Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 97 (2006) 217–222
Food Chemistry
www.elsevier.com/locate/foodchem

Raw *Millefiori* honey is packed full of antioxidants

Manuela Blasa, Manila Candiracci, Augusto Accorsi, Maria Piera Piacentini, Maria Cristina Albertini, Elena Piatti *

Istituto di Chimica Biologica "Giorgio Fornaini", Universitá degli Studi di Urbino, Via Saffi 2, 61029 Urbino, Italy

Received 13 December 2004; accepted 7 March 2005

Abstract

Total polyphenols, flavonoids and antioxidant power of raw honey samples from two of the most common Italian varieties, i.e., *Millefiori* and *Acacia*, were evaluated. Phenolic content, expressed as caffeic acid equivalents, ranged from 12.5 to 17.5 mg/100 g and from 3 to 11 mg/100 g in *Millefiori* and *Acacia* honeys, respectively. All *Millefiori* samples exhibited the highest flavonoid concentration being between 1.23 and 2.93 mg catechin equivalents (CE)/100 g honey. Total flavonoids in 100 g *Acacia* honeys were in the range of 0.45–1.01 mg CE. *Acacia* honeys had lower total antioxidant power, as assessed by ferric reducing/antioxidant power assay, than *Millefiori*. The relationship between phenolic content and antioxidant power was discussed. Comparative experimental analysis was performed with an artificial honey and processed honeys. Raw *Millefiori* honey is rich in both amount and variety of antioxidant substances, and its inclusion in the diet may be recommended to complement other polyphenol sources.

Keywords: Raw honey; *Millefiori*; *Acacia*; Polyphenols; Flavonoids; FRAP; Antioxidant property

1. Introduction

Honey is the natural substance produced by honeybees, *Apis mellifera*, in almost every country of the world. Raw honeys are usually produced by small farms and left in their natural state without undergoing processing (e.g., filtration and heat treatment).

Honey is essentially a concentrated aqueous solution of inverted sugar, but it also contains a very complex mixture of other saccharides, enzymes, amino and organic acids, polyphenols, carotenoid-like substances, Maillard reaction products, vitamins, and minerals (Gheldof, Wang, & Engeseth, 2002). As collected from the honeycomb, raw honey contains extraneous matter that is removed to make it marketable on a large scale (Wang, Gheldof, & Engeseth, 2004). On account of its high nutritional value (303 kcal/100 g honey) and fast absorption of its carbohydrates, honey is a food suitable for humans of every age. Moreover, it is particularly recommended for children and sportsmen, and by itself or associated to appropriate therapies, honey can help to improve the organism efficiency of the elderly and invalids.

Previous studies indicate that honey may be used for the treatment of skin wounds and various gastrointestinal diseases (Postmes, van den Bogaard, & Hazen, 1993). This beneficial role was attributed to both antibacterial and anti-inflammatory properties of honey regarding high osmolarity, acidity and content of hydrogen peroxide and non-peroxide components (Weston, 2000). According to White, Subers, and Schepartz (1963) the antimicrobial agent is predominantly hydrogen peroxide the concentration of which is determined by relative levels of glucose oxidase, synthesized by the bee, and catalase, originating from flower pollen (Weston, 2000).

* Corresponding author. Tel.: +39 0722 305242; fax: +39 0722 320188.
E-mail address: e.piatti@uniurb.it (E. Piatti).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.03.039

Among minor honey constituents, polyphenols such as flavonoids and phenolic acids, may function as natural antioxidants in our diet. In recent years, considerable interest has been focussed on dietary antioxidants in reference to their protective effect against oxidative damage (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Jialal & Grundy, 1993). Current opinion suggests that oxidative and free radical-mediated reactions are implicated in degenerative processes related to aging (Ames, Shigenaga, & Hagen, 1993) and various diseases such as cancer, atherosclerosis and diabetes (Gutteridge & Halliwell, 1994). Reactive oxygen species (ROS) are constantly generated in vivo either through cell respiration or induced by exogenous sources such as pollution, ionizing radiation and drugs (Briviba & Sies, 1994). Living organisms protect themselves from oxidative damage by endogenous antioxidant defence systems (Ames et al., 1993) or by dietary antioxidants widely distributed in natural foods (Rice-Evans, Miller, & Paganga, 1997; Weisburger, 1999). There are many studies about the polyphenol content and antioxidative effects of fruits, vegetables and beverages (Rice-Evans et al., 1997; Weisburger, 1999); in contrast, hardly any work has been reported with honey. Honey, as a source of antioxidants, has been reported to be effective against enzymatic browning of fruits and vegetables (Chen, Mehta, Berenbaum, Zangeri, & Engeseth, 2000), oxidative deterioration of some foods (McKibben & Engeseth, 2002) and in controlling the growth of or eliminating foodborne pathogens (Taormina, Niemira, & Beuchat, 2001).

Available literature indicates that no previous antioxidant power studies have been done on Italian honeys; thus, the objective of this work was to evaluate antioxidant activity of two representative Italian raw honeys obtained from beekeepers belonging to the same geographical region (central Italy) as a function of their sensorial characteristics and content of total polyphenols and flavonoids. Comparative experimental analysis was performed with an artificial honey and processed honeys.

2. Materials and methods

2.1. Chemicals and honey samples

2,4,6-Tri(2-pyridyl)-*s*-triazine (TPTZ) and polyvinylpolypyrrolidone (PVPP) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals and reagents used were of analytical grade.

Eight samples from two of the most common Italian honeys were used in this study; they are named *Millefiori* (multifloral honey, MH) and *Acacia* (unifloral honey, *Robinia pseudoacacia*, AH). All of these samples, collected from four beekeepers in the same geographical area (Marche, central Italy), were obtained from the Associazione Marchigiana Apicoltori (AMA) with a guarantee of genuineness and known history. Samples were harvested in 2003 and they have not been subjected to thermic treatments or to pasteurization. Each beekeeper supplied honey samples from both sources. Processed multifloral and *Acacia* honeys (PMH and PAH, respectively) were purchased from the local market. All honey samples were stored in darkness at room temperature to minimize any alterations. An artificial honey, which serves as a control, was prepared according to Taormina et al. (2001). Sensorial characteristics of undiluted honeys were achieved by subjective assessment.

2.2. Determination of content of total phenolics and flavonoids

The concentration of total phenolics and total flavonoids in diluted raw and processed honeys (10%, w/v in 75 mM phosphate buffer, pH 7.0) was determined according to Singleton, Orthofer, and Lamuela-Raventos (1999) and to Kim, Jeong, and Lee (2003), respectively. To eliminate the interference of reducing sugars, present in large amounts in whole honey, a blank was prepared according to Gheldof et al. (2002) with some modification. Briefly, 0.1 g of insoluble PVPP was added to 5 ml of 75 mM phosphate buffer, pH 7.0, and moisturized at 4 °C for 24 h. The suspension was centrifuged at 3000 rpm for 10 min and the supernatant was discarded. Five milliliters of a honey solution (1 g honey in 10 ml of the same phosphate buffer) was added to the pellet, stirred for 30 min at 30 °C and then filtered. The filtered solution was used as blank. Total phenolics were expressed as mg caffeic acid equivalents (CAE)/100 g honey and total flavonoids as mg catechin equivalents (CE)/100 g. Samples were analyzed in four replications.

2.3. Determination of the total antioxidant power (FRAP assay)

The antioxidant capacity of raw, processed and artificial honeys (10%, w/v in 0.25 M phosphate buffer, pH 7.2) was estimated using the ferric reducing/antioxidant power (FRAP) assay according to the procedure described by Benzie and Strain (1999). Briefly, aliquots of 200 μl sample were mixed with 1.8 ml fresh FRAP reagent solution (2.5 ml of a 10 mM TPTZ solution in 40 mM HC1 plus 2.5 ml of 20 mM $FeCl_3$ and 25 ml of 0.3 M acetate buffer, pH 3.6) in spectrophotometer cuvettes. Absorbance was measured at 593 nm after incubation at 37 °C for 10 min and centrifugation at 10,000 rpm for 10 min. The final results were expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mM $FeSO_4$ used as the standard solution. All tested samples were replicated four times.

2.4. Statistical analysis

Results are presented as a mean values ± standard deviation. Statistical analysis between experimental results are based on Student's *t* test. Significant differences were statistically considered at the level of $P < 0.05$.

3. Results and discussion

Italy has the peculiarity of having extended areas without intensive agricultural development. The nectar of wild blossoms on hill zones as well as on mountain zones, gives rise to honey with more richness in its organoleptic characters and in its nourishing values, Italian honeys are selected from regions with favourable climatology, which becomes evident in their low moisture, high density and a stability which guarantees their very long shelf life, without risk of fermentation neither changes in its properties. The great variety of blossoms allows honeys to display a wide range of colors and the large quantity produced permits an offer of consecutive and uniform lots.

Raw honeys utilized in this study were not submitted to thermal treatments or to pasteurization or any other operations to alter natural composition. Undiluted samples were noticeably different from each other with regard to sensorial characteristics (Table 1). Raw multifloral honeys differed from raw unifloral samples with regard to color, granularity and viscosity, the former showing dark color, solid crystallization and thickness, and the latter light color, transparent appearance and thinness. Processed honeys were very thin and free from visible crystals, independently of the floral source, but PAH was very light-colored and PMH had medium color.

The total phenolic content of 100 g honey ranged from 12.5 to 17.5 mg CAE and from 3 to 11 mg CAE for *Millefiori* (MH*n*) and *Acacia* (AH*n*), respectively (Fig. 1). Our results are similar to those of honey from other floral sources concentrations of which were reported to be in the range of 4.6–45.6 mg of gallic acid equivalents (GAE) per 100 g of honey (Gheldof et al., 2002). The same study reported that total phenols of *Acacia* honey were 4.6 mg GAE/100 g. Polyphenol levels of MH was about 2.2-fold higher than that of AH. The averages of total phenolic contents of MH and AH were significantly different at a level of $P = 0.001$. In comparison to raw honey, the total content of phenolics of processed honey is approximately 3.2 and 4.3 times lower for processed *Millefiori* honey (PMH) and processed *Acacia* honey (PAH), respectively. MH4 and MH1 showed a phenolic level about 3.6-fold and 2.6-fold higher than PMH, respectively; AH4 and AH1 showed about 3.8 and 1.05 times higher phenolic content than PAH, respectively.

The total flavonoid content of the raw compared to processed honey varieties is reported in Fig. 2. All MH*n* samples exhibited the highest flavonoids content, being in the range of 1.23–2.93 mg CE/100 g honey. The concentration of total flavonoids in 100 g AH*n* was in the range of 0.45–1.01 mg CE. The averages of total flavonoid content of MH*n* and AH*n* honeys were different at a significance level of $P < 0.05$. There were no significant differences among various samples of both MH*n* and AH*n*.

In summary, all samples of honey tested contain polyphenols. Flavonoids represented 12.85 ± 3.34% and 11.75 ± 4.72% of MH*n* and AH*n* polyphenols, respectively. It is worth to pointing out that the unprocessed, darker, opaque, honeys contained the highest levels of total phenolics.

Table 1
Appearance of raw and processed honeys

Variety	Sensorial characteristics
Raw *Acacia* honey	
AH1	Light color, clear, thin
AH2	Medium color, clear, thin
AH3	Very light color, clear, very thin
AH4	Medium color, slightly granular, thin
Raw *Millefiori* honey	
MH1	Dark color, granular, thick
MH2	Medium color, granular, very thick
MH3	Very dark color, very granular, very thick
MH4	Dark color, granular, very thick
Processed *Acacia* honey (PAH)	Very light color, clear, very thin
Processed *Millefiori* honey (PMH)	Medium color, clear, very thin

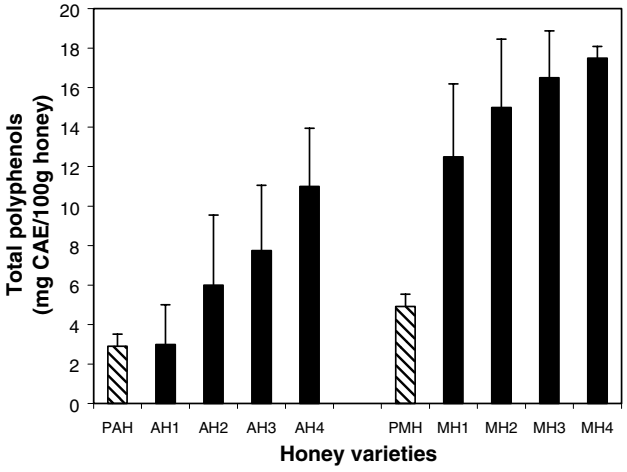

Fig. 1. Contents of total polyphenols in raw and processed honeys. The data are displayed with means ± standard deviation (bars) of four replications. Total phenolic contents of *Millefiori* (PMH and MH*n*) and *Acacia* (PAH and AH*n*) honeys were significantly different at $P < 0.05$.

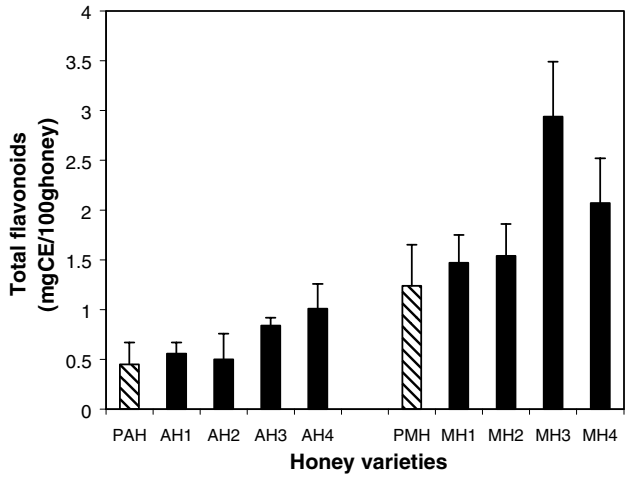

Fig. 2. Contents of total flavonoids in raw and processed honeys. The data are displayed with means ± standard deviation (bars) of four replications. Total flavonoid contents of *Milleflori* (PMH and MH*n*) and *Acacia* (PAH and AH*n*) honeys were significantly different at $P < 0.05$.

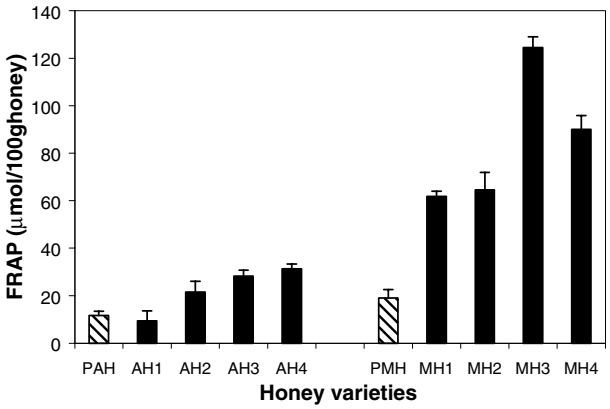

Fig. 3. Antioxidant power (FRAP) of raw and processed honeys. The data are displayed with means ± standard deviation (bars) of four replications. FRAP values of *Millefiori* (PMH and MH*n*) and *Acacia* (PAH and AH*n*) honeys were significantly different at $P < 0.05$.

It is currently assumed that the negative effect on humans of various environmental stresses is often mediated by the generation of ROS. Protection against ROS is provided by endogenous antioxidants (Ames et al., 1993) or by an array of different compounds contained in the diet (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). Several polyphenols have been reported to quench ROS and inhibit in vitro oxidation of low-density lipoproteins and therefore reduce thrombotic tendency (Heim, Tagliaferro, & Bobilya, 2002). Phenolics may inhibit cycloxygenase and lipoxygenase and thereby modulate arachidonic acid metabolism (Alanko et al., 1999). A variety of plant flavonoids have also been shown to be antimutagenic and anticarcinogenic both in vitro and in vivo (Hertog, Hollmann, & Katan, 1992).

Many of the honey polyphenols are known to have antioxidant activity (Aljadi & Kamaruddin, 2004; Al-Mamari, Al-Meeri, & Al-Habori, 2002). Previous works reported that the FRAP assay is a valid method to determine the antioxidant properties of food beverages (Benzie & Strain, 1999). Therefore, we used the same procedure to evaluate the antioxidant power of diluted honey samples and compare it to an artificial honey. As reported in Fig. 3, all AH*n* in this study had lower FRAP values than MH*n* at a significance level of $P < 0.05$. Our results are consistent with those of other authors which demonstrated a strong correlation between honey color and antioxidant power, with darker and more crystallized honeys having stronger antioxidant activity than lighter and transparent honeys (Chen et al., 2000; Frankel, Robinson, & Berenbaum, 1998; Taormina et al., 2001). By comparing the four raw AH with PAH, FRAP values showed significant differences except between AH1 and AH2 vs. PAH. The total antioxidant activity of raw MH, that we evaluated in the range of 61.75 to 124.5 μmol/100 g, was significantly different from PMH ($P < 0.001$). FRAP activity of the artificial honey at the dilution used in the test was undetectable, therefore indicating that antioxidant compounds are responsible for FRAP values of honey samples (Fig. 3). Positive correlations between flavonoid content and the FRAP values ($r^2 = 0.983$, in Fig. 4(a)) and between polyphenols and FRAP values ($r^2 = 0.938$, in Fig. 4(b)) were observed. As shown in Fig. 4(a), while there is a linear relationship between total flavonoid content and total antioxidant power, FRAP activity increases abruptly when the polyphenol concentration is above approximately 11 mg/100 g honey. Thus, apparently an exponential relationship between FRAP value and polyphenol content was observed (Fig. 4(b)). Indeed, the higher FRAP value of MH compared to that of AH correlates with the honey color that would originate, almost in part, from flower pigments such as carotenoids, many of which are antioxidants (Taormina et al., 2001). The observation that darker colored honeys was more affected by processing than light ones in terms of reduction in antioxidant power, confirms the findings of Wang et al. (2004).

The health benefits of honey depends on its quality, and the quality varies greatly on the bases of the types of flowers used by the bees (Frankel et al., 1998). Raw honey contains copious amounts of compounds, such as flavonoids and other polyphenols, which may function as effective natural antioxidants. Pollen, nectar and propolis are the main sources for the honey antioxidants (Weston, 2000). The processing of honey often removes many of the phytonutrients found in raw honey as it exists in the hive (Wang et al., 2004). This study shows that raw *Millefiori* honey can add many health-giving antioxidants to the diet. Although there is not enough evidence to assert that honey is a food of primary importance for human diet, anyhow its consumption

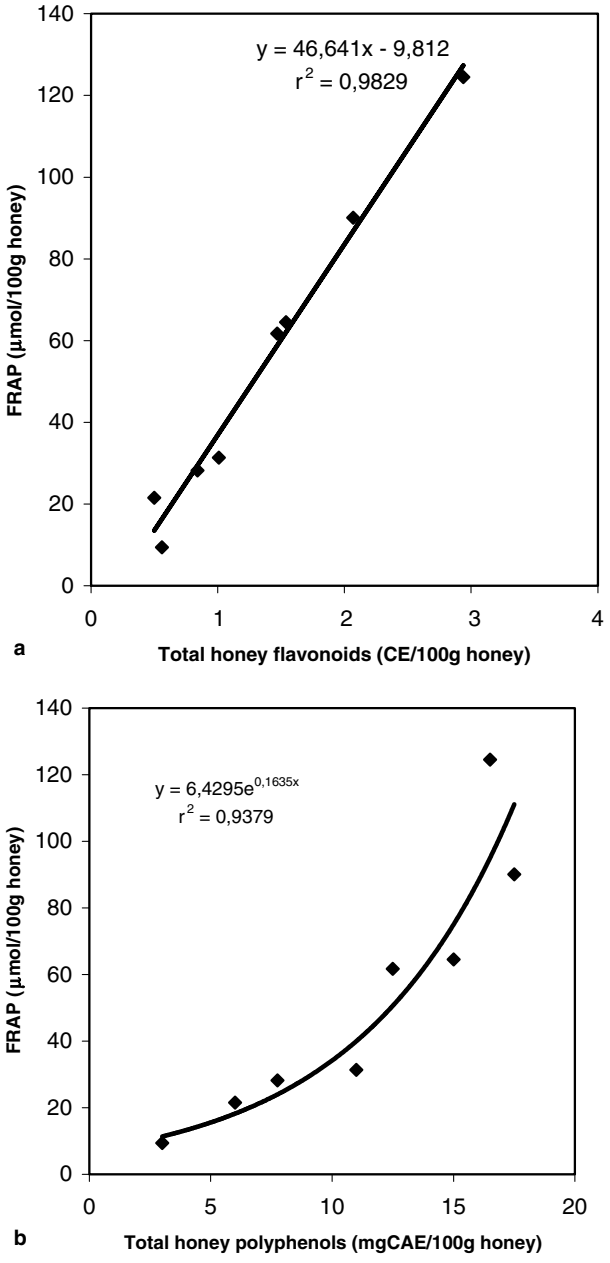

Fig. 4. Correlation between total flavonoids (a) or total polyphenols (b) and antioxidant power. There is a positive linear correlation ($r^2 = 0.983$) between flavonoids and FRAP and a positive exponential correlation ($r^2 = 0.938$) between phenolics and FRAP.

can be recommended to complete other polyphenol sources, such as vegetables and fruits, acting as "chemopreventive" in the onset of degenerative diseases (Greenvald, Kelloff, Burch-Whitman, & Kramer, 1995).

References

Alanko, J., Riutta, A., Holm, P., Mucha, I., Vapaatalo, H., & Metsä-Ketalä, T. (1999). Modulation of arachidonic acid metabolism bv phenols: relation to their structure and antioxidant/prooxidant properties. *Free Radical Biology and Medicine, 26*(1–2), 193–201.

Aljadi, A. M., & Kamaruddin, M. Y. (2004). Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chemistry, 85*, 513–518.

Al-Mamari, M., Al-Meeri, A., & Al-Habori, M. (2002). Antioxidant activities and total phenolics of different types of honey. *Nutrition Research, 22*, 1041–1047.

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidant, antioxidant, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences of the United States of America, 90*, 7915–7922.

Benzie, I. F. F., & Strain, J. J. (1999). Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology, 299*, 15–27.

Briviba, K., & Sies, H. (1994). Non enzymatic antioxidant defence systems. In B. Frei (Ed.), *Natural antioxidant in human health and disease* (pp. 107–128). New York: Academic Press.

Chen, L., Mehta, A., Berenbaum, M., Zangeri, A. R., & Engeseth, N. J. (2000). Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. *Journal of Agricultural and Food Chemistry, 48*, 4997–5000.

Frankel, S., Robinson, G. E., & Berenbaum, M. R. (1998). Antioxidant capacity and correlated characteristics of 14 unifloral honeys. *Journal of Apicultural Research, 37*, 27–31.

Gheldof, N., Wang, X.-H., & Engeseth, N. J. (2002). Identification and quantification of antioxidant components of honeys from various floral sources. *Journal ofAgricultural and Food Chemistry, 50*, 5870–5877.

Greenvald, P., Kelloff, C., Burch-Whitman, C., & Kramer, B. S. (1995). Chemoprevention. *CA: A Cancer Journal for Clinicians, 45*, 31–49.

Gutteridge, J. M. C., & Halliwell, B. (1994). Free radicals and antioxidants in aging and disease: fact or fantasy. In *Antioxidants in nutrition, heath, and disease* (pp. 111–135). Oxford, UK: Oxford University Press.

Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships. *Journal of Nutritional Biochemistry, 13*, 572–584.

Hertog, M. G. L., Feskens, E. J., Hollman, P. C., Katan, M. B., & Kromhout, D. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet, 342*, 1007–1011.

Hertog, M. G. L., Hollmann, P. C. H., & Katan, M. B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in The Netherlands. *Journal of Agricultural and Food Chemistry, 40*, 2379–2383.

Jialal, I., & Grundy, S. M. (1993). Effect of supplementation with alpha tocopherol, ascorbate and beta-carotene on low-density lipoprotein oxidation. *Circulation, 88*, 2780–2786.

Kim, D.-O., Jeong, S. W., & Lee, C. Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry, 81*, 321–326.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition, 79*, 727–747.

McKibben, J., & Engeseth, N. J. (2002). Honey as a protective agent against lipid oxidation in ground turkey. *Journal of Agricultural and Food Chemistry, 50*, 592–595.

Postmes, T., van den Bogaard, A. E., & Hazen, M. (1993). Honey for wounds ulcers and skin graft preservation. *Lancet, 341*, 756–757.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*(4), 152–159.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and

antioxidants by means of Folin–Ciocalteau reagent. *Methods in Enzymology, 299*, 152–178.

Taormina, P. J., Niemira, B. A., & Beuchat, L. R. (2001). Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. *International Journal of Food Microbiology, 69*, 217–225.

Wang, X.-H., Gheldof, N., & Engeseth, N. J. (2004). Effect of processing and storage on antioxidant capacity of honey. *Journal of Food Science, 69*(2), 96–101.

Weisburger, J. H. (1999). Mechanisms of action of antioxidants as exemplified in vegetables tomatoes and tea. *Food and Chemical Toxicology, 37*(9/10), 943–948.

Weston, R. J. (2000). The contribution of catalase and other natural products to the antibacterial activity of honey: a review. *Food Chemistry, 71*, 235–239.

White, J. W., Subers, M. H., & Schepartz, A. I. (1963). The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose–oxidase system. *Biochimica et Biophysica Ada, 73*, 57–70.