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Full Length Research Paper

Evaluation of xylanases from *Aspergillus niger* and *Trichoderma* sp. on dough rheological properties

Daniella Valeri, André Moreni Lopes* and Adalberto Pessoa-Júnior

Department of Biochemical and Pharmaceutical Technology, School of Pharmaceutical Sciences, University of São Paulo-FCF/USP, Brasil.

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Although starch is the main polysaccharide used in the fermentation of bread dough, wheat flour also contain some non-starch polysaccharides such as pentosans or hemicelluloses, which may contribute up to 3% of the total polysaccharide content of the flour. Despite being present in relatively low amounts, pentosans and hemicelluloses play an important role in dough rheology and bread properties. The aim of this work is to understand how the xylanases from *Aspergillus niger* and *Trichoderma* sp. influence dough rheology, such as elasticity, extensibility, strength and stability. When the extensograph parameters such as extensibility (E) and elasticity (R) were determined, it was possible to note that all dosages of xylanase from *A. niger* were capable of decreasing the dough elasticity in 21%. Depending on the dosage, the xylanase from *Trichoderma* sp. can decrease dough stability in the mixing and, consequently, the mixing time during the process. An increased dosage of *Trichoderma* xylanase decreased the elasticity in 32% and increased the extensibility by 8% following 45 min. It was also observed that raising dosages of *Trichoderma* xylanase in flour content affected the dough rheology more significantly than raising dosages of *A. niger* xylanase.

Key words: Xylanase, dough rheological properties, bread, *Aspergillus niger, Trichoderma* sp.

INTRODUCTION

In general, wheat is characterized by its high carbohydrate content of about 70%, low protein content (9 to 13%) and its small quantities of lipids, fiber and minerals. The carbohydrates are composed of starch and cellulose, with small quantities of sugar, hemicelluloses or pentosans (Pyler, 1988; Simões et al., 2009). The hemicelluloses are heteropolysaccharide consisting of D-xylose, L-arabinose, D-mannose, D-glucose and D-glucuronic acid, which may be acetylated or methylated (Singh et al., 2003). The arabinoxylans are the major non-starch polysaccharides of the cell walls of wheat grain. They consist of a linear chain of units of β-D-xylanopyranosyl connected by α (1,4) links, which may be mono substituted at the O-3 or disubstituted at the O-3 and O-2 with α-L-arabinofuranosil (Saunier and Ortiz-Ordaz, 2005) units, for L-arabinose, glucuronic acid, ferulic acid, p-coumaroyl, D-galactose, acetyl and L-arabinose (Subramaniyan and Prema, 2002; Collins et al., 2006; Berrin and Juge, 2008; Ahmed et al., 2009).

Even in small quantities, pentosans and hemicelluloses play an important role in the rheology of dough and properties of bread, because they are capable of absorbing 10 times their weight in water (Monfort et al., 1997). The role of arabinoxylans in dough and in the process of bread making is well described, as well as their capacity to decrease viscosity, to connect to water and their capacity for oxidative gelation (Redgwell et al., 2001).

The physical properties of the dough depend on the water content, quality of flour and structure. They are affected by different or common factors with the firmness

*Corresponding author. E-mail: andreml@usp.br. Tel: 55-11-3091-3862. Fax: 55-11-3815-6386.

of the dough, depending on the level of humidity, length of rest time and damaged starch content, where adhesion depends on the last two and cohesion on the first. Adhesion of the dough is sometimes desirable and necessary for some products, but too much water can make the dough sticky. The adhesion properties are often identified as stickiness, which is proportional to the work required to overcome the forces of adhesion, and which is also affected by proteolytic and amylolytic enzyme activities, protein composition, content of water-extractable pentosans and the quantities of fat and sugar in the recipe. The components of stickiness seem to be the gliadins, certain specific proteins or ferulic acids are linked to hexoses by an ester bond, as occurs in arabinoxylans (Anaya and Jimenez, 1998).

Endo-β-1,4-D-xylanases (1,4-β-xylan xylohydrolase, EC 3.2.1.8) hydrolyze glucosidic bonds within the xylan, which results in a decrease in the degree of polymerization of the substrate (Reilly, 1981; Sunna and Antranikian, 1997). The use of xylanases in the baking process leads to changes in the rheology of the dough such as dough development time, consistency, extensibility and resistance to breakage. These changes can be visualized in the final product, the bread, as improved quality, especially in relation to the volume and crumb structure (Sorensen, 2003; Várnaia et al., 2010).

An optimum dose of xylanase is defined as one that provides better performance to the properties of bread without causing sticky dough. Xylanase dosages provide more oven-rise and volume, the gluten is strengthened and the dough displays more elastic characteristics. With overdoses, the dough becomes stickier and the properties of the gluten make the dough more viscous than elastic, which does not provide increased volume or oven-rise (Si, 1997; Okunowo et al., 2010). The addition of xylanase increases the extensibility of the dough in a dose-dependent manner (Primo-Martín et al., 2005).

Moulds, such as *Trichoderma* and *Aspergillus*, are the most efficient producers of xylanolytic enzymes among the fungi (Romanowska et al., 2006). The aim of this study is to evaluate the different actions of the enzymes on flour dough rheology and draw a parallel with the characteristics that the enzymes provide to the dough.

MATERIALS AND METHODS

Commercial xylanase enzymes were derived from the culture of *Aspergillus niger* by DSM, Delft, The Netherlands and *Trichoderma* sp. were derived from Shin Nihon Chemical Co. Ltd., Anjyo, Japan. These enzymes were the objects of this study. Alvalade AN wheat flour was acquired from the Anaconda Mill in São Paulo, Brazil. Practical tests of baking and rheology tests of the dough were performed. The tests were performed on the same batch. Equipment for rheological analysis of flour are: Bradender farinograph and extensograph, from Brabender Laboratory of Cereals of the Faculty of Food Engineering, Unicamp, under the responsibility of Prof. Yoon Kil Chang.

Methods for the evaluation of pure wheat flour and wheat flour with enzyme

Farinograph

The following properties of pure wheat flour and wheat flour containing xylanases were determined following the methodology of the American Association of Cereal Chemists 54-21 (AACC, 1995): water absorption capacity, arrival time, dough peak time, dough stability, departure time and mixing tolerance index associated with the flour and its mixture with enzymes.

Extensograph

This method was used to evaluate the rheological characteristics of the dough obtained at different periods of rest. Thus, it was possible to evaluate the influence of fermentation time on the performance of the flour and the effect of enzymes in it. The method recommended by AACC 54-10 (1995) was modified: instead of using the same dough for 3 resting times of 45, 90 and 135 min, a second dough was produced for each time. Thus, it was possible to perform all readings. The method evaluated the strength of the dough measured by an energy curve, its resistance to extension or elasticity (R), the maximum resistance (Rm), extensibility (E) and proportional number (D = R/E), which indicates the behavior of the dough, regarding its tendency to shrink or flow.

RESULTS AND DISCUSSION

Flour rheology studies

As shown in Table 1, the dough peak time (PT), which is the time to reach peak or maximum dough consistency and indicates the relative strength of the flour, is not affected by the enzyme from *A. niger*, while small doses of the xylanase from *Trichoderma* sp. (30 and 60 U/kg flour) decreased development time by 1 min. Increased dosage of *Trichoderma* xylanase in flour resulted in a decrease of peak time which is about 1.5 min. This decrease did not mean that the strength of the flour fell, but indicated the decreasing time to beat the dough to arrive at the formation of gluten, that is, the dough became looser and more malleable. However, this decrease is not regarded as desirable by bakers because they believe that the flour is weak.

Stability (S) is the parameter most used in the farinograph analysis, because it indicates the time that refers to the tolerance of the flour to over-beating or under-beating. It is the interval between the time of arrival and departure from the line of 500 UF. As can be seen in Table 1, the xylanase from *A. niger* did not affect the stability of the flour. Xylanase derived from the fermentation of *Trichoderma* sp. decreased the stability of flour from 90 U/kg flour, because it lowered the stability by 1 min with 90 U/kg flour and by 2 min with dosages of 180 and 240 U/kg flour. Greater stability facilitates flour mixing, which indicates that the xylanase from *A. niger* should be used in flours in which this parameter should

Table 1. Results of the farinograph tests.

Sample	Dosage of enzyme (U/kg$_{flour}$)	Abs (%)	AT (min)	PT (min)	DT (min)	S (min)
Pure flour	0	57.9	1.20	13.0	17.5	16.30
An	60	57.9	1.25	12.5	17.0	15.75
An	80	58.5	1.25	13.0	19.0	17.75
An	120	59.3	1.30	13.3	17.8	16.50
An	160	59.4	1.00	12.7	17.7	16.70
An	240	59.2	1.20	13.0	17.5	16.30
Ts	30	59.1	1.00	12.0	17.5	16.50
Ts	60	59.3	1.20	12.0	17.5	16.30
Ts	90	59.0	1.20	11.5	16.5	15.30
Ts	120	58.4	1.00	11.0	14.5	13.50
Ts	180	58.0	1.10	11.0	15.5	14.40
Ts	200	58.0	0.80	10.5	15.0	14.20

An, *Aspergillus niger*; *Ts*, *Trichoderma* sp.; Abs, water absorption capacity; AT, arrival time; PT, peak time; DT, departure time; S, stability.

not be changed and *Trichoderma* xylanase in those where there is a need to reduce stability.

In general, it can be seen from Table 2 that the maximum resistance to extension Rm - which represents the elasticity of the dough, decreased as the working time increased even for the pure flour, and the dough extensibility (E) increased with working time. Factor D, which corresponds to Rm/E and represents an index of gluten behavior, also decreased with time, as the extensibility increased and elasticity decreased with working time. Crescent dosage of xylanase from *A. niger* did not alter the increase in extensibility in a significant way. Therefore, we can infer that the decrease of factor D with increasing dosages of xylanase from *A. niger* is due more to the decrease in elasticity than to the increase in extensibility. As the xylanase did not act on the gluten to decrease elasticity, the theory of action of xylanase proposed by Hilhorst et al. (1999) and Rouau and Moreau (1993), that the xylanases act on xylans that obstruct the gluten, makes sense because the xylans would make it more rigid, increasing the resistance to extension, not due to the presence of high-gluten, but due to the presence of the xylans that stiffen. With the action of xylanase, the gluten would become free and less rigid, which would decrease Rm.

Regarding the xylanase from *Trichoderma* sp., increasing the dosage decreased the elasticity of the dough, that is, the resistance to extension. Comparing the xylanase from *A. niger*, the dosage of only 30 U/kg flour of xylanase from *Trichoderma* sp. roughly corresponds to the drop in Rm when using 180 U/kg flour of *A. niger* xylanase (Table 2). Comparing the two xylanases, one can infer that the xylanase from *Trichoderma* sp. has a greater effect in decreasing the elasticity, in lowering dosages and with more intensity than that of *A. niger*. It may be observed that the extensibility of the dough is increased by increasing the dosage of xylanase from *Trichoderma* sp. In comparison with the xylanase from *A. niger*, the xylanase from *Trichoderma* sp. showed a trend of increasing the extensibility with time and with increasing dosage. At 135 min, extensibility reached 196 mm with a dose of 200 U/kg flour, while the dough with the addition of *A. niger* xylanase reached 180 mm with a dose of 240 U/kg flour.

It may also be observed that the addition of xylanase from *Trichoderma* sp. decreased factor D significantly at the three analysis times and with increasing dosage. For the time of 45 min, D fell from 3.64 for pure flour to levels lower than 3 with the lower dosage of 30 U/kg. At 90 min of analysis, D fell from 2.80 to levels lower than 2 for all dosages, decreasing with increasing dosage. At 135 min, D fell from 2.39 to values below 1.5 for dosages of 180 and 200 U/kg flour. Regarding the addition of xylanase from *A. niger,* no dosage decreased D to levels lower than 1.5 at 135 min of analysis. For the time of 90 min, the xylanase from *A. niger* did not decrease the D to levels below 2 and at 45 min, the mean value of D was above 3 for all dosages, excluding the dough with the addition of 240 U/kg flour. These data suggest that the xylanase from *Trichoderma* sp. is more efficient in decreasing the maximum resistance, the D factor and in increasing the extensibility.

In this study, the evaluation of the performance of xylanases derived from the fermentation of *A. niger* and *Trichoderma* sp. in the industrial baking process was tested. Regarding the specific objective of assessing the differences in action of the enzymes on dough rheology, one can say that the xylanase derived from the fermentation of *Trichoderma* sp. is more active in flour because it alters the rheological measurements of the

Table 2. Results of the extensograph tests.

Xylanase from	Activity U/kg flour	45 min				90 min				135 min			
		R (BU)	Rm (BU)	E (mm)	D (Rm/E)	R (BU)	Rm (BU)	E (mm)	D (Rm/E)	R (BU)	Rm (BU)	E (mm)	D (Rm/E)
Pure flour	0	510	750	140	3.6	455	640	163	2.8	365	560	153	2.4
An	60	485	715	144	3.4	410	603	156	2.6	328	500	179	1.8
An	80	500	725	149	3.4	380	565	165	2.3	350	493	173	2
An	120	412	603	144	2.9	305	477	168	1.8	302	430	170	1.8
An	160	440	633	140	3.2	355	556	168	2.1	317	503	181	1.8
An	240	390	590	153	2.6	325	495	153	2.1	280	445	180	1.6
Ts	30	435	629	147	3	340	536	172	2	310	459	171	1.8
Ts	60	422	638	162	2.6	318	470	168	1.9	280	445	184	1.5
Ts	90	383	569	152	2.5	310	465	173	1.8	273	427	184	1.5
Ts	120	367.5	525	156	2.4	310	468	169	1.8	288	430	182	1.6
Ts	180	365	528	152	2.4	297	440	165	1.8	236	377	178	1.3
Ts	200	380	510	142	2.7	295	415	152	1.9	210	340	196	1.1

An, *Aspergillus niger*; *Ts*, *Trichoderma* sp.; R, resistance; Rm, maximum resistance; E,extensibility; D, proportional number.

dough more intensely. For example, it can be observed that this xylanase decreases stability in farinograph measurements and reduces the peak time of the dough. This may mean that, in baking, the enzyme is more aggressive and might be used in smaller dosages. The enzyme from *A. niger* did not affect these parameters. The xylanase from *Trichoderma* sp. can also be considered the most aggressive and active in flour from the extensograph results. This enzyme effects a greater reduction on the resistance of the dough, reduces the proportional number of D and increases the extensibility to a greater extent. The enzyme from *A. niger* moderately modified these parameters. These results may divide the market and/or the situation in which the enzymes can be used. For example, xylanase from *Trichoderma* sp. may be specified for flours that have high D or processes that need to greatly decrease the viscosity and consistency of the dough, such as "cracker" type biscuits. The xylanase from *A. niger* could be used in less rigid, more balanced flour, that is, in flours with lower D values. For example, xylanase from *A. niger* is suitable for all kind of breads: buns, French style bread, tin bread and bread for hot dog. Xylanase from *Trichoderma* is suitable for crackers, wafers, biscuits and products that need extensible dough. The xylanases improve the kneading of the dough, the stability to fermentation, oven spring and volume of the bread, and cut open the French style breads.

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