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Floral-type identification and quality evaluation of some honey types

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Floral identification of six of the most commonly used honeys was investigated and their chemical characteristics were reported and compared with the Saudi Standards on honey. Microscopic examination confirmed the origin of the honey claimed by the manufacturer. 'Sugar-feed' honey was significantly ($P < 0.05$) the lowest in moisture and pH, but the highest in sucrose, while 'Buck thorn-sidir' was the highest in ash and pH, but the lowest in sucrose. Water-insoluble solids (WIS) as well as fructose/glucose ratio (F/G) values were nearly the same for all samples. Higher diastase activity (DIA) was found in 'Buck thorn-Zaarorah' followed by 'Pot marigold-kateefah'. However, DIA in 'Alfalfa-Berseem Higazi' was below the limit set by the Saudi Standard. Honey samples, except 'Buck thorn-Sidir' and 'Buck thorn-Zaarorah', exceeded the maximum level of hydroxymethyl furfural (HMF) set by the Saudi Standard. Individual mineral contents varied among samples and were present in abundance, particularly, phosphorus and potassium. Vitamins were generally very low and ascorbic acid was only high in 'Buck thorn-Zaarorah'.

INTRODUCTION

Honey is the sweet viscous substance elaborated by the honey bee from the nectar of plants. It is a very important energy food and is used as an ingredient in virtually hundreds of manufactured foods, mainly in cereal based products, for sweetness, colour, flavour, caramelization, pumpability and viscosity (LaGrange & Sanders, 1988).

The physical properties and chemical composition of honey have been published by many workers (White *et al.*, 1962; Siddiqui, 1970; Doner, 1977; Mesallam & El-Shaarawy, 1987; LaGrange & Sanders, 1988). The composition depends highly on the types of flowers utilized by the bee as well as regional and climatic conditions.

Some types of commercially available honey in Saudi Arabia are essentially monofloral. These include alfalfa, citrus, pot-marigold (Kateefah) and Buck thorn (Zaarorah and Sidir). Consumers in Saudi Arabia prefer honey produced from Sidir (Buck thorn) and they believe that this type of honey is superior to other types produced locally or imported from other countries around the world. Therefore, this study was conducted to investigate some of the most popular types of honey marketed in the country in terms of floral identification utilized by the bee and chemical evaluation of the products.

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MATERIALS AND METHODS

Sample collection

Commercial honey samples were purchased from a local market, Riyadh, Saudi Arabia. Representative samples were drawn and kept refrigerated until needed for analysis.

Identification of honey samples

Procedures by Wodehouse (1959), Moore and Webb (1978), and Musa (1989) were consulted for floral identification. Five grams of crude honey were centrifuged ($2000 \times g$) and pollen grains were expelled. With the aid of a brush, pollen grains were spread on a slide, a drop of water was added and impurities were removed. Pollen grains were then dehydrated in a series of alcohol solutions, 50, 70, 90 and 100%, where a drop of each concentration was put on the slide for 2 min. A drop of xylene was added and left for one minute. Slide mounting with glycerol was prepared for microscopic examination and compared with the reference for identification.

Chemical analysis

Moisture (refractometer, Atago, 2520-E02, Japan), ash (AOAC, 1984), total soluble solids (Abbe refractometer, Bausch and Lombo No. 33.45.71), water insoluble solids (WIS), pH (pH meter, Sargent-Wetch pH 6000), total acidity (meq acid/kg sample), diastase activity, and hydroxymethyl furfural (SASO, 1978*b*) were determined. Reducing sugars and sucrose were determined (AOAC, 1984) and minerals were wet-ashed (AOAC, 1984) and determined using an atomic absorption spectrophotometer (1100 B Perkin-Elmer). Phosphorus was separately determined using a modified molybdenum blue method (Abu-Lehia, 1987). Vitamins were determined according to AOAC (1984) using an LS-2B filter fluorometer (Perkin-Elmer).

High-pressure liquid chromatography (HPLC)

Sugars separation (fructose and glucose) was performed on a 0·39 cm × 30 cm carbohydrate column (Waters, Milford, MA). The mobile phase (15% water and 85% acetonitrile, HPLC grade) was introduced by a delivery pump Model 501 at a flow rate of 1·5 ml/min. The system was attached to an injector (U6K) through which a 5 μl sample was injected. The peak areas for the calibration curves and for the calculations of sugar amounts in the honey samples were measured by 820 maxima program (Waters, Milford). Sample preparation and chromatographic procedure were conducted as described in AOAC (1984).

Statistical analysis

Data were statistically analyzed using the analysis of variance (Steel & Torrie, 1980) and the differences among the means were determined for significance at 5% level using Duncan's New Multiple Range Test and SAS computer programs (SAS, 1982).

RESULTS AND DISCUSSION

The microscopic examination of flora in honey types confirmed the identity of honey source indicated by the manufacturers as listed in Table 1.

Moisture content varied due to the type of honey (Table 1). However, the difference in moisture content was not significant between 'Buck thorn-Zaarorah' and 'Pot marigold-Kafeefah' honey and also no significant difference was found between 'Citrus-Mawalih' honey and 'Buck thorn-sidir' honey in the moisture content. The moisture content for all samples was low due to the increase in the sugar content of the samples. 'Sugar-feed' honey had the lowest water content compared with the other samples. Mesallam and El-Shaarawy (1987) found that the moisture content for some locally produced honey in Saudi Arabia ranged from 13·8 to 15·6%. However, their study did not mention the type of honey. The difference in moisture content between clover honey (16·3%) and cotton honey (16·8%) was not significant as reported by El-Sherbiny and Rizk (1979). White (1978) stated that normally ripened honey has a moisture content below 18·6%. The amount of water in honey is of major importance to its stability against fermentation and granulation. It is obvious that all samples in this study had low moisture contents and in that they complied with the requirements of SASO (Table 2).

The ash content in honey types studied differed widely as shown in Table 1. 'Buck thorn-Sidir' honey had the highest ash content when compared with the other types. However, no significant difference was found between ash content of 'Buck thorn-sidir' and 'Buck thorn-kateefah' honey types. On the other hand, the ash content of 'Buck thorn-sidir' honey was seven times than that of 'citrus' honey. Honey normally has a low ash content and this depends on the material collected by the bees during foraging. Mesallam and El-Shaarawy (1987) reported 0·09 to 0·36% in some locally produced honey in Saudi Arabia. El-Sherbiny and Rizk (1979) found that the ash content of cotton honey (0·291%) was three times that of clover honey (0·096%). This confirmed our finding in that ash content depends on the floral type used by bees. All samples studied complied with the requirements of SASO (Table 2).

Water-insoluble solids content are shown in Table 1. This fraction represents suspended wax particles, insect and vegetable debris of honey (Rodgers, 1979). All samples were below the limit of 0·5% in pressed honey and 0·1% in other kinds set by SASO (Table 2).

Reducing sugars, mainly fructose and glucose, represented the largest portion of honey composition while sucrose content was below 6% in all honey samples (Table 3). Such levels are above the minimum reducing sugars of 65% and below the maximum sucrose of 10% set of SASO (Table 2). The latter limit was set to discourage heavy sugar feeding of the bees and possible adulteration of honey by the direct addition of sucrose (Rodgers, 1979). The ratio of fructose to glucose (F/G) (Table 3) indicates the tendency of crystallization; a higher or lower ratio is for liquid or granulated honey, respectively (Austin, 1958).

Total acidity varied significantly among honey types (Table 3). However, total acidity of 'citrus' honey was not significantly different from that of 'Buck thorn-sidir' honey. 'Buck thorn-Kateefah' honey had the highest total acidity, whereas 'sugar-feed' honey had the lowest value. Honey regulations depend on total acidity which can indicate the history of honey and possible alcohol fermentation and production of acetic acid by bacterial action (Rodgers, 1979). Despite the variation in total acidity among honey types studied, all total acidity values were within the limits of SASO (Table 2) except that of the 'Buck thorn- kateefah' type. Generally these values were also lower than those reported by Mesallam and El-Shaaraway (1987) for some locally produced honeys. El-Sherbiny and Rizk (1979) found that total acidity was higher in cotton honey than in clover honey. This indicates the influence of floral type in total acidity.

Table 1. Moisture, ash and water-insoluble solids (%) in honey types

Sample Number	Honey types			Moisture[a]	Ash[a]	WIS[a,b]
	Scientific name	English name	Common name			
1	*Rhamnus* spp.	Buck thorn	Zaarorah	17·50a	0·25ab	0·014
2	*Citrus* spp.	Citruses	Mawalih	16·50b	0·06c	0·014
3	*Calendula* spp.	Pot-marigold	Kateefah	18·20a	0·39a	0·003
4	—	Sugar-feed	—	13·40d	0·28ab	0·020
5	*Medicago* spp.	Alfalfa	Berseem Higazi	15·50c	0·14bc	0·035
6	*Zyziphus* spp.	Buck thorn	Sidir	16·60b	0·43a	0·0135

[a] Means not followed by the same letters are significantly different ($p < 0·05$)
[b] WIS=Water insoluble solids.

Differences among honey types in the pH values were not widely significant (Table 3). 'Buck thorn-Sidir' followed by 'Alfalfa-Barseem Higazi' had significantly higher pH values (5·4 and 4·8, respectively) than the other samples. The pH of a honey is not directly related to the free acidity because of the buffering action of the various acids and minerals present. White (1978) found that the pH of honey varied from 3·42 to 6·10.

Diastase is the most heat resistant enzyme in honey; therefore, it is usually used as an indicator of overheating. The level of diastase is dependent upon the source of honey. Honey from citrus as well as honeys produced in warmer climates contain naturally low levels of diastase (LaGrange & Sanders, 1988). The highest diastase activity was found in 'Buck thorn-Zaarorah' and all honey types except 'Alfalfa-Berseem Higazi' exceeded the limit set by SASO (Table 2). High diastase activity in some honeys indicated the absence of pasteurization of honey.

Honey sugars, particularly dextrose and fructose, are affected by temperature during extracting, liquefying or clarifying, or by age during storage. The result is the production of 5-hydroxymethyl furfuraldehyde (HMF) (Rodgers, 1979). A trace of HMF (10 mg/kg) is naturally present in honey, but a large increase of the HMF content could be due to overheating or to the adulteration of honey with commercial invert sugar (Crane, 1980). On the other hand, LaGrange and Sanders (1988) stated that honey produced in subtropical climates has a high HMF value, which exceeds 40 ppm, which is the maximum standard for HMF in the EEC. Table 3 shows the significant differences among samples in HMF content. 'Buck thorn-Sidir' honey has the

Table 2. Requirements of honey types set by Saudi Arabian Standards Organization (SASO, 1978*a*)

	Requirement	Honey type
Moisture	Not > 23%	Heather honey (calluna) and clover honey (*Trifolium* spp.)
	Not > 21%	other kinds
Ash	Not > 1·0%	Honeydew honey, blends of honeydew honey and blossom honey
	Not > 0·60%	other kinds
Water-insoluble solid content	Not > 0·5%	Pressed honey
	Not > 0·1%	other kinds
Reducing sugar content	Not < 65%	Blossom honey
	Not < 60%	honey and blossom honey
Sucrose content	Not > 10%	Honeydew honey, blends of honeydew, Blossom honey, Pubinia Lavender and Bonksia marziesii honeys
	Not > 6%	other kinds
Acidity	Not > 40% or (meq/kg)	
Diastase activity	Not < 3 (Goth scale)	Low natural enzyme content (e.g. citrus)
	Not < 8 (Goth scale)	other kinds
Hydroxymethyl furfural	Not > 15 mg/kg	Low natural enzyme content (e.g. citrus)
	Not > 40 mg/kg	other kinds

Table 3. Sugar contents, TA, PH, DIA and HMF of honey types

Sample number[a]	Sugar contents			TA meq/1000g	PH	DIA No.	HMF mg/kg
	Sucrose %	Reducing sugar	Fructose / Glucose				
1	3·74b[b]	76·8b	1·38	31·40b	4·20c	37·00a	9·6e
2	3·53bc	78·1ab	1·22	13·75d	4·01c	9·00d	32·6d
3	3·23c	77·6ab	1·18	49·00a	3·85c	33·00b	63·35c
4	5·95a	79·2a	1·32	5·35e	4·20c	18·75c	163·2b
5	1·89d	79·4a	1·31	18·05c	4·77b	6·80d	400a
6	1·85d	78·5ab	1·24	15·60d	5·40a	20·00c	7·7e

TA = Total acidity; DIA = Diastase activity; HMF = Hydroxymethylfurfural.
[a] Sample number (1–6) corresponds to those shown in Table 1.
[b] Means not followed by the same letter are significantly different ($p < 0.05$).

lowest value, whereas, 'Alfalfa-Berseem Higazi' honey had the highest value. Values exceeding 40 mg/kg set by SASO (1978*a*) could be due to high room temperatures storage which occurs in Saudi Arabia. Gonnet and Lavie (1963) indicated that room temperature storage led to an increase in HMF, whereas cool storage retarded it.

The mineral types varied among the honey samples studied. Phosphorus and potassium were the highest of all minerals (Table 4). Mesallam and El-Shaarawy (1987) found that potassium and sodium were the highest of all minerals in some honey produced in Saudi Arabia. However, they did not determine the phosphorus content in their study. If phosphorus was not taken into account our results agreed with their finding in that potassium and sodium existed in high concentration in the honey. The effect of floral type in mineral contents was also studied by El-Sherbiny and Rizk (1979) who stated that cotton honey contained higher mineral concentrations than clover honey. 'Buck thorn-Sidir' honey was higher in potassium and sodium. 'Buck thorn-zaarorah' honey was higher in phosphorus and iron, and 'sugar-feed' honey was higher in calcium compared with the other types of honey (Table 4).

Honey samples showed low levels of riboflavin and ascorbic acid and trace amounts of thiamin (Table 4). However, 'Buck thorn-zaarorah' type had a much higher concentration of ascorbic acid. Honey has measurable amounts of some vitamins, but at such low levels that they have no nutritional significance (White, 1978). Rahmanian *et al.* (1970) found that some Iranian honeys contained high concentrations of ascorbic acid (75–150 mg/100 g). The widely conflicting reports of the ascorbic acid content of honey have been mentioned by White (1978) and this could be due to interfering materials in the chemical determination.

In conclusion, the chemical characteristics of the honey types investigated in this study generally agreed with the Saudi standards. The preference of Saudi consumers to 'Buck thorn-sidir' honey is not generally justified as far as the chemical characteristics is concerned. However, the reason for the preference of 'Buck thorn' by Saudi consumers could be due to the taste and to the belief that this honey is naturally produced in the mountains and can cure many diseases.

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Table 4. Some minerals and vitamins in honey types

Sample number[a]	Minerals (ppm)					Vitamins (mg/100 g)		
	Ca	Fe	Na	K	P	Thiamin	Riboflavin	Ascorbic acid
1	5·16c[b]	8·39a	54·0d	254c	13150a	—[c]	0·03c	42·4a
2	2·82d	4·96d	10·0f	40·1f	5165c	—	0·05b	0·00f
3	13·6b	6·97b	96·9b	793b	6475b	—	0·01d	1·73d
4	27·7a	1·70e	79·6c	201d	4405c	—	0·03c	2·06c
5	3·52d	1·80e	46·1e	75·1e	3080d	—	0·08a	0·91e
6	1·50e	5·50c	133a	979a	6700b	0·01	0·06b	3·94b

[a] Sample number (1–6) corresponds to those shown in Table 2.
[b] Means not followed by the same letter are significantly different ($p < 0.05$).
[c] Trace.

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