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A Novel Maltopentaose-Producing Amylase as a Bread Antistaling Agent

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Abstract A maltopentaose-producing amylase (G5-amylase) from *Bacillus megaterium* KSM B-404 was applied to retard bread retrogradation. Retrogradation rates were determined by differential scanning calorimetry. Gel permeation chromatography determined changes in maltooligosaccharide composition and the molecular weight profiles of carbohydrate fractions. The baking process produced maltopentaose and maltotriose by the hydrolysis of starch molecules into small units. Amylose and amylopectin degradation as well as maltooligosaccharides produced by the enzyme were likely responsible for retarding starch retrogradation. Overall, addition of G5-amylase reduced the starch retrogradation rate, and was as effective as Novamyl®, a commercial enzyme.

Keywords: Bread, maltopentaose-producing amylase (G5-amylase), retrogradation.

Introduction

Starch retrogradation is a complex phenomenon, the detailed mechanisms of which are not yet well understood. In general, researchers have proposed that starch retrogradation results from physical changes of amylose and/or amylopectin in starch from a swollen, gel-like state to a more crystalline state, one of the most undesirable reactions during storage of bakery products. Numerous studies have attempted to find ways to stop or slow the retrogradation process. These studies have led to the development of various enzymes, emulsifiers, oligosaccharides, and polysaccharides intended to retard the staling process and improve bread quality (1-7).

Several reports have indicated that adding amylases to bread formulations significantly prevents bread from becoming too firm during storage (1-4, 8). Although many researchers have suggested explanations for the antistaling mechanisms of amylases (8-10), the process remains unclear. Boyle and Hebeda (9) proposed that amylases hydrolyze branched amylopectin chains into smaller units, thus preventing starch recrystallization. Researchers have also reported that the antistaling effect of amylases is related to the size of the dextrins produced (10-13). Because antistaling amylases have been shown to break links in amylose and amylopectin, they may also promote the formation of the amylopectin-lipid complex and decrease bread retrogradation rates (5). In this study, we isolated a maltopentaose-producing amylase (G5-amylase) and investigated its potency as an antistaling agent in bread.

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Materials and Methods

Purification of amylases The main maltopentaose-producing amylase (G5-amylase) was isolated from *Bacillus megaterium* KSM B-404, expressed in *Bacillus subtilis* LKS88 (14), and purified using a modified version of the process used by Park *et al.* (15). The protein was fractionated by acetone precipitation and purified further through a Q-sepharose column chromatography (FPLC) system (Amersham Pharmacia Biotech, Uppsala, Sweden).

Enzyme characteristics Reducing sugar was determined by the dinitrosalicylate (DNS) method (16), allowing an assay of G5-amylase activity. A mixture of 1% (w/v) soluble starch (0.25 ml) in 50 mM KH_2PO_4/NaOH buffer (pH 6.0) and 0.2 ml of the same buffer was prewarmed at 50°C for 5 min. Next, 0.05 ml of the enzyme solution was added to the prewarmed solution and incubated for 30 min. The reaction was terminated by the addition of 1.5 ml of dinitrosalicylic acid solution followed by boiling for 5 min and cooling under running tap water. Absorbance was measured at 575 nm. One unit of activity was defined as the amount of enzyme producing 1 mol of reducing sugar (with maltose as the standard) for one min.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) A Purified enzyme sample was electrophoresed on a discontinuous polyacrylamide gel containing SDS according to methods described by Laemmli (17). The separating gel was prepared in the standard way: a 10% (w/v) acrylamide gel with a stock solution of 33.5% (w/v) acrylamide containing 0.3% (w/v) N,N'-methylene bisacrylamide. The standard molecular weight size marker was composed of myosin (MW 205,000), β-galactosidase (MW 116,000), phosphorylase b

(MW 97,000), bovine serum albumin (MW 66,000), egg albumin (MW 45,000), and carbonic anhydrase (MW 29,000).

Matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry analysis MALDI-TOF mass spectrometry (Voyager-DE, Perceptive Biosystem, Boston, MA, USA) was used to identify the molecular mass spectrum of the enzyme. Sinapinic acid (3, 5-Dimethoxy-4-hydroxycinnamic acid) was prepared for the matrix, and bovine serum albumin was used for calibration analysis. One microliter of purified enzyme was mixed with the same volume of prepared matrix and dropped on a sample plate where it was allowed to dry until it crystallized. The sample plate was loaded into the Voyager-DE Biospectrometry workstation for analysis of the molecular weight of the enzyme.

Bread-making Loaves of bread used in this study were baked in an automatic home bread-maker (SHB-600, Samsung Co., Seoul, South Korea) using White Pan Bread Mix II (Cheiljedang Co., Seoul, South Korea) and stored at 4°C in polyethylene bags. The loaves were divided into three groups depending on the treatment of the dough: a control group; a group supplemented with 0.02% (w/w) Novamyl® (Novo Nordisk, Bagsvaerd, Denmark), a commercial antistaling maltogenic amylase; and a group treated with 1000 units of G5-amylase. The quantity of enzyme used had been predetermined by sensory evaluation to judge the optimum quality produced in bread texture, volume, and appearance (data are not shown).

Starch retrogradation in bread Each group consisted of four loaves of bread; the retrogradation rate in each group was measured by the DSC method described in Kweon *et al.* (5). Samples were stored at 4°C and assayed after 3 and 7 days using the DSC 120 (Seiko Instruments Inc., Chiba, Japan). Bread samples (10 mg) were placed in aluminum pans and then heated from 20-130°C at 5°C/min. The enthalpy of the endothermic peak was used as the retrogradation index (5).

Maltooligosaccharide composition of bread Bread crumbs (10 g) and 100 ml of distilled water were mixed and stirred vigorously for 1 hr at room temperature. The mixture was placed in a centrifuge at 10,000 rpm for 10 min; the supernatant was diluted with distilled water (1:99, v/v) and then filtered through a 0.45 μm filter (Gelman Sciences, East Hills, NY, USA). Samples were analyzed by high-performance ion chromatography (HPIC) using a CarboPac PA1 (Dionex Co., Sunnyvale, CA., USA) and an electrochemical detector (ED 40; Dionex Co.). Filtered samples were eluted with a linear gradient of solvent containing 600 mM sodium acetate (0-30% [v/v]) and 150 mM NaOH.

High molecular weight carbohydrates in bread To analyze changes in the high molecular weight carbohydrates in breads, HPLC was performed with a Sugar KS-804 column (0.8 × 30 cm, Showa Denko K.K., Tokyo, Japan) using distilled water as an elutant. Bread samples were prepared using a slightly modified version of the method described by Lin *et al.* (18): breadcrumbs were dehydrated using acetone, and lipids were removed using an ethanol-benzene mixture. The dehydrated, defatted bread powder was suspended in 90% DMSO and stirred for 3 to 4 h at 55°C. The resulting supernatants were used in the analysis.

Results and Discussion

Preparation of G5-amylase G5-amylase was purified by acetone precipitation of a *B. subtilis* LKS88 culture broth and Q-Sepharose column chromatography; the yield was 7.7%, and the purification fold was 3.9. The MALDI-TOF mass analysis (Fig. 1) estimated the molecular weight as 55367 Dalton, similar to weights reported for maltopentaose-producing amylase from *Bacillus cereus* (19).

Hydrolysis pattern of G5-amylase A hydrolysis pattern of the purified enzyme was examined using 1% soluble starch as a substrate and was analyzed by HPIC as described above. As the reaction progressed, different patterns of oligosaccharide composition were produced, but the predominant compositions were maltotriose and maltopentaose. After 24 hours, hydrolysis had produced glucose (6.3%), maltose (11.5%), maltotriose (23.2%), maltotetraose (4.8%), and maltopentaose (54.2%; Fig. 2).

Effects of enzyme treatment on starch retrogradation in bread The retrogradation rate differed significantly (p

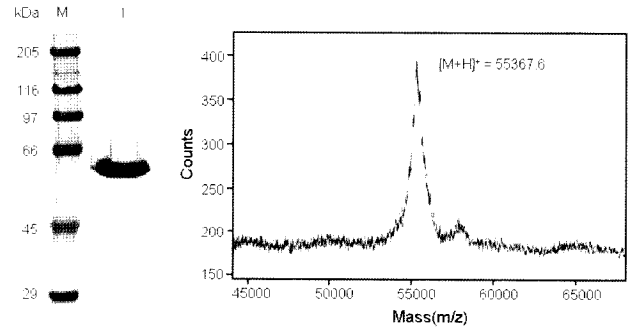

Fig. 1. SDS-PAGE and MALDI-TOF mass analysis of purified G5-amylase. Lane M: size marker, Lane I: purified enzyme. Molecular weight was estimated as 55367 Dalton.

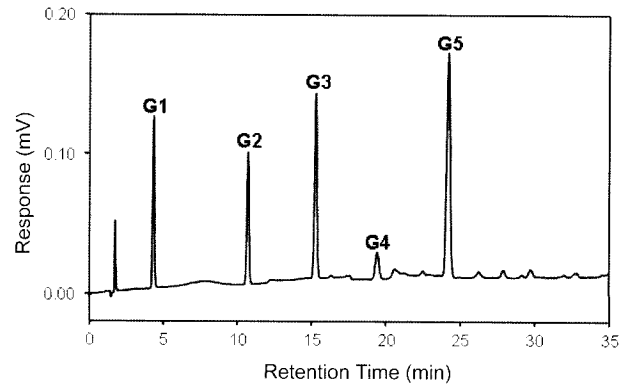

Fig. 2. High-performance ion chromatography (HPIC) analysis of maltooligosaccharides produced from soluble starch by G5-amylase.

<0.05) between the control and enzyme-treated breads (Fig. 3). Adding G5-amylase to the bread mix retarded starch retrogradation throughout the storage period to a degree comparable with that of the commercial enzyme Novamyl®.

Maltooligosaccharide and carbohydrate composition in bread Enzyme-treated bread contained more maltooligosaccharides than the control group (Fig. 4) and produced mainly maltose, maltotriose, maltotetraose, and maltopentaose. The significant amount of maltose observed in the control group was likely a result of yeast fermentation during baking (2). Profile comparison indicated that adding amylases produced maltooligosaccharides larger than maltotriose; these results are similar to the starch hydrolysis pattern described above. Similar levels of maltotriose and maltotetraose were observed in samples treated with both Novamyl® and G5-amylase, whereas maltopentaose was observed only in breads treated with G5-amylase, indicating superior antiretrogradation in this group. Researchers have generally concurred that maltooligosaccharides act as antiretrogradation agents (2, 9, 10, 12, 20-22), and studies have confirmed that maltopentaose has the greatest retarding effect (12, 21).

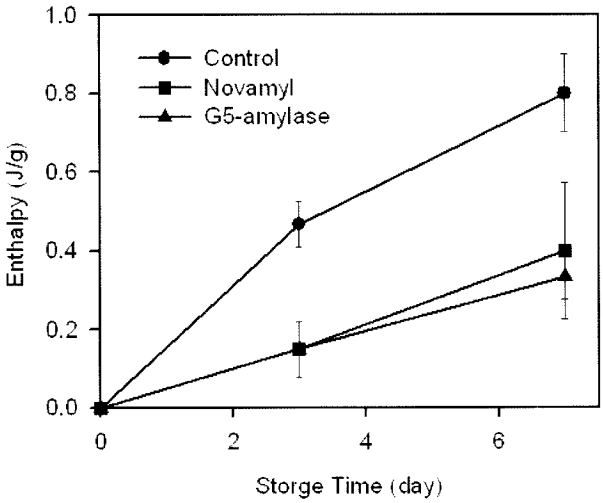

Fig. 3. Retrogradation rates during storage of bread treated with various enzymes.

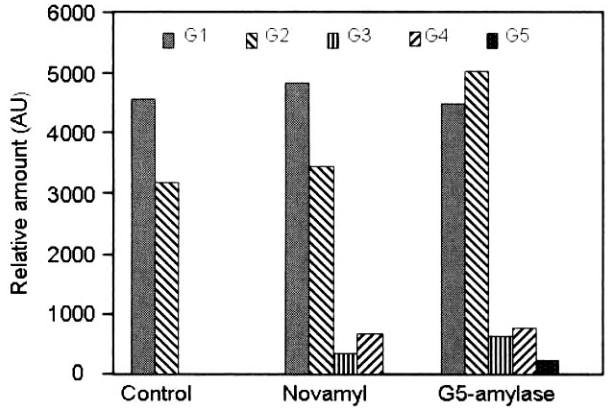

Fig. 4. Maltooligosaccharide compositions in bread loaves treated with various enzymes. G1: glucose, G2: maltose, G3: maltotriose, G4: maltotetratose, G5: maltopentaose.

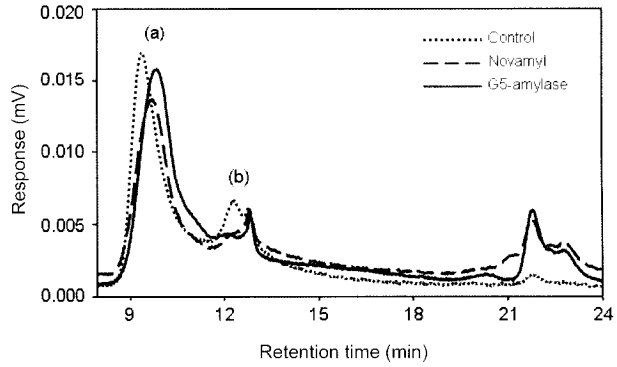

Fig. 5. Gel permeation chromatography of carbohydrate fractions in bread. Peaks in region (a) indicate amylopectin fractions; peaks in region (b) indicate amylose fractions.

Hygroscopicity (23) and the temperature of glass transition (24, 25) appear to affect the extent of antiretrogradation, but the underlying mechanisms are still under discussion.

The enzyme treatment caused significant changes in the molecular weight distribution of starch in the bread (Fig. 5). As expected, enzyme supplementation lowered the molecular weight. Boyle and Hebeda (9) reported a significant reduction of molecular weight in starch and theorized that the partial hydrolysis of starch could inhibit its recrystallization. A rapid reorganization of amylose components, followed by a slow recrystallization of amylopectin components, causes starch retrogradation (26). The results of this study indicate that modification of the molecular sizes of amylose and amylopectin using enzyme treatments can effectively retard recrystallization (Fig. 3). The almost identical molecular distributions created by G5-amylase and Novamyl® treatments suggest that G5-amylase could be used in industrial applications as a new antistaling agent.

Acknowledgments

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References

1. Gujral HS, Haros M, Rosell CM. Starch hydrolyzing enzymes for retarding the staling of rice bread. Cereal Chem. 80: 750-754 (2003)
2. Min BC, Yoon SH, Kim JW, Lee YW, Kim YB, Park KW. Cloning of novel maltooligosaccharide-producing amylases as antistaling agents for bread. J. Agric. Food Chem. 46: 779-782 (1998)
3. Lee SH, Kim YW, Lee S, Auh, JH, Yoo SS, Kim TJ, Kim JW, Kim ST, Rho HJ, Choi JH, Kim YB, Park KH. Modulation of cyclizing activity and thermostability of cyclodextrin glucanotransferase and its application as an antistaling enzyme. J. Agric. Food Chem. 50: 1411-1415 (2002)
4. Haros M, Rosell CM, Benedito C. Effect of different carbohydrases on fresh bread texture. Eur. Food Res. Technol. 215: 425-430 (2002)
5. Kweon MR, Park CS, Auh JH, Cho BM, Yang NS, Park KH.

Phospholipid hydrolysate and antistaling amylase effects on retrogradation of starch in bread. J. Food Sci. 59: 1072-1076 (1994)
6. Stampfli L, Nerste B. Emulsifier in bread making. Food Chem. 52: 353-360 (1995).
7. Armero A, Collar C. Crumb firming kinetics of wheat breads with antistaling additives. J. Cereal Sci. 28: 165-174 (1998)
8. Hebeda RE, Bowles LK, Teague WM. Developments in enzymes for retarding staling of baked goods. Cereal Food World 35: 453-457 (1990)
9. Boyle PJ, Hebeda RE. Antistaling enzymes for baked goods. Food Technol. 44: 129 (1990)
10. Martin ML, Hoseney RC. A mechanism of bread firming. II. Role of starch hydrolyzing enzymes. Cereal Chem. 68: 503-507 (1991)
11. Akers AA, Hoseney RC. Water soluble dextrins from α-amylase treated bread and their relationship to bread firming. Cereal Chem. 71: 223-226 (1994)
12. Duran E, Leon A, Barber B, de Barber C. Effect of low molecular weight dextrins on gelatinization and retrogradation of starch. Eur. Food Res. Technol. 212: 203-207 (2001)
13. Leon A, Duran E, de Barber C. Firming of starch gels and amylopectin retrogradation as related to dextrin production by α-amylase. Z Lebensm. Unters. Forsch. A. 205: 131-134 (1997)
14. Son HJ. Expression of the amylase gene from *B. megaterium* KSM B-404 in *B. subtilis* LKS88 and the maltopentaose production. MS thesis, Korea University, Seoul, Korea (1999)
15. Park JW, Kim BJ, Lee JW, Kim YB. Purification and characterization of maltopentaose-producing amylase from *Bacillus megaterium* KSM B-404. Kor. J. Microbial. Biotechnol. 30: 352-358 (2002)
16. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31: 426-428 (1959)
17. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685 (1980)
18. Lin W, Linback DR. Changes in carbohydrate fractions in enzyme-supplemented bread and the potential relationship to staling. Starch 42: 385-394 (1990)
19. Yoshigi N, Chikano T, Kamimura M. Characterization of maltopentaose-producing bacterium and its cultural conditions. Agric. Biol. Chem. 49: 2379-2383 (1985)
20. Palacios HR, Schwarz PB, Appolonia BLD. Effects of α-amylase from sources on the firming of concentrated wheat starch gels: Relationship to bread staling. J. Agric. Food Chem. 52: 5987-5994 (2004)
21. Smits ALM, Kruiskamp PH, van Soest JJG, Vilegenthart JFG. The influence of various small plasticizers and malto-oligosaccharides on the retrogradation of (partly) gelatinized starch. Carbohydr. Polym. 51: 417-424 (2003)
22. Sugiyama H, Nitta T, Horii M, Motohashi K, Sakai J, Usui T, Hisamichi K, Ishiyama J. The conformation of α-(1.4)-linked glucose oligomers from maltose to maltoheptaose and short-chain amylose in solution. Carbohydr. Res. 325: 177-182 (2000)
23. Donnelly BJ, Fruin JC, Scallet BL. Reactions of oligosaccharides. III. Hygroscopic properties. Cereal Chem. 50: 512-519 (1973)
24. Auh JH, Park KH. Estimating the glass transition of oligosaccharides mixtures through the state diagram. Food Sci. Biotechnol. 14: 301-303 (2005)
25. Kalichevsky MT, Jaroszkiewicz EM, Blanshard JMV. A study of the glass transition of amylopectin-sugar mixtures. Polymer 34: 346-358 (1993)
26. Miles MJ, Morris VJ, Orford PD, Ring SG. The roles of amylose and amylopectin in the gelatinization and retrogradation of starch. Carbohydr. Res. 135: 271-281 (1985)