*Food Chemistry* **48** (1993) 209–212
*Analytical Methods Section*

# Adulteration of honey with high-fructose corn syrup: Detection by different methods

E-S.M. Abdel-Aal, H. M. Ziena & M. M. Youssef*

*Department of Food Science and Technology, Faculty of Agriculture, University of Alexandria, El-Chatby, Alexandria, 21526, Egypt*

(Received 17 June 1992; revised version received 21 January 1993; accepted 26 January 1993)

Pure honey was deliberately adulterated with high-fructose corn syrup (HFCS) at levels of 10%, 20%, 30%, 40%, and 50% (w/w). Sugar composition as a fingerprint was determined by HPLC for all samples. The following compositional properties were determined for pure and adulterated honey: moisture, total soluble solids, nitrogen, apparent viscosity, hydroxymethylfurfural (HMF), ash, sodium, calcium, potassium, proline, refractive index and diastatic activity.

Statistical analysis revealed that the following compositional properties were highly significantly negatively correlated with sugar composition: dry matter, apparent viscosity, sodium, potassium, proline, and nitrogen. In contrast, ash, calcium, HMF, and moisture were highly significantly positively correlated with sugar composition for pure and adulterated honey. Accordingly, such simple tests can be applied as good indicators for detecting the adulteration of honey with HFCS at adulteration levels ranging from 10% to 50%.

## INTRODUCTION

According to Recommended European Regional Standards, honey is the sweet substance produced by honey bees from the nectar of blossoms or from secretion of or on living parts of plants, which they collect, transform and combine with specific substances and store in honeycomb (Martin, 1979). However, honey is frequently adulterated with relatively cheap high-fructose corn syrup (HFCS) (Croft, 1987; Swallow & Low, 1990).

Detection of honey adulteration is difficult and depends on the composition of the honey or on the presence of a specific component in the adulterant. Many analytical methods have been used to detect the adulteration of honey with HFCS. These methods include viscosity (Pechhacker & Sölkner, 1987); TLC (White & Robinson, 1983; Clarke, 1988); GLC and GC–MS (Frangipane *et al.*, 1984; Serra Benvehi & Gomez Pojuelo, 1986); HPLC (Sangiorgi, 1988; Lipp & Ziegler, 1989; Swallow & Low, 1990); electrophoresis (Marshall & Williams, 1987); staple-isotope-ratio analysis (SIRA) by means of MS (White & Winters, 1989) and $^{13}$CNMR spectroscopy (Low *et al.*, 1988).

The present work was undertaken to develop a simple and inexpensive method(s) that is (are) correlated well with HPLC as a 'fingerprint' method for detecting the adulteration of honey with HFCS. Such a finding will be practically important, especially in developing countries, where sophisticated analytical instruments are not available for routine analysis.

* The author to whom correspondence should be addressed.



## MATERIALS AND METHODS

### Materials

An authentic sample of clover honey was collected directly from the comb of honey bees by straining it through a No. 40 sieve (size of opening 0·420 mm). Honey was thoroughly mixed and strained through cheese cloth to remove foreign matter. High-fructose corn syrup (HFCS) of 55% fructose was kindly provided by the National Company for Maize Products, 10th of Ramadan City, Egypt.

### Deliberate adulteration

Honey was deliberately adulterated with 10, 20, 30, 40 and 50% HFCS (w/w). The adulterated samples were heated in a water bath at up to 35°C to ensure thorough mixing.

### Analytical methods

Both pure honey and HFCS were analyzed along with the adulterated samples for comparison.

Refractive index and total soluble solids were measured at 20°C by an Abbé refractometer (Digital Refractometer Atago, Germany). Moisture content was computed from the refractive index by utilizing the appropriate tables given by Martin (1979).

The apparent viscosity was assessed with a rotary viscometer (Type RN, VEB MLW Prüfgerate Werk, Germany). The apparent viscosity was computed as follows:

$$\eta = KN\alpha$$

where $\eta$ = apparent viscosity in centipoise; $K$ = cylinder constant; $N$ = speed factor and $\alpha$ = pointer deflection.

Fructose, glucose, maltose, trisaccharides and polysaccharides were identified and determined by high-performance liquid chromatography (HPLC) by using a Waters HPLC (M 510), USA. The pressure applied was 500 psi and the column used was carbohydrate Aminex at 85°C. The refractive-index detector (Atago, Model Rx1000, Japan) was used to monitor the column effluent. Hydroxymethylfurfural (HMF) was determined spectrophotometrically as outlined by Martin (1979). The diastatic activity was based on starch hydrolysis (AOAC, 1980) as 300/time to a value of absorbance at 660 nm = 0·235. Nitrogen was determined by the Kjeldahl method by using a Kjeltec Auto Analyzer (Model 1030—Tecator) as described by Egan *et al.* (1981).

A weighed sample was ignited in a muffle furnace at 550°C to a constant weight for ash determination (AOAC, 1980). Sodium, calcium and potassium were determined by using the Flame Analyzer (Gallenkamp, UK) according to the AOAC (1980).

Proline was determined spectrophotometrically by using ninhydrin in methyl cellosolve, and absorbance was read at 512 nm. A standard curve using pure proline was constructed (Martin, 1979).

### Statistical analysis

Data were subjected to analysis of variance and multiple-correlation analysis as outlined by Steel and Torrie (1980). Further multiple comparison of means for each constituent or property was conducted by Duncan's multiple-range test.

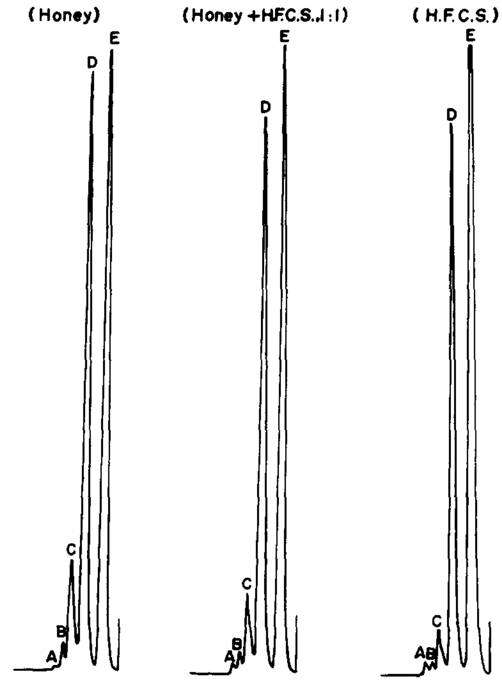


**Fig. 1.** High-performance liquid chromatogram of honey, mixture of honey and high-fructose corn syrup (HFCS, 1:1) and HFCS (A = polysaccharide; B = trisaccharide; C = maltose; D = glucose; E = fructose).

## RESULTS AND DISCUSSION

### Sugar composition

Data in Table 1 and Fig. 1 indicate that the fructose content of pure honey (47·6%) increased gradually as the percentage of HFCS adulterant was increased. In contrast, contents of both glucose and maltose were inversely proportional to the percentage of HFCS adulterant. Fructose, glucose, and maltose constitute about 76·8% of honey composition on a wet basis, the individual contents ranging from 27·3 to 44·3%, from 22·0 to 40·8%, and from 2·7 to 16·0% for fructose, glucose, and maltose, respectively (Martin, 1979). However, the levels of trisaccharides and polysaccharides did not exhibit a clear-cut trend with respect to adulteration with HFCS, despite the significant differences in their contents for pure honey and HFCS.

It is worth mentioning that the fructose/glucose ratio ranged between 1·10 and 1·42 for pure honey and HFCS, respectively. Moreover, this ratio was increased by raising the percentage adulteration with HFCS.

**Table 1. Carbohydrate contents of honey, adulterated honey with high-fructose corn syrup (HFCS), and HFCS**

| Adulteration (%) | Fructose (%) | Glucose (%) | Maltose (%) | Trisaccharide (%) | Polysaccharide (%) | *F/G* ratio |
|---|---|---|---|---|---|---|
| 0·0 (Honey) | 47·6$^{f}$ | 43·4$^{a}$ | 7·4$^{a}$ | 1·5$^{a}$ | 0·1$^{f}$ | 1·10$^{f}$ |
| 10 | 48·4$^{e}$ | 42·9$^{b}$ | 7·0$^{b}$ | 1·5$^{a}$ | 0·2$^{e}$ | 1·13$^{e}$ |
| 20 | 49·2$^{d}$ | 42·7$^{b}$ | 6·6$^{c}$ | 1·2$^{c}$ | 0·2$^{e}$ | 1·15$^{d}$ |
| 30 | 50·0$^{c}$ | 42·2$^{c}$ | 6·2$^{d}$ | 1·3$^{b}$ | 0·3$^{d}$ | 1·18$^{c}$ |
| 40 | 50·9$^{b}$ | 41·7$^{d}$ | 5·8$^{e}$ | 1·1$^{d}$ | 0·5$^{b}$ | 1·22$^{b}$ |
| 50 | 51·2$^{b}$ | 41·6$^{d}$ | 5·3$^{f}$ | 1·1$^{d}$ | 0·4$^{c}$ | 1·23$^{b}$ |
| HFCS | 56·2$^{a}$ | 39·5$^{e}$ | 3·0$^{g}$ | 0·6$^{e}$ | 0·7$^{a}$ | 1·42$^{a}$ |

*F/G* ratio: fructose/glucose ratio.
Means in a column not sharing the same superscript are significantly different at $p < 0{\cdot}05$.

**Table 2. Chemical composition of honey, honey adulterated with high-fructose corn syrup (HFCS) and HFCS**

| Adulteration (%) | Moisture (%) | Nitrogen (%) | Proline mg per 100 g | HMF mg $kg^{-1}$ | Ash (%) | K mg $kg^{-1}$ | Na mg $kg^{-1}$ | Ca mg $kg^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| 0·0 (honey) | 18·4$^{e}$ | 0·06$^{a}$ | 76·8$^{a}$ | 12·8$^{g}$ | 0·17$^{e}$ | 269$^{a}$ | 59·5$^{a}$ | 52·6$^{g}$ |
| 10 | 18·6$^{e}$ | 0·04$^{b}$ | 72·9$^{b}$ | 16·8$^{f}$ | 0·19$^{e}$ | 240$^{b}$ | 54·0$^{b}$ | 58·6$^{f}$ |
| 20 | 18·8$^{e}$ | 0·04$^{b}$ | 69·1$^{c}$ | 19·8$^{e}$ | 0·22$^{d}$ | 221$^{c}$ | 49·0$^{c}$ | 67·5$^{e}$ |
| 30 | 19·3$^{d}$ | 0·04$^{b}$ | 62·7$^{d}$ | 21·2$^{d}$ | 0·25$^{c}$ | 203$^{d}$ | 45·4$^{d}$ | 74·0$^{d}$ |
| 40 | 19·8$^{c}$ | 0·03$^{c}$ | 61·4$^{e}$ | 25·0$^{c}$ | 0·26$^{c}$ | 185$^{e}$ | 41·6$^{e}$ | 77·2$^{c}$ |
| 50 | 20·6$^{b}$ | 0·03$^{c}$ | 47·4$^{f}$ | 27·3$^{b}$ | 0·30$^{b}$ | 166$^{f}$ | 37·3$^{f}$ | 82·0$^{b}$ |
| HFCS | 23·0$^{a}$ | 0·01$^{d}$ | 25·9$^{g}$ | 46·4$^{a}$ | 0·39$^{a}$ | 66·4$^{g}$ | 27·3$^{g}$ | 114·5$^{a}$ |

HMF: hydroxymethylfurfural.
Means in a column not sharing the same superscript are significantly different at $p<0·05$.

## Chemical composition

The moisture content of pure honey (18·4%) increased gradually as percentage of HFCS adulterant was raised (Table 2). However, the 50% adulteration level possessed a moisture content of 20·6%, which is still less than the critical moisture level (21%) from the standpoint of the keeping quality of honey (Mesallam & El-Sharawy, 1987).

Some variations were traced with regard to the nitrogen content of pure honey, adulterated honey and pure HFCS (Table 2). In contrast, the proline content of the above commodities exhibited a clear-cut trend, since it was inversely proportional to the percentage of HFCS used as an adulterant (Table 2).

The point of interest is that the hydroxymethylfurfural (HMF) content increased gradually as the HFCS percentage was raised. This increment was such that the HMF content for honey adulterated with 50% HFCS was more than twice that for pure honey (Table 2). This is in accordance with the observation of Doner (1977), who reported that the level of HMF in fresh honey is normally very low but increased with the addition of invert sugar.

The ash content of honey (0·17%) increased gradually as the percentage of HFCS adulterant was increased. However, the highest ash content (0·30%), exhibited by the 50% adulteration level, is still less than the corresponding value (0·39%) for pure HFCS (Table 2).

Potassium contents were 269 and 66 mg $kg^{-1}$ for pure honey and pure HFCS, respectively. Increasing the HFCS percentage as adulterant resulted in a gradual decline in potassium content. The 50% adulteration level showed a potassium content of 166 mg $kg^{-1}$. Moreover, the same trend was also true with respect to the sodium content, since it declined from 59·5 to 37·3 mg $kg^{-1}$ for pure honey and adulterated honey with 50% HFCS, respectively. The calcium content, contrary to that of potassium, was proportional to the percentage of added HFCS. The calcium content amounted to 82·0 mg $kg^{-1}$ for the 50% adulteration level as compared with 52·6 and 114·5 mg $kg^{-1}$ for pure honey and pure HFCS, respectively (Table 2).

## Physical properties and diastatic activity

Data presented in Table 3 explore some variations concerning both total solids and refractive indices for pure honey, deliberately adulterated honey with HFCS and pure HFCS. Pure honey possessed the highest apparent viscosity as compared with HFCS and any of the adulterated-honey samples. It is worth mentioning that, as the adulteration level with HFCS was increased, the apparent viscosity decreased. For instance, the apparent-viscosity value for the 50% adulteration level did not exceed 14·6% of that for pure honey (Table 3).

The diastatic-activity number of the pure honey used in the present study was quite high (136). This can be attributed to the direct collection of honey from the comb. The diastatic-activity number decreased gradually as the percentage of HFCS adulterant was raised. Notwithstanding this, pure HFCS did not possess any diastatic activity, indicating that the syrup was subjected to heat treatment during manufacture. Such a treatment was severe enough to inactivate the enzymes completely.

## Statistical analysis

From the standpoint of 'carbohydrate composition', pure honey exhibited the lowest content of fructose and polysaccharides. Moreover, pure honey possessed the lowest fructose/glucose ratio, and this ratio was significantly different from the corresponding values for adulterated honey and HFCS. In contrast, the levels of glucose, maltose and trisaccharides had significantly higher values than those for adulterated-honey samples and HFCS (Table 1).

**Table 3. Some physical properties and diastatic activity of honey, honey adulterated with high-fructose corn syrup (HFCS) and HFCS**

| Aduleration (%) | Total soluble solids (%) | Refractive index | Apparent viscosity (cp) | Diastatic-activity number |
|---|---|---|---|---|
| 0·0 (honey) | 80·1$^{a}$ | 1·4905$^{a}$ | 179032$^{a}$ | 136$^{a}$ |
| 10 | 79·9$^{ab}$ | 1·4901$^{a}$ | 112312$^{b}$ | 60$^{b}$ |
| 20 | 79·6$^{ab}$ | 1·4894$^{a}$ | 78952$^{c}$ | 50$^{c}$ |
| 30 | 79·2$^{bc}$ | 1·4882$^{ab}$ | 60150$^{d}$ | 40$^{d}$ |
| 40 | 78·7$^{c}$ | 1·4868$^{bc}$ | 36240$^{e}$ | 35$^{e}$ |
| 50 | 78·0$^{d}$ | 1·4850$^{c}$ | 26152$^{f}$ | 26$^{f}$ |
| HFCS | 75·6$^{e}$ | 1·4788$^{d}$ | 7846$^{g}$ | 0·0$^{g}$ |

Means in a column not sharing the same superscript are significantly different at $p<0·05$.

**Table 4. Correlation coefficients between carbohydrate composition and different compositional properties of honey, adulterated honey, and HFCS**

| Property | Fructose (*F*) | Glucose (*G*) | Maltose | Trisaccharides | Polysaccharides | *F/G* ratio |
|---|---|---|---|---|---|---|
| DA | −0·79* | 0·81* | 0·80* | 0·78* | −0·82* | −0·77* |
| Viscosity | −0·81* | 0·83* | 0·82* | 0·83* | −0·87* | −0·79* |
| RI | −0·98** | 0·98** | 0·99** | 0·95** | −0·93** | −0·99** |
| TSS | −0·98** | 0·98** | 0·99** | 0·95** | −0·93** | −0·98** |
| Ca | 0·99** | −0·99** | −0·99** | −0·98** | 0·95** | 0·99** |
| Na | −0·95** | 0·96** | 0·96** | 0·95** | −0·93** | −0·94** |
| K | −0·99** | 0·99** | 0·95** | 0·97** | −0·95** | −0·99** |
| Ash | 0·98** | −0·98** | −0·97** | −0·96** | 0·93** | 0·97** |
| HMF | 0·99** | −0·99** | −0·99** | −0·97** | 0·94** | 0·99** |
| Proline | −0·98** | 0·97** | 0·99** | 0·94** | −0·92** | −0·97** |
| Nitrogen | −0·94** | 0·94** | 0·94** | 0·91** | −0·94** | −0·92** |
| Moisture | 0·98** | −0·97** | −0·99** | −0·95** | 0·93** | 0·99** |

*Significantly correlated at $p < 0{\cdot}05$.
**Significantly correlated at $p < 0{\cdot}01$.

It is worth mentioning that pure honey possessed the highest content of nitrogen, proline, potassium and sodium. However, pure honey exhibited the lowest content of hydroxymethylfurfural (HMF), ash, moisture and calcium as compared with the adulterated-honey samples and HFCS (Table 2).

The point of interest is that, up to 20% level of adulteration of honey with HFCS, honey samples could not be significantly differentiated by either total soluble solids or refractive indices. On the other hand, pure honey showed the highest apparent viscosity and diastatic-activity number, with significant differences among all the samples (Table 3).

Data presented in Table 4 indicate that sugar composition, which was determined by HPLC and considered as a fingerprint for honey, was found to be significantly correlated with many compositional properties of honey. Reductions in dry matter, viscosity, sodium, potassium, proline and nitrogen can be taken as good indicators for the adulteration of honey with HFCS. Moreover, the increases in ash, calcium, hydroxymethylfurfural (HMF) and moisture contents can be considered as simple and rapid tests for adulteration levels ranging between 10% and 50%.

In the light of data presented here, it can be concluded that both diastatic activity and apparent viscosity can be taken as key differential criteria to detect the adulteration of honey with HFCS. HFCS exhibited no diastatic activity and very low apparent viscosity in contrast to honey. The other constituents evaluated in the present study could only be used under specific circumstances (i.e. comparison with standard honey) owing to the wide ranges of such constituents for natural honey (Martin, 1979).

## REFERENCES


AOAC (1980). Official Methods of Analysis, 13th edition. Association of Official Analytical Chemists, Washington, D.C., USA.

Clarke, M. A. (1988). Sugars and sugar products. *J. Assoc. Off. Anal. Chem.*, **71**, 89–90.

Croft, L. R. (1987). Stable isotope mass spectrometry in honey analysis. *Trends in Anal. Chem.*, **6**, 206–9.

Doner, L. W. (1977). The sugars of honey: a review. *J. Sci. Food Agric.*, **28**, 443–56.

Egan, H., Kirk, S. R. & Sawyer. R. (1981). *Pearson's Chemical Analysis of Foods*, 8th edn. Churchill Livingstone, Edinburgh, UK.

Frangipane, G., Incitti, S. & Petrucci, M. (1984). Legal quality standards of honey. *Rass. Chim.*, **36**, 11–14.

Lipp, J. & Ziegler, H. (1989). Procedure for detection of adulteration of honey. German Federal Republic Patent Application DE 3723190 AI. Food Science and Technology Abstract, 21 (1989), 8V5.

Low, N. H., Brisbane, T., Bigam, G. & Sporns, P. (1988). Carbon-13 nuclear magnetic resonance for the qualitative and quantitative analysis of structurally similar disaccharides. *J. Agric. Fd. Chem.*, **36**, 953–7.

Marshall, T. & Williams, K. M. (1987). Electrophoresis of honey: characterization of trace proteins from a complex biological matrix by silver staining. *Anal. Biochem.*, **167**, 301–3.

Martin, P. G. (1979). *Manuals of Food Quality Control: 3-Commodities*. Food and Agriculture Organization of the United Nations, Rome, Italy.

Mesallam, A. S. & El-Shaarawy, M. I. (1987). Quality attributes of honey in Saudi Arabia. *Food Chem.*, **25**, 1–11.

Pechhacker, H. & Solkner, I. (1987). Studies on the question of sensory evaluation of honey. *Mitt. Klosterneuburg Rebe und Wein, Obstbau und Früchteverwert.*, **37**, 254–7. Food Science and Technology Abstract, 20 (1988), 8L55.

Sangiorgi, E. (1988). Survey on isoglucose adulteration of honey. *Industr. Aliment.*, **27**, 442–4.

Serra Benvehi, I. & Gomez Pojuelo, A. (1984). Determination of adulteration in honey. *Aliment. Equiposy Tecnol.*, **5**, 143–7. Food Science and Technology Abstract, 19 (1987), 8L146.

Steel, R. G. D. & Torrie, T. H. (1980). *Principles and Procedures of Statistics*. McGraw-Hill, New York, NY, USA.

Swallow, K. W. & Low, N. H. (1990). Detection of adulteration of honey with high fructose corn syrup and beet invert sugar using HPLC. Paper presented at 33rd Annual Conference, Canadian Institute of Food Science and Technology, 3–6 June, 1990, Saskatoon, Saskatchewan, Canada.

White, J. W., Jr & Robinson, F. A. (1983). $^{13}C/^{12}C$ ratios of citrus honeys and nectars and their regulatory implications. *J. Assoc. Off. Anal. Chem.*, **66**, 1–3.

White, J. W. & Winters, K. (1989). Honey protein as internal standard for stable carbon isotope ratio detection of adulteration of honey. *J. Assoc. Off. Anal. Chem.*, **72**, 907–11.