

ORIGINAL RESEARCH ARTICLE

Long-term changes of naturally produced honey depending on processing and temperature

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Online Supplementary Material

Table S1. Pollen analysis of the honey samples (pollen >3%/honey; DIN-Norm-10760, 2002).

Honey	Plant (lat.)	Pollen %
H ₁	<i>Brassica</i> spp.	40.60
	<i>Salix</i>	30.00
	<i>Aesculus</i>	11.20
	<i>Prunus/ Prus</i> spp.	7.80
	<i>Aceraceae</i>	3.20
H ₂	<i>Brassica</i> spp.	44.05
	<i>Salix</i>	17.93
	<i>Prunus/ Pirus</i> spp.	17.35
	<i>Rhamnus</i>	7.02
	<i>Aesculus</i>	6.43
	<i>Myosotis</i>	4.68
H ₃	<i>Brassica</i> spp.	61.35
	<i>Rubus</i> spp.	11.75
	<i>Vicia</i> spp.	11.16
	<i>Trifolium repens</i>	5.38
	<i>Rhamnus</i>	3.78
H ₄	<i>Trifolium repens</i>	39.73
	<i>Tilia</i> spp.	17.61
	<i>Brassica</i> spp.	13.70
	<i>Rubus</i> spp.	5.28
	<i>Centaurea cyanus</i>	5.09
	<i>Raphanus</i> spp.	4.89
	<i>Sinapis</i> spp.	3.13

Table S2. Changes of moisture in 24 months storage in glass jars dependent on temperature (T_1 - T_3), processing (M_1 - M_3) and honey (H_1 - H_4); mean and standard deviation.

Month			0	6	12	18	24
H_1	T_1	M_1	17.0	16.9±0.03	16.8±0.01	16.8±0.02	16.9±0.06
		M_2	17.0	16.9±0.06	16.9±0.02	16.7±0.03	16.7±0.14
		M_3	17.0	16.9±0.05	17±0.04	16.8±0.01	16.9±0.06
	T_2	M_1	17.0	16.9±0.06	16.9±0.01	16.5±0.01	16.1±0.04*
		M_2	17.0	16.9±0.05	16.9±0.03	16.5±0.02	16.5±0.06*
		M_3	17.0	16.9±0.04	16.9±0.02	16.6±0	16.4±0.06*
	T_3	M_1	17.0	17.0±0.03	17±0.02	16.9±0	17.0±0.04
		M_2	17.0	17.0±0.03	17±0	16.8±0.01	17.0±0.02
		M_3	17.0	17.0±0.05	17.1±0	16.8±0.02	17.0±0.04
H_2	T_1	M_1	17.8	17.6±0.04	17.7±0.02	17.6±0.01	17.6±0.15
		M_2	17.8	17.7±0.07	17.7±0	17.5±0.01	17.6±0.09
		M_3	17.8	17.7±0.04	17.8±0.02	17.7±0	17.6±0.07
	T_2	M_1	17.8	17.5±0.05	17.7±0.02	17.4±0.04	16.9±0.11*
		M_2	17.8	17.6±0.05	17.7±0	17.4±0.01	17.2±0.02*
		M_3	17.8	17.5±0.03	17.8±0.02	17.2±0	17.1±0.22*
	T_3	M_1	17.8	17.6±0.04	17.8±0	17.6±0.02	17.6±0.2
		M_2	17.8	17.6±0.03	17.8±0.02	17.6±0.02	17.6±0.09
		M_3	17.8	17.6±0.06	17.8±0.04	17.7±0	17.7±0.16
H_3	T_1	M_1	16.3	16.2±0.14	16.2±0.04	16.2±0.04	16.4±0.05
		M_2	16.3	16.4±0.05	16.2±0	16.1±0.02	16.2±0.04
		M_3	16.3	16.4±0.04	16.2±0.04	16.1±0.07	16.3±0.02
	T_2	M_1	16.3	16.4±0.13	16.2±0.02	15.9±0.15	15.6±0.04*
		M_2	16.3	16.2±0.08	16.2±0.02	16.0±0.04	15.7±0*
		M_3	16.3	16.4±0.09	16.2±0	15.9±0.12	15.9±0.10*
	T_3	M_1	16.3	16.3±0.13	16.2±0.02	16.2±0.06	16.5±0.09
		M_2	16.3	16.4±0.04	16.2±0	16.2±0.05	16.4±0.05
		M_3	16.3	16.45±0.02	16.2±0.04	16.3±0.06	16.4±0.06

H ₄	T ₁	M ₁	17.3	17.3±0.02	17.3±0.02	17.1±0	17.0±0.06
		M ₂	17.3	17.4±0.02	17.4±0.02	17.1±0	17.2±0
		M ₃	17.3	17.4±0.08	17.3±0.02	17.0±0.05	17.1±0.06
	T ₂	M ₁	17.3	17.3±0.12	17.4±0.02	17.0±0.18	16.8±0.07*
		M ₂	17.3	17.3±0.04	17.2±0.02	17.0±0.05	16.9±0.02*
		M ₃	17.3	17.3±0.09	17.2±0	16.8±0.21	16.6±0.11*
	T ₃	M ₁	17.3	17.3±0	17.3±0.02	17.3±0.02	17.4±0.05
		M ₂	17.3	17.3±0.06	17.4±0.02	17.3±0.02	17.3±0.02
		M ₃	17.3	17.4±0.04	17.4±0.02	17.3±0.04	17.6±0.05

Note: * indicate statistical differences of T₂ at p<0.001 after 24 month in the group of each honey (H₁, H₂, H₃ or H₄) to T₁ and T₃ (Anova; Tukey's multiple comparisons test).

Table S3: Changes of physico-chemical data after 24 months storage in glass jars dependent on temperature (T_1 - T_3), processing (M_1 - M_3) and honey (H_1 - H_4); mean and standard deviation.

			Invertase Activity	HMF	Fructose	Glucose	Sucrose	pH
H_1	Start		97.3±0.23	2.5±1.31	41.1±0.28	33.7±0.45	1.60±0.07	3.83±0.06
	T_1	M_1	79.8±0.78 ⁺	6.9±0.08	40.6±0.18	34.2±0.01	0.73±0.26	3.83±0.07
		M_2	80.4±0.15 ⁺	8.5±1.10	41.1±0.08	33.8±0.29	0.49±0.13	3.80±0.03
		M_3	79.0±1.17 ⁺	8.9±0.35	40.4±0.52	34.0±0.40	0.71±0.06	3.81±0.05
	T_2	M_1	61.6±0.06 ⁺⁺	21.8±9.2 ⁺⁺	41.0±0.70	32.3±0.76	0.76±0.14	3.85±0.04
		M_2	60.8±0.93 ⁺⁺	24.3±3.9 ⁺⁺	41.3±0.98	32.7±1.01	0.57±0.34	3.85±0.04
		M_3	60.2±0.95 ⁺⁺	22.3±2.2 ⁺⁺	40.9±0.90	32.5±0.78	0.46±0.02	3.84±0.07
	T_3	M_1	76.1±2.27	9.1±1.46	41.8±0.43	33.4±0.74	0.56±0.31	3.82±0.05
		M_2	75.9±1.51	9.1±4.67	40.3±0.30	33.9±0.03	0.75±0.05	3.81±0.01
		M_3	72.6±0.87	9.0±3.49	40.7±0.27	33.8±0.43	0.74±0.07	3.80±0.00
H_2	Start		91.5±0.36	3.5±0.90	41.6±0.01	33.4±0.07	1.60±0.09	3.75±0.05
	T_1	M_1	75.1±1.47 ⁺	6.9±0.78	40.4±0.18	34.1±0.59	0.83±0.05	3.77±0.07
		M_2	75.5±1.27 ⁺	8.6±1.21	40.5±0.12	33.9±0.16	0.66±0.09	3.79±0.01
		M_3	75.8±1.02 ⁺	9.9±1.53	40.3±0.18	33.4±0.07	0.85±0.15	3.79±0.03
	T_2	M_1	56.9±0.76 ⁺⁺	21.5±3.7 ⁺⁺	40.5±0.10	33.4±0.03	0.58±0.14	3.80±0.01
		M_2	59.1±1.34 ⁺⁺	22.9±2.1 ⁺⁺	41.1±0.55	32.2±0.52 [*]	0.57±0.03	3.76±0.06
		M_3	57.4±1.32 ⁺⁺	22.5±1.5 ⁺⁺	39.3±0.85	32.7±0.23 [*]	0.50±0.06	3.77±0.05
	T_3	M_1	69.4±0.32	9.1±2.42	40.3±0.45	33.7±0.62	0.69±0.10	3.75±0.06
		M_2	72.7±0.49	12.1±1.2	40.7±0.58	33.1±0.69	0.84±0.18	3.75±0.03
		M_3	68.6±1.17	10.9±0.1	40.2±0.52	33.6±0.72	0.77±0.20	3.76±0.06
H_3	Start		144.0±2.36	3.3±0.38	40.2±0.13	32.4±0.08	0.30±0.17	4.07±0.02
	T_1	M_1	123.6±0.40 ⁺	6.2±0.16	40.2±0.13	33.4±0.14	0	4.07±0.09
		M_2	121.3±1.44 ⁺	6.1±0.39	40.3±0.02	33.3±0.12	0	4.08±0.01
		M_3	122.3±0.91 ⁺	7.1±0.14	39.9±0.01	33.2±0.16	0	4.06±0.01
	T_2	M_1	93.3±1.61 ⁺⁺	17.8±0.9 ⁺⁺	39.6±0.05	31.6±0.08 [*]	0	4.13±0.06
		M_2	92.4±1.08 ⁺⁺	20.6±0.5 ⁺⁺	39.8±0.26	31.6±0.22 [*]	0	4.09±0.02
		M_3	90.7±0.83 ⁺⁺	20.8±0.4 ⁺⁺	39.7±0.23	31.4±0.04 [*]	0	4.11±0.02
	T_3	M_1	116.2±1.95	7.1±0.42	40.7±0.07	32.9±0.08	0	4.03±0.02
		M_2	115.3±0.76	7.4±0.05	40.5±0.24	32.4±0.04	0	4.05±0.02
		M_3	112.4±2.17	8.1±0.07	40.1±0.07	32.4±0.17	0	4.05±0.03
H_4	Start		139.0±2.13	1.8±0.18	37.2±0.01	31.1±0.01	0.50±0.07	5.03±0.02

T ₁	M ₁	124.0±1.08 ⁺	3.1±0.10	37.5±0.16	31.7±0.07	0	4.99±0.05
	M ₂	123.9±0.82 ⁺	3.9±0.26	37.6±0.01	32.2±0.33	0	5.00±0.05
	M ₃	123.0±1.65 ⁺	3.6±0.03	37.9±0.06	31.9±0.14	0	4.99±0.05
T ₂	M ₁	91.6±0.51 ⁺⁺	8.9±0.10 ⁺⁺	37.0±0.15	29.1±0.12*	0	5.09±0.03
	M ₂	90.4±0.15 ⁺⁺	11.4±0.1 ⁺⁺	36.9±0.09	29.4±0.01*	0	5.08±0.03
	M ₃	92.2±0.12 ⁺⁺	10.9±0.5 ⁺⁺	37.9±0.13	28.3±0.11*	0	5.05±0.04
T ₃	M ₁	119.5±1.18	3.7±0.10	37.2±0.07	31.4±0.19	0	5.01±0.04
	M ₂	118.3±0.64	4.4±0.04	37.2±0.10	30.6±0.23	0	4.99±0.02
	M ₃	118.0±1.04	4.3±0.02	37.5±0.10	30.5±0.23	0	4.99±0.03

Note: glucose:* indicate statistical differences at $p<0.0001$ after 24 month in the group of each honey (H₁, H₂, H₃ or H₄) to the starting value. Invertase, HMF: ++ indicate statistical differences of T₂ at $p<0.0001$ after 24 month in the group of each honey (H₁, H₂, H₃ or H₄) to T₁ and T₃. + indicate statistical differences of T₁ at $p<0.05$ after 24 month in the group of each honey (H₁, H₂, H₃ or H₄) to T₃ (Anova; Tukey's multiple comparisons test).

Table S4. Organoleptic properties of the honey samples stored in containers after two years depending on honey type, processing and temperature.

Honey	Temperature	Processing	Ferm.	Color	Crystals	Phase separation	Frosting
H ₁	T ₁	M ₁	n	3	3	3	y
		M ₂	n	3	3	3	n
		M ₃	n	3	3	3	y
	T ₂	M ₁	n	2	2	2	y
		M ₂	n	2	3	1	n
		M ₃	n	2	2	2	y
	T ₃	M ₁	n	2	1	2	y
		M ₂	n	2	3	1	n
		M ₃	n	2	1	2	y
H ₂	T ₁	M ₁	n	3	3	3	y
		M ₂	n	2	3	3	n
		M ₃	n	3	3	3	y
	T ₂	M ₁	y	2	2	2	y
		M ₂	y	2	3	1	n
		M ₃	y	2	2	2	y
	T ₃	M ₁	n	2	1	2	y
		M ₂	n	2	3	1	n
		M ₃	n	2	1	2	y
H ₃	T ₁	M ₁	n	3	2	3	y
		M ₂	n	3	3	3	n
		M ₃	n	3	3	3	y
	T ₂	M ₁	n	1	2	3	y
		M ₂	n	1	2	2	n
		M ₃	n	1	2	3	y
	T ₃	M ₁	n	2	1	3	y
		M ₂	n	2	2	2	n
		M ₃	n	2	2	3	y
H ₄	T ₁	M ₁	n	3	1	3	y
		M ₂	n	3	3	3	n
		M ₃	n	3	2	3	y
	T ₂	M ₁	n	1	1	3	y
		M ₂	y	1	3	1	n
		M ₃	n	1	1	3	y
	T ₃	M ₁	n	2	1	3	y
		M ₂	n	2	1	3	y
		M ₃	n	2	1	3	y

	M ₂	n	2	3	l	n
	M ₃	n	2	l	3	y

Scoring: Color: 3=original, light beige-yellow; 2=beige-yellow; l=light brown; crystals: 3=fine, 2=like sand, l=coarse; phase separation: 3=no, 2=wet surface, l=2 phases; frosting, fermentation (Ferm.): n=no, y=yes.