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Application of Microbial α-amylase as Food Additives in Bakery Product (Bread). A mini Review

Mohamed Ismael Ahmed[1,2]*, Zhang Lianfu[1], Amer Mahdi[1,3]
[1]State key Laboratory of Food Science and Technology & School of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi 214122, P. R.
[2]Department of of Food Science and Technology.Nyala Technical College, South Darfur State, Sudan.
[3]Deparatment of Food Science and Technology, Faculty of Agriculture, Sana'a University, Sana'a, Yemen.
Tel: +86 18800574016 E-mail address: mohamedis75@yahoo.com

***Abstract* – The α- amylase enzyme is used in bakery processing as improver the rheological properties of bread. The α- amylase hydrolyze the starch. the high maltose level as a final end – product from the starch and malto-oligosaccharides, and the unique action pattern of the enzyme indicate unusual maltose forming system . The amount of free liquid was larger and of lower viscosity in dough containing α-amylase. After the addition of enzyme in the dough for the bread – bakery process, the bread volume increasing and kept its softness longer during storage than the bread had no enzyme. This review focuses on the use of these enzymes in industrial applications on bakery products and rheological properties of the products.**

***Keywards* – α-Amylases, Enzyme Production, Bacterial and Fungal Amylase, Bakery Products.**

I. INTRODUCTION

α-amylases (E.C.3.2.1.1) are enzymes that catalyses the hydrolysis of internal α-1,4-glycosidic linkages in starch in low molecular weight products, such glucose, maltose and maltotriose units(1, 2). Amylases are among the most important enzymes and are of great significance for biotechnology, constituting a class of industrial enzymes having approximately 25% of the world enzyme market (3,4). They can be obtained from several sources, such as plants, animals and microorganisms. Today a large number of microbial amylases are available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industry. The amylases of microorganisms have a broad spectrum of industrial applications as they are more stable than when prepared with plant and animal α-amylases (5). The major advantage of using microorganisms for the production of amylases is the economical bulk production capacity and the fact that microbes are easy to manipulate to obtain enzymes of desired characteristics (1).

II. STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF α -AMYLASE

The α -amylase belongs to a family of endo-amylases that catalyses the initial hydrolysis of starch into shorter oligosaccharides through the cleavage of α -D- (1-4) glycosidic bonds (6). Neither terminal glucose residues nor α -1,6- linkages can be cleaved by α -amylase (7). The end products of α-amylase action are oligosaccharides with varying length with an α-configuration and α - limit dextrins (8), which constitute a mixture of maltose, maltotriose, and branched oligosaccharides of 6–8 glucose units that contain both α -1,4 and α -6 linkages (7). Others amylolytic enzymes participate in the process of starch breakdown, but the contribution of α- amylase is the most important for the initiation of this process (9). The amylase has a three-dimensional structure capable of binding to substrate and, by the action of highly specific catalytic groups, promote the breakage of the glycoside links (10). The human α-amylase is a classical calcium-containing enzyme composed of 512 amino acids in a single oligosaccharide chain with a molecular weight of 57.6 kDa (7). The active site (substrate-binding) of the α-amylase is situated in a long cleft located between the carboxyl end of the A and B domains. The calcium (Ca^{2+}) is situated between the A and B domains and may act in the stabilization of the three-dimensinoal structure and as allosteric activator. Binding of substrate analogs suggest that Asp206, Glu230 and Asp297 participate in catalysis (11). The substrate-binding site contains 5 subsites with the catalytic site positioned at subsite 3. Substrate can bind to the first glucose residue in subsite 1 or 2, allowing cleavage to occur between the first and second or second and third glucose residues (7).

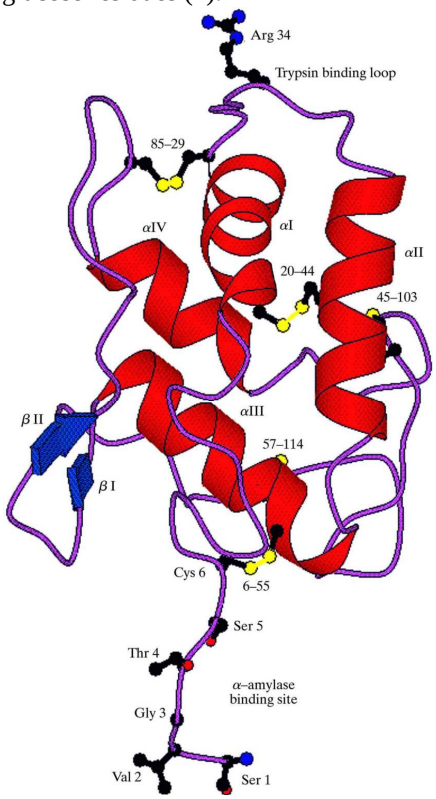

Fig. 1. Ribbon diagram of RATI indicating secondary structures and binding sites for trypsin and α-amylase.

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III. α-AMYLASE PRODUCTION

The production of α-amylase by submerged fermentation (SmF) and solid state fermentation (SSF) has been investigated and depend on a variety of physicochemical factors. SmF has been traditionally used for the production of industrially important enzymes because of the ease of control of different parameters such as pH, temperature, aeration and oxygen transfer and moisture(12, 13). SSF systems appear promising due to the natural potential and advantages they offer. SSF resembles the natural habitat of microorganism and is, therefore, the preferred choice for microorganisms to grow and produce useful value added products. SmF can be considered as a violation of their natural habitat, especially of fungi (14). Fungi and yeast were termed as suitable microorganisms for SSF according to the theoretical concept of water activity, whereas bacteria have been considered unsuitable. However, experience has shown that bacterial cultures can be well managed and manipulated for SSF processes (15). There are others advantages of SSF over SmF, including superior productivity, simpler technique, lower capital investment, lower energy requirement and less water output, better product recovery and lack of foam build up, besides it is reported to be the most appropriate process for developing countries. Recently, researches evaluated whether SSF is the best system for producing enzymes. They found that SSF is appropriate for the production of enzymes and other thermolabile products, especially when higher yields can be obtained when compared to SmF (12). The optimization of fermentation conditions, particularly physical and chemical parameters, are important in the development of fermentation processes due to their impact on the economy and practicability of the process (16). The role of various factors, including pH, temperature, metal ions, carbon and nitrogen source, surface acting agents, phosphate and agitation have been studied for α-amylase production. The properties of each α-amylase such as thermostability, pH profile, pH stability, and Ca-independency must be matched to its application. For example, α-amylases used in starch industry must be active and stable at low pH, but at high pH values in the detergent industry. Most notable among these are the composition of the growth medium, pH of the medium, phosphate concentration, inoculum age, temperature, aeration, carbon source and nitrogen source (12,17). The physical and chemical parameters of α-amylases from bacteria and fungi have been widely studied and described (1). The properties of some amylases from microorganisms has been shows in Table 1 and 2.

Table 1. Properties of bacterial α-amylases [18]

Microorganism	Fermentation	pH optimal/ stability	Temperature optimal/ stability	Molecular weight (kDa)	Inhibitors
Bacillus amyloliquefaciens	SmF	7	33 °C	–	–
Chromohalobacter sp. TVSP 101	SSF	7.0 - 9.0	65 °C	72	–
Caldimonastaiwanensis sp. nov.		7	55 °C	–	Galactose, malate, malonate, sucrose and acetate
Halobacillus sp MA-2	SmF	7.5 - 8.5	50 °C	–	Cd^{2+}, Cu^{2+}
Haloarculahispânica		6.5	50 °C	43.3	EDTA
Bacillus sp. I-3	SmF	7	70 °C	–	EDTA, HgCl2
Bacillus sp. PN5	SmF	10	60 °C	–	NH_4Cl
Bacillus sp. PS-7	SSF	6.5	60 °C	71	–
Bacillus subtilis	SSF	7	37 °C	–	–
Bacillus subtilis DM-03	SSF	6.0–10.0	50 °C	–	–
Bacillus subtilis KCC103	SmF	6.5	37 °C	–	–
Bacillus sp. KCA102		71	57.5 °C	–	–
Bacillus sp. AS-1	SSF	6.5	50 °C	–	–
Bacillus subtilis JS-2004	SmF	7	50 °C	–	Co^{2+}, Cu^{2+}, Hg^{2+} Mg^{2+}, Zn^{2+}, Ni^{2+}, Fe^{2+}, and Mn^{2+}
Bacillus sp. IMD 435.	SmF	6	65 °C		glucose, fructose
Bacillus subtilis	SmF	7	135 °C	–	–
Bacillus caldolyticus DSM405	SmF	5.0-6.0	70 °C	–	–
Bacillus sp. Ferdowsicous		4.5	70 °C	53	Hg^{2+}, Zn^{2+} and EDTA
Halomonasmeridiana	SmF	7	37 °C	–	Glucose
Rhodothermusmarinus	SmF	6.5 - 7	85 °C	–	-
Bacillus sp. KR-8104		4.0 - 6.0	70-75 °C	59	-
Bacillus licheniformis GCBU-8	SmF	7.5	40 °C	–	-
Bacillus subtilis		6.5	135 °C	–	-
Bacillus dipsosauri DD1		6.1	60 °C	80	Zn^{2+} and Cd^{2+}
Nocardiopsis sp.		5	70 °C	–	–
Geobacillusthermoleovorans		7	70 °C	–	–
Lactobacillus fermentum Ogi E1		5	30 °C	–	–
*Lactobacillus manihotivorans*LMG 18010T	SmF	5.5	55 °C	135	Ni2+, Cu2+, Hg2+, Fe3+ and Al3+

Table 2. Properties of Fungi and Yeast α-amylases[18]

Microorganism	Fermentation	pH optimal/ stability	Temperature optimal/ stability	Molecular weight (kDa)	Inhibitors
*Thermomyceslanuginosus*ATCC 58160	SSF	6	50 °C	–	–
*Thermomyceslanuginosus*ATCC 200065		6	50 °C	–	–
Aspergillusniger	SSF	5.5	70 °C	–	–
Aspergillus sp. AS-2	SSF	6	50 °C	–	–
Aspergillusniger UO-1	SmF	4.95	50 °C		Cu^{2+}, Hg^{2+} and Zn^{2+}
Aspergillusniger ATCC 16404	SmF	5.0 / 6.0	30 °C	–	–
Aspergillusoryzae		5.0 – 9.0	25-35 °C	–	–
Aspergillusoryzae CBS570.64	SSF	7	35 °C	–	–
Aspergillusoryzae NRRL 6270	SSF		30 °C	–	–
Aspergillusoryzae CBS 125-59	SSF	6	30 °C		
Aspergillusfumigatus	SmF	6	30 °C	–	–
Aspergilluskawachii		3	30 °C	108	–
Cryptococcus flavus		5.5	50 °C	75	Hg^{2+}, Fe^{2+} and Cu^{2+}
Penicilliumfellutanum	SmF	6.5	30 °C	–	–
Pycnoporussanguineus	SmF	7	37 °C	–	Glucose, maltose
Pycnoporussanguineus	SSF	5	37 °C	–	–
Mucor sp.		5	60 °C	–	EDTA
Saccharomyces kluyveri YKM5		5	30 °C	–	–

IV. Influence of Improvers (α- Amylase) On The Baking Properties

In the 19th century it was established that the inclusion of flours made from sprout-damaged grains into wheat doughs increased the volume of the baked goods. Some years later, flour from artificially sprouted grains (malt) was used as source of amylase. Today microbial amylase preparations are used as well as malt flour. Amylases have two important effects on the volume of wheat based bakery items. During the dough phase, amylases partly degrade the damaged starch to fermentable sugars. These, in turn, will be converted into alcohol and carbon dioxide by the yeast and ultimately contribute to the leavening of the dough. The main effect of the α-amylases, however, takes place during the baking process when the gas bubbles in the dough expand because of the temperature increase (oven spring). This thermal expansion is counteracted by the increasing viscosity of the starch which is simultaneously absorbing water, swelling and partially gelatinising. Selective use of amylases can decrease the viscosity of the starch enabling greater expansion of the gas bubble at the start of the baking process. Amylases also have an effect on the browning of the crust (bloom). Dextrins and sugars formed during the enzymatic degradation of starch give rise to the formation of a brown colour during baking and the typical bread flavour develops as a result of the reaction between these ingredients and other dough components. Finally, the starch quality also influences the staling of baked goods. With selective use of amylases, the starch structure can be altered and the shelf life of the baked goods prolonged. Flour also contains waterinsoluble hemicelluloses originating from the walls of the grain cell. The absorbed water migrates into the starch during the baking process causing a decrease in viscosity and resulting in an improved oven spring and higher volume for the baked goods (19). The quantities, taste, aroma and porosity of the bread are improved by using the enzyme in the flour. More than 70 % bread in U.S.A, Russia and European countries contain α-amylase. Amylases play important role in bakery products(20). For decades, enzymes such as malt and fungal alpha-amylases have been used in bread-making. The significance of enzymes is likely to raise as consumers insist more natural products free of chemical additives. Larger loaves with softer texture are obtained when dough contains either fungal or bacterial α-amylases. Fungal α-amylases facilitate proofing under ambient conditions while bacterial α-amylases act at higher temperatures and enhance loaf expansion during baking. The fungal α-amylases have been used in amounts of w300-400 mg per 100 g flour (21). Due to the ease of use and benefits observed on finished product qualities, mixtures of ascorbic acid and fungal α-amylases are widely used in bakeries in Australia as a way to boost the bread-making qualities of local flours (discussion with Mr. Robert Millard, Bakery Training Instructor, Polytechnic West, Perth, Australia). It is thought that fungal α-amylases depolymerise damaged starch and reduce its ability to bind moisture, thus allowing more moisture to be available for gluten hydration (22). Microscopic observation has shown that starch granules disintegrate in doughs mixed with α-amylase, possibly due to extensive hydration and swelling (23). Depolymerisation also facilitates the production of dextrin or fermentable sugars, which in turn facilitates the production of carbon dioxide by yeast (24). Thus, more gas is produced and the loaves are larger. Beneficial effects of α-amylases on quality (texture) have also been reported for chapattis (25). However, this cannot be explained by greater gas production as chapattis are not made with yeast. Hence it is not clear if the observed improvement in finished product qualities arising from addition of α-amylases occurs due to production of excess gas during

fermentation or from changes in dough strength resulting from improved gluten hydration. It is not simple to design studies incorporating yeasted doughs that decouple these two potential mechanisms. Such an opportunity is available when analysing the effects of fungal α-amylases on chemically leavened doughs, since the gas production is independent of production of simple sugars resulting from degradation of starch by α-amylases(26).

V. CONCLUSIONS

The α-amylase play and ever increasing role in baking industries has for manyyears and a number of microbial sources exist for the efficient production of this enzyme, but only a few selected strains of fungi and bacteria meet the criteria for commercial production.

The addition of α-amylases to the dough chemically leavened doughs leads to the formation of bigger loaves with softer crumbs.

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