Aging of Honey

M. Teresa Sancho, Soledad Muniategui, José F. Huidobro,* and J. Simal Lozano

Area de Nutrición y Bromatología, Facultad de Farmacia, 15706 Santiago de Compostela, La Coruña, Spain

Diastase number and hydroxymethylfurfural content were determined for 115 honeys at 4, 16, and 28 months after their extraction. The mean values for all samples show linear relations between diastase number and the logarithm of the time and between the logarithm of the hydroxymethylfurfural content and the time. In these honeys, the long-term effects of heat and storage affect the increase in hydroxymethylfurfural content to a greater degree; however, given the nature of some samples, the diastase number is the limiting factor for freshness in the short term. The factors determining the freshness date of the honeys were also studied and suggestions made for a quality control rule.

INTRODUCTION

The composition of honey changes with time, which leads to darkening and loss of aroma and flavor.

The diastase number and the hydroxymethylfurfural (HMF) content are the two parameters used to evaluate the freshness of this food and together can be used to judge the processing and storage conditions of honey.

The Codex Alimentarius Commission (1969), on the basis of work by Hadorn et al. (1962) and Renner and Duisberg (1968), has set minimum values for diastase number (considering honeys with low natural diastase activity as a special case) and maximum values for the HMF content: "Degree of freshness after treatment and mixing: Diastase activity: no less than 8 on Gothe's scale. Honeys with low enzymatic content, no less than 3 on Gothe's scale, as long as hydroxymethylfurfural content does not exceed 15 mg/kg. Hydroxymethylfurfural: No more than 40 mg/kg."

The present colorimetric method for diastase number determination using spectrophotometry, according to Schade et al. (1958), Hadorn (1961), and White (1959 and 1964), is based on Kiermeier and Koberlein's (1954) development of Gothe's (1914) original method and forms the official method of the Codex Alimentarius Commission (1969), the AOAC (1984), and Spain (BOE, 1986). The procedure has formed part of work published by Dahle et al. (1983), Huidobro and Simal (1984a), Dustman et al. (1985), and Thrasyvoulou (1986).

To determine HMF content, White (1979) proposed a spectrophotometric method in the ultraviolet, where the absorption of the sample is measured against that of a control in which the carbonyl bond in HMF has been removed by the addition of sodium bisulfite. It gives results much in keeping with those of Winkler (1955); direct ultraviolet measurements give much higher values. White's (1979) method is official in the AOAC (1984) and in Spain (BOE, 1986). Huidobro and Simal (1984b) showed White's (1979) method to be more precise than Winkler's (1955). The HMF content has been measured by others (Pichler et al., 1984; Dustmann et al., 1985; Chepurnoi et al., 1987). Marini and Righi (1985), Wootton and Ryall (1985), and other scientists have determined the HMF content by HPLC method.

Variations of HMF contents and diastase number during storage have been studied by Vorwohl (1980), Pichler et al. (1984), Dustmann et al. (1985), Bosch and Serra (1986), Thrasyvoulu (1986), Chepurnoi et al. (1987), and others.

Invertase content is more sensitive and invertase reacts more accurately to overstorage, heat, and other physical influences than diastase (Dustmann et al., 1985). In this work, only the diastase number has been determined, because the Codex Alimentarius Commission (1969) and different honey standards legislations have set diastase as the only enzyme for evaluating the degree of freshness of honey.

The three purposes of this work have been (1) to study the aging of honeys stored at room temperature, (2) to estimate the out of freshness date according to the limits for diastase number and HMF content set by the Codex Alimentarius Commission (1969), and (3) to suggest new limits of diastase number and HMF content for a Basque honey quality norm bearing in mind the current legislation (Codex Alimentarius Commission, 1969).

MATERIALS AND METHODS

Samples. The study was carried out on 115 honeys of the Basque Country (Spain), provided by the beekeepers. The samples were harvested in September 1987. The pollen analysis of these honeys showed that 35 samples were Ericaceae honeys (numbers 1, 2, 3, 7, 10, 11, 13, 41, 46–48, 64, 70, 72–79, 81, 83, 87, 93, 94, 97–99, 101, 102, 104, and 106–108), 17 samples were *Eucalyptus* sp. honeys (numbers 4, 8, 9, 15, 16, 18, 19, 21, 23, 25, 26, 29, 31–34, and 36), 5 samples were Leguminosae type *Trifolium* L. sp. honeys (numbers 35, 91, 109, 110, and 113), 2 samples were *Castanea sativa* Miller honeys (numbers 12 and 61), 1 sample was Rosaceae type fruit-bearing honey (number 49), 1 sample was *Rubus* sp. honey (number 56), 1 sample was *Lotus* sp. honey (number 84), 1 sample was Ericaceae and Leguminosae type *Trifolium* L. sp. honey (number 92), with 47.5% of Ericaceae pollen and 45% of Leguminosae type *Trifolium* L. sp. pollen, and 52 samples were polyfloral honeys (Sancho et al., 1991).

The samples were unpasteurized, although some had been heated to facilitate their extraction. The honeys were analyzed in January 1988, January 1989, and January 1990. The average storing temperature in the laboratory was 20 °C (15–25 °C).

Diastase Number. The procedure of Schade et al. (1958), modified by Hadorn (1961) and White (1959 and 1964), was used. The diastase induced hydrolysis in a starch solution (Merck Art. 1252), which fulfills the requirements of the method [AOAC (31.162–31.167), 1984; BOE, 1986]. Solutions were thermostated in a Haake-type NBS ultrathermostatic bath. Absorption was followed using a Hitachi 100-60 UV–vis double-beam spectrophotometer and a Hanhart 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

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Table I. Diastase Numbers on Gothe's Scale Measured at 4, 16, and 28 Months after Extraction (DN 4, DN 16, and DN 28, Respectively) for the Honeys of the Basque Country (Spain), the Type of Fit (Linear, Logarithmic, or Asymptotic) for the Evolution of the Parameter, and the Time Calculated for the Diastase Number of Each Sample To Reach 8 (T_8)

sample	DN 4	DN 16	DN 28	type	T_8, years	sample	DN 4	DN 16	DN 28	type	T_8, years
1	30.9	14.7	13.6	logarithmic	3.5	61	16.9	13.2	13.1	logarithmic	>5
2	27.1	13.3	7.2	logarithmic	2.2	62	13.8	13.5	9.1	asymptotic	2.6
3	21.2	3.1	1.0	logarithmic	1.1	63	26.8	17.3	14.5	logarithmic	>5
4	20.4	16.7	13.4	linear	4.2	64	27.6	14.9	7.3	logarithmic	2.4
5	23.7	15.7	14.7	logarithmic	>5	65	23.6	20.3	15.4	linear	4.2
6	31.3	17.8	8.4	logarithmic	2.7	66	21.5	21.3	20.3	linear	>5
7	17.1	10.3	6.2	logarithmic	1.8	67	27.4	26.8	24.4	linear	>5
8	18.9	17.7	16.4	linear	>5	68	24.7	23.1	20.0	linear	>5
9	22.5	19.9	16.7	linear	>5	69	17.2	13.4	12.7	logarithmic	>5
10	5.2	3.4	1.7	linear	0.0	70	12.5	11.6	3.3	asymptotic	1.8
11	9.8	4.3	1.9	logarithmic	0.5	71	25.7	10.2	7.7	logarithmic	2.0
12	11.4	9.8	7.3	linear	2.1	72	20.6	9.7	7.2	logarithmic	1.9
13	20.6	9.2	6.0	logarithmic	1.7	73	22.2	10.9	9.8	logarithmic	2.6
14	26.3	13.7	13.6	logarithmic	4.1	74	13.8	4.1	2.9	logarithmic	0.8
15	30.1	17.2	14.3	logarithmic	4.5	75	6.6	3.8	1.6	linear	0.0
16	19.2	16.4	15.1	logarithmic	>5	76	15.7	8.7	6.5	logarithmic	1.6
17	33.4	20.4	19.6	logarithmic	>5	77	34.4	21.2	13.1	logarithmic	4.1
18	37.6	24.0	22.2	logarithmic	>5	78	15.3	3.5	3.3	logarithmic	0.9
19	22.3	16.4	16.1	logarithmic	>5	79	33.3	20.5	8.0	linear	2.3
20	21.1	18.5	14.5	linear	4.4	80	25.1	20.2	16.0	linear	4.1
21	33.7	20.7	19.3	logarithmic	>5	81	18.4	12.1	4.7	linear	1.9
22	28.9	17.1	13.2	logarithmic	4.3	82	31.2	22.9	12.8	logarithmic	>5
23	30.0	21.6	19.7	logarithmic	>5	83	18.2	7.6	4.8	logarithmic	1.4
24	50.1	19.6	16.5	logarithmic	3.2	84	19.3	16.5	13.3	linear	4.1
25	20.8	14.6	12.6	logarithmic	>5	85	27.4	27.0	19.7	asymptotic	3.9
26	20.9	17.8	16.1	logarithmic	>5	86	27.6	27.2	20.3	asymptotic	>5
27	31.9	25.5	21.2	logarithmic	>5	87	26.4	18.3	15.3	logarithmic	>5
28	37.1	20.4	20.3	logarithmic	>5	88	40.7	39.1	30.9	asymptotic	>5
29	21.7	18.8	15.9	linear	>5	89	16.7	16.4	9.5	asymptotic	2.6
30	21.9	18.0	14.3	linear	4.0	90	26.1	22.1	14.0	linear	3.4
31	24.7	19.1	16.7	logarithmic	>5	91	21.1	18.2	6.9	linear	2.4
32	51.6	16.9	16.6	logarithmic	2.9	92	12.5	3.7	2.3	logarithmic	0.7
33	21.0	17.6	16.9	logarithmic	>5	93	35.1	26.4	24.7	logarithmic	>5
34	33.7	23.4	18.8	logarithmic	>5	94	16.7	4.1	3.2	logarithmic	1.0
35	20.5	12.5	8.5	logarithmic	2.6	95	19.1	12.6	11.4	logarithmic	4.8
36	36.9	25.1	19.8	logarithmic	>5	96	46.2	38.8	33.5	logarithmic	>5
37	36.9	23.3	18.5	logarithmic	>5	97	14.7	5.1	2.2	logarithmic	0.9
38	14.6	1.8	1.7	logarithmic	0.8	98	41.3	33.5	30.9	logarithmic	>5
39	35.3	22.8	16.6	logarithmic	>5	99	44.8	34.9	30.9	logarithmic	>5
40	26.4	17.4	16.8	logarithmic	>5	100	26.4	21.4	17.8	logarithmic	>5
41	15.8	7.4	2.4	logarithmic	1.1	101	15.7	11.9	4.8	linear	1.9
42	28.8	15.0	12.0	logarithmic	3.3	102	26.2	15.8	9.5	logarithmic	3.0
43	58.7	30.3	25.1	logarithmic	>5	103	31.8	30.5	29.7	logarithmic	>5
44	27.0	21.2	18.6	logarithmic	>5	104	13.7	6.5	5.0	logarithmic	1.1
45	14.3	13.3	11.0	linear	4.3	105	32.5	29.6	23.7	linear	>5
46	22.1	11.4	6.3	logarithmic	2.0	106	24.3	20.2	16.9	logarithmic	>5
47	19.7	8.8	5.3	logarithmic	1.6	107	19.2	18.2	14.7	linear	>5
48	17.1	7.6	3.3	logarithmic	1.2	108	25.9	24.5	21.0	linear	>5
49	43.9	21.3	13.9	logarithmic	2.5	109	27.7	25.9	24.5	logarithmic	>5
50	40.7	28.0	22.4	logarithmic	>5	110	23.6	15.1	13.2	logarithmic	>5
51	17.1	12.8	12.7	logarithmic	>5	111	33.8	29.3	28.5	logarithmic	>5
52	20.3	15.0	13.3	logarithmic	>5	112	34.1	29.1	26.5	logarithmic	>5
53	34.9	26.9	17.0	logarithmic	>5	113	34.0	28.0	27.1	logarithmic	>5
54	23.1	18.8	18.4	logarithmic	>5	114	34.5	33.4	25.6	asymptotic	4.6
55	15.0	14.3	13.5	linear	>5	115	35.7	35.2	30.5	asymptotic	>5
56	27.1	21.5	17.5	logarithmic	>5						
57	28.4	22.0	20.3	logarithmic	>5	$\bar{x}$	25.6	18.1	14.5		
58	16.5	16.3	11.3	asymptotic	3.2	S_{n-1}	9.61	8.16	7.74		
59	31.9	30.0	24.5	asymptotic	>5	V_m	5.2	1.8	1.0		
60	30.7	30.2	21.7	asymptotic	3.9	V_M	58.7	39.1	33.5		

taken for the absorbance to reach 0.235 was estimated with the absorbances at 5, 5, and 10 or 5, 10, and 15 min, depending on the activity.

Hydroxymethylfurfural. Determination is as per White's (1979) method. Honey samples are divided into two clarified aliquots. Water is added to one, and the ultraviolet absorption in the solution due to the presence of HMF is then read against that of a solution in which this absorption had been destroyed by the addition of sodium bisulfite solution to break the double conjugated bond responsible [AOAC (31.152–31.155), 1984; BOE, 1986].

A Hitachi 100-60 UV–vis double-beam spectrophotometer was used.

RESULTS AND DISCUSSION

Tables I and II, respectively, show the 4-, 16-, and 28-month values of diastase number and HMF content for each sample.

(A) Aging Study. All of the 4-month values lie within the legal requirements as to diastase number and HMF content; at 16 months only one sample (number 38, diastase number less than 3) fails the norm. At 28 months, 61 honeys were not fresh.

The values of diastase number show three types of temporal behavior: (a) linear relation between diastase number and logarithm of time [79 samples (68.7%) in

Table II. Hydroxymethylfurfural (HMF) Content (mg/kg) Measured at 4, 16, and 28 Months after Extraction (HMF 4, HMF 16, and HMF 28, Respectively) of the Samples and the Time (Years) for Each Sample To Reach a Value of 40 mg/kg of HMF (T_{40})

sample	HMF 4	HMF 16	HMF 28	T_{40}	sample	HMF 4	HMF 16	HMF 28	T_{40}
1	10.6	19.9	42.7	2.3	61	8.5	11.0	13.3	>5
2	0.0	6.5	23.6	2.7	62	14.4	26.9	49.6	2.0
3	0.0	2.1	19.8	2.6	63	18.8	37.2	57.6	1.6
4	5.3	15.7	36.3	2.4	64	0.0	4.7	22.3	2.7
5	3.4	12.6	35.6	2.4	65	8.1	16.3	26.1	3.0
6	5.3	15.7	49.4	2.2	66	6.5	22.5	46.3	2.1
7	3.8	20.6	34.8	2.3	67	2.9	10.8	26.9	2.6
8	9.5	13.7	69.5	2.1	68	9.8	26.8	53.7	1.9
9	4.3	28.5	37.5	2.1	69	7.0	12.4	36.2	2.6
10	0.9	3.6	24.8	2.7	70	0.0	7.8	34.3	2.4
11	0.1	5.1	30.0	2.3	71	3.1	12.7	38.7	2.3
12	7.0	18.9	44.8	2.2	72	8.9	18.8	47.2	2.2
13	13.0	27.7	48.4	2.0	73	1.4	7.8	25.4	2.6
14	7.6	17.4	32.1	2.6	74	0.0	0.0	19.0	2.6
15	3.5	10.0	28.5	2.7	75	0.0	0.5	24.1	2.5
16	3.6	12.2	26.0	2.7	76	0.9	8.0	25.7	2.5
17	1.7	7.9	18.3	2.9	77	0.6	8.3	24.8	2.5
18	5.6	14.8	30.2	2.6	78	0.0	1.8	14.8	2.8
19	3.3	9.1	18.9	3.1	79	0.0	2.1	15.0	2.8
20	8.7	21.1	40.8	2.3	80	2.0	8.9	23.7	2.7
21	5.3	15.9	41.3	2.3	81	0.0	0.0	8.9	3.0
22	7.8	18.5	46.0	2.2	82	0.0	5.6	18.6	3.0
23	9.4	23.8	47.2	2.1	83	0.0	0.5	16.6	2.6
24	5.9	15.3	29.3	2.7	84	4.3	11.2	29.1	2.7
25	0.1	4.6	16.6	2.5	85	2.1	6.0	16.5	3.2
26	7.8	18.9	41.0	2.3	86	2.0	7.5	17.9	3.0
27	6.0	21.5	50.1	2.1	87	1.2	8.2	22.3	2.6
28	8.0	18.7	54.5	2.0	88	4.2	15.4	28.0	2.6
29	3.0	12.4	34.2	2.4	89	5.4	9.9	42.4	2.4
30	7.9	18.1	40.1	2.3	90	0.7	6.6	28.6	2.4
31	8.6	18.6	41.5	2.3	91	0.0	8.8	28.9	2.6
32	7.1	17.2	40.1	2.3	92	0.0	2.3	21.1	2.6
33	2.8	8.8	21.9	2.8	93	0.0	0.5	6.3	3.1
34	9.9	20.6	45.7	2.2	94	0.0	4.9	27.4	2.6
35	9.2	25.2	50.4	2.0	95	1.3	6.4	28.4	2.5
36	5.3	15.2	32.8	2.5	96	5.3	11.6	28.9	2.7
37	5.3	15.8	43.1	2.3	97	0.0	2.3	22.5	2.6
38	2.1	6.7	37.6	2.4	98	4.0	11.0	25.7	2.8
39	12.0	22.9	43.0	2.2	99	2.4	15.0	40.5	2.2
40	11.1	17.9	39.2	2.5	100	3.8	10.3	29.8	2.6
41	0.8	1.4	21.3	2.9	101	6.0	19.3	39.9	2.3
42	8.0	15.8	45.6	2.3	102	0.3	5.6	29.7	2.4
43	6.0	16.1	48.1	2.2	103	9.6	23.4	47.1	2.1
44	5.7	12.0	34.8	2.5	104	1.4	8.2	23.6	2.6
45	12.8	33.4	76.5	1.6	105	12.4	29.7	48.9	1.9
46	1.6	6.2	23.3	2.7	106	1.5	6.9	30.2	2.5
47	0.8	5.6	24.7	2.6	107	1.0	1.9	10.9	3.6
48	0.0	0.4	17.4	2.6	108	1.4	7.3	15.1	3.0
49	0.0	3.9	22.5	2.7	109	8.4	19.9	46.1	2.2
50	1.2	4.3	17.8	3.0	110	2.9	19.7	28.6	2.4
51	24.1	39.1	57.2	1.5	111	5.8	15.5	41.7	2.3
52	5.1	19.5	31.2	2.4	112	7.1	10.8	34.1	2.7
53	4.4	13.3	35.9	2.4	113	1.9	5.9	25.4	2.7
54	9.3	24.2	48.3	2.1	114	6.5	14.9	42.7	2.3
55	10.3	26.5	61.8	1.8	115	0.0	0.0	3.2	4.5
56	5.9	16.6	32.9	2.5					
57	5.9	19.6	31.7	2.5	$\bar{x}$	4.7	13.1	33.2	
58	4.1	14.0	42.0	2.3	S_{n-1}	4.33	8.37	13.16	
59	6.7	17.1	33.8	2.5	V_m	0.0	0.0	3.2	
60	5.2	17.9	38.3	2.3	V_M	24.1	39.1	76.5	

keeping with the relation White (1967) found in honeys of the United States (indicated as logarithmic in Table I]; (b) linear relation between diastase number and time [25 samples (21.7%), indicated as linear in Table I]; (c) rapid drop in diastase number between 4 and 16 months followed by a small decrease, this latter behavior fitting a straight line of small gradient [11 samples (9.6%), indicated as asymptotic in Table I].

Most samples (96.5%) show a linear relation between the logarithm of HMF content and time. This relation is similar to that found by Hadorn and Kovacs (1960) for Mexican and Guatemalan honeys but is not in keeping with the linear relation between HMF content and time these authors found in Californian honeys, White et al. (1964) found in honeys of the United States, and Huidobro and Simal (1984b) found in Galician (Spain) honeys. Only four (numbers 7, 9, 52, and 108) fit a linear relation between HMF content and time (Table II).

After the SPSSX (1986) statistical package was applied, no relations were found between floral origin of samples and diastase number and between floral origin of samples and HMF content.

(B) Estimating the Date by which the Honeys Are Not Legally Fresh. We have considered the time taken to reach the legal limits for each honey analyzed (T_8 and T_{40} in Tables I and II, respectively).

The lines fitted for each honey (diastase number and HMF content) were used to predict the length of time needed to reach the legal limits for both parameters of aging.

For the diastase number (Table I), the samples were treated differently according to the type of behavior (linear, logarithmic, or asymptotic). Only in 8 samples would the diastase number take less than 1 year to reach the legal limit of 8. In 22 samples (19.1%), the diastase number would take less than 2 years to reach the limit of 8.

For the HMF content (Table II), only one group of samples was considered given that only 4 did not fit the general logarithmic relation. The limit for this parameter (40 mg/kg) would be reached in 97 honeys between 2 and 3 years; hence, HMF content appears to be the limiting parameter in honey freshness.

According to these results the time of 2 years could be considered the out of freshness date for Basque honeys.

(C) Proposed Values of Diastase Number and HMF Content for a Possible New Regulation for Higher Quality Honeys. A regulation governing quality should set limits of diastase number and HMF content for both immediately after extraction (4 months) and the year following processing; the former is the period in which honeys are normally packaged, the latter the time over which most honeys stay fresh.

(*a*) *Recently Extracted Honeys* (*DN 4 and HMF 4 in Tables I and II, Respectively*). It is interesting to note that only two samples (numbers 51 and 75) have a diastase number between 3 and 8. Moreover, these samples have HMF contents of less than 1 mg/kg, which makes it convenient to set the maximum limit for this sort of honey at 3 mg/kg of HMF.

In the honeys having a diastase number greater than 8, the HMF content exceeds 15 mg/kg in samples 51 and 63 (two of the group that had been heated during extraction). Hence, this is suggested as the limit in the quality regulation.

(*b*) *After 1 Year* (*DN 16 and HMF 16 in Tables I and II, Respectively*). In this period only one sample has a diastase number less than 3 (number 38), and of the 13 honeys (numbers 3, 10, 11, 41, 48, 74, 75, 78, 83, 92, 94, 97, and 104) with a diastase number between 3 and 8, the highest HMF content found was 8.2 mg/kg (number 104), which could warrant a change of the present limit of 15 mg/kg of HMF (Codex Alimentarius Commission, 1969) in honeys with diastase numbers between 3 and 8 to 10 mg/kg.

Samples 3, 11, 38, 48, 74, 78, 83, 92, 94, 97, and 104 cannot be considered honeys with low diastasic activity because at 4 months their diastase numbers were greater than 8.

Two samples contain more than 30 mg/kg of HMF (numbers 45 and 51). It is interesting to note that these are in the group heated during extraction; hence, in the quality regulation a maximum limit of 30 mg/kg of HMF could be adopted.

CONCLUSIONS

(1) In the honeys from the Basque Country (Spain), the long-term effects of heating and storage have more influence over the increase of hydroxymethylfurfural. In some honeys, however, given their nature, the short-term limit of freshness is the diastase number.

(2) In terms of the limits established by the Codex Alimentarius Commission (1969), the out of freshness date for 80% of the honeys could be set at 2 years after packaging; hence, it would be good practice for honey to be eaten within the year following its extraction since it easily satisfies the regulations.

(3) In unpasteurized Basque Country (Spain) honey the following limits are considered to be adequate for a regulation governing quality:

(*a*) After extraction and mixing: diastase number, no less than 8 on Gothe's scale (for honeys of low enzyme content, no less than 3 on Gothe's scale, as long as HMF content does not exceed 3 mg/kg); hydroxymethylfurfural, no more than 15 mg/kg.

(*b*) Within the year following packaging: diastase number, no less than 8 on Gothe's scale (for honeys of low enzyme content, no less than 3 on Gothe's scale, as long as HMF content does not exceed 10 mg/kg); hydroxymethylfurfural, no more than 30 mg/kg.

(4) Pasteurized honeys require a separate study taking into consideration their processing and conservation.

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