ผลของการใช้โอโซนร่วมกับยูวี-ซีหรือละอองลอยจากสารละลายไฮโดรเจนเปอร์ออกไซด์ในการกำจัดเชื้อเอสเชอริเชียโคไลและเอสเปอร์จิลลัสไนเจอร์ที่ปนเปื้อนในโรงงานอุตสาหกรรมก๋วยเตี๋ยว

EFFECTS OF COMBINED OZONATION AND UV-C TREATMENT OR VAPORED HYDROGEN PEROXIDE FUMIGATION TO INACTIVATE *ESCHERICHIA COLI* AND *ASPERJILLUS NIGER* CONTAMINANTS IN RICE NOODLE INDUSTRY

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาสภาวะที่เหมาะสมในการทำลายเชื้อในการผลิตแป้งที่ใช้ในอุตสาหกรรมเส้นก๋วยเตี๋ยวโดยการใช้โอโซนร่วมกับยูวีที่มีต่อปริมาณแบคทีเรียทั้งหมด ยีสต์/รา และอีโคไลที่ปนเปื้อนในน้ำแป้งจากกระบวนการผลิตเส้นก๋วยเตี๋ยว และเพื่อหาสภาวะที่เหมาะสมของไฮโดรเจนเปอร์ออกไซด์ที่ใช้ในการฆ่าเชื้อในห้องที่ใช้ในการผลิตเส้นก๋วยเตี๋ยว

เชื้ออีโคไลและเชื้อแอสเปอร์จิลลัส ไนเจอร์ ถูกนำมาใช้ทดลองเพื่อเป็นต้นแบบของเชื้อแบคทีเรียและเชื้อรา ทั้งนี้ต้นแบบของกระบวนการฆ่าเชื้อร่วมกันของเครื่องกำเนิดโอโซน (2 ลิตรต่อนาที) และยูวี (45 วัตต์) โดยทำการทดสอบระบบกับน้ำแป้ง ใช้ปริมาณเริ่มต้นของเชื้ออีโคไลและเชื้อแอสเปอร์จิลลัส ไนเจอร์ ที่ประมาณ 7 ล็อกโคโลนีต่อมิลลิลิตร ในการทดลองอัตราส่วนของแป้งต่อน้ำที่ทำการศึกษาถูกเตรียมที่ร้อยละ 0, 20, 45, 75 และ 100 น้ำแป้งที่ปริมาตร 15 ลิตรถูกเติมเชื้ออีโคไล หรือเชื้อแอสเปอร์จิลลัสที่ 7 ล็อกโคโลนีต่อมิลลิลิตร ปริมาตร 200 มิลลิลิตร โดยอัตราการไหลของมวลเป็น 0.3 กิโลกรัมต่อวินาที ในระหว่างทำการฆ่าเชื้อมีการเก็บตัวอย่างที่เวลา 0, 5, 10, 20, 30 และ 40 นาที ปริมาณของแบคทีเรียทั้งหมด เชื้ออีโคไลและยีสต์/รา ถูกวิเคราะห์ด้วยตัวอย่างปริมาตร 1 มิลลิลิตร

ผลการวิจัยพบว่ารูปแบบการลดลงของปริมาณของแบคทีเรียทั้งหมด ยีสต์/ราและอีโคไลขึ้นกับเวลาและอัตราส่วนของแป้งต่อน้ำ โดยเมื่อใช้ระบบโอโซนร่วมกับยูวีเป็นเวลา 40 นาที ปริมาณแบคทีเรียทั้งหมด ยีสต์/รา และอีโคไล ลดลงไปได้จนถึง 0 ล็อกโคโลนีต่อมิลลิลิตรที่ความเข้มข้นของน้ำแป้งร้อยละ 20 สำหรับความเข้มข้นของน้ำแป้งมากกว่าร้อยละ 20 การใช้โอโซนร่วมกับยูวีสามารถที่จะลดปริมาณแบคทีเรียทั้งหมดลงได้ 3 - 2 ล็อกโคโลนีต่อมิลลิลิตร ตามลำดับ อย่างไรก็ตามในการทดลองกับน้ำแป้งที่ไม่ได้ผ่านการเจือจาง ระบบโอโซนและยูวีที่ประดิษฐ์ขึ้นในงานวิจัยนี้ไม่สามารถลดปริมาณแบคทีเรียทั้งหมด ยีสต์/รา และอีโคไลได้ เนื่องจากน้ำแป้งมีการตกตะกอน มีความขุ่นและความหนืดสูงมาก การทดลองพ่นไอระเหยไฮโดรเจนเปอร์ออกไซด์ที่ความเข้มข้นร้อยละ 1 - 5 ในห้องทดสอบขนาด 1 ลูกบาศก์เมตรพบว่า ไอระเหยไฮโดรเจนเปอร์ออกไซด์สามารถลดปริมาณอีโคไลภายใน 2 นาทีจาก 10^9 ถึง 10^5 โคโลนีต่อมิลลิลิตร หรือ 4 ล็อก จากการทดลองแสดงให้เห็นว่าใช้เวลาน้อยในการกำจัดเชื้อแบคทีเรียจากการกระจายของละอองไฮโดรเจนเปอร์ออกไซด์ ไอละอองลอยดังกล่าวยังมีประสิทธิภาพในการฆ่าเชื้ออีโคไลและยังสามารถลดจำนวนของโคโลนีเชื้อราเมื่อความเข้มข้นของละอองลอยไฮโดรเจนเปอร์ออกไซด์สูงขึ้น ความเข้มข้นของไฮโดรเจนเปอร์ออกไซด์ร้อยละ 3 และ 5 ที่เวลาการกระจายของละอองลอย 4 – 6 นาที สามารถที่จะเพิ่มความสามารถในการทำลายแอสเปอร์จิลลัส

ดังนั้นการใช้ไอระเหยไฮโดรเจนเปอร์ออกไซด์ในการฆ่าเชื้อพบว่าสามารถที่จะลดการปนเปื้อนของแบคทีเรียและราในห้องทดลอง โดยไฮโดรเจนเปอร์ออกไซด์สามารถระเหยได้ง่ายและไม่เสถียร สามารถแตกตัวและสลายด้วยการเปลี่ยนสภาพเป็นน้ำและออกซิเจน ไม่ก่อให้เกิดสารพิษตกค้างต่อผู้บริโภคและสิ่งแวดล้อม และสำหรับการใช้โอโซนร่วมกับยูวีถูกพบว่ามีความเหมาะสมในการฆ่าเชื้อตัวอย่างที่มีความขุ่นและมีตะกอนลักษณะเป็นน้ำแป้ง โดยมีประสิทธิภาพฆ่าเชื้อดีกว่าการใช้ยูวีหรือโอโซนอย่างใดอย่างหนึ่ง ทั้งนี้การใช้โอโซนร่วมกับยูวีสามารถที่จะทำลายเชื้ออีโคโลและเชื้อราแอสเปอร์จิลลัสไนเจอร์ได้เป็นอย่างดี

คำสำคัญ: การใช้โอโซนร่วมกับยูวี-ซี ไอระเหยไฮโดรเจนเปอร์ออกไซด์ การยับยั้ง เชื้อเอสเชอริเชีย โคไล เชื้อแอสเปอร์จิลลัส ไนเจอร์

Abstract

The ability of ozonation and UV-C applied to eradicate total plate count, yeast/mold and *Escherichia coli* contamination were tested on a flour slurry and to study the application of vapored hydrogen peroxide (VHP) for sanitizing the chamber.

Method: A prototype system, consisting of ozone generator (2 L/min) and UV sterilizer (45 W), was fabricated and tested on flour slurry systems using the same initial *E. coli* and *A. niger* concentration. In this experiment, the ratios of flour slurry to water were prepared at 0, 20, 45, 75 and 100%. The flour slurry (15 L) were spiked with *E. coli* or *A. niger* at 7 log CFU/mL approximately 200 mL. The circulation mass flow rates were fixed at 0.3 kg/s. Sampling time intervals were 0, 5, 10, 20, 30 and 40 min. Total plate count (TPC), yeast/mold and *E. coli* was achieved by taking 1 mL aliquot of sample.

Result: The result showed the destruction profiles of TPC, yeast/mold and *E. coli* counts as a function of time and ratio of flour slurry. After Ozone and UV treatments for 40 min, the TPC, yeast/mold and *E. coli* counts were reduced to 0 log CFU/mL in the 20% of flour slurry. For the flour slurry concentrations higher than 20%, the ozone with UV treatment was able to bring down the microbial counts to only 3 - 2 log reductions, respectively. However, the ozone with the UV treatment was not able to reduce

TPC, yeast/mold and *E. coli* counts at the 100% of flour slurry treatment because it has high solid loading, turbidity and viscosity. In all treatments with 1-5% hydrogen peroxide (H_2O_2), there was an instant drop of *E. coli* numbers within the first 2 min from 10^9 to 10^5 CFU/mL or 4 log reductions. It was shown that even at shorter time periods could eliminate organisms as long as H_2O_2 was well distributed and maintained at an effective concentration to destroy *E. coli* cells. The number of the fungal colonies decreased when the concentration of VHP were increased. When the addition of H_2O_2 at 3 and 5% concentrations at exposure times of 4-6 min produced incremental increases in *A. niger* destruction.

Conclusion: The combined ozone/UV treatments have been shown to be more appropriate treatments for cloudy and turbid medium, like flour slurry. The use of combined treatment was able to achieve zero *E. coli* and *A. niger* count. For fumigation with VHP, we found that VHP decontamination reduce bacterial and fungal contamination in chamber. H_2O_2 is easily evaporated or destroyed after use (readily decomposing into water and oxygen), has no unpleasant lingering odor, and poses minimal safety problems for workers if handled properly.

Keywords: Combined Ozonation and UV-C Treatment, Vaporized Hydrogen Peroxide, Inactivate, *Escherichia coli Aspergillus niger*

Introduction

Noodle, a traditional staple food in many Asian countries, has been consumed for thousands of years. It is becoming increasingly popular worldwide for its convenience, nutritional quality, and palatability [1]. Generally, rice noodles are prepared from flour, water and various optional ingredients. Rice flour is kneaded in the presence of water to form dough and is then sheeted, compounded, steamed and cut to form a noodle strands. For noodle industry, flour raw material always contains different natural micro-flora and fauna [2]. During production delay or storage, bacterial cells can multiply resulting in increasing number of viable cells that lead to flour slurry spoilage. To prolong the fermentation of rice flour slurry for noodle production, the rice flour slurry need to destroy spoilage microorganisms and to prevent further microbial growth. For food industry, the microbiological quality of food is commonly assessed by determining TPC and yeast/mold [3, 4]. The TPC provides a wealth of valuable information regarding freshness and hygienic conditions of food and raw materials and production sanitation, controlled by many food regulatory guidelines. Especially, *E. coli* is usually as indicates poor hygienic practice in handling and production operations, inadequate storage and post-process contamination [5, 6]. The presence of *E. coli* O157:H7 bacteria, in particular, can cause a lethal illness called *E. coli* enteritis and a wide range of symptoms (e.g., diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome). The needs to improve both quality and safety of food products have driven the industry to innovate new processes to achieve all desirable qualities and minimal affect the operating cost of the whole production. An application of non-thermal disinfection technology by combining ozone and ultraviolet-C (UV-C) was developed and shown effective in wash water for the fresh-cut vegetable industry [7]. The successful implement of this technology should have the least impact to the physicochemical properties of the flour slurry while the growth of spoilage microbes was ceased. The application of ozone with UV technology serves as a good combination

for disinfection and/or oxidation of drinking water and food products with respect to micro pollutant and microbial destruction [8]. The hydroxyl radicals in the ozone/UV process were generated by photocatalytic reaction [9]. Its synergistic effects in sequential or combined disinfection processes were shown to be beneficial in improving disinfection effectiveness, reducing operating costs and decreasing disinfection by product formation [3].

Not only the bacteria can growth in the flour slurry but also several literatures suggested that bacteria have the ability to multiply and attach to both engineered plastic and metal surfaces (e.g., polystyrene, polypropylene, and stainless steel) [10, 11]. At the presence of high microbial counts, cross-contamination allows the transfer of microorganisms (bacteria, virus, parasites, or fungi) from a contaminated item to a non-contaminated item. Unfortunately, people in the food production line occasionally have little awareness of surfaces and equipment with high microbial contamination which is considered a high risk factor to transmission and the occurrence of sporadic foodborne outbreaks [12, 13]. High bacterial and yeast/mold contamination on any food processing areas can signify poor sanitation practices and generate detrimental consequences on human health. For indoor working space or food production environment, enriched samples of cultures showed confluent growth of bacteria and molds (e.g., Bacillus species bacteria, Mucor, Penicillium, and Aspergillus species mold) [14]. Although many of these microorganism were considered common microflora and have a strong ecological link with human food supplies, some were found to be an opportunistic reason for infections of humans. For example, excessive inhalation of *A. niger*, cause severe lung problems in humans [15, 16]. Traditional fumigation with formaldehyde is effective in disinfecting indoor areas, hospitals, and food processing environment. Formaldehyde fumigation is toxic and harmful for human, animals and the environment [17, 18]; however, it is still ubiquitously applied to disinfect surfaces, especially in resource-poor settings. H_2O_2 vaporization has recently been explored and tested to be a good fumigant replacement of formaldehyde [19]. The use of VHP is coupled with ultrasonic aerosolization to create and disperse a disinfectant aerosol to disinfect food processing surfaces as well as difficult-to-reach areas, especially overhead surfaces, cracks and crevasses of food equipment and so on. This technique has been proven safer, less irritating and requires shorter exposure times. This H_2O_2 fumigation have constituted a promising technology since many industries incorporated H_2O_2 solution into their routine applications ranging from wastewater treatment to microbial disinfection [20]. As a highly active biocide, H_2O_2 exhibits antimicrobial activity through the generation of hydroxyl free radicals that penetrate the cell wall to attack lipids, proteins, and DNA [21]. Owing to its non-selective biocidal property, it can inhibit viruses, spores, and fungi as well as bacteria and does not react with the organic matter to form the toxic residues [22, 23]. The reaction is terminated by forming benign by-products (e.g., oxygen and water) [24]. The AOPs employed included H_2O_2, O_3 and UV-C. The individual and combined effects of these AOPs parameters directly influence the OH• generated and consequently the degree of microbial destruction. There was a limited evidence about the efficacy of OH• mist and its effectiveness over the common sanitary indicator both bacteria and mold were observed and investigated [25].

Objectives

In this paper, the application of ozone and UV combination was performed on turbid rice flour slurry that is commonly used to prepare rice noodles. The goal was to prolong the fermentation of rice flour slurry for noodle production by using the optimal condition. The successful implement of this technology should have the least impact to the physicochemical properties of the flour slurry while the growth of spoilage microbes was ceased. Moreover, this paper aimed to demonstrate the effectiveness of highly oxidizing mist in destroying artificially spiked *E.coli* and *A. niger* contaminant on surfaces and to determine the optimal condition of hydroxyl radical aerosolization for high-risk area fumigation. The use of AOPs is coupled with ultrasonic aerosolization to disperse the highly reactive free radicals in the form of fine mist for sanitization purposes and surface disinfection.

Methods

Combined ozonation and UV-C treatment to inactivate microbiological contaminants in rice noodle industry

Microorganisms and culture preparation

E. coli; glycerol stock of *E. coli* strain (DMST 4609 received from Department of Medical Science of Thailand) was thawed and recovered in 100 mL of Tryptic Soy Broth (TSB, Difco, USA) for incubated 24 h at 37 $^{\circ}$C to reach the final cell density at 7 log CFU/mL.

A. niger; Single colony of *A. niger* (TISTR 3012 received from Department of Medical Science of Thailand) was grown in Potato Dextrose Broth (PDB, Difco, USA) and incubated to reach the final cell density at 7 log CFU/mL.

Ozone/UV/Ozone+UV treatment

UV treatment

The ultraviolet sterilizer allowed the liquid sample to flow into annular space between quartz sleeve and inside chamber wall and ensure uniform exposure of suspended microorganisms to lethal ultraviolet rays. The UV germicidal lamps (9047A481 Hanovia lamp 45 W, YUP OEM, China) operated at the radiation peak of 254 nm.

Ozone treatment

Ozone gas was generated using a water-cooled ceramic ozone tube enabling the production of ozone gas from oxygen at 16-34 g/h. The ozone flow rate was set at 2 L/min and the ozone gas was dissolved in liquid using a Venturi mixer. The final dissolved ozone concentration was validated at 4-15 ppm.

Ozone/UV treatment

The prototype ozone/UV reactor was a closed circulation system with the sample liquid transferred from the reservoir using a centrifugal sanitary pump through a Venturi gas liquid mixer followed by two sets of UV sterilizers in series (Figure 1). The holdup volume in the piping system was estimated at 5 L. The

mass flow of the pump can be adjusted using an inverter. This prototype equipment can be configured to operate using ozone and UV sterilizer independently or ozone/UV combination.

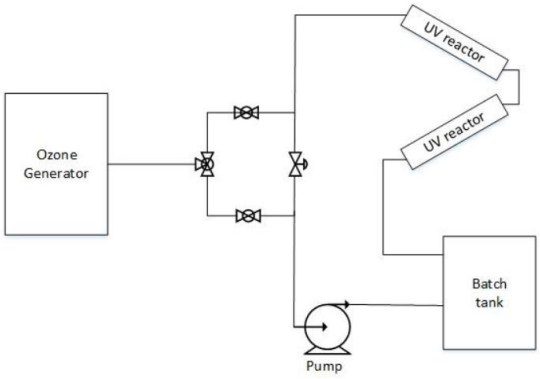


Figure 1 Schematic diagram of the Advanced Oxidation Processes (AOPs) prototype.

Flour slurry preparation and spiking

The flour slurry was mixed with water to prepare 0, 20, 45, 75, 100% (v/v) to reach the final volume of 15 L. The flour slurry samples were spiked individually with 200 mL of 7 log CFU/mL *E. coli* and *A. niger*.

Treatment conditions

The 15.2 L solution was placed in the reservoir of the Ozone/UV/Ozone+UV system in Figure 1. The circulation mass flow rates were fixed at 0.3 kg/s due to the physical constraint of the prototype equipment for different treatments of UV, ozone and ozone/UV. Sampling time intervals were 0, 5, 10, 20, 30 and 40 min.

Enumeration

TPC, yeast/mold and *E. coli* enumeration was achieved by taking 1 mL aliquot of sample and preparing serial dilution. Sample dilutions were then plated on Plate Count Agar (PCA, Difco, USA), Chromocult® coliform agar (CCA, Difco, USA) and Potato Dextrose Agar (PDA) and incubated at 37 ºC for 24 h. The colony count was performed using the micro-inoculation technique [26].

Fumigation with H_2O_2 to inactivate *Escherichia coli* and *Aspergillus niger*

Fumigation with H_2O_2

The chamber model

The experiments were conducted in a 1x1 m^3 chamber (Figure 2) containing a 250 W ultrasonic atomizer with 10 head was installed in the center of the 1 cubic meter chamber. Some circulation fans were also placed inside the cube to disperse the H_2O_2 vapor evenly.

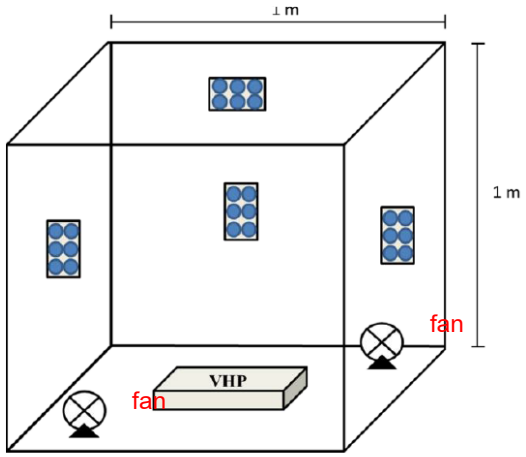


Figure 2 The chamber model of fumigation and 6 - micorewell plate setup insides this model chamber.

Sample preparation and spiking

E. coli culture was prepared in shake tubes using Tryptic Soy Broth (TSB), for *A. niger* using Potato Dextrose Broth (PDB) and incubated to reach the final cell density at 10^9 and 10^7 CFU/mL respectively. Serial dilution was done to achieve the desired initial cell concentration at 10^9 to 10^5 CFU/mL. Each testing strain was aseptically transferred into the surface of corresponding agar media in the 6-micorewell plate. Plate Count Agar (PCA) and Potato dextrose agar (PDA) were used to cultivate *E. coli and A. niger* colonies, respectively. Each inoculation volume containing 30 µL of microbial sample at was spreaded on the agar surface by glass beads

Fumigation technique

As shown in Figure 2, the inoculated agar plates at 7 log CFU/mL were attached to the side and top surfaces of the chamber. At 3 liters per hour vaporization capacity of the ultrasonic atomizer, the concentration of H_2O_2 solution was varied from 0, 1, 3, to 5%. The H_2O_2 vaporization times were varied at 0, 2, 4, 6, 8, 10, 15, and 20 min.

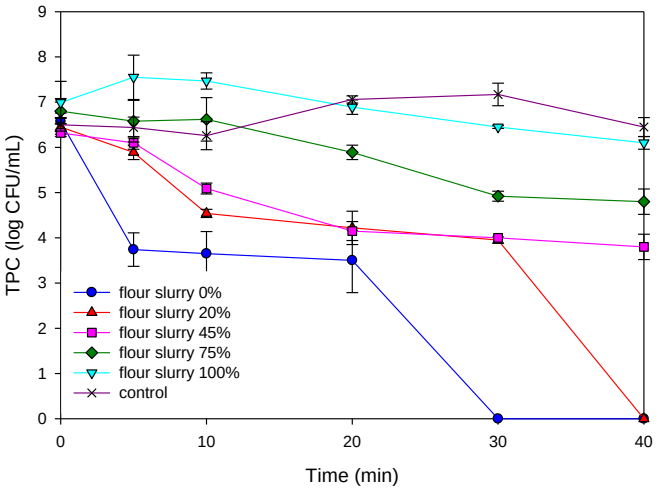
Enumeration

The viable cell count after fumigation was performed by incubating the top and side inoculated plates for overnight at 37 $^{\circ}$C for bacterial count and 5-7 days at 30 $^{\circ}$C for mold.

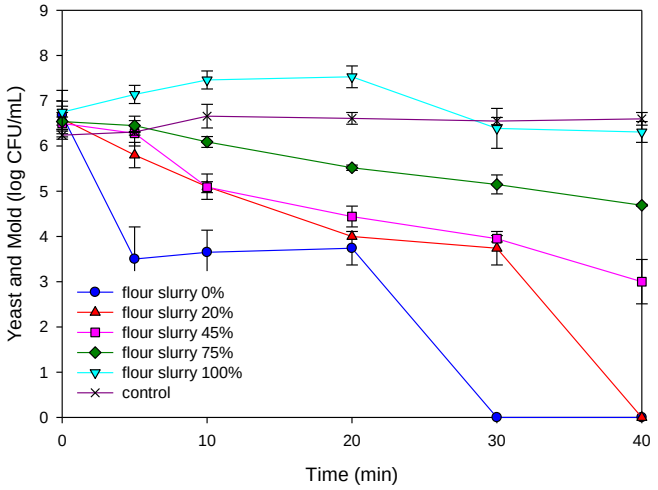
Results

Combined ozone/UV treatment on spiked flour slurry (TPC, yeast/mold and *E. coli*)

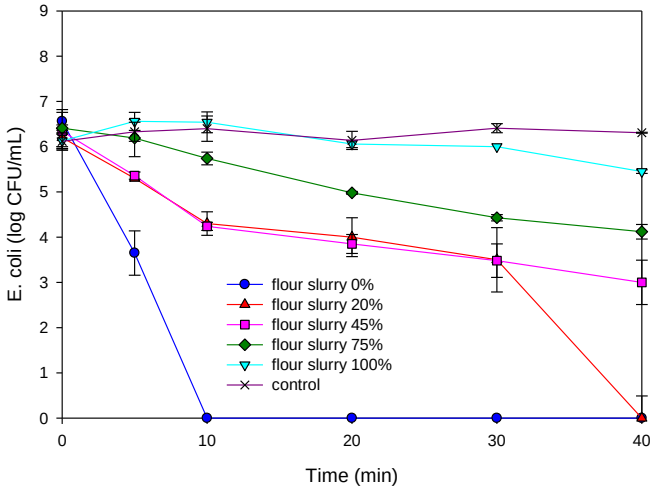
The microbial destruction efficiency of the combined ozone/UV process is typically higher than the additive removal efficiencies of ozone and UV alone [27].



(a) Total Plate Count



(b) Yeast and Mold



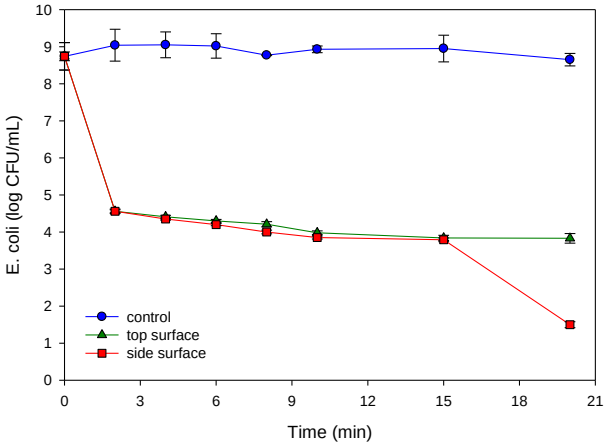
(c) *E. coli*

Figure 3 Effect of Ozone/UV treatment on the TPC (a), yeast/mold (b) and *E. coli* (c) viable cell counts at various concentrations (0, 20, 45, 75 and 100%) of flour slurry at mass flow rate 0.3 kg/s.

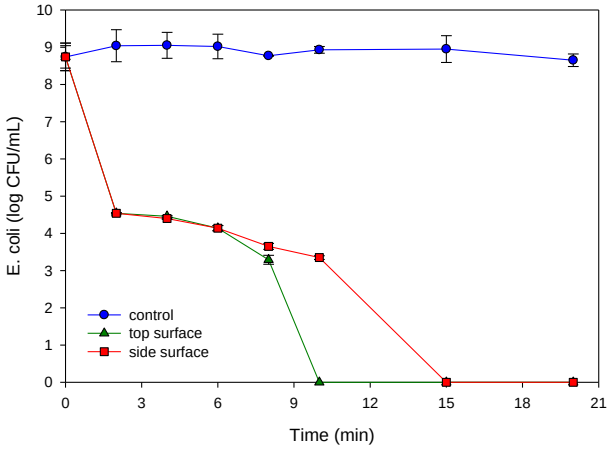
Figure 3 showed the destruction profiles of TPC, yeast/mold and *E. coli* counts as a function of time and ratio of flour slurry. After Ozone and UV treatments for 40 min, the TPC, yeast/mold and *E. coli* counts were reduced to 0 log CFU/mL in the 20% of flour slurry. For the flour slurry concentrations higher than 20%, the ozone with UV treatment was able to bring down the microbial counts to only 3 and 2 log reductions, respectively. However, the ozone with the UV treatment was not able to reduce TPC, yeast/mold and *E. coli* counts at the 100% of flour slurry treatment because it has high solid loading, turbidity and viscosity.

Effect of hydrogen peroxide concentrations on the efficacy of vaporized hydrogen peroxide to destroy *E. coli*

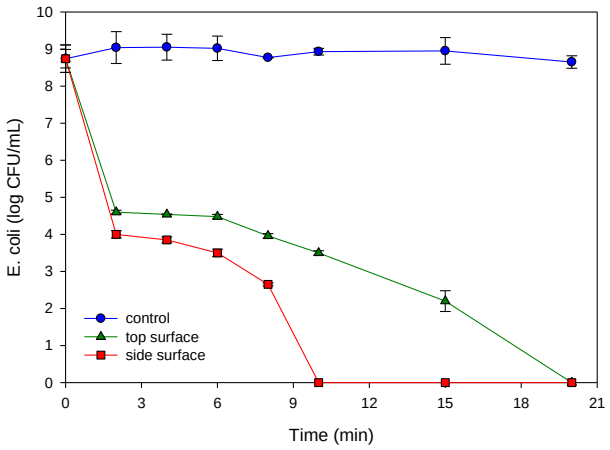
The survivability of *E. coli* to various concentrations of H_2O_2 was shown in Figure 4 using the initial cell at 10^9 CFU/mL. The 1 m^3 chamber was constructed using plastic boards to retain the H_2O_2 vapor. In all treatments with 1-5% H_2O_2, there was an instant drop of *E. coli* numbers within the first 2 min from 10^9 to 10^5 CFU/ml or 4 log reductions.



(a) 1% H_2O_2



(b) 3% H_2O_2



(c) 5% H_2O_2

Figure 4 Growth of *E. coli* using various concentrations of H_2O_2 on cell density 10^9 CFU/mL (a) 1% H_2O_2 (b) 3% H_2O_2 (c) 5% H_2O_2

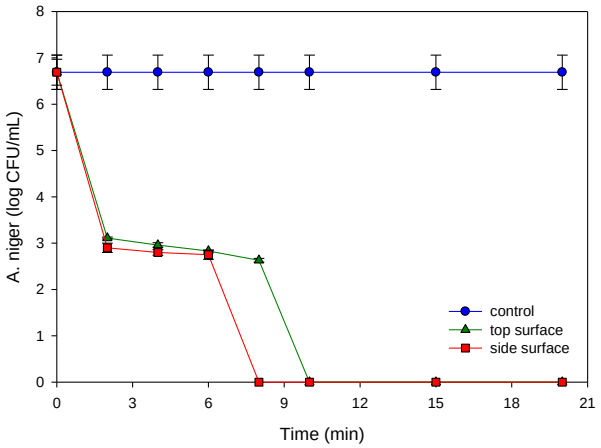
What is the adequate concentration of the H_2O_2 solution to generate effective H_2O_2 vapor? The results of 1% H_2O_2 (Figure 4a) decreased slightly during 2-15 min. Increasing the vaporized hydrogen peroxide concentration to 3% in the test chamber produced a high level of bacterial destruction (Figure 4b), with a 8 log reduction in as little as 15 min of exposure. The highest concentration of H_2O_2 solution was 5% H_2O_2 (Figure 4b). The result showed that there was an instant drop of *E. coli* numbers within 15 min similar results were obtained by using 3% H_2O_2 vapor. Further, results suggest that there is no substantial benefit in using higher concentrations of hydrogen peroxide above 3%.

Effect of various concentrations of the vaporized hydrogen peroxide on *A. niger* survival

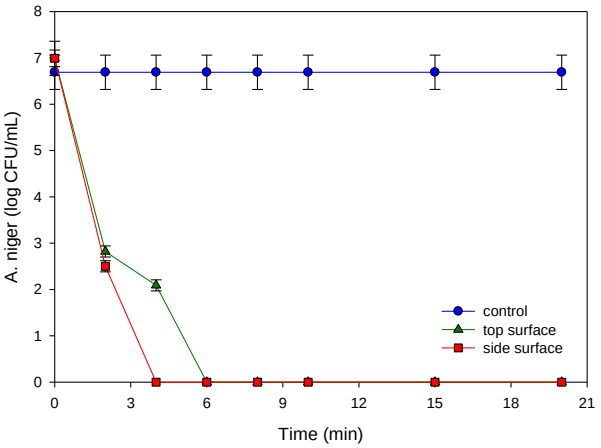
The Figure 5 shows the profiles of *A. niger* at 0-20 min after hydrogen peroxide treatment. At the all concentrations of vaporized hydrogen peroxide decontamination were achieved a 7 log reduction after 10 minutes (Figure 5). At 2 min, 5% H_2O_2 caused 7 log reductions while 3% and 1% H_2O_2 were necessary to obtain 5 and 4 log reductions respectively (Figure 5). The lowest concentration (1% H_2O_2) was reduced

to undetectable number within 10 min. The results showed that the number of the fungal colonies decreased when the concentration of vaporized hydrogen peroxide were increased (Figure 5).

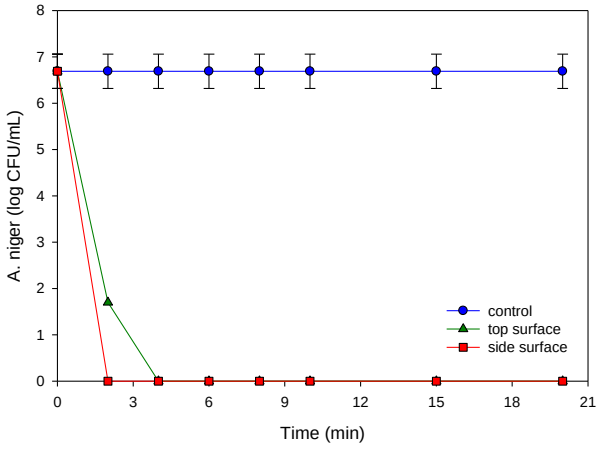
When the addition of hydrogen peroxide at 3 and 5% concentrations at exposure times of 4-6 min produced incremental increases in *A. niger* destruction.



(a) 1% H_2O_2



(b) 3% H_2O_2



(c) 5% H_2O_2

Figure 5 Growth of *A. niger* using various concentrations of hydrogen peroxide on cell density 10^7 CFU/mL (a) 1% H_2O_2 (b) 3% H_2O_2 (c) 5% H_2O_2

Conclusions and Discussion

The combined ozone/ UV has considerable potential to reduce microbial contamination in flour slurry samples (Figure 3). In Figure 3, our setting of the AOP prototype may be appropriate to treat such a high flour concentration. Thai Asia Rice factory as the noodle prototype factory which was transfer the technology in destroying microorganisms, this factory prepared the ratio of flour slurry to water approximately 20% (v/v). At this condition, the ozone with UV treatment tend to be able to bring down the microbial counts to 3 - 2 log reductions, respectively. However, the ozone with the UV treatment was not able to reduce TPC, yeast/ mold and *E. coli* counts at the 100% of flour slurry treatment because it has high solid loading, turbidity and viscosity. Our setting of the AOP prototype may not be appropriate to treat such a high flour concentration. Presumably
more hydroxyl radicals can be generated by the energy supplied by UV radiation interacting with dissolved O_3 [28]. The overall reaction of hydroxyl production can be presented as follows:

$$O_3 + H_2O \rightarrow^{hv} 2OH^{\cdot} + O_2 \qquad \text{.....(1)}$$

High concentration of OH radical in Equation (1) from the ozone/ UV system enabled faster and more powerful oxidative rupture or disintegrations leading to leakage of the intracellular contents [29] to cell membranes as proposed earlier in the effect of ozone. Together with UV radiation and background of high ozone concentration, this proposed ozone/ UV scheme was highly effective to reduce spoilage microorganism such as *E. coli* in a flour slurry system. “Glaze and others [30] and Peyton and Glaze [31] studied the different steps involved in the mechanism of this hydroxyl generation process. This system emphasizes a high synergic effect between the ozone and the UV radiation being activated simultaneously. Ozone/ UV treatment has been shown to affect the physicochemical parameters (turbidity, organic matter, and temperature) of the vegetable wash water. In fact, an ozone/ UV treatment was effective as the photocatalytic disinfection method for microbial inactivation of vegetable wash water [7].

Common AOPs techniques (e.g., H_2O_2, O_3 and/or UV-C) can be utilized to activate free radicals, especially hydroxyl radicals (OH•). Although each single process by itself has been reported to cause the sufficient destruction of microorganism and oxidation of organic and inorganic compounds, the combined effects of these techniques enable better efficacy [32]. The underlining mechanism of AOPs is a two-step process (i.e., the formation of powerful free radicals and the reaction of these oxidants with other reactants). Figure 4, other synergistic effect can be observed by combination of vaporized hydrogen peroxide with UV irradiation [33]. Application of UV irradiation alone requires a relatively long time and thus its combination with vaporized hydrogen peroxide can significantly shorten the operations.

The fumigation chamber should be airtight, and should be equipped with a fan to circulate the vaporized hydrogen peroxide during fumigation. Without a mini fan, the bacterial destruction was low at 1.37 ± 0.11 log CFU/mL, whereas adding the mini fans increased the kill to 7.1 ± 0.55 log CFU/mL. Current literature data indicate that the vaporized hydrogen peroxide process is a multi-parameter problem, the effectiveness of which is mainly influenced by the concentration of gaseous hydrogen peroxide, temperature, relative humidity, and condensation of hydrogen peroxide on the decontaminated surfaces.

Figure 5, H_2O_2 probably exerts its effect by producing destructive hydroxyl-free radicals that may attack lipid membranes, DNA and other cell components. Hydroxyl radicals may affect the spore core, oxidize thiol groups in proteins and enzymes of viruses and bacteria, and act on ribosomes in fungi [34]. Fungal spores are commonly responsible for cross contamination in laboratories, *Aspergillus spp*. being one of the more frequent culprits. *Aspergillus spp*. is killed when exposed to VHP. Other fungi are also killed when exposed to VHP, including *Penicillium spp*., *Alternaria spp*. and *Candida spp*. [35]

The combined ozone/UV treatments have been shown to be appropriate treatments for cloudy and turbid medium, like flour slurry. The use of combined treatment was able to achieve zero TPC, yeast/mold and *E. coli* count within 40 min in the 20% (v/v) of flour in water ration according to the routine productions and local consumptions from our collaborative noodle factory (Thai Asia Rice). This technology can be applied in the step of flour slurry to prolong the fermentation of rice flour during the preparation that may delay due to the production processing (i.e., power off, limited worker).

Treated or reconditioned flour slurry by ozone/UV may be a reliable alternative resource for flour slurry reuse. However, these options require continuous monitoring of efficiency and quality parameters of the flour slurry in order to guarantee an optimal performance of the treatment. Thus, for practical application of ozone/UV at noodle industry level, it would be necessary to determine the physicochemical characteristics of each type of water and flour slurry, including turbidity, and then the length of the treatments that ensure death of microorganisms. For fumigation with VHP, we found that VHP decontamination reduce bacterial and fungal contamination in chamber. H_2O_2 is easily evaporated or destroyed after use (readily decomposing into water and oxygen), has no unpleasant lingering odor, and poses minimal safety problems for workers if handled properly. An additional study of VHP is needed to establish the situations in which the different technologies are most beneficial and cost effective. Several studies reported that the VHP was highly effective against various microorganisms including bacteria, yeast, fungi, viruses, bacteria spores and prions [36, 37].

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