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Variation of apparent ethanol content of unspoiled northwestern Spanish honeys during storage

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Abstract

This paper describes, for the first time, the variation of apparent ethanol content during storage of 33 honey samples. Moisture content was determined by measuring refractive index at 20°C. Apparent ethanol content was determined in a double cuvette, according to a modification of the Boehringer–Mannheim enzymatic method. Four different types of apparent ethanol evolution were observed: constant increment, increment followed by decrease, values oscillation and constant decrease.

Keywords: Honey; Apparent ethanol; Moisture content; Evolution; Storage; Galicia, Spain

1. Introduction

It is generally agreed that all honeys contain osmophilic (sugar-tolerant) yeasts in greater or lesser amounts, which could lead honey to ferment. Ethanol is a honey fermentation product, together with carbon dioxide and several volatile and non-volatile acids (Marvin, Peterson, Fred & Wilson, 1931).

Ethanol content of honey can increase during fermentation, and this is normally related to moisture content (Fabian & Quinet, 1928; Lochhead, 1933; Stephen, 1946) and crystallization (Dyce, 1931).

Cremer and Riedmann (1964, 1965) observed that in unfermented honeys, aliphatic alcohols constituted about half of the volatile compounds and included small quantities of ethanol (Duisberg, 1967).

Huidobro et al. (1994) summarized the different gas-chromatographic methods employed for ethanol determination in honeys. These authors determined primary normal alcohols as apparent ethanol in several unfermented honeys, by developing a modification of Boehringer–Mannheim (1989) enzymatic method.

The purpose of this paper is to establish, for the first time, apparent ethanol evolution values during a year of storage.

2. Material and methods

2.1. Samples

The work was carried out on 33 samples labelled "Producto Galego de Calidade-Mel de Galicia" from Galicia (northwestern Spain). The samples were harvested in autumn 1996 and stored in darkness at room temperature. Thirty-one honeys were floral and two honeys were from a mix of floral and honeydew sources. Microscopic analysis showed that the honeys were unspoiled by yeasts (Terradillos, Muniategui, Sancho, Huidobro & Simal-Lozano, 1994).

2.2. Procedure

Moisture contents of the honeys were estimated from their refractive indices at 20°C, by reference to Wedmore's (1955) revised Chataway (1932) tables (AOAC, 1990), this being the officially designated method in Spain (BOE, 1986). An ATAGO RX-1000 digital refractometer,

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comprised of a sapphire prism, a data processor and a wide-screen liquid-crystal display, was used to determine the refractive index. The sample was deposited on the prism with a plastic spoon, and removed using a pre-washed chamois and distilled water from a polyethylene wash-bottle to clean the surface of the prism. The refractometer was maintained at 20±0.1°C by circulating water from an ultrathermostatted water bath.

The content of primary normal alcohols in honeys, as apparent ethanol content, was determined by using the Boehringer–Mannheim (1989) UV-Test Ref. No. 176 290. In the presence of the enzyme alcohol dehydrogenase, ethanol is oxidized to acetaldehyde with the concomitant reduction of nicotinamide-adenine dinucleotide. The equilibrium of this reaction lies on the side of ethanol and nicotinamide-adenine dinucleotide. It can, however, be completely displaced under alkaline conditions and by entrapment of the acetaldehyde formed. In the presence of the enzyme aldehyde dehydrogenase, acetaldehyde is oxidized to acetic acid with another stoichiometric reduction of nicotinamide-adenine dinucleotide. The amount of reduced nicotinamide-adenine dinucleotide formed is stoichiometric with half the amount of apparent ethanol. Reduced nicotinamide-adenine dinucleotide is determined by means of absorbance. A modification of the Boehringer–Mannheim (1989) method was used (Huidobro et al., 1994) in which the solution of honey was employed directly, without clarification. Removal of interferences was achieved by using the solution of honey with the solution of aldehyde dehydrogenase in the reference cuvette. Then, water was added to the reference cuvette and alcohol dehydrogenase suspension to the sample cuvette. The blanks were measured, following the same procedure, with redistilled water instead of sample solution. The modified enzymatic method displayed coefficients of variation of less than 1.75% (depending on ethanol concentration) and performed well in recovery experiments (recovery 100.1%). This method is highly specific for linear primary alcohols since nonlinear alcohols do not contribute, significantly, to apparent ethanol content. Secondary, tertiary, and aromatic alcohols do not react with the enzymes used in this method.

Apparent ethanol content of honey samples was determined four times in a year. Analyses were developed at 90, 180, 270 and 360 days after extraction of samples from the honeycomb. Honeys were stored in darkness and the storing temperature in the laboratory was 20°C (15–25°C).

3. Results and discussion

The results of determination of primary normal alcohols, as apparent ethanol of the 33 samples analyzed, are shown in Table 1, together with the moisture contents of the honeys. At the end of the Table 1, mean value, standard deviation and minimum and maximum values are included.

Table 1
Moisture (%) and variation of apparent ethanol contents (mg/kg) at 90, 180, 270 and 360 days after extraction of honey samples from the honeycomb

		Apparent ethanol (mg/kg)			
Sample No.	Moisture (%)	90	180	270	360
1	15.8	15.0	21.1	20.3	17.7
2	16.0	41.0	17.3	18.4	14.9
3	16.3	15.4	23.2	23.2	18.3
4	16.5	23.7	19.0	19.3	15.3
5	16.7	23.1	31.0	30.6	28.3
6	16.8	23.2	33.4	33.3	28.5
7	16.8	15.7	22.1	20.0	17.3
8	16.9	35.3	46.8	45.8	39.2
9	16.9	13.5	21.3	19.3	15.4
10	16.9	18.7	29.8	30.9	27.7
11	16.9	16.4	32.3	45.9	37.1
12	17.0	19.0	25.0	24.1	20.3
13	17.1	24.9	30.9	30.0	24.5
14	17.2	13.5	21.7	19.7	19.2
15	17.2	35.8	46.7	48.0	41.9
16	17.2	25.6	31.6	31.8	29.3
17	17.2	36.7	43.8	45.5	36.8
18	17.2	13.6	20.6	21.2	16.8
19	17.3	26.1	32.4	32.7	27.6
20	17.4	30.5	43.8	43.1	39.1
21	17.4	29.2	40.3	40.5	34.3
22	17.5	44.2	62.1	63.7	58.2
23	17.5	32.2	44.0	44.4	29.1
24	17.6	41.9	59.4	65.9	61.9
25	17.6	35.3	51.4	50.9	45.7
26	17.8	142	198	187	173
27	18.0	32.0	37.6	36.4	31.8
28	18.1	26.5	39.0	40.5	40.7
29	18.3	31.9	19.9	17.7	14.7
30	18.5	50.1	63.9	54.4	46.2
31	18.7	25.5	45.7	68.5	209
32	18.8	31.7	43.4	43.7	36.7
33	18.8	42.4	56.2	69.9	81.7
Mean	17.3	31.3	41.0	42.0	41.8
S.D.	0.746	22.2	31.1	30.3	41.5
V_{min}	15.8	13.5	17.3	17.7	14.7
V_{max}	18.8	142	197	187	209

Moisture content (mean value) was 17.3% (15.8–18.8%). These honey moisture contents show that fermentation is rare under normal storage conditions, which has been corroborated by microscopic analysis.

In the literature, we have not found values for evolution of primary normal alcohols, as apparent ethanol, in unfermented honeys, so we have no data for comparison.

In these honeys, neither initial nor final apparent ethanol content was related to moisture content.

In the first analysis, carried out 90 days after extraction of honey from the honeycomb; only the value of sample

number 26 is far different from the rest. Apparent ethanol content of sample number 26 may be due to the quantity of osmophilic (sugar-tolerant) yeasts of this honey.

In the second analysis, carried out 180 days after extraction of honey from the honeycomb, only three samples (Nos. 2, 4 and 29) showed an apparent ethanol content lower than those of the first analysis. The other samples showed an important increase.

In the third analysis, carried out 270 days after extraction of honey from the honeycomb, the mean values as well as the maxima and minima were in the same range as those found in the previous analysis. Only samples 11 and 33 showed a significant increase in their apparent ethanol contents. Sixteen honey samples showed a slight apparent ethanol increase. Sample number 3 showed the same apparent ethanol content as in the previous analysis. The other sixteen samples showed a decrease in their apparent ethanol contents (especially samples 26 and 30). This decrease can be interpreted as a reutilization of ethanol by the honey microorganisms (Barnett, Payne & Yarrow, 1990).

In the last analysis, carried out 360 days after extraction of honey from the honeycomb, thirty honey samples showed a lower apparent ethanol content than in the previous analysis and only three samples showed an increase (Nos. 28, 31 and 33).

With regards to the apparent ethanol content in time, four types of evolution were observed (Fig. 1):

1. Apparent ethanol content increases and then decreases (Fig. 1a). This initially rather surprising behaviour occurred in most (82%) of the samples. A plausible explanation for it may be found in the work of Barnett et al. (1990), who pointed out that among the yeasts isolated from honey, that were collected in 1978 by White (White, 1978, specifically in the Chapter "Storage of Honey" in which honey fermentation is discussed), are several that can derive acetic acid from ethanol. This process may account for the decrease in ethanol content latterly observed for most of the honeys studied in the present work.
2. Significant and constant increase of the apparent ethanol content (Fig. 1b). This behaviour is followed by 9% of samples (Nos. 28, 31 and 33). In these honeys, moisture contents are higher than

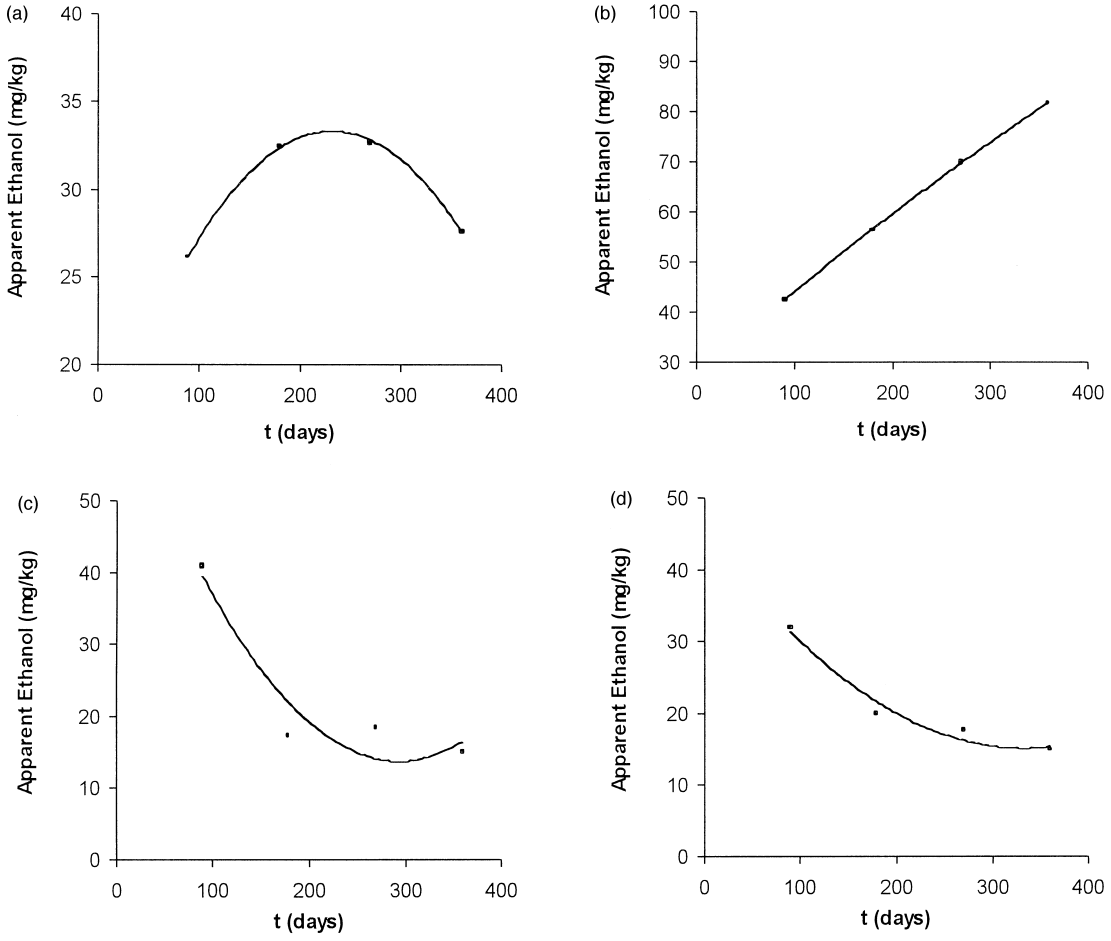

Fig. 1. Four different types of apparent ethanol evolution in Galician (northwest Spain) honey samples.

18.0%. The ethanol content of three of the six samples, with moisture content higher than 18.0%, evolved in this way, suggesting that, not only the nature of the yeasts present in the honey, but also its moisture content, are important factors increasing apparent ethanol content.

3. Apparent ethanol content oscillates (samples 2 and 4), decreasing, then increasing slightly, and finally decreasing again (Fig. 1c). This behaviour (followed by 6% samples) suggests the symbiotic coexistence of fermenting yeasts and alcohol-consuming yeasts in the honeys. The predominant yeast varied in the course of the study.
4. Apparent ethanol content decreases steadily (3% samples), as in sample 29 (Fig. 1d). In this honey, ethanol-consuming yeasts already predominate 90 days after extraction of honey from the honeycomb.

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