Modelling of Starch Gelatinisation and Liquefaction with the Enzyme Liquozyme

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Abstract. In this paper the influence of the enzyme Liquozyme Supra concentration (0.4-1.2 kg/t dry substance DS) and gelatinisation time (3-11 min.) on the liquefied starch quality is investigated. The composition in sugars with different Degrees of Polymerisation (DP) as DP1 (glucose), DP2 (maltose), DP3 (maltotriose), DP4 (maltotetrose) and oligosaccharides with DP≥5 is analysed by using HPLC with RI detector. The products obtained have different DP, the main components being oligosaccharides with DP≥5, followed by small amount of DP3+DP4 and much smaller concentrations of DP1; no DP2 is produced by this enzyme. Two quality criteria: the percent of oligosaccharides with DP≥5 and the percent of DP1 are proposed, in order to appreciate gelatinisation + liquefaction for the obtaining of various syrups in the next step of starch hydrolysis, the saccharification. For the production of maltose syrups, the optimal conditions at gelatinisation + liquefaction in the domains tested are 0.4 Kg/t DS enzyme and 11 min. gelatinisation time; no conditions are imposed for the production of glucose syrups; at the obtaining of dextrose syrups, gelatinisation and liquefaction are to be realised with maximal enzyme concentration (1.2 kg/t DS) and maximal gelatinisation time (11 min.).

Keywords: gelatinisation, liquefaction, degree of polymerisation DP, Liquozyme.

INTRODUCTION

Starch hydrolysates are obtained through starch gelatinisation followed by the enzymatic hydrolysis in two main steps called liquefaction and saccharification by using different enzymes and conditions, with the obtaining of products as maltose syrups, glucose syrups or dextrose syrups.

Starch is gelatinised by steam treatment at 105-110°C under pressure of the starch slurry (Van der Maarel et al., 2002). The efficiency of the gelatinisation process is appreciated by the denaturation degree of the starch granule measured through rheology (Mironescu et al., 2011).

Gelatinised starch is then liquefied; liquefaction refers to the process by which a quasi-solid product becomes to behave like a liquid and is realised with enzymes, this term being used for other foods, as dough, where different enzymes transform it in a less viscous product (Ognean et al., 2011). In the case of starch gels, α-amylases of different origins are used at liquefaction, the resulted products being classically characterised by the Dextrose Equivalent (DE) which represent the total number of reducing sugars; actually, the characterisation of the starch hydrolysates is based on the composition in sugars with different Degrees of Polymerisation (DP) as DP1 (glucose), DP2 (maltose), oligosaccharides with DP≥3 as maltotriose (DP3), maltotetrose (DP4) (Mironescu et al., 2008).
Gelatinisation and a primary liquefaction are often carried out simultaneously by adding the enzyme in the starch slurry before gelatinisation, followed by a secondary liquefaction with the enzyme already added at gelatinisation. The number of enzymes used at starch liquefaction increases constantly with the evolution of the microbial biotechnology, big producers as Novozymes or Danisco producing today new enzymes able to degrade starch in different conditions. In order to help the industry to choose the right enzyme for the obtaining of a hydrolysis product with defined characteristics, the authors developed a database and a decision support system, called ENZYSSYS (Mironescu, 2002). Previous works enlarged the database with enzymes used for liquefaction (Mironescu et al., 2008) (Mironescu et al., 2009) and saccharification (Mironescu et al., 2007). For example, experiments with Thermamyl 120 L as liquefaction enzyme were made, the results showing that in the liquefied starch higher concentrations of this enzyme and smaller liquefaction time increased the quantity of products with DP≤3, whereas smaller quantities of enzyme Thermamyl 120 L and higher time increase the proportion of products with DP>7 (Mironescu et al., 2008). Also, the composition in hydrolysis products with DP≥4 in the liquefied starch has a strong influence on the saccharification products; for the obtaining of maltose syrups the content of DP3 and DP4 must be very small (Mironescu et al., 2009).

The aim of this research is to analyse the liquefaction process at the use of the enzyme Liquozyme Supra. The research intend to establish the influence of the enzyme dose and gelatinisation time on the sugars composition of the liquefied starch and to determine the optimal conditions to obtain a product adequate for saccharification, in order to obtain maltose syrups, glucose syrups or dextrose syrups. The process quality is appreciated by measuring the DP using High-Pressure Liquid Chromatography (HPLC) with RI detection.

MATERIALS AND METHODS

Corn starch with the characteristics: Dry Substance (DS) = 13.5%; Protein content = 0.32% DS; Fat content = 0.68% DS; Ash = 0.86% DS was used.

Liquefaction. An industrial installation working on the low-temperature principle was used. The liquefaction was realised with the enzyme Liquozyme Supra from Novozymes. The liquefaction conditions were: Concentration of starch suspension 18°Beaume; Concentration of enzyme 0.4-1.2 kg/t DS starch; pH=5.5; Calcium ions 5 ppm.; Gelatinisation temperature (in jet cooker) 107°C; Gelatinisation + primary liquefaction time = 3-11 min.; Secondary liquefaction temperature 85°C; Liquefaction time 2 h. These conditions were chosen by respecting the recommended parameters for the low-temperature liquefaction process given by Novozymes (***)

The quality of the liquefaction process was differently assessed in time by determining sugars composition (and subsequently DP) using HPLC, as described in (Mironescu et al., 2007). Briefly, aqueous solutions with 5% starch hydrolysate were used; the injection volume was 80µl. A separation column type SP0810 (Shodex) with Pb counterions was used and the detection was by RI. The working temperature in the column was 96°C, and the temperature in the detector was 60°C. Water was used as eluent. The method used allows the qualitative and quantitative analysis of DP, with identification of compounds with DP1 (glucose), DP2 (maltose), DP3+DP4 (a peak corresponding to maltotriose+ maltotetrose) and DP≥5 (a peak corresponding to compounds with higher molecular mass).

Experiments were realised by using a rotatable experimental design (Tab. 1) with two variable process parameters: the quantity of enzyme and the gelatinisation time, identified
as being very important by other studies (Mironescu et al., 2008). The parameters variation limits are given in Tab. 2.

### Tab. 1

**Rotatable experimental design**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>(x_0)</th>
<th>(x_1)</th>
<th>(x_2)</th>
<th>(x_1x_2)</th>
<th>(x_1^2)</th>
<th>(x_2^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-1</td>
<td>+1</td>
<td>-1</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
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<td>1</td>
<td>+1</td>
<td>-1</td>
<td>-1</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
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<td>1</td>
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<td>+1</td>
<td>+1</td>
<td>+1</td>
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<td>0</td>
<td>2</td>
<td>0</td>
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<tr>
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<td>-1.41</td>
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<td>0</td>
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<td>0</td>
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<tr>
<td>7</td>
<td>1</td>
<td>0</td>
<td>+1.41</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
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<td>0</td>
<td>-1.41</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(x_1\)- gelatinisation time; \(x_2\)- enzyme dose

### Tab. 2

**Parameters variation limits**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>(x_1) (minutes)</td>
<td>-1.41  -1  0  +1  +1.41</td>
</tr>
<tr>
<td>(x_2) (kg/t DS)</td>
<td>3  4  7  10  11</td>
</tr>
</tbody>
</table>

### Tab. 3

**RESULTS AND DISCUSSION**

In Table 3 is presented the composition in sugars of the liquefied products, cumulated on four types of sugars.

**Sugar composition in the liquefied starch**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>DP(\geq 5)</th>
<th>DP3+DP4</th>
<th>DP2</th>
<th>DP1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83.83</td>
<td>15.161</td>
<td>0</td>
<td>1.000</td>
</tr>
<tr>
<td>2</td>
<td>96.143</td>
<td>3.079</td>
<td>0</td>
<td>0.746</td>
</tr>
<tr>
<td>3</td>
<td>84.324</td>
<td>15.048</td>
<td>0</td>
<td>0.619</td>
</tr>
<tr>
<td>4</td>
<td>95.544</td>
<td>3.876</td>
<td>0</td>
<td>0.580</td>
</tr>
<tr>
<td>5</td>
<td>93.886</td>
<td>5.382</td>
<td>0</td>
<td>0.813</td>
</tr>
<tr>
<td>6</td>
<td>95.985</td>
<td>3.579</td>
<td>0</td>
<td>0.413</td>
</tr>
<tr>
<td>7</td>
<td>83.653</td>
<td>14.966</td>
<td>0</td>
<td>1.381</td>
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<tr>
<td>8</td>
<td>91.513</td>
<td>8.147</td>
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<td>0.340</td>
</tr>
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<td>9</td>
<td>90.601</td>
<td>8.662</td>
<td>0</td>
<td>0.737</td>
</tr>
<tr>
<td>10</td>
<td>89.572</td>
<td>9.697</td>
<td>0</td>
<td>0.697</td>
</tr>
<tr>
<td>11</td>
<td>88.891</td>
<td>10.327</td>
<td>0</td>
<td>0.765</td>
</tr>
<tr>
<td>12</td>
<td>88.563</td>
<td>10.704</td>
<td>0</td>
<td>0.712</td>
</tr>
<tr>
<td>13</td>
<td>90.341</td>
<td>8.876</td>
<td>0</td>
<td>0.774</td>
</tr>
</tbody>
</table>
As expected, the main hydrolysis products are the oligosaccharides with higher molecular mass (DP ≥ 5); the enzyme allows the obtaining of a small quantity of maltotetraoses and maltotrioses. No maltose (DP2) is produced and the concentration of glucose is very small.

Comparing this enzyme with another enzyme usually used at liquefaction, Termamyl 120L (which was also investigated by the authors in (Mironescu et al., 2009)), Liquozyme Supra is more suitable for the obtaining of maltose syrups, because gives much smaller percent of maltotriose, maltotetrose and glucose at liquefaction.

Based on the experimental design presented in Table 1, evolution models for two groups of sugars (with DP ≥ 5 and DP1) are made. The models for the hydrolytic process were considered as polynomial functions of second degree. The functions obtained after data processing and application of the coefficient signification test are:

\[
y_1 = 89.73 - 1.52 x_2 - 5.29 x_1 \cdot x_2 + 2.04 x_1^2 - 1.26 x_2^2
\]

\[
y_2 = 0.74 + 0.14 x_1 + 0.21 x_2 + 0.06 x_1 \cdot x_2 - 0.055 x_1^2 + 0.055 x_2^2
\]

where: \(y_1\) – concentration of DP ≥ 5, %; \(y_2\) – glucose concentration, %.

The analysis of the two functions shows that beside the two analysed parameters, the process is considerable influenced by other factors, too, which appear in the free term. In both cases, the quantity of enzyme has a high influence on the formation of the two types of products, but its action is negative on the obtaining of DP ≥ 5 and positive on the obtaining of glucose. Gelatinisation time alone has no influence on the products DP ≥ 5 but has a positive influence on the obtaining of DP1. The parameters combined action influences positively the formation of DP ≥ 5 and negatively the formation of DP1.

The two obtained functions are presented in Fig. 1 and 2 in the analysed interval and the parameters for which the functions are minimised or maximised are assessed.

Fig. 1. Variation of dextrin’s content with enzyme concentration and gelatinisation time

The optimal conditions for the process command are found at the intersection of the zones where the functions are minimal. The analysis of the graphical representation of function 1 (Fig. 1) allow to establish that in the analysed interval the function has a minimum placed between two extremes, one for small enzyme concentration and small gelatinisation time and the other for high treatment time and high enzyme concentration. Minimal values
for the oligosaccharides with higher polymerisation degree are obtained in a large interval of
time and enzyme concentration, but which have to be correlated.

Depending on the syrups intended to be obtained at saccharification, the conditions at
gelatinisation + liquefaction are to be established. For the obtaining of maltose syrups, the
quantity of oligosaccharides with DP≥5 has to be high (Mironescu et al., 2009); so, in this
case, the optimal conditions at the work with Liquozyme Supra are: gelatinisation time is 11
min. or even longer and the concentration of enzyme is minimal (0.4 Kg/t DS for the
conditions tested) or very short gelatinisation time (100 seconds) and a high concentration of
enzyme (1.3 kg/t DS).

No special conditions are imposed at gelatinisation and liquefaction for the obtaining
of glucose syrups, because the enzyme used in saccharification is able to cut very well the
oligosaccharides with DP≥5 but also the products with DP≤5.

For the obtaining of dextrose syrups, the saccharification get faster if the number of
oligosaccharides with DP≥5 is smaller, meaning short gelatinisation time and small quantity
of enzyme or long gelatinisation time and high quantity of enzyme. Taking into account that
with the increase of the gelatinisation time the number of DP3+DP4 should increase (and so
the saccharification becomes easier), the conditions for the obtaining of dextrose syrup are:
long gelatinisation time (11 min. or longer) and high quantity of enzyme (1.3 Kg/t DS).

Fig. 2 presents the function describing the relation between enzyme concentration,
gelatinisation time and the quantity of glucose in the liquefied product.

A minimum can be obtained by working with 0.5-0.8 kg enzyme /t DS and
gelatinisation time of 100 seconds, conditions imposed for the obtaining of maltose syrups.
This condition is not well correlated with the other results regarding the content in DP≥5. An
intermediate solution would be to work with 0.4 Kg/t DS enzyme for 740 seconds
gelatinisation time. The maximal glucose content is obtained for long gelatinisation time (620
to 700 seconds) and high enzyme concentration (1.2-1.3 Kg/t DS), conditions which are
adequate for the obtaining of glucose syrups.
CONCLUSIONS

Gelatinisation followed by liquefaction are very important at the starch-based syrups obtaining. At the use of the enzyme Liquozyme Supra, hydrolysis products with different DP are obtained, the main components being oligosaccharides with DP≥5, followed by small amount of DP3+DP4 and traces of DP1. No maltose is produced.

In this work we propose two quality criteria: percent of DP≥5 and percent of DP1 in order to appreciate gelatinisation + liquefaction for:

- The obtaining of a liquefaction product with high content of oligosaccharides with DP higher as 5 and reduced content of glucose, suitable for the production of maltose syrup. The optimal conditions are: 0.4 Kg/t DS enzyme Liquozyme Supra and more than 11 min. gelatinisation time.
- The obtaining of a liquefaction product with reduced content of oligosaccharides with DP higher as 5 and higher content of glucose, suitable for the production of dextrose syrup. The optimal conditions are: 1.2 Kg/t DS enzyme and 11 min. or longer gelatinisation time.

Acknowledgments. This work was co-financed from the European Social Fund through Sectoral Operational Programme Human Resources Development 2007-2013, project number POSDRU/89/1.5/S/63258 “Postdoctoral school for zootechnical biodiversity and food biotechnology based on the eco-economy and the bio-economy required by eco-san-genesis”.

REFERENCES