**Additional Files**

**Supplementary Table 1:** Chromatography conditions for carbohydrate analysis on the Dionex system. Elution protocols for mono- and di-saccharides and for oligo-saccharides maltose, melibiose, and those of three sugars or greater using a Dionex PA1 column and guard column at a flow rate of 1mL•min-1.

|  |  |  |  |
| --- | --- | --- | --- |
| **Mono-, and some di-saccharides** | **Time**  **(min)** | **Eluant stock solutions: A and D: H2O; B: 250 mM NaOH; C: 250mM NaOH and 1M NaOAc.** | **Flow rate**  **mL•min-1** |
| Isocratic | 0-40.0 | 46.4% A; 7.2% B; 0%C; 46.4% D | 1.0 |
| Wash | 40.1-50.0 | 25% A; 0% B; 50%C; 25% D | 1.0 |
| Wash | 50.1-60.0 | 0% A; 100% B; 0%C; 0% D | 1.0 |
| Re-equilibrate | 60.1-70.0 | 46.4% A; 7.2% B; 0%C; 46.4% D | 1.0 |
|  |  |  |  |
| **Melibiose, maltose, and RFOs** | **Time**  **(min)** | **Eluant stock solutions: A and D: H2O; B: 250 mM NaOH; C: 250mM NaOH and 1M NaOAc.** | **Flow rate**  **mL•min-1** |
| Isocratic | 0-30 | 20.3% A; 58.4% B; 1%C; 20.3% D | 1.0 |
| Wash | 30.1-35.0 | 0% A; 0% B; 100%C; 0% D | 1.0 |
| Re-equilibrate | 35.1-45.0 | 20.3% A; 58.4% B; 1%C; 20.3% D | 1.0 |

**Supplementary Table 2:** Carbohydrate and elution time with the conditions related in the Supplementary Table 1. Components of carbohydrate standards for neutral carbohydrates and raffinose family oligosaccharides and elution times on HPLC using a CarboPac PA1 column and guard.

|  |  |  |  |
| --- | --- | --- | --- |
| **Neutral sugar protocol** | | **RFO sugar protocol** | |
| **Sugar identity** | **Elution Time**  **(min)** | **Sugar identity** | **Elution Time**  **(min)** |
| Glycerol | 1.68 | 2-deoxyglucose | 3.33 |
| *myo*-Inositol | 1.79 | Fructose | 4.81 |
| Glycerol-O--D-galactopyranoside | 2.33 | Glucosamine | 6.08 |
| Galactinol | 2.54 | Sucrose | 7.19 |
| Unknown 2 | 2.72 | Melibiose | 4.81 |
| Sorbitol | 2.89 | Raffinose | 11.66 |
| Mannitol | 3.38 | Stachyose | 12.55 |
| Trehalose | 3.89 | Maltose | 13.61 |
| 2-deoxyglucose | 9.68 | Verbascose | 15.17 |
| Glucosamine | 12.74 |  |  |
| Galactose | 14.42 |  |  |
| Glucose | 15.50 |  |  |
| Fructose | 20.10 |  |  |
| Sucrose | 25.05 |  |  |
| Melibiose | 27.60 |  |  |

**Supplementary Table 3:** Chromatography conditions for carbohydrate analysis on the Waters system. Elution protocols for mono- and di-saccharides using Waters hydrophilic interaction liquid chromatography (HILIC) on a Waters Aquity UPLC at 30 °C and a flow rate of 0.35 mL•min-1.

|  |  |  |  |
| --- | --- | --- | --- |
| **Mono-, and some di-saccharides** | **Time**  **(min)** | **Eluant stock solutions: Eluent A: H2O** (Fisher Optima) **+ 0.1% formic acid** (Fisher)**; Eluent B: Acetonitrile** (Fisher Optima) **+ 0.1% formic acid.** | **Flow rate**  **mL•min-1** |
| Isocratic | 0-1.00 | 20% A; 80%B | 0.35 |
| Linear gradient | 1.01  to  11.00 | 20% A; 80%B  to  50% A; 50% B | 0.35 |
| Isocratic | 11.01-12.50 | 50% A; 50% B | 0.35 |
| Linear gradient | 12.51  to  13.50 | 50% A; 50% B  to  20% A; 80%B | 0.35 |
| Re-equilibrate | 23.50 | 20% A; 80%B | 0.35 |

**Supplementary Table 4:** Carbohydrate, retention time, observed ion, and monoisotopic mass with conditions as in Supplementary Table 3. Identities of select carbohydrates analyzed using HILIC separation on the Waters mass spectrometer system, showing retention time, [M-H] ion and the monoisotopic mass of neutral molecule.

|  |  |  |  |
| --- | --- | --- | --- |
| **Sugar identity** | **Retention**  **Time (min)** | **Observed ion**  **[M-H]** | **Monoisotopic mass of neutral molecule** |
| Ribitol | 2.66 | 151.0601 | 152.0685 |
| Mannitol | 3.36 | 181.0713 | 182.0790 |
| Glycerol-O--D-galactopyranoside | 3.70 | 253.0920 | 254.1002 |
| *myo*-Inositol | 5.14 | 179.0553 | 180.0634 |
| Unknown 2 | 5.88 | 415.1456 | 416.1530 |
| Galactinol | 6.79 | 341.1084 | 342.1162 |

**Supplementary Table 5:** Stepwise prediction of glycerol-O--D-galactopyranoside (GG).

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Analysis of Variance | | | | | | | | | | | | |
| **Source** | | **DF** | | | **Sum of Squares** | | **Mean Square** | | **F Value** | | **Pr > F** | |
| Model | | 3 | | | 1134217 | | 378072 | | 1525.86 | | <.0001 | |
| Error | | 20 | | | 4955.54539 | | 247.77727 | |  | |  | |
| Uncorrected Total | | 23 | | | 1139173 | |  | |  | |  | |
| **Variable** | | **Parameter Estimate** | | | **Standard Error** | | **Type II SS** | | **F Value** | | **Pr > F** | |
| Unknown 2 | | 0.03247 | | | 0.00210 | | 59112 | | 238.57 | | <.0001 | |
| Mannitol | | -1.05204 | | | 0.35832 | | 2135.92000 | | 8.62 | | 0.0082 | |
| Fructose | | -0.15990 | | | 0.02686 | | 8782.69822 | | 35.45 | | <.0001 | |
| Summary of Stepwise Selection | | | | | | | | | | | | |
| **Step** | **Variable Entered** | | **# of Vars In** | **Partial R-Square** | | **Model R-Square** | | **C(p)** | | **F Value** | | **Pr > F** |
| 1 | Unknown 2 | | 1 | 0.9820 | | 0.9820 | | 60.4023 | | 1197.94 | | <.0001 |
| 2 | Fructose | | 2 | 0.0118 | | 0.9938 | | 9.0994 | | 39.84 | | <.0001 |
| 3 | Mannitol | | 3 | 0.0019 | | 0.9956 | | 2.6360 | | 8.62 | | 0.0082 |

No intercept in model. All variables left in the model are significant at the 0.05 level. No other variable met the 0.05 significance level for entry into the model.

Model Glycerol-O--D-galactopyranoside = + 0.03(Unknown 2) - 1.05(Mannitol) - 0.16 (Fructose) + error.

**Supplementary Table 6:** Stepwise prediction of Unknown 2.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Analysis of Variance | | | | | | | | | | |
| **Source** | | **DF** | | | **Sum of Square** | | **Mean Square** | | **F Value** | **Pr > F** |
| Model | | 4 | | | 3662963775 | | 915740944 | | 5772.92 | <.0001 |
| Error | | 19 | | | 3013912 | | 158627 | |  |  |
| Uncorrected Total | | 23 | | | 3665977686 | |  | |  |  |
| **Variable** | | **Parameter Estimate** | | | **Standard Error** | | **Type II SS** | | **F Value** | **Pr > F** |
| Glycerol-O--D- galactopyranoside | | 26.24429 | | | 1.74366 | | 35935478 | | 226.54 | <.0001 |
| Mannitol | | 59.98302 | | | 10.56455 | | 5113659 | | 32.24 | <.0001 |
| Trehalose | | -12.86540 | | | 4.45628 | | 1322145 | | 8.33 | 0.0094 |
| Fructose | | 7.60083 | | | 1.11332 | | 7393703 | | 46.61 | <.0001 |
| Summary of Stepwise Selection | | | | | | | | | | |
| **Step** | **Variable Entered** | | **# of Vars In** | **Partial R-Square** | | **Model R-Square** | | **C(p)** | **F Value** | **Pr > F** |
| 1 | Glycerol-O--D- galactopyranoside | | 1 | 0.9820 | | 0.9820 | | 286.151 | 1197.94 | <.0001 |
| 2 | Fructose | | 2 | 0.0157 | | 0.9977 | | 19.9640 | 144.54 | <.0001 |
| 3 | Mannitol | | 3 | 0.0011 | | 0.9988 | | 3.1452 | 18.68 | 0.0003 |
| 4 | Trehalose | | 4 | 0.0004 | | 0.9992 | | -0.9975 | 8.33 | 0.0094 |

No intercept in model. R2 is redefined. All variables left in the model are significant at the 0.05 level. No other variable met the 0.05 significance level for entry into the model.

Model Unknown 2 = 26.24(Glycerol-O--D-galactopyranoside) + 59.98(Mannitol) -12.87(Trehalose) + 7.60(Fructose) + error.