Evolution of Invertase Activity in Honey over Two Years

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Invertase activity is a good parameter for evaluating honey freshness. Invertase activity evolution was determined on 57 fresh, unheated, commercially purchased Galician (northwestern Spain) floral honey samples. All honeys were stored in darkness at room temperature for up 24 months and analyzed each 6 months so as to determine the invertase activity evolution tendency for the first time. Invertase activity analysis was carried out according to Siegenthaler's method and in a simple assay, the latter showing a good precision (coefficient of variation between 0.35 and 0.66%). Initial invertase activity mean value was 163.9 (48.4−251.0) μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min. After application of the SPSS statistical package, the values of invertase activity showed five types of temporal behavior: exponential (56% of samples), linear (25% of samples), logarithmic (11% of samples), inverse (5% of samples), and quadratic (3% of samples). Linear regression equations were used to predict the invertase activity at 6, 12, 18, and 24 months from the initial Galician honeys' invertase activities; no statistical differences were found between experimental data and the activities calculated from the linear regression equations.

Keywords: *Honey; invertase; sucrase; α-glucosidase; evolution; Galicia, Spain*

INTRODUCTION

Invertase catalyzes the main reaction that ripens nectar to honey. White and Maher (*1*) revealed the α-glucosidase nature of honey invertase, confirmed in 1967 by White and Kushnir (*2*). Invertase is produced in the honeybee's hypopharyngeal glands (*3*).

Invertase allows the bees to make a very concentrated solution of sugars, which resists fermentation, being a high-energy foodstuff that takes up a minimum area in the honeycomb (*3*, *4*).

Invertase activity, together with diastase activity and hydroxymethylfufural content, is a honey quality control parameter. In honeys made by *Apis mellifera*, invertase activity measurement has commercial interest because invertase is related to honey freshness and both heat and storage conditions (*4*, *5*).

Invertase activity should be determined instead of or as well as diastase activity. The reasons can be summarized as follows: invertase measurement is simpler than diastase determination, invertase is more sensitive to heat than diastase (*6*), and invertase should be present in honey in higher quantities than diastase, because bees have to add invertase to both nectar and honeydew.

Aldcorn et al. (*7*) and Sporns et al. (*8*) have determined the invertase activity using the Siegenthaler's method (*9*). They carried out the assay in duplicate but did not discuss the method's variation coefficient.

Invertase activity decrease has been studied by Takenaka and Echigo (*10*) and Ivanov (*11*) at 6 months, by Echigo et al. (*12*) at 7 months, by Rychlik and Federowska (*13*) and Ivanov (*11*) at 12 months, by White et al. (*6*) at 18 months, and by Krauze and Krauze (*14*) at 24 months.

With regard to invertase evolution, in four honeys Piro et al. (*15*) determined initial invertase activity as well as invertase activity at 7, 14, 30, and 60 days. Gonnet (*16*) studied 2 years of invertase evolution in seven samples analyzed once a year. Neither Gonnet (*16*) nor Piro et al. (*15*) described the invertase activity decrease tendency, which would be very interesting. However, diastase decrease tendencies were described by Sancho et al. (*17*) in Basque (northern Spain) honeys.

It seems to be necessary to develop an invertase activity evolution study with significant number of samples, at shorter periods of time, which cover the whole range of honey commercial periods and usual storage conditions.

The purposes of this work have been to study the precision of Siegenthaler's (*9*) method in the range of values for honey invertase activity, to study the influence of storage influence on invertase activity during 2 years, analyzing this enzyme each 6 months, and, finally, to establish the invertase decrease tendency for each sample during storage by trying to predict the invertase activity at 6, 12, 18, and 24 months from the invertase initial activity.

MATERIALS AND METHODS

Samples. This study was carried out on 57 floral, unheated honeys from Galicia, a region with typical oceanic climate in northwestern Spain (Figure 1). All of the samples bore the label "Producto Galego de Calidade-Mel de Galicia" (*18*), which

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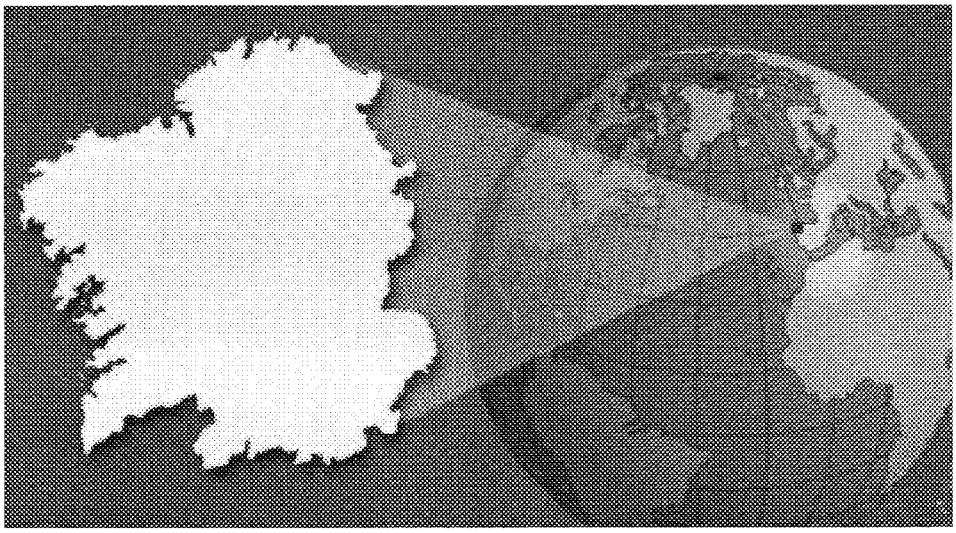

Figure 1. Geographical location of Galicia in northwestern Spain.

Table 1. Study of the Precision of the Determination of Invertase Activity (Micromoles of 4-Nitrophenyl-α-D-Glucopyranoside Hydrolyzed per Kilogram of Honey per Minute) of Honey

	sample A (low activity)	sample B (medium activity)	sample C (high activity)
	57.0	154.8	250.9
	56.5	154.7	252.5
	57.2	153.4	250.1
	57.1	154.7	250.4
	56.4	154.8	249.6
	57.3	153.0	249.3
	56.7	154.0	250.6
	57.5	152.9	250.3
	56.9	154.9	250.2
	57.4	153.1	251.0
mean	57.0	154.0	250.5
SD[a]	0.374	0.846	0.880
% CV[b]	0.66	0.55	0.35

[a] Standard deviation. [b] Coefficient of variation.

guaranteed the origin. The samples, which represented all of the Galician producers of honeys labeled in this way, were harvested in autumn 1995 and were kept in darkness at the laboratory, where the temperature averaged 20 °C (15−25 °C). The botanical origin of the samples was determined according to the Louveaux et al. (*19*) procedure, after the honey sediment had been treated and dyed according to the method of Terradillos et al. (*20*). The honeys were of the following species: *Castanea sativa* (2); *Eucalyptus* sp. (18); *Rubus* sp. (4); and 33 polyfloral honeys.

Analytical Methods. Invertase activity was measured according to the method of Siegenthaler (*9*), based on the spectrophotometric measurement of 4-nitrophenol, which is formed by the reaction of honey invertase with 4-nitrophenyl-α-D-glucopyranoside, used as substrate. Other honey components do not interfere if the appropriate solution is used (*7*, *9*).

RESULTS AND DISCUSSION

Repeatability. The precision (Table 1) was established by measuring the invertase activity of 10 different solutions. Preparing 10 different honey solutions of the same honey sample step by step has carried out the repeatability from 3 samples with low, medium, and high activities, respectively.

The precision (%CV) of the invertase method, determined at the first time in a simple assay, ranged between 0.35 and 0.66%. Therefore, it is unnecessary to carry out the analytical procedure in duplicate (*9*) or in triplicate (*21*) because the determination of the invertase activity in a simple assay is sufficiently accurate (CV% ≤ 0.66).

Effects of 2 Years of Storage on the Invertase Activity of the Galician Honeys Analyzed and Curve Estimation of the Invertase Activity during Storage. Table 2 shows for each honey sample the sample number, the botanical origin, the measured invertase activity in micromoles of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed per kilogram of honey per minute at initial time and at 6, 12, 18, and 24 months, and the best type of fit for the evolution of the invertase activity.

To compare our data with those obtained in previous studies carried out by other researchers at the same storage conditions (darkness and room temperature), it is necessary to give the results in the same units. Sometimes, invertase activities are given in sucrose hydrolysis units (sucrase number). The sucrase number (*22*) expresses grams of sucrose hydrolyzed per 100 g of honey in 1 h at 40 °C. The sucrase number can be converted into micromoles of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed per kilogram of honey per minute by multiplying by 7.35 (*9*). The 4-nitrophenol was measured via spectrophotometric analysis at 400 nm. Absorbance at 400 nm results are converted into micromoles of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed per kilogram of honey per minute by multiplying by 158.94 (*9*).

The mean value for initial invertase activity was 163.9 μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min with a minimum activity of 48.4 μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min and a maximum activity of 251.0 μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min. These invertase activities were higher than those found in floral honeys by Aldcorn et al. (*7*), with a mean value of 136.8 (83.1−202.2) μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min, by Dustmann et al. (*23*), 98.7 (12.7−217.0) μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min, by Krauze and Zalewski (*24*), 121.3 (53.7−207.4) μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min, by Sporns et al. (*8*), 76.0 (8.0−237.0)

Table 2. Changes in Invertase Activity during 2 Years of Storage and the Type of Fit for the Evolution of the Parameter

		invertase activity (μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min)					
sample	plant origin	initial	6 months	12 months	18 months	24 months	type
1	*Castanea sativa*	239.8	180.3	161.4	149.8	141.4	logarithmic
2	*Castanea sativa*	249.6	220.5	194.9	172.2	152.2	exponential
3	*Eucalyptus* sp.	96.0	87.3	78.7	70.0	61.4	linear
4	*Eucalyptus* sp.	112.8	101.9	91.1	80.2	69.4	linear
5	*Eucalyptus* sp.	109.2	100.2	91.2	82.2	73.2	linear
6	*Eucalyptus* sp.	173.0	103.2	90.1	85.5	41.1	inverse
7	*Eucalyptus* sp.	195.1	196.7	179.5	143.6	88.7	quadratic
8	*Eucalyptus* sp.	118.8	110.9	103.0	95.1	87.3	linear
9	*Eucalyptus* sp.	141.8	131.2	120.5	109.9	99.3	linear
10	*Eucalyptus* sp.	176.8	158.3	139.8	121.3	102.8	linear
11	*Eucalyptus* sp.	146.9	130.5	114.1	97.7	81.2	linear
12	*Eucalyptus* sp.	144.0	128.3	114.3	101.9	90.8	exponential
13	*Eucalyptus* sp.	107.8	98.4	89.1	79.8	70.5	linear
14	*Eucalyptus* sp.	91.5	74.1	60.0	48.6	39.4	exponential
15	*Eucalyptus* sp.	63.1	42.6	28.8	19.4	13.1	exponential
16	*Eucalyptus* sp.	165.0	148.8	134.1	120.9	108.9	exponential
17	*Eucalyptus* sp.	150.2	131.0	111.8	92.5	73.3	linear
18	*Eucalyptus* sp.	173.9	154.2	136.8	121.3	107.6	exponential
19	*Eucalyptus* sp.	132.1	105.5	97.1	91.9	88.1	logarithmic
20	*Eucalyptus* sp.	154.2	140.4	127.9	116.4	106.0	exponential
21	*Rubus* sp.	70.3	32.2	20.1	12.6	7.3	logarithmic
22	*Rubus* sp.	207.7	163.2	118.8	74.4	30.0	linear
23	*Rubus* sp.	100.5	78.5	61.3	47.9	37.5	exponential
24	*Rubus* sp.	206.7	183.2	162.3	143.9	127.5	exponential
25	multifloral	146.3	114.1	103.9	97.6	93.1	logarithmic
26	multifloral	179.8	148.7	123.0	101.8	84.2	exponential
27	multifloral	173.5	155.1	138.6	123.9	110.8	exponential
28	multifloral	133.1	100.0	89.5	83.0	78.4	logarithmic
29	multifloral	158.0	158.7	144.3	114.8	70.2	quadratic
30	multifloral	177.1	156.5	135.9	115.4	94.8	linear
31	multifloral	216.7	191.5	166.3	141.1	115.9	linear
32	multifloral	165.4	109.1	100.6	96.5	53.4	inverse
33	multifloral	108.5	86.3	68.6	54.6	43.4	exponential
34	multifloral	110.4	89.7	72.9	59.2	48.1	exponential
35	multifloral	231.7	205.0	178.4	151.7	125.0	linear
36	multifloral	197.7	173.8	152.7	134.2	118.0	exponential
37	multifloral	154.4	135.4	118.8	104.3	91.5	exponential
38	multifloral	130.2	105.2	85.0	68.7	56.5	exponential
39	multifloral	184.8	160.0	138.5	120.0	103.9	exponential
40	multifloral	119.2	101.5	86.5	73.7	62.8	exponential
41	multifloral	118.2	95.1	76.5	61.5	49.5	exponential
42	multifloral	48.4	15.9	8.0	5.5	0.0	inverse
43	multifloral	153.9	124.4	100.6	81.4	65.8	exponential
44	multifloral	195.6	160.2	131.2	107.4	87.9	exponential
45	multifloral	163.1	134.6	106.1	77.6	49.1	linear
46	multifloral	168.8	142.0	119.6	100.7	84.7	exponential
47	multifloral	193.1	172.3	153.9	137.4	122.6	exponential
48	multifloral	181.1	150.5	125.1	104.0	86.4	exponential
49	multifloral	240.9	210.9	181.0	151.1	121.2	exponential
50	multifloral	218.2	192.4	169.6	149.5	131.8	exponential
51	multifloral	236.1	208.4	183.9	162.3	143.3	exponential
52	multifloral	244.9	187.8	169.6	158.5	150.5	logarithmic
53	multifloral	171.1	149.8	131.1	114.8	100.4	exponential
54	multifloral	181.6	155.5	133.2	114.1	97.7	exponential
55	multifloral	232.6	205.9	182.3	161.4	142.9	exponential
56	multifloral	232.4	205.2	181.3	160.1	141.4	exponential
57	multifloral	251.0	220.4	193.6	170.1	149.4	exponential
mean		163.9	139.0	120.6	104.2	87.2	
SD[a]		49.5	46.8	42.8	38.7	36.9	
%CV[b]		30.2	33.7	35.5	37.1	42.3	
minimum		48.4	15.9	8.0	5.5	0.0	
maximum		251.0	220.5	194.9	172.2	152.2	

[a] Standard deviation. [b] Coefficient of variation.

μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min, by Huidobro et al. (*25*), 128.3 (68.7−225.4) μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min, and by Ortiz and Subrá (*26*), 114.0 (64.8−170.8) μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min. Also, the present result for invertase activity was higher than that obtained by Krauze and Zalewki (*24*) in honeydew, 146.4 (5.0−278.1) μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min.

The mean value for 6 month invertase activity was 139.0 μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min with a minimum activity of 15.9 μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min and a maximum activity of 220.5 μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of

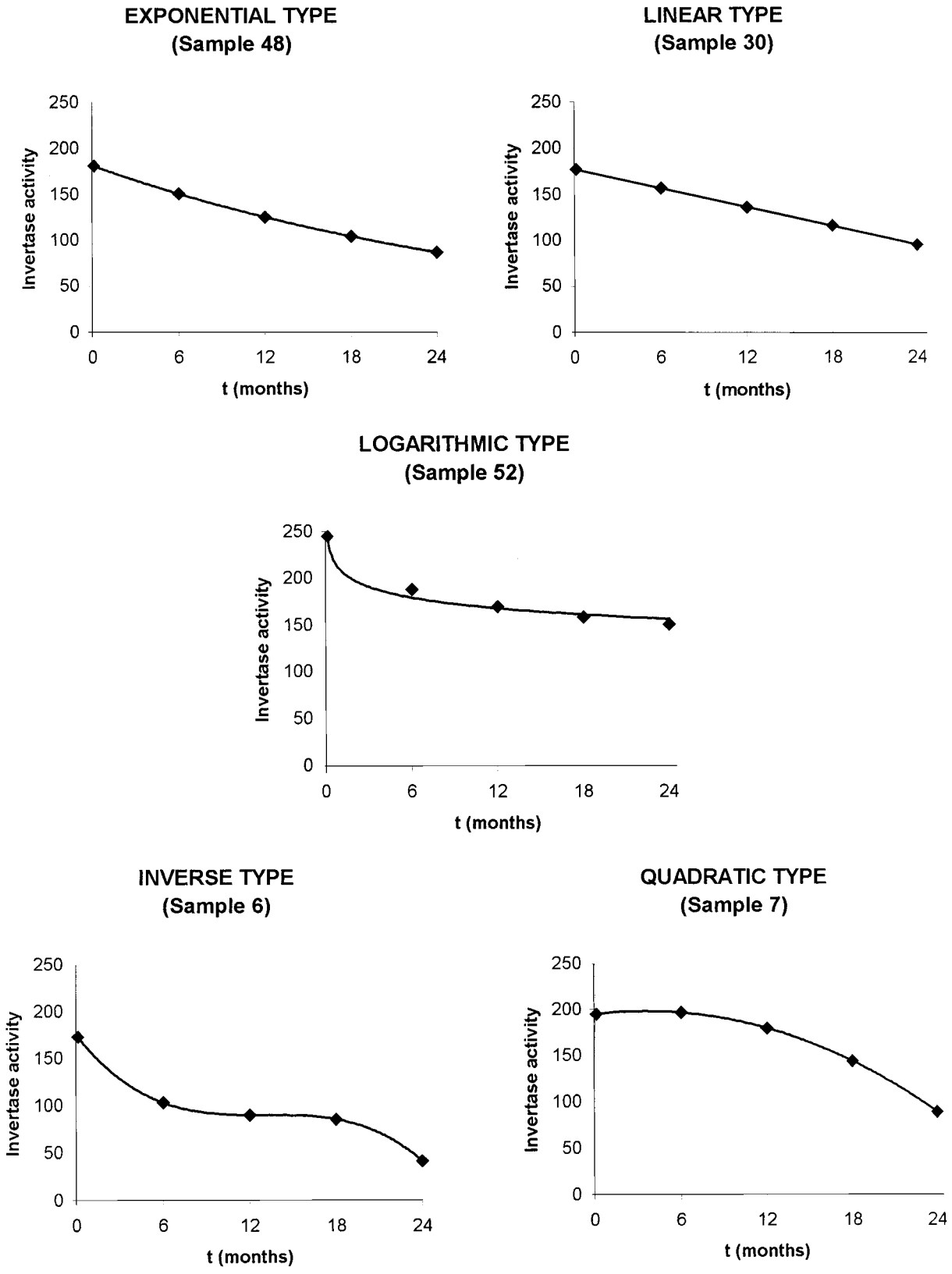

Figure 2. Five different types of invertase activity (micromoles of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/minute) evolution in Galician (northwestern Spain) honey samples.

honey/min. The data reported by Takenaka and Echigo (*10*) and Ivanov (*11*) at 6 months of storage and by Echigo et al. (*12*) at 7 months of storage data are given in percentage; therefore, our results cannot be compared with those invertase activity data.

The mean value for 12 month invertase activity was 120.6 μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min with a minimum activity of 8.0 μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min and a maximum activity of 194.9 μmol

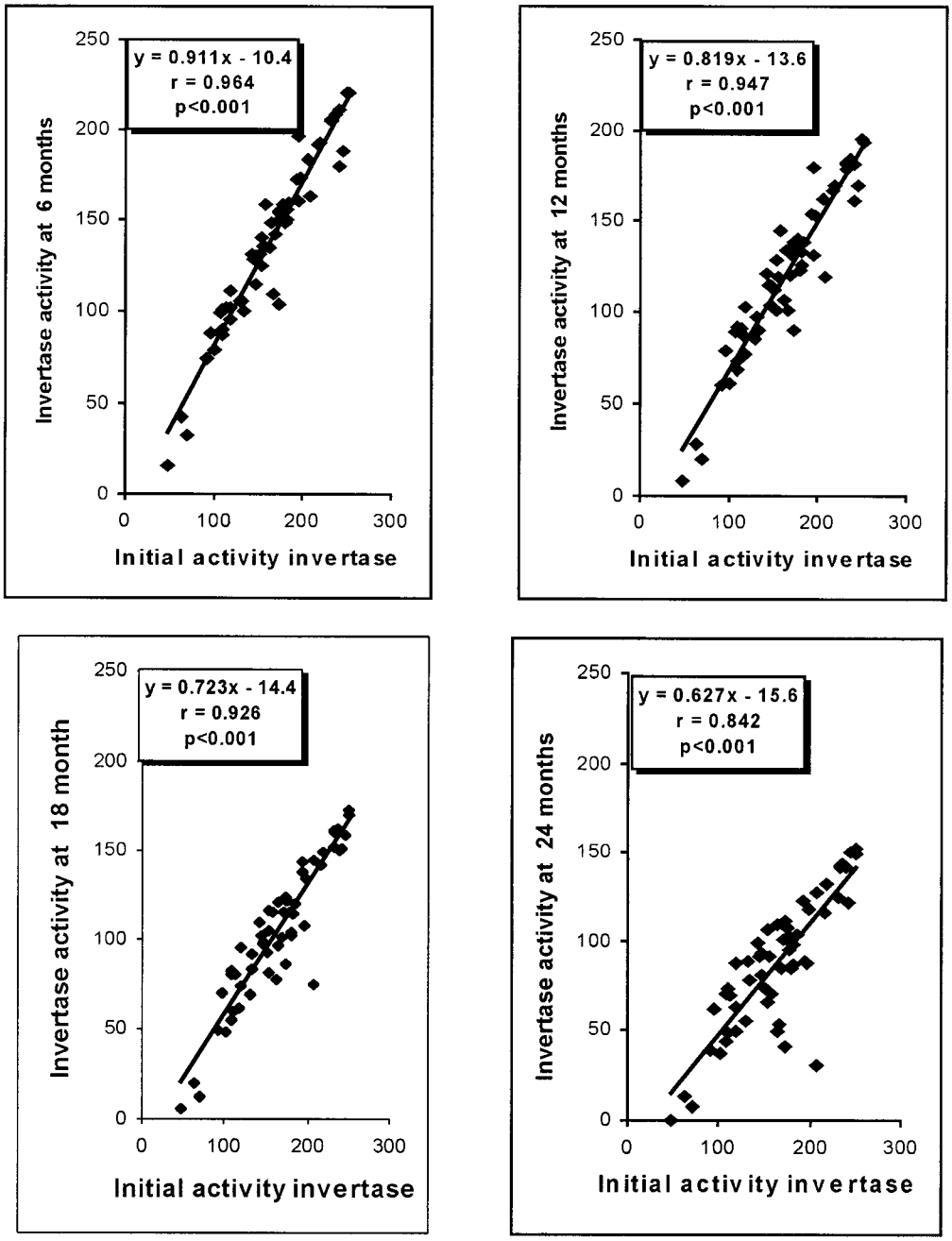

Figure 3. Relationship between initial invertase activity (micromoles of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/minute) and invertase activities at 6, 12, 18, and 24 months.

of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min. Our data cannot be compared with the invertase decrease given by Rychlik and Federowska (*13*) and Ivanov (*11*) in percentage at 12 months of storage.

The mean value for 18 month invertase activity was 104.2 μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min with a minimum activity of 5.5 μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min and a maximum activity of 172.2 μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min. The mean and maximun values were higher than those obtained by White et al. (*6*) in three samples stored at 21 °C, 48.6 (16.1−67.9) μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min.

The mean value for 24 month invertase activity was 87.2 μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min with a minimum activity of 0.0 μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min and a maximum activity of 152.2 μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min. This invertase activity was similar to the activity obtained by Krauze and Krauze (*14*) in honeydew, 103.0 (3.6−162.2) μmol of 4-nitrophenyl-α-D-glucopyranoside hydrolyzed/kg of honey/min.

After the SPSS (*27*) statistical package was applied, the values of invertase activity showed five types of temporal behavior: exponential (32 samples, 56%), linear (14 samples, 25%), logarithmic (6 samples, 11%), inverse (3 samples, 5%), and quadratic (2 samples, 3%) (Figure 2).

Prediction of Invertase Activity at 6, 12, 18, and 24 Months from the Initial Invertase Activity. We

Table 3. Invertase Activities at 6, 12, 18, and 24 Months

sample	INV6E[a]	INV6T[a]	INV12E	INV12T	INV18E	INV18T	INV24T	INV24E
1	180.3	208.1	161.4	182.7	149.8	159.0	141.4	134.8
2	220.5	217.1	194.9	190.8	172.2	166.1	152.2	141.0
3	87.3	77.1	78.7	65.0	70.0	55.0	61.4	44.6
4	101.9	92.4	91.1	78.8	80.2	67.2	69.4	55.1
5	100.2	89.1	91.2	75.8	82.2	64.6	73.2	52.9
6	103.2	147.3	90.1	128.1	85.5	110.7	41.1	92.9
7	196.7	167.4	179.5	146.1	143.6	126.7	88.7	106.8
8	110.9	97.9	103.0	83.7	95.1	71.5	87.3	58.9
9	131.2	118.8	120.5	102.5	109.9	88.1	99.3	73.3
10	158.3	150.7	139.8	131.2	121.3	113.5	102.8	95.3
11	130.5	123.5	114.1	106.7	97.7	91.8	81.2	76.5
12	128.3	120.8	114.3	104.3	101.9	89.7	90.8	74.7
13	98.4	87.8	89.1	74.7	79.8	63.6	70.5	52.0
14	74.1	73.0	60.0	61.3	48.6	51.8	39.4	41.8
15	42.6	47.1	28.8	38.1	19.4	31.2	13.1	24.0
16	148.8	140.0	134.1	121.5	120.9	104.9	108.9	87.9
17	131.0	126.5	111.8	109.4	92.5	94.2	73.3	78.6
18	154.2	148.1	136.8	128.8	121.3	111.4	107.6	93.5
19	105.5	110.0	97.1	94.6	91.9	81.1	88.1	67.2
20	140.4	130.1	127.9	112.7	116.4	97.1	106.0	81.1
21	32.2	53.7	20.1	44.0	12.6	36.4	7.3	28.5
22	163.2	178.9	118.8	156.5	74.4	135.8	30.0	114.7
23	78.5	81.2	61.3	62.7	47.9	58.3	37.5	47.4
24	183.2	178.0	162.3	155.6	143.9	135.1	127.5	114.0
25	114.1	122.9	103.9	106.2	97.6	91.4	93.1	76.2
26	148.7	153.5	123.0	133.6	101.8	115.6	84.2	97.2
27	155.1	147.7	138.6	128.5	123.9	111.1	110.8	93.2
28	100.0	110.9	89.5	95.4	83.0	81.9	78.4	67.9
29	158.7	133.6	144.3	115.8	114.8	99.9	70.2	83.5
30	156.5	151.0	135.9	131.4	115.4	113.7	94.8	95.5
31	191.5	187.1	166.3	163.8	141.1	142.3	115.9	120.3
32	109.1	140.3	100.6	121.8	96.5	105.2	53.4	88.1
33	86.3	88.5	68.6	75.3	54.6	64.1	43.4	52.4
34	89.7	90.2	72.9	76.8	59.2	65.4	48.1	53.6
35	205.0	200.7	178.4	176.1	151.7	153.2	125.0	129.7
36	173.8	169.8	152.7	148.3	134.2	128.6	118.0	108.4
37	135.4	130.3	118.8	112.8	104.3	97.3	91.5	81.2
38	105.2	108.3	85.0	93.0	68.7	79.8	55.5	66.1
39	160.0	158.0	138.5	137.7	120.0	119.2	103.9	100.3
40	101.5	98.2	86.5	84.0	73.7	71.8	62.8	59.2
41	95.1	97.3	76.5	83.2	61.5	71.1	49.5	58.5
42	15.9	33.7	8.0	26.1	5.5	20.6	0.0	14.7
43	124.4	129.8	100.6	112.4	81.4	96.9	65.8	80.9
44	160.2	167.9	131.2	146.6	107.4	127.0	87.9	107.1
45	134.6	138.2	106.1	120.0	77.6	103.5	49.1	86.7
46	142.0	143.4	119.6	124.6	100.7	107.7	84.7	90.3
47	172.3	165.6	153.9	144.5	137.4	125.2	122.6	105.5
48	150.5	154.6	125.1	134.7	104.0	116.6	86.4	98.0
49	210.9	209.1	181.0	183.6	151.1	159.8	121.2	135.5
50	192.4	188.4	169.6	165.1	149.5	143.4	131.8	121.3
51	208.4	204.8	183.9	179.7	162.3	156.3	143.3	132.5
52	187.8	212.8	169.6	186.9	158.5	162.7	150.5	138.0
53	149.8	145.5	131.1	126.5	114.8	109.3	100.4	91.7
54	155.5	155.1	133.2	135.1	114.1	116.9	97.7	98.3
55	205.9	201.6	182.3	176.8	161.4	153.8	142.9	130.3
56	205.2	201.4	181.3	176.7	160.1	153.7	141.4	130.2
57	220.4	218.3	193.6	191.9	170.1	167.1	149.4	141.8
mean	139.0	139.0	120.6	120.5	104.2	104.2	87.2	87.2

[a] E, experimental activities; T, calculated activities with the linear regression equations.

Table 4. Analysis of Data for Invertase Activity Comparison in Samples Measured at 6, 12, 18, and 24 Months with Invertase Activity Values from Figure 3 Linear Regression Equations (*t* Test; *27*)

		paired differences							
					Cl				
pair		mean	SD	SE of mean	lower	upper	t	gl	two-tail sig
1	INV6E[a]−INV6T[a]	6.421E−03	12.496	1.655	−4.407	4.420	0.004	56	0.997
2	INV12E−INV12T	5.437E−03	13.811	1.829	−4.873	4.883	0.003	56	0.998
3	INV18E−INV18T	2.873E−03	14.716	1.949	−5.195	5.201	0.001	56	0.999
4	INV24E−INV24T	2.705E−04	20.046	2.655	−7.080	7.080	0.000	56	1.000

[a] E, experimental activities; T, calculated activities with the linear regression equations.

have determined with the SPSS (*27*) statistical package linear regression equations to predict the invertase activity at 6, 12, 18, and 24 months from the initial invertase activity.

It is interesting that relationships have been found between invertase activities from different times. There are good and very significative (significance level = 99%) correlation coefficients between the initial invertase activity and activities at 6, 12, 18, and 24 months (Figure 3).

The *t* test from the SPSS (*27*) statistical package has been applied to compare experimental data obtained at 6, 12, 18, and 24 months with data obtained from Figure 3's linear regression equations (Table 3). Statistical differences at a confidence level of 99% have not been found between pairs of both data (Table 4). The *t* value obtained in all cases was lower than that tabulated, 2.677. This is important because at least for the 1995 Galician honey harvest, invertase activity values at any time have been able to be calculated by starting from the initial invertase activity data, saving time, work, and reagents.

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