

## CHANGES IN SUGARS OF CLEAR HONEYS KEPT IN GLASS JARS DURING STORAGE

### SÜZME BALLARIN CAM KAVANOZDA DEPOLANMALARI SIRASINDA ŞEKERLERDE MEYDANA GELEN DEĞİŞMELER

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**SUMMARY:** Influence of storage temperature and duration on the composition of sugars of honey determined by HPLC on honey samples stored at room temperature in the dark or light, in an incubator at 35°C or in a refrigerator at 4°C, were investigated. In general a decrease in monosaccharides and an increase in di- and trisaccharides were observed during the storage. The samples stored at 35°C showed significant changes, but samples stored at 4°C showed a little change in sugar composition.

**ÖZET:** Balın şeker kompozisyonu üzerine depolama sıcaklığı ve sürenin etkisinin araştırıldığı bu çalışmada, oda sıcaklığında aydınlıkta ve karanlıkta, 35°C'de ve 4°C'de depolanan bal örneklerindeki şekerler Yüksek Basınç Sıvı Kromatografisi (HPLC) kullanılarak belirlenmişlerdir. Genel olarak depolama süresi boyunca monosakkaritlerde azalma, di ve trisakkaritlerde artma gözlenmiştir. 35°C'da depolanan örneklerde önemli farklar belirlenirken, 4°C'da depolanan örneklerde çok küçük bir değişme görülmüştür.

#### INTRODUCTION

Honey is the sweet substance produced by honey bees from the nectar of blossoms or from secretions of on living parts of plants, which they collect, transform and combine with specific substances, and store in honey combs (CAC, 1969). Sugars are the major constituents of honey. Glucose and fructose together account for 85-95 % of honey carbohydrates. Besides glucose and fructose, honey contains protein, aminoacids, enzymes, organic acids, mineral substances, pollen and other substances (CRANE, 1975). According to many research, honeys include glucose, fructose, sucrose, turanose, maltose, isomaltose, melibiose, kojibiose, nigerose, melezitose, erlose, késtose, raffinose (DONER, 1977).

Factors influencing the composition of honey are the source of nectar, climate, soil, ripening time and the period and conditions of storage. More complex sugars have formed along the period of storage. WHITE et al (1961) reported that monosaccharides decreased and oligosaccharides increased in honey stored at 18-30°C for 12 months. The increase in the oligosaccharides in honey in caused by enzyme activity and acid reversion. Extensive changes in sugar composition occurred when the honey samples stored at room temperature.

Decrease in glucose and fructose were observed as 13 % and 5.5 %, respectively during two years. The reducing disaccharide sugars, stated as maltose have been increased to the average 69 % of the amount initially present (DONER, 1977).

HAREMI (1978) studied on the effect of prolonged storage at 50°C. It was observed that honeys had dark colour and sucrose, melezitose and total sugar content decreased and turanose content increased.

In this study, the changes in mono-, di- and trisaccharides in honey due to the storage conditions and time were investigated by HPLC. HPLC study conditions were adapted from THEAN and FUNDERBURK (1977), LOMBARD et al (1984) and BOGDANOV and BAUMANN (1988).

#### MATERIALS AND METHODS

Honeydew and floral honey samples provided from beekeepers were stored at four different conditions (at room temperature at light or dark; at 35°C; at 4°C) for 9 months.

Honey sugars were determined by HPLC at 3 months intervals. Determination of sugars in honey by HPLC is as follows; 10 g of the honey was weighed into 50 ml volumetric flask and added 10 ml acetonitrile and completed with water to mark. Diluted honey was filtered through 0.45 µm membrane

filter and injected directly into the chromatograph. Samples are eluted from a  $\mu$ -Bondapak-NH<sub>2</sub> column with acetonitrile-water (83+17, v/v) and quantitated with refractive index detector. pH of water used for mobil phase was buffered to pH 7 to prevent the separation of glucose peak as  $\alpha$  and  $\beta$  anomers. The ratios of sugars on HPLC chromatogram were calculated by dividing the area of the each peak to the total area of the peaks detected.

## RESULTS AND DISCUSSION

Sugars are eluted from NH<sub>2</sub> column according to their molecular weight. Among the sugars which have the same molecular weight, ketose sugars eluted from the column before aldose sugars. Ten different sugars; fructose, glucose, sucrose, turanose, maltose, isomaltose, gentiobiose, melibiose, melezitose, raffinose were determined in honeydew honeys and floral honeys. Table 1 shows the initial sugar contents of honeydew and floral honey samples.

Table 1. Initial sugar contents of honeydew and floral honey samples (%)

	Honeydew honey	Floral honey
Fructose	47.76	48.90
Glucose	43.75	42.95
Sucrose	1.69	1.55
Turanose	1.45	1.40
Maltose	1.57	1.63
Isomaltose	1.17	0.83
Gentiobiose	0.33	0.34
Melibiose	0.78	0.87
Melezitose	0.75	0.87
Raffinose	0.67	0.58

Table 2. The Changes in sugar content of honeys stored at different conditions 9 months along (%)

		at room temp.			
		in light	in dark	at 35°C	at 4°C
HONEYDEW HONEY	Decreasing in monosaccharides	3.83	3.49	5.26	1.56
	Increasing in di- and trisacc.	43.04	38.29	57.91	15.10
FLORAL HONEY	Decreasing in monosaccharides	3.75	3.21	6.40	1.56
	Increasing in di- and trisacc.	43.66	37.30	73.23	17.97

Monosaccharides: Glucose + Fructose

Di- and trisaccharides: Sucrose + Turanose + Maltose + Isomaltose + melibiose + melezitose + raffinose

an active transglucosylase enzyme in the honey may also result in accumulation of oligosaccharide material (WHITE et al., 1961). Since the temperature of the incubator is close to the optimum temperature of enzyme activity, the most significant changes were occurred in samples stored at 35°C. On the other hand, fructose in honey undergoes to degradation to hydroxymethyl furfural by long standing in mild acidic media.

Changes in samples stored at room temperature (light or dark) seem to be closed to each other but the changes in samples at light are higher than the samples at dark as statistically ( $p < 0.1$ ).

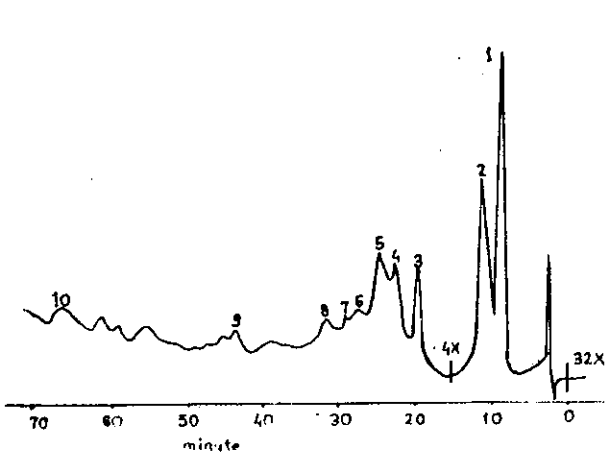
High Pressure Liquid Chromatograms of honeydew and floral honeys are shown in Figure 1 and 2. During the period of 9 months a decrease in monosaccharides (glucose+fructose) and an increase in di- and trisaccharides (sucrose + turanose + maltose + isomaltose + gentiobiose + melibiose + melezitose + raffinose) were observed (Table 2).

Table 2. shows the changes of sugar composition after 9 months storage. The most significant changes were occurred in the samples stored at 35°C.

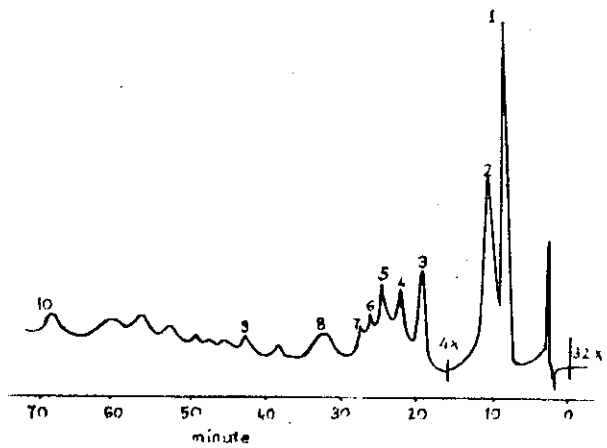
A decrease of 6.40 % and 5.26 % in the monosaccharides and an increase of 73.23 % and 57.91 % in the di- and trisaccharides of floral and honeydew honey samples, respectively, were found, when they were stored at 35°C.

Data were commented on statistically by the analysis of variance. Variability due to storage time and conditions was calculated and tested statistically and the differences were found significant ( $p = 0.01$ ).

The main reason for change in sugars is activities of the enzymes and acid reversion in honey. A high sugar concentration and a considerable acidity over a period of time would promote combination of monosaccharides (reversion). The presence of



1) Fructose      2) Glucose      3) Sucrose  
 6) Isomaltose    7) Gentiobiose    8) Melibiose



4) Turanose      5) Maltose  
 9) Melezitose    10) Raffinose

Figure 1. HPLC Chromatogram of honeydew honey

Figure 2. HPLC Chromatogram of floral honey

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