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A rapid and sensitive method for the determination of high-fructose corn syrup (HFCS) in honey

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Abstract. The authors developed a new, sensitive and rapid liquid chromatographic method suitable for the determination of concentrations of high-fructose corn syrup (HFCS) as low as 1% in honey. In this investigation, we determined the oligosaccharides formed in the course of the enzyme hydrolysis of starch, and which cannot occur in honey by means of natural processes. Since the high-performance liquid chromatography (HPLC) method does not require the removal of other sugars present in honey, the procedure is also appropriate for the quantitative determination of such sugars.

Keywords and phrases: high-fructose corn syrup (HFCS), high-performance liquid chromatography (HPLC), adulterated honey, glucose, fructose, disaccharides, oligosaccharides.

1 Introduction

Honey was the first sweetening substance known to man. Due to the extensive production and use of beet and cane sugar, the nutritional importance of honey is now derived mainly from its high biological value. The quantity collected by honey bees (*Apis mellifera*) may vary considerably from year to year, depending upon weather conditions. Honey can only be produced by natural means and in limited quantities – hence its relatively high price. For the purpose of increasing the quantity of honey available, it is often adulterated with alien substances of high sugar content. Since for many years the particular sugar composition of honey could not be imitated, the detection of adulteration was relatively simple. However, by the application of enzyme chemistry on the industrial scale, it is now possible to produce sweetening substances of sugar composition almost identical to that of honey. One of the cheapest and most commonly used of these is the high-fructose corn syrup (HFCS), produced from maize starch by means of enzyme hydrolysis.

In the production of HFCS, the gelatinized and liquidized maize starch is first hydrolysed with amylase enzyme to DP 10-15 dextrins, subsequent to which 94-96% is transformed into glucose by glucoamylase. By the effect of the glucose isomerase enzyme, the so-termed 42HFCS of maximum 47-48% fructose content (in practice, approximately 42%) is formed. By the application of ion exchange enrichment, 90VEFCS (very enriched fructose corn syrup) of fructose content as high as 90% can be produced from this compound. Production of corn syrup of any level of fructose content between 42% and 90% is possible from the above-mentioned products (*Comprehensive Biotechnology*, 1985). Since the dry-matter content and constituency of HFCS are similar to those of honey, while its price is much lower, HFCS can be used effectively in the adulteration of honey. The detection of HFCS is substantially more difficult than that of traditional substances of high sugar content; therefore, the development of a more advanced analytical method has become necessary.

2 Materials and methods

2.1 Examination procedures

In the *Official Methods of Analysis of AOAC International* (1995), two main examination procedures are recommended for the detection of HFCS adulteration of honey. One of these exploits the differences between the stable carbon isotope ratios of honey and HFCS (*Zieglet et al.*, 1979; *White* & *Robinson*,

1983), while the other one detects oligosaccharides present in HFCS by means of a thin layer chromatographic method (*Kushnir*, 1979; *White et al.*, 1979). Since the determination of the isotope ratio is very costly and substantial development in the level of equipment available for separation technology has been achieved in recent years, liquid and gas chromatographic procedures have gained increasing degrees of prominence. These exploit the fact that – due to the technology involved in the manufacture of HFCS – approximately 1% sugar of higher saccharide number remains in the end-product, which does not occur in unadulterated honey. The presence of such marker compounds (fingerprint oligosaccharides, or FOS) constitutes unambiguous evidence of adulteration. Such procedures are sufficiently sensitive and in other ways suitable for the detection of concentrations as low as a few per cent of HFCS mixed into honey. Nevertheless, the sample preparation required before measurements can be taken with the relevant instruments is not highly suitable for rather lengthy routine quality control procedures. In consequence of this, the development of a rapid, sensitive and non-labour intensive examination procedure is necessary. The HPLC method outlined below, which was developed in the authors' laboratories, is appropriate for the detection of HFCS in concentrations as low as 1% and for the analysis of as many as 70 samples a day. It is also suitable for the quantitative determination of fructose and glucose in honey and the group determination of disaccharides and trisaccharides.

2.2 Experimental materials and equipment

The Supelco oligosaccharide kit (cat. no. 4-7265), disaccharide kit (cat. no. 4-7268) and monosaccharide kit (cat. no. 4-7267) were used for the identification of the fingerprint oligosaccharides (FOS) and for the qualitative and quantitative determination of other sugars occurring in honey. Pure HFCS and honey, guaranteed unadulterated, were applied for the quantitative determination of HFCS and the mapping of the calibration curve.

2.3 Method

Some years ago, the examination procedure outlined below was officially approved by the Deutscher Akkreditierungs Rat, under the designation HERPAI#MEZ.001, for the detection of high-fructose corn syrup adulteration of honey. Statistical analysis of the data obtained from the measurements was performed by means of the BORWIN chromatographic software, version 1.21.60 (JMBS DEVELOPPEMENTS, Grenoble, FRANCE).

2.3.1 Appliance

Pump: Jasco PU-980
Autosampler: Jasco AS-950-02
Column thermostat: Merck 7350
Detector: Merck RI-71

2.3.2 Sample preparation

Sampling and homogenization were performed in accordance with the stipulations of AOAC 920.180. In each case, 1g (accurate to 0.1mg) of the sample to be examined was measured into a 10 ml volumetric flask and dissolved in HPLC water with the assistance of a test-tube agitator; the vessel was then filled up to the mark. The dissolved sample was filtered through a Millex-GV_{13} 0.22μ hydrophilic membrane filter (cat. no. SJGV013NS) and injected from this onto the HPLC. For the purpose of the examination of the shape of the FOS calibration curve, a 50-point calibration range from 1 mg/ml to 100 mg/ml concentration was prepared using pure HFCS. This, taking into account the preparation of the honey samples, corresponds to adulteration levels in the honey, ranging from 1% to 100%.

2.3.3 Chromatographic parameters

Column: Supelcogel Ca $300 * 7.8$ mm, (cat. no. 5-9305-U), with Supelguard Ca $50 * 4$ mm (cat. no. 5-9306-U) guard column
Column temperature: 70 °C
Eluent: water, Milli-q Ultra pure water system
Flow rate: 0.5 ml/min

An Upchurch A-$101 \times 2\mu$ in-line filter was positioned after the pump for the in-line filtration of the eluent. In the interest of protecting the analytical column, subsequent to injection, the sample was filtered through an Upchurch A-$102 \times 0.5\mu$ in-line filter. This was changed when the pressure of the pump exceeded the initial value of 10 bars. The guard column was changed after every 100 injections.

3 Results and discussion

On the comparison of a chromatogram for guaranteed pure honey and one for HFCS (*Figure 1*), a characteristic difference observed was the peak for FOS, present in the isosugar, at a retention time of 7.76 minutes. The shape of the peak indicates that not only one molecule is eluted at this retention time,

but also several dextrin molecules of similar structure. These molecules are larger than DP7, which can be concluded from the comparison of the retention times of oligosaccharide standards; that is, on the column used for analysis, the larger molecules are eluted first.

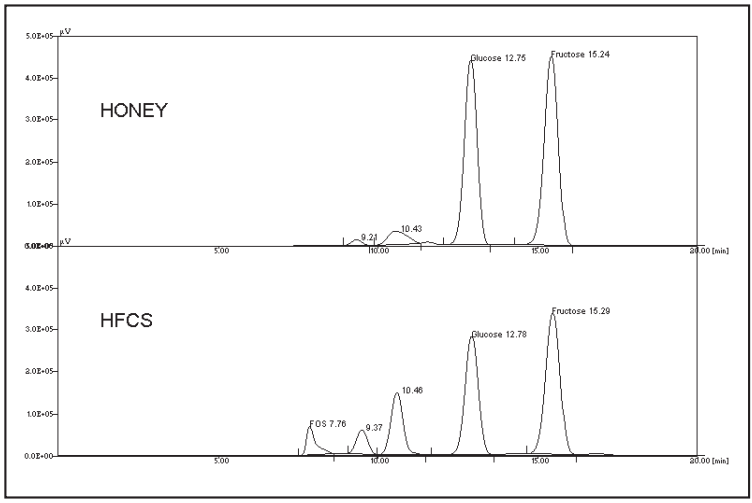

Figure 1: Chromatogram for pure honey and HFCS

The dimensions of the area beneath the FOS peak were taken as the base for the determination of HFCS content. The calibration curve was proved linear for the concentration range examined (*Figure 2*).

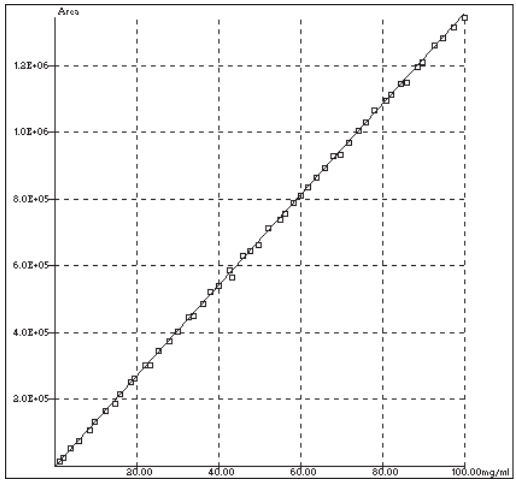

Figure 2: Calibration curve for FOS

Component: FOS, Model: Y = AX Nb of points: 50
A =13518.6233. Correlation = 0.99987
Standard error Vy = 911.6224. Mean % error = S1.134

Statistical analysis of the method is given in *Table 1.*

Table 1: Statistical analysis of fingerprint oligosaccharides (FOS) by means of the Herpai#mez.001 method of measurement

Concentration w%	**n**	**Measured conc. w% mean**	**Recovery %**	**Std. dev. precision**	**%RSD**	**Accuracy**
0 **blank**	9	0.10		0.11	110.87	0.10
1.0	11	0.81	81.0	0.06	7.34	0.19
5.0	11	4.05	81.0	0.09	2.21	0.95
10.0	11	8.32	83.2	0.08	0.96	1.68
30.0	11	26.72	89.1	0.21	0.79	3.28
50.0	11	46.76	93.5	0.23	0.49	4.24
Mean			85.6	0.13		

Limit of detection calculated from calibration curve $(Y = Ax)0.20w\%$
$k * s/A$ where $k = 3$, s = standard error of calibration curve $Vy = 911.6224$
Limit of determination calculated from blank $0.43w\%$
$y + k * s$; where $y = 0.1$ mean concentration of blank, $k = 3, s = 0.11$ Std. dev. of blank
r calculated $r = 2.83\ \sigma_r = 0.36$; where $r = 0.13$
R calculated $R = 2.83\sigma_R = 0.85$; where $\sigma_R = 0.30$

The possibility of the separation of glucose (rt: 12.7 min) and fructose (rt: 15.2 min) on the baseline can be seen clearly; they do not load over the column, and thus there also exists the possibility for the quantitative determination of these. A concentration range of 10 to 50 mg/ml was used for the glucose and fructose calibration curves (Figures 3 and 4), which was proved to be linear. On the HFCS chromatogram, a sharp peak can be seen at a retention time of 10.4 minutes, this being the peak for maltose derived from the incomplete hydrolysis of the maize starch. At the corresponding point on the honey chromatogram, a flat, broader peak is visible, which originates from several molecules. This is resulted from the fact that honey contains a number of different disaccharides and among these a substantial quantity of saccharose. The trisaccharides are eluted from the column prior to these,

at a retention time of 9.3 minutes. It may therefore be concluded that the procedure developed is appropriate for the detection of HFCS concentrations as low as 1% in honey. The advantages of the method are its simple sample preparation procedure and the modest equipment requirement, which allow large quantities of samples to be examined in series. A single chromatographic analysis enables HFCS adulteration to be ascertained and fructose as well as glucose content – important to the quality of honey – to be determined. Although no precise result is provided, conclusions can also be drawn with regard to saccharose adulteration. The disadvantage of the method is that the ion exchange columns required for the measurements are still relatively expensive and a great deal of care is necessary in their application.

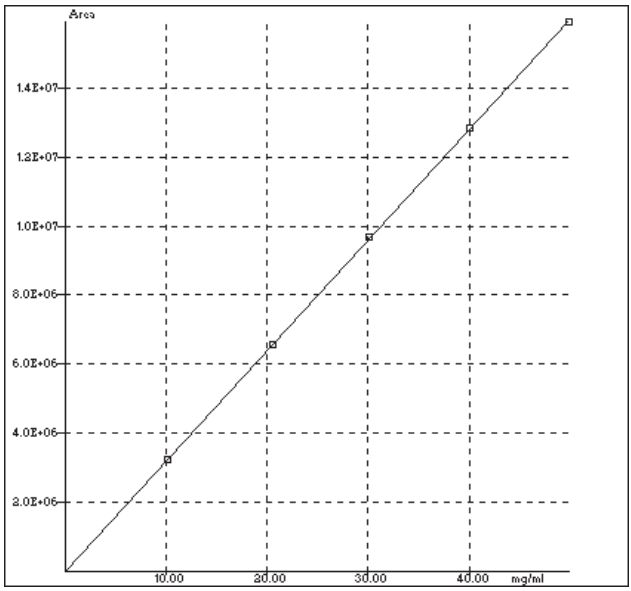

Figure 3: Calibration curve for glucose

Component: Glucose model: $Y = AX$, No. of points: 5. $A = 319844.6318$. Correlation $= 0.99997$. Standard error $Vy = 21336.4215$. Mean % error$= 0.373$.

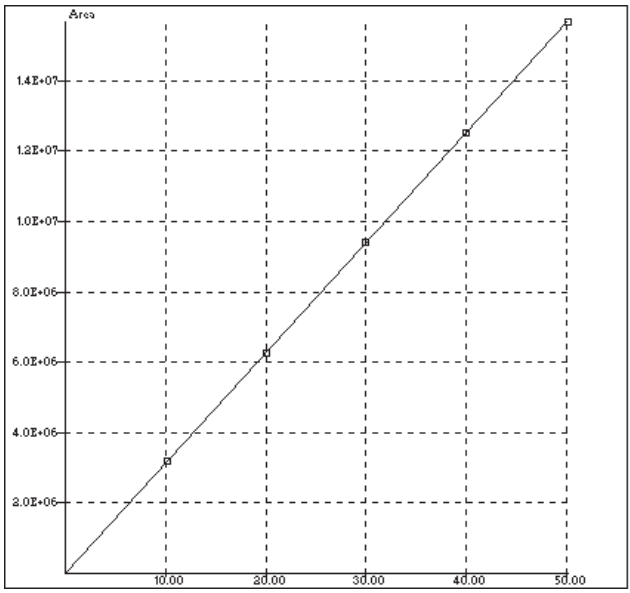

Figure 4: Calibration curve for fructose

Component: Fructose model: $Y = AX$. No. of points: 5. $A = 313119.6087$. Correlation = 0.99998. Standard error $Vy = 17035.8673$. Mean % error= 0.331.

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