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An Optimized Attempt for Dextrose Equivalent Evaluation of Corn Syrups from HPLC-Data

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Introduction

The dextrose equivalent «DE», defined as the percentage of reducing sugars expressed as glucose (dextrose) and calculated as the % of dry matter, is an important element of the characterization of corn syrups allowing their classification.

Even if the sugar's spectrum is known, the DE determination is still useful as it gives a global image of the extent of the hydrolysis of the corn syrups.

The titrimetric and gravimetric methods (1-3) for the DE determination are rather difficult and time-consuming. It is therefore evident that an evaluation of the DE by a HPLC analysis of corn syrups is advantageous as long as the results are close to those of the standard methods.

Kiser and Hagy (4) made a first attempt at calculating DE's from HPLC-data using an Aminex 50W-X4 column, calcium form. Due to the fact that the response factors of the refraction index (RI)-detector are very close for the different components (5-7), they showed that the calculation of the DE is possible if the total area of all the chromatographic peaks obtained is normalized to 100%. The normalized value of the area of each peak also directly gives the percentage of each component against the total sugars content.

The theoretical DE of each glucose polycondensation homologue (oligomer) is given by its own concentration multiplied by a conversion factor that represents the ratio of the molecular masses of the glucose and that of the oligomer in question. The sum of these DE gives the total DE value of the analyzed corn syrup.

Unfortunately, in reality it is not possible to simplify the calculation to such an extent. There are some drawbacks to overcome, notably:

- The chromatographic separation is not perfect. The baseline between the peaks is not always reached.
- The response factors of the chromatographic detector are effectively not quite the same for glucose and its condensing oligomers.
- In the titrimetric and gravimetric methods of DE determination, the real DE of each homologue is different from the theoretical one. This deviation

is due to differences in the reaction conditions or to inherent differences in the stoichiometry of the reaction (8).

Kiser and Hagy observed that the results calculated with the theoretical conversion factors do not correlate well with those obtained by the titrimetric method. To obtain an improved agreement with the titrimetric results, they use a set of factors given by *Commerford and Scallet* (8). The latter authors have experimentally determined by *Lane and Eynon* titration (1) the relative reducing values of the glucose oligomers up through the polycondensation degree (DP) of 6. With this improvement, *Kiser and Hagy* took into account only the differences in the stoichiometry of the titrimetric reaction. This may be the reason why their DE values calculated taking into account the areas of only the first three homologues individually correlate better with the titrimetric DE than the DE calculated with the individual area of the first six homologues.

We believe that the DE determination from the HPLC-data obtained with Aminex type columns could be improved if the individual percentages of the largest number of oligomers were taken into account. To verify this, we established one reliable transformation of the theoretical DE conversion factors to obtain a better agreement between the calculated DE values and the titrimetric or gravimetric ones. This optimized set of DE conversion factors together with the HPLC results obtained using a chromatographic column of improved performance helped us to reach our goal.

Theoretical background

The theoretical DE conversion factors giving the ratios between the molecular masses of the glucose and of an oligomer can be calculated according to the following formula:

$$f_n = \frac{1}{0.9 n + 0.1} \quad (I)$$

where: n represents the number of units of polycondensed glucose (DP);
0.9 is the ratio between the glucose molecular mass and that of the glucose minus one unit of water;
0.1 is the ratio between the water molecular mass and that of glucose.

We tried to find a more suitable set of factors derived from these theoretical ones according to the following basic theory:

The small change in the response factor of the RI-detector from one homologue to the next must follow a regular relationship since they are related and the molecular change consists of the addition of a supplementary glucose unit and the elimination of a water molecule. Similarly, the stoichiometry of the reducing reactions used in the gravimetric or titrimetric DE determination must change from one homologue to another in the same regular manner.

In order to take this detector response factor and stoichiometry modification into account, we raised all theoretical DE conversion factors to one power slightly different from 1.0:

$$f_n = \left(\frac{1}{0.9 n + 0.1} \right)^{(1-a)} = (0.9 n + 0.1)^{-(1-a)} \quad (II)$$

This correction «a» must be independent of the chromatographic parameters. In fact, in empirical experiments, we noted that the practical conversion factors must still be greater than the theoretical ones. Consequently, «a» must be positive and smaller than 1.

The chromatographic separation, i.e. the retention time, is a function of DP. For the Aminex type columns, the retention time decreases with increasing DP, while the overlapping of the peaks increases. These phenomena must follow a similar relationship to the former ones and their influence on the DE conversion factors must similarly be considered.

A supplementary correction «b» will take into account this incomplete separation:

$$f_n = (0.9 n + 0.1)^{-(1-a-b)} \quad (III)$$

The correction *b* is also small and depends on the type of chromatographic column used as well as on the range of the DE values of the analyzed corn syrups. The *b* value can change during use of the column, depending on its age. The better the separation, the smaller *b* becomes until it is negligible. A better separation also allows a larger number of oligomers to be taken into account. Furthermore, the input to the DE of the last, unsolved part of the chromatogram becomes less important.

Since the corrections *a* and *b* are unknown and have different origins depending on the chromatographic system selected, as well as on the standard DE determination method used for comparison, the formula (III) may be written:

$$f_n = (0.9 n + 0.1)^{-M} \quad (IV)$$

where $M = 1-a-b$.

This is equivalent to raising the theoretic factors to the *M*-th power. To determine *M* for a range of corn syrups for a given chromatographic separation, using a standard method for comparison, we turned to a simple BASIC program to calculate the DE with different exponents *M*.

Finally, we tested our method with 18 corn syrups with DE's ranging from 35 to 45.

Experimental conditions

Chromatographic supply

Column: Aminex HPX-42A (30cm, ϕ 7.8mm) Bio-Rad Laboratories
Pump: Perkin-Elmer serie 2
Detector: RI-Perkin Elmer LC-25
Thermostatic system of the column: Jones, model 7930
Thermostatic system of the detector: HAAKE DK 12
Integrator: HP 3390 A

Sample preparation

Dissolve 5 g of corn syrup in 50 ml hot water (~ 60 °C), cool the solution then filtrate on a 0.45 μ m membrane.

Chromatographic conditions

Elution: Bi-distilled water kept at about 70 °C. Stir slowly on a hot plate stirrer to remove dissolved gas.
Flow: 0.6 ml/min
Injected volume: 20 μ l
Temperature of column: 85 °C
Temperature of detector: 30 °C
Integration of all peaks to the baseline (see fig. 1).

Results and discussion

The Aminex HPX-42A column allows the separation of corn syrups up to DP 10 as shown by *Scobell* and *Brobst* (9, 10).

The results of the chromatograms obtained from 14 different corn syrups are shown in table 1. The values are expressed in % area of peaks corresponding to DP 1 to DP 10 and to the sum higher than DP 10, normalized to 100% (total area of the chromatogram). These corn syrups come from different commercial sources and are used to manufacture confectionery. For the 14 corn syrups, the DE's have been calculated from the percentages area by multiplying by the theoretical conversion factors as well as by the empirical factors (4, 8). For the calculation with the theoretical factors, the factor 0.1 was used for the total area of peaks higher than DP 10 while for the empirical factors, the factor 0.16 was used for the total area of peaks higher than DP 6. The results are shown in table 2.

Table 1. Percentages of the surfaces of the chromatographic peaks corresponding to the glucose oligomeres in different corn syrups

| Homo- logue | Corn syrups No | | | | | | | | | | | | | |
|----------------|----------------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| DP 1 | 19.0 | 4.1 | 8.4 | 5.8 | 7.2 | 6.2 | 7.5 | 5.8 | 20.3 | 18.3 | 11.7 | 17.6 | 16.3 | 14.9 |
| DP 2 | 13.7 | 31.1 | 25.6 | 28.4 | 24.4 | 28.8 | 23.0 | 29.3 | 14.1 | 12.8 | 25.1 | 15.3 | 12.5 | 16.9 |
| DP 3 | 11.7 | 17.6 | 14.5 | 18.5 | 16.6 | 17.4 | 15.7 | 16.8 | 11.8 | 10.9 | 12.2 | 11.7 | 11.1 | 10.7 |
| DP 4 | 9.8 | 8.6 | 7.5 | 9.5 | 9.3 | 8.8 | 9.3 | 7.7 | 9.7 | 9.2 | 7.9 | 9.1 | 9.7 | 8.4 |
| DP 5 | 8.0 | 3.8 | 4.7 | 5.7 | 5.0 | 4.8 | 5.5 | 4.1 | 7.8 | 7.6 | 4.3 | 7.6 | 8.1 | 5.9 |
| DP 6 | 6.4 | 2.2 | 3.6 | 2.9 | 3.2 | 2.8 | 3.1 | 2.6 | 6.2 | 6.3 | 3.0 | 6.3 | 6.7 | 6.2 |
| DP 7 | 5.3 | 2.1 | 3.4 | 2.5 | 2.9 | 2.6 | 2.8 | 2.7 | 5.0 | 5.3 | 3.1 | 5.2 | 5.6 | 5.9 |
| DP 8 | 4.2 | 1.9 | 3.1 | 2.6 | 2.8 | 2.5 | 2.3 | 2.6 | 4.0 | 4.2 | 2.5 | 4.1 | 4.6 | 3.4 |
| DP 9 | 3.6 | 2.2 | 2.7 | 2.9 | 2.8 | 2.3 | 2.3 | 2.3 | 3.3 | 3.6 | 1.1 | 3.5 | 3.9 | 2.4 |
| DP 10 | 2.1 | 1.8 | 1.1 | 2.0 | 2.3 | 1.5 | 2.2 | 0.9 | 1.4 | 2.1 | — | 2.2 | 2.4 | 1.0 |
| >DP 10 | 16.2 | 24.6 | 25.4 | 19.2 | 23.5 | 22.3 | 26.3 | 25.2 | 16.4 | 19.7 | 29.1 | 17.4 | 19.1 | 24.3 |

Table 2. DE in corn syrups

| Corn syrup No | Gravimetric method Reference values | Calculation from HPLC results with | |
|------------------|--|------------------------------------|------------------------|
| | | theoretical factors | empirical factors * |
| 1 | 40.5 | 39.6 | 42.7 |
| 2 | 35.0 | 33.8 | 38.2 |
| 3 | 36.2 | 34.7 | 38.8 |
| 4 | 36.2 | 34.9 | 39.0 |
| 5 | 35.2 | 33.9 | 38.0 |
| 6 | 35.9 | 35.0 | 39.2 |
| 7 | 34.4 | 33.4 | 37.6 |
| 8 | 35.3 | 34.3 | 38.6 |
| 9 | 41.8 | 40.9 | 44.0 |
| 10 | 39.2 | 38.3 | 41.5 |
| 11 | 38.1 | 36.8 | 40.8 |
| 12 | 39.7 | 38.9 | 42.1 |
| 13 | 37.8 | 36.6 | 39.9 |
| 14 | 37.3 | 36.5 | 40.0 |

* as given by Kiser and Hagy (7).

Even though the DE's of the corn syrups chosen only range between 35 and 43 DE, it must be mentioned that the ratios between the sugars components were variable. This is illustrated by comparing the chromatograms of figures 1 and 2. These correspond to samples No 4 and 14 of corn syrups having similar DE values.

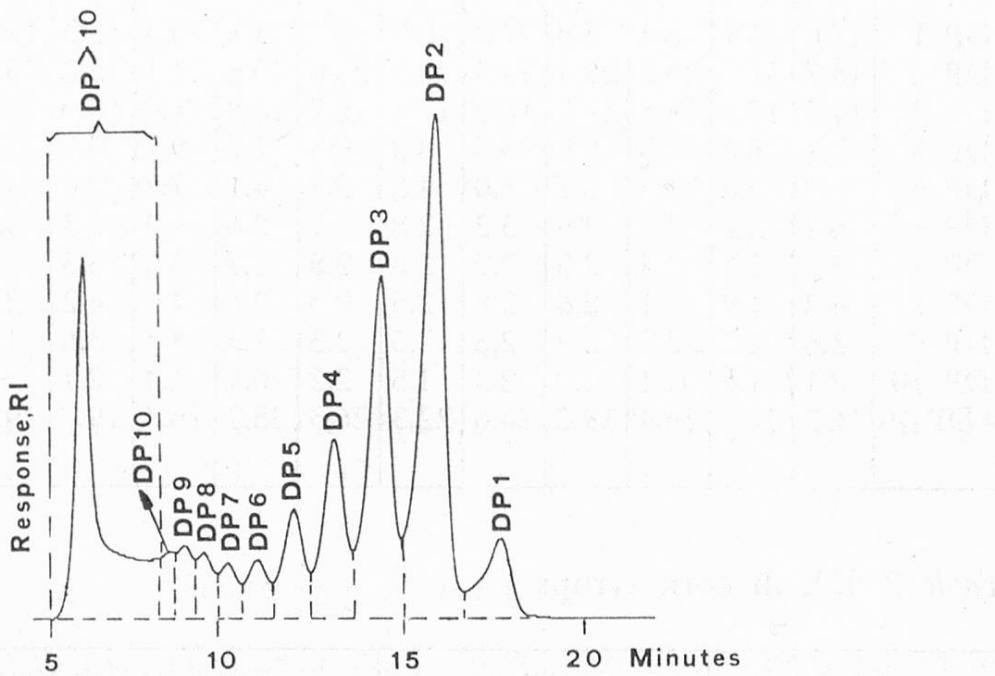


Fig. 1. HPLC separation of glucose and its oligomers for the high-maltose corn syrup No 4. Operating conditions as indicated in the experimental section

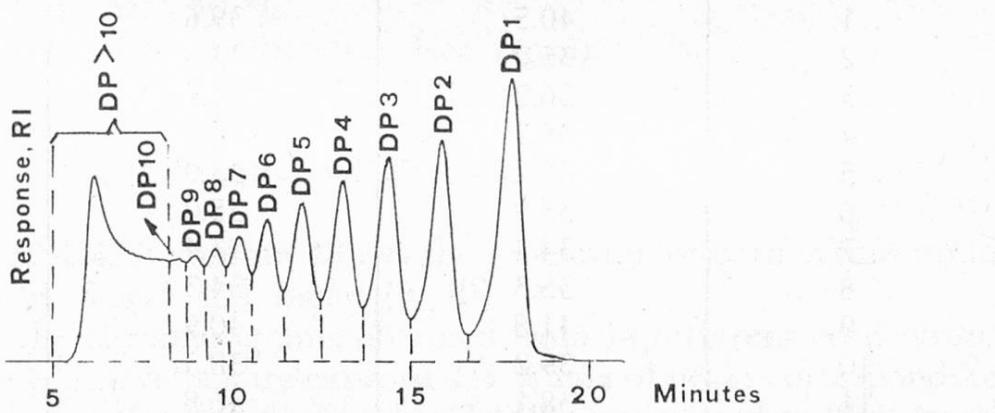


Fig. 2. HPLC separation of glucose and its oligomers for the acid hydrolyzed corn syrup No 13. Operating conditions as indicated in the experimental section

In table 2, it can be noted that for all corn syrups analyzed, the DE results are clearly too high when calculated with empirical factors compared to the reference values and slightly too low when calculated with theoretical factors. Several sets of factors have been used and are derived from the theoretical ones according to formula (IV).

For the exponent M all values between the initial value 0.90 and the final value 1.00 have been taken with an incrementation of 0.01. These calculations were performed for all the 14 corn syrups mentioned above and the results are shown in table 3.

Table 3. DE calculated with the formula: (theoretical factors) $M \cdot \% \text{ surface of peaks}$

| M | DE for corn syrups No | | | | | | | | | | | | | |
|------|-----------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| 1 | 39.6 | 33.8 | 34.7 | 34.9 | 33.9 | 35.0 | 33.4 | 34.3 | 40.9 | 38.3 | 36.8 | 38.9 | 36.6 | 36.5 |
| 0.99 | 39.8 | 34.0 | 34.9 | 35.1 | 34.1 | 35.2 | 33.7 | 34.5 | 41.1 | 38.5 | 37.0 | 39.1 | 36.8 | 36.7 |
| 0.98 | 40.0 | 34.3 | 35.2 | 35.4 | 34.4 | 35.4 | 33.9 | 34.8 | 41.3 | 38.7 | 37.2 | 39.3 | 37.0 | 36.9 |
| 0.97 | 40.2 | 34.5 | 35.4 | 35.7 | 34.6 | 35.7 | 34.1 | 35.0 | 41.5 | 38.9 | 37.4 | 39.5 | 37.2 | 37.1 |
| 0.96 | 40.5 | 34.8 | 35.6 | 35.9 | 34.9 | 35.9 | 34.3 | 35.3 | 41.7 | 39.1 | 37.6 | 39.7 | 37.5 | 37.3 |
| 0.95 | 40.7 | 35.0 | 35.8 | 36.2 | 35.1 | 36.2 | 34.6 | 35.5 | 41.9 | 39.3 | 37.8 | 40.0 | 37.7 | 37.5 |
| 0.94 | 40.9 | 35.3 | 36.1 | 36.5 | 35.3 | 36.5 | 34.8 | 35.8 | 42.2 | 39.5 | 38.0 | 40.2 | 37.9 | 37.7 |
| 0.93 | 41.1 | 35.5 | 36.3 | 36.7 | 35.6 | 36.7 | 35.0 | 36.0 | 42.4 | 39.8 | 38.2 | 40.4 | 38.1 | 38.0 |
| 0.92 | 41.4 | 35.8 | 36.6 | 37.0 | 35.8 | 37.0 | 35.3 | 36.3 | 42.6 | 40.0 | 38.4 | 40.6 | 38.4 | 38.2 |
| 0.91 | 41.6 | 36.1 | 36.8 | 37.3 | 36.1 | 37.2 | 35.5 | 36.5 | 42.8 | 40.2 | 38.7 | 40.9 | 38.6 | 38.4 |
| 0.90 | 41.9 | 36.3 | 37.0 | 37.6 | 36.4 | 37.5 | 35.8 | 36.8 | 43.1 | 40.4 | 38.9 | 41.1 | 38.8 | 38.6 |

Since our chromatographic separation was satisfactory up to DP 10, the value 0.1 has been taken as the multiplying factor for the percentage of area corresponding to the remaining unseparated oligomers (DP>10).

By comparing the calculated DE values with those determined by the reference gravimetric method of table 2, it was concluded that the best agreement between the two methods is obtained using the exponent 0.95. This is valid for corn syrups with DE's of between 35 and 45 using the chromatographic conditions given in the experimental section.

Basic program for DE calculation

The program adapted for a chromatographic integrator HP 5880 A is as follows:

```

1Ø REM*****DEXTROSE EQUIVALENT*****
2Ø INPUT "NUMBER OF SOLVED PEAKS ",P
3Ø DIM D(2Ø)
4Ø FOR N=1 TO P
5Ø PRINT "DP ";N;
6Ø INPUT ",D(N)
7Ø NEXT N
8Ø INPUT "REMAINING DP ",R
9Ø INPUT "FACTOR FOR REMAINING ",B

```

```

100 INPUT "UPPER LIMIT OF ELEVATION ",C
110 INPUT "LOWER LIMIT OF ELEVATION ",E
120 FOR M=E TO C STEP 0.01
130 A=0
140 FOR N=1 TO P
150 A=A+D(N)/(0.9*N+0.1)^M
160 NEXT N
170 H=A+R*B
180 PRINT "FOR ELEVATION TO POWER ",M;" DE=";H
190 NEXT M
200 END

```

Remarks for users:

- To set the dimension in line 30, a value equal or superior to the number P of the separated peaks must be chosen.
- The loop between lines 40 and 70 is to introduce the percentages of the surfaces of the resolved peaks according to the level of polycondensation DP .
- In line 80 the remaining percentage of the area of peaks corresponding to the upper unseparated oligomers (R) is set and in line 90 the multiplying factor B used to calculate the contribution to the DE of the percentage of the unseparated oligomers. This factor decreases with the number of the separated peaks and was, in our case, 0.1.
- In lines 100 und 110, the interval of the values for the exponent M of the calculation formula (IV) for the factors is chosen. Each value of the exponent gives a DE value.
- By comparing the calculated DE values with those obtained from a standard method for several referenced corn syrups, the best exponent value can be established. This exponent must then be introduced in the program both for the lower and the upper limit in lines 100 und 110. This program can finally be used for the routine analysis of corn syrups in the referenced DE range.

Conclusions

The determination of the DE , from HPLC analysis data obtained using Aminex type ion-exchanger columns, gives results very close to those obtained by a standard gravimetric or volumetric method if a simple calculation program is applied. The program allows the DE to be calculated using a modified set of factors for multiplying the areas of each chromatographic peak. These factors are derived mainly from the theoretical factors corresponding to the ratio of the masses raised to a convenient power. They are thus adjusted to the real conditions of the chromatographic separation as well as to those of the comparative method. Since the HPLC method does not depend either on

water determination of the analysed corn syrup or on sample weighing, the method is not only faster, but also more reproducible. The good agreement between the results obtained with those of a gravimetric or volumetric method used as reference, together with the mentioned advantages, justify the replacement of the standard method by the HPLC method for the control of corn syrups during manufacturing. A periodical calibration with established standards in the useful DE range is evidently recommended.

Acknowledgement

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Summary

The present work describes a method for the determination of the dextrose equivalents of corn syrups from the HPLC analysis data obtained with Aminex type ion-exchanger columns. The DE conversion factors used for multiplying the areas of the chromatographic peaks are derived from the theoretical ones corresponding to the ratios of the masses of the component versus those of glucose.

They are selected with the help of a calculation programme in BASIC language to take into account the chromatographic separation and the standard DE determination method used.

The analysis of 14 corn syrups showed the good agreement of the results obtained with this new method and the reference results (gravimetric method). Besides its simplicity and rapidity, this method has the advantage of having a better reproducibility than the standard method.

Zusammenfassung

In der vorliegenden Arbeit wird eine Methode zur Ermittlung der Dextrose-Equivalent-Werte (DE) der Glucosesirupe vorgestellt, aufgrund von HPLC-Analysedaten mit Ionenaustauschersäulen vom Typ Aminex. Die DE-Umrechnungsfaktoren, die zur Multiplikation mit den chromatographischen Peak-Flächen dienen, sind von den theoretischen Faktoren abgeleitet, die die Massenverhältnisse zwischen Glucose und den entsprechenden Komponenten darstellen.

Diese Faktoren werden mit Hilfe eines in BASIC-Sprache programmierten rechnerischen Vorganges gewählt, um die chromatographische Trennung und die zum Vergleich genommene Standard-DE-Bestimmungsmethode zu berücksichtigen.

Die durch diese Methode gewonnenen Analysewerte von 14 Glucosesirupen zeigen eine gute Übereinstimmung mit denen der gravimetrischen Vergleichsmethode auf. Die Methode ist nicht nur einfach und schnell durchzuführen, sondern weist auch eine bessere Reproduzierbarkeit als die der Standard-DE-Bestimmung auf.

Résumé

Ce travail présente une méthode de détermination des valeurs de dextrose équivalent (DE) de sirops de glucose, basée sur les résultats issus de l'analyse HPLC des sucres à l'aide d'une colonne de type Aminex. Les facteurs de conversion utilisés pour multiplier les surfaces des pics chromatographiques sont dérivés des facteurs théoriques des rapports des masses des composants vis-à-vis de celle du glucose.

Ils sont choisis à l'aide d'un système de calcul programmé en langage BASIC pour tenir compte de la séparation chromatographique et de la méthode de référence de détermination du DE employé.

L'analyse de 14 sirops de glucose a montré la bonne concordance des résultats obtenus par cette nouvelle méthode avec ceux de références (méthode gravimétrique). Enfin, cette méthode, en plus de sa simplicité et de sa rapidité, présente l'avantage d'une meilleure reproductibilité que les méthodes traditionnelles.

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