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Preparation of immobilized glucose oxidase and its application in improving breadmaking quality of commercial wheat flour

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ABSTRACT

Preparation of immobilized glucose oxidase (GO) on chitosan (CS)-sodium tripolyphosphate (TPP) and its application in improving breadmaking quality of commercial wheat flour were investigated. The optimum conditions for GO immobilization were: viscosity of CS: 700 cP, ratio of CS to TPP (w/w): 5 to 1, and GO concentration 100 U/mL. The obtained CSTPP-GO was 5 μm-diameter particle with a pseudo-spherical shape. By addition of CSTPP-GO (400 U/kg flour) and fungal α-amylase (62.5 U/kg flour), bread springiness slightly increased from 0.923 to 0.940, specific volume of crumb increased by 13.48% and hardness decreased by 19.22%, compared to addition of $KBrO_3$ (60 mg/kg flour). It could be concluded that CSTPP-GO combined with fungal α-amylase had potential application in improving breadmaking quality of commercial wheat flour.

1. Introduction

Bread is an important food in human diet in many countries and contributes substantially to the daily intake of carbohydrates, dietary fibre, protein, minerals and B vitamins (Belitz, Grosch, & Schieberle, 2004, chap. 15). The functional breadmaking properties of wheat greatly depend on the gluten proteins (Wieser & Kieffer, 2001). However, cultivar differences, insect damage and environmental, genetic and post-harvest conditions of wheat may affect gluten properties and wheat processing quality (Georget, Underwood-Toscano, Powers, Shewry, & Belton, 2008; Lukow, White, & Sinha, 1995; Wrigley & Bietz, 1988). In order to overcome deficiencies in breadmaking quality (loaf volume, crumb structure, shelf-life, flavour and colour) of wheat flour, various bread improvers, such as oxidants, reductants, emulsifiers and enzymes, have been added (Haros, Rosell, & Benedito, 2002; Martinez-Anaya, 1996; Moayedallaie, Mirzaei, & Paterson, 2010).

Potassium bromate ($KBrO_3$) has been widely used in the baking industry for a long time as an effective improver for improving loaf volume, crumb structure and texture (Kohman, Hoffman, Godfrey, Ashe, & Blake, 1916). $KBrO_3$, a slow acting oxidant, oxidizes free thiol groups generating small –S–S– compounds and bromide. It is active during later stages of fermentation and baking. However, its application has been gradually restricted and even forbidden in many countries because of the potential relationship between bromate intake and cancer (Kurokawa et al., 1986; Ranum, 1992). Therefore, it is an urgent challenge to find the alternatives for $KBrO_3$.

Enzymes are attractive, safe alternatives to $KBrO_3$. Trials of enzymes such as transglutaminase (EC 2.3.2.13) (Beck, Jekle, Selmair, Koehler, & Becker, 2011), laccase (EC 1.10.3.2) (Selinheimo, Autio, Kruus, & Buchert, 2007), hexose oxidase (EC 1.1.3.5) (Gül, Özer, & Dizlek, 2009) and glucose oxidase (EC 1.1.3.4) (Bonet et al., 2006; Rasiah, Sutton, Low, Lin, & Gerrard, 2005) have been studied in breadmaking. They are able to improve different aspects of bread qualities such as reinforcing gluten structure, strengthening dough stability, increasing specific volume, texture, elasticity and water holding capacity (Martínez-Anaya & Jiménez, 1997; Miller & Hoseney, 1999; Zhu, Rinzema, Tramper, & Bol, 1995).

Fungal (*Aspergillus niger*) Glucose oxidase (GO) is one of the most effective alternative to $KBrO_3$ (Hanft & Koehler, 2006; Vemulapalli, Miller, & Hoseney, 1998). In the presence of oxygen, GO catalyses the oxidation of β-D-glucose to D-gluconic acid with the release of hydrogen peroxide (H_2O_2). The H_2O_2 promotes the formation of disulphide linkages in the gluten networks and consequently improves dough handling properties, better gas retention capability and bread quality, especially for commercial wheat flour (Poulsen & Høstrup, 1998; Steffolani, Ribotta, Pérez, & León, 2010; Vemulapalli & Hoseney, 1998). However, GO is unable to replace $KBrO_3$ due to some deficiencies including fast

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oxidation at the initial stage in dough development, low stability in flour and thermal-inactivation in the baking stage. Fast oxidation will cause rapid release of H_2O_2 at the beginning stage, resulting in over-developed dough and low volume and poor crumb of bread (Primo-Martín, Wang, Lichtendonk, Plijter, & Hamer, 2005; Rakotozafy et al., 1999). Immobilization is an effective method to control the release of H_2O_2 and improve the stability of GO. According to Tzanov, Costa, Gubitz, and Cavaco-Paulo (2002), immobilized GO can control the release of H_2O_2 and be used for at least 5 times for textile bleaching. But there are few people applying the immobilized GO to control the release of H_2O_2 in bakery. Wang, Zhu, and Zhou (2011) has proved that GO immobilized in alginate–chitosan microcapsules has good storage stabilities with 70.4% GO activity remained after over 2 months. This investigation has suggested a promising way to prepare potential breadmaking improver by immobilization of GO.

Many researchers (Dash, Chiellini, Ottenbrite, & Chiellini, 2011; Park, Saravanakumar, Kim, & Kwon, 2010) have focused on drug delivery by Chitosan-TPP nanoparticles. In the present study, CSTPP-immobilized GO was prepared as the breadmaking improver. Immobilization conditions including the viscosity of chitosan, ratio of chitosan to TPP (w/w) and the concentration of GO were optimized. The H_2O_2-release profile and appearance of immobilized GO were characterized. Finally, the improvement of bread quality with the addition of CSTPP-GO was examined.

2. Materials and methods

2.1. Materials

Commercial wheat flour (moisture 13.8%, ash 0.42%, protein 10.2% w/w) was obtained from Wuxi Rongguo Flour Mill (Wuxi, Jiangsu, China). Glucose oxidase (10,000 U/g) Gluzyme mono 10000 BG was generously gifted by Novozymes (Bagsvaerd, Denmark). Butter, active dry yeast, commercial sugar and salt were obtained from the local market (Wuxi, Jiangsu, China). Food-grade chitosans (deacetylation degree 90%) with different viscosity (100, 700, 1200, 2200 cP, respectively) were purchased from Pan'an Chitosan Product Co. (Pan'an, Zhejiang, China). Sodium tripolyphosphate (analytical grade) was obtained from Sinopharm Chemical Reagent (Beijing, China). Potassium bromate was obtained from Shantou Xilong Chemical Factory (Shantou, Guangdong, China). All the other chemicals were of analytical grade. All solutions were prepared with distilled water.

2.2. Immobilization of GO on CS-TPP microparticles

Immobilization of GO on CS-TPP microparticles was performed by ionotropic gelation according to the reported method (Jonassen, Kjøniksen, & Hiorth, 2012; Shu & Zhu, 2002) with slight modifications.

Chitosan (0.5% w/v) was dissolved in 1% (w/v) acetic acid aqueous solution and insoluble impurities were removed by filtration. TPP aqueous solution (2.5 mg/mL) was filtered to remove impurities by 0.45 μm membrane before it was added dropwise into the chitosan solution by a constant flow pump with thorough stirring at room temperature. Subsequently, GO in 0.1 mol/L pH 5.6 phosphate buffer was added into the chitosan-TPP solution and the mixture solution was stirred for another 1 h. At last, the chitosan-TPP-GO suspension was then spray dried to obtain the TPP cross-linked chitosan microspheres loaded with GO. The spray drying conditions were: 180 ± 5 °C inlet temperature, 70 ± 5 °C outlet temperature and 10 mL/min liquid flow rate. The dried product was finally collected and stored in a desiccator with silica gel at room temperature.

2.3. Enzyme assay

The activities of free GO and CSTPP-GO were measured using indirect oxidation of o-dianisidine by horseradish peroxidase (HRP). The assays were performed according to the method developed by Bergmeyer (Bergmeyer & Grabi, 1988, chap. 2; Simpson, Jordaan, Gardiner, & Whiteley, 2007). Reaction mixtures contained 0.006% (w/v) o-dianisidine·2HCl in 0.1 mol/L pH 7.0 potassium phosphate buffer, 10% (w/v) β-D-glucose in distilled water and 60 U/mL HRP in 0.1 mol/L pH 7.0 potassium phosphate buffer. They were mixed immediately prior to assaying for GO in the ratio 2.4:0.5:0.1, respectively. The reaction was initiated by the addition of 0.1 mL of GO (0.15–0.2 U/mL), and ended with 3 mL of the final reagent (0.5 mol/L H_2SO_4) 5 min later. The absorbance of the reaction was read at 436 nm against a blank consisting 0.1 mL pH 7.0 potassium phosphate buffer replace GO. One unit of GO activity was defined as the amount of enzyme that catalyses the conversion of 1 μmol β-D-glucose to D-gluconic acid and H_2O_2 per minute at 25 °C and pH 7.0.

The enzyme activity recovery was calculated using Eq. (1).

$$\text{Enzyme activity recovery}\,(\%) = \frac{\text{Calculated CSTPP-GO concentration}}{\text{Theoretical GO concentration}} \times 100 \quad (1)$$

2.4. Characterization of CS-TPP GO

2.4.1. Microstucture of CS-TPP GO

Scanning electron microscopy (SEM) was used to characterize the surface and shape of the spray-dried CSTPP-GO microparticles. The samples were coated for 70 s under an argon atmosphere with gold–palladium (sputter-coater) and examined with a scanning electron microscope (Quanta 200F, FEI, Hillsboro, OR, USA).Photographs were taken at a high voltage of 15.0 kv and a magnification of 1.2 or 2 or 5 or 10 or 15 k. The working distance was 15.9 nm.

2.4.2. Stability of CS-TPP GO

The stability of the CS-TPP GO was determined by the enzyme activity which was investigated every week during 3 months.

2.4.3. In vitro release of hydrogen peroxide

The hydrogen peroxide (H_2O_2) released from enzymatic catalysis was analyzed by colorimetric method (Graf & Penniston, 1980).

2.5. Breadmaking procedure and bread analysis

2.5.1. Breadmaking

The breadmaking basic formulation used included: 100 g commercial wheat flour, 15 g commercial sugar, 1 g salt, 1.5 g active dry yeast, 57.3 g water (optimum level), 8 g butter. And other additives such as free GO 400 U/kg wheat flour, or $KBrO_3$ 60 mg/kg wheat flour, or CSTPP-GO 400 U/kg wheat flour were added in the formulation according to the different experiments, respectively. These additives were added before the mixing step. The ingredients (wheat flour, sugar, salt, yeast and additives) were blended for 5 min in a mixer and then water was added and mixed with all the ingredients to develop dough. Afterwards, butter was added into the dough and mixed until dough development under the optimal conditions. The resulting dough was allowed to rest for 15 min for the first fermentation at 25 °C and 75% relative humidity. After first fermentation, the dough was divided into 100 g pieces, rounded, moulded and placed in baking pans. The pieces were then proofed for 90 min at 38 °C and 85% relative humidity and baked at 210 °C for 15 min. Bread was removed from the pans and cooled at room temperature, then stored at room temperature in plastic bags for measurement and further analysis.