FARINOGRAPHIC EFFECTS OF SEVERAL COMMERCIAL XYLANASES ON LOW EXTRACTION WHEAT FLOUR

— research paper —

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Abstract: The xylanases changes the viscosity and the pentosans content of flour extracts and also the rheology of dough. The changes of dough rheology prepared with low extraction flours are well correlated with the changes of arabinoxylans content in flour extracts. The farinographic characteristics of dough were determined after a resting period of 40 minute. The correlations were weaker and fewer for the dough prepared with resting but still significant.

Keywords: xylanase, dough rheology, soluble arabinoxylans, insoluble arabinoxylans

INTRODUCTION

In wheat flour, arabinoxylans (AX) represent only a small fraction expressed as flour component, but they play an important role during breadmaking, even it is not well understood yet. The total AX and water extractable arabinoxylans (WEAX) content in flour is 1.37 ÷ 2.06 and 0.54 ÷ 0.68 according to Izydorsczyk et al. (Izydorcszyk et al., 2007) and 1.66 ÷ 1.86 and respective 0.7 ÷0.83 according to Hashimoto (Hashimoto et al., 1987).

The most important property of all AX, no matter they are soluble or insoluble, is their great capacity to bind water. One gram of dry AX can bind

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15 grams of water (Bushuk, 1966). Despite AX represent 2 ÷ 3% from flour weight, another research (Autio, 2006) shows that they are responsible for binding 25% from the water in dough. Some researchers (Linko et al., 1997) are more reserved and estimate the amount of water bounded to be 6.5 times greater than their weight. The quantity of water bounded by AX is great and the way haw is managed these water could have a great impact on breadmaking.

WEAX have a great capacity to increase viscosity of solutions (Udy, 1956), (Izydorcszyk et al., 1992) (Vinkx et al., 1996), (Izydorcszyk et al., 1998) (Autio, 2006) and to decrease surface tension. These properties have important implication in breadmaking and its stability is a key factor in baking. According to Gan et al. (Gan et al., 1995) the fluid fraction of dough plays an important role in gas cell sealing. If the viscosity of aqueous phase of dough has high viscosity, it could seal the pore of gas cell during expansion and do not allow the gas to escape and the gas cell to collapse. This hypothesis is sustained by the fact of that WEAX have a positive effect on loaf volume, while the water unextractable arabinoxylan (WUAX) have a negative effect and stimulate coalescence by disrupting the cell wall film.

Xylanases are enzymes able to hydrolyze AX; depending on the bound spitted, they can be endo- or exoxylanases and hydrolyse the xylan backbone of AX or can be arabinofuranosidase or ferulic acid esterase. In breadmaking β-xylosidase is used, which is able to split the β-1→4 linkage between two xylose units from AX central backbone. The high molecular AX are converted in smaller AX which have lower capacity to increase viscosity. If xylanases have a greater specificity to WEAX, these lead to lower viscosity of dough liquid phase and its softening. If xylanases have a great specificity for WUAX, they are converted to WEAX and the viscosity of dough is increased. In this way the WUAX with bad effects in baking are converted to WEAX with good effects.

The xylanase activity in untreated and normal wheat flour is very small (Popper et al., 2006); it is assumed 15 to 20% of AX is hydrolyzed by xylanases and this activity is responsible for variability of water loosed in dough for different wheat varieties. Based to their specificity, the exogenous xylanases have different effects in dough and can be used to manipulate the rheological properties of dough according with necessities imposed by technology or final product characteristics.

In this study we are looking to find a correlation between the capacities of different xylanases to modify the flour extracts viscosity and to increase their AX content. Also, the dough's farinographic changes are analysed.

MATERIALS AND METHODS

Materials

Three commercial enzymatic preparation of xylanase was used for this study:
- Depol 333P from Biocatalysts Ltd. UK, with 265.8 IRV/g (Inverse Reciprocal Viscosity);
- Veron 393 provided by AB Enzymes GmbH, with 3.7 IRV/g;
- Xila L from Belpan, with 13.8 IRV/g endo-xylanase

at a pH 5.5.

The flour, unsupplemented, was purchased from a local mill (Cibin Mill, from Sibiu) and had 13.1% moisture, 29.6% wet gluten and 0.41% ash d.b.

Methods

The xylanase activity was determined by a viscometric method proposed by Megazyme with soluble wheat xylan as substrate, at pH 5.5. For measuring the liquefying capacity of xylanases, 5 g of flour was vigorously mixed with 25 ml of water and an amount of xylanase to reach 25 IRV/100 kg of flour was added. The mix was kept at 30ºC with constant stirring in a water bath and after that was centrifuged for 10 minutes at 1000xg. The viscosity of supernatant was determined with an Ubbelohde type glass viscometer. To measure the capacity to solubilize WUAX was determined the amount of AX from supernatant by orcinol method described by Hashimoto et al. (Hashimoto et al., 1987) and modified by Delcour et al. (Courtin and Delcour, 1998). Previously, 1 ml of extract was diluted with 14 ml of water and 1 ml of this dilution was analyzed. The results were compared with the control sample, prepared in the same way, but without xylanase.

The rheological characteristics of dough with and without xylanases were determined by AACC Method 54-21 (AACC Approved Methods, 1995). Because the time of action for xylanases during kneading is very short the time of action was increased according to Bordei et al. (Bordei et al., 2007). After the dough reach the peak the mixer was stopped for 40 minutes and after that the test resumed. Development time represent the time from the beginning of test at the moment to reach the second peak (after resting period) and 40 minutes was subtracted. In the same way, the dough stability and time to break down were determined. The dough elasticity was estimated as the farinographic curve width at the maximum peak and at 5 minutes after that (Bordei at all, 2007) (Weipert, 2006). The degree of softening was measured at 12 minutes after the second peak.

RESULT AND DISCUSSION

The farinographic characteristic of dough with and without xylanase was determined and the results are presented in Table 1. The xylanase was added to achieve 25 IRV xylanase activity /100 kg of flour. The dough consistency was changed very little at enzymatic preparations addition. The changes are in relation with enzymes capacity to modify aqueous extracts of flour. Veron 393 preparation decrease the extracts viscosity and also the dough consistency while Xila L had the highest capacity to increase the consistency and also the flour extract's viscosity.
The Depol 333 P and Xila L preparation decreased dough development time, while Veron 393 preparation increased it. Maybe the preparation modified AX from flour and some water was liberated into the dough so the consistency decrease and time to reach equilibrium between dough constituents increase. This additional time to reach equilibrium increase the mechanical degradation of dough by kneading and the rheological characteristics of dough was worsened.

Table 1. Farinographic characteristics of doughs with added xylanases

Enzyme preparation / **Dough characteristics**	**Control**	**Depol 333**	**Veron 393**	**Xila L**
Consitency, F.U.	492	490	483	498
Absorption, %	57.8	57.8	57.8	57.8
Dough development time, min	2.4	1.9	2.7	2
Stability, min	10	9.2	8.9	9.3
Tolerance index., F.U.	13	12	17	13
Time to breakdown, min.	10.1	10	10	9.8
Drop-off (AACC), F.U.	27	31	29	31
Dough softening (ICC), F.U.	47	51	55	52
Dough elasticity at peak, F.U.	81	98	85	84
Dough elasticity at 5 minutes after peak, F.U.	85	85	78	82

The dough stability of dough was decreased by xylanases addition, the highest effect was observed at dough prepared with Veron 393. Surprising, only Veron 393 preparation increased MTI (mixing tolerance index), from 13 F.U. to 17 F.U. and the others preparation have o or even a small improved effect (Depol 333 P).
The dough prepared with xylanase became less consistent in time and the highest softening effect was observed at dough with Veron 393 added. At the

maximum consistency, the dough elasticity expressed at curve's width, was increased by xylanases, but at 5 minutes the elasticity of dough decreased and Veron 393 addition leads to dough with elasticity inferior than control.
The dough changes were small because a short time to action for enzyme but it can be observed differences in haw different xylanases act on dough. To put in evidence the effect of xylanases the dough kneading was stopped after it reach the maxim of consistency for 40 minutes and after the kneading was resumed and the farinograme recorded. The results are presented in Table 2. For preparation Depol 333 P was prepared a dough with a xylanase activity about 200 IRV/100 kg of flour.

Table 2. Farinographic characteristics of doughs with added xylanases when the kneading was interrupted

Enzyme preparation / Dough characteristics	Con-trol	Depol 333 P	Veron 393	Xila L	Depol 333 P 200 IRV
Consitency, F.U.	507	526	508	526	531
Absorption, %	57.8	57.8	57.8	57.8	57.8
Dough development time, min	2.2	2.2	1.7	2.2	2.2
Stability, min	1	0.8	0.4	1.5	1
Tolerance index., F.U.	84	91	84	85	95
Time to breakdown, min.	42.3	42.2	44.1	42.2	42.3
Dough softening (ICC), F.U.	108	117	106	110	118
Dough elasticity at peak, F.U.	112	112	97	109	106
Dough elasticity at 5 minutes after peak, F.U.	43	51	42	49	48

The dough consistency increased after 40 minutes of resting in the case of dough with Depol 333 P and Xila L while the consistency of dough with Veron 393 preparation was almost the same as the control. The addition of Veron 393 decreased the development time while the others xylanase did not changed this. Dough stability decreased had a great variability with preparation used. The preparation Veron 393 had the most decreasing effects while the Xila L preparations increased, i.e. improved the dough stability.
The farinograme width at 5 minutes after peak was increased just for the dough with Depol 333 P and Xila L preparations.
From these data we can presume that the Veron 393 have a great specificity on WEAX ant by the hydrolysis of them the viscosity of aqueous phase from dough decreased so the consistency and stability of dough decreased. The

preparations Depol 333 P and Xila L have a great specificity for WUAX and convert them in WEAX which lead to a great stability and consistency. When the xylanase activity was increased from 25 IRV/100 kg of flour to 200 IRV/100 kg of flour the rheology of dough was increased and that sustain this hypothesis.

Some correlations between the capacities of xylanases to modify the viscosity or xylan content of flour's aqueous extracts and the variation of rheological characteristics of dough could be observed. The variations were determined by reporting the differences between value of sample and value of control to the control value. The data was analyzed and some correlations were observed between farinographic variations of rheological characteristics of dough and mostly with variation of AX content in extracts. In the case of analyzed flour the capacity of xylanases to convert WUAX in WEAX had a great impact on rheological characteristics than the capacity to modify WEAX. The data are presented in Figure 1 and Figure 2.

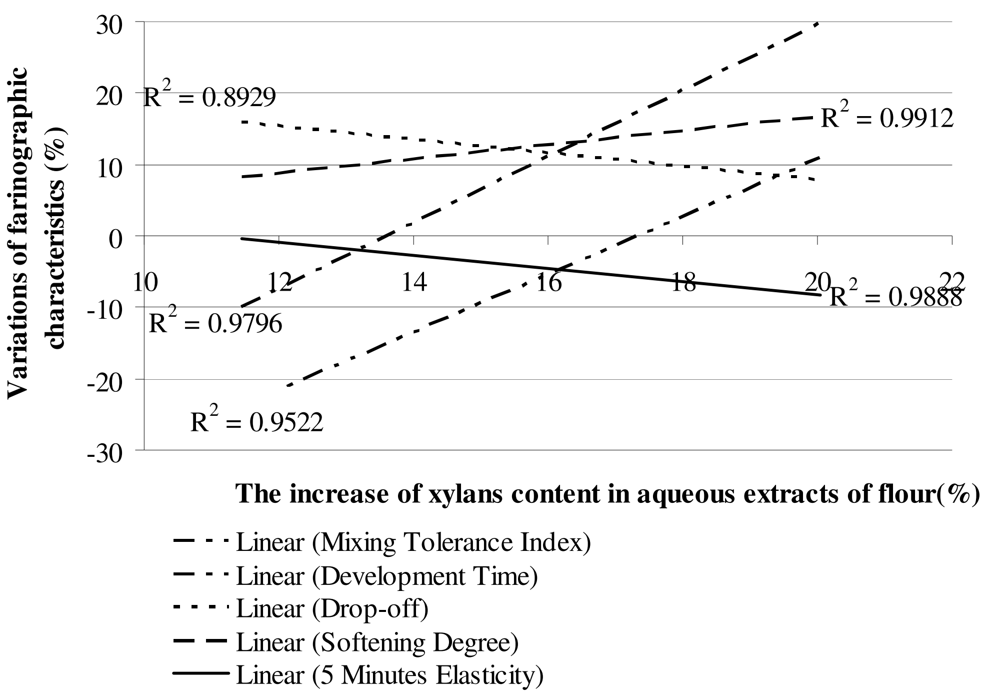

Figure 1. The correlation between the variation of rheological characteristic of dough and the variation of xylans content in extract

When doughs were prepared without interruption of kneading the variations of dough characteristics have been correlated well ($R^2 > 0.9$) with the variation of pentosans content in flour extracts. The increasing content of AX in extracts could be correlated with conversion oh WUAX in WEAX. The capacity to modify viscosity did not correlate well with variation of dough characteristics which mean the viscosity of liquid fraction from dough have a smaller influence on dough rheology than the interaction between fragments of cell wall and gluten. The xylanase modify the WUAX which are located in cell wall of wheat seeds and so are modified the characteristics of these.
Just the drop-off of dough was improved when the AX was solubilized. All other characteristics (Elasticity, Mixing Tolerance Index, Degree of Softening) were depreciated when a large quantity of AX was solubilized.
A good correlation ($R^2 = 0.9786$) with viscosity variations was observed for the time to break down. Time to break down increases when the viscosity of extracts increases (data not show). This confirms the important role of fluid fraction on dough rheology and the stabilization of dough by increasing the viscosity of this.
The correlation was weaker in the cases of doughs prepared with interruption but some of these are still significant ($R^2 > 0.7000$), the data are presented in Figure 2.

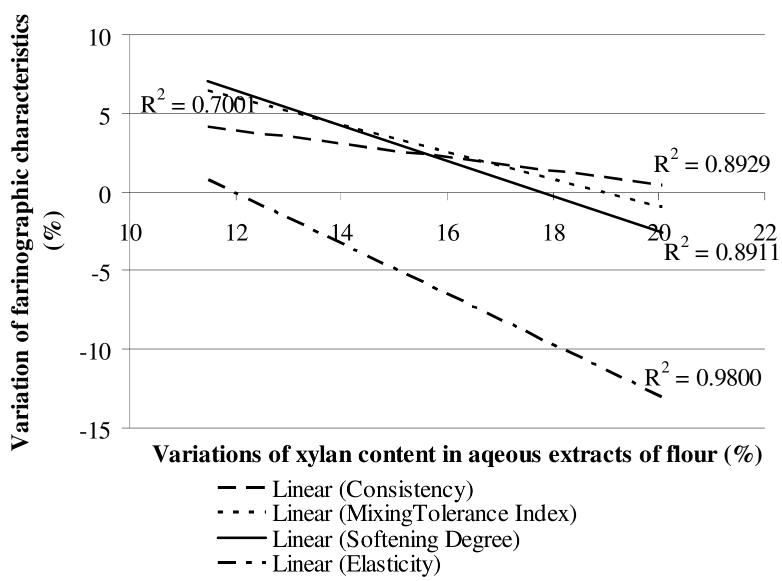

Figure 2. The correlation between the variation of rheological characteristic of dough and the variation of xylans content in extract for the dough prepared with an interruption

The consistency of doughs with xylanases was higher than the control at low quantity of AX solubilized and decreased when the content of AX in extracts increased but the consistency remain higher than control. The tolerance at mixing was greatly affected by xylanase addition but at high level of WUAX converted the mixing tolerance was improved. The softening of dough was higher at lower solubilisation rate but at high solubilisation rate the dough became harder than the control after 12 minutes of mixing.

CONCLUSIONS

Some correlation could be established between the changes of farinographic characteristics of dough and the changes produced by xylanase in aqueous extracts of flour. For the low extraction flour used in this experiment the changes of farinographic characteristics are well correlated with the changes of AX content in flour extracts. These correlations are weaker in the cases of doughs prepared with a 40 minutes interruption of kneading.

The conversion of WUAX in WEAX seems to be much important for the dough rheology than the changes of viscosity. This conversion could be assimilated also with the cell wall modification.

The correlation of dough rheology changes with changes of pentosans content of extracts could be used as a tool for research to predict the effects of different xylanases or to determine the amount of xylanases needed for bread formulation. Some reserves must be taken because to obtain different levels of AX solubilisation and viscosity changes we used different xylanases and the number of experiments was reduced. This hypothesis must be verified by further experiments in which different flours must be used and different levels of xylanases too.

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