Mathematical model of dependence of factors for chromatographic separation of fructose from glucose-fructose syrup

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Abstract. The aim of this study is to determine the optimal parameters for the separation of glucose-fructose syrup sugars into glucose, fructose, and oligosaccharides. A mathematical model of chromatographic separation of glucose-fructose syrup (GFS) was constructed. During the optimization of the chromatographic separation process, it was found that the most significant parameter affecting the purity of the fructose yield is the dry matter content. In the course of research, it was found that the dry matter content of 30% is optimal for separating glucose-fructose syrup, at separating the use of this parameter made it possible to obtain a carbohydrate composition with a content of fructose 93%, glucose 5% and oligosaccharides 2%. This is the highest yield of fructose when separated by ion exchange resins of company "Purolite" brand PCR641Ca.

Keywords: glucose-fructose syrup, fructose, ion chromatography.

1. Introduction

Using chromatographic systems to separate sugars, such as fructose and glucose, is a fairly effective and feasible method (Mostafazadeh et al., 2011). Chromatographic separation of glucose and fructose in the production of high-fructose corn syrup (HFCS) is one of the most commonly used in chromatography (Ganetsos and Barker, 1993).

Classically, the separation of sugars is carried out using resins in the form of calcium, while the separation of sugar/non-sugar is achieved using resins in the form of potassium. Resins in the form of calcium simultaneously produce two types of separation. First, the resin acts as a molecular sieve. Large molecules that cannot penetrate the resin granules are more or less excluded because of their size. Second, the separation is due to the difference in the stability of the sugar complex with calcium: only polyols and some sugars (fructose, galactose) are able to form such a complex. Other components (sucrose, glucose) do not form a complex with the resin, which explains the separation (Paillat et al., 2000).

Among the main and socially important food products, sugar and sugary substances are one of the first places, and, at the current level of purchase power of the population, they are the most affordable products in the diet of people (Uglov et al., 2017). Alternative natural sources of edulcorating components are sugary starch products: glucose, maltose, glucose-fructose syrups, maltodextrins and others that are obtained from corn, wheat, tapioca, triticale, sorghum, etc. (Polumbrik et al., 2016).

Glucose-fructose syrup is a plant ingredient used in the food industry, mainly derived from wheat and corn. Glucose-fructose syrup has a composition similar to table sugar (also called “sucrose”), which is obtained from sugar cane or beet. Sucrose and glucose-fructose syrups consist of glucose and fructose, but in different proportions (https://starchinfood.eu/ingredient/glucose-fructose-syrup/). GFS
production is highly dependent on the local sugar industry and on agricultural raw materials (Vuilleumier, 1993), (kiran ,2020).

GFS is obtained from corn starch by first hydrolyzing the starch into glucose in an enzymatic manner, which is then partially converted to a fructose solution containing 42% fructose (HFCS-42) (Palazzi et al., 1999). GFS is cheaper than beet and cane sugar. Large-scale implementation of GFS in the food industry of developed countries began in the late 1960s (Ventura et al., 2011). Sucrose, GFS, and crystalline fructose give the body comparable energy: calories are 3.9 kcal/g, 3.7 kcal/g, and 3.6 kcal/g, respectively. GFS contain both glucose and fructose, most glucose and fructose are in the free form of monosaccharides. GFS contains a small amount of residual glucose oligosaccharides (White, 2014).

GFS production was made possible by parallel developments in refining, isomerization, and separation technologies in the 1960s. Fructose contributes many useful physical and functional properties to foods and beverages, including sweetness, improved taste, moisture, color and flavor development, reduced freezing point, and osmotic stability. GFS is widely used in carbonated beverages, baked goods, canned fruits, jams and jellies, as well as in dairy products. The use of crystalline fructose and crystalline fructose syrup has recently expanded from pharmaceutical and specialty foods to basic foods and beverages (Hanover and White, 1993).

Glucose-fructose syrups need special storage conditions, which exclude the possibility of glucose crystallization, increasing the product color or its infection. Glucose-fructose syrup can be stored for a long time at a temperature of 25-30°C. At temperatures below 25°C, the process of glucose crystallization begins (when the content of dry matter (DM)=70% saturation occurs at 27°C). At temperatures above 30°C, color increases over time due to the thermal decomposition of monosugars, as well as their reaction with amino acids. In factories, for syrup storage, stainless steel tanks with autonomous heating are used. Sometimes storage containers are placed in an isolated room where the temperature is maintained at 35-34°C. This eliminates the need for heating elements and isolation of individual containers. It should be noted that in mixtures of glucose-fructose syrup (42% fructose) with sucrose, there is no probability of crystallization due to the greater solubility of the mixture of components compared to the solubility of each of them separately. At a temperature from 22.8 to 50°C, the maximum solubility is a syrup with a sucrose content of 33-47%, so the storage of such syrups is simplified (Guljuka, 1985).

Since 1978, the United States, Japan, and other countries have started producing syrups with a higher fructose content and, consequently, a lower glucose content. These are so-called second-generation syrups. High-fructose syrups are obtained mainly by chromatographic separation of ordinary glucose-fructose syrup into fructose and glucose in a special separation column, where ion-exchange resins in Ca2+form are used as an adsorbent. Having an affinity for the adsorbent, fructose is retained on the resin. Glucose and polysaccharides pass at high speed through the adsorbent layer. Subsequent elution with 230 deposited desorbent produces an enriched fructose fraction containing up to 90% fructose. Enriched glucose syrup contains up to 89% glucose. The glucose fraction is returned to isomerization, and the fructose fraction is mixed with the original glucose-fructose syrup after purification with special ion-exchange resins. In this case, a syrup with a fructose content of 55% or higher is obtained (Guljuka, 1985). Due to its advantages, high-fructose syrup (HFS) is currently the leader among sweeteners in the food industry, especially in the beverage industry (White, 2014). First, these are relatively stable beverages with a low pH and require less processing costs. In addition, it has a high tendency to change color, which is useful in the manufacture of baked products. In addition, the sugars are already pre-dissolved and resistant to crystallization and growth of microorganisms as a traditional method of preservation. It should be noted that fructose is the sweetest dietary sugar, which is almost 1.2 times sweeter than sucrose (Hanover and White, 1993).

Now the most popular and effective way to separate glucose-fructose syrup is chromatography. In Finland, “SuomenSokeri” started producing pure crystalline fructose for the first time in the world. This technology is based on a method for distributing invert syrup into fructose and glucose fractions using a chromatographic method. Using this technology, the world's largest “Kjurofin” plant was built in the United States [https://nomnoms.info/poluchenie-glyukozo-fruktoznynh-siropov-i-fruktozy/].
The use of the high-performance liquid chromatography method allows monitoring changes in the composition of carbohydrates and organic acids of the grain wort, and promptly identifying and eliminating the causes that lead to a decrease in the quality of the finished product. The developed new methods for determining the mass concentration of carbohydrates (maltose, glucose, fructose) and organic acids (acetic, lactic) are based on the processes of ligand exchange and ionic excretion (Medrish et al., 2016).

Usually, cation exchange resin (Ca2) with pure water as an eluent are used at separation (Verhaar and Kuster, 1981).

In the “Ca-pillar” system, the main function of ion exchangers is to immobilize Ca2, whereas separation is the result of different complexing abilities of polyols with Ca2. However, the degree of crosslinking of divinylbenzene is also important; polymer sugars are better separated at low degrees, and monomeric and dimeric sugars at high crosslinking degrees (Salah, 2006).

Chromatographic processes for separating sugars use layers of cation exchange, which are in the form of calcium (Ca2), which is known to form a weak complex with fructose, which leads to a predominant slowdown of fructose, while glucose is carried away with the mobile phase (Barker and Joshi, 1991). The advantages of these processes are low-cost eluent, high performance and stability of column, and complete elution of all added sugars (Angyal et al., 1979).

Fructose distribution coefficients depend on temperature; fructose retention is lower in the chromatographic column at a higher temperature (Viaird and Lameloise, 1992). In practical use, calcium forms of ion-exchange adsorbents are preferred because of their low cost and non-toxic nature (Lloyd and Nelson, 1984).

Significant research has been conducted on the separation of GFS on ion-exchange resins in the world, but there is insufficient data on the separation of GFS 42 into glucose and fructose by “Purolite” resins of PCR641 brand. The main purpose of these studies is mathematical model of the process of separating GFS 42 used to produce a syrup with a high fructose content.

2. Mathematical model

To construct a mathematical model of the chromatographic separation of GFS, which is a regression equation used rotatable plan of the second order (Box plan) when the number of factors to equal 3, the number of experiments is not more than 20, the number of experiments at the zero point was 6 and the number of coefficients is 10.

A mathematical and statistical method was used as a mathematical apparatus and a system of regression equations was obtained that models the relationship of the most preferred optimality criterion with the rest.

Specific factors that determine specific production conditions were used as optimality criteria that affect the process of chromatographic separation of GFS. In this regard, it is advisable to adjust the system of regression equations in accordance with the factors taken.

The regression equation of the mathematical model of separation in the chromatographic column will have the form:

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 + b_{11} x_1^2 + b_{22} x_2^2 + b_{33} x_3^2 \quad (1)$$

where $b_i (i = 0, 1, \ldots, 33)$ represent the regression coefficients of the model.

3. Experimental part

3.1 Materials

Glucose-fructose syrup containing 52% glucose, 42% fructose and 6% oligosaccharides was used as the starting product. For the separation of glucose-fructose syrup, the method of separation with a strong-acid cation-exchange resin PCR641Ca of the company “Purolite International Ltd” (Great Britain) was used. In chromatographic separation, the following parameters were monitored: DE concentration in glucose-fructose syrup, separation temperature, and mobile phase speed. Dependent variables are the volume of elution (ml) and time (min) for the fructose-rich fraction, the return fraction, and the glucose-rich fraction, as well as the concentration of glucose and fructose in each of the fractions.
**Description of resin**

PCR641Ca is a resin consisting of polystyrene cross-linked with divinylbenzene, the functional group is sulfonic acid. The resin is presented in the form of spherical grains, the diameter was 310 mm with a total exchange capacity of 1.5 g-eq/l (32.8 Kilogram/cubic foot) (H+ form) and a maximum operating temperature of 120 °C. The functional group of the resin was sulfonic acid, and the ionic form in Ca\(^{2+}\).

**3.2 Experiments in chromatographic packed column**

**Process of wetting the resin**

A glass column with a length of 20 cm and a diameter of 3 cm was used as a device for chromatography. The resin slightly retains glucose with its layer in the calcium-fructose complex. In this way, fructose will remain bound to the resin until the flow of eluting water weakens their bond by eluting fructose with water.

A weighted amount of resin (1 g/ml of column content) was placed in a separating chromatographic column. PCR641Ca resin separates fructose from glucose by molecular elimination. A peristaltic eluent pump (Buchi Chromatography B-688 Pump) was connected and deionized water was pumped through a column to wet the resin. In a resin-filled container, water was filled to activate the resin, swell it, and form uniform pores between the grains, after which the water was drained.

**The separation steps**

Glucose-fructose syrup (eluate) was diluted in deionized water to a dry matter content of 30-50% and passed through a 2-mm cellulose filter, then the solution was heated to a temperature of 40 to 48 °C. Volume of the syrup solution added into the column was 5% of BV. The eluent was deionized water and heated to a temperature higher than the column temperature to remove any dissolved gases that were released.

The resulting solution of glucose-fructose syrup was fed into a column with flow rates from 4 to 8 milliliters per minute. After the batch of syrup solution, the calculated amount of deionized water (elution) was fed to the column with the same flow rate equal to the flow rate of glucose-fructose syrup. The elution time was calculated from the time when the water pump was turned on. The solution flowing out of the column was sent to the fraction collector (Buchi B-684 Fraction Collector) at a speed of 60 s per tube. When the separation was complete, the column was washed with deionized water at a flow rate of 10-15 milliliters per minute.

The collected effluents from the column (in 60-second portions) were analyzed using high-performance liquid chromatography (HPLC) to determine the carbohydrate content.

**3.3 Sample analysis**

In the obtained samples, the carbohydrate composition was analyzed for the content of glucose in the syrup. The fructose concentration was determined by high-performance liquid chromatography (Waters HPLC “Waters Corporation”, USA) with a differential refractometric detector Waters 2414 using a column (Aminex HPX-87H (300*7.8 mm), Bio-Rad Labs, USA). The studies were performed under the following parameters: flow rate: 0.6 ml/min; detector: refractive index (RI), 40°C; column temperature: 60°C; mobile phase: 5 mmol/l H2SO4; injection: 10 µl; preprocessing of the sample: dilution.

**4. Results and discussions**

**4.1 Mathematical model and optimization of the chromatographic separation process**

During experimental studies of chromatographic separation, samples of glucose-fructose solution were selected and analyzed. To build a mathematical model, the following factors were established: the heating temperature of GFS (T, °C), the GFS feed rate to the chromatographic column (V, gal/min), and DM content (DM,%). All these factors influence the optimization criteria – the purity of fructose yield in solution (n,%).

At the beginning, the intervals and levels of variation of the parameters taken were coded, which are presented in Table 1. The experiment planning matrix is shown in Table 2.
Table 1. Coding of intervals and levels of the variation of input factors

| Factors | Variation levels | Variation intervals |
|---------|------------------|---------------------|
| natural coding | -1.68 | -1 | 0 | +1 | +1.68 |
| T, °C | $x_1$ | 40 | 42 | 44 | 46 | 48 |
| V, gal/min | $x_2$ | 4 | 5 | 6 | 7 | 8 |
| DM,% | $x_3$ | 30 | 35 | 40 | 45 | 50 |

Table 2. Matrix of rotatable planning of experimental studies of the chromatographic separation

| No. | Coded values | Natural values | Experimental values |
|-----|--------------|----------------|---------------------|
| $x_1$ | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| $x_2$ | - | - | - | 42 | 5 | 35 | 88 |
| $x_3$ | - | + | - | 42 | 7 | 35 | 84 |
| $x_1$ | - | + | + | 42 | 7 | 45 | 84 |
| $x_2$ | + | - | - | 46 | 5 | 35 | 85 |
| $x_3$ | + | - | + | 46 | 5 | 45 | 85 |
| $x_1$ | + | + | - | 46 | 7 | 35 | 85 |
| $x_2$ | + | + | + | 46 | 7 | 45 | 85 |
| $x_3$ | -1.68 | 0 | 0 | 40 | 6 | 40 | 85 |
| $x_1$ | +1,68 | 0 | 0 | 48 | 6 | 40 | 88 |
| $x_2$ | 0 | -1,68 | 0 | 44 | 4 | 40 | 84 |
| $x_3$ | 0 | +1,68 | 0 | 44 | 8 | 40 | 90 |
| $x_1$ | 0 | 0 | -1,68 | 44 | 6 | 30 | 85 |
| $x_2$ | 0 | 0 | +1,68 | 44 | 6 | 50 | 93 |
| $x_3$ | 0 | 0 | 0 | 44 | 6 | 40 | 88 |
| $x_1$ | 0 | 0 | 0 | 44 | 6 | 40 | 86 |
| $x_2$ | 0 | 0 | 0 | 44 | 6 | 40 | 87 |
| $x_3$ | 0 | 0 | 0 | 44 | 6 | 40 | 87 |
| $x_1$ | 0 | 0 | 0 | 44 | 6 | 40 | 87 |

Table 3. shows the values of confidence intervals of the chromatographic separation optimization criteria.

Table 3. Values of confidence intervals of the optimization criterion for $y$ (n, %)

| Chromatographic separation | Parameter | Confidence intervals |
|----------------------------|-----------|----------------------|
| Fructose yield purity | n, % | y | $\pm 0.84$ | $\pm 0.56$ | $\pm 0.54$ | $\pm 0.73$ |

The coefficient of the regression equation is significant in cases where its absolute value is greater than the confidence interval. Otherwise, it is considered insignificant and can be excluded from further consideration of the mathematical model.

When comparing confidence intervals from Table 3 with regression coefficients from Table 4, it can be said that the effects of the interaction of input factors are negligible, and they can be neglected.

Substituting the resulting encoded values in the regression equation (1), then the equation of the fructose yield purity in the solution will have the form:
After decoding the equation, we get the regression equation for natural values of the factors:

\[ y = 86.80348128 - 0.28987x_1 + 0.002928x_2 + 0.447984x_3 + 1.625x_4x_2 - 1.375x_3 + 0.125x_3x_3 - 0.83975x_1^2 - 0.12174x_2^2 + 0.218646x_3^2 \]

Table 4. Coefficients of regression equations of the yield parameters for \( y (n, \%) \)

| Optimization criterion | Coefficients | Yield of finished product |
|------------------------|--------------|---------------------------|
| Fructose yield purity, % | For coded values of factors | | |
| \( b_0 \) | 86.80348128 | | |
| \( b_1 \) | -0.28987 | | |
| \( b_2 \) | 0.002928 | | |
| \( b_3 \) | 0.447984 | | |
| \( b_{12} \) | 1.625 | | |
| \( b_{13} \) | -1.375 | | |
| \( b_{23} \) | 0.125 | | |
| \( b_{11} \) | -0.48695 | | |
| \( b_{22} \) | -0.83975 | | |
| \( b_{33} \) | 0.218646 | | |
| | For natural values of factors | | |
| \( B_0 \) | -189.3959 | | |
| \( B_1 \) | 11.48293 | | |
| \( B_2 \) | -26.6759 | | |
| \( B_3 \) | 5.110737 | | |
| \( B_{12} \) | 0.8125 | | |
| \( B_{13} \) | -0.1375 | | |
| \( B_{23} \) | 0.02500 | | |
| \( B_{11} \) | -0.12174 | | |
| \( B_{22} \) | -0.83975 | | |
| \( B_{33} \) | 0.008746 | | |

Figure 1. Three-dimensional model in space that characterizes the dependence of \( y_i = f(T, V) \) temperature and syrup feed rate
At the stage of optimization of chromatographic separation, the concentration of dry matter (DM) of the supplied glucose-fructose syrup solution in the separation column is of particular interest, as a characteristic that determines one of the main parameters that affect the fructose content purity in the solution at the outlet of column. The dependences (Figures 1-3) of influence of the main parameters (temperature, solution feed rate and dry matter content) of the separation are obtained that the fructose yield purity decreases with increasing temperature and increasing the solution feed rate to the separation column, the value of the solution feed rate close to 4 ml/min is observed the highest fructose yield purity. Figure 2 shows that at the lowest DM of 30°C, the yield of pure fructose in the solution increases. The three-dimensional model of Figure 3 shows how the solution feed rate and the dry matter content affect the fructose yield purity in the solution. In this model, it is clearly seen that when the dry matter content exceeds 50%, the separation process is worse, as shown in Figure 2. When the minimum and
maximum value of the solution feed rate is equal to 4 and 8 milliliters per minute, the yield of fructose decreases. From the model of Figure 3, it can be concluded that the most optimal value is the speed equal to 6 ml/min and the DM content equal to 30%. The maximum fructose yield purity was 93% of the fructose content in the solution.

4.2 High-performance liquid chromatography
At the end of optimization of the chromatographic separation process, control separation experiments were performed using the obtained parameters. After that, the resulting separated solution was analyzed. The obtained data on the carbohydrate composition of the separated solution were compared with the data on the carbohydrate composition of GFS.

![Figure 4. Chromatogram of carbohydrate composition of GFS before chromatographic separation](image)

The chromatogram of GFS carbohydrate composition (Figure 4) shows that the glucose content was 52%, fructose 42%, and other sugars (oligosaccharides) 6%.

![Figure 5. Chromatogram of the carbohydrate composition of solution after chromatographic separation of GFS at the DM content of 50% (a) and 30% (b)](image)

During the optimization of the chromatographic separation process, it was found that the most significant parameter affecting the purity of the fructose yield is the dry matter content. In this regard, control experiments were performed in several repetitions with different dry matter content. The chromatograms (Figure 5 (a) and (b)) show the carbohydrate composition of high-fructose syrup after GFS separation. Figure 5 (a) shows a chromatogram of the carbohydrate composition of the experiment at a 50% DM content, which shows that the yield of fructose was 85%, glucose 12% and oligosaccharides 3%. In order to increase the yield of pure fructose in the syrup, the DM content was lowered to 30% and separated. The chromatogram of Figure 5 (b) shows the carbohydrate composition...
of the syrup divided at a 30% DM content. The carbohydrate composition showed a fructose content of 93%, glucose of 5% and oligosaccharides of 2%. When the DM content decreases below 30%, the glucose contained in the solution does not have time to react with the resin. This in turn increases the concentration of fructose in the solution. This is the highest yield of fructose when separated by “Purolite” cationic resins of PCR641Ca brand.

5. Conclusion
Fructose is supplied as a solution, and in this case its degree of purity must be at least 80%, so the fructose solution obtained from the column by chromatographic method is a ready-made solution that meets the requirements of commercial quality. During the chromatographic separation of GFS to obtain high-fructose syrup, a number of experiments were conducted using ion-exchange resins of PCR641Ca brand of company “Purolite”. From the results, it became clear that fructose reacts more strongly than glucose, which means that it leaves the column a little later. It follows that this method can be used to obtain a fraction saturated with fructose from glucose-fructose syrup for the food industry. One of the problems, as it turned out, is the concentration of syrup, that is, the content of dry matter in the syrup. For the purpose of separation, DM content was lowered to 30%. It is also important to apply the solution to the resin surface. Optimization of chromatographic separation parameters was carried out by constructing a mathematical model. The model represented the dynamics of the column behavior with varying input optimization parameters from the zero point.

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