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# Flavonoids from Italian Multifloral Honeys Reduce the Extracellular Ferricyanide in Human Red Blood Cells

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In this study we investigated some biological properties of flavonoids recovered in the aqueous (AqE) and ether (EtE) extracts from four Italian multifloral honeys. In particular, a cell-free assay was employed to detect direct reduction of ferricyanide, whereas an assay using intact human erythrocytes was used to measure the ability to donate electrons to a trans-plasma membrane oxidoreductase. It was found that the AqE displays greater "in vitro" ferricyanide-reducing activity than the EtE but, unlike the latter, is virtually ineffective in the cell-based assay. Uptake studies employing high-performance liquid chromatography/mass spectrometry (HPLC/MS) showed that the different results were explained by the inability of AqE components to cross the erythrocyte plasma membrane and by the excellent uptake of EtE flavonoids, which, once within the cell, donate electrons to the membrane oxidoreductase to efficiently reduce extracellular oxidants. The latter property appears to depend on the content of ether-soluble flavonoids in the starting honeys.

**KEYWORDS: Flavonoids; honey; antioxidants; trans-plasma membrane oxidoreductase; ferricyanide.**

## INTRODUCTION

Flavonoids are polyphenol compounds, widely distributed in plant foods, exerting a variety of beneficial effects including vasodilation as well as antiinflammatory, antiviral, antioxidant, and anticarcinogenic activities (*1*−*3*). Although many of the biological actions of these polyphenolic compounds have been attributed to their antioxidant activity (*4*−*6*), growing information suggests that flavonoids, and/or their metabolites, may exert their effects by displaying modulatory actions in the cells (*7*−*10*).

Flavonoids are the major functional components of honey, used since ancient times not only as a food or flavorful sweetener but also for traditional folk medical preparations. During the past decade, the use of honey for therapeutic purposes has been re-evaluated in a more scientific setting (*11*) and several properties were identified. They include antibacterial (*12*) and antiinflammatory effects (*13*) as well as stimulation of wound and burn healing (*14*). In addition, honey provides some beneficial effects in patients affected by gastric ulcers and gastritis (*15*).

Honey also displays a significant antioxidant activity, preventing oxidative reactions in foods, such as lipid peroxidation (*16*, *17*) and enzymatic browning of fruits and vegetables (*18*, *19*). Recent studies demonstrate a strong correlation between the content of phenolic compounds in honeys from various floral sources and their antioxidant capacity (*11*, *20*).

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We recently reported a novel mechanism whereby selected flavonoids, in particular myricetin, fisetin, quercetin (and its methylated derivatives), kaempferol, luteolin, taxifolin, and morin, may promote their beneficial effects (*21*−*23*). These flavonoids, while efficiently taken up by human red blood cells (RBCs), may act as substrates for a trans-plasma membrane oxidoreductase (PMOR) and thus promote the reduction of extracellular oxidants. PMOR activity is indeed a potentially important strategy that, in addition and/or in alternative to the more conventional mechanisms [e.g., those mediated by glutathione (GSH), scavenging enzymes, antioxidants, etc.], might participate in cell defense against extracellular oxidative stress (*24*). This mechanism should be particularly important for those cells, such as RBCs, that are constantly exposed to circulating oxidative stressors. A major role for PMOR activity in maintaining a reduced plasma environment was indeed recently proposed (*25*).

The present study was performed with the aim of gathering more information on the biological effects of phenolic compounds present in three raw honeys from Marche, a region of central Italy, and a blended and processed honey obtained after pasteurization of a mixture of Italian and imported honeys. The aqueous and ether fractions obtained after methanol extraction of the honey samples were analyzed for their flavonoid content and their ability to directly reduce the oxidant ferricyanide (FIC). Finally, we measured the accumulation of selected flavonoids in human RBCs and the ensuing stimulation of the PMOR activity resulting in the reduction of extracellular oxidants.

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## MATERIALS AND METHODS

**Materials.** Flavonoid standards, 1,10-phenanthroline, and ethyl acetate were purchased from Sigma−Aldrich Chemie (Steinheim, Germany). Isorhamnetin and tamarixetin were purchased from Extrasynthese (Z.I. Lyon Nord, France). $K_3Fe(CN)_6$, $FeCl_3$, citric acid, $NaH_2PO_4$, and acetonitrile (HPLC-grade) were Carlo Erba products (Milan, Italy). The multifloral honeys (MH1, MH2, and MH3) used in this study are raw honeys, all coming from Marche (a central Italy region) and were supplied directly by the beekeepers. These honeys, all granular, thick, and with a dark color (MH2>MH1>MH3), were harvested in 2005 and have not been subjected to heat treatments or to pasteurization. MH4, a multifloral honey (clear, thin, and medium-colored) purchased in a local market, was a mixture of Italian and imported honeys subjected to pasteurization processes. The honey samples were stored at room temperature.

**Preparation of Phenolic Extracts of the Honey.** Flavonoids were extracted from the whole honey by use of a nonionic polystyrene resin (Amberlite XAD-2), as reported by Gheldof et al. (*26*), with minor modifications. Briefly, a honey acidified solution (100 g in 500 mL of water, pH 2.1) was poured in a column previously packed with XAD-2 resin (25 × 1.8 cm). A 300 mL volume of acidified water was used to wash out the sugars and polar compounds. Prior to collection of the phenolic compounds, the column was washed with 300 mL of neutral water. Then phenolic compounds were eluted with 300 mL of methanol, concentrated by rotary evaporation (40 °C), and dissolved in 5 mL of water. This solution was extracted three times with 5 mL of diethyl ether to further separate flavonoids on the basis of their hydrophobicity. The more hydrophobic ether fraction (EtE) is treated with anhydrous $Na_2SO_4$ for 30 min and then filtered, concentrated, and dissolved in 150 $\mu$L of dimethyl sulfoxide (DMSO). The residual aqueous phase after ether extraction is named AqE. Both fractions were subdivided into aliquots and stored at −80 °C until further analysis. Just before use, an aliquot of honey EtE or AqE was taken and diluted with PBS to reach the concentrations reported in the figures.

Total flavonoid content was measured by a colorimetric assay according to Zhishen et al. (*27*) and is expressed as milligrams of flavonoid /100 g of honey, on the basis of a calibration curve performed with catechin.

**Measurement of FIC Reduction by AqEs and EtEs.** Increasing amounts of honey AqEs or EtEs (final concentrations ranging from 0.1 to 2.0 g of honey/mL of solution) were added to a 1 mM FIC solution in PBS (PBS = 150 mM NaCl and 5 mM $Na_2HPO_4$ in deionized water, adjusted to pH 7.4). After a 30 min incubation at 37 °C, ferrocyanide (FOC) formation was determined as detailed by Avron and Shavit (*28*), with 1,10-phenanthroline as an indicator and the absorption measured at 510 nm ($\epsilon$ = 10 500 $M^{-1}$ $cm^{-1}$) against a blank. Appropriate blanks were performed for both AqEs and EtEs (values were very similar).

**Human RBCs.** Human venous blood from healthy volunteers was obtained by venipuncture and anticoagulated with heparin. The RBCs were used immediately after sampling. The blood was centrifuged at 1861.5*g* for 10 min at 4 °C. After removal of plasma, buffy coat, and the upper 15% of the RBCs, the remaining RBCs were washed twice with cold PBS and then resuspended as described below.

**Incubation of Human RBCs with AqEs or EtEs from Different Types of Honey.** The EtEs (stored in DMSO at −80 °C) were diluted in PBS, thereby lowering the DMSO concentration in the RBC suspensions to less than 0.5% (v/v). Control samples were performed in the presence of DMSO under the same experimental conditions. Packed RBCs (10% v/v) were incubated (15 min) in PBS (37 °C) containing increasing concentrations of honey extracts. After treatments, the cell suspensions were immediately centrifuged at 1861.5*g* and the RBCs were washed twice with at least 50 volumes of PBS and then processed as indicated below.

**Measurement of FIC Reduction by Human RBCs.** FIC reduction was estimated as reported by Avron and Shavit (*28*). After exposure to the honey EtEs or AqEs, RBCs were washed twice with PBS and resuspended (10% v/v) in PBS + 2.5 mM adenosine containing 1 mM FIC, dissolved immediately prior to use. The suspensions were incubated for 30 min at 37 °C and then centrifuged at 1861.5*g* at 4 °C. The resulting supernatants were assayed for their FOC content with 1,10-phenanthroline as an indicator and absorption measured at 510 nm ($\epsilon$ = 10 500 $M^{-1}$ $cm^{-1}$) against a blank. Appropriate blanks were performed for both AqEs and EtEs (values were very similar). EtE- and AqE-dependent FIC-reducing activity was determined upon subtraction of basal FIC-reducing activity detected in the untreated samples.

**Table 1.** Total Flavonoid Content of EtE and AqE[a]

| honey | EtE | AqE |
|---|---|---|
| MH1 | 2.9 ± 0.29 | 1.24 ± 0.08 |
| MH2 | 2.76 ± 0.33 | 1.52 ± 0.18 |
| MH3 | 2.45 ± 0.37 | 1.08 ± 0.14 |
| MH4 | 0.58 ± 0.1 | 0.50 ± 0.08 |

[a] Total flavonoid contents are expressed as milligrams in 100 g of honey. All data are the means ± SD of at least five independent determinations.

When used, 4-(hydroxymercuri)benzoic acid (PHMB) was added to the RBC suspension 15 min before the addition of FIC. After a further 30 min of incubation at 37 °C, the cell suspensions were centrifuged and the appearance of FOC was measured as reported above.

**HPLC/MS Analysis of EtE Flavonoids Taken Up by Human RBCs.** After incubation of the human RBC suspensions with honey EtE as described above, the samples were centrifuged at 1861.5*g* for 5 min and the supernatants were collected. The packed RBCs were then extensively washed with PBS and lysed with cold bidistilled water. Both samples (extracellular milieu and RBC lysate) were extracted three times with ethyl acetate. All the samples were taken to dryness by rotary evaporation, redissolved in DMSO, and diluted with methanol just before HPLC analysis. The HPLC system (Waters Alliance 2795) was coupled with a photodiode array detector (Waters 2996 PDA), followed by an electrospray mass spectrometer detector (ESI-MS) (Waters-Micromass ZQ) worked by Mass Lynx 4.0 SP4 software. The ESI-MS analyses were performed in a positive mode under the following conditions: source and desolvation temperature 100 and 250 °C; capillary and cone voltage 3.0 kV and 60 V; cone and desolvation flow (nitrogen gas) 40 and 400 L/h.

HPLC analysis was performed on a 25 cm × 4.6 mm Discovery C-18, 5 m column (Supelco, Bellefonte, PA) equipped with a Supelguard Discovery C-18 guard column (2 cm × 4 mm, 5 $\mu$m). A modified version of the analytical HPLC method from Day et al. (*29*) was used. Solvents A (0.1% formic acid) and B (acetonitrile) were run at a flow rate of 1 mL/min. The running gradient was adjusted to 17% B (2 min), increasing to 25% B (5 min), 35% B (8 min), 50% B (5 min), and then 100% B (10 min), followed by a reequilibration at 17% B (15 min). All solvents were HPLC-grade (Aldrich−Sigma) and water was purified via a Millex Q-plus system (Millipore).

Calibration curves of the main flavonoids analyzed were linear within the range investigated (0.5−100 $\mu$M) and the method was reproducible and precise (within-day and between-day coefficient of variation below 10%).

## RESULTS

**Evaluation of in Vitro Reducing Activity of EtEs or AqEs from Multifloral Honeys.** The total flavonoid content of the EtE and AqE obtained from three raw multifloral honeys (MH1−3) was both similar and remarkably greater than that observed in the processed MH4 honey (**Table 1**). In addition, the EtE contained approximately twice the amount of flavonoids detected in the AqE from MH1−3, whereas an analogous distribution was found in extracts from MH4.

The results illustrated in **Figure 1** indicate that, contrary to what might be expected from their relative flavonoid contents, the AqE (**a**) was in general (i.e., with the exception of MH3) remarkably more effective than the EtE (**b**) in promoting chemical reduction of FIC. Extracts from MH3 were equally active despite a 2.5-fold difference in their flavonoid content.

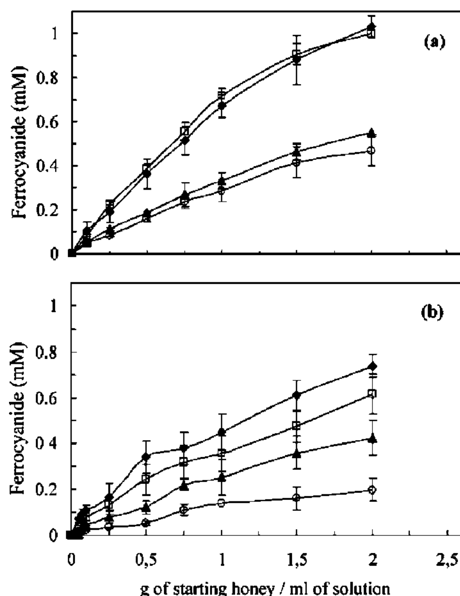



**Figure 1.** Direct FIC reduction by honey AqEs and EtEs. FIC (1 mM) in PBS (pH 7.4) was incubated for 30 min at 37 °C with the honey AqEs (**a**) or EtEs (**b**) (◆, MH1; □, MH2; ▲, MH3; ○, MH4) obtained from the amounts of honey reported in the abscissa. Ferrocyanide formation was assayed as reported under Materials and Methods. All data are the means ± SD calculated from at least three independent determinations.

MH1 and MH2 samples were the most effective, and MH4 samples the least effective, as reductants in the FIC assay.

**Human Erythrocyte FIC-Reducing Activity Promoted by EtEs or AqEs from Multifloral Honeys.** FIC is a mild oxidant that, while unable to penetrate the plasma membrane, is readily reduced by the PMOR activity. Although the physiological intracellular electron donors for this enzyme are ascorbic acid and NADH (*30*−*33*), our recent findings indicate that specific flavonoids effectively promote PMOR activity in human RBCs (*21*−*23*). We therefore investigated the effect of extracts from multifloral honeys on the ability of human RBCs to reduce extracellular oxidants. For this purpose, RBCs were first exposed for 15 min to increasing amounts of EtEs or AqEs and then extensively washed and incubated for 30 min in fresh PBS supplemented with 1 mM FIC. The results illustrated in **Figure 2** indicate that none of the AqEs was able to promote reduction of the extracellular oxidant. The FIC-reducing activity was, however, significantly enhanced by the EtEs, with the following order of potency: MH1 > MH2 = MH3 > MH4. These findings are in good correlation with the in vitro FIC-reducing activity (**Figure 1**) and with the total flavonoid content (**Table 1**). It should be noted that reduction of FIC was largely mediated by erythrocyte PMOR and not by flavonoids and/or other FIC-reducing molecules released in the extracellular milieu. Indeed, PHMB, a potent PMOR inhibitor (*34*−*38*), caused a dose-dependent inhibition of the FIC reduction (**Figure 3**), and HPLC analysis of the supernatants from cells exposed to the EtEs and then postincubated for 30 min in drug-free PBS revealed the absence of detectable flavonoids (not shown).

In contrast with the above results, exposure of human RBCs to the AqEs failed to promote the reduction of extracellular FIC (**Figure 2**), most likely as a consequence of the poor cellular uptake of the AqE components. This notion is supported by the observation that the extracellular flavonoid levels in the medium of RBCs exposed to AqE (corresponding to 2.0 and 4.0 g of honey/mL of RBC suspension) from MH1−4 remain constant up to 60 min (not shown).

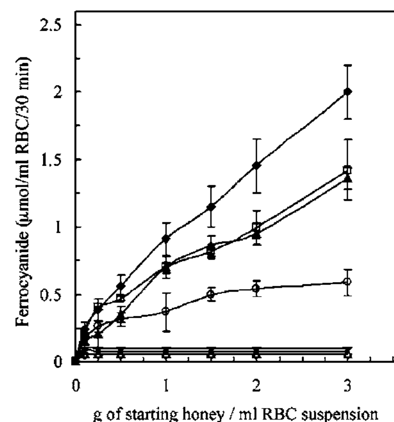



**Figure 2.** Effect of AqEs and EtEs on the RBC-dependent FIC reduction. Human RBCs were incubated for 15 min with increasing amounts of EtEs and AqEs. Values in the abscissa indicate the starting amounts of honey from which the extracts were obtained (for EtE, ◆, MH1; □, MH2; ▲, MH3; and ○, MH4; for AqE, –, MH1; ◇, MH2; △, MH3; and *, MH4). Data are the means ± SD calculated from at least three independent determinations.

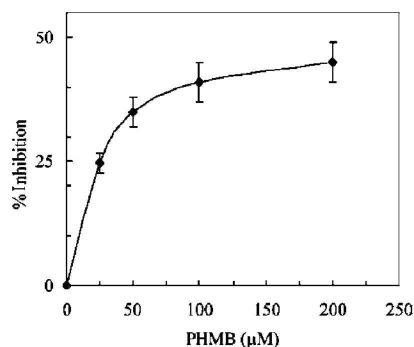



**Figure 3.** Effect of PHMB on the rate of FIC reduction by human RBC preloaded with EtE. Human RBCs were incubated for 15 min with MH1 EtE (corresponding to 1.5 g of starting honey). The effect of PHMB is expressed as percent inhibition of the control sample without PHMB. Data are the means ± SD calculated from at least three independent determinations.

**Intra/extracellular Distribution of Polyphenolic Compounds from EtEs.** The chromatographic profile obtained by HPLC/MS analysis of MH1 EtE is shown in **Figure 4a**. The main flavonoids identified were luteolin (A), quercetin (B), apigenin (D), fisetin (E), kaempferol (F), isorhamnetin (G), acacetin (J), tamarixetin (K), chrysin (N), and galangin (O), and their structures are illustrated in **Figure 5**.

The nature of these compounds was confirmed by UV and MS response as well as by chromatographic comparison with the authentic standards. Peak C corresponds to $C_{16}H_{12}O_7$. While more information is needed for its identification, we suspect that peak C corresponds to a methoxy derivative of kaempferol, such as 8-methoxykaempferol. This would be consistent with the analytical results collected, that is, retention time, molecular weight and formula, and lipophilic properties. In addition, the presence of 8-methoxykaempferol in other honey samples is documented in numerous studies (*39*, *40*). Peaks H, I, L, and M were not identified and likely correspond to compounds with molecular characteristics different from flavonoids.

Chromatograms obtained from the EtEs from MH2−4 (not shown and **Figure 4b**) showed basically the same peaks

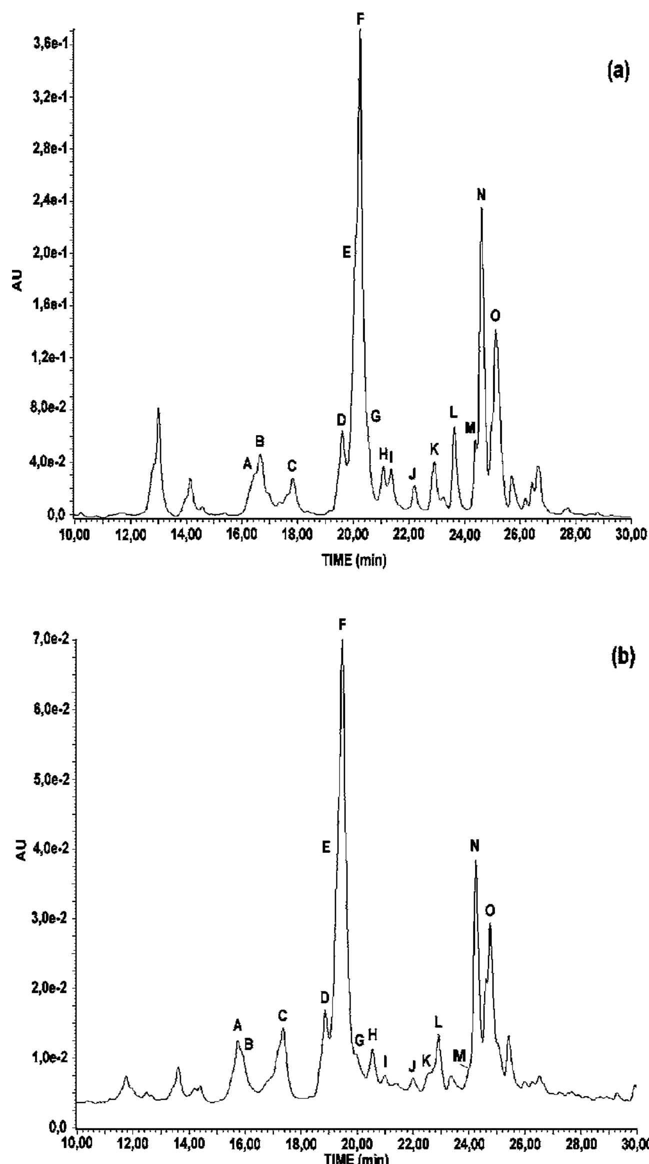



**Figure 4.** HPLC chromatograms of MH1 (**a**) and MH4 (**b**) EtEs. The EtEs obtained as described under Materials and Methods were dissolved in DMSO, diluted in methanol, and analyzed by HPLC/MS. Peaks: luteolin (A), quercetin (B), 8-methoxykaempferol (?) (C), apigenin (D), fisetin (E), kaempferol (F), isorhamnetin (G), acacetin (J), tamarixetin (K), chrysin (N), and galangin (O). Peaks H, I, L, and M are unidentified compounds.

observed in the MH1 EtEs, and the relative amount of each of the identified flavonoids was dependent on the flavonoid content of the starting honey (**Table 1**). **Figure 4b** shows the chromatogram from the MH4 EtE, containing the lowest flavonoid content (**Table 1**), and provides a good example for this inference.

RBC uptake of EtE flavonoids was evaluated by using an approach previously described (*23*) that involves ethyl acetate extractions of cell lysate and medium, solvent evaporation, and sample dissolution in DMSO/methanol just prior to HPLC analysis. It should be noted that this procedure, as shown by HPLC/MS analyses of the honey EtEs before and after ethyl acetate extraction and solvent evaporation, does not affect the concentration of the different flavonoids in the sample.

**Figure 6** shows the chromatographic profiles obtained by processing RBCs and medium after a 15 min incubation with MH1 EtE. It can be seen that most of the luteolin (A), quercetin (B), isorhamnetin (G), and acacetin (J) was taken up by the

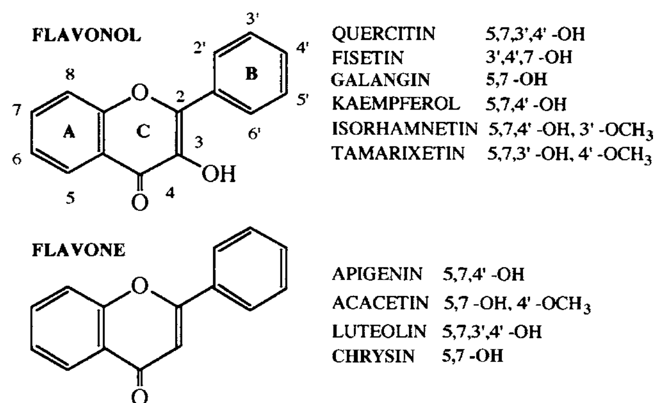



**Figure 5.** Structures of the main MH1–4 EtE flavonoids.

RBCs. While a lower uptake was observed for the unidentified flavonoid corresponding to peak C, apigenin (D), fisetin (E), kaempferol (F), tamarixetin (K), chrysin (N), and galangin (O), the fraction of each of these flavonoids taken up by the cells was nevertheless greater than that remaining in the extracellular milieu. The outcome of experiments by using EtEs from MH2−4 was similar when data were normalized to the flavonoid content in the EtEs (not shown).

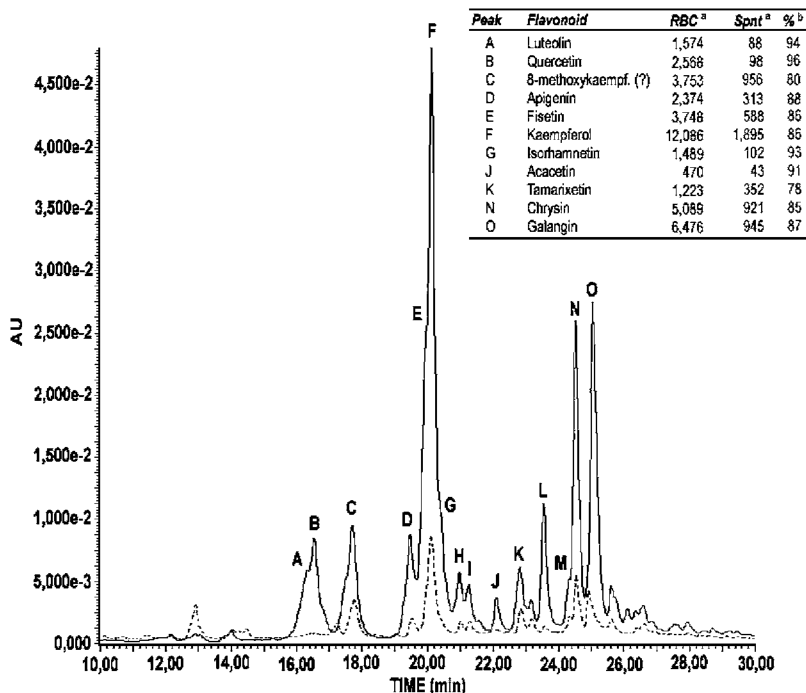
## DISCUSSION

The EtEs and AqEs from the three raw honeys (MH1−3) displayed a significantly greater flavonoid content than that from the industrial honey (MH4) (**Table 1**) obtained after pasteurization of a mixture of honeys produced in Italy and in other countries. In addition, the EtEs contained approximately twice the amount of the flavonoids detected in the AqEs from MH1−3, whereas no difference was found in MH4.

In apparent contrast with the above observations, we found that the AqEs were in general more potent than the EtEs in directly reducing the oxidant FIC (**Figure 1**). The greater reducing power despite the lower flavonoid content is, however, in keeping with previous findings by Gheldof et al. (*26*) and can be readily explained by the high content of water-soluble phenolic polymers and sugars. Indeed, Ferreres et al. (*41*) used an identical extraction procedure and demonstrated the presence of dark phenolic polymers and contaminant sugars in the aqueous phase. HPLC/MS analysis of the AqEs, performed in our laboratory, did not reveal the presence of flavonoid aglycons or phenolic acids as chlorogenic, caffeic, coumaric, pherulic, and elagic acids (not shown). This is consistent with the lipophilic nature of most aglycon flavonoids that are indeed recovered in the EtEs (**Figure 4**). We therefore attribute the high reducing power of the AqEs to the presence of contaminant sugars, phenolic polymers, and also flavonoids bound to carbohydrates that, while detected by the flavonoid quantification method of Zhishen et al. (*27*), are not revealed by the chromatographic analysis.

HPLC/MS analyses showed that the EtEs are particularly rich in a variety of flavonoids including quercetin, luteolin, kaempferol, fisetin, isorhamnetin, acacetin, chrysin, apigenin, galangin, and tamarixetin (**Figure 4**). These flavonoids were in general well taken up by RBCs exposed to the EtEs (**Figure 6**), and most of them quantitatively accumulates within the cells because of their ability to bind to hemoglobin (*22*, *23*). These findings are in agreement with our previous results from experiments with commercial flavonoids (*23*).

The intracellular accumulation of the flavonoids was associated with enhanced reduction of extracellular FIC (**Figure 2**),




| *Peak* | *Flavonoid* | *RBC*[a] | *Spnt*[a] | *%*[b] |
|---|---|---|---|---|
| A | Luteolin | 1,574 | 88 | 94 |
| B | Quercetin | 2,568 | 98 | 96 |
| C | 8-methoxykaempf. (?) | 3,753 | 956 | 80 |
| D | Apigenin | 2,374 | 313 | 88 |
| E | Fisetin | 3,748 | 588 | 86 |
| F | Kaempferol | 12,086 | 1,895 | 86 |
| G | Isorhamnetin | 1,489 | 102 | 93 |
| J | Acacetin | 470 | 43 | 91 |
| K | Tamarixetin | 1,223 | 352 | 78 |
| N | Chrysin | 5,089 | 921 | 85 |
| O | Galangin | 6,476 | 945 | 87 |

**Figure 6.** Flavonoid uptake by human RBCs exposed to MH1 EtE. Human RBCs were incubated for 15 with MH1 EtE (corresponding to 0.5 g of starting honey/mL of erythrocyte suspension). After centrifugation, the cells were washed twice with at least 50 volumes of PBS and lysed with cold bidistilled water. Flavonoids were extracted from either the RBC lysate (RBC,—) or supernatant (Spnt,•••) with ethyl acetate, taken to dryness by rotary evaporation, redissolved in DMSO, and diluted with methanol just before HPLC analysis, as described under Materials and Methods. (Inset) Fraction of each flavonoid taken up by the cells with respect to its total amount. [a]Peak area. [b]Percentage of flavonoid recovered in RBC lysate with respect to total flavonoid content (extra- + intracellular contents).

most likely mediated by PMOR activity. Previous studies from other laboratories (*36*, *37*) noted that the sulfhydryl reagent PHMB fails to completely inhibit PMOR activity stimulated by ascorbic acid. This might be due, as suggested by VanDuijn et al. (*36*), to some PHMB-insensitive oxidoreductase mediating in concert with the PHMB-sensitive enzyme extracellular FIC reduction. Alternatively, it might be speculated that a proportion of the oxidoreductase, while potentially sensitive to PHMB, is in fact resistant because it is inaccessible to the sulfhydryl reagent. Incomplete inhibition of PMOR activity by PHMB after stimulation with selected flavonoids was also noted in our previous studies (*21*), and this event was not associated with the release of the flavonoid. We can therefore rule out the possibility of direct FIC reduction mediated by extracellular flavonoids. However, we cannot exclude the possibility of direct FIC reduction mediated by flavonoids accumulated in the membrane fraction. This interesting possibility is consistent with the notion that, while mostly bound to intracellular proteins, a significant fraction of quercetin (15%) is bound to the RBC membranes (*22*).

Thus, while most of the extracellular FIC reduction induced by the intracellular fraction of the flavonoids is mediated by the activity of PMOR, some FIC might nevertheless be directly reduced by the membrane-associated flavonoids. This hypothesis, however, needs to be validated experimentally.

It is well-established that the electron-donating ability of flavonoids depends on the position and degree of hydroxylation (*42*−*44*) and that the reducing activity of the flavonoids is enhanced by the following structural features: (i) the hydroxyl groups at 3 and 5 positions, (ii) the 2,3-double bond in conjugation with a 4-oxo group, and (iii) the 3′- and 4′-hydroxyl groups on the B ring. As shown in **Figure 5**, most of the flavonoids recovered in the EtEs (**Figure 4**) present all these structural features, supporting the enhanced PMOR activity observed (**Figures 1** and **2**).

The MH1−3 honey EtEs are more active in donating electrons to human erythrocyte PMOR than honey (MH4), as shown in **Figure 2**. These findings are in good correlation with the total flavonoid concentration (**Table 1**) and with the HPLC/MS analyses (**Figure 4**) showing a significantly lower polyphenolic content in MH4 EtE. Having tested only one type of pasteurized honey, we cannot draw general conclusions except that this process, when it leads to a decrease in the fraction of lipid-soluble polyphenols, will cause a loss of those flavonoids more easily adsorbed by RBCs and consequently a lower PMOR-dependent reduction of extracellular oxidants. The AqEs were on the one hand excellent reductants (**Figure 1a**) and on the other hand poorly taken up by the RBCs, so that virtually no stimulation of PMOR activity was observed (**Figure 2**). This may also suggest poor absorption of the AqE components and, eventually, poor distribution, which would lead to their accumulation in the plasma to promote direct reduction of extracellular oxidants.

We may therefore conclude that the RBC-dependent reduction of extracellular oxidants is a potentially important beneficial effect that might be added to the list of previously established beneficial effects of honey (*11*−*20*). This novel effect is mediated by various lipid-soluble flavonoids that act as substrates for the PMOR activity, providing an efficient way to maintain a redox state in plasma (*45*, *46*). These flavonoids as well as those contained in AqEs might also be important for their direct reducing effects.

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