THE CONSISTOGRAPHIC DETERMINATION OF ENZYME ACTIVITY OF PROTEASE ON THE WAFFLE

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ABSTRACT
The purpose of this research is to analyse the action of protease, an exogenous enzyme used in different dosages on the waffle dough. Proteases hydrolyze the peptide bond which are found in the protein molecules, preferable at the level of positive aminoacids, which explains their reduced action on the wheat flour proteins where the basic aminoacids are in low proportion. In dough, this leads to weakening of the gluten chains. To avoid weakening the gluten structure, which result in an uneven texture of the dough structure, protease has to be added to the dough in the mixing phase. In these conditions, it will not reduce mixing time because the enzyme has not enough time to hydrolyze more gluten. The waffle doughs contain small amounts of water and fat, which makes them dense and therefore difficult to laminate layers. The addition of proteolytic enzymes allows adjustment of the rheological characteristics of dough according to the needs of the technological process. The consistograph method used in this study shows the rheological characteristics of the waffle dough presenting the effects caused by protease in: a low dosage, a correct dose and an overdose. The dough sample that has a low dose of protease does not have a significant improvement on the quality of the dough. On the other hand to much protease influences the dough handling during the technological process due to a wet and sticky content of the dough which leads to a low porosity, high humidity and a higher water activity. Using the correct dose of protease has positive effects on freshness, on the porosity and color of the crust.

KEY WORDS: waffles, protease, consistographic method

INTRODUCTION
In the technological process of waffles, protease is used for processing strong gluten flours with high resistance and elasticity and low extensibility. Dough elasticity is improved when using protease in a correct dose and it is reduced at higher doses.

In comparison with chemical reducing agent such as sulfite, the use of proteolytic enzymes results in shorter polypeptide formation from the gluten, without affecting the concentration of free groups - SH or -SS- groups. The sulfite reacts rapidly in the dough and every molecule reacts only once, but the enzymatic activity of protease is slow and continuously until it is destroyed in the oven. The quantity of the
The consistographic determination of enzyme activity of protease on the waffle
dough which has been weakened by the enzyme is depending not only on the quantity of the protease but also on the time allowed for the action of the protease on the dough. Proteases have the ability to catalyze the breaking of CO-NH peptide bonds from protein molecules, and their degradation products (Mencinicopschi et al., 2008).
The role of proteases is not only to reduce dough consistency and elasticity but also to influence the texture of the waffle sheets. Addition of proteases improves the dough handling and enhances volume and texture of the waffles.

MATERIAL AND METHOD
Samples preparation
The research has been conducted on dough samples which contain wheat flour 650, salt, water, yeast and protease. The first dough sample $W_0$ does not contain any protease – this sample is considered the blank sample. The second dough sample $W_1$ contains 5g/100kg protease, the third dough sample $W_2$ contains 10g/100kg protease and the fourth dough sample $W_3$ contains 25g/100kg protease.
The protease used is: ALPHAMALT PRO – commercial enzyme preparation which contains protease (Muhlenchemie, Germany).
The consistograms have been determined with the Alveo-Consistograph and the results for the sample have been recorded by Alvoelink. The following tests were performed: the constant hydration test (CH) and adapted hydration test (AH).
In order to make the adapted hydration it is necessary to perform the constant hydration first. From each sample is weighted 250 g flour and it is determined the moisture with the analytical balance. A 2.5% NaCl solution is prepared and placed into the burette of the consistograph. After 1 minute of the mixing phase the device is stopped and cleaned on the side walls of the kneading space, being careful not to touch the sensor and not longer than 2 minutes for cleaning. Then the mixing phase is restarted and after 250 seconds of mixing the consistograph indicates the quantity of sample that will take part in the next test and the salt solution required to determine the adapted hydration. The adapted hydration test starts by weighing the suitable quantities of flour and the required amount of water indicated, then the device is started again according to the first test and after 480 seconds the consistograph stops.

Methods of analysis
The consistency of dough, the water absorption capacity of flour and the changes in the process of kneading dough have been determined by the consistographic method. The following indicators were obtained from the consistogram: • flour moisture ($H_2O$); • maximum pressure ($Pr\ MAX$); • hydration potential of the flour (HYDRA); • time to reach the maximum pressure ($Pr\ T\ MAX$); • tolerance or dough stability (TOL); • pressure drop compared to $Pr\ Max$ after 250 seconds or the degree of softening of the dough after 250 seconds ($D\ 250$); • pressure drop compared to $Pr\ Max$ after 450 seconds or the degree of softening of the dough...
RESULTS

The consistogrames of the dough samples \(W_0\) (no protease), \(W_1\) (protease 5g/100kg), \(W_2\) (protease 10g/100kg) and \(W_3\) (protease 25g/100kg) are represented in Fig. 1, Fig. 2, Fig. 3 and Fig. 4.

Based on the time to reach maximum pressure (\(T_{Pr\ MAX}\)) and dough tolerance (\(TOL\)) the flours can be classified as:

- Weak flour: \(T_{Pr\ MAX}\) between 1-3 min, \(TOL\) between 1 - 4 min;
- Medium flour: \(T_{Pr\ MAX}\) between 3-8 min, \(TOL\) between 4 - 5 min;
- Strong flour: \(T_{Pr\ MAX}\) between 8-15 min and \(TOL\) between 10-15 min.[1]

The consistogram from Fig. 1 represented the blank sample \(W_0\) (no protease).

It can be seen that the time at which the maximum pressure is reached (\(TPR_{MAX}\)) is 167s and the tolerance of the dough (\(TOL_{W_0}\)) is 289s. The pressure drop compared to \(Pr_{MAX}\) after 250s and after 450s is not so high (181 or 503 mb) and water absorption capacity is 54.5%. These indicators are corresponding to the optimal conditions for medium flour, which can be used more for bread making and not for waffle production. The good enzymatic activity from the dough influences the waffle sheets making them thicker, with low porosity and hard structured. Also the baking time increased due to the high viscosity which creates as well a very hard handling process of the dough.

The consistogram of the Sample \(W_1\) (protease 5g/100kg) is shown in Fig. 2 and what we can notice is that there is a decrease in the following indicators: the time
at which the maximum pressure is reached has decreased with 25s, the stability of the dough has also been reduced with 24s and water absorption capacity has decrease with1.5%. If we look on the degree of softening at 250 and 450 seconds we can see an increase to 238 and to 622 mb. These indicators show a small improvement of the dough characteristics, but they correspond to the conditions for a medium flour and the dough sample cannot be used for waffle production.

The consistogramme from Fig. 3 shows the results of the Sample W₂ (protease 10g/100kg) and the consistogramme from Fig. 4 represents the Sample W₃ (protease 25g/100kg). These two samples show the characteristics of weak flours due to low enzymatic activity on the gluten chain. This effect is caused by the protease addition which has decreased the viscosity and the water activity. If we compare these samples we notice that Sample W₂ has the best characteristics for waffle production suggesting that the 10g/100kg protease is the proper dosage which creates a more porous waffle sheet obtained in a shorter backing time. The dose used in Sample W₃ caused a much higher reduction of $\text{TPRMAX}_W$ and the $\text{TOL}_W$ that resulted in wet and sticky content which created a final product with an abnormal porosity.

In Table 2. there are presented the characteristics of dough samples obtained by consistographic method.

The proper rheological characteristics for waffle production in comparison with the blank sample W₀ are shown in dough sample W₂(protease 10g/100kg) more exactly in the time for complete hydration (TPRMAX) which decreased with 41s, the tolerance of the dough (TOLW) that shows a decrease of 71s, D250W₂ has increased with 187mb and D450W₂ also increased with 175mb. If we compare the results from sample W₂(protease 10g/100kg) with the other two samples: W₁ (protease
5g/100kg) and W₃ (protease 25g/100kg), we see a decrease in the time for complete hydration (TPRMAXₓ) and the tolerance of the dough (TOLₓ) at a level that corresponds to the condition for waffle production. These conditions are not achieved nor by W₁ (protease 5g/100kg) due to low dosage of protease that does not have a significant improvement on the quality of the dough or by sample W₃ (protease 25g/100kg) that has an overdose of protease creating a much thinner and sticky layer in the oven very hard to handle in the manufacturing process. Adjusting the dosage of protease in accordance with the characteristics of the flour used for waffle production creates a dough which becomes thin and elastic, with a good stability and an uniform pore structure, improving also freshness and the texture of waffle.

Sample W₂ (protease 10g/100kg)
Results
H₂Oₓ = 14.10%
HYDHAₓ = 51.8% b 15%H₂O
PRMAXₓ = 2010 mb
TPRMAXₓ = 126 s
TOLₓ = 218 s
D₂₅₀ₓ = 368 mb
D₄₅₀ₓ = 678 mb
WACₓ = 52.9 % b 15%H₂O

Sample W₃ (protease 25g/100kg)
Results
H₂Oₓ = 14.10%
HYDHAₓ = 52.5% b 15%H₂O
PRMAXₓ = 2125 mb
TPRMAXₓ = 98 s
TOLₓ = 272 s
D₂₅₀ₓ = 368 mb
D₄₅₀ₓ = 685 mb
WACₓ = 53.8 % b 15%H₂O

FIG. 7. The consistogramme for sample W₂ (protease 10g/100kg)

FIG. 8. The consistogramme for sample W₃ (protease 25g/100kg)
TABLE 2. Consitograph results of the dough samples: W₀ (no protease), W₁ (protease 5g/100kg), W₂ (protease 10g/100kg), W₃ (protease 25g/100kg)

<table>
<thead>
<tr>
<th>Sample</th>
<th>W₀ (no protease)</th>
<th>W₁ (protease 5g/100kg)</th>
<th>W₂ (protease 10g/100kg)</th>
<th>W₃ (protease 25g/100kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O(%)</td>
<td>14.10</td>
<td>14.10</td>
<td>14.10</td>
<td>14.10</td>
</tr>
<tr>
<td>HYDHA(%b 15% H₂O)</td>
<td>51.3</td>
<td>52.2</td>
<td>51.8</td>
<td>52.5</td>
</tr>
<tr>
<td>PRMAX(mb)</td>
<td>1902</td>
<td>2099</td>
<td>2010</td>
<td>2125</td>
</tr>
<tr>
<td>TPRMAX(s)</td>
<td>167</td>
<td>142</td>
<td>126</td>
<td>98</td>
</tr>
<tr>
<td>TOL(s)</td>
<td>289</td>
<td>265</td>
<td>218</td>
<td>272</td>
</tr>
<tr>
<td>D250(mb)</td>
<td>181</td>
<td>238</td>
<td>368</td>
<td>261</td>
</tr>
<tr>
<td>D450(mb)</td>
<td>503</td>
<td>622</td>
<td>678</td>
<td>685</td>
</tr>
<tr>
<td>WAC(%)</td>
<td>54.5</td>
<td>53.0</td>
<td>52.9</td>
<td>53.8</td>
</tr>
</tbody>
</table>

CONCLUSIONS

The results of the consistographic tests show improvements on the texture, color, flavor, water activity and stability of freshness of the waffle products if it is added at the correct dosage of protease in waffle dough. The structure of the waffle is presented with an increased porosity of the waffle sheet and a developed filigree structure. The low viscosity of the dough has positive effects on the baking process which is done uniformly and in a shorter time. The low moisture and a low water activity in the waffle sheet leads to a longer freshness time reducing the risk of developing microorganism especially fungi, mycotoxins and even Salmonella. The color and flavor of the waffle sheet are improved showing a uniform texture of waffle with a softer core, fine and pore structure. Research conducted with consistograph method at optimal concentrations of proteases show that the enzyme improves the plastics proprieties of the dough, which makes the dough easier to handle during the technological process.

REFERENCES