agric. Food Chem.* **1994**, *42*, 1060−1063.

(26) Bedinghaus, A. J.; Ockerman, H. W. Antioxidative Maillard reaction products from reducing sugars and free amino acids in cooked ground pork patties. *J. Food Sci.* **1995**, *60*, 992−995.

(27) Smith, J. S.; Alfawaz, M. Antioxidative activity of Maillard reaction products in cooked ground beef, sensory and TBA values. *J. Food Sci.* **1995**, *60* (2), 234−236.

(28) Yen, G. C.; Hsieh, P. P. Antioxidative activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction products. *J. Sci. Food Agric.* **1995**, *67* (3), 415−420.

(29) Bersuder, P.; Hole, M.; Smith, G. Antioxidants from a heated histidine-glucose model system. I. Investigation of the antioxidant role of histidine and isolation of antioxidants by high-performance liquid chromatography. *J. Am. Oil Chem. Soc.* **1998**, *75* (2), 181−187.

Received for review June 19, 2001. Revised manuscript received October 19, 2001. Accepted October 31, 2001. This research was supported in part by a Johnathan Baldwin Turner Undergraduate Research Scholarship (to J.M.), and the antioxidant data collection was supported in part by the National Honey Board from a related funded project.

JF010820A