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Preliminary contribution to the characterisation of artisanal honey produced on the island of Ireland by palynological and physico-chemical data

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Abstract

Fifty artisanal honey samples from the island of Ireland were collected over two consecutive harvest seasons, providing two sets of 25 samples each. Parameters measured for years 1 and 2 were water content, pH, electrical conductivity, ash content, free acidity, lactonic acidity, total acidity and mineral content; those from year one had melissopalynological and those from year two had HMF analyses performed. Evidence from all parameters tested is consistent with the fact that they were generally of floral origin.

Keywords: Honey; Geographical characterisation; Melissopalynology; Physico-chemical parameters

1. Introduction

According to the Council of the European Union (2002), ''honey is the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants, which the bees collect, transform by combining with specific substances of their own, deposit, hydrate, store and leave in honeycombs to ripen and mature''. Honey consists essentially of sugars, predominantly fructose and glucose, other substances such as organic acids, enzymes (EU, 2002) and solid particles, mainly consisting of pollen, traces of wax and variable amounts of sugar-tolerant yeast (Anklam, 1998). On account of its sweetness, honey has been a highly valued food item since primitive times (Davies, 1976). As with any biological material, the specific composition depends highly on the type of flowers visited by the bees, as well as on the climatic conditions in which the plants grow (Abu-Tarboush, Al-Kahtani, & El-Sarrage, 1993). At present, there are about 2000 beekeepers in Ireland, managing 22,000 colonies of bees (Bennett, 2000). Ireland has few common plant species, only about 200 (Preston, Pearman, & Dines, 2002); however, this does not practically limit the variation in flavour and quality of honeys produced, since honey from any given floral source can vary as a result of seasonal climatic variations or geographical location (Anklam, 1998).

In order to identify the botanical and geographical origin of a given honey sample, it is necessary to determine the physicochemical (moisture, ash content, conductivity, acidity) and biological properties (melissopalynology) of that sample (Foldházi, Amtmann, Fodor, & Ittzés, 1996; Krauze & Zalewski, 1991; Mateo & Bosch-Reig, 1998; Sánchez, Huidobro, Mato, Muniategui, & Sancho, 2001; Soria, González, de Lorenzo, Martinez-Castro, & Sanz, 2004). Comparison of the composition of an unknown honey with typical ranges from a collection of identified, authentic honeys may then permit identification. Melissopalynology (pollen identification)

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has been the standard method for determining the floral origin of honey but this technique is tedious and has some limitations (Hermosin, Chicón, & Dolores Cabezudo, 2003). A particular difficulty is that melissopalynology requires previous knowledge of pollen morphology and specialised professional personnel to achieve reliable results (Cometto, Faye, Di Paola Naranjo, Rubio, & Aldo, 2003).

This present study is part of a larger project, which is investigating methods to discriminate between Irish honey and honey from other geographical locations. During the course of this project, it became apparent that, while much has been published on the characterisation of honey produced in a number of countries (De Rodriguez, Sulbarán de Ferrer, Ferrer, & Rodriguez, 2004; Golob & Plestenjak, 1999; Pérez-Arquillué, Conchello, Ariño, Juan, & Herrera, 1995; Soria et al., 2004; Terrab, Díez, & Heredia, 2003a, 2003b; Thrasyvoulou & Manikis, 1995), little corresponding information on the characterisation of honey produced in Ireland has been available in the literature. The purpose of the work reported in this paper was therefore to provide some baseline compositional data on a small number ($n = 50$) of Irish artisanal honeys as a first contribution to rectifying this deficiency. While this work was not specifically aimed at relating the composition of these honeys to EU standards (2002), comparisons have been made between the results of analyses performed and values for these analyses contained in EU legislation. In accordance with published work in other countries, analyses were performed to quantify moisture and ash contents, conductivity, acidity, pH and mineral content. Palynological analyses and hydroxymethylfurfural (HMF) determination, were also performed on a subset ($n = 25$) of these samples.

2. Materials and methods

2.1. Samples

The present study was carried out using 50 honey samples. Honeys were collected over two harvest seasons, providing two sets of 25 samples. The first set was collected during the 2000–2001 season (year 1) while the second was collected during the 2002–2003 season (year 2); all samples were analysed in 2003. The samples were stored in screw-capped plastic or glass sample jars at room temperature until required for analysis.

The thermal history of the two sample sets differed somewhat. Those sampled earlier, year 1, had undergone two periods of warm-holding (40 °C overnight) while the second set, year 2, had not undergone any warm-holding prior to analysis. As HMF is a freshness parameter (Schade, Marsh, & Eckert, 1958), adversely affected by elevated heating and storage treatments, it was thought futile to include year 1 HMF results.

2.2. Palynological analysis

Palynological analysis was restricted to samples collected during the 2000–2001 season only. It was performed by microscopical analysis according to the method of Lutier and Vaissière (1993).

2.3. Physicochemical parameters

Samples were prepared for analysis according to AOAC (1990) method 920.180. Moisture was determined with an Abbé refractometer reading at 20 °C, using the Wedmore table (AOAC, 1990). The following parameters were determined according to AOAC methods (1990): ash, electrical conductivity, free, lactonic and total acidity and pH. Hydroxymethylfurfural (HMF) was measured by a spectrophotometric method (AOAC, 1990). Mineral content was determined by ashing samples (AOAC, 1990), adding 5 ml of 0.1 N HCl to the ash of each sample and stirring the mixture on a heating plate to almost complete dryness. Ten ml of HCl (0.1 N) were added to the almost dry mixture and the solution brought up to 50 ml with distilled water. It was necessary to add 1.5 ml of lanthanum (0.1% as chloride) to solutions for calcium and magnesium determination in order to suppress other elements, which might affect results (Perkin–Elmer, 1994). Mineral content (mg/100 g) for each honey sample was then determined using an atomic absorption spectrometer (AA analyst 200 system, Perkin–Elmer, USA.)

Analysis of each sample was carried out in duplicate for each test except for mineral analysis; insufficient sample prevented a duplicate determination being performed in this case. To determine the repeatability of each test procedure, the standard deviation between duplicate (SDD) estimations was calculated. Mean and standard deviation values were also calculated for each parameter.

3. Results and discussion

3.1. Palynological analysis

Results of the qualitative pollen analysis for the 25 honey samples from year 1 are summarised in Table 1; all results are given as percentages of the total pollen content in each sample. Overall, 43 pollen types (present at levels $\geqslant 1\%$) were identified from the 25 honey samples analysed. An accurate count could not be made for sample 21 because of the very low content of pollen grains detected. This sample appeared to have been fil-

Table 1
Pollen analysis of honey samples ($n = 25$) from 2000 to 2001 season

Pollen type	Sample No.																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Acer spp.		1	2	1	2					2	1	*p*		*p*	5	1	*p*	4	4	2		6	4	2	4
Heracleum spondylium					1													*p*						*p*	
Other Apiaceae			8																						
Ilex aquifolium									*p*						1	*p*			*p*	*p*					
Catalpa sp.							1	*p*																	
Myosotis						85																			
Buddleya globosa					1				*p*							2									
Cistus sp																						1			
Carduus spp.							1	*p*	1			*p*			1		*p*							*p*	
Brassica spp.	3		*p*	2	8	1	*p*	1	2	2		1	*p*		2	9	1	3	1	2					
Rorippa sp.					3	1		*p*	1			1			*p*		*p*	2					*p*		
Empetrum nigrum														1									*p*		
Cytisus scoparius	6	3	2	1		1	1	1			1			1	1	4	4	6	2	6		1	2	2	1
Lotus spp.	1	3	4	1	2		1	16	*p*	1		38		6	*p*				7	1		1	*p*	14	2
Medicago lupulina		11	14	16	4		5		9	9	25	16	7	22	18	23	7	6	5	6		3	11	3	1
Trifolium pratense	18																					*p*			
Trifolium repens type		28	40	33	29	5	71	38	60	51	40	30	78	20	32	26	69	48	60	42		42	45	59	67
Ulex spp.			*p*		1																				
Quercus spp.	1	*p*	2						1	1					2	2		2		1		1	*p*		*p*
Castanea sativa														8	*p*			*p*		*p*		*p*			*p*
Hypericum spp.			1	*p*	1									*p*											
Aesculus hippocastanum	1			*p*	1				*p*		*p*				*p*										
Allium sp.	*p*	1	1				*p*			*p*					*p*	1							1		
Moraceae											1									*p*		*p*			*p*
Poaceae		1	1		1		1		1									1	1					*p*	*p*
Ligustrum vulgare					2	*p*		1				1					1		*p*						
Montia fontana								4	*p*																
Ranunculus spp.									1		*p*					*p*									
Anemone nemorosa					2	2			*p*	1		*p*	*p*			8	2	*p*		*p*		1			*p*
Caltha palustris				4	8	1	2	1	1	1			1	1	*p*	1			*p*						
Filipendula ulmaria	1	2	1	6	6		3	2	7	8	6	2	3	13	3	5	5	9	3	4		17	8	2	3
Malus spp.	*p*		4	1			1		1	*p*	*p*			1	3			5	2	*p*					
Prunus spp.		6	*p*	*p*	*p*				*p*	*p*		*p*	*p*		4	3		4	3	11		4	1	2	13
Rosa type								2																	
Geum sp.	1																								
Rosa spp.									*p*	*p*					1	2	2	1					*p*		
Rubus fruticosus	64	38	17	24	25	3	10	30	11		22	6	9	26	22	11	6	8	9	12		20	24	14	6
Potentilla sp.										*p*	*p*						1					*p*			
Other Fragaria sp.				8	1																				
Fragaria vesca												2			*p*	*p*								*p*	
Other Rosaceae		2	*p*							20															
Salix spp.	1	2	1	2			*p*	2	*p*	1				*p*	*p*	*p*	1	*p*		10		*p*	2		1
Verbena officinalis							2																		
Total (%) pollen	97	98	98	99	98	99	99	98	96	97	96	97	98	99	95	98	99	99	97	97	0	97	98	98	98

Results presented as percentages of total pollen.
(p = values below 1%).

tered excessively. The number of pollen types (present at levels $\geqslant 1\%$) constituting the total pollen content of each sample ranged between five (sample 13) and eighteen (sample 5).

Trifolium repens was the dominant pollen type in 19 of the 25 honeys. It was present in a total of 23 samples from a minimum of 5% to a maximum of 78% of total pollen. *T. repens* (white clover) is a very common plant on the island of Ireland and its presence in Irish honey in large amounts is to be expected. *Trifolium pratense* (red clover) was present at a significant level (18% of total pollen) in sample no. 1 (Table 1).

Rubus fructicosus (blackberry) was the second most abundant pollen type identified, being the dominant pollen in three of the 25 honeys tested. It was also found to be present in 23 samples, but in slightly lower amounts than *T. repens*, with values ranging from 3% to 64% of all pollen types present. Such large differences in percentage pollen content can be attributed to a number of factors: the amount of pollen present in the nectar

can be very variable, pollen can be filtered out in the bee's honey sac (Maurizio, 1975), or the bee may take pollen without taking nectar (Anklam, 1998).

Pollens from a number of other species were present in a large number of the honey samples, albeit at generally low levels. These included *Brassica* spp. (cabbage family), *Lotus* spp. (e.g. *Lotus corniculatus* or Bird's foot trefoil), *Malus* spp. (e.g. crab apple), *Prunus* spp. (e.g. blackthorn, wild cherry or dwarf cherry) and *Acer* species (e.g. sycamore or maples). A number of specific plant varieties were similarly represented, namely *Cytisus scoparius* (scotch broom), *Filipendula ulmaria* (meadowsweet) and *Caltha palustris* (marsh marigold). *Medicago lupulina* (black medick) was also present in 21 of the 25 honeys, at levels ranging from 1% to 25% of total pollen. All of these are relatively common plants in Ireland and their presence in the artisanal honeys is to be expected.

Some unusual pollen types were identified in this sample set. Sample 6 contained a large percentage of pollen (>85%) from a single species (*Myosotis*; forget-me-not). It was the only sample to contain pollen from this plant. It displayed both the highest ash content (0.36%) and the highest conductivity measurement (0.40 mS/cm) for year 1 results, along with the lowest free acidity measurement (39.9 meq/kg). *Catalpa* is an ornamental woody plant not common in Ireland and pollen from this plant was present in two samples (samples No. 7 and 8) although at very low levels, (1 and <1%, respectively). Sample No. 12 was found to contain mostly *Lotus* spp. (Bird's foot trefoil).

On the basis of this small sample set, therefore, it would appear that *Apis mellifera* bees in Ireland feed mainly on a diet of nectar from white clover and blackberry.

3.2. Physicochemical parameters

Table 2 summarises the mean, standard deviation, range and standard deviations between duplicates of the data obtained from analysis of the selected physicochemical parameters. Frequency distributions of each of these parameters are shown in Fig. 2(a)–(h). Table 3 summarises the mean, standard deviation and range of the data obtained for the minerals selected for analysis.

Moisture content (% w/w) is a parameter which depends on climatic conditions, season of the year and degree of maturity of any given honey sample (White, 1975). High moisture content renders honey liable to fermentation, spoilage and flavour loss, resulting in a significant decrease in quality (Costa et al., 1999). EU regulations (2002) require that not more than 20% moisture be present in any sample.

Honeys from year 1 had an average moisture content in the range 15.6% to 18.8% w/w, indicating optimum harvesting and a good degree of maturity. Year 2 moistures ranged from 16.3% to 20.6%, with one sample slightly above the upper limit (20%) laid down in Council Directive 2001/110/CE (EU, 2002). Overall, year 2 samples had slightly higher moistures than year 1, but both sets, when averaged, were within the prescribed limits. The histogram for moisture distribution illustrated in Fig. 2(a) approximates well to a normally distributed variable with a median value in the range 17.0–17.6%. The overall mean value of 17.6% w/w was high when compared to floral honeys from central Spain (16% w/w; Iglesias, de Lorenzo, del Carmen Polo, Martin-Alvarez, & Pueyo, 2004) but is similar to the mean result (17.5% w/w) obtained by Terrab et al. (2003a) when investigating Moroccan *Eucalyptus* honeys.

Table 2
Summary of honey physicochemical parameters

Samples	Moisture (% w/w)	Ash (% w/w)	Conductivity (mS/cm)	Free acidity (meq/kg)	Lactonic acidity (meq/kg)	Total acidity (meq/kg)	HMF (mg/kg)	pH
Year 1 (2000–2001), n = 25								
Range	15.6–18.8	0.07–0.36	0.17–0.40	23.8–42.1	0.2–14.9	26.8–55.9	–	3.85–4.28
Mean	17.2	0.2	0.3	32.6	4.5	37	–	4.1
SD	0.7	0.1	0.1	5.3	2.8	6.3	–	0.1
SDD	0.3	0.02	0.01	0.9	3	3.3	–	0.1
Year 2 (2002–2003), n = 25								
Range	16.3–20.6	0.03–0.46	0.11–0.48	17.1–50.9	0.3–6.3	21.2–52.4	0.4–37.3	3.75–4.61
Mean	18.0	0.2	0.3	32.6	2.1	34.7	7.0	4.1
SD	1.1	0.1	0.1	10.0	1.7	9.7	8.6	0.2
SDD	0.2	0.03	0.01	1.6	1.6	1.5	1.4	0.09
All data (Year 1 and 2), n = 50								
Range	15.6–20.6	0.03–0.46	0.11–0.48	17.1–50.9	0.2–14.9	21.2–55.9	0.4–37.3	3.75–4.61
Mean	17.6	0.2	0.3	32.7	3.4	36.1	7.0	4.1
SD	1.0	0.1	0.1	7.9	3.4	8.7	8.6	0.2
SDD	0.3	0.03	0.01	1.3	2.3	2.4	1.4	0.1

Table 3
Summary of honey mineral content (mg/100 g of honey)

Samples	Fe	Cu	Zn	Ca	Mg	Mn	Na	K
Year 1 (2000–2001), n = 25								
Range	0.17–1.32	0.14–0.23	0.16–0.85	7.93–15.15	1.89–5.33	0.17–1.02	6.01–15.8	41.0–69.3
Mean	0.7	0.2	0.3	11.3	3.2	0.4	10.2	55.5
SD	0.3	0.0	0.2	2.1	0.8	0.2	2.7	7.7
Year 2 (2002–2003), n = 25								
Range	0.25–3.63	0.10–0.23	0.17–2.25	7.49–17.54	2.01–3.93	0.09–1.00	4.13–19.6	44.7–71.4
Mean	1.0	0.2	0.7	10.8	3.0	0.4	9.3	57.7
SD	0.6	0.0	0.5	2.4	0.5	0.2	4	7.1
All data (Year 1 and 2), n = 50								
Range	0.17–3.63	0.10–0.23	0.16–2.25	7.49–17.5	1.89–5.33	0.09–1.02	4.13–19.6	41.0–71.4
Mean	0.8	0.2	0.5	11.1	3.1	0.4	9.8	56.6
SD	0.5	0.0	0.5	2.3	0.7	0.2	3.5	7.5

Ash content is a quality criterion of particular relevance for honey of stated botanical origin (White, 1978); blossom honeys have a lower ($\leqslant$0.6%) ash content than honeydew honeys ($\leqslant$1.2%). Blossom, nectar or floral honey is honey which is produced from the nectar of plants, whereas honeydew honey is that which is obtained mainly from the excretions of plant sucking insects (*Hemiperta*) on the living part of plants or secretions of living parts of plants (EU, 2002). All honeys analysed in this work had ash contents below 0.6%, indicating that they were more likely to be of floral than honeydew origin. In this study it was found that samples containing high levels of pollen from *T. repens* primarily gave lower ash contents. Samples 7, 13 and 17 (year 1) exhibited ash contents of 0.07, 0.16 and 0.11% w/w with corresponding *T. repens* pollen levels of 71%, 78% and 69%. While the frequency distribution of ash values (Fig. 2(b)) exhibits an approximately equal occurrence of ash values in the range 0.05–0.26%, the average and standard deviation ash content values obtained for honeys in each year were identical.

Electrical conductivity varies with botanical origin (Terrab et al., 2003b); floral honeys should have conductivity values below than 0.8 mS/cm, while honeydew should have values over 0.8 mS/cm. As all samples had conductivity measurements below 0.8 mS/cm; this again suggests that honeys collected in this work were of floral origin. Conductivity values ranged from 0.17 to 0.40 mS/cm (year 1) and 0.11 to 0.48 mS/cm (year 2); the distribution of these values in the total sample set (Fig. 2(c)) suggests a normal distribution with a small number of higher conductivity samples. The mean conductivity value of 0.3 mS/cm, obtained for the 50 samples in this study, is similar to the published values for Spanish honeys of 0.25 and 0.21 mS/cm, respectively, (Serra Bonhevı́ & Granados Tarrés, 1993).

Piazza, Accorti, and Persano Oddo (1991) have previously reported the existence of a linear relationship between ash content and electrical conductivity of honeys. Confirmation of this relationship, in the Irish honeys analysed, is revealed following linear regression analysis of these two variables. The regression model is characterised by a correlation coefficient R, equal to 0.88 and a standard error of prediction equal to 0.04. This regression is shown graphically in Fig. 1.

Acidity in honey is calculated as free, lactonic and total acidity. Free acidity is due to the presence of organic acids, particularly gluconic acid, which are in equilibrium with the corresponding lactones and some inorganic ions such as phosphate or sulphate. Lactonic acidity is considered as the acidity reserve when the honey becomes alkaline and total acidity is the sum of free and lactonic acidities (Terrab, Dıez, & Heredia, 2002). EU regulations (2002) specify a free acidity of not more than 50 milli-equivalents acid per 1000 g (meq/kg). The average values for free acidity in samples from year 1 were between 23.8 and 42.1 meq/kg but, in year 2, one value (50.9 meq/kg) very slightly exceeded EU limits. The mean free acidity values for the remaining year 2 honeys ranged from 17.1 to 50.9 meq/kg. Lactonic acidity ranges were from 0.2 to 14.9 meq/kg in year 1 and 0.3 to 6.3 meq/kg in year 2 samples. Total acidity ranged from 26.8 to 55.9 meq/kg and 21.2 to 52.4 meq/kg in year 1 and year 2, respectively, in agreement with reported data for honeys from other geographical locations (Costa et al., 1999; Kaushik, Joshi, & Gupta, 1993; Pérez-Arquillué, Conchello, Ariño, Juan, & Herrera, 1994). Variation in total acidity has been attributed to harvest season (De Rodriguez et al., 2004). Frequency distributions of these three acidity measurements are shown in Fig. 2(d) (free acidity), 2(e) (total acidity) and 2(f) (lactonic acidity) and they exhibit some interesting differences. Free acidity values display a normal distribution while lactonic acidity reveals an asymmetric distribution with frequencies tailing off from the highest value at the lowest acidity range. Total acidity is a

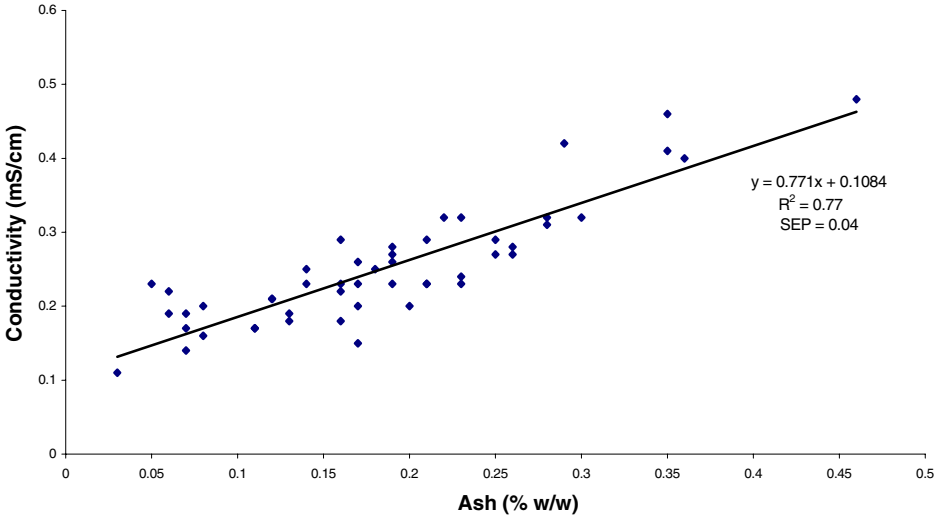

Fig. 1. Linear regression of ash content (% w/w) and conductivity (mS/cm).

summation of lactonic and free acidity so that it exhibits a distribution which is slightly skewed in favour of higher acidity ranges.

The HMF content of honey is an indicator of freshness (Schade et al., 1958). It is well known that heating of honey results in the formation of HMF, which is produced during acid-catalysed dehydration of hexoses, e.g. fructose and glucose (Belitz & Grosch, 1999). White (1994) proposed the HMF level as the only reliable heating/storage index in honey. Unfortunately, year 1 honeys had undergone some heat abuse prior to analysis, and so were not tested for HMF levels. Year 2 honeys received no heat treatment and experienced only a very short storage time prior to analysis i.e., a maximum of 6 months. No sample in this group exceeded EU regulations (2002), with values ranging from 0.4 to 37.3 mg/kg (SD equal to 8.6). Honey pH values are of great importance during extraction and storage as they influence texture, stability and shelf-life (Terrab et al., 2003a). Values recorded for this parameter in the current study ranged from 3.85 to 4.28 (year 1) and 3.75 to 4.61 (year 2). However, average pH values in each year's samples were identical at 4.1; standard deviations of 0.1 and 0.2, respectively, were recorded; the histogram illustrating these results (Fig. 2(h)) resembles a normal distribution. These pH values indicate that the honeys tested were most likely of floral origin, since honeydew honeys generally have a higher ash content than floral, resulting in honey with less active acidity and therefore a higher pH (White, 1978). The pH values recorded were similar to those obtained by Iglesias et al. (2004) for floral honeys collected in central Spain, for which a mean value of 3.9 was obtained. Similar results were also found by Persano Oddo, Piazza, Sabatini, and Accorti (1995) for Italian unifloral honeys.

Apart from the nutritional significance of minerals and the fact that they affect colour (Vorlová & Čelechovska, 2002), mineral content is also an important indicator of possible environmental pollution and a potential indicator of geographical origin of honey (Anklam, 1998). In this study, a total of eight elements were quantified, namely iron, copper, zinc, calcium, magnesium, manganese, sodium and potassium. Potassium, quantitatively, was the most abundant mineral found; it accounted for 68.6% of total minerals. Studies on honey from other geographical locations also showed potassium to be the most abundant element, albeit in greater amounts than those found in these Irish honeys. Rodriguez-Otero, Paseiro, Simal, and Cepeda (1994) found potassium (150 mg/100 g) to be the most abundant element in honeys from Galicia, Spain. Serra Bonhevı and Granados Tarrés (1993) found very large amounts of potassium (937 mg/100 g) in ling heather (*Calluna vulgaris* (L) Hull) honey produced in Spain in comparison to these Irish honeys. Calcium, sodium and magnesium levels in this study occurred at average values of 11.1, 9.8 and 3.1 mg/100 g honey, respectively. Magnesium levels were lower than those found by Terrab et al. (2003b) in Moroccan citrus honeys, for which an average value of 21.0 mg/100 g was detected. Average values for iron, zinc, manganese and copper were slightly lower than those found in the literature. Moroccan citrus honeys (Terrab et al., 2003b) contained average iron, zinc, manganese and copper levels of 6.83, 1.91, 0.45 and 0.58 mg/100 g, respectively compared to Irish levels of iron (0.8 mg/100 g), zinc (0.5 mg/100 g), manganese (0.4 mg/100 g) and copper (0.2 mg/100 g). One interpretation of this difference is that all honeys of Irish origin may be less exposed to industrial pollution than

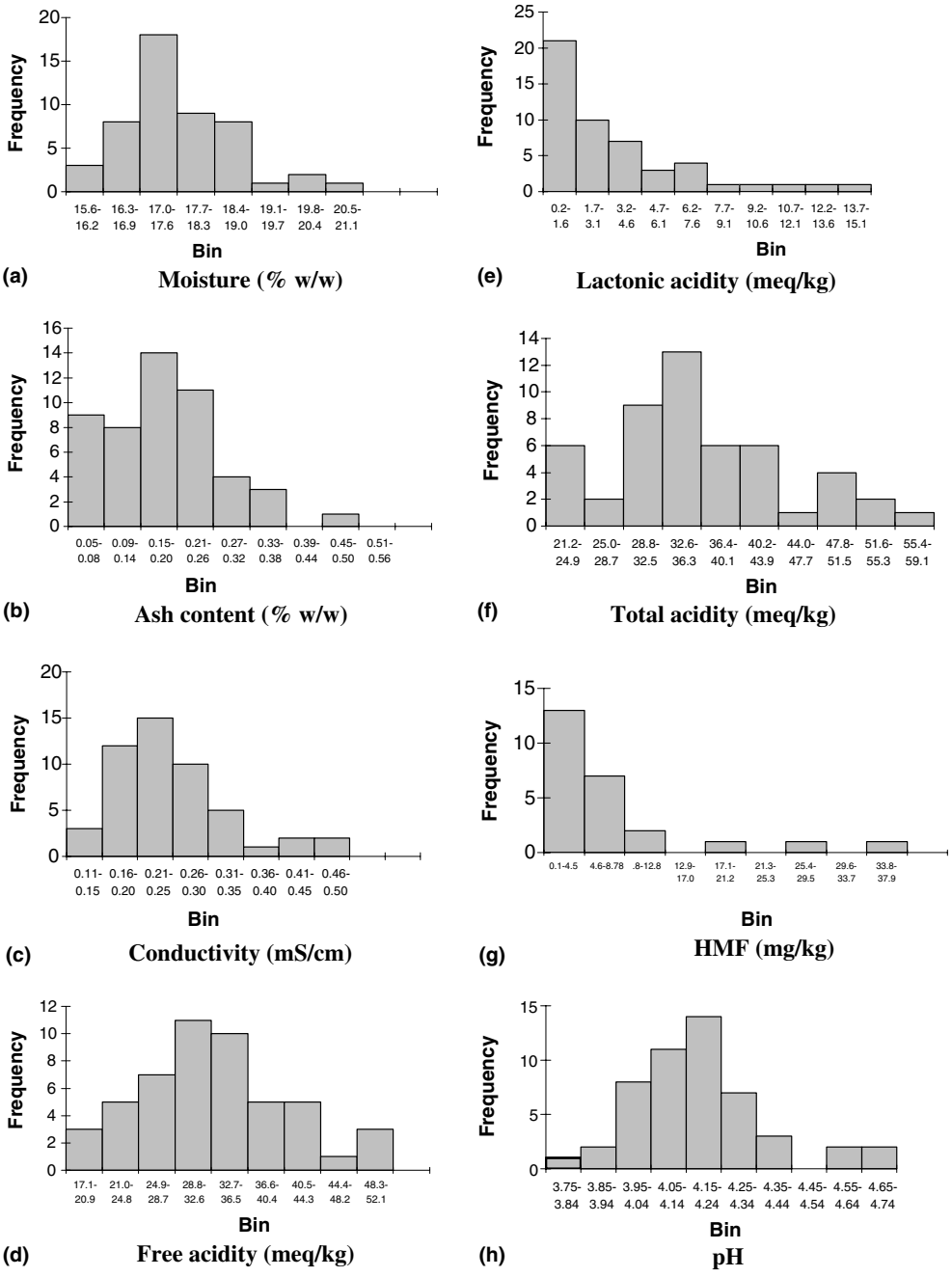

Fig. 2. Frequency distribution of physico-chemical parameters in honey samples.

those from other geographical locations; such an interpretation would be consistent with the predominantly rural character of the entire island of Ireland.

4. Conclusion

Although this work was a preliminary and limited investigation into the characterisation of Irish artisanal honeys, evidence from all parameters measured indicates that they were generally of floral origin. Not all quality and compositional parameters included in the EU honey directive (2002) were investigated. However results for the parameters tested were within the EU limits.

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