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Characterization of Commercial Honeys Produced in Australia, Malaysia, New Zealand And Thailand, Based on Physicochemical, Colour, Volatile And Antioxidant Activity Parameters

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Abstract: *The objective of the present study was to provide information for different honey types (multifloral, mixed eucalyptus, wild flower, and thyme) produced in Australia, Malaysia, New Zealand and Thailand, based on physicochemical parameters (including colour), volatile compounds, and antioxidant activity. For this purpose, five commercial honeys were collected during harvesting year 2015-2016. Physicochemical parameters (EC, ash, pH, moisture, FA, LA, L/FA, salinity, TDS) were determined using official methods of analysis, while colour attributes and antioxidant capacity were determined using CIE recommendations and spectrophotometric assays, respectively. Finally, volatile profile was estimated using HS-SPME-GC/MS.*
Results showed that honey samples analyzed met the criteria set by regulatory standards, and exhibited variations in physicochemical parameters, colour attributes, radical scavenging activity, and volatile content, according to geographical origin. Thyme honey from New Zealand, recorded the higher radical scavenging activity rate, and possessed a rich volatile pattern.
On the basis of the results obtained, it is possible to distinguish different types of commercial honeys produced in specific countries, using a data set of several physicochemical parameters.

Keywords: *commercial honey; physicochemical parameters; volatiles; colour intensity; radical scavenging activity; characterization*

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I. Introduction

Magical, divine, or nutritional could be some words to describe honey. A favorable and traditional food commodity that enhances the history of many civilizations. It is a highly concentrated solution of a complex mixture of sugars produced by honeybees, via collecting nectar from different flowers or honeydew secretions, after the addition of enzymes (mainly invertase and glucose oxidase) produced in their hypopharyngeal glands. It also contains small amounts of other nutrients such as minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids, and other phytochemicals. The latter compounds are responsible for the antioxidant activity of honey [1-3].

Over the past 30 years, there are numerous research studies in the literature that highlight chemical composition and properties of several honey types produced in different countries, Chemical composition of honey, and thus, its properties (i.e. aroma, colour, antioxidant activity), has been shown to be affected by botanical and geographical origin [2,4,5,6,7]. As a matter of fact, these variations in the chemical composition and properties of honey, has enabled a flourishing research on authentication, using instrumental and conventional methods [8-10] in combination with statistical analyses. Based on the above, the aim of the present study was to evaluate several physicochemical parameters, volatile compounds, and the antioxidant activity of commercial honeys from different countries in an effort to test whether determined parameters, could provide information about honey origin.

II. Experimental

2.1. Honey samples

A total of 5 honey samples were purchased from a local supermarket in Singapore harvesting year 2015-2016. All samples were packaged, shipped via airplane to the laboratory and maintained at 4±1°C until analysis. The botanical and geographical origin of samples analyzed, along with some nutritional data provided by the production company, is given in Table 1.

Table 1. Nutritional data (as labeled in the package) of the five commercial honey samples

Geographical Origin	Botanical Origin	Trademark	Total carbohydrates (g/100g)	Calories (kcal/100g)	Na (mg/100g)	Fe (mg/100g)	Vitamin C (mg/100g)	Sucrose (g/.100g)
(1) Australia	Blend of eucalyptus and ground flora	Capilano	np	np	np	np	np	np
(2) Malaysia	Multiflower	Green House Pure Honey	85	320	6	np	np	np
(3) Malaysia	Multiflower	Hosen Quality Pure Honey	80	350	20	10	30	5
(4) New Zealand	Thyme	New Zealand Honey Co.	85	353	7	np	np	np
(5) Thailand	Wild flower	Honey Farm	np	np	np	np	np	np

Np: not provided, cholesterol:0 mg, saturated fat:0 g, total fat:0 g, dietary fiber:0 g, calories from fat:0 g, Na: sodium, Fe: iron.

2.2. Reagents, solutions, and consumables

2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (Germany). Methanol and acetate buffer ($CH_3COONa•3H_2O$) were purchased from Merck (Darmstadt, Germany). Sodium hydroxide and hydrochloric acid (37%) used for the determination of free and lactonic acidity were purchased from Sigma Aldrich (Germany). Potassium chloride (0.1M) was obtained from Hanna (HI 7031, Hanna Instruments, Inc.,Woonsocket, USA). Whatman filters (CAT. No. 6780-2504, UK) with a pore size of 0.45 μm, were used to remove any solid particles prior to spectrophotometric measurements.

2.3. Physicochemical parameters

Physicochemical parameters such as EC, pH, moisture, free acidity (FA), lactonic acidity (LA), total acidity (TA), as well as the ratio of lactonic to free acidity (L/FA) were determined according to harmonized methods of international honey commission [11]. The conductivity meter (Delta OHM, model HD 3456.2, Padova, Italy) was calibrated with a 1413 μS/cm standard solution of 0.1M potassium chloride at 20 °C. Results were expressed as mS/cm. Each sample was run in triplicate (n=3).

2.4. Determination of ash content

The ash%, (w/w) was calculated on the basis of the results of electrical conductivity [12].

2.5. Salinity and total dissolved solids (TDS)

Salinity and total dissolved solids of a 20% (w/v) honey solution in distilled water, were measured at 20 °C using a Delta OHM, model HD 3456.2, conductimeter (Padova, Italy) with 4-ring and 2-ring conductivity/temperature probes. Temperature was measured by 4 wire Pt100 and 2 wire Pt1000 sensors by immersion. The probe was calibrated automatically resorting to the 1413 μS/cm conductivity standard solution (Hannah Instruments, Inc., Woonsocket, USA). Results were expressed as g/L and mg/L, respectively. Each sample was run in triplicate (n=3).

2.6. Determination of colour attributes: (*L, *a**, *b**), and colour intensity: ABS_{450}-ABS_{720}**

Colour parameters (*L**, *a**, *b**) were determined according to CIE recommendations [13], as shown in previous study [10]. Colour intensity was determined according to previous studies [1, 2]. The net absorbance was defined as the difference between spectrometric absorbance at 450 and 720 nm. Results were expressed as mAU. Each sample was run in triplicate (n=3).

2.7. HS-SPME-GC/MS analysis

Headspace volatile compounds were extracted from honey, using a divinyl benzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber 50/30 μm (Supelco, Bellefonte, PA, USA). Prior to use, the fiber was conditioned following the manufacturer's recommendations. Test samples were prepared daily prior to HS-SPME-GC/MS analysis. Blank runs were carried out before sample analysis to make sure that there was no contamination that could cause memory effects. Details on identification, semi-quantification, along with the GC/MS instrumentation and analysis conditions are given in a previous study [10]. Each sample was run in duplicate (n=2).

2.8. *In vitro* estimation of radical scavenging activity (% RSA)

2.8.1. Preparation of DPPH free radical standard solution

A standard solution of DPPH 15.98 x 10^{-5} mol/L (M) was prepared by dissolving 0.0063g of the free radical [DPPH•] in 100 mL methanol as described previously [2].

2.8.2. Preparation of DPPH free radical calibration curve

A calibration curve of concentration versus absorbance of [DPPH•] was prepared as follows: The 15.98x10^{-5} M solution of [DPPH•] was diluted with the addition of methanol. The resulting solutions were vortexed, left in the dark (until measurements were made) and their absorbance was measured in a UV/VIS Spectrometer (SHIMADJU, UV-1280) at λ_{max} of 517 nm. The calibration curve of absorbance (y) versus concentration (x) of [DPPH·] was expressed by the following equation $y = 0.032x + 0.0068$; $R^2 = 0.9999$ (Eq.1). Parameters such as the % decrease in [DPPH·] free radical absorbance (% RSA), % decrease in [DPPH·] free radical concentration, % [DPPH·] Remaining of the mixture obtained by the addition of honey solution when the reaction reached plateau (2h), were estimated by the above calibration curve.

2.8.3. Determination of radical scavenging activity

In order to estimate the *in vitro* radical scavenging activity of commercial honeys from Australia, Malaysia, New Zealand, and Thailand an aqueous honey solution was used, as described in a previous study [2].

2.9. Statistical analysis

In order to test the differences between the investigated parameters, with respect to honey origin, *T-test* was applied at the confidence level $p<0.05$. Statistical treatment of data was performed using the SPSS v.22.0 statistics software.

III. Results And Discussion

3.1. Physicochemical parameter and radical scavenging activity values of commercial honeys

In Table 2 are summarized the physicochemical parameter and radical scavenging activity values of the commercial honeys analyzed.

Table 2. Physicochemical parameter and radical scavenging activity values (average±SD) of the five commercial honey samples analyzed

Geogrsphical Origin	EC (µS/cm)	pH	TDS (mg/L)	Salinity (g/L)	Ash (mg/100g)	Moisture g/100g)	FA (meq/kg)	LA (meq/kg)	TA (meq/kg)	L/FA	*L** (lightness)	*a** (greenness)	*b** (yellowness)	A_{450}- A_{720} (mAU)	%RSA
(1)Australia	369±3	4.13±0.03	184±2	0.19±0.01	212±2	17.50±0.10	15.50±0.16	6.80±0.07	22.30±0.22	0.44	75.61±1.51	-2.93±0.06	10.91±0.22	144±1	7.59±0.08
(2)Malaysia	83±1	3.88±0.02	41±0.4	0.04±0.002	48±0.4	17.35±0.15	10.00±0.10	7.10±0.07	17.10±0.17	0.71	79.85±1.60	-1.78±0.04	2.84±0.06	43±0.44	na
(3)Malaysia	86±1	3.89±0.02	43±0.4	0.04±0.002	50±0.4	17.60±0.16	3.00±0.03	6.30±0.06	9.30±0.09	2.10	79.42±1.47	-1.57±0.03	3.40±0.07	46±0.50	4.05±0.04
(4)New Zealand	453±4	4.33±0.03	225±3	0.23±0.01	260±2	16.80±0.10	14.00±0.14	5.50±0.06	19.50±0.20	0.39	76.06±1.50	-4.88±0.10	20.76±0.42	205±2	23.76±0.24
(5) Thailand	210±2	3.85±0.02	105±1	0.11±0.01	121±1	18.00±0.15	8.00±0.80	6.00±0.06	14.00±0.90	0.75	79.32±1.46	-1.96±0.04	3.83±0.08	19±0.20	4.73±0.05
t	3.220	43.116	3.224	3.156	3.235	89.517	4.535	22.332	7.313	2.799	85.561	-4.30±	2.431	2.569	2.162
df	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
p	*0.032*	*<0.001*	*0.034*	*0.034*	*0.032*	*<0.05*	*0.011*	*<0.001*	*0.002*	*0.049*	*<0.001*	*0.013*	*0.072*	*0.062*	*0.199*

Na: no antioxidant activity measured. Every value is the average of three replicates (n=3). SD: standard deviation. Significant differences at the confidence level $p<0.05$ as indicated by *T-test (t: T-test values*, df: degrees of freedom).

Water represents the second major constituent of honey. Water content or moisture (g/100g) is related to different factors such as: botanical and geographical origin of the nectar, soil and climatic conditions (rainfall, relative humidity), season of harvesting, degree of maturation, beekeeper's manipulation during period of harvesting, as well as extraction, processing and storage conditions, respectively [14]. Honeys from different botanical origins, may have different moisture content, as in the present study. It is a quality parameter, related to honey shelf life. In the European regulation [15], the upper limit for moisture content in honey is adopted to be <20g/100g. A higher moisture content may result to an undesirable fermentation, due to osmophilic (sugar tolerant) yeasts and moulds. On the other hand, moisture content may affect several properties of honey such as colour, crystallization, viscosity, flavour, and density [16]. The moisture content in honeys analyzed was below

20g/100g, while it was in general agreement with Moroccan honeys [8], lower as compared to Mexican honeys [6], and higher than Greek pine honeys [2], respectively.

pH value of honey usually ranges between 3.20 and 4.50 in nectar or blossom honeys and 5.0 to 6.0, in honeydew honeys.. On the other hand, pH is not directly related to the acidity of honey, due to the presence of salts and numerous minerals [8]. Present pH values were typical of blossom honeys, being in the range reported previously for honeys originating from Greece, Mexico, Thailand and New Zealand [2, 8, 17, 18]. EC of honey is owed to its ions, organic acids and proteins and is related to the ash content [12]. Thus, the higher the above mentioned content, the higher the resulting conductivity. It is often used as the dominant criterion in the quality control of honey, in order to distinguish blossom from honeydew honeys. EC values in blossom honeys should be below 0.80 mS/cm [15]. All honeys analyzed met this criterion. Low EC values were recorded for Malaysian and Thailand honeys. However, EC values of thyme honey from New Zealand and Australian mixed eucalyptus honey, were in general agreement with previously reported results involving Mexican and New Zealand honeys [6, 18].

Ash content (g/100g) is a measure of quality that evaluates the mineral content present in honey. The mineral content may be indicative of geographical origin or environmental pollution since the content depends on the type of soil used for the growth of plants, from which the nectar was collected from honeybees [7].

FA (meq/kg) is an important parameter related to the freshness and the deterioration of honey. It is characterized by the presence of organic acids in equilibrium with lactones, internal esters and some inorganic ions (phosphates, sulfates and chlorides) as reported by Belay et al. [19]. The maximum value set by the EU [15], is 50.00 meq/kg. Higher values may be indicative of fermentation of sugars into organic acids. However, the presence of different organic acids, geographical origin and harvest season may also affect honey's acidity [7]. FA values were lower compared to the upper limit set by the EU [15]. What is remarkable, is the very low free acidity values in honey no.3 from Malaysia and no.5 from Thailand, which are the lowest values recorded until now [7,8,10-18].

LA (reverse acidity when honey becomes alkaline) and TA (sum of free and lactonic acidity) recorded variations according to the geographical and botanical origin of commercial honeys (Table 2). The same holds, for L/FA ratio. In addition, variations in LA and L/FA ratio have been also reported previously in the case of Moroccan and Turkish blossom honeys [7, 8] according to geographical and botanical origin. The L/FA ratio in Malaysian honey no.3 is the higher ever determined [8, 10].

TDS is a measure of the combined content of all inorganic and organic substances present in honey in molecular, ionized or micro-granular (colloidal solution) suspended forms. TDS content (mg/L) was lower as compared to commercial honeys produced in Algeria [20]. Finally, to the best of our knowledge, data on salinity are scarce. From the results obtained, it is clear that honey has a rather low salt content. Taking into consideration data provided by producers, that is no detected cholesterol, saturated fat, or total fat, along with the low salt content provided in the present study, honey is a special nutritious food.

3.2. Colour attributes of commercial honeys

CIE system uses three parameters to evaluate colour in several foodstuffs: colour parameter *L** corresponds to degree of brightness, parameter *a** (positive values) corresponds to degree of redness, *a** (negative values) to degree of greenness, parameter *b** corresponds to yellowness of colour (when positive) and to blueness of colour (when negative). This system stimulates the human eye observation when irritated with a specific colour. The colour of honey usually reflects: a) the content of pigments with antioxidant properties (carotenoids, phenolic acids, flavonoids, etc.), b) nectar source and pollen content, along with c) mineral content [21]. In addition processing of honey (i.e. thermal treatment, ultrasonication) affects honey colour [17]. Results showed that significant differences ($p<0.001$) in colour parameters were observed according to geographical origin (and consequently to botanical origin) among honeys analyzed (Table 2). *L** values were higher as compared to previous studies dealing with honeys from Thailand (longan flower, lychee flower, wildflower) [17], Mexican (cacao, citrus, multifloral, etc.) [6] and Greek (pine) honeys [10]. However, *a** and *b** colour parameters were much lower as compared to honeys from Thailand and Mexico, respectively [6, 17]. Colour intensity (ABS_{450}-A_{720}), was used to evaluate the contribution of coloured phytochemicals (carotenoids, flavonoids, etc.) to the overall honey colour. Such pigments are related to botanical origin of honey [1, 2]. Thyme honey from New Zealand recorded the highest colour intensity values (Figure 1).

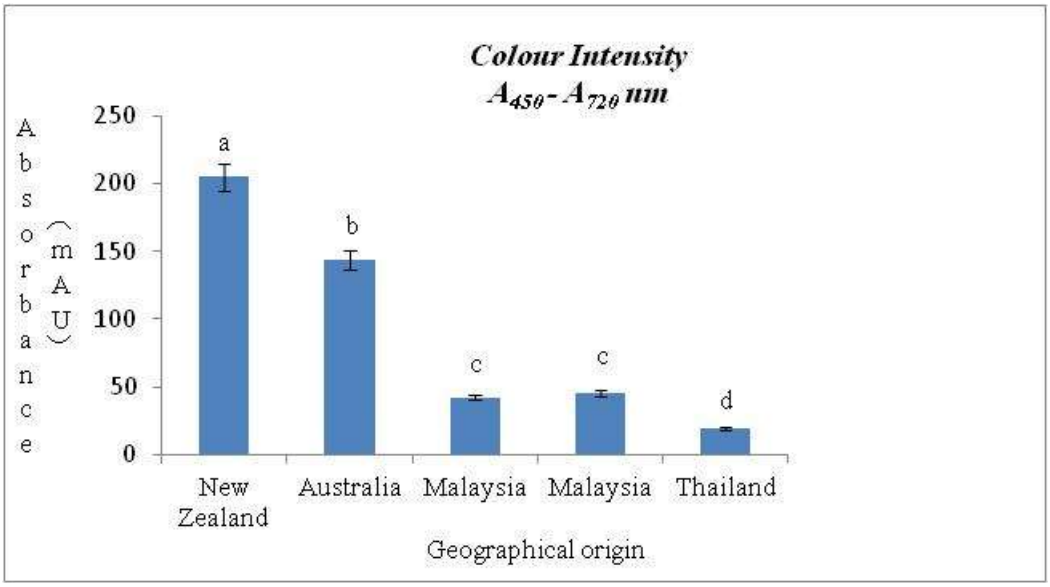

Figure 1. Colour intensity of commercial honeys according to botanical and geographical origin. Different letters (a, b, c, d) indicate statistically significant differences at the confidence level $p<0.05$.

Furthermore, the obtained results for thyme honey and wildflower honey from Thailand, are in very good agreement with the results of Beretta et al.[1] involving sylia, dandelion and chicory honeys (light colored honeys), respectively. However, values obtained from the 5 samples analyzed are lower than those reported for dark coloured honeys such as the Italian strawberry tree, Greek pine, and the Slovenian chestnut honey, respectively [1, 2, 22]. Similar variations in colour intensity (net absorbance ($Abs_{560}-Abs_{720}$), were also reported by Vela et al. [23], in a study involving Spanish honeys of different floral origins (nectar and honeydew). Respective average values (mAU) were lower in nectar honeys and higher to honeydew honeys, in very good agreement with the results of the present study. Finally, the reported ABS_{450} values for 9 Malaysian honey samples, of different botanical origins were in the range of 170-741 mAU, being higher than Malaysian, Australian, and Thailand honeys analyzed [24]. All above indicative research studies, carried out in different parts of the world (geographical origin), point out the impact of botanical origin on the colour attributes of honey.

3.3. Radical scavenging activity (% RSA) of commercial honeys

The aqueous solution of thyme honey from New Zealand, recorded the higher rate of radical scavenging activity, as compared to the other honey samples analyzed. Such a scavenging rate of [DPPH•] free radical, is in very good agreement with the results reported for Spanish light coloured honeys (nectar honeys), and Greek *Thymus capitatus* L. honeys, respectively [2, 23]. Similarly, Australian, Malaysian, and Thailandian honeys recorded a lower antioxidant ability as compared to honeys from Lithuania (linden honey) Malaysian (Borneo Tropical honey), Greek (orange blossom honeys), respectively [2, 24, 25].

In Figure 2, is plotted the radical scavenging activity of thyme honey from New Zealand, as reflected by the [DPPH•] free radical remaining rate (%), after the addition of honey water soluble antioxidants (mg/L).

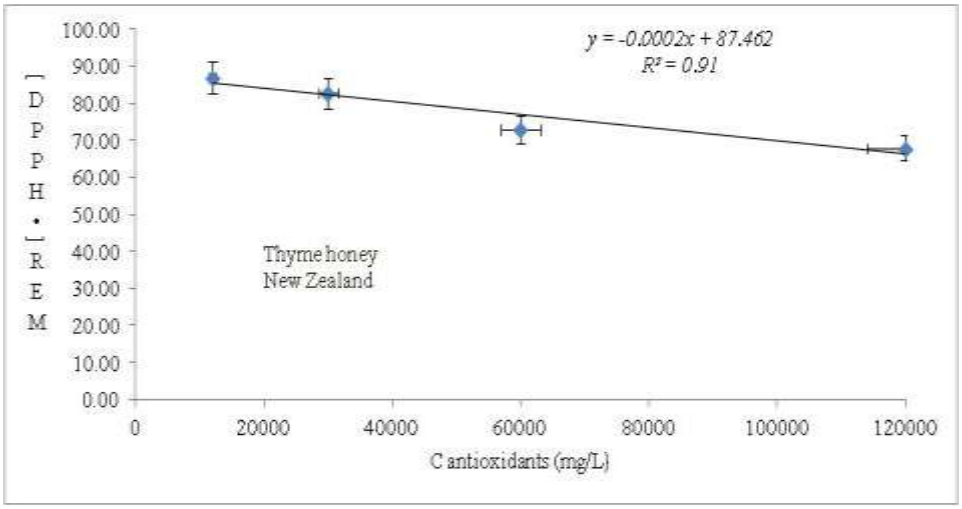

Figure 2. Radical scavenging activity (% RSA) of thyme honey aqueous solution from New Zealand.

Using above equation, *y=-0.0002x+87.462* (Eq.2), it was possible to estimate the EC_{50} value, namely the concentration of added antioxidants (mg/L) to the [DPPH•] solution, that can cause 50% radical scavenging inhibition. Respective EC_{50} value for thyme honey from New Zealand, corresponding to mg of water soluble antioxidants present in honey/0.1mL of aqueous honey solution added to the [DPPH•] free radical solution, was 18.73 mg/0.1mL.It should be stressed that, the lower the EC_{50} value, the higher the resulting antioxidant ability. Since the radical scavenging activity of Australian, Malaysian, and Thailand honey samples were lower than 10%, the EC_{50} values were not calculated/considered. Present EC_{50} values for New Zealand's thyme honey, are within the range of those reported previously regarding Greek thyme honey [2], while are lower as compared to values reported previously for Portuguese (*Erica* spp. honey) and Italian dandelion honey, respectively [1,26].

3.4. Volatile compounds of commercial honeys

Sixty three volatile compounds were identified and semi-quantified using the internal standard method [10]. These compounds comprise different classes: acids, esters, alcohols, aldehydes, ketones, terpenoids, norisoprenoids, hydrocarbons, benzene derivatives, furan and puran derivatives, etc. In Table 3 are listed the aforementioned compounds according to geographical and botanical origin of honeys.

Table 3. Volatile compounds (average±SD values, mg/kg) of commercial honeys

Number of compounds	RT	Volatile compounds	Australia	Malaysia-Green	Malaysia-Hosen	New Zealand	Thailand	KI[a]	Method of identification[b]
		Acids							
1	5.61	formic acid	nd	nd	0.59±0.11	nd	0.28±0.02	<800	MS
2	7.17	acetic acid	nd	nd	nd	nd	0.66±0.12	<800	MS
3	10.87	butanoic acid	nd	nd	nd	0.16±0.05	nd	<800	MS
4	16.24	hexanoic acid	nd	nd	nd	0.17±0.01	nd	951	KI/MS
5	19.81	3,5,5-trimethylhexanoic acid	nd	nd	0.09±0.02	nd	0.12±0.02	1110	KI/MS
6	30.57	tetradecanoic acid	nd	nd	0.04±0.01	nd	nd	1732	KI/MS
7	33.83	hexadecanoic acid	nd	nd	0.07±0.01	nd	nd	1932	KI/MS
		Alcohols							
8	7.37	2-methyl-1-propanol	nd	0.34±0.01	0.51±0.03	nd	nd	<800	MS
9	10.30	3-methyl-1-butanol	0.11±0.03	0.33±0.02	nd	nd	nd	<800	MS
10	10.43	2-methyl-1-butanol	nd	0.27±0.05	0.23±0.01	nd	nd	<800	MS
11	17.77	2-ethyl-1-	nd	nd	nd	nd	0.11±0.02	1017	KI/MS

		hexanol							
		Aldehydes							
12	8.21	3-methyl-butanal	0.21±0.06	nd	nd	nd	nd	<800	MS
13	8.47	2-methyl-butanal	0.13±0.04	nd	nd	nd	nd	<800	MS
14	13.13	furfural	0.92±0.30	nd	0.65±0.10	nd	0.54±0.10	828	KI/MS
15	16.40	5-methyl-furfural	nd	nd	0.05±0.01	nd	0.38±0.04	958	KI/MS
16	16.60	benzaldehyde	0.78±0.23	nd	nd	nd	nd	966	KI/MS
17	18.45	benzeneacetalde hyde	0.43±0.06	nd	0.12±0.01	0.61±0.06	0.19±0.01	1047	KI/MS
18	20.62	lilac aldehyde C	nd	nd	nd	0.90±0.01	nd	1149	KI/MS
19	20.93	lilac aldehyde D	nd	nd	nd	0.43±0.08	nd	1164	KI/MS
20	21.99	5-hydroxymethyl-2-furancarboxaldeh yde (HMF)	0.15±0.01	nd	4.62±0.19	nd	3.10±0.16	1218	KI/MS
21	27.03	8-octadecenal	nd	nd	nd	nd	0.16±0.01	1501	KI/MS
		Benzene derivatives							
22	11.42	methyl-benzene	0.15±0.01	nd	nd	nd	nd	<800	MS
23	17.98	Benzene, 1-methyl-4-(1-methylethyl)	0.06±0.01	nd	nd	nd	nd	1026	KI/MS
24	19.96	benzeneethanol	nd	nd	0.16±0.02	nd	nd	1117	KI/MS
		Dioxolanes							
25	10.11	2,4,5trimethyl-1,3-dioxolane	nd	nd	0.06±0.01	nd	nd	<800	MS
		Esters							
26	6.99	acetic acid ethyl ester	nd	0.23±0.01	0.57±0.03	nd	nd	<800	MS
27	9.95	butanoic acid methyl ester	0.08±0.03	nd	0.06±0.01	nd	0.04±0.01	<800	MS
28	11.46	2-methyl-butanoic acid methyl ester	nd	nd	nd	0.17±0.04	nd	<800	MS
29	15.26	hexanoic acid methyl ester	nd	nd	nd	1.04±0.24	nd	910	KI/MS
30	15.41	3-hexenoic acid methyl ester,(Z)	nd	nd	nd	0.64±0.37	nd	916	KI/MS
31	19.78	octanoic acid methyl ester	nd	nd	nd	0.62±0.15	nd	1108	KI/MS
32	21.74	3-nonenoic acid methyl ester	nd	nd	nd	0.04±0.01	nd	1205	KI/MS
33	21.79	nonanoic acid ethyl ester	0.12±0.09	nd	nd	nd	nd	1207	KI/MS
34	22.67	acetic acid,2-phenylethyl ester	nd	1.21±0.11	5.68±1.36	nd	nd	1253	KI/MS
35	23.43	4-decenoic acid methyl ester	nd	nd	nd	0.16±0.10	nd	1293	KI/MS
36	23.47	2,4-decadienoic acid, methyl ester,(2E,4Z)	nd	nd	nd	0.10±0.01	nd	1295	KI/MS
37	23.67	decanoic acid methyl ester	nd	nd	nd	0.07±0.01	nd	1306	KI/MS
		Hydrocarbons							
38	7.76	4-methyl-1,3-pentadiene	nd	nd	nd	0.35±0.01	nd	<800	MS
39	12.13	octane	0.67±0.44	nd	0.17±0.02	0.45±0.04	0.12±0.02	800	KI/MS
40	14.74	nonane	0.29±0.01	nd	0.07±0.01	0.16±0.00	0.05±0.01	900	KI/MS
41	17.13	decane	0.12±0.01	nd	0.08±0.01	nd	0.04±0.01	1000	KI/MS
42	20.16	2,6-dimethyl-1,3,5,7-octatetraene, E,E	nd	nd	nd	0.05±0.01	nd	1127	KI/MS
		Ketones							
43	8.80	1-hydroxy-2-propanone	nd	nd	0.17±0.01	nd	nd	<800	MS
44	9.76	3-hydroxy-2-butanone	0.12±0.08	nd	nd	nd	nd	<800	MS
45	14.47	2-heptanone	nd	nd	nd	0.14±0.01	nd	879	KI/MS
46	14.80	1,3-dihydroxy-2-	nd	nd	1.49±0.01	nd	nd	892	KI/MS

		propanone							
47	23.87	1-(2-aminophenyl)-ethanone	nd	nd	nd	0.09±0.01	nd	1317	KI/MS
48	25.34	3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one	nd	nd	nd	0.04±0.01	nd	1499	KI/MS
		Norisoprenoids							
49	20.22	isophorone	0.17±0.01	nd	nd	nd	0.04±0.02	1130	KI/MS
50	20.57	2-cyclohexane-1,4,-dione	nd	nd	nd	nd	0.04±0.01	1147	KI/MS
51	20.70	2-hydroxy-3,,5-trimethyl-2-cyclohexenone	0.11±0.01	nd	nd	nd	nd	1153	KI/MS
		Puran and furan compounds							
52	9.54	2,5-dimethylfuran	nd	nd	0.44±0.05	nd	nd	<800	MS
53	13.61	2-furanmethanol	nd	nd	0.75±0.05	nd	nd	846	KI/MS
54	16.83	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	nd	nd	1.38±0.10	nd	0.99±0.10	976	KI/MS
55	19.25	2-furancarboxylic acid, hydrazide	nd	nd	0.39±0.20	nd	nd	1084	KI/MS
56	25.91	2-methyl-5-(1,1,5-trimethyl)-5-hexenyl-furan	nd	nd	nd	0.99±0.03	nd	1433	KI/MS
		Sulfur compounds							
57	10.79	dimethyl disulfide	0.13±0.04	nd	nd	nd	nd	<800	MS
		Terpenoids							
58	15.92	cyclooctane, 1,4-diol	nd	nd	nd	0.10±0.01	nd	938	KI/MS
59	18.98	cis-linalool oxide	1.02±0.08	nd	nd	0.12±0.01	0.26±0.06	1071	KI/MS
60	19.32	trans-linalool oxide	nd	nd	nd	0.20±0.04	nd	1087	KI/MS
61	19.41	linalool	0.16±0.04	nd	nd	0.23±0.04	nd	1091	KI/MS
62	19.51	Ho-trienol	nd	nd	nd	12.55±2.74	0.43±0.05	1095	KI/MS
63	22.17	a-4-dimethyl-3-cyclohexene-1-acetaldehyde	nd	nd	nd	0.04±0.01	nd	1227	KI/MS
		t	3.444	1.797	2.638	1.630	2.257		
		df	63	63	63	63	63		
		p	*0.001*	*0.077*	*0.010*	*0.108*	*0.027*		

RT: retention time (min). Every value is the average of two replicates (n=2), SD: standard deviation. [a] KI: Experimental Kovats index values using hydrocarbons being naturally present in honey. [b] Method of identification: MS, identification by comparison with MS data in Wiley 7 NIST 2005 mass spectral library/ KI, identification by comparison of Kovats index with the literature cited or included in the Wiley library. Class of compounds is presented alphabetically; nd: not detected. *T-test* was carried out at the significance level $p<0.05$ *(t: T-test values*, df: degrees of freedom).

More than 600 volatile compounds have been identified in honeys originating from different geographical and botanical origins [9, 27], and comprising a complex mixture of several chemical structures serving as the '' aroma agents''. Volatile compounds may originate from the transformation of plant aroma compounds by the bees' metabolism, from heating or handling during honey processing and storage, from microbial or environmental contamination, respectively [7].

Honey aroma, along with colour and taste, are one of the most important parameters that affect the acceptability of the product by consumers. On the other hand, several volatile markers have been used to determine the geographical and botanical origin of a given honey [9, 10, 28] using chemometrics. What is remarkable is that, despite the limited samples analyzed, hence, there are distinguishable variations *($p<005$)* in the total volatile content, according to geographical origin (and botanical) of commercial honeys. It should also be mentioned, that numerous of these volatiles have been identified in several honey types from different parts of the world [6, 7, 9, 10, 27, 29]. It is worth mentioning, the high content of Hotrienol and lilac aldehydes (C,D)

in thyme honey from New Zealand, as compared to the other commercial honeys analyzed, and to previous studies involving Mexican, Turkish, Spanish, and Croatian blossom honeys [6, 7, 9, 29]. In Figure 3, is given a typical chromatogram of thyme honey from New Zealand pointing out (numbered according to Table 3) the volatile compounds determined.

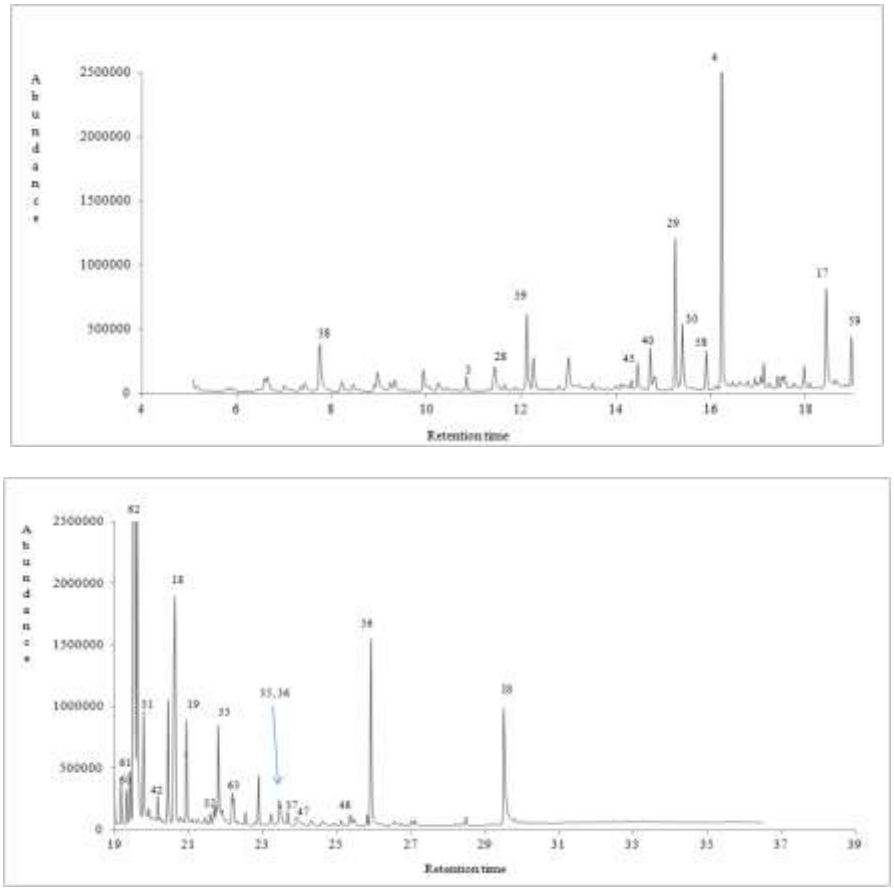

Figure 3. A typical chromatogram of commercial thyme honey from New Zealand. Possible volatile markers are numbered according to Table 3. IS: internal standard (benzophenone).

Furthermore, honey from Malaysia (no.3) contained several volatile compounds (formic acid, tetradecanoic acid, hexadecanoic acid, 2,4,5-trimethyl-dioxolane, acetic acid, 2-phenylethylester, 1-hydroxy-2-propanone) that did not determined in the other commercial honey samples analyzed.

The same holds for wildflower honey from Thailand, in which butanoic acid, 2-ethyl-1-hexanol, 8-octadecenal, and 2-cyclohexane-1,4-dione, were not determined in any other commercial honey sample. Using total volatile content (sum of individual compounds presented in Table 3) according to geographical origin of commercial honeys, and applying *T-test* ($p<0.05$), distinguishable differences were observed for honeys from Australia ($p=0.001$), Malaysia (Hosen trademark, $p=0.010$), and Thailand ($p=0.027$), respectively. In conclusion, such a distinguishable trend, gives a positive remark for future research with a larger number of commercial honey samples from the specific regions, and the application of advanced multivariate analysis to highlight specific key volatile markers of geographical and botanical origin [10, 28].

IV. Conclusions

Today there is an increasing demand from consumers, exporters, and even producers for unique agricultural products. For example, commercial honey with a documented identity, as supported by regulatory standards [15], records a higher price in the international or domestic markets. Thus, the use of different parameter analysis has the potential to provide useful information about the origin and properties of commercial honey.

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