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Rheological Effects of Some Xylanase on Doughs from High and Low Extraction Flours

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Abstract

The xylanases are widely used in breadmaking with positive effects on bread quality but how they act in doughs is not fully understand yet. The aim of this study is to determine how different xylanolytic preparations modify the rheology of dough prepared from low and high extraction flours and the correlation between the rheological changes induced in dough and the viscosity and xylan content of flour extracts. Four flours, two white and two black, and three xylanolytic preparation was used in study. The rheological characteristics of dough were measured with the Extensograph. The xylan content and viscosity of flour extracts with xylanase were determined. In doughs from white flours xylanases increased the energy, maximum resistance and extensibility while in doughs from black flours decreased the energy and maximum resistance and increased the extensibility. The extensographic effects of xylanases were compared with their capacity to modify the viscosity and xylan content of aqueous flours extracts. The changes of extensographic indicators are well correlated with the changes of xylan content of extracts for white flour while for black flour correlations were observed with the changes of extracts viscosity. The capacity of xylanases to modify the viscosity of extract and convert the insoluble xylans in soluble xylans could be used to predict the performance of xylanases.

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Keywords: flour; arabinoxylans; xylanases; rheology

1. Introduction

Arabinoxylans (AX), formerly named hemicelluloses or pentosans, are polysaccharides widely spread in cell wall of plant and are mainly composed of a backbone formed by molecule of D-xylopiranose linked β(1→4) substituted with units of arabinofuranose [1]. Some arabinose units are esterified with ferulic acid. In wheat kernel AX occur in cell wall of cells from starchy endosperm but also in cells from

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aleuronic layer or outer layer of seed. AX are minor components of wheat flour, they represent 1.37% to 2.06% [2] or 1.66% to 1.86% [3]. The AX content of white flours is lower than black flours because the participation of outer layer of kernel to flours, the AX content of bran is 19.38% [2] while Maes and Delcour report AX content in commercial bran about 20-25% [4].

The AX from wheat flour are characterized by their extractability (solubility) in water as water extractable arabinoxylans (WEAX) or water unextractable arabinoxylans (WUAX). Despite AX are just a minor component of wheat flours they play an important role during breadmaking. The most important function of AX, WEAX or WUAX, is their capacity to bind large amount of water in dough. One gram of AX could bind 15g of water according to Bushuk [5], 10g according to Autio [6] or just 6.5g of water according to Linko et al [7]. The water bounded in dough has a great impact in dough rheology, especially if it is possible to mobilize it through xylanase hydrolysis. The WEAX have positive effects because the WEAX form solutions with a high viscosity [6-10]. The aqueous phase from dough is a viscous solution which stabilizes the dough porosity by sealing the gas cells [11] and the gas retention is improved and loaf specific volume increased. According to the same theory the cell wall fragment disrupt the gas cell walls and stimulate the coalescence of pores and gas release. The WUAX present in cell wall fragment reduce the gas retention in dough and specific volume of breads.

The conversion of WUAX with negative effects in WEAX with positive effects it is supposed to have positive effects on breads characteristics. The conversions could be realised with enzymes which are very efficient tools in food industry due their specificity of substrates and reactions. On wheat AX could act several enzymes, endo-β-(1,4)-D-xylanases, β-xylosidases, α-L-arabinofuranosidases, ferulic acid esterases [13]. From these only endo-β-(1,4)-D-xylanases (usually named xylanases) are largely used in breadmaking due their capacity to split the xylan backbone of AX with formation of two molecules with different properties. If the hydrolysed AX are WUAX the effects it is presumed to be positive by their conversion and if WEAX are hydrolysed the effects will be negative because the AX obtained have negative or no effects [14, 15]. Different xylanases have different effects on AX in term of substrate specificity and product of hydrolysis with different effects on breadmaking [16]. The technological effects of xylanases depend on their specificity for WUAX and the ability to form AX with high molecular weight and capacity to increase the viscosity of liquid phase of dough. A wide range of xylanolytic preparation is now available for breadmaking and more and more sources of xylanases are tested.

Xylanases from different sources will act different in dough, with different degree of solubilisation of AX and will increase or decrease the viscosity of liquid phase from dough. The degree of solubilisation of WUAX could be evaluate by measuring the increases of AX content in four extracts prepared with xylanases toward the extracts without xylanases and the impact on WEAX could be evaluated by measuring the viscosity of flour extracts. In ours previous studies we observed a good correlation between the changes of dough and bread properties and AX solubilisation for white wheat flours and with viscosity changes of extracts for black wheat flours [17-19]. In this study we evaluate the extensographic effects of some commercial xylanase on dough rheology.

2. Materials & Methods

Three commercial enzymatic preparation of xylanase was used for this study, Depol 333P, from Biocatalysts Ltd, UK, Veron 393 provided by AB Enzymes GmbH and Xila L from Belpan with 265.8 IRV/g (Inverse Reciprocal Viscosity), 3.7 IRV/g and respectively 13.8 IRV/g endo-xylanase at a pH 5.5. The unsupplemented flours were purchased from a local mill (Cibin Mill, from Sibiu). For the experiment were used two white flours coded F1 and F2 and two black flours coded F3 and F4. The flours F1 and F2 had 13.1% and 13.5% moisture, 29.6% and respective 29.8% wet gluten and 0.41% and respective 0.45% ash d.b. while the black flours F3 and F4 had 13.6% and respective 14.1% moisture, 1.30 and 1.25% ash d.b. and 27.8 and respective 28.1% wet gluten.

The xylanase activity was determined by viscometric method proposed by Megazyme with soluble wheat xylan (medium viscosity) as substrate, at pH 5.5.

To measure 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. The mix was kept at 30ºC with constant stirring for 30 minutes in a water bath and after that was centrifuged for 10 minutes at 1000 x g. The viscosity of supernatant was determined after 5 minutes for temperature equilibration with a Ubbelohde type glass viscometer. To measure the capacity to solubilise WUAX was determined the amount of AX from supernatant by orcinol method described by Hashimoto et all [3] and modified by Delcour et all [20]. 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 probe, prepared in the same way but without xylanase.

The rheological characteristics of dough with and without xylanases were determined by AACC Method 54-10 [21].

3. Results & Discussion

The rheology of dough prepared with xylanase was evaluated using Extensograph at 45, 90 and 135minutes. The doughs were prepared with commercial xylanases added to reach 25 IRV/100 kg flour, the dosage was choose to be close to the dosages recommended by the producers. We used to measure the activity of enzymatic preparation the IRV method because this method measured the endoxylanase activity and the substrate is AX from wheat. The method had a negative aspect because the AX used as substrate are soluble and are hydrolysed easier by enzyme with specificity for soluble AX.

In table 1 are presented the results of rheological evaluation of doughs prepared with white flours.

Table 1. Rheological parameters of doughs prepared with white flours

Flour	Time	Preparation	Energy [cm^2]	R_5 [UB]	Extensibility [mm]	R_{max} [U.B.]	Ratio R_5/E	Ratio R_{max}/E
F1	45 min.	Control	76	256	168	320	1.5	1.9
		Xila L	84	258	171	346	1.5	2
		Veron 393	83	257	177	330	1.4	1.9
		Depol 333P	86	263	175	347	1.5	2
	90 min.	Control	81	259	170	341	1.5	2
		Xila L	89	270	175	362	1.5	2.1
		Veron 394	89	263	184	340	1.4	1.9
		Depol 333P	88	266	175	358	1.5	2
	135 min.	Control	73	228	174	295	1.3	1.7
		Xila L	82	234	181	320	1.3	1.8
		Veron 395	75	218	181	295	1.2	1.6
		Depol 333P	74	237	171	311	1.4	1.8
F2	45 min.	Control	83	251	175	334	1.4	1.9
		Xila L	89	246	186	340	1.3	1.8
		Veron 393	85	232	185	327	1.3	1.8
		Depol 333P	92	270	180	360	1.5	2
	90 min.	Control	81	268	164	353	1.6	2.2
		Xila L	92	288	172	384	1.7	2.2
		Veron 394	85	250	175	355	1.4	2
		Depol 333P	87	278	167	376	1.7	2.2

	135 min.	Control	77	245	169	325	1.5	1.9
		Xila L	83	254	175	338	1.4	1.9
		Veron 395	77	214	181	308	1.2	1.7
		Depol 333P	82	269	166	352	1.6	2.1

On white flours the xylanases induced a small increase of dough energy. The pattern of action in time is similar for the two flours evaluated. At 45 minutes the effect is strongest and in time the effects is diminished. The Xila L preparations had a constant effect in time while for the others preparations the energy decrease in time. The energy of doughs prepared with black flours and xylanases (results presented in table 2) is smaller than the control. The Xila L preparation had the smallest decrease of energy. It is observed an improvement of energy of doughs with xylanases when the time of action is higher (135 min).

Table 2. Rheological parameters of doughs prepared with black flours

Flour	Time	Preparation	Energy [cm^2]	R_5 [UB]	Extensibility [mm]	R_{max} [U.B.]	Ratio R_5/E	Ratio R_{max}/E
F3	45 min.	Control	57	215	150	260	1.4	1.7
		Xila L	56	210	154	244	1.4	1.6
		Veron 393	50	180	159	212	1.1	1.3
		Depol 333P	53	207	150	240	1.4	1.6
	90 min.	Control	60	257	144	288	1.8	2
		Xila L	57	228	149	260	1.5	1.8
		Veron 394	59	220	156	261	1.4	1.7
		Depol 333P	61	253	145	288	1.7	2
	135 min.	Control	58	278	132	311	2.1	2.4
		Xila L	61	248	149	284	1.7	1.9
		Veron 395	59	228	152	268	1.5	1.8
		Depol 333P	58	250	143	282	1.7	2
F4	45 min.	Control	60	185	181	221	1	1.2
		Xila L	58	186	177	215	1	1.2
		Veron 393	55	174	179	206	1	1.1
		Depol 333P	54	182	167	214	1.1	1.3
	90 min.	Control	62	208	169	246	1.2	1.5
		Xila L	57	191	170	226	1.1	1.3
		Veron 394	59	187	178	222	1	1.2
		Depol 333P	57	212	158	248	1.3	1.6
	135 min.	Control	60	213	164	246	1.3	1.5
		Xila L	62	192	181	233	1.1	1.3
		Veron 395	56	186	174	219	1.1	1.3
		Depol 333P	57	214	157	248	1.4	1.6

The preparation Xila L and Depol 333 P determined a small increases of the resistance at constant extension for the doughs prepared with white flours while preparation Veron 393 decrease the R_5, especially for flour F2. Similar behaviour was observed for black flours, flour F3 was more sensitive to xylanase action.

The maximum resistance of doughs from white flours are improved. Preparation Veron 393 had the smaller positive effect and decrease the maximum resistance for the dough prepared from flour F2. The doughs prepared from black flour with xylanase had maximum resistance smaller than the control, the use of preparation Veron 393 lead to smallest resistance and Depol 333 P higher.

The extensibility of doughs from white flours increases when xylanases were added. The highest effects were observed when Veron 393 was used while preparations Depol 333 P had lowest effect. The extensibility of doughs from black flour F3 was higher or very close to control at 45 minutes while for flour F4 the extensibility was smaller. For all black flours the extensibility increases in time. Again preparation Depol 333 P had the worst effect on extensibility in doughs with black flours.

The xylanolytic preparations used had similar effect on the ratio R/E, R_5/E or R_{max}/E.

From the results presented in tables 1 and 2 we observed a similar pattern in action of xylanases uses in experiments but the flours had different sensitivity to xylanases, differences are explained due differences of the structure of AX or the inhibitors present in wheat flours.

We determined the variations of extensographic characteristics as differences between the probe with xylanase and control. The same evaluations were made for viscosity and arabinoxylans content of aqueous extract of flours.

We try to establish some correlations between changes induced by xylanase preparations on the rheological of doughs and on the flour extract, viscosity modification and solubilisation of AX expressed as the increases of pentosans content in extracts. Different xylanases have different effect on doughs rheology and different effect on viscosity and AX content of flour extract due their specificity for WUAX or WEAX and the product of hydrolysis obtained. From the data analysis we observed a good correlation between the variation of extensographic characteristics of doughs prepared from white flour F1 and the solubilisation of AX expressed as the percentage increases of AX (pentosans) in wheat flour extracts. The correlation coefficient R^2 vary from 0.7200 to 0.9231 for dough energy, resistance at constant extension (R_5), maximum resistance and ratio R/E (data shown in figure 1). No correlation could be observed between extensibility and solubilisation of AX at 45 minutes. At 90 and 135 minutes the correlations decreased. All correlations were negative, the extensographic characteristic decreased when the solubilisation of AX was higher. At small solubilisation of AX the energy and resistances of dough were higher.

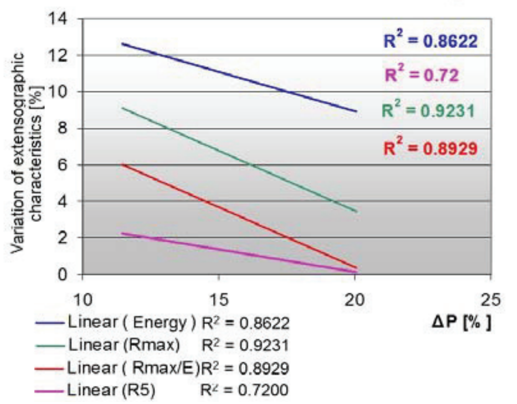

Fig. 1. The linear correlation between extensographic characteristics and pentosans content in flour extract for the white flour F1

On black flour F3 all xylanolytic preparations induced a decrease of the viscosity of aqueous extracts while on white fours F1 Xila L and Depol 333 P induced an increase of viscosity in extracts and preparation Veron 393 induced a decrease of viscosity.

When the data were analyzed no correlation between extensographic characteristics of dough from black flours and variations of pentosans content in extracts was observed. The correlations were observed with variations of viscosity of extracts (presented in figure 2).

Good and very good linear correlations were observed between the extensographic characteristics and variation of viscosity. The factor of correlation R-squared vary from 0.7659 to 0.9997. Only the extensibility of dough (E) presented an inverse correlation, the extensibility of dough increases when the viscosity decreases. This behaviour is predictable and expected. The others correlations also were predictable because we expected to observe a decrease of maximum resistance, resistance at constant extension and energy when the viscosity of extracts decrease. The correlation of ratio R_{max} / E also was positive; the decreases of maximum resistance were higher than the increases of dough extensibility.

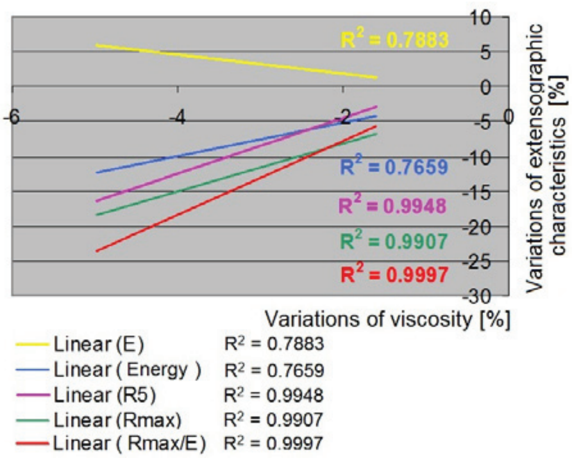

Fig. 2. The linear correlation between extensographic characteristics and pentosans content in flour extract for the black flour

4. Conclusion

The data presented suggested that the changes induced by xylanases on flour extracts as changes of viscosity and arabinoxylans content could be used to predict the changes of doughs rheology. This conclusion is confirmed by other previous study conducted by us [17-19]. For the white flours the changes of arabinoxylans had a greater impact on dough rheology than the changes of viscosity. We presumed that the changes cell wall fragment rich in WUAX have a greater impact dough rheology than the changes of viscosity. For the black flours the changes of cell wall fragment have a smaller effect in dough rheology because they are in greater amounts in black flours than in white flours and small modifications don't have a noticeable impact. The changes of viscosity have a greater effect on black flours. The liquefying of liquid phase from dough slightly increases the extensibility of dough and decreases the energy and resistance of doughs.

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