Food Control 42 (2014) 220–224

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

A rapid detection method for microbial spoilage of agro-products based on catalase activity

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ARTICLE INFO

Article history:
Received 31 October 2013
Received in revised form
11 February 2014
Accepted 14 February 2014
Available online 24 February 2014

Keywords:
Catalase
Agro-product
Microbial count
Detection method

ABSTRACT

Microbial spoilage of agro-products should be monitored for the quality and safety of food. In this study, we successfully developed a catalase detection device employing an oxygen sensor to monitor the microbial spoilage of milk and stored grain samples. The results showed that catalase activities correlated well (>0.99) with the microbial counts of four pure fungi and aerobic bacteria that commonly cause spoilage of stored grains and milk, respectively. The correlation coefficient between catalase activity and microbial counts reached 0.99 in stored grain samples and 0.96 in milk samples, and the changes in the microbial counts could be effectively monitored by detection of catalase activities during the microbial spoilage of stored wheat and milk. The detection of catalase activity could be accomplished in 10 min to reveal mold counts in agro-products, and it showed higher sensitivity than the conventional agar plate method. Therefore, the proposed catalase activity-based method could be rapidly and sensitively applied to the detection of microbial spoilage of agro-products.

1. Introduction

Microbial spoilage of agro-products is a persistent problem worldwide. Until now, the detection and control of microorganisms related to food safety has attracted increasing attention from food manufacturers and supervisors (Schothorst, Zwietering, Ross, Buchanan, & Cole, 2009). To meet the requirements of production of high-quality food, the number and activity of microorganisms in raw materials during their production, storage, and transportation need to be controlled. For instance, for the production of certain flour foods, the maximum allowed counts of thermophiles and *Bacillus* spp. have been specified (Berghofer, Hocking, Miskelly, & Jansson, 2003). In addition, to ensure the quality of dairy products, microbial counts in raw milk have been discriminatively restricted for the manufacture of different types of products. All these demand for rapid and sensitive detection methods of microbial counts and their activities in the manufacturing process of agro-products, from the raw material to the end product.

Conventional methods rely on the cultivation of microbes for their multiplication to visible colonies, which is very time-consuming and labor-intensive to meet the demands of the food industry (Rosmini, Signorini, Schneider, & Bonazza, 2004). In recent years, several rapid detection methods have been developed to monitor the presence of microorganisms in agro-products such as PCR-based methods (Fernández-No et al., 2011; Settanni & Corsetti, 2007), ATP bioluminescence methods (Valat et al., 2003), and catalase activity-based methods (Hirvi & Griffiths, 1998; Sippy, Luxton, Lewis, & Cowell, 2003) that are used for the analysis of mold, bacteria, and pathogens in agro-products. Among these, the catalase activity-based method is a convenient detection method. It is mainly based on the capability of catalases to hydrolyze H_2O_2 into water and oxygen. To date, a diversity of catalase activity-based detection methods have been developed, involving the use of biosensors (Serra et al., 2008), electrochemical strategies (Sippy et al., 2003), and Pasteur pipette techniques (Rebecca & Vivian, 2011), which display a great potential to detect microbial counts and their activities in agro-products.

In this study, we developed a rapid catalase activity-based method employing an oxygen biosensor and investigated its feasibility to detect microbial spoilage of agro-products in comparison with the conventional agar plate method. Moreover, the relationship between catalase activity and microbial counts in

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http://dx.doi.org/10.1016/j.foodcont.2014.02.029
0956-7135/

agro-products and the effect of microbial growth on catalase activity were studied.

2. Materials and methods

2.1. Materials and chemical

Commercial wheat, corn, and rice were purchased from a local market. Milk was sampled from a diary plant in Zhengzhou. *Aspergillus glaucus*, *Aspergillus candidus*, *Aspergillus flavus*, *Penicillium cyclopium*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus thermophilus* were conserved in our laboratory. All other analytical reagents were obtained from general commercial suppliers.

2.2. Microbiological analysis of samples

Milk samples (1 mL) were diluted 10^2–10^6 times and plated on Luria–Bertani (LB) medium containing 1% yeast extract (OXOID), 0.5% tryptone (OXOID), 1% sodium chloride, and 2% agar powder. After overnight incubation at 37 °C, the number of colonies was counted. For identification of mold counts and microfloral species from cereal grains, 25 g of each sample was withdrawn and added to 225 mL of sterile distilled water in a 1-L flask bottle, which was shaken for 30 min on a flatbed shaker at 300 excursions per minute to completely dissolve the microorganism in the grain samples. The sample solution was diluted 10^2–10^6 times, and 1 mL of each diluted solution was plated on modified Czapek's medium (3% sucrose, 6% NaCl, 0.1% K_2HPO_4, 0.3% $NaNO_3$, 0.05% KCl, 0.05% $MgSO_4 \cdot 7H_20$, and 2% agar powder) and incubated at 28 °C for 7 d. The mold count was calculated at day 5, and the microfloral species in wheat were identified using a microscope at day 7 (Cai, Zhang, Zhai, Huang, & Wei, 2013). Mold species with counts >20% of the total mold counts in grain samples were considered as dominant molds. The moisture content of the grain samples was determined by placing 3 g of wheat in a hot-air oven for 3 h at 105 °C (GB/T 5497–1985; National Standard of China). The moisture content of wheat was calculated as the weight loss percentage of the oven-dried wheat.

2.3. Detection device and catalase activity assay

The catalase detection device designed employed an oxygen sensor, and it mainly consisted of an oxygen biosensor, heater, heat sensor, magnetic stirrer, 1-L reactor, control center, and display screen (Fig. 1). The heater, heat sensor, and magnetic stirrer components provided a stable reaction condition, and the oxygen sensor detected oxygen in the reacting solution, which was released from H_2O_2 by catalase and produced an electric current. The sample for assaying the catalase activity was centrifuged at 300 rpm for 10 min at 30 °C, and the catalase activity was determined automatically and showed on a display screen. For the assay of the catalase activity in grains, 100 g of grain sample was washed with 160 mL of sterile distilled water in a 1-L flask bottle for three times. At each washing step, the sample was shaken on a flatbed shaker at 300 excursions per minute for 5 min to completely dissolve the microorganisms in the sample. The washing eluate was recovered and diluted to 500 mL for the analysis of catalase activity. The diluted eluate was added to the reactor, and 10 mL of H_2O_2 was added to start the reaction. One unit of catalase activity was defined as the amount of enzyme releasing oxygen from H_2O_2 to produce a 1-μA electric current in the oxygen sensor under the assay conditions. For assay of catalase activity in milk, 500 mL of milk was added to the reactor, and the reaction was carried out as described above.

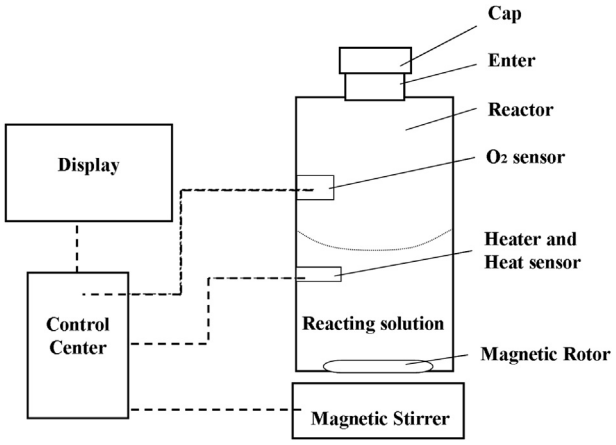

Fig. 1. The schematic diagram of catalase activity detection device.

Table 1
Linear correlation coefficients of spore counts of diluted *A. glaucus*, *A. candidus*, *A. flavus*, *P. cyclopium* detected by the conventional agar plate method.

Mold	Mold count ($\times 10^4$ cfu/mL)				r^a
	100%	75%	50%	25%	
A. glaucus	371.5 ± 11.1	179.5 ± 10.7	94.3 ± 4.1	33.3 ± 3.6	0.93
A. candidus	352.0 ± 17.5	136.3 ± 9.0	82.0 ± 2.5	22.0 ± 2.1	0.88
A. flavus	364.0 ± 10.4	139.3 ± 7.8	75.7 ± 4.7	22.7 ± 2.7	0.87
P. cyclopium	400.3 ± 10.5	186.0 ± 5.1	112.3 ± 2.8	32.7 ± 2.7	0.93

[a] r is the linear correlation coefficient.

2.4. Statistical analysis

All experiments were performed in triplicate. Analysis of variance and correlation coefficient of catalase activity and microbial count were analyzed using SPSS 17.0 (SPSS, Chicago, Illinois, USA). Least significant difference (LSD) method was used to compare the changes of catalase activities and microbial counts.

3. Results and discussion

3.1. The effect of the microbial counts on catalase activity

Most fungi are catalase-positive microorganisms (Hansberg, Salas-Lizana, & Domínguez, 2012). Because it was proposed that a good correlation coefficient exists between catalase activity and fungal counts, the catalase activity-based method could be applied to rapidly detect microbial spoilage of agro-products. Therefore, a catalase detection device employing an oxygen sensor was designed as described above, using which the catalase activity could be qualitatively detected under a stable reaction condition. The integration of the oxygen sensor, heater, heat sensor, and magnetic stirrer improve the reproducibility of this method as compared with the method using Pasteur pipettes (Kang, Dougherty, Clark, & Costello, 2002), the performance of which might be significantly affected by the ambient temperature. To validate the feasibility of the device in detecting catalase in microorganisms, four molds (i.e., *A. glaucus*, *A. candidus*, *A. flavus,* and *P. cyclopium*) were selected to evaluate the correlation between microbial counts and catalase activity. These molds commonly exist in agro-products naturally, and their growth always has a significant effect on the quality of grains (Cai, Wang, & Huang, 2012; Filtenborg, Frisvad, & Thrane, 1996; Lanier, Richard, & Heutte,