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Different forms of maleic and fumaric acids (*cis* and *trans* of 2-butenedioic acid) in honey

Silvia Suárez-Luque[a], Inés Mato[a], José F. Huidobro[a,*], Jesús Simal-Lozano[a], Juan C. García-Monteagudo[b]

[a]*Facultad de Farmacia, Departamento de Química Analítica, Nutrición y Bromatologia, Área de Nutrición y Bromatologia, Universidad de Santiago, 15782 Santiago de Compostela, Galicia, Spain*

[b]*Facultad de Farmacia, Departamento de Química Física, Universidad de Santiago, 15782 Santiago de Compostela, Galicia, Spain*

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Abstract

The total contents of maleic and fumaric acids (*cis* and *trans*-2-butenedioic acids, respectively) were quantified by a high performance liquid chromatography method, in 50 floral honeys of Galicia (north-western Spain). Honey pH, activity coefficients, dissociation constants of the acids (K_1 and K_2), and the molar concentrations of the forms of maleic and fumaric acids naturally present in honey ($[AH_2]$, $[AH^-]$ and $[A^{2-}]$) have been calculated for the first time. The contents of maleic and fumaric acids can be determined either as total maleic and fumaric acids ($[AH_2]$) or as total maleate and fumarate ($[A^{2-}]$), but there are other forms of these acids in honey. Therefore the calculation of the forms of the maleic and fumaric acids present would illuminate their origin at honey pH. The predominant acid form depends on honey pH value. Maleic acid was quantifiable in 44 honeys. The $[AH^-]$ form was found as a major component in all samples. Fumaric acid was quantifiable in 49 honeys. The $[A^{2-}]$ form was found as a major component in most honeys (28 samples) and the $[AH^-]$ was predominant in 21 samples. No honey analysed had a $[AH_2]$ form as predominant. Although maleic and fumaric acids have the same molecular weight and they are both dicarboxylic acids, their pH relationships differ. The relationships between maleic and fumaric acids and honey pH and between the total content of maleic and fumaric acids and their forms in honey have been calculated.

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Keywords: Honey; Maleic acid; Fumaric acid; Forms; pH

1. Introduction

Organic acids comprise a small proportion of honey (0.5%) and they can be used as indicators of deterioration on account of storage, aging or even to measure purity and authenticy (White, 1978). Organic acids are also components of the honey flavour (White, 1979a). Acidity of honey contributes, with other substances, such as inhibine or high concentration of sugars, to preserve this foodstuff against micro-organism spoilage (White, 1979b).

Maleic and fumaric acids are both dicarboxylic acids and have the same molecular weight. Nervertheless they have different structural formulas. Maleic acid is the *cis*-(Z)-2-butenedioic acid and fumaric acid is the *trans*-(E)-2-butenedioic acid (Fig. 1).

These acids are naturally present in honey. Although their concentrations in honey are very low, their determination is important. The fumaric acid content is related to the content of citric acid and could be useful for characterizing different honey types (Talpay, 1988). However, the content of maleic acid is not related to the content of citric acid. So differences in structure determine differences in properties.

Maleic and fumaric acids are dicarboxylic acids. The relationship between the acid forms and their salts depends on honey pH, the total content of maleic and fumaric acids, the ionic power and the dissociation constants of the acids (K_1 and K_2; Weast, 1981). Therefore, three forms of maleic and fumaric acids may be found in honey: $[AH_2]$, $[AH^-]$ and $[A^{2-}]$. The total contents of these acids are necessary to determine these forms.

* Corresponding author. Tel.: +34-981-594626; fax: +34-981-594912.

E-mail address: qnhuidob@usc.es (J.F. Huidobro).

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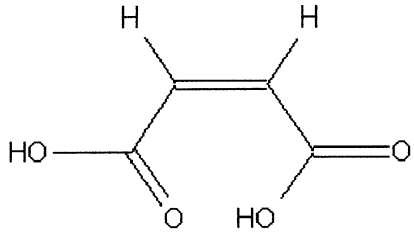
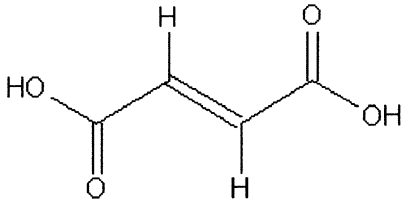


Cis 2-butenedioic acid (Z) $K_1 = 1.48 \times 10^{-2}$
Maleic acid $K_2 = 8.51 \times 10^{-7}$



Trans 2-butenedioic acid (E) $K_1 = 9.33 \times 10^{-4}$
Fumaric acid $K_2 = 3.63 \times 10^{-5}$

Fig. 1. Structural formulas of the maleic and fumaric acids and their dissociation constants (Weast, 1981).

The purpose of this paper is to determine the pH and the total contents of maleic and fumaric acids. Moreover, the molar concentrations of three forms of these acids have been calculated according to honey pH, the total content of the acids and the dissociation constants of the acids. We attempt to establish possible relationships between them and the honey pH.

2. Material and methods

2.1. Samples

The work was carried out on 50 floral samples from Galicia (north-western Spain). The samples, which were labelled "Producto Galego de Calidade-Mel de Galicia" (Diario Oficial de Galicia, 1989), were harvested in autumn 1997 and stored in darkness at room temperature until the analysis.

2.2. Reagents

Analytical standard-grade maleic and fumaric acids were obtained from Sigma (Sigma Chemical Co., St. Louis, MO, USA). Stock standard solutions were obtained by dissolution of acids in Milli-Q water and stored at 4 °C for 1 month. The Milli-Q water was purified by passage through a Compact Milli-RO and Milli-Q water system from Millipore, Milford, MA, USA. Working standard solutions were prepared daily by dilution with Milli-Q water. Metaphosphoric acid, sulfuric acid and sodium hydroxide pellets were analytical-reagent grade and supplied by Merck KGaA, Darmstadt, Germany.

The samples were filtered through cellulose membrane filters Whatman (0.45 μm, Cat No. 7000 0002, Whatman Inc, Clifton, NJ, USA) and the solid phase extraction was achieved with a Waters Accell Plus QMA ion-exchange cartridge Part No. WAT020545 (Waters Associates, Inc. Milford, MA, USA).

The eluent was filtered with membrane filters Phenomenex (0.45 μm, AFO-0504, California, USA).

2.3. Apparatus

Chromatographic analyses were carried out using a Waters liquid chromatograph equipped with a Waters ILD on-line degasser, a Waters 600E pump, a Waters 717 plus autosampler and a Waters 996 diode-array UV detector (Waters Associates, Inc. Milford, MA, USA). The detector signals were recorded on a Chromatography Data System Millennium 32®. The column was a Spherisorb ODS-2 S5 (particle size 5 μm; 250×4.6 mm I.D).

A Crison micropH 2002 pH meter (Crison Instruments S.A., Alella, Barcelona, Spain) and a Selecta Agimatic-S magnetic stirrer (Selecta, Abrera, Barcelona, Spain) were also used.

2.4. Methods

2.4.1. pH

The AOAC (1990) method was employed.

2.4.2. Maleic and fumaric acids

An HPLC method was used to determine the contents of maleic and fumaric acids in honey (Suárez-Luque et al., 2002).

These acids were removed from honey by using a solid-phase extraction procedure with anion exchange cartridges. The chromatographic separation was achieved with one Spherisorb ODS-2 S5 column thermostatted at 25 °C. Metaphosphoric acid (pH 2.20) was used as mobile phase at a flow-rate of 0.7 ml/min. Dicarboxylic acids were detected with a UV–vis detector (215 nm).

The precision results in honey samples analysed showed repeatability and reproducibility with coefficients of variation $\leqslant 3.11\%$ and $\leqslant 4.59\%$, respectively. The recoveries of the acids in honey samples analysed were 93.4% for maleic acid and 94.4% for fumaric acid. Under the optimum conditions the quantification limits were 0.65 μmol/kg for maleic acid and 0.22 μmol/kg for fumaric acid.

2.4.3. Calculations and statistical treatment

The molar concentrations of the forms of both acids ($[AH_2]$, $[AH^-]$ and $[A^{2-}]$) were calculated according to the honey pH, the total acids content (M), the activity

Table 1
pH, total content of maleic and fumaric acids (μmol/kg of honey) and the content of the forms of maleic and fumaric acids (μmol/kg of honey) of 50 honeys analysed

Sample	pH	Maleic acid (μmol/kg)				Fumaric acid (μmol/kg)			
		Total acid	$[AH_2]$	$[AH^-]$	$[A^{2-}]$	Total acid	$[AH_2]$	$[AH^-]$	$[A^{2-}]$
1	4.16	ND	ND	ND	ND	5.17	0.0856	2.19	2.89
2	3.95	2.21	0.0087	2.16	0.041	3.62	0.124	1.93	1.56
3	4.07	1.57	0.0047	1.53	0.038	3.62	0.0834	1.71	1.82
4	4.46	4.15	0.0048	3.91	0.242	20.3	0.109	5.65	14.6
5	4.28	1.84	0.0033	1.77	0.070	14.5	0.157	5.35	8.96
6	4.27	2.96	0.0054	2.84	0.109	17.40	0.196	6.52	10.7
7	4.08	2.58	0.0073	2.51	0.063	5.69	0.125	2.65	2.91
8	4.01	4.52	0.0153	4.41	0.097	3.27	0.0908	1.64	1.54
9	4.17	3.23	0.0075	3.12	0.099	2.15	0.0344	0.90	1.22
10	4.31	3.23	0.0054	3.09	0.135	2.67	0.0255	0.92	1.72
11	4.29	1.75	0.0031	1.68	0.070	3.02	0.0311	1.07	1.91
12	4.07	1.02	0.0030	0.99	0.025	0.52	0.0119	0.24	0.26
13	4.21	ND	ND	ND	ND	4.05	0.0572	1.61	2.39
14	3.99	4.80	0.0171	4.68	0.099	4.74	0.140	2.45	2.14
15	4.08	3.96	0.0115	3.85	0.101	3.27	0.0714	1.54	1.66
16	3.80	3.60	0.0198	3.54	0.046	4.48	0.244	2.71	1.53
17	3.89	2.40	0.0106	2.35	0.038	2.41	0.0981	1.36	0.95
18	3.95	2.40	0.0094	2.34	0.044	3.79	0.130	2.04	1.62
19	4.41	4.80	0.0063	4.55	0.245	33.3	0.221	10.2	22.9
20	4.12	4.70	0.0122	4.56	0.128	6.72	0.127	3.04	3.55
21	3.66	1.02	0.0076	1.00	0.009	2.07	0.167	1.35	0.55
22	3.86	2.49	0.0118	2.44	0.036	3.02	0.137	1.75	1.13
23	3.86	3.14	0.0149	3.07	0.047	1.90	0.0843	1.10	0.71
24	3.94	2.77	0.0111	2.70	0.049	2.93	0.103	1.59	1.23
25	3.85	4.52	0.0219	4.44	0.066	1.38	0.0635	0.80	0.51
26	3.76	ND	ND	ND	ND	1.55	0.0942	0.95	0.51
27	3.74	ND	ND	ND	ND	0.34	0.0220	0.22	0.11
28	3.59	1.20	0.0106	1.18	0.010	0.60	0.0585	0.40	0.14
29	3.89	35.4	0.160	34.7	0.567	11.3	0.465	6.41	4.41
30	3.82	5.07	0.0270	4.98	0.072	2.93	0.148	1.74	1.04
31	3.99	2.40	0.0085	2.34	0.048	5.17	0.155	2.69	2.33
32	4.03	3.51	0.0114	3.42	0.079	10.1	0.260	5.02	4.80
33	4.41	ND	ND	ND	ND	2.84	0.0189	0.85	1.98
34	3.84	4.33	0.0215	4.25	0.062	ND	ND	ND	ND
35	3.90	6.45	0.0283	6.32	0.105	1.64	0.0656	0.92	0.65
36	4.11	3.78	0.0100	3.67	0.099	3.53	0.0694	1.61	1.85
37	4.27	ND	ND	ND	ND	8.18	0.0926	3.02	5.07
38	4.11	40.2	0.107	39.1	1.04	4.74	0.0946	2.18	2.46
39	4.27	2.49	0.0046	2.39	0.095	5.34	0.0602	2.00	3.28
40	4.77	1.02	0.0005	0.90	0.112	2.84	0.0044	0.45	2.39
41	4.54	3.78	0.0035	3.52	0.255	4.48	0.0174	1.06	3.40
42	3.89	3.32	0.0150	3.25	0.053	1.72	0.0715	0.97	0.68
43	3.93	2.31	0.0092	2.26	0.040	1.12	0.0401	0.61	0.47
44	4.63	1.84	0.0014	1.69	0.149	59.3	0.163	12.4	46.7
45	4.60	4.43	0.0036	4.08	0.349	59.0	0.180	13.1	45.7
46	4.62	3.60	0.0029	3.30	0.296	30.2	0.0867	6.54	23.5
47	5.23	1.29	0.0002	0.95	0.342	31.1	0.0065	1.97	29.1
48	5.00	4.15	0.0012	3.43	0.724	18.8	0.0106	1.96	16.8
49	5.27	4.33	0.0006	3.11	1.22	35.3	0.0060	2.06	33.2
50	4.38	1.47	0.0020	1.40	0.068	30.7	0.228	10.0	20.4
Mean	4.17	4.68	0.0148	4.49	0.175	9.97	0.105	2.89	6.98
S.D.	0.37	7.44	0.0277	7.27	0.255	13.9	0.0841	3.06	11.4
V_{min}	3.59	1.02	0.0002	0.90	0.009	0.34	0.0044	0.22	0.11
V_{max}	5.27	40.2	0.160	39.1	1.22	59.3	0.465	13.1	46.7

S.D.: standard deviation; ND: no detectable.

coefficients (γ_{AH^-} and $\gamma_{A^{2-}}$) calculated by the Debye–Hückel equation, and the dissociation constants of the acids (K_1 and K_2; Fig. 1), as:

$$\frac{[AH^-]}{[AH_2]} = \frac{K_1}{\gamma_{AH^-} \times 10^{-pH}}$$

$$\frac{[A^2]}{[AH^-]} = \frac{K_2 \times \gamma_{AH^-}}{10^{-pH} \times \gamma_{A^{2-}}}$$

$$[AH_2] + [AH^-] + [A^{2-}] = M$$

Statistical analysis of the data was performed using the SPSS v. 10.0.6 statistical package for Windows (1999).

3. Results and discussion

The contents of maleic and fumaric acids can be determined either by total acid ($[AH_2]$) or total salt, but they were not found only as these forms in honey. So the calculation of the forms of the acids can illuminate how the acids were really found at honey pH. Table 1 shows the pH, the molar concentrations of total maleic and fumaric acids and the molar concentrations of the three forms ($[AH_2]$, $[AH^-]$ and $[A^{2-}]$) of the acids in honeys analysed. The decreasing order of the molar concentration (μmol/kg of honey) of these forms in honey were $[AH^-] > [A^{2-}] > [AH_2]$ for maleic acid and $[A^{2-}] > [AH^-] > [AH_2]$ for fumaric acid.

Maleic acid was quantifiable in 44 honey samples of 50 analysed. Fumaric acid was quantifiable in 49 honey samples. Table 2 lists the distributions of the forms of these acids in honey samples analysed.

Fig. 2(A) shows relative percentages of three forms of the maleic acid at honey pH. The $[AH^-]$ form is predominant in all samples. In Fig. 2(B) it is also obvious that when $pH < 4.05$, the predominant fumaric acid form was $[AH^-]$ and if $pH > 4.05$ the predominant form was $[A^{2-}]$. Therefore, the predominant form of maleic and fumaric acids in honey can be determined, knowing only the honey pH.

For maleic acid, a honey $pH < 1.54$ would be necessary for the $[AH_2]$ form to be predominant. A $pH < 2.74$ would be necessary in the case of fumaric acid. To the authors' knowledge, the lowest pH reported in floral honeys (White et al., 1962) is 3.42, so the $[AH_2]$ form can never be the major form of maleic and fumaric acids in honey.

Differences between *cis*-2-butenedioic acid (maleic acid) and *trans*-2-butenedioic acid (fumaric acid) were found in their correlation with pH. There was no correlation between honey pH and total maleic acid (Fig. 3A). Nervertheless, honey pH and total fumaric acid had a correlation (Fig. 3B).

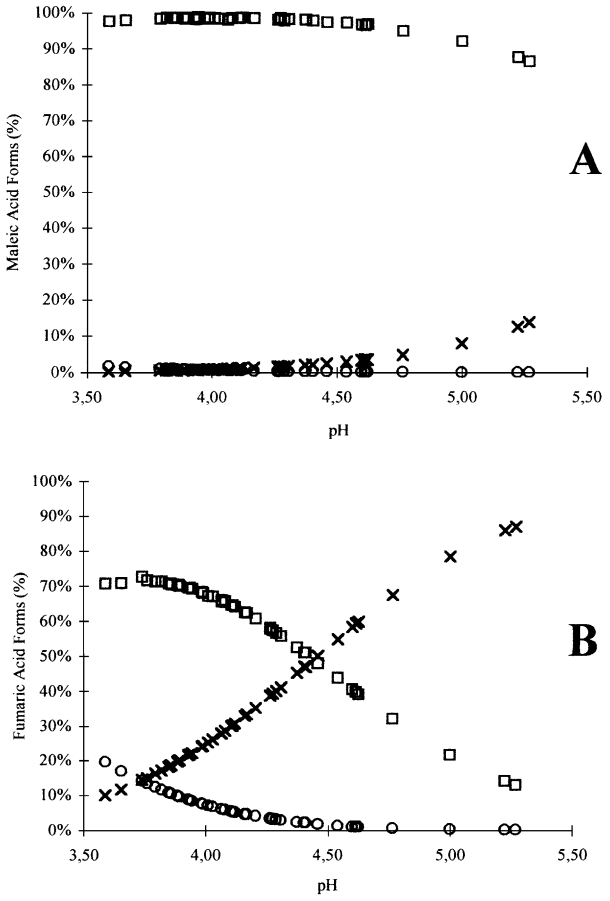


Fig. 2. Relative percentages of the three forms: $[AH_2]$ (○), $[AH^-]$ (□) and $[A^{2-}]$ (x), of the maleic acid (A) and fumaric acid (B) at honey pH.

Table 2
Distribution of the forms of maleic and fumaric acids in honey samples analysed

	Maleic acid (No. of samples)			Fumaric acid (No. of samples)		
	Major form	2nd form	Minor form	Major form	2nd form	Minor form
$[AH_2]$	0	1	**43**	0	0	**49**
$[AH^-]$	**44**	0	0	21	**28**	0
$[A^{2-}]$	0	**43**	1	**28**	21	0
Total of samples	44	44	44	49	49	49

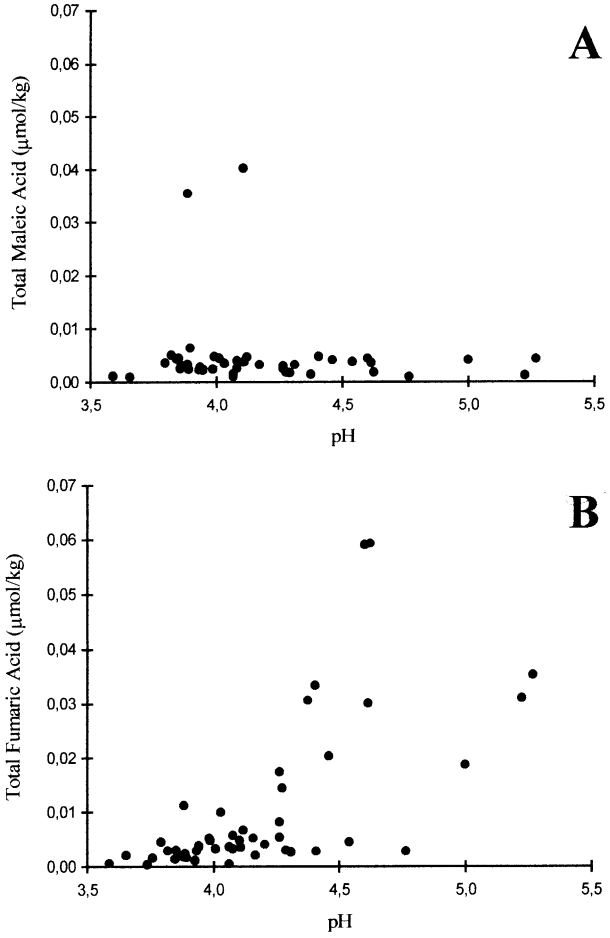


Fig. 3. Relationship of pH vs total maleic acid (A) and pH vs total fumaric acid (B) in μmol/kg of honey.

4. Conclusions

The predominant form of maleic and fumaric acids in honey can be determined knowing only the honey pH.

The [AH^-] form of maleic acid (*cis*-2-butenedioic acid) was predominant in all honeys. For fumaric acid (*trans*-2-butenedioic acid), the [A^{2-}] form was also found as a major component in most honeys (28 samples) but the [AH^-] was predominant in 21 samples. No honey analysed had the [AH_2] form as predominant.

Although maleic and fumaric acids have the same molecular weight, differences between them were found in their correlation with pH. Total fumaric acid had a correlation with honey pH but there was no correlation between honey pH and total maleic acid.

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