Maillard reaction and the process of yelloowing of the glucose syrup extracted from potatoes, corn and millet

G. Bujanca, D. Stefan, F. Berbentea, L. Geogescu, A. Rinovetz
Banat’s University of Agricultural Sciences and Veterinary Medicine Timisoara, Romania

Abstract
Both the glucose syrup and solid glucose process of “yellowing” can be considered as a special example of the Millard reaction (a reaction which takes place in the absence of enzymes and which changes the color into brown). Compounds with an amino nitrogen composition- which help in the formation of melanoidinic compounds- originate in the protean impurities resulted from processed starch. As far as the glucose syrup and solid glucose is concerned, there were noticed some dependence relations between the starch and sulphur dioxide contents and the intensity of the color during heating. It is advisable that the glucose syrup color and the glucose syrup process coloring be represented and indicated with the help of the extinction coefficients. In order to make a comparison, we determined the extinction coefficients of the standard iodine and potassium bichromate solutions, mainly used in the creation of patterns in the glucose syrup color.

Keywords: glucose syrup, melanoidinic compounds

1. Introduction
Kroner and Kothe (Kroner, 1999) have shown in their test concerning the color modification of the glucose syrups, the action of the simplest protean substance, which is glycine, on the color modification of the raw thin syrup (Kroner, 1999). The mentioned authors admit that the conditions necessary for this secondary reaction exist only in case of a more advanced converting (over 60%) and that these are not yet shown for the syrups with a low converting. This concept is true as long as there is a differentiation between colorings occurred at “converting”, during the acid hydrolyze of the starch and those presented in the final syrup or that occur after a longer period of storage. The colorings that occur during the acid hydrolyze of the raw thin syrup become more obvious as the converting is more advanced. These colorings can not be entirely avoided not even if the best quality raw material is being processed.

Yellowing, which is the formation of yellow colorings in the final glucose syrup, represents only a particular case of the reaction that takes place between the carbon hydrants and the protean substances. The reaction has been described in detail by Maillard in 1912 and has lead to the so called melanoidinic compounds (Enders 1998).

The undesired formation of the melanoidinic compounds take place, for example, in case of coloring in brown, considered to be of non-enzymatic nature and are noticed after a long period of storage of some vegetal food products, preserved by drying, especially at different types of vegetables dried. The darkening of the molasses color when processing the beet sugar as well as the browning of the bee honey that occurs after storage for a longer period of time are also placed in the area of undesired formation of the melanoidinic compounds.

Corresponding author: e-mail address: bujanca_gabriel_tpatm@yahoo.com
As far as the obtaining of the colorings in the food products technology is concerned, the first step in this phenomenon is the formation of the melanoidinic compounds. The real intensity of the color of different shades can be obtained in this area only through adding adequate quantities of ammonia salts in the mass of hot sugar. As far as the complex mechanism of the reaction that leads to the formation of melanoidinic compounds is concerned, there is no clear data even though numerous studies have been conducted. The first step in the reaction could be considered the formation of the so-called Schiff bases through the reaction between the sugar carbonyl group and the amino group from the protean substances that takes place by separating a water molecule and probably by passing through a primary, additional product:

\[
\text{CO} + \text{H}_2\text{N} \rightarrow > \text{C} = \text{N} - + \text{H}_2\text{O} \quad (1)
\]

This compound has the priority of entering afterwards in other reactions (condensations). The first step of the reaction series leads to colorless compounds while the actual formation of the melanoidinic compounds, that is the formation of the colorings with a darker shade, occurs only later. Thus, for the coloring occurrence there are necessary free and reactive carbonyl groups as they exist in reducible sugar contents (dextrose, maltose) and amino reactive groups from the protean substances and from their decomposition products, down to the last step which is the ammonia. First of all we are interested in the large quantity of the protean substances content from the starch types that can be considered for producing the glucose syrup, such as the potato, corn, and millet and wheat starch. The quality commercial standards stipulate a maximum content of 0,5% of protean substances in the cereal starch. For the potato starch the maximum content of protean substances is not established as these substances can be relatively easy separated from the actual starch in the potato juice in comparison with the protean substances from the cereals.

Due to this, the highly content of protean substances from the potato flour, which represent the primary product in producing the glucose, occur only rarely.

Lately, approximately 100 samples from different types of commercial starch have been analyzed by Polshenke, Ruggeberg and Lindemann (Polshenke 2000). They found an average content of approximately 0,1% protean substances in the potato starch and a content of approximately 0,3% in the corn and wheat starch. The content of protean substances from the millet starch has been established by us, at 0,24%.

During the industrial acid hydrolyze of the starch, not the entire quantity of the protean substances passes in the unconcentrated syrup because as Kroner and Wegner (Kroner 1993) have shown, a part of these substances take part even to the neutralization of the raw syrup and separate simultaneously with the neutralization mud. The protean substances that remain in the glucose syrup are in a very low quantity and their determination through common methods (Kjeldahl) is prevented by the carbon hydrants that play a ballast role. Parow (Parow 1995) established at his time the content of the protean substances in 10 different samples of glucose syrup obtained from the corn and found values between 0,19 and 0,39%, with an average value of 0,26%. Tryller (Tryller 1995) mentions in an earlier research a content of 0,0014% ammonia in the potato starch. The authors of the present article have found through own researches contents of protean substances that varied between 0,074 and 0,088%.

If we consider “the content of the protean substances” of the glucose syrup by taking into account these values, we establish that the unmodified proteins from the initial starch are not important anymore.

For the Kjedahl determination we must dose the entire amount of the compounds that contain amino nitrogen as a result of the protean substances during the acid hydrolyze.
2. Materials and methods

The working procedure is the following: In a cone-shaped recipient of 100 ml with a wide opening we dissolve approximately 20g of glucose syrup using 20 ml of water and adding 25 ml of sodium hydrate concentrated solution (33%). The recipient is covered with a clock glass which has on the lower part a strip of wet red indicator paper. The reaction is accelerated by slight heating (on a water bath, with a temperature between 40 – 45°C) so that after a few minutes we can observe a visible blue coloring of the indicator paper. The maximum coloring is reached usually within 15 – 30 minutes. The reaction is carried out quicker and more intense for the cereal starch than for the potato glucose syrup.

The intense fish smell that the analyzed tests often have indicated from the beginning that as far as the volatile substances are concerned in the mixture there is not only ammonia but also organic bases. The presence of the primary and tertiary amines in the syrup from the starch converting has occurred probably even earlier.

The absolute quantity of all these nitrate compounds is very low, especially for the potato glucose syrup and can be determined with the help of Kjeldahl method with some difficulties, as it was shown above. The distillate is caught in the 0,02 acid and a burette for semi-microdeterminations (according to DAB 6) is used for back titration.

Yet, the ammonium salts can be easily indicated with the help of the Nessler reactive, so that the idea of colorimetrically establishing the content of the ammonium salts from the glucose syrup with the Nessler reactive occurred without any difficulty having as base the dependence between the protean substances and ammonia quantities. We have considered that the Urbach method for establishing the ammonia from the urea is the most appropriate for this identification.

This method needs first of all fixing the ammonium salts with the help of a special permutite and removing all the carbon hydrants that represent a ballast. Afterwards a highlighting with an alkaline solution and the colorimetical determination of the free ammonia is performed.

2g Of special permutite (according to Folin, for establishing the ammonia) are washed twice with water that does not contain ammonia in a 200 ml graded balloon, and then with diluted acetic acid (2%) and then again twice with water. We wait until the substance is settled and we decant as complete as possible the washing liquids (each time around 75 – 100 m). Than we add 10g of glucose syrup diluted in 10 ml of water and we shake it for 10 minutes, during which the ammonium salts from the syrup are fixed on the permutite.

The adherent permutite portions fall to bottom while washing the recipient, they should be left to depose, then we rinse them twice in order to remove the dissolved carbon hydroxide. Then we add approximately 150 ml water in the balloon to liberate the starch linked to the permutite when dissolved into 10 ml solution, 10% sodium hydroxide; we then shake it thoroughly and add 20 ml fresh Arabic gum solution 1% (protective colloid) and then, with a dropper, we add exactly 4 ml of Nessler reactive; then we shake it thoroughly and don’t move it for 15 minutes in order to determine its red yellowish color which can be determined with the help of the Pulfrich photometer (kitchen sink of 50 mm and a filter of S 47). In order to calculate the determination data it was established a standard curve by using a pure ammonium chloride. It is absolutely necessary that each and every determination be accompanied by a witness test. It is possible that the solution used in the measuring process may turn into yellowish red after adding the Nessler reactive, but it must never become unclear. If it the solution becomes unclear, this is a sign that basic mixture was wrong and that’s why the procedure must be repeated.
By resorting to this basic method, we managed to determine the starch content from a series of tests undergone on glucose syrup and solid glucose purchased from the market. The solid glucose was added in order to complete the determination, because this is also a case in which the determination of the product’s color and the modification of the color represent a major interest. As expected, we found reduced quantities of starch - between 0.6 and 2.1 mg% starch (Table 1, column 6). Unfortunately, the initial starch wasn’t available in any of these cases and because of this fact we weren’t able to make the necessary comparison.

According to the research results, there weren’t obvious discrepancies between the starch quantities. First and foremost, it was noticed that the lowest values of starch content were not found, as expected, in the glucose syrups extracted from potatoes, but in the cereals’ starch. That’s why, it is very likely that the best explanation for this phenomenon would be that the proteins from potatoes and cereals present different degrees of sensitivity in the process of hydrolysis; it can also be possible that the protem substances absolute content from the raw material play a certain role in this process.

As it has already been mentioned, the thick syrup goes through a process of “sulphitation” in order to avoid not only a possible process of coloring in final stage of the concentration, but also to avoid a possible yellowing of the color of the syrup during storage. In the past it was widely known and accepted that the sulphuric acid had a specific effect on the harmful iron salts-it reduced the content from trivalent iron to bivalent iron. This conceptions regarding the role of the sulphuric acid, is no longer valid nowadays. According to the latest research, the sulphur dioxide has an inhibiting effect on the melanoidinic compounds. Their effect on melanoidinic compounds can be easily demonstrated by testing a sample of pure dextrose solution and a starch with the simplest composition namely, the glycine.

Consequently, a heated cone-shaped recipient with a polished cork put on boiling water, was filled up with 20 g dextrose solution and 5g glycine dissolved in 100 ml water. At well established periods of time, certain samples were taken from the solution. These samples were instantly cooled down so that extinction coefficient could be determined with the help of the Pulfrich photometer and S47 filter.

The same procedure was repeated (this time by adding 0.1% SO2 in the basic solution), so that the inhibition effect of this reactive could be measured. The solution which didn’t go through the sulphitation process, turned into dark brown; after adding the SO2, a bright yellow pale coloring could have been noticed. It is widely known that the non enzymatic reaction - which is responsible for changing the color into brown, which are, in fact, the melanoidinic compounds- depends on the temperature it is exposed to; that’s why, it usually takes place at higher temperatures.

This phenomenon presents a practical importance if we take into consideration the fact the recipients filled with glucose syrup are usually stored in the processing factory while they are still warm. The main reason why it we resort to such solution is because in this way the recipients are emptied easily and special processes of heating would no longer be necessary.
The temperature effect on the yellowing phenomenon helped us to replace the old method of storage (which took a longer period of time) with a relatively short process of heating.

The glucose syrup tests were put in dark colored recipients and boiled at a temperature reaching 1050. The quantity of reactive compounds containing amino nitrogen determines the coloring degree. To measure the coloring resulted in the process, the glucose syrup was dissolved into water so that the concentration contains 50% of dry substance (determined with the help of the refractometer); nevertheless, the extinction coefficient from the same solution was determined with the help of a Pulfrich photometer.

The extinction coefficient (K) represents the direct value of the color intensity of a solution which results from the following equation:

\[ \log \frac{J_0}{J} = K \cdot s \]  

According to which “Jo” represents the intensity of the light used in or to irradiate the solution; “J” represents its intensity after penetrating the solution and “s” represents the thickness of the solution layer. The extinction coefficient is calculated for one layer of solution with 1 cm thickness and a certain light wave. Consequently, the comparative determinations should always be carried out within the same spectral domain.

In order to complete our determinations we used a S 47 filter with a spectral weight center of 4650, indicated by Urbach. In order to obtain the same values, K value was recalculated according to the glucose syrup, containing 80% dry substance (table 1, column 8-10). It is absolutely necessary that the glucose syrup solutions used for measuring the extinction should be crystal clear because, in the yellowing process.

That’s why, such solutions may lead to measurement errors caused by a process of light dispersion (an apparently too high extinction).

Our attention was drawn on the repeated and difficult to avoid periods when the liquid becomes unclear, a phenomenon cause by the gesso combinations and protein substances.

3. Results and discussions

It was demonstrated that the glucoses with the lowest degree of sulphitation, with a medium content of nitrogen and in a very advanced conversing stage, have gone through the most intensive process of coloring. The lowest absolute coloring could have been noticed to millet glucoses, with the lowest degree of sulphitation, (nr.19 and 12). The most pronounced medium coloring could have been noticed to corn glucose syrups which contained a relatively high quantity of nitrogen and a medium quantity of sulphur dioxide.

The moment the glucose syrups extracted from potatoes starch are being analyzed in order to establish their main features, we should first and foremost take into account the fact that their very pale yellowish coloring from the beginning, darkened during the heating test.

By resorting to two examples, on one hand to glucose syrup extracted from potatoes’ starch and on the other, to glucose syrup extracted from millet’s starch (table 2), it was demonstrated that during a longer process of storage at normal temperature, the “free” starch showed a measurable decrease, which became more obvious when the quantity of SO2 from the glucose syrup was reduced. It appears that in this case we can talk about the first step of the reaction which results into the formation of melanoidinic compounds. The formation of melanoidinic compounds is a phenomenon which exclusively determines the glucose syrup coloring. The fact that the glucose syrup goes through a process of coloring is also demonstrated by their characteristic fluorescence, also present in the intensively colored tests, examined in the day light; this feature proved to be even more intensive in the ultraviolet light. Enders is the one to claim that this fluorescence is one of the main features of melanoidinic compounds.
As regards the example presented above it becomes questionable whether the creation of patterns in the glucose syrup color can any longer be considered satisfactory. It's true that it may seem quite univocal to characterize high quality glucose as “clear and colorless” because the refraction of a perfectly clear and colorless glucose used in the preparation of candies, rarely reaches such a high level to be able to qualify the product obtained as authentic crystal glucose syrup. Still, even its pale color shades usually qualified as “yellow color shading” “yellowish” “half white” are first of all exclusively subjective. In order to make a comparison and to establish the differences we decided to compare it with the color of a highly dissolved iodine solution.

According to this comparison we will indicate the differences with the following qualifying: “half white”, “bright yellow”, “medium yellow”, “dark yellow”. By resorting to a Pulfrich photometer, it was determined not only the color of some standard iodine solutions, but also indicated their extinction coefficient as it can be viewed in Table 3. In order to complete and determine the colored samples (1-5) we resorted to the same determination method of the extinction coefficient of the highly dissolved potassium bichromate solutions. By comparing the extinction coefficients from table 3 and 4 with the values from table 1, column 8, it can easily be established the differences between the colors of the glucose syrups. This comparison demonstrated that there are some tests which, when analyzed together with colorless glucooses, according to Preuss iodine ladder should be characterized as colored with an intensive dark yellow. In relation to this, it is important to take into account the fact that the glucose syrups used in the determination of the values from table 1, have been prepared in exceptional fabrication conditions.

---

Table 1 Composition, color and degree of coloring of the glucose syrups and of the solid glucose

| Number | Re-searched material | Year in which it was produced | Concentration | Converting degrees | NH\(_4\) mg% | SO\(_2\) mg% | Extinction coefficient (K) calculated in comparison to the 80% dry substance
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose syrup from potatoes</td>
<td>1999</td>
<td>45</td>
<td>37,7</td>
<td>1,5</td>
<td>0,51</td>
<td>1,87</td>
</tr>
<tr>
<td>2</td>
<td>Glucose syrup from potatoes</td>
<td>2000</td>
<td>45</td>
<td>33,6</td>
<td>1,6</td>
<td>0,05</td>
<td>0,44</td>
</tr>
<tr>
<td>3</td>
<td>Glucose syrup from potatoes</td>
<td>2000</td>
<td>45</td>
<td>35,6</td>
<td>1,6</td>
<td>0,10</td>
<td>0,99</td>
</tr>
<tr>
<td>4</td>
<td>Glucose syrup from potatoes</td>
<td>2000</td>
<td>46</td>
<td>37,0</td>
<td>1,6</td>
<td>0,02</td>
<td>0,45</td>
</tr>
<tr>
<td>5</td>
<td>Glucose syrup from corn</td>
<td>2001</td>
<td>45</td>
<td>37,7</td>
<td>2,0</td>
<td>0,02</td>
<td>1,29</td>
</tr>
<tr>
<td>6</td>
<td>Glucose syrup from corn</td>
<td>2001</td>
<td>45</td>
<td>32,8</td>
<td>2,1</td>
<td>0,02</td>
<td>1,23</td>
</tr>
<tr>
<td>7</td>
<td>Glucose syrup from corn</td>
<td>2001</td>
<td>46</td>
<td>33,9</td>
<td>1,3</td>
<td>0,002</td>
<td>1,11</td>
</tr>
<tr>
<td>8</td>
<td>Glucose syrup from corn</td>
<td>2001</td>
<td>45</td>
<td>35,6</td>
<td>1,4</td>
<td>4,00</td>
<td>1,1</td>
</tr>
<tr>
<td>9</td>
<td>Glucose syrup from corn</td>
<td>2001</td>
<td>46</td>
<td>31,1</td>
<td>1,0</td>
<td>0,03</td>
<td>0,23</td>
</tr>
<tr>
<td>10</td>
<td>Glucose syrup from millet</td>
<td>2001</td>
<td>44</td>
<td>44,7</td>
<td>1,1</td>
<td>0,05</td>
<td>0,94</td>
</tr>
<tr>
<td>11</td>
<td>Glucose syrup from millet</td>
<td>2001</td>
<td>44</td>
<td>38,1</td>
<td>0,9</td>
<td>0,09</td>
<td>0,64</td>
</tr>
<tr>
<td>12</td>
<td>Glucose syrup from millet</td>
<td>2001</td>
<td>46</td>
<td>37,0</td>
<td>1,0</td>
<td>0,04</td>
<td>0,22</td>
</tr>
<tr>
<td>13</td>
<td>Glucose syrup from millet</td>
<td>2001</td>
<td>43</td>
<td>37,9</td>
<td>1,0</td>
<td>0,21</td>
<td>2,41</td>
</tr>
<tr>
<td>14</td>
<td>Solid glucose</td>
<td>2000</td>
<td>-</td>
<td>73,5</td>
<td>1,4</td>
<td>0,89</td>
<td>3,96</td>
</tr>
<tr>
<td>15</td>
<td>Solid glucose</td>
<td>2001</td>
<td>-</td>
<td>67,6</td>
<td>1,8</td>
<td>0,80</td>
<td>4,73</td>
</tr>
</tbody>
</table>

Table 2. The reduction of the starch (free) in comparison with the sulphate dioxide, when storing the glucose syrup.

<table>
<thead>
<tr>
<th>Sublayer</th>
<th>SO(_2) mg%</th>
<th>In the beginning</th>
<th>NH(_3) mg% - after one year of storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose syrup extracted from potatoes</td>
<td>2,7</td>
<td>1,6</td>
<td>0,6</td>
</tr>
<tr>
<td>Glucose syrup extracted from millet</td>
<td>14,2</td>
<td>1,0</td>
<td>0,9</td>
</tr>
</tbody>
</table>
Our proposal would be that, when establishing new quality norms for the glucose syrup, it is advisable to mention all indications regarding the color by maintaining the extinction coefficient and by indicating a “coloring” method for establishing its quality. Nevertheless, it is important to avoid as much as possible any kind of modifications in the color of the sugar contents (modifications which would inevitably take place when exposed to high temperatures).

4. Conclusions

- It was demonstrated that the lowest values in what concerns the starch content were to be found in the cereal starch syrups and not, as expected, in the glucose syrups extracted from potatoes.

- We usually resort to the sulphitation of the thick syrup in order to avoid not only the coloring of the concentration in final stage, but also to avoid the yellowing during storage of the finished syrup.

- The non-enzymatic reaction, which changes the color of the melanoidinic compounds into brown, depends on the temperature which is exposed to and consequently, the reaction takes place at high temperatures.

- The most intensive coloring was noticed to glucoses with the lowest degree of sulphitation, with the highest degree of conversion and with an intermediary content nitrogen.

- The lowest absolute coloring was noticed to millet glucoses exposed to a process of intensive sulphitation.

- The most pronounced medium coloring, was noticed in the composition of the glucose syrup extracted from corn; the composition contained a relatively high quantity of nitrogen and an medium one of sulphur dioxide.

References

Compare Enders C., too: Contributions to the melanoidinic compounds scientific research, Klloid Ztschr. 85 (1998), 74.

Kroner W. and Wegner H.: *Raw syrups purification when neutralizing those resulting from the conversion with muriatic acid*, Vorratspflege and Lebensmittelforsch, 6 (1993), 76.

Parow E.: *The research of the glucose syrup from corn starch*, Ztschr. Spirit Ind. 45 (1992), 229.

Pelshenke P., Ruggerberg and Lindmann E.: *Results obtained after researching some important starches*, Die Starke, 2(200) 153.


Tryller H.: *The acid and its determination from the potatoes flour and the glucose syrup extracted from potatoes*, Ztschr. Spirt. Ind. 48 (1995), 44.