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Review article

The Role of Thermostable Xylanase Enzymes in Bread Making

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Abstract

Bread, an intensively consumed food product should be optimized to minimize the staling and therefore waste. Xylanases, a group of enzymes are able to get rid of bread staling and it can be widely isolated from a group of fungi, bacteria or yeast. This review focuses on the main characteristics, producers and the recent textural assistance of thermostable xylanases in bakery industry.

Keywords: Xylanase, Bread, Enzyme, Dough stability.

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INTRODUCTION

Bread is one of the main food items in the world. Not only the poor but also the rich consume it at a great extent. Enzymes, the biological catalysts find their way via frequent applications in the industry. Over the course of several decades, the biotechnological use of xylanolytic enzymes has increased and become applicable to many industries. Xylanases are reported to be used in many industrial fields including food, dairy, baking, pulp, paper and animal feeding and mostly of microbial origin and filamentous fungal type is the most common form, moreover bacterial and yeast xylanases are also present (Raveendran et al, 2018, Sharma et al., 2018).

Xylanase enzyme functions not only increase the volume, but also provides a more flexible dough structure in bakery products. By this action the bread is both more stable and machinable. A noticeable improvement in crumb structure is maintained. The thermostable xylanases have recently gained interest due to the high oven temperature while baking. In addition, the combined use of xylanases with other enzymes improve the texture and organoleptic properties of the bread(Kumar et al., 2017).

Xylanases were reported to speed up the baking process by breaking down the polysaccharides present in dough(Courtin and Delcour, 2002). In this review it is aimed to collect the general information on thermostable xylanases, as well as help use them in baking industry in a high quantity.

Producer organisms

It is known that filamentous fungi which produce xylanases, are particularly important in baking industry, as since they produce more xylanases than bacteria or yeast into the medium they are obtained from. Thermophilic fungi are the most attractive group among these fungi because they can able to secrete thermostable xylanases(Li et al., 1993; Li et al., 2006). Chaetomium spp. are claimed to have thermophilic xylanases with a temperature optima interval of 60-70˚ C. Large scale applications of xylanases are also possible other than bread making, many different sources were reported including fungi(*Aspergillus terreus* S9) and current studies have implied that waste materials, including wheat bran, corn cob powder, mushroom compost, cotton cake and almond hulls allow confluent growth and therefore production of xylanase by this fungi(Sharma et al., 2020).

From different filamentous fungi xylanase can be isolated. One example is studied by Yegin et al(2018). *Aureobasidium pullulans* strain no: NRRL Y-2311-1 was found to produce an extremophilic xylanase on wheat bran and it was subjected to trial in bakery products at the first instance, and its performance was tested against the common xylanases used in the world in order to compare its efficiency.comparison. The extensographic and farinographic characteristics of the bread and dough were analyzed and the new xylanase increased the water absorption, development time and stability of the dough and a reduced amount of dough softening degree and mixing tolerance index at a dosage of 100 U/100 g flour was observed. When compared, the other common enzymes do not help increasing

the dough extension. The specific volume of bread was found to be noticeably improved (30%) by *A. pullulans* xylanase (125 U/100 g flour), as well as providing a normal moisture content (35-40%), and reducing the crumb firmness. The cohesiveness was slightly improved and a noticeable reduction in springiness and gumminess were obtained in all three xylanases, offering this enzyme a candidate for utilization in industry(Li et al., 1993; Li et al.,1996). A recombinant *Aspergillus niger* (strain XEA) displays an endo-1,4-beta-xylanase (EC 3.2.1.8) characteristics, was provided by DSM Food Specialities B.V. designed for use in baking and brewing processes. The allergenicity, genetic and oral toxicity tests indicated no any doubts for use, as well as for the allergy with the food enzyme did not indicate a genotoxic concern by European Food Safety Association, testing the availability of this enzyme to baking industry.

Table 1. Thermophilic fungi producing xylanases isolated in the recent three years.

Type of enzyme	Organisms
β-Xylanase	*Thermotoga naphthophila*(Hamid and Aftab, 2019)
Xylanase	*Myceliophtora heterothallica*(Simoes et al., 2019)
Endo-1,4-beta-xylanase (XynA)	*Thermotoga neapolitana*(Benedetti et al., 2019), Orpinomyces sp. PC-2 (Passarinho et al., 2019), *Pycnoporus sanguineus* BAFC 2126 (Niderhaus et al., 2018)
GH 11 xylanase (Xyn 10 A)	*Malbranchea cinnamomea (Basotra et al., 2018)*
GH10 xylanase (Xyn10A)	*Aspergillus fumigatus Z5* (Miao et al.,2018)

A bacterial strain, *Bacillus subtilis* LMG S-27588 was also considered to produce a xylanase enzyme(endo-1,4-beta-xylanase: 4-beta-D-xylan xylanohydrolase; EC 3.2.1.8) through genetic modification by Puratos N. V. according to Silano et al.(2018) and an *Aspergillus niger* xylanase(Silano et al., 2020) with no genotoxic and allergenic doubts and it is claimed by European Food Safety Association that this enzyme can be applicable to bakery production with no concern.

Another bacteria,a thermoacidophilic Alicyclobacillus spp. was reported to produce a high pH adaptable xylanase, displaying ultimate stability, isolated from a hot spring in Yunnan province of China and it was also expressed in *E.coli* for further processing(Bai et al.,2010).

During the prefermentation process of wheat bran by *Kluvyeromyces marxianus*, Zhang et al. observed that this yeast can produce different enzymes, including xylanase, ferulate esterase, endoglucanase, exoglucanase, and beta glucosidase and this release is ended up in positively improved performance of dough during bread making(Zhang et al., 2019). The destroying process of wheat bran on the gluten network, together with fermentation and enzymatic hydrolysis by xylanase, elevated the polyphenols and soluble arabinoxylans of wheat bran(Zhang et al., 2018).

Characteristics of Xylanases

Xylanases are glycoside hydrolase enzymes that genetically possess single-chain glycoproteins. They catalyze the hydrolysis of glycosidic bonds in complex sugars. Xylanases have a pH value between 4.5 and 6.5. They are also active at temperatures between 40 and 60 ° C. These enzymes are produced by some microorganisms to separate the xylanes (substrate), a main component of semi-cellulose. The three main enzymes, endoxylases, exoxylanases, and-oxlosidases act synergistically. They are necessary for the breakdown of the Xylan backbone in hemicellulosis. Endoxylanases cleave the -1.4 ligaments of the Xylan spine. Exoxylases randomly hydrolyze the -1.4 Xylan bond from the non-reducing interior, releasing xylooligosaccharides. Xylanases, which can be produced by different sources, differ for temperature, pH, etc. requirements under optimum operating conditions. In the food industry, xylanases are used for bread making (dough conditioning), corn starch production, juice and wine purification, as well as for alcoholic fermentation. One of the most common and traditional foods in the world is bread. Bread has a close bond with enzymes. Enzymes such as malt and fungal alpha-amylases have been used for years in bread making. Enzymes have increased in importance as a result of changes in the bakery industry and increased demand for more natural products. Enzymes have gained great importance in bread making as they increase the flexibility, workability, stability, loaf volume and crumb structure of the dough and improve the quality of the dough. Enzymes such as proteases, xylanases and cellulases directly or indirectly increase the strength of the gluten network, thus increasing the quality of bread. Due to their water absorption capacity and their interaction with gluten, xylans have an important role in bread quality. There is much evidence in the literature that the increase in volume, decrease in stickiness and increase in shelf life in bread making are obtained as a result of the use of xylanases. Xylanases, which are preferably effective on water unextractable arabinoxylan(WU-AX) and weakly active on water extractable arabinoxylan(WE-AX), are most suitable for bread making. This is because xylanases remove insoluble arabinoxylans that interfere with the formation of the gluten network, thus leading to high molecular weight resolution arabinoxylanes, which leads to increased viscosity and eventually increases dough stability. One of the most common causes of deterioration in bread quality is usually the staling. Xylanase reduces the water absorption of whole wheat flour and increases loaf volume and crumb softness by hydrolysing arabinoxylans.At the same time this enzyme increases proof height, reported as another effect on dough. By these functions xylanases work well to retard staling. Also the crumb hardness is claimed to be decreased when intermediate xylanase levels combined with oxidants used in bread making(Tebben et al., 2018). The polysaccharides other than starch abundant in the wall of bran and germ is said to be a problem leading to a decrease of the quality of bread making (Autio, 2006). While the dough is mixed, there is a competition between arabinoxylans and gluten during binding to water (Labat, Rouau, & Morel, 2002; Li et al., 2012). Xylanase mainly hydrolyzes the xylan backbone of water unextractable arabinoxylan, reduces its molecular size and decrease the water holding capacity (Gruppen, Kormelink, & Voragen, 1993). Consequently more gluten hydration takes place,

resulting better developed gluten matrix, therefore bread making capability is increased. Among many factors contributing to shelf life, moisture loss is an important factor affecting the freshness of bread. The higher the moisture loss, the faster the hardening of the bread. Xylanase hydrolyzes the semi-cellulose into smaller fractions with better water retention capacity, resulting in delayed water redistribution between different components. The moisture retention capacity of these ingredients is responsible for the freshness of the loaves of bread (Harris and Ramalingam, 2010).

Thermostability is another important concern of these enzymes since they are prone to high temperature during processing, especially in bread making. Many recent studies are present on thermostable xylanases, e.g. one study is on the isolation of xylanases from *Thermotoga maritima*(Yang et al., 2020), another is on *Streptomyces* spp.(Liu et al.,2020), *Malbranchea pulchella*(Ribeiro et al., 2014). Martins et al. (2020) stated that by thermostable enzyme use, the polysaccharide biomass is going to be easily saccharified at high temperatures and as well as lowering the reaction period, ascend the mass transfer and the substrate's viscosity is increased, moreover process optimization and low cost will associate.

Bread staling

This process is an unwanted issue according to the consumer attitude. It occurs in two types: the microbiological spoilage leading to staling and the other is the sequential chemical and physical changes leading to firmness of crumb. Mould spoilage happens due to post processing contamination, in addition bacteria such as *Bacillus spp.* leading to rope formation. Various yeast species are also responsible for staling processes. The physicochemical changes include the firmness of crumb, loss of freshness and sensory parameters including flavor and texture. Basically the starch transformation, starch-gluten interaction and moisture redistribution takes place in bread staling.

In the crust staling moisture redistribution is observed. Water travels from the crumb to crust or its air adsorption takes place therefore the crust of bread is hydrated. The crumb's staling is more complex, the inner part of the crumb is firmer than its outside. Since the crumb is made up of water, gluten and starch, in this matrix starch and protein makes a combination and leads to staling together. The retrogradation of starch occurring here is the main reason for the staling (Katina et al.,2006).

Effect of Xylanase on the physical properties of bread

Recent studies showed that xylanase has an impact on many different physical properties of bread and other components. According to Liu et al.(2020) xylanase has been applicable to the whole wheat bun to provide improvement of the crumb structure. Its effect on the dissolved components of the dough liquor was studied and it was found that the efficiency of the dough liquor was increased. Not only the water-extractable arabinoxylan level but also the foaming capacity and the foam stability under heat treatment was elevated(Liu et al., 2020). When xylanase was added to the wheat dough, it was found

that the water-retention capacity of water unextractable arabinoylan was decreased, releasing of water was associated to this process. Since water is redistributed between the flour components, the use of xylanase gained extra importance(Leys et al., 2020). A study performed by Scarton et al. (2020) was on the effect of lime juice used together with fungal xylanase in labelpan bread making. It was claimed that crumb softness and a long shelf life, with no use of bread additives were observed by using lime juice and this enzyme together(Scarton et al., 2020). Other than thermostable forms, xylanases are also applicable to frozen bread doughs since these kind of doughs reflect not only low amount of bread volume but also poor textural properties related to the weakened dough and decreased viable yeast counts. Carbohydrate active property of xylanases can improve the textural characteristics, since a study was shown to decrease the crumb hardness, independently or in combination with alpha amylase(Kim and Yoo, 2020). More studies are required in different countries to observe the effects, for example Chinese steamed bread is very commonly consumed, in traditional means in China, but up to now no effect of xylanases were studied on this bread (Su et al., 2005).

Buksa(2020) pointed out that the presence of arabinoxylan in rye flour requires the presence of appropriate xylanase activity. In this way, the availability of the pentoses in arabinoxylan is provided, leading a high dough acidification, more acetic acid synthesis and more lactic acid content. Since the arabinoxylans are reported to be the main non-starch carbohydrates in cereals, the influence of enzymes on the water extractable arabinoxylans were studied by Çetiner et al.(2020) and it was found that the water extractable arabinoxylan was elevated in whole-wheat breads by xylanases. In order to increase the daily intake of total fiber in bread, the combination of xylanases and the lactic acid bacteria fermented milling side-products such as bran; obtained from pigment containing wheat genera such as emmer was recommended (Pontonio et al., 2020). A study carried out by Xue and coworkers(2020) indicated that the thermostable form of xylanases, isolated from *Thermotoga maritima*, together with another enzyme, arabinofuranosidase was used to treat wheat bran, not only lead to the hydrolytic degree improvement of wheat bran, but also the antioxidative capacity, as well as the oil and water-retention capacities were increased. The degree of extension and softening, water absorbance, developing time were descended but on the other hand, the extensibility, time of stability, porosity and sensory properties were increased. The combination was found to be increasing the specific volume, cohesiveness and springiness but decreasing the crumb firmness, chewiness and gumminess characteristics of breads. Besides the thermsotability will provide advantage in baking as well, more than the action during kneading and proofing of the dough. For instance, a beta-xylanase isolated from *Thermotoga naphthophila* is reported to cause breaking of complex carbohydrates into monosaccharides, helping high temperature processing by its hyperthermophilic nature (Hamid and Aftab, 2019).

According to the study of Tozatti et al.(2019), when compared with chemical oxidizers, enzymes such as the glucose oxidase and a fungal xylanase (Grindamyl S 250) help improvement of not only the

strength but also the handling of dough. Leys et al.(2019) claimed that when the mutated wild type of *Bacillus subtilis* was used to observe the sensitivity behavior of inhibitory action for the functionality of its xylanase enzymes during bread making, it was found that the available water content was risen up related to starch-starch and starch gluten interaction, therefore the volume of the loaves were improved, which was determined by the extensional viscosity analysis of the dough by the xylanase activity.

On the other hand, Zhang et al.(2019) pointed out that when the enrichment of bread by fractional arabinoxylan use, isolated from wheat bran by drying, for fortification, the use of xylanase showed that no any improvement of the quality of loaves were observed together with arabinoxylan use.

Another study, performed by Passarinho et al.(2019) focused on the recombinant expression of Xylanases (xynA) isolated from the anaerobic fungi Orpinomyces spp. PC-2 strain, which was found to be highly active, therefore a strong recommendation of its use in the bakery industry for whole-wheat flour containing the polysaccharide xylan. A small wild type xylanase (SWT) and the small mutant xylanase (SM2) (V108A, A199T) were expressed in *Escherichia coli*, after purification, characterization, and testing for the ability, following the hydrolysis of whole wheat flour and use in dough processing. Both of the purified enzymes retained high specific activity against oat spelt xylan and wheat arabinoxylan, and the activity reached at the peak point at pH 3-7 and 60° C.

Tebben et al.(2018) declared that water absorption of the loaves was decreased, but the loaf volume was bigger than the control group when xylanase was added to the dough. It also helped formation of soft crumb by the hydrolysis of arabinoxylans.

Conclusion

The frequent and huge demand of enzyme utilization is always in an increasing trend, so novel enzymes and new sources must be screened out in different industries. Xylanases are the group of enzymes, recently gained interest in the industry and it has been declared that the xylanase utilization potential is going to rise, as since recent findings indicate good properties in dough, as well as focusing on the use of thermostable forms from different sources and in bakery products from the physical point of view, one of the indicators of bread staling.

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