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Microbial decontamination of wheat grain with superheated steam

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ABSTRACT

Wheat grain was treated with superheated steam (SS) for microbial decontamination purpose at various steam temperature (110–200 °C), processing time (10–80 s) and velocities (7.5 and 15.0 m^3/h) with or without tempering. The decontamination was efficient at high SS velocity. The residual levels of bacteria decreased rapidly during the first 10–40 s especially at high SS-treatment temperatures, and then followed by a 'tailing' period. High temperatures (170 or 200 °C) killed *Bacillus* spp. more effectively than low temperatures (110 or 140 °C) did. After a SS treatment at 200 °C, with 15.0 m^3/h for 80 s, approximate 99.9% of total bacteria and 81.8% of *Bacillus* spp. were reduced, respectively. Moulds were completely removed by SS-processing at 110 °C or higher temperatures, for 30 s or longer. Moisture tempering could increase the levels of microorganisms in grain, while promoted the microbial decontamination activity. Short time SS-processing on raw wheat kernels could be an efficient method to inactivate the microorganisms and moulds. SS should be applied to clean wheat between the processes of milling and tempering for producing clean wheat flour.

1. Introduction

Cereal grains are the most important food commodity for the worlds population and can represent up to 80% of the diet in some cultures (Maga, 1978; Olsson, Börjesson, Lundstedt, & Schnürer, 2000). Grains are often contaminated with microorganisms and moulds during harvesting, transportation and storage, which decrease the safety and quality of the grains (Dack, 1961; Li, Li, Luo, & Yoshizawa, 2002). For example, the lactic and coliform bacteria can make wet mash of grains suffering acid fermentation (Laca, Mousia, Díaz, Webb, & Pandiella, 2006). Moulds are the most important spoilage organisms in cereal grains as they can not only reduce the nutritional value, but also decrease the germination properties, cause dry matter loss, heating, off-odours, and in the worst case, form mycotoxins and allergenic spores (Olsson et al., 2000; Schnürer, Olsson, & Börjesson, 1999). *Bacillus* spp. is hard to be eliminated during food processes because of its spore-forming ability. Moreover, the spores may then germinate if the grains are left at room temperature and even at refrigeration temperatures (Iurlina, Saiz, Fuselli, & Fritz, 2006).

Milling can reduce most of the microbial contamination (Laca et al., 2006), but part of the microorganisms and moulds can still remain in flour and have strong influence on the ultimate quality of milling end products (Berghofer, Hocking, Miskelly, & Jansson, 2003). Moreover, when whole wheat flour products are getting more and more popular, a huge amount of microorganisms and moulds existing in the bran make the whole grain flour deteriorated easily. In order to acquire clean wheat flour to meet the demand of consumers and industries, and to improve the storage quality of wheat flour and its products, the microbial decontamination process of wheat grain is needed in many cases.

Beside conventional hot air drying which is considered to be time and energy consuming, several methods including ozone (Tiwari et al., 2010), antimicrobial agents (Periago & Moezelaar, 2001), yeast (Ädel Druvefors & Schnürer, 2005), pulsed light (Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2010; Zhang, Oh, Cisneros-Zevallos, & Akbulut, 2013), gamma (Aziz, Souzan, & Shahin Azza, 2006) and infrared radiation (Andrejko, Grochowicz, Goździewska, & Kobus, 2011; Krishnamurthy, Khurana, Soojin, Irudayaraj, & Demirci, 2008) have been used to investigate the reducing of microorganisms and moulds in grain. However, limited effects, residual of chemicals and irradiation are main problems to prevent

Abbreviations: SS, superheated steam; SD, standard deviation; CFU, colony-forming unit.

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them from usage. Superheated steam (SS) pasteurization as an emerging technology has recently attracted a lot of attention for its advantages. During SS processing, large amount of heat transferred to food when steam condenses on food surfaces, which rapidly increases the surface temperature (Ban, Yoon, & Kang, 2014; James, Göksoy, Corry, & James, 2000). And the reverse process of moisture transfer that condensation followed by evaporation of moisture on the materials produces characteristic food-processing property of SS (Bari et al., 2010; Iyota et al., 2005). SS processing is more efficient than saturated steam and hot air processing as SS has a higher enthalpy (Ban et al., 2014). The treatment generates and performs quickly in an oxygen-free environment (Bari et al., 2010), in which the foodstuff can maintain its natural physicochemical properties (Wu et al., 2014). Recently, SS has been used to inactivate *Salmonella* inoculated on raw almonds (Bari et al., 2010), *Listeria innocua* inoculated on chicken skin (Kondjoyan & Portanguen, 2008), *Escherichia coli* O157:H7 and *Listeria monocytogenes* biofilms on polyvinyl chloride and stainless steel (Ban et al., 2014). It also reduced effectively the viability of *Geobacillus stearothermophilus* spores (Head, Cenkowski, Holley, & Blank, 2008) and deoxynivalenol concentration in grain (Pronyk, Cenkowski, & Abramson, 2006). However, the inactivation effect on total bacteria, *Bacillus* spp. and moulds in grain has rarely been studied.

The objective of this study was to investigate the microbial decontamination effect of SS processing on wheat grain at various SS temperature, velocity, processing time and tempering conditions so as to inactivate microorganisms of wheat flour.

2. Materials and methods

2.1. Materials

The wheat (Xinmai-26) was harvested and obtained from Xinliang grain processing Corporation in June, 2014 (Henan, China). The initial moisture content of the wheat grain was 12.1% (w.b).

2.2. Tempering of wheat kernels

The moisture content of the wheat kernels were adjusted to 16.0% (w.b) according to the method of Head, Cenkowski, Arntfield, and Henderson (2010). A pre-determined amount of sterile distilled water was added to wheat kernels in a sterilized glass jar. The jar was then sealed and tumbled for at least 60 s. For up to 1 h after the addition of water, the jar was periodically tumbled for 60 s at 10 min intervals. Then the jars were kept at 4 °C overnight, to ensure even distribution of the added moisture across individual wheat grain.

2.3. Superheated steam processing

A continuous SS processing system developed by Laboratory of Cereal Science at China Agricultural University was used in this study (Fig. 1). Saturated steam was generated by a boiler (1). Flow rate of the steam was controlled by a valve (2). The steam from the boiler was heated to a desired temperature in a superheater (3), delivered through steam conveying pipe (4) to steam distributing system (5) and (6) which distribute on both of upside and downside of the metal mesh conveyor belt. There were lots of small holes on the steam distributing system. SS was sprayed to the sample tray (8) from both sides. When SS come out of the processing chamber, it was directed to a condenser (9). Wheat grain (200 g) were scattered on the metal mesh sample tray and conveyed into the processing chamber. The processing time was controlled by setting the speed of conveyor belt. The temperature in the processing chamber was measured using digital thermocouples (TES, TES-1310, Taiwan, China). Control panel (12) was used to monitor temperature data and control the temperature of SS as well as the speed of conveyor belt.

Processing was conducted at atmospheric pressure in the SS chamber at four nominal steam temperatures (110, 140, 170, and 200 °C) and two steam velocities (7.5 and 15.0 m^3/h), and the processing time ranged from 10 to 80 s. The mass was recorded before and after processing. Final moisture content of sample was calculated by taking the change in mass during processing (Cenkowski, Pronyk, Zmidzinska, & Muir, 2007).

After processing, wheat samples were cooled at room temperature. About 150 g samples were ground to whole wheat flour using a high speed grinding mill (Huanyatianyuan, HY-04A, China). The mill had been cleaned thoroughly with ethanol before the process to minimize the contamination of microorganisms from the equipment. All the flour samples were sealed in aseptic bags and refrigerated at 4 °C until usage.

2.4. Analysis of microbial concentration

About 25 g of whole wheat flour was aseptically transferred to a stomacher bag, and 225 mL of sterilized physiological saline solution was added and stomached for 2 min. One milliliter of this homogenate was successively diluted 1:10 in sterilized physiological saline solution until the required bacterial concentration was reached. And 0.1 mL aliquots of homogenate or diluted suspension were inoculated on nutrient agar plates and incubated at 37 °C (Laca et al., 2006). Total bacterial content in the samples was quantified by counting the colony-forming units (CFU) after 48 h. To quantify the number of moulds colony-forming units, 0.1 mL aliquots of homogenate or diluted suspension were inoculated on malt extract agar supplemented with 100 ppm of chloramphenicol (MEAC). Mould colony forming units were determined after 5 days of incubation at 25 °C (Laca et al., 2006; Petersson & Schnürer, 1999). *Bacillus* spp. was enumerated by spread-plating homogenates and dilutions onto dextrose tryptone agar. The plates were incubated at 37 °C for 72 h. The colonies were selected and confirmed by Gram staining and observing the shape and position of spores (Berghofer et al., 2003; Iurlina et al., 2006).

2.5. Statistical analysis

The results were reported as mean ± standard deviation (SD). Error bars indicated standard deviation of three measurements. Data was analyzed by analysis of variance (ANOVA) using SPSS version 17.0 (SPSS Institute, Chicago, USA) and the separation of means was tested by Duncan's multiple range test at a probability level of $P < 0.05$.

3. Results and discussion

3.1. Effect of superheated steam velocity on microbial decontamination

The survivals of total bacteria (Fig. 2a), moulds (Fig. 2b) and *Bacillus* spp. (Fig. 2c) in wheat kernels after SS-treatment at two velocities were shown in Fig. 2. Both residual concentrations of bacteria, moulds and *Bacillus* spp. were decreased with the increase of processing time. However, the decreasing rates of bacteria (Fig. 2a), moulds (Fig. 2b) and *Bacillus* spp. (Fig. 2c) at 15.0 m^3/h were higher than that at 7.5 m^3/h. Higher SS velocity induced a larger amount of condensed steam and a stronger gas radiation, and hence made the grain temperature increased more quickly and had a higher efficiency of microbial inactivation. As the SS velocity of 15.0 m^3/h was more efficient in eliminating bacteria, moulds and *Bacillus* spp., it was used in the following experiments.