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Quality evaluation of Portuguese honey

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Abstract

The present work was conducted to evaluate the quality of 25 brands of honey commercially available on the Portuguese market, in a total of 50 samples. The brands included unifloral and multifloral honeys, which were studied and botanically typified. Carbohydrate composition was determined by HPLC-RI to evaluate the contents of monosaccharides fructose and glucose; the disaccharides saccharose, maltose and trehalose and of the trisaccharide melizitose.

5-Hydroxymethyl-2-furfuraldehyde (HMF) was quantified by HPLC-UV and other physicochemical quality parameters were also carried out according to the European (Directive 74/409/EC) and Portuguese Regulations (Decreto-lei No. 131/85, 1985) in order to determine moisture, ash content, diastase activity, free acidity, free acidity and water-insoluble solids. All samples were organoleptically and microscopically examined.

Only 13 brands were found to meet all major national and international specifications, the remaining 12 did not agree with one or more of the analysed parameters (HMF, diastase activity, or carbohydrate composition); such observations may reflect an inadequate manufacturing or storing process and a certain ageing of these honeys.

Keywords: Honey; Sugars; Hydroxymethylfurfural; Physicochemical analysis

1. Introduction

Codex Alimentarius Commission (1981) defines honey as ''the natural sweet substance produced by honey bees from nectar of blossoms or from secretions of living parts of plants or excretions of plant sucking insects on the living part of plants, which honey bees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature''. Honey is produced in almost every country of the world and it is very important energy food. Honey cannot be considered a complete food by human nutritional standards, but it does offer potential as a dietary supplement. For infants, senior citizens and invalids, honey can be more easily digested and a more palatable carbohydrate food than saccharose by itself.

Several types of honey are produced in Portugal. Sugars represent the largest portion of honey composition (95–99% of the honey solids). Fructose and glucose are the most abundant sugars found, but others are usually mentioned, namely, saccharose, maltose, trehalose and elizitose. Its composition depends highly on the types of flowers used by the bees, as well as regional and climatic conditions (LaGrange and Sanders, 1988). The honey of world commerce varies greatly in quality, and its quality is assessed largely on the basis of colour, flavour and density.

Adulteration of honey is possible, so its quality must be controlled analytically with the aim of guaranteeing the genuity and preserving the consumer from commercial speculation. The present work was conducted to investigate the quality of 25 brands of honey commercially available on the Portuguese market, in a total of 50 samples. Carbohydrate composition was determined by HPLC-RI to evaluate the monosaccharides, fructose and glucose; the disaccharides, saccharose, maltose, and trehalose; and the trisaccharide, melizitose. Saccharose content is important to detect heavy sugar feeding of the bees or adulteration by direct addition of saccharose. The ratio fructose to glucose was also evaluated because it has been suggested that it can be used to typify honey samples from different origins, moreover it may indicate the tendency of honey to crystallise (Abu-Tarboush et al., 1993).

Honey quality can also be affected by heating during the extracting, liquefying or clarifying processes or by ageing during storage with production of 5-hydroxymethyl-2-furfuraldehyde (HMF). This compound was quantified by

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HPLC-UV. Some other physicochemical quality parameters were also investigated according to the European Legislation (Directive 74/409/EC), Official Portuguese Methods (NP-1307, Decreto-lei No. 131/85, 1985) and Codex Alimentarius Commission (1981) in order to determine moisture, ash content, diastase activity, free acidity and water-insoluble solids.

2. Materials and methods

2.1. Reagents

All reagents used were pro analysis 5-Hydroxymethyl-2-furfuraldehyde and sugars were from Sigma (Darmstadt, Germany). Methanol was gradient grade from Merck (St. Louis, MO, USA).

2.2. Sample collection

Twenty-five brands of commercial honey were randomly purchased from the Portuguese market, including 18 brands produced in different regions of Portugal, three brands from Australia, two brands from Spain, one brand from Greece and one labelled as from different countries. Two different bottles of each brand were analysed in a total of 50 samples.

The 18 brands of Portuguese honey produced in Portugal included some unifloral honeys (rosemary honey (*Rosmarinus officinalis* L.), heather honey (*Calluna*, *Erica* sp.) and sunflower honey (*Helianthus annus* L.) and multifloral honeys. Imported honeys were all multifloral.

2.3. Analysis of sediment for the identification of honey samples

The 25 brands of honey were classified according to their botanical origin using the method of Louveaux et al. (1978) as described elsewhere (Perez-Arquillué et al., 1994). Pollen grains were microscopically observed and compared with the reference for identification. Botanical classification was achieved when the pollen spectrum contained >45% of the corresponding dominant pollen. However, the pollen spectrum of rosemary honey often contains as little as 20% of *Rosmarinus* (Perez-Arquillué et al., 1994).

2.4. Physicochemical analysis

The samples of honey were analysed according to the Official Portuguese Methods (NP-1307, Decreto-lei No. 131/85, 1985) and Codex Alimentarius Commission (1981) in order to determine moisture, ash content, diastase activity, free acidity and water-insoluble solids. (Two replicate analyses were made from each sample to obtain the reported data.)

Moisture in honey was determined with an Abbe refractometer reading at 20°C obtaining the corresponding % moisture from the Chatway Table (Chatway, 1935) revised and updated Codex Alimentarius Commission (1981).

Ash percentage was measured by calcination, overnight in a furnace at 550°C, to constant mass.

Diastase activity was measured using a buffered solution of soluble starch, which fulfils the requirements of the method (Codex Alimentarius Commission, 1981), and honey which was incubated in a thermostatic bath at 40°C. Absorption was followed using a JENWAY 6105 UV/VIS spectrophotometer and a chronometer. Using regression (without using the data point at zero minutes), lines were fitted to the absorption data and the diastase number was calculated from the time taken for the absorbance to reach 0.235. For samples of low diastase activity the regression was made on the basis of the last three data points to improve the linear correlation.

In samples of high diastase activity the time taken for the absorbance to reach 0.235 was estimated with absorbances at 5 and 10, or 5, 15, and 20 min, depending on the activity. Results were expressed (as Gothe degrees) as ml of 1% starch hydrolysed by an enzyme in 1 g of honey in 1 h (Codex Alimentarius Commission, 1981).

2.5. High-performance liquid chromatography (HPLC)

The chromatograhic analyses for determination of HMF and sugars were carried out in a Gilson high performance liquid chromatograph equipped with a pump 305, a 7125 Rheodyne injector, a refractive index detector RI 132, a variable wavelength 118 UV detector and a 712 Controller Software.

For the determination of HMF a C_{18} reversed phase column, isocratic elution with methanol/water (10:90) was used, at a flow rate of 1.0 ml/min and UV detection at 280 nm.

The chromatographic separation of sugars was achieved in an amine bonded phase column (Spherisorb NH_2), using acetonitrile/water (84:16) as mobile phase, at a flow rate of 1.0 ml/min and refractive index detection (Ferreira et al., 1998).

The sample preparation in both determinations was easy, involving only dissolution in deionized water and filtration through W42 paper and thereafter, through 0.2 μm filter paper.

2.6. Organoleptical quality

In addition to the identification of honey type by microscopical analysis of sediment, the honey samples were subjected to a sensory panel. The colour, aspect, smell and flavour of each sample were qualitatively analysed.

2.7. Statistical analysis

Data are represented as the mean ± standard deviation.

Table 1
Classification of honey brands according to the respective label

Brand	Honey type	Origin
1	Multifloral	Australia
2	Multifloral	Portugal
3	Multifloral	Spain
4	Multifloral	Portugal
5	Multifloral	Portugal
6	Multifloral	Spain
7	Multifloral	Australia
8	Rosemary (*Rosmarinus officinalis* L.)	Portugal
9	Multifloral	Portugal
10	Multifloral	Portugal
11	Rosemary (*Rosmarinus officinalis* L.)	Portugal
12	Rosemary (*Rosmarinus officinalis* L.)	Portugal
13	Multifloral	Portugal
14	Multifloral	Australia
15	Multifloral	Portugal
16	Rosemary (*Rosmarinus officinalis* L.)	Portugal
17	Multifloral	Portugal
18	Rosemary (*Rosmarinus officinalis* L.)	Portugal
19	Multifloral	Many countries
20	Heather/Rosemary (*Calluna, Erica* sp.)/ (*Rosmarinus*)	Portugal
21	Heather (*Calluna, Erica* sp.)	Portugal
22	Heather (*Calluna, Erica* sp.)	Portugal
23	Sunflower (*Helianthus annus*)	Portugal
24	Heather (*Calluna, Erica* sp.)	Portugal
25	Multifloral	Greece

The results were statistically analysed by analysis of variance (ANOVA) methodology followed by Fisher's PLSD test. Differences were considered significant for $P < 0.05$.

3. Results and discussion

The microscopic examination of flora in honey types confirmed the identity of the honey source indicated by the manufacturers as listed in Table 1, except for brands 22 and 23, where pollen grains of *Erica* sp. and *Helianthus annus* L., respectively, were present, but not as the dominant pollen.

Tables 2 and 3 show the results expressed as mean (±SD) obtained from the physicochemical and carbohydrate composition analyses of honey samples, respectively.

No significant differences between the results obtained for the two bottles of each sample were obtained when determined by ANOVA methodology (differences were considered significant for $P < 0.05$).

The average moisture content ranged from 13.6 to 19.2% while current EC regulations (Directive 74/409/EC) and Portuguese legislation (Decreto-lei No. 131/85, 1985) require <21% moisture in honey for safety against fermentation.

Table 2
Analysis of some physicochemical parameters (mean ± SD) in samples of honey

Brands[a]	Moisture (%)	Free acidity (meq/kg)	Ash content (%)	Diastase activity (°C)	Water insoluble (%)	HMF (mg/kg)
1	15.4 ± 0.0	13.2 ± 0.6	0.2 ± 0.0	12 ± 1	0.01 ± 0	6.2 ± 0.2
2	16.4 ± 0.2	22.6 ± 0.0	0.2 ± 0.1	9 ± 0	0.02 ± 0	34.8 ± 1.3
3	17.3 ± 0.0	17.1 ± 0.3	0.2 ± 0.1	13.0 ± 0	0.02 ± 0	35.3 ± 0.2
4	14.9 ± 0.1	16.2 ± 0.5	0.1 ± 0.0	13 + 1	0.04 ± 0.02	9.1 ± 0.9
5	17.3 ± 0.1	15.7 ± 0.3	0.3 ± 0.7	3 ± 0	0.02 ± 0	471 ± 15.6
6	16.8 ± 0.2	25.4 ± 1.6	0.2 ± 0.0	11 ± 1	0.06 ± 0.02	74.0 ± 6.6
7	15.3 ± 0.0	12.0 ± 0.5	0.1 ± 0.0	2 ± 0	0.02 ± 0	49.9 ± 0.8
8	14.9 ± 0.1	18.3 ± 0.6	0.1 ± 0.0	14 ± 1	0.02 ± 0	15.4 ± 0.3
9	15.7 ± 0.0	13.6 ± 2.4	0.1 ± 0.0	4 ± 1	0.01 ± 0	52.5 ± 27.3
10	16.0 ± 0.1	19.8 ± 0.1	0.3 ± 0.1	9 ± 0	0.02 ± 0	16.9 ± 0.0
11	15.5 ± 0.0	31.2 ± 1.2	0.3 ± 0.0	3 ± 1	0.12 ± 0.01	57.5 ± 6.4
12	13.6 ± 0.0	13.9 ± 1.5	0.1 ± 0.0	10 ± 1	0.03 ± 0.01	29.0 ± 0.0
13	19.2 ± 0.0	17.0 ± 1.5	0.1 ± 0.0	13 ± 0	0.02 ± 0.01	20.3 ± 0.6
14	16.5 ± 0.3	16.4 ± 0.7	0.1 ± 0.0	7 ± 1	0.01 ± 0	94.9 ± 0.1
15	15.9 ± 0.0	28.8 ± 1.1	0.3 ± 0.0	8 ± 0	0.02 ± 0	19.1 ± 0.0
16	16.1 ± 0.0	19.9 ± 0.1	0.1 ± 0.0	9 ± 0	0.03 ± 0.1	62.5 ± 2.8
17	14.7 ± 0.4	13.0 ± 0.9	0.1 ± 0.0	5 ± 0	0.05 ± 0	46.9 ± 2.2
18	16.0 ± 0.0	16.9 ± 0.1	0.1 ± 0.0	5 ± 0	0.02 ± 0	59.1 ± 0.9
19	17.6 ± 0.1	14.7 ± 0.3	0.2 ± 0.0	18 ± 0	0.06 ± 0.04	12.5 ± 0.5
20	15.5 ± 0.0	19.7 ± 0.1	0.3 ± 0.0	17 ± 0	0.10 ± 0.06	1.7 ± 0.0
21	16.8 ± 0.0	38.7 ± 0.9	0.4 ± 0.0	22 ± 2	0.05 ± 0.03	70.8 ± 1.7
22	17.0 ± 0.1	29.3 ± 0.3	0.3 ± 0.0	3 ± 0	0.16 ± 0	66.3 ± 0.1
23	16.5 ± 0.3	16.7 ± 2.8	0.1 ± 0.0	2 ± 0	0.03 ± 0.01	145.5 ± 2.1
24	17.3 ± 0.3	21.5 ± 0.4	0.4 ± 0.0	18 ± 0	0.04 ± 0	8.7 ± 0.2
25	15.7 ± 0.0	25.3 ± 0.8	0.5 ± 0.0	15 ± 0	0.03 ± 0.01	13.5 ± 0.3

[a]Twenty-five brands were analysed, two samples of each brand in a total of 50 samples. Results are expressed as mean ± SD of the two samples.

Table 3
Analysis of sugar composition (mean ± SD) in honey

Brands[a]	Fructose (g/kg)	Glucose (g/kg)	Saccharose (g/kg)	Maltose (g/kg)	Threalose (g/kg)	Melizitose (g/kg)	Ratio Fructose/Glucose
1	398 ± 6.4	309 ± 9.9	14.5 ± 5.4	Traces	Traces	Traces	1.29
2	393 ± 0.0	295 ± 2.1	7.97 ± 0.60	Traces	Traces	Traces	1.33
3	370 ± 23.3	331 ± 11.3	7.30 ± 3.70	Traces	Traces	N.D.	1.12
4	362 ± 9.2	304 ± 1.4	19.9 ± 7.3	N.D.	N.D.	N.D.	1.19
5	314 ± 1.4	306 ± 2.8	Traces	N.D.	23.2 ± 4.3	N.D.	1.03
6	364 ± 6.4	343 ± 1.4	7.90 ± 1.50	N.D.	N.D.	N.D.	1.06
7	320 ± 6.4	274 ± 1.4	80.6 ± 2.3	N.D.	Traces	N.D.	1.17
8	395 ± 1.4	330 ± 5.7	N.D.	N.D.	Traces	N.D.	1.20
9	388 ± 8.5	328 ± 8.5	5.60 ± 0.70	N.D.	N.D.	N.D.	1.18
10	365 ± 1.4	305 ± 12.7	44.9 ± 2.6	10.6 ± 2.1	N.D.	N.D.	1.20
11	354 ± 16.3	323 ± 6.4	N.D.	N.D.	N.D.	N.D.	1.10
12	376 ± 5.7	314 ± 2.8	3.70 ± 0.18	N.D.	N.D.	N.D.	1.20
13	381 ± 6.4	363 ± 7.8	N.D.	N.D.	N.D.	N.D.	1.05
14	390 ± 12.0	295 ± 2.8	Traces	N.D.	23.0 ± 2.8	N.D.	1.32
15	356 ± 4.2	319 ± 3.5	N.D.	N.D.	13.5 ± 1.2	N.D.	1.12
16	377 ± 10.6	315 ± 0.0	6.60 ± 0.70	N.D.	Traces	N.D.	1.20
17	340 ± 12.0	279 ± 1.4	79.8 ± 10.9	N.D.	N.D.	N.D.	1.22
18	393 ± 8.5	300 ± 1.4	N.D.	N.D.	N.D.	N.D.	1.31
19	360 ± 7.1	322 ± 2.8	Traces	N.D.	N.D.	N.D.	1.12
20	354 ± 4.2	298 ± 1.4	11.6 ± 1.3	N.D.	13.4 ± 1.3	N.D.	1.19
21	354 ± 4.2	307 ± 3.5	Traces	N.D.	21.7 ± 2.3	Traces	1.15
22	344 ± 13.4	334 ± 11.3	Traces	N.D.	13.4 ± 4.7	Traces	1.03
23	363 ± 15.6	304 ± 7.1	Traces	N.D.	Traces	N.D.	1.19
24	371 ± 2.1	280 ± 0.7	7.10 ± 2.80	Traces	Traces	N.D.	1.33
25	350 ± 0.7	313 ± 5.9	N.D.	N.D.	9.60 ± 0.90	N.D.	1.12

[a]Twenty-five brands were analysed, two samples of each brand in a total of 50 samples. Results are expressed as mean ± SD of two samples. N.D., not detected.

Free acidity was, likewise, within limits (below 40 meq/kg) indicating an absence of undesirable fermentations. The range of values for ash content (0.1–0.5%) fell within the limit allowed for floral honeys (0.6%) and indicated the clearness of honey samples and possibly lack of adulteration with molasses.

With respect to the content of water-insoluble solids, this fraction represents suspended wax particles, and/or insect and vegetable debris in honey. All samples were below the limit of 0.5% in pressed honey and 0.1% in other types set by the EC Directive.

Diastase activity and hydroxymethylfurfural content are widely recognised parameters used for the evaluation of honey freshness. Legal regulations of the EC set a minimum value of eight on Gothe's scale for diastase activity and a maximum HMF content of 40 mg/kg. Only 13 honey brands (1, 2, 3, 4, 8, 10, 12, 13, 15, 19, 20, 24 and 25) showed an appropriate diastase number ranging from 8 to 22°C and HMF content below the allowable limit of 40 mg/kg; both parameters indicated the high degree of freshness of such honey. Nine other honey brands (5, 7, 9, 11, 14, 17, 18, 22 and 23) did not fall within the European legal regulation, neither for diastase number nor for HMF. The three remaining honey brands (6, 16 and 21) showed an appropriate diastase number but the HMF content exceeded the allowable limits.

Reducing sugars, mainly fructose and glucose, represent the largest portion of honey composition, but small quantities of other sugars are also present such as saccharose, maltose, trehalose, melizitose and others. The number obtained from adding the percentages of fructose, glucose plus maltose was above 65%, the minimum limit set by the EC for reducing sugars, in all except three brands (5, 7, and 17). The mean percentage of saccharose for two of these brands (7 and 17) was above the allowable limit of 5%, which is the maximum limit proposed by Directive 74/409/EC. The percentage of the trisaccharide melizitose was low, indicating that these are nectar honeys. The analysis of sediment showed in all samples a very low honeydew indication which explains the low melizitose content found in the samples.

The fructose/glucose ratios were widely distributed (1.03–1.33), indicating the variety of floral sources from which the honey samples originated.

With respect to the organoleptical quality, the colour of multifloral honeys varied from light amber to nearly black. The flavour of these honeys varied even more than the colour, some honeys appeared to have only a simple sweetness and attenuated aroma, others were mild, spicy, fragrant, aromatic, bitter, harsh, medicinal or occasionally objectionable. Some brands presented aroma and flavour typical of caramel (5, 11, 14, 21 and 23) which was in agreement with the high levels of HMF that each brand contained.

Rosemary honey was light-coloured, white after

crystallisation and smelled of rosemary flowers with some balsamic odour. The natural crystallisation was fine to medium coarse. This honey was light-flavoured at first taste, and tasted of wet flour after a while in the mouth (Consuelo Perez-Arquillué et al., 1994). All rosemary brands (8, 12, 16 and 18) presented these characteristics except brand 11, which had a dark amber colour and a slightly different flavour, but the analyses of sediment revealed it as a rosemary honey, owing to the predominance of pollen grains of *Rosmarinus officinalis*.

Heather honey samples (brands 21, 22 and 24) presented a dark colour, a typical strong flavour and variable crystallisation (Jackson and Burns, 1978). Brand 22 presented an attenuated flavour, which was in agreement with the sediment analyses where the pollen grains of *Ericacae* were not predominant in this brand.

4. Conclusions

The chemical characteristics of 13 of the 25 honey brands analysed in this study completely agree with the European (Directive 74/409/EC) and Portuguese regulations (NP-1307, Decreto-lei No. 131/85, 1985), indicating adequate processing, good maturity and freshness.

Twelve other honey brands did not satisfactorily agree in all characteristics established in Portuguese and European standards relative to the diastase number, HMF content or carbohydrate composition, although the other physico-chemical parameters were within the range of the allowable limits. All such observations may reflect inadequate manufacture or storage and a certain ageing of these honeys.

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References

Abu-Tarboush, H.M., Al-Kahtani, H.A., & El-Sarrage, M.S. (1993). Floral-type identification and quality evaluation of some honey types. *Food Chem., 46*, 13–17.

Chatway, H.D. (1935). Honey tables showing the relationship between various hydrometer scales and refractive index to moisture content and weight per gallon of honey. *Can. Bee J., 43*, 215.

Codex Alimentarius Commission-CAC (1981). Vol.II, 1st ed. Codex Stan. 12.

Decreto-lei No. 131/85 (Portuguese Legislation) 29 April 1985.

Directive 74/409/EC, relating to harmonisation of legislation concerning honey, J.O.E.C., No. L291-19/10/74.

Ferreira, M.R.P., Alves, A., & Ferreira, M.A. (1998). Estudo Comparativo de Metodos de avaliaçao de humidade, cinzas e açucares em mel. *Rev. Port. Farm. , 39*, 119–122.

Jackson, G.I., & Burns, E.E. (1978). Quality honey. *Texas Agric. Progress, 24*, 21–22.

LaGrange, V., & Sanders, S.W. (1988). Honey in cereal-based new food products. *Cereal Food World, 33*, 833–838.

Louveaux, J., Maurizio, A., & Vorwohl, G. (1978). Methods of melissopalynology. *Bee World, 59*, 139–157.

Official Portuguese Methods. NP1307, NP 1308, NP 1309.

Perez-Arquillué, C., Conchello, P., Arino, A., Juan, T., & Herrera, A. (1994). Quality evaluation of Spanish rosemary (*Rosmarinus officinalis*) honey. *Food Chem., 51*, 207–210.