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Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity

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Abstract

Several honey samples (27) from Burkina Faso were analyzed to determine their total phenolic, flavonoid and proline contents as well as their radical scavenging activity. These samples consisted of 18 multifloral, 2 honeydew and 7 unifloral honeys, derived in the latter cases from flowers of Combretaceae, *Vitellaria*, *Acacia* and *Lannea* plant species. The total phenolic contents varied considerably with the highest values obtained for honeydew honey. Similarly, much variation was seen in total flavonoid and proline content, with *Vitellaria* honey having the highest proline content. *Vitellaria* honey was also found to have the highest antioxidant activity and content. The correlation between radical scavenging activity and proline content was higher than that for total phenolic compounds. This suggests that the amino acid content of honey should be considered more frequently when determining its antioxidant activity.

Keywords: Honey; Burkina Faso; Antioxidants, Radical scavenging activity; DPPH; Phenolics; Flavonoids; Proline

1. Introduction

Honey has been used since the earliest times. It is widely appreciated as the only concentrated form of sugar available worldwide (FAO, 1996) and is also used as a food preservative (Cherbuliez, 2001; Cherbuliez & Domerego, 2003). Lately, the physiological functioning of different foods including honey has received much attention. Antioxidants, for example, play an important role in food preservation and human health by combating damage caused by oxidizing agents e.g., oxygen. Natural and synthetic antioxidants have a long history as preservatives in food (The National Honey Board, 2003), where they specifically retard deterioration, rancidity or discoloration due to oxidation caused by light, heat and some metals. These antioxidants, which act as preservatives because of their antioxidative activity (Antony, Han, Rieck, & Dawson, 2000; Cherbuliez, 2001; Ferreres, Garcia-Vigera, Tomas-Lorente, & Tomas-Barberan, 1993; Gheldof, Wang, & Engeseth, 2002; Nagai, Sakai, Inoue, Inoue, & Suzuki, 2001; The National Honey Board, 2003; Vit, Soler, & Tomas-Barberan, 1997), include both enzymatic (e.g., catalase, glucose oxidase) and non-enzymatic substances (e.g., organic acids, Maillard reaction products, amino acids, proteins, flavonoids, phenolics, α-tocopherol, flavonols, catechins, ascorbic acid and carotenoids) (The National Honey Board, 2003).

Many authors have studied the phenolic and flavonoid contents of honey to determine if a correlation exists

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with floral origins (Amiot, Aubert, Gonnet, & Tacchini, 1989; Ferreres, Tomas-Barberan, Gil, & Tomas-Lorente, 1991; Gil, Ferreres, Ortiz, Subra, & Tomas-Barberan, 1995; Martos et al., 2000; Tomas-Barberan, Martos, Ferreres, Radovic, & Anklam, 2001; Vit et al., 1997) and also to determine the presence of antimicrobial activity (The National Honey Board, 2003; Snow & Manley-Harris, 2004).

It has been demonstrated that some amino acids also have antioxidant properties (Wu, Shiau, Chen, & Chiou, 2003). Proline content, which varies considerably between different honeys, is also a measure of the level of total amino acids present. It can also serve as an additional determinant of quality and in some cases also as a criterion for estimating the maturity of honey as well as an indicator for detecting sugar adulteration (Bogdanov, 1999).

Many methods for determining the antioxidative activity in honey have been used, e.g., determination of active oxygen species (viz. the superoxide anion, peroxyl and hydroxyl radicals), their radical scavenging ability (Gheldof & Engeseth, 2002; Gheldof et al., 2002; Kefalas, Gotsiou, & Chougoui, 2001; Nagai et al., 2001; Taomina, Niemira, & Beuchat, 2001), the 1,1-diphenyl-2-picrylhydrasyl (DPPH) antioxidant content (Chen, Mehta, Berenbaum, Zangerl, & Engeseth, 2000) and enzymatic or non-enzymatic measurements of lipid peroxidation inhibition (Chen et al., 2000; McKibben & Engeseth, 2002; Nagai et al., 2001). The antioxidant activities of various foods have been accurately and rapidly determined using DPPH, which can be used for both solid or liquid samples and is also not specific for any particular antioxidant alone.

The purposes of the present study were to determine the total phenolic, flavonoid and proline contents of several Burkina Fasan honey samples as well as their antioxidant levels. In addition a correlation between the latter and total phenolic, flavonoid and proline contents was also sought.

2. Materials and methods

2.1. Honey samples

Twenty seven honey samples (Table 1) were collected for this study. Seventeen samples (1, 2, 3, 4, 5, 10, 11, 12, 13, 14, 17, 18, 23, 24, 25, 26, 27) were from apiarists, three (7, 8, 9) from the Fada Beekeeping Cooperative (east of Burkina Faso), two (15, 16) from the apiculture research center (CPFRA; Centre de Production, de Formation et de Recherche en Apiculture) and five (6, 19, 20, 21, 22) were commercial honeys. The different samples were collected between December 2002 and July 2003 from different geographical regions (eastern, western, south-western and central parts) of Burkina Faso (BF).

Qualitative microscopic analysis and frequency determination of the classes of pollen grains in the honey samples were done as described (Moar, 1985). The different pollen morphologies were compared with that published (Association des Palynologues de Langue Française, 1974; Bonnefille & Riollet, 1980) and with reference slides from the Laboratory of Biology and Ecology, University of Ouagadougou. All the samples were stored between 0 and 4 °C.

2.2. Estimation of total phenolic and flavonoid contents

The Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999) was used to determine total phenolic content. Each honey sample (5 g) was diluted to 50 ml with distilled water and filtered through Whatman No. 1 paper. This solution (0.5 ml) was then mixed with 2.5 ml of 0.2 N Folin–Ciocalteu reagent (Sigma–Aldrich Chemie, Steinheim, Germany) for 5 min and 2 ml of 75 g/l sodium carbonate (Na_2CO_3) (Labosi, Paris, France) was then added. After incubation at room temperature for 2 h, the absorbance of the reaction mixture was measured at 760 nm against a methanol blank (CECIL CE 2041 spectrophotometer 2000 series from CECIL instruments, Cambridge, England). Gallic acid (Sigma–Aldrich Chemie, Steinheim, Germany) (0–200 mg/l) was used as standard to produce the calibration curve. The mean of three readings was used and the total phenolic content was expressed in mg of gallic acid equivalents (GAE)/100 g of honey.

The total flavonoid content was determined using the Dowd method as adapted by Arvouet-Grand, Vennat, Pourrat, and Legret (1994). Briefly, 5 ml of 2% aluminium trichloride ($AlCl_3$) (Labosi, Paris, France) in methanol (Fluka Chemie, Switerland) was mixed with the same volume of a honey solution (0.01 or 0.02 mg/ml). Absorption readings at 415 nm (CECIL CE 2041 spectrophotometer 2000 series) were taken after 10 min against a blank sample consisting of a 5 ml honey solution with 5 ml methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with quercetin (Sigma–Aldrich Chemie, Steinheim, Germany) (0–50 mg/l) as the standard. The mean of three readings was used and expressed as mg of quercetin equivalents (QE)/100 g of honey.

2.3. Estimation of proline content

The proline content was determined by using a colour comparison after applying ninhydrin, with a proline standard. The content was expressed as a proportion to the mass of honey tested. The proline content was determined using the method of Ough as adapted by Bogdanov (1999). A 0.5 ml solution of honey (0.05 g/ml) was

Table 1
A compilation of data from 27 honey samples obtained in Burkina Faso

Sample no.	Floral origin (% of pollen)	Harvest date	Geographical origin in Burkina Faso	Total phenolic content (mg GAE/ 100 g ± SD)	Total flavonoid content (mg QE/ 100 g mg ± SD)	Proline content (mg/kg ± SD)	RSA: IC_{50} (mg/ml ± SD)	AEAC (mg/ 100 g ± SD)	QEAC (mg/ 100 g ± SD)
1.	Multifloral	July 2003	Fada, eastern BF	83.80 ± 3.35	2.86 ± 0.63	629.2 ± 32.5	9.60 ± 1.40	22.12 ± 0.17	10.87 ± 0.03
2.	*Combretaceae* (64.9%)	July 2003	Fada, eastern BF	59.67 ± 1.35	1.61 ± 0.03	870.0 ± 23.6	10.40 ± 0.50	23.40 ± 0.74	11.31 ± 0.28
3.	*Acacia* (59.2%)	July 2003	Fada, eastern BF	93.43 ± 0.87	6.14 ± 0.35	790.4 ± 69.1	10.53 ± 0.65	17.50 ± 0.05	9.43 ± 0.01
4.	Multifloral	July 2003	Fada, eastern BF	56.47 ± 1.61	3.92 ± 0.14	931.0 ± 54.1	6.90 ± 0.53	27.50 ± 0.65	11.61 ± 0.32
5.	Honeydew	July 2003	Fada, eastern BF	113.05 ± 1.10	1.85 ± 0.10	1216.6 ± 15.7	4.93 ± 0.23	32.38 ± 0.05	13.94 ± 0.15
6.	Multifloral	July 2003	Fada, eastern BF	61.49 ± 1.87	2.15 ± 0.17	911.7 ± 16.8	6.00 ± 0.52	28.70 ± 1.88	13.08 ± 0.19
7.	Multifloral	July 2003	Fada, eastern BF	62.04 ± 0.53	8.35 ± 0.16	687.6 ± 19.8	13.43 ± 1.12	17.56 ± 0.02	8.59 ± 0.02
8.	Honeydew	December 2002	Fada, eastern BF	114.75 ± 1.30	3.62 ± 0.46	797.1 ± 14.1	4.37 ± 0.10	24.80 ± 0.04	12.46 ± 0.01
9.	Multifloral	April 2003	Fada, eastern BF	69.43 ± 1.24	4.61 ± 0.39	437.8 ± 23.0	12.38 ± 1.53	19.05 ± 0.02	9.51 ± 0.01
10.	Multifloral	July 2003	Gonsé, central BF	74.39 ± 0.90	4.50 ± 0.04	755.7 ± 17.8	7.00 ± 0.50	29.40 ± 4.44	12.29 ± 0.51
11.	Multifloral	July 2003	Gonsé, central BF	63.37 ± 0.90	7.13 ± 0.11	609.3 ± 19.4	10.43 ± 0.31	19.46 ± 0.03	10.22 ± 0.01
12.	Multifloral	February 2003	Gonsé, central BF	43.41 ± 0.00	1.39 ± 0.29	1320.4 ± 13.0	29.13 ± 1.50	10.20 ± 0.59	4.27 ± 0.03
13.	*Vitellaria* (81.4%)	February 2003	Gonsé, central BF	76.10 ± 0.56	0.90 ± 0.16	1593.5 ± 46.8	2.43 ± 0.08	57.72 ± 0.00	31.01 ± 0.03
14.	*Vitellaria* (90.1%)	February 2003	Gonsé, central BF	83.53 ± 0.25	1.14 ± 0.04	2169.4 ± 18.4	1.63 ± 0.03	63.60 ± 0.00	32.29 ± 0.03
15.	*Lannea* (94.5%)	May 2003	CPFRA, central BF	42.96 ± 0.63	0.37 ± 0.00	890.8 ± 21.3	23.53 ± 0.40	11.27 ± 0.02	5.35 ± 0.01
16.	Multifloral	May 2003	CPFRA, central BF	57.63 ± 0.49	0.17 ± 0.07	973.7 ± 3.3	15.40 ± 0.00	17.34 ± 0.03	7.88 ± 0.01
17.	*Combretaceae* (82.8%)	July 2003	Pabré, central BF	52.08 ± 0.31	0.88 ± 0.17	1090.5 ± 38.9	17.97 ± 1.44	16.34 ± 0.25	6.89 ± 2.02
18.	Multifloral	July 2003	Pabré, central BF	32.59 ± 0.48	0.41 ± 0.07	890.8 ± 27.0	28.00 ± 0.56	12.43 ± 0.16	5.09 ± 0.03
19.	Multifloral	July 2003	Banfora, south-western BF	79.99 ± 0.11	3.00 ± 0.22	634.4 ± 15.4	6.55 ± 0.51	34.27 ± 0.59	15.55 ± 0.24
20.	Multifloral	July 2003	Banfora, south-western BF	81.44 ± 0.29	1.03 ± 0.56	637.9 ± 9.1	6.52 ± 0.30	32.57 ± 0.56	15.16 ± 0.28
21.	Multifloral	July 2003	Banfora, south-western BF	90.84 ± 0.54	2.21 ± 0.03	715.3 ± 34.2	5.03 ± 0.06	37.87 ± 0.26	17.30 ± 0.27
22.	Multifloral	July 2003	Banfora, south-western BF	93.66 ± 0.44	1.67 ± 0.26	753.6 ± 18.7	6.42 ± 0.28	32.14 ± 1.23	15.96 ± 0.71
23.	*Vitellaria* (84.8%)	July 2003	Gaoua, south-western BF	100.39 ± 1.29	2.70 ± 0.17	1968.1 ± 39.7	1.37 ± 0.03	65.86 ± 0.10	33.34 ± 0.21
24.	Multifloral	July 2003	Gaoua, south-western BF	86.07 ± 2.98	0.65 ± 0.07	1153.8 ± 8.7	6.97 ± 0.45	25.87 ± 0.54	12.67 ± 0.62
25.	Multifloral	July 2003	Gaoua, south-western BF	65.69 ± 0.19	3.22 ± 0.80	787.1 ± 19.8	11.80 ± 0.36	18.36 ± 0.71	7.93 ± 0.29
26.	Multifloral	July 2003	Gaoua, south-western BF	84.82 ± 0.58	1.22 ± 0.03	1243.1 ± 27.0	9.60 ± 1.40	18.48 ± 0.50	8.35 ± 0.03
27.	Multifloral	July 2003	Gaoua, south-western BF	85.07 ± 0.41	1.67 ± 0.13	1258.4 ± 26.2	10.40 ± 0.50	16.28 ± 0.10	6.96 ± 1.68
	Mean ± SD			74.38 ± 20.54	2.57 ± 2.09	989.5 ± 407.4	10.60 ± 7.30	27.04 ± 14.68	12.94 ± 7.74

Phenolic, flavonoid and proline contents as well as radical scavenger activity (IC_{50}) and antioxidant contents (AEAC and QEAC) were determined. AEAC, ascorbic acid equivalent antioxidant content; BF, Burkina Faso; CPFRA, Centre de Production et de Formation et de Recherche en Apiculture; GAE, gallic acid equivalent; IC_{50}, 50% inhibitory concentration; QEAC, quercetin equivalent antioxidant content; RSA, radical scavenger activity; SD, standard deviation; % of pollen, Percentage pollen grains of a specific plant species.

mixed with 1 ml of formic acid (80%) (Labogros, France), 1 ml of ninhydrin (Labosi, Paris, France) solution (3% in ethylene glycol monomethylether, from Labosi, Paris, France) and shaken vigorously for 15 min. The mixture was placed in a boiling water bath for 15 min and transferred to a 70 °C bath for 10 min. A 5 ml solution of 50% 2-propanol (Fluka Chemie, Switzerland) in water was then added and the mixture was left to cool and the absorbance determined (510 nm), 45 min after removal from the 70 °C water bath. Water was used as the blank and 0.032 mg/ml solution of proline (Labosi, Paris, France) was used as the standard solution. Proline concentration in mg/kg of honey was calculated as follows: Proline (mg/kg) = $(E_s/E_a) \times (E_1/E_2) \times 80$, where E_s is the absorbance of the sample solution; E_a is the absorbance of the proline standard solution (average of 3 readings); E_1 is the mg of proline used for the standard solution; E_2 is the weight of honey in grams; 80 is the dilution factor. The mean of three readings was used.

2.4. Radical scavenging activity and antioxidant content

The scavenging activity of honey samples for the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured as described (Velazquez, Tournier, Mordujovich de Buschiazzo, Saavedra, & Schinella, 2003) with some modifications. Honey samples were dissolved in methanol at a concentration of 2.65–170 mg/ml, and 0.75 ml of each sample was mixed with 1.5 ml of DPPH (Fluka Chemie, Switzerland) in methanol (0.02 mg/ml), with methanol serving as the blank sample. The mixtures were left for 15 min at room temperature and the absorbances then measured at 517 nm. Quercetin (0–50 mg/l) and ascorbic acid (Labosi, Paris, France) (0–40 mg/l) were used as positive controls. The radical scavenging activity was calculated as follows as: % Inhibition = [(blank absorbance − sample absorbance)/blank absorbance] × 100. The mean of three IC_{50} (concentration causing 50% inhibition) values of each honey sample was determined graphically.

The antioxidant content was evaluated as described (Chen et al., 2000), with some modifications. Honey samples were dissolved in methanol (0.02 or 0.04 g/ml) and 0.75 ml of each was mixed with 1.5 ml of a 0.02 mg/ml solution of DPPH in methanol. The mixtures were left for 15 min at room temperature and the absorbances then measured (517 nm). The blank sample consisted of 0.75 ml of a honey solution with 1.5 ml of methanol. The antioxidant content was determined using standard curves for ascorbic acid (0–10 μg/ml) and quercetin (0–6.25 μg/ml). The means of three values were obtained, expressed as mg of ascorbic acid equivalent antioxidant content (AEAC) per 100 g of honey and mg of quercetin equivalent antioxidant content (QEAC) per 100 g of sample.

2.5. Statistical analysis

Correlation coefficients (R) to determine the relationship between two variables were calculated using MS Excel software (CORREL statistical function).

3. Results

Pollen analysis was used to specifically identify 7 unifloral (2 Combretaceae, 3 *Vitellaria*, 1 *Acacia* and 1 *Lannea*), 2 honeydew and 18 multifloral honey samples (Table 1).

3.1. Phenolic, flavonoid and proline contents

Total phenolic content (mg of GAE/100 g of honey) varied from 32.59 to 114.75 mg with a mean of 74.38 ± 20.54 mg (Table 1) using the standard curve of gallic acid ($R^2 = 0.9990$).The total phenolic content varied from 32.59 in a multifloral honey (sample 18) to 93.66 mg in honeydew honey.

Using the standard curve generated by quercetin ($R^2 = 0.9999$), the total flavonoid content of honey samples (mg of QE/100 g) varied from 0.17 to 8.35 mg with a mean value of 2.57 ± 2.09 mg (Table 1) with the highest and the lowest levels observed in multifloral honeys. A low correlation ($R = 0.11$) was shown between total phenolic and total flavonoid content.

The proline content (mg/kg) varied from 437.8 to 2169.4 with a mean of 989.5 ± 407.4 (Table 1). The highest proline contents were observed in *Vitellaria* honeys (samples 13, 14 and 23) with the lowest in a multifloral honey (sample 9).

3.2. Radical scavenging activity and antioxidant content

The results of DPPH radical scavenging activity (RSA) and the antioxidant content of different honey samples are summarized in Table 1. The IC_{50} values ranged from 1.63 to 29.13 mg/ml. The highest DPPH RSAs were found in all *Vitellaria* honeys following by honeydew honeys while the lowest was observed in a multifloral honey. The IC_{50} values were 0.87 ± 0.06 and 1.8 ± 0.43 μg/ml, respectively, for quercetin and ascorbic acid.

Using the standard curves of ascorbic acid ($R^2 = 0.9986$) and quercetin ($R^2 = 0.9851$), it was shown that higher antioxidant contents were observed in *Vitellaria* honeys viz. 31.01, 32.29 and 33.34 mg QEAC/100 g, and 57.72, 63.6 and 65.86 mg AEAC/100 g, for samples 13, 14, 23, respectively. The antioxidant contents in multifloral honeys varied from 4.27 to 17.30 mg QEAC/100 g and from 10.20 to 37.87 mg AEAC/100 g. The ratio of AEAC to QEAC was 2 for all samples.

The correlation between the two sets of antioxidant contents was 0.993. The same correlation existed between $1/IC_{50}$ and QEAC ($R = 0.95$) and between $1/IC_{50}$ and AEAC ($R = 0.95$). The correlation between RSA and total phenolics was 0.5, between RSA and proline content it was 0.75, while between RSA and total flavonoids there was a negative correlation.

4. Discussion

Several investigators have found phenolic and flavonoid compounds in honey (Amiot et al., 1989; Ferreres et al., 1991; Gil et al., 1995; Martos et al., 2000; Tomas-Barberan et al., 2001; Vit et al., 1997). The total phenolic content of certain honey samples has also been previously determined (Amiot et al., 1989; Kefalas et al., 2001). The phenolic content of the 27 honey samples that we analyzed are similar to the average values found for some French and Greek honeys (Amiot et al., 1989; Kefalas et al., 2001), although we showed that honeydew honeys showed relatively higher levels of phenolic compounds (113.05 and 114.75 mg GAE/100 g). We also found that *Vitellaria* honey had a high phenolic content (86.67 ± 12.45 mg GAE/100 g).

The mean values for total flavonoids were 2.57 ± 2.09 mg of QE/100 g and are similar to those for European honeys e.g., *Eucalyptus* honey (2–2.5 mg QE/100 g), sunflower and rape honey (1.5–2 mg QE/100 g), fir, lavender, ivy and *Acacia* honey (0.5–1 mg QE/100 g), arbutus and chestnut honey (less than 0.5 mg QE/100 g) (Amiot et al., 1989; Martos et al., 2000). The *Acacia* honey from Burkina Faso had a total flavonoid content of 6.14 mg QE/100 g which is considerably higher than that of French honeys (less than 1 mg QE/100 g). The flavonoid content of honey has usually been determined by HPLC using amberlite XAD-2 columns for extraction. In this study, we used a spectrophotometric quantification of flavonoids with aluminum chloride, which has previously been described for the quantification of flavonoids in propolis extracts (Arvouet-Grand et al., 1994; Chang, Yang, Wen, & Chern, 2002). Chang et al. (2002) showed that the real content of total flavonoids must be the sum of flavonoid contents determined by the aluminum chloride method which is specific only for flavones and flavonols, and by the 2,4-dinitrophenylhydrazine method that is specific for flavanones. Propolis-derived flavanones (pinocembrin and pinobanksin) were found in significant levels in European and New Zealand honeys (Tomas-Barberan et al., 2001). This means that by using the aluminum chloride method alone, one will underestimate the content of total flavonoids. This also partly explains the low correlation ($R = 0.11$) observed between the total amount of flavonoids and the total amount of phenolic compounds. A lower correlation between total phenolic and total flavonoid content was also found by Miliauskas, Venskutonis, and Van Beek (2004) during some RSA analyses of medicinal and aromatic plant extracts. This could also be explained by the presence of some chemical groups of amino acids and proteins that can also react with Folin–Ciocalteu reagent.

Published analyses have revealed that various honeys contain 11–21 free amino acids with proline predominating (White & Doner, 1980). The content of proline is an indication of the quality of honey and is also an indication of adulteration when it falls below a value of 183 mg/kg (Bogdanov et al., 1995; Bogdanov, 1999). All the honey samples we studied had good proline levels of up to 183 mg/kg, indicating absence of adulteration. Proline is the most abundant amino acid in honey and is used as a standard to quantify amino acid content.

The results of DPPH RSA analyses demonstrated that the most active radical scavengers were found in *Vitellaria* honey, followed respectively by honeydew, *Acacia*, *Combretaceae* and *Lannea* honeys. The antioxidant content which is a measure of RSA, was inversely proportional to IC_{50}. *Vitellaria* honey had the higher antioxidant content followed by honeydew honey. Quercetin had on average a twofold greater level of active RSA than ascorbic acid. This was confirmed by the ratio of AEAC to QEAC. Quercetin and ascorbic acid RSA were more than a thousand fold higher than those of *Vitellaria*.

A good correlation was observed between the QEAC and the AEAC tests ($R = 0.99$) and also between the RSA ($1/IC_{50}$) and the two antioxidant tests ($R = 0.95$). The correlation between total phenolics and QEAC ($R = 0.5$) was the same as that between total phenolics and AEAC ($R = 0.5$), neither of which were highly significant. The same moderate correlation was shown between the DPPH RSA results and total phenolic levels, suggesting that phenolic compounds were likely to be contributing to the RSA of these honeys. Others studies on plant extracts have confirmed that such exists (Miliauskas et al., 2004). It is known that where similar phenolic levels occur, these do not necessarily correspond to the same antioxidant responses. The response of phenolics in the Folin–Ciocalteu assay also depends on their chemical structure (Atoui, Mansouri, Boskou, & Kefalas, 2005). This means the RSA of a sample cannot be predicted on the basis of its total phenolic content. In the case of honey, the antioxidant capacity is the result of the combined activity of a wide range of compounds including phenolics, peptides, organic acids, enzymes, Maillard reaction products and possibly other minor components (Gheldof et al., 2002).

The flavonoid contents in our study, however, showed negative correlations with $1/IC_{50}$ ($R = -0.10$), QEAC ($R = -0.11$) and with AEAC ($R = -0.14$). This

can be partly explained by the underestimation that occurs when using the aluminum chloride method for total flavonoid quantification. Others authors have also found a low correlation ($R = 0.32$) between plant flavonoid levels and RSA (Miliauskas et al., 2004). It is known that only flavonoids of a certain molecular structure, particularly those with a certain hydroxyl position, will determine the antioxidant properties present. In general, these properties depend on the ability to donate hydrogen or electrons to a free radical. The same authors found that flavonols had a higher correlation with antiradical activity in plant extracts than flavonoids.

In our study, the best correlation was observed between proline content and RSA ($R = 0.75$). Amino acids are one of the antioxidant components in honey. The antioxidant activity of some free amino acids (histidine, taurine, glycine, alanine) and their combinations have already been shown (Wu et al., 2003). A detailed examination of free amino acids in honey and their RSA is required to understand the specific contribution of proline.

5. Conclusion

This study showed that the 27 samples of Burkina Faso honey contained phenolic compounds, flavonoids and proline of good quality. *Vitellaria* honey, with the highest proline content, had the most active radical scavenger activity and the highest AEAC and QEAC values of all samples. Honeydew honey had the highest amount of total phenolic compounds and possessed good radical scavenger activity. The RSA of *Acacia* honey was higher than that of *Combretaceae* and *Lannea* honeys. The correlation between RSA and proline content was higher than that between RSA and total phenolic content. Further investigations are needed to evaluate the RSA of individual free amino acids as well as the RSA of phenolic and flavonoid extracts from honey.

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