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Original article

Determination and Comparison of Melissopalynological and some Chemical Characters of raw and Processed Honeys

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Abstract. Honey produced by honeybees is presented to the consumer thanks to its high nutritional values. Honey obtained without any filtration or thermal process is called raw honey. However, it is offered to the consumer through various processes for reasons such as preventing crystallization, inhibition microbial growth and extending shelf life. While the during the filtration process honeys lose some of their pollen content and heat treatment applications can affect the chemical and nutritional values of honey. These processes affect the natural structure of honey. In this study, we aimed to analyze and compare of the botanical and chemical structures of raw and processed honeys. Therefore, the melissopalynological, sugar, moisture, invertase, diastase, proline and HMF contents of 20 raw honey (RH) supplied from the beekepers and 20 processed honey (PH) collected from the shelves were investigated and evaluated statistically. As a result, it has been observed that there are significant differences between the total pollen count, moisture %, F/G, HMF, diastase, invertase and proline values of raw honey and processed honey.

Keywords: Raw honey, processed honey, melissopalynology, chemical composition.

Introduction

Honey is gained from plant nectars, plant secretions or insects secretions (feed on the sap of flowering plants) by honeybees and matured in the hive (1). It is divided into two groups as flower honey and honeydew honey. If it is obtained from plant nectar is called flower honey, whereas obtained from plant secretion or insect secretion is called honeydew honey (2, 3).

Honey obtained without processing after matured from the honeycomb is called raw honey (4). It contains more than 200 ingredients such as sugar (70-80%), water (10-20%), organic acids, minerals, oils, vitamins, proteins, pigments, phenolic compounds and free amino acids (2, 5, 6). Monosaccharides (glucose, fructose) constitute a large part of the sugar content (80%) of honey. In addition, 25 types of oligosaccharides (disaccharides, trisaccharides, tetrasaccharides, etc.) are also found in varying proportions (7, 8). Thanks to the organic acids (butyric acid, citric acid, formic acid, gluconic acid, etc.) in its structure, it has an average pH of 3.9. Enzymes (invertase, diastase, glucose oxidase, catalase, acid phosphate) from the hypopharyngeal glands and salivary secretions of honeybees play an important role in the transformation of nectar into honey and digestion of carbohydrates. While diastase provides the breakdown of starch grains in dextrin and maltose, invertase is effective in breaking down sucrose in nectar and converted into fructose and glucose (5, 9). Prolin, which is a free amino acid, is found in varying proportions in honey depending on its vegetable source. The amount of proline is important in determining the total amino acid level and quality of honey (10).

The chemical content of honey varies depending on many factors such as the botanical origin, the climate, geographical features, collecting and the storage conditions (2, 11, 12). Therefore, it is important to know the botanical origin while evaluating the chemical properties of honey (13). Determining the botanical origin by examining the pollen in honey with melissopalinology is used for many years (13). Pollen grains in honey can categorize by calculating of the percentages of pollen taxa (14). Flower honeys may exhibit monofloral or polyfloral features depending on their plant origin (15, 16). Honeys with more than 45% of pollen belonging to the same taxa generally monofloral, and are generally named according to their botanical source (15). Also, the total pollen number (TPN) in honey gives important information about the quality of honey (17).

Thanks to acidity level, water content and sugar concentration, raw honey has a natural inhibitory effect against yeast and bacteria growth (18).

Due to its rich chemical and nutritional content, raw honey has been used as a therapeutic and nutrient for humans for centuries (8). Studies have demonstrated the beneficial and therapeutic properties of consuming honey on the human body such as antitumoral, anticarcinogenic, antimicrobal, antioxidant, hepatoprotective and tissue regenerating effects, etc. (8, 19, 20).

The sugars and water ratio in the honey cause to crystallize naturally. When water molecules release glucose or the glucose/fructose ratio changes crystallization occurs at honey (21). Processed honey is subjected to various processes in order to prevent crystallization, the inhibition of microbial growth and to extend the shelf life. After process and heat treatment various changes are observed in the enzyme and chemical structure of honeys and nutritional values of the honey is lost (22, 23). In addition to this, filtration is also carried out in order to remove foreign substances (bee and plant parts, wax etc.) in processed honey. Unfortunately, this filtration process also causes pollen number loss and reduces the quality of honey (24).

Although there are scientific publications and regulations on processed honey, information about raw honey is limited. In this study, it was aimed to compare the mellissopalynological and chemical characters (sugar, moisture, invertase, diastase, proline and HMF contents) properties between raw and processed honey samples.

Material and Methods

Collecting of honey samples

Raw honey samples used in this study were obtained from beekeepers in Turkey. Processed honeys were selected randomly from the markets.

Melissopalynological Analyses

Pollen slides prepared according to the Özkök et al. (25, 26) methods and were examined and counted under a Nicon Eclipse E400 light microscope. Özkök et al. (25) method was used to find the Total Pollen Number (TPN) and Honeydew Elements Number (HDE) in the honey samples. Beside this, botanical origin was found in the honey samples. Özkök et al. (26) method was used for this method and the minimum number of 300 pollen was counted and determined. Botanical origins were determined by making use of existing literature and reference preparations (13, 27). The density of plant taxa was expressed according to the percentages of pollen detected and counted. Corvucci et al. (14) method was followed for this. According to this, pollen taxa groups categorized as dominant (45% ≤ x), secondary (16-44%), minor (4-15%) and trace (3% ≤ x).

Chemical Analyses

Moisture analysis

The moisture percentage (%) content of the honey samples were determined using the Lega Refractometer HB90 according to the AOAC method (28).

Diastase analysis

Diastase Number was determined according to the TS 3036 method (29). A certain amount of honey was kept at a constant temperature by mixing a certain concentration of starch solution. Starch hydrolysis is carried out by the effect of diastase enzyme in honey. After the hydrolysis event whose conditions and duration are specified in this experiment, the

remaining non-hydrolyzed starch is converted into a colored complex by treatment with iodine solution. Starch solutions of different volumes are subjected to the same process to calculate the volume of starch solution that 1 g of honey can fully hydrolyze.

Proline analysis

Proline content of honey samples were determined according to the IHC (30) method and VWR spectrophotometer uv-3100pc was used. The analysis is based on the principle of measuring the absorbance of the colored complex of proline in honey with ninhydrin in an acidic environment after the addition of 2-propanol to the blind sample at maximum wavelength.

Invertase analysis

Invertase content of honey samples were determined according to IHC invertase method (30) and VWR spectrophotometer uv-3100 pc is used.

Hydroxymethylfurfural (HMF) analysis

Hydroxymethylfurfural (HMF) content of honey samples were determined according to IHC HMF method (30) and VWR spectrophotometer uv-3100pc is used.

Sugar analysis

The sugar profile of honey samples were determined according to DIN 10758 (31) method by VWR-Hitachi Chromaster HPLC-RID.

Statistical Analyses

IBM® SPSS® Statistics 23 software was used to perform statistical analysis. The suitability of variables to normal distribution was examined by visual (histogram and probability graphs) and analytical (Kolmogrov-Smirnov/Shapiro-Wilk tests) methods. Descriptive statistics were given using median and IQR for non-normally distributed variables. Differences in TPS values between raw and processed honeys were tested with Mann–Whitney U tests for independent samples. Results with a *P* value below 0.05 were considered statistically significant.

Results and Discussion

The botanical origin of honey affects its chemical structure, so knowing the botanical origin is very important (32). Pollen grains in honey can categorize into four groups by calculating of the percentages of pollen taxa. The frequency classes of pollen grains grouped as dominant (D) (>45%), secondary (S) (16-44%), minor (M) (4-15%) and trace (T) (<3%) (14, 26). Flower honeys may exhibit monofloral or polyfloral features depending on their plant origin (15, 16). Honeys with more than 45% of pollen belonging to the same taxa classified as monofloral, and are generally named according to their botanical source (15, 26). However, according to the pollen production potential of plants, this rate may change for some taxa. For example, in monofloral chestnut honey, *Castanea sativa* pollen should be at least 70-90% (15, 20). In citrus honey at least 10% *Citrus* spp. pollen; in acacia honey 15% *Acacia* spp. pollen are enough (1, 26).

Total number of pollen in honey gives information about honey quality and adulteration. Based on TPN values honeys can seperate to 5 groups. According to this, Group I: TPN <20.000; Group II: 20.000 <TPN <100.000; Group III: 100.000 <TPN <500.000; Group IV: 500.000 <TPN <1.000.000 and Group V: TPN >1.000.000 (17, 33). If the total number of pollen is less than 20000 and more than 100.000.000, adulteration should be suspected (17, 32).

In previous studies conducted by different researchers, TPN values were found in the 1247-110192 (34); 2416-92632 (35); 2071-82005 (36); 888-722419 (20) value ranges.

In this study, botanical origins and TPN of 20 raw honeys and 20 processed honeys were determined. 33 pollen taxa were found at the raw honey samples. Accordingly, 11 of 20 raw honey samples showed monofloral, 6 of them were multifloral and 3 of them were pine honey properties (Table 1). TPN of raw honeys varried between 31158 and 259773 (Table 1; Figure 1). On the other hand, 31 pollen taxa were found at the

processed honey samples and 9 of 20 processed honeys were defined as monofloral, 8 of them are multifloral and 3 of them are pine honey. TPN of processed honeys varried between 5575 and 76895. It was also determined under the microscope that two of the processed honey (PH12, PH15) contained artifical pollen grains (Table 2; Figure 1, 2).

In Turkish Food Codex, filtered honey described as 'Honey whose pollen content is significantly reduced during the removal of foreign organic or inorganic

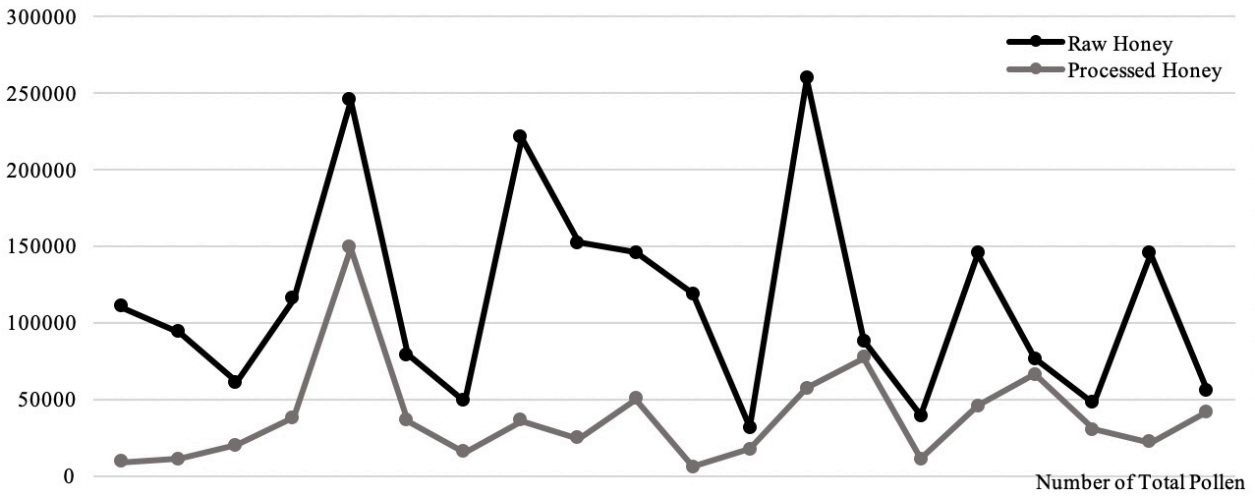

Figure 1. TPN values of raw and processed honeys

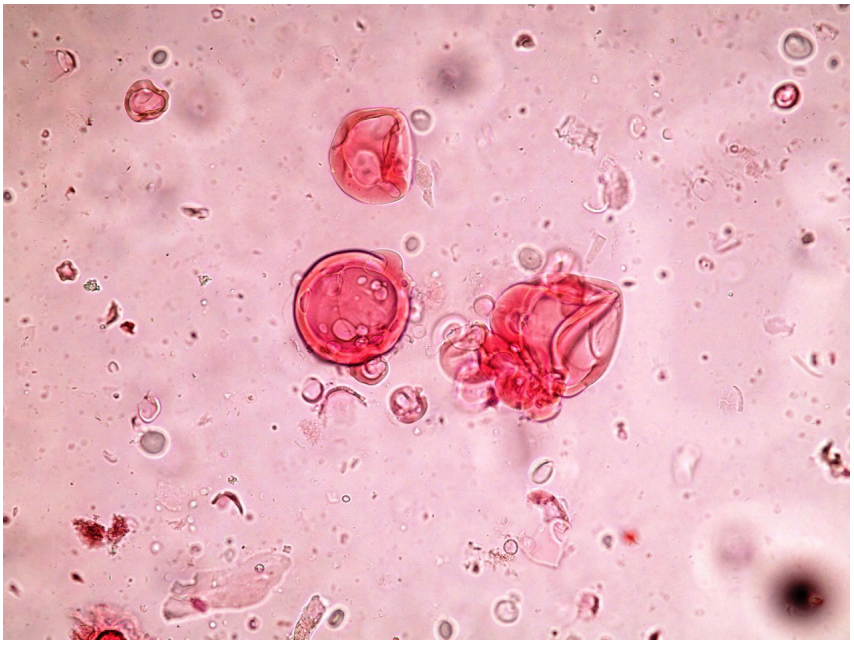

Figure 2. Artificial pollen grains in processed honeys (PH12, PH15)

Table 1. Melissopalynological Analysis Results of Raw Honey Samples

Pollen Taxa	Raw Honey Samples Plant Pollen Percentages (%)																			
	RH1	RH2	RH3	RH4	RH5	RH6	RH7	RH8	RH9	RH10	RH11	RH12	RH13	RH14	RH15	RH16	RH17	RH18	RH19	RH20
Anchusa spp.	-	3.19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	HDE 43974	HDE 198690	HDE 46165
Apiaceae	7.69	6.38	31.74	3.79	-	-	-	-	-	7.07	6.42	-	2.58	5.97	-	1.29	2.15			
Asteraceae	-	5.31	41.26	31.64	36.66	23.52	8.33	-	61.22	46.9	18.34	22	-	-	0.74	12.98	-	TPN 47161	TPN 144990	TPN 54822
Astragalus spp.	-	-	-	-	35	-	-	-	-	7.07	47.70	-	87.09	-	82.83	-	-			
Berberidaceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.89	-			
Brassicaceae	-	-	0.79	-	5	1.17	-	-	4.08	10.61	1.83	-	-	2.98	-	-	-			
Caryophyllaceae	1.09	-	-	1.26	-	-	-	-	3.40	0.88	3.66	-	-	-	-	-	-	HDE/ TPN 0.93	HDE/ TPN 1.37	HDE/ TPN 0.84
Castanea sativa	-	-	-	-	-	-	-	95	-	-	-	-	-	-	-	-	91.39			
Centaurea spp.	-	-	8.73	1.26	-	5.88	-	-	-	17.69	-	-	7.09	14.92	-	-	-			
Cephalaria spp.	-	-	-	-	-	-	25	-	-	-	-	-	-	1.49	-	-	-			
Chenopodiaceae	43.95	5.31	-	1.26	-	3.52	-	-	6.8	0.88	-	12	-	-	-	-	-			
Cichorium spp.	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-			
Cistaceae	-	-	-	2.53	6.66	1.17	-	-	0.68	3.53	-	-	-	-	-	3.89	-			
Fabaceae	15.38	21.27	2.38	18.98	5	15.29	-	-	10.20	-	-	8	-	67.16	-	64.93	4.30			
Geraniaceae	-	-	-	-	-	1.17	-	-	-	-	1.83	-	-	-	-	-	-			
Hedysarum spp.	2.19	-	-	2.53	-	-	-	-	-	-	-	-	0.64	-	-	-	-			
Lamiaceae	1.09	-	1.58	7.59	1.66	12.94	-	-	-	1.76	7.33	-	0.64	-	-	7.79	-			
Liliaceae	-	-	4.76	1.26	-	-	-	-	-	-	-	-	-	-	2.23	1.29	-			
Lotus spp.	-	15.95	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Malvaceae	-	4.25	-	1.26	-	3.52	-	-	-	-	-	50	-	-	1.49	-	-			
Plantago spp.	-	-	0.79	1.26	3.33	1.17	50	-	8.16	-	3.66	-	-	-	5.22	-	-			
Poaceae	-	5.31	0.79	8.86	-	28.23	-	-	-	-	0.91	8	-	1.49	1.49	1.29	-			
Polygonaceae	-	29.78	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Rhododendron spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.07			
Rosaceae	21.97	3.19	6.34	7.59	-	1.17	-	-	5.44	1.76	0.91	-	-	5.97	4.47	2.59	1.07			
Rumex spp.	-	-	-	-	-	1.17	-	-	-	-	-	-	-	-	-	-	-			
Salix spp.	-	-	-	-	-	-	-	-	-	0.88	5.5	-	-	-	-	-	-			

(Continued)

Pollen Taxa	Raw Honey Samples Plant Pollen Percentages (%)																			
	RH1	RH2	RH3	RH4	RH5	RH6	RH7	RH8	RH9	RH10	RH11	RH12	RH13	RH14	RH15	RH16	RH17	RH18	RH19	RH20
Sanguisorba spp.	-	-	-	2.53	-	-	-	-	-	-	0.91	-	-	-	0.74	-	-			
Scabiosa spp.	2.19	-	0.79	6.32	-	-	-	-	-	-	0.91	-	-	-	0.74	-	-			
Taraxacum spp.	4.39	-	-	-	1.66	-	16.66	1	-	0.88	-	-	-	-	-	-	-			
Teucrium spp.													1.93	-	-	-	-			
Thymus spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Tilia spp.	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-			
Honey Type	MTH	MTH	MTH	MTH	MTH	MTH	MNH	MNH	MNH	MNH	MNH	MNH	MNH	MNH	MNH	MNH	MNH	PH	PH	PH
TPN	110468	93512	60573	115593	245274	78777	48330	220744	152145	145901	118945	31158	259773	87715	38664	144990	76080	47161	144990	54822

-Pollen frequency of the pollen taxa: Dominant (D) (>45%), Secondary (S) (16-44%), Minor (M) (4-15%) and Trace (T) (<3%)-MTH: Multifloral Honey

- MNH: Monofloral Honey

- PH: Pine Honey

- TPN: Total Pollen Number

-HDE: Honeydew Elements

Table 2. Melissopalynological Analysis Results of Processed Honey Samples

Pollen Taxa	Processed Honey Samples Plant Pollen Percentages (%)																			
	PH1	PH2	PH3	PH4	PH5	PH6	PH7	PH8	PH9	PH10	PH11	PH12	PH13	PH14	PH15	PH16	PH17	PH18	PH19	PH20
Apiaceae	-	-	-	18.18	-	-	-	-	3.5	-	-	-	3.3	2.94	6.66	12.12	-	HDE	HDE	HDE
Asteraceae	20	-	-	22.72	15.90	33.33	18.18	-	8.77	62.5	12.5	28.57	27.47	5.88	73.33	56.06	5.55	23722	12388	43295
Astragalus spp.	-	-	-	-	-	-	-	-	57.89	-	-	-	-	-	-	-	-			
Berberidaceae	-	-	-	-	-	-	-	-	-	11.25	-	-	-	-	-	-	-			
Brassicaceae	-	-	-	-	1.13	-	9.09	-	3.5	-	-	4.76	1.09	-	-	1.51	-	TPN	TPN	TPN
Campanula spp.	-	-	-	-	1.13	-	-	-	-	1.25	-	-	-	-	-	-	-	30049	21827	41730
Caryophyllaceae	-	-	-	-	1.13	-	-	5.26	-	1.25	-	-	-	-	-	-	-			
Castanea sativa	-	14.28	35.29	-	45.45	-	-	-	12.28	-	-	-	-	-	-	-	-	HDE/ TPN	HDE/ TPN	HDE/ TPN
Centaurea spp.	20	-	-	-	2.27	-	-	10.52	-	-	-	-	-	-	-	15.15	11.11	0.78	0.56	1.03

(Continued)

Pollen Taxa	Processed Honey Samples Plant Pollen Percentages (%)																			
	PH1	**PH2**	**PH3**	**PH4**	**PH5**	**PH6**	**PH7**	**PH8**	**PH9**	**PH10**	**PH11**	**PH12**	**PH13**	**PH14**	**PH15**	**PH16**	**PH17**	**PH18**	**PH19**	**PH20**
Cephalaria spp.	-	-	-	-	-	-	-	5.26	-	2.5	-	-	-	-	-	-	-			
Chenopodiaceae	-	-	-	2.27	1.13	-	18.18	-	1.75	-	-	-	-	-	-	3.03	-			
Cichorium spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Cistaceae	-	-	5.88	4.54	-	11.66	-	21.05	-	-	-	-	2.2	8.82	13.33	-	11.11			
Echium spp.	-	9.52	5.88	-	1.13	-	-	-	-	-	-	-	-	-	-	1.51	-			
Ericaceae	-	-	-	6.81	-	-	-	-	-	-	-	-	-	-	6.66	-	-			
Fabaceae	-	19.04	23.52	40.90	6.81	15	27.27	-	-	2.5	25	47.61	3.3	70.58	-	9.09	61.11			
Hedysarum spp.	40	-	-	-	-	-	-	26.31	-	2.5	-	-	-	5.88	-	-	-			
Lamiaceae	-	23.80	8.82	-	-	1.66	-	-	1.75	-	62.5	14.28	60.44	-	-	-	-			
Liliaceae	-	-	2.94	-	1.13	3.33	-	10.52	-	-	-	-	-	5.88	-	-	-			
Lotus spp.	-	-	-	-	-	16.66	-	-	-	-	-	-	-	-	-	-	-			
Myosotis spp.	-	19.04	14.70	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Onobrychis spp.	-	-	-	-	6.81	16.66	-	21.05	-	5	-	-	-	-	-	-	5.55			
Plantago spp.	-	-	2.94	-	-	-	-	-	-	6.25	-	-	-	-	-	-	-			
Poaceae	-	-	-	-	5.68	-	9.09	-	-	-	-	4.76	2.2	-	-	1.51	-			
Rosaceae	-	-	-	2.27	3.40	-	18.18	-	-	-	-	-	-	-	-	-	-			
Rumex spp.	-	-	-	-	1.13	1.66	-	-	-	-	-	-	-	-	-	-	-			
Salix spp.	20	-	-	2.27	-	-	-	-	-	-	-	-	-	-	-	-	-			
Taraxacum spp.	-	-	-	-	2.27	-	-	-	-	2.5	-	-	-	-	-	-	5.55			
Thymus spp.	-	-	-	-	-	-	-	-	-	2.5	-	-	-	-	-	-	-			
Tilia spp.	-	-	-	-	3.40	-	-	-	-	-	-	-	-	-	-	-	-			
Trifolium spp.	-	14.28	-	-	-	-	-	-	10.52	-	-	-	-	-	-	-	-			
Honey Type	MTH	MTH	MTH	MTH	MTH	MTH	MTH	MTH	MNH	MNH	MNH	MNH	MNH	MNH	MNH	MNH	MNH	PH	PH	PH
TPN	8476	10379	19821	37389	149035	35927	15125	35560	24314	49709	5575	17096	57178	76895	10562	45612	66044	30049	21827	41730

-Pollen frequency of the pollen taxa: Dominant (D) (>45%), Secondary (S) (16-44%), Minor (M) (4-15%) and Trace (T) (<3%)

-MTH: Multifloral Honey

-MNH: Monofloral Honey

-PH: Pine Honey

-TPN: Total Pollen Number

-HDE: Honeydew Elements

substances by filtration (37). In accordance with this information pollen taxa number and total pollen number (TPN) of raw honeys were higher than the processed honey. Also, a significant difference TPN was showed with statistical analysis between the raw honey and processed honey (p <0.001, Mann-Whitney U test). So, it is possible to say that significantly pollen loses in the honey during the filtration process.

The moisture, diastase, invertase, proline, HMF contents and nutritional properties of honeys are affected by factors such as temperature and storage (22, 23). Especially during processes such as pasteurization and filtration, honey is exposed to thermal treatment (38).

According to Turkish Food Codex and European (CEU) Standards, the moisture content of honey should be below 20% (37, 39). Fermentation in honey with high moisture content is negatively affect the quality and shelf life of honey (40).

The moisture values of the raw honeys were determined 16.57% on average. On the other hand, the moisture values were found at processed honeys 15.85% on average. Moisture values of all honey samples were found below 20%, which is the maximum moisture value determined by the Turkish Food Codex (37) and European Standards (39).

A large part (75-80%) of honey consists of sugars (5). The sugars in honey vary according to the plant source. Sucrose from happening nectar or plant secretions is convert into invert sugars (glucose, fructose) thanks to the enzymes of honeybees during the maturation of honey (9). According to Turkish Standards (2012/58), the amount of sucrose in honey should not be more than 5% and the inverted sugars content should not be less than 60%. F + G value should not exceed 60 g/100 g in flower honey and 45 g/100 g in honeydew honey. Also F/G ratio is important for adulteration. F/G rate should be 0.9–1.4 ranges for flower honey and 1.0–1.4 for honeydew honey (37). In order to determine honey quality and sugar adulteration, it has been suggested by various researchers to determine sucrose values. However, it has been understood that the rate of sucrose and inverted sugars in honey will change with honeybee enzymes, and sugar analysis alone was not sufficient for adulteration and quality (41). Additionaly processing procedures affect the honey' sugar compositions.

In this study sugar profile of raw and processed honeys were determined. Sucrose content was found average 0.7% in raw honeys, while it was found average 0.8% in processed honeys. The sucrose contents of raw honey and processed honey were found in accordance with the limits set in the Turkish Food Codex. F+G content was determined average 57.59% in raw honey and average 63.2% in processed honey (Table 2; Figure 3). F/G ratio was determined average 1.11 in raw honeys and average 1.29 in processed honeys. There was statistically significant difference between F/G values in raw and processed honeys (p= 0.039; Mann-Whitney U test).

Umarani et al. (42) found the sucrose average content as 1.73% in raw honeys and average content in as 5.01% in processed honeys. In our study we found sucrose results lower than Umarani's results. De Rodríguez et al. (43) found average values of fructose 40.5%, glucose 34.7%, sucrose 3.4% and F/G 1.27 in the honey samples. Also this study results are compatible with our results. Can et al. (44) investigated chemical profiles of Turkish unifloral, multifloral and honeydew honeys. According to this study, unifloral honeys' fructose content 38.76%, glucose 36.85% on average. Sucrose generally were not found in unifloral honeys. Fructose content 32.35%, glucose content 25.07% and sucrose content 0.91% were found in average for multifloral honeys. Honeydew honeys' (oak and pine honeys) fructose content 41.54%, glucose 33.56% and sucrose content were found 0.36%. Similarly, sucrose was generally not found in unifloral raw honey used in our study. The sucrose averages of these honeys were determined as 0.1%. Sugar results of that study are also close to our results.

Hydroxymethyl-furfural (HMF) is a cyclic aldehyde and almost absent in fresh and unprocessed honey (45). Thermal treatment and long-term storage lead to the conversion of sugars in honey to Hydroxymethyl-furfural (HMF) as a result of Maillard reaction (46). F/G ratio in honey is also among the factors affecting HMF formation. Since fructose is morethan glucose, high F/G ratio at a certain acidity

level (average pH 4.6) can accelerate HMF formation According to Turkish Standarts (2012/58) (48) HMF content should be below the 40 mg/kg.

HMF values of raw honeys were determined between 1.2 mg/kg (RH20) and 8.1 mg/kg (RH10), an average of 4.0 mg/kg, and the values complied with the limits specified in the Turkish Food Codex. When the HMF Content of processed honeys was evaluated, it was determined as an average of 20.59 in the range of between 6.3 mg/kg (PH15) to 38.4 mg/kg (PH10) (Table 2; Figure 2). Processed honeys HMF values were also found within the specified limits. According to the statistical analysis results, a significant difference was observed between the HMF content of raw and processed honey. Accordingly, it is possible to say that HMF values can be affected if proper temperature values are not applied during the process applied to honey (p <0.00; Mann-Whitney U test).

In the study conducted by Umarani et al. (42), the chemical contents of 3 processed honey and 2 raw honey were investigated. HMF content of raw honey was determined as 2.63 mg/kg and HMF content of processed honey was determined as 17.43 mg/kg on average. These results are compatible with the results of HMF in our study and show that HMF value is high in processed honey (Table 3, Figure 3).

Proline is the most abundant amino acid in honey and it can be found in different proportions depending on the botanical origin of the honey and bee species (10, 49). It has been used for distinguish between natural and adulterated honeys and is affected the thermal process (30, 49). In European Union, the proline content is determined as 180 mg/kg. In the Turkish Food Codex value of proline should be at least 180 mg/kg for *Canola* spp., *Citru*s spp., *Lavender* spp., *Eucalyptus* spp. honeys, and should be at least 300 mg/kg for flower honey and pine honeys (37, 41).

The proline mean value in raw honey was determined as 620.6 mg/kg and in the processed honey was determined as 421.96 mg/kg. The proline value, of two processed honeys (PH6, PH10) were found below the determined limits (Table 3; Figure 3). Proline values of raw honeys were found higher than processed honeys. Statistical analysis also confirmed that there was a significant difference between the proline values of raw and processed honeys (p= 0.029; Mann-Whitney U test).

Invertase and diastase are enzymes in the hypopharyngial glands of honey bees and they are added to honey by bees. It is effective in the conversion of sucrose to glucose and fructose. Diastase is effective in the conversion of starch grains to maltose and dextrin (5, 9). Since they are both in enzyme structure, they may undergo structural degradation under temperature applications and may be affected by long-term storage conditions (50). It is known that invertase is more sensitive to heat than diastase. It has been observed in some reseachers that it is a more preferred parameter in quality and adulteration studies than diastase. In addition, the amount of diastasis in honey is less than invertase (5, 23, 30). Turkish Food Codex has determined the number of diastase in honey to be at least 8 DN (37).

The invertase value was determined as 188.30 U/kg in raw honey and as 56.39 U/kg in processed honey on average. The invertase value of 14 honeys from the processed honeys were found under 75 U/kg. The diastasis value of all raw honey samples was found above 8 DN and the average value was 14.1 DN. Thus, all examples are compatible with the Turkish Food Codex. On the other hand, the diastase values of the processed honeys were found 13.6 DN on average and 4 of these honeys were found under 8 DN and were not compatible with the limits (Table 3; Figure 3). The results obtained show that invertase and diastase values can be negatively affected after the procedure. A statistically significant difference was observed between the invertase and diastase values of raw and processed honey (p <0.00 (invertase), p= 0.019 (diastase); Mann-Whitney U test).

In the study of ahin et al. (23), the enzyme contents of the raw honeys were evaluated after the heat treatment. Invertase values were found initially in the range of 167.00 to 135.742 U/kg. After heat application invertase values were determined between 150.260 and 119.79 U/kg. Similarly, in this study, it was observed that the invertase values of the honeys were negatively affected as a result of the temperature application.

Table 3. Chemical contents of raw and processed honey samples

Sample Name		Moisture (%)	HMF (mg/kg)	Diastase Number (DN)	Invertase (U/Kg)	Proline (mg/kg)	Sucrose (%)	Fructose + Glucose (%)	Fructose/ Glucose (F/G)
Raw Honey	RH1	16.6	3.1	13.5	176.1	484.0	1.0	63.6	1.20
	RH2	17.1	4.8	10.9	133.5	401.2	0.6	66.0	1.17
	RH3	15.4	3.8	22.7	276.7	519.0	0.1	64.2	1.34
	RH4	15.6	2.9	10.9	187.9	331.1	1.0	62.6	1.26
	RH5	16.4	4.8	10.9	162.1	458.5	0.3	67.7	1.24
	RH6	16.9	5.0	11.9	114.4	436.2	0	0.0	0.0
	RH7	16.3	3.3	11.4	230.3	426.7	0.1	52.8	1.16
	RH8	18.8	3.5	17.9	130.3	1168.6	0	0.0	0.0
	RH9	17.3	1.9	13.2	229.5	544.5	0.3	66.2	1.20
	RH10	17.8	8.1	19.2	254.3	808.8	0.0	68.0	1.31
	RH11	15.7	4.6	10.9	135.1	687.8	0.3	69.1	1.24
	RH12	16.2	3.3	17.2	151.0	764.2	0.1	69.6	1.20
	RH13	15.3	5.8	11.9	178.0	576.3	0.1	66.9	1.34
	RH14	16.4	4.8	10.9	162.1	458.5	0.3	67.7	1.24
	RH15	16.3	1.5	11.9	298.0	420.3	0.3	62.7	1.26
	RH16	16.9	5.4	10.9	174.8	582.7	0.1	69.6	1.23
	RH17	15.9	4.8	22.7	176.4	831.0	0.0	64.9	1.27
	RH18	17.1	2.7	16.7	252.9	818.3	1.2	59.2	1.20
	RH19	17.4	2.9	13.2	209.8	811.9	3.4	61.4	1.19
	RH20	16.9	1.2	13.2	132.7	490.3	5.0	53.5	1.24
Processed Honey	PH1	16.4	22.3	8.8	26.9	452.6	0.3	479.5	0.06
	PH2	16.1	32.6	9.4	119.2	316.9	0.0	436.1	0.38
	PH3	16.1	30.7	9.4	106.5	445.8	0.0	552.3	0.24
	PH4	15.8	17.3	6.5	31.0	341.5	1.4	372.5	0.09
	PH5	14.1	12.5	8.1	73.1	427.3	1.1	500.4	0.17
	PH6	14.6	16.9	4.0	22.4	194.2	2.3	216.6	0.12
	PH7	16.5	26.5	8.1	23.0	382.1	0.4	405.1	0.06
	PH8	16.6	28.8	12.5	54.5	586.9	0.0	641.37	0.09
	PH9	17.6	27.8	7.5	15.9	464.8	0.6	480.7	0.03
	PH10	14.1	38.4	3.0	21.3	251.5	0.8	272.8	0.08
	PH11	16.6	7.7	47.6	9.5	406.9	0.0	416.4	0.02
	PH12	15.1	17.1	23.8	19.1	478.5	0.7	497.6	0.04
	PH13	16.5	27.6	9.4	81.1	427.3	2.2	508.4	0.19
	PH14	15.4	21.7	12.5	100.1	384.4	3.6	484.5	0.26
	PH15	16.1	6.3	45.5	57.2	411.0	0.7	468.2	0.14
	PH16	15.6	6.7	9.4	47.0	396.7	0.7	443.7	0.12
	PH17	16.8	28.4	13.5	96.0	704.2	0.0	800.2	0.14
	PH18	15.0	10.0	8.1	98.7	333.4	0.3	432.1	0.30
	PH19	16.0	21.1	12.8	77.9	575.8	0.1	653.6	0.14
	PH20	16.1	11.3	12.5	47.4	457.5	0.2	504.9	0.10

Oddo et al. (11) also showed that the enzyme contents of honey can be affected by conditions such as botanical origin and climatic conditions. In their study, botanic origins, invertase and diastase activities of 499 honeys were determined. They found the highest invertase and diastase values at the honeydew honeys (*Metcalfa* spp., *Abies* spp.). However, in our study, invertase and diastase values were changing to the honey types (Table 3, Figure 3). Also Belay et al. (51) showed that the enzyme content is statistically affected by botanical origin (p <0.01). In that study invertase values of honeys were detected ranged from 1.9 to 38.40 IN. Diastase activity of honeys were varried from 3.91 to 13.6 (DN) according to botanical origin. Can et al. (44) determined diastase content of Turkish honeys. Diastase content of unifloral honeys were detected 8.98 DN on average. Honeydew honeys (Oak and Pine honeys) had 11.05 DU and multifloral honeys had 9.07 DN on average. This results are compatible for Turkish Standarts. In our study, we found higher diastase results.

According to statistical analysis results, 95% confidence level, TPN (p <0.00), moisture % (p= 0.029), F/G (p= 0.039), HMF (p <0.00), diastase (p= 0,019), invertase (p <0.00), proline (p= 0.029) values were found to be significant between raw and processed honeys. At this point, it has been observed that the filtration process in honey causes pollen losses and the heat applications negatively affect the chemical properties of honey.

Conclusion

As a result of this study, it has been observed that processed honey contains less pollen compared to raw honey and raw honeys are richer in terms of pollen quality. When evaluated chemically, it was observed

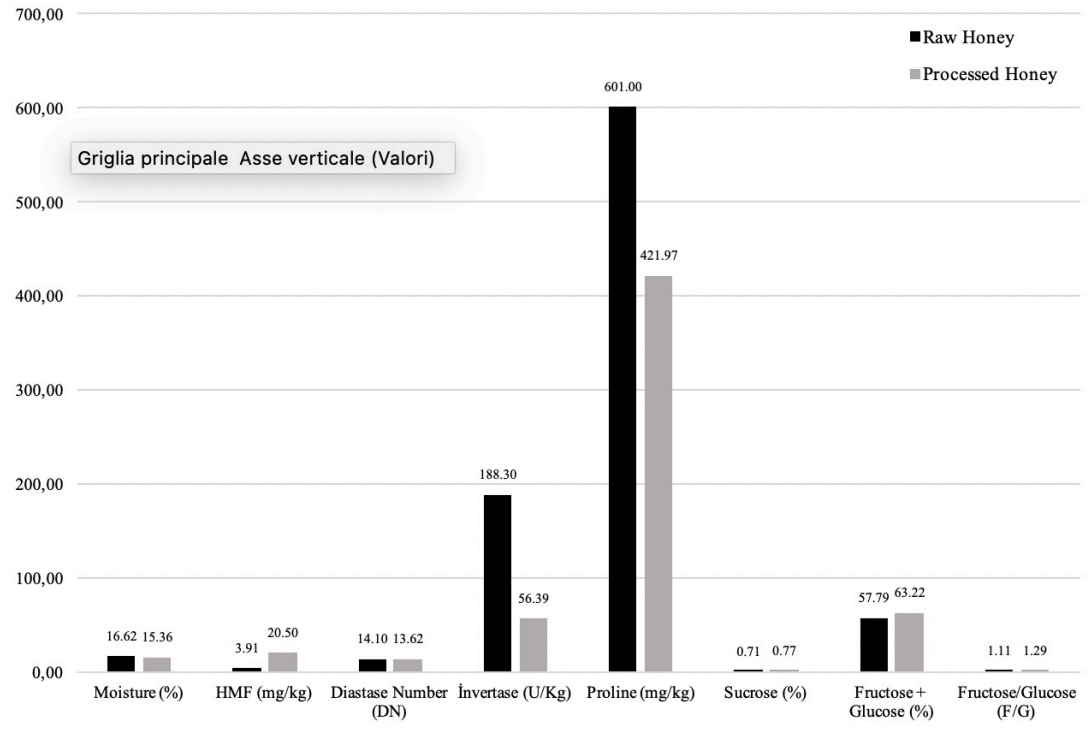

Figure 3. Average data of chemical analysis results of honey samples.

that raw honey and processed honey showed significant differences in F/G ratios, diastase, invertase, proline and HMF values. It is possible to say that the filtration process applied during the process causes pollen losses in the honey samples. In addition, the amount of HMF increases as a result of heat treatment in honey samples, and diastase, invertase and proline values are negatively affected. For this reason, consuming honey in its raw form, which is intact, is very important for human health.

However, although there are regulations regarding processed honey, limits regarding the quality characteristics of raw honey have not been determined. In this case, it causes problems in the sale of raw honey. Beside that, there are limited number of studies on raw honeys in the literature. Furthermore, studies should be done to identify the differences between raw honey and processed honey and contribute to international honey standardization.

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References

1. Anklam E. A review of the analytical methods to determine the geographical and botanical origin of honey. Food Chem 1998; 63(4): 549–562.
2. Sorkun K, Yılmaz B, Özkırım A, Özkök A, Gençay Ö, Bölükbaşı D. Yaşam Için Arılar. TAB 2012; 36–56.
3. Clemson A. Honey and Pollen Flora. New South Wales, Australia. Inkata Press 1985; 10–263.
4. Blasa M, Candiracci M, Accorsi A, Piacentini M P, Albertini M C, Piatti E. Raw Millefiori Honey is packed full of antioxidants. Food Chem 2006; 97(2): 217–222.
5. Özkök A. Arı Ürünleri-I. Türkiye'de Hızla Büyüyen Sektör: Arı Ürünlerine Genel Bir Bakış. Edited by Aslı Özkök, Hacettepe Üniversitesi, Ankara 2018; 49-55.
6. White J, Crane E. Honey a Comprehensive Survey. Edited by Eva Crane, Morrison and Gibb Ltd 1975: 194–206.
7. White J, Landis W. Beekeping in the United States Agriculture Handbook Honey Composition and Properties. 1980; 124–335.
8. Krell R. Value-added Products from Beekeeping. FAO AGRICULTURAL SERVICES BULLETIN No. 124 Food & Agr. Org 1996.
9. Vorlova L, Pridal A. Invertase and diastase activity in honeys of Czech Provenience. Acta Universitatis Agriculturae et Silviculturae Sbornik Mendelovy Zemedelske a Lesnicke Mendelianae Brunensis 2002; 5: 57–66.
10. Von Der Ohe W, Dustmann J, Von Der Ohe K. Proline as a criterion of honey maturity. Dtsch Lebensm Rundsch 1991; 87: 383–6.
11. Oddo L P, Piazza M G, Pulcini P. Invertase activity in honey. Apidologie 1999; 30(1): 57–65.
12. Abdel-Aal E M, Ziena H, Youssef M. Adulteration of honey with high-fructose corn syrup: Detection by different methods. Food Chem 1993; 48(2): 209–212.
13. D'Albore G R. Textbook of Melissopalynology. Apimondia 1997; 139–288.
14. Corvucci F, Nobili L, Melucci D, Grillenzoni F V. The Discrimination of honey origin using melissopalynology and Raman spectroscopy techniques coupled with multivariate analysis. Food Chem 2015; 169: 297–304.
15. Terrab A, González A G, Díez M J, Heredia F J. Characterisation of Moroccan unifloral honeys using multivariate analysis. Eur Food Res Technol 2003; 218(1): 88–95.
16. Soares S, Amaral J S, Oliveira M B P, Mafra I. Improving DNA Isolation from honey for the botanical origin identification. Food Control 2015; 48: 130–136.
17. Feller-Demalsy M-J, Parent J, Strachan A A. Microscopic analysis of honeys from manitoba, Canada. J. Apic. Research 1989; 28(1): 41–49.
18. Postmes T, Van Den Bogaard A, Hazen M. Honey for wounds, ulcers, and skin graft preservation. The Lancet 1993; 341(8847): 756–757.
19. Subrahmanyam M. Topical application of honey in treatment of burns. Br J Surg 1991; 78(4): 497–498.
20. Mayda N, Özkök A, Sorkun K. Some characteristic properties of chestnut and rhododendron honeys in Turkey. Hacettepe J. Biol. Chem 2018; 46(1): 135–145.
21. Alam M S, Sharma D, Sehgal V, Arora M, Bhatia S. Development and evaluation of low cost honey heating-cum-filtration system. J Food Sci Technol 2014; 51(11): 3476–3481.
22. Ribeiro G P, Villas-Bôas J K, Spinosa W A, Prudencio S H. Influence of freezing, pasteurization and maturation on Tiúba honey quality. LWT 2018; 90: 607–612.
23. ahin H, Kolaylı S, Beykaya M. Investigation of variations of invertase and glucose oxidase degrees against heating and timing options in raw honeys. J Chem 2020; 2020: 1–7.
24. Ruoff K, Bogdanov S. Authenticity of honey and other bee products. Apiacta 2004; 38(38): 317–327.
25. Özkök A, Yüksel D, Sorkun K. Chemometric evaluation of the geographical origin of Turkish pine honey. Food and Health 2018; 4(4): 274–282.

26. Özkök A, Özenirler Ç, Canli D, Mayda N, Sorkun K. Monofloral features of Turkish honeys according to mellissopalynologic, total phenolic acid and total flavonoid content. GUJS 2018; 31(3): 713–723.
27. PalDat. Palynological Database. [cited 15th March 2019]; Available from: www.paldat.org,Faegri.
28. AOAC Officlal Method 969.38. moisture in honey. 1969; 729.
29. Turkish Stadart Institution. Honey standart. ICS 67.180.10. 2002.
30. Bogdanov S, Martin P, Lullmann C. Harmonised Methods of the International Honey Commission. Swiss Bee Research Centre, FAM, Liebefeld 2002.
31. Deutsches Institut Tür Normung, Fructose, Glucose, Sucrose, Turanose and Saccharides Determination of Maltose Content. DIN 10758:1997–05. 1997.
32. Sorkun K. Türkiye'nin nektarlı bitkileri, polenleri ve balları. Palme Yayıncılık. 2008; 1–9.
33. Maurizio A. Microscopy of honey. A Comprehensive Survey of Honey. Edited by Crane. London, Heinemann. 1975.
34. Özenirler Ç, Mayda N, Çelemli Ö G, Özkök A, Sorkun K. Paketli balların botanik orijin tespiti ve mikroskobik analizleri: Etiket bilgileri ile içerik uyumluluğunun karşılaştırılması. Gıda 44(5): 861–865.
35. Keskin , Mayda N, Keskin M, Özkök A. Bilecik ballarının melissopalinolojik ve kimyasal özellikleri açısından değerlendirilmesi. Gıda 45(2): 275–289.
36. Gürbüz S, Gençay Çelemli Ö, Özenİrler Ç, Mayda N, Özkök A, Sorkun K. Melissopalnological analysis of honey samples collected from ırnak city. U Bee J 2019; 19(2).
37. Turkish Food Codex. Honey Notification 2012/58. T.C. Ministry of Agriculture and Rural Affairs 2012.
38. Turhan K. Effects of thermal treatment and storage on hydroxymethylfurfural (HMF) content and diastase activity of honeys collected from Middle Anatolia in Turkey. in Innovations In Chemical Biology 2009; 233–239.
39. Küçük M, Kolaylı S, Karaoğlu , Ulusoy E, Baltacı C, Candan F. Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chem 2007; 100(2): 526–534.
40. Stephen W. The relationship of moisture content and yeast count in honey fermentation. Scientific Agriculture 1946; 26(6): 258–264.
41. Cavrar S, Yıldız O, ahin H, Karahalil F, Kolaylı S. Comparison of physical and biochemical characteristics of different quality of Turkish honey. U Bee J 2013; 13(2).
42. Umarani S, Eswaran V U, Keerthika E, Mathumitha K, Elakkiya S, Bhargava H. A Relative Study on the chemical composition amongthe pure and branded honey types collected from diverse sources of Tamilnadu, India. World Appl. Sci. J. 2015; 33(3): 401–408.
43. De Rodríguez G O, De Ferrer B S, Ferrer A, Rodríguez B. Characterization of honey produced in Venezuela. Food Chem 2004; 84(4): 499–502.
44. Can Z, Yildiz O, Sahin H, Turumtay E A, Silici S, Kolayli S. An Investigation of Turkish honeys: Their physico-chemical properties, antioxidant capacities and phenolic profiles. Food Chem 2015; 180: 133-141.
45. Bath P K, Singh N. A Comparison between *Helianthus annuus and Eucalyptus lanceolatus honey*. Food Chem 1999; 67(4): 389–397.
46. Tosi E, Ciappini M, Re E, Lucero H. Honey thermal treatment effects on hydroxymethylfurfural content. Food chem 2002; 77(1): 71–74.
47. Lee H S, Nagy S. Relative reactivities of sugars in the formation of 5-hydroxymethylfurfural in sugar-catalyst model systems 1. J Food Process Pres 1990; 14(3): 171–178.
48. Directive Council 110/EC of 20 December 2001 Relating to Honey. OJEC 2001; 10(12.1): 2002.
49. Başoğlu F N, Sorkun K, Löker M, Doğan C, Wetherilt H. Saf ve sahte balların ayırt edilmesinde fiziksel, kimyasal ve palinolojik kriterlerin saptanması. Gıda 1996; 21(2).
50. D'Arcy B. High-power ultrasound to control of honey crystallisation. Rural Industries Research and Development Corporation 2007; 7–145.
51. Belay A, Haki G D, Birringer M, Hannelore B, Young-Chul L, Kyung-Tack K, Kaleab B, Samuel M Enzyme activity, amino acid profiles and hydroxymethylfurfural content in Ethiopian monofloral honey. Journal of Food Science and Technology 2017; 54 (9): 2769–2778.

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