Study on the Sterilization of Grain Surface Using UV Radiation
—Development and Evaluation of UV Irradiation Equipment—

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Abstract
The aim of this study was to control microorganisms that cause grain degradation using ultraviolet (UV) sterilization, as a method that is eco-friendly and safe for storage without the need for postharvest application. In order to obtain practical ultraviolet sterilization, we manufactured recirculating grain sterilization equipment that uses UV irradiation. We investigated applying UV sterilization directly to microorganisms that adhere to the surface of the wheat, and then checked the quality. Sterilization tests indicated that the required sterilization time to obtain a 90% sterilization rate was 6.3 h for bacteria and 5.6 h for mold using 254 nm wavelength and 97 W/m^2 UV irradiance. The germination and amylograph tests suggested that quality was minimally affected by UV irradiation in this range.

Discipline: Postharvest technology / Agricultural machinery
Additional key words: grain quality, storage

Introduction

Food safety has become an important factor in our lives, and it is necessary to think about agricultural products hygiene consistent with the idea of "from farm to table." However, in recent years, when considering the global environment and human health, postharvest applications have tended to prohibit or decrease product hygiene globally.

Since grain is dried after harvest, water activity is low, enabling us to control microorganism increases. Therefore, grain storage is generally better when compared to fresh food like vegetables and meat. Moreover, we did not regard it as a serious problem because there are very few pathogenic bacteria on dried grain and we do not eat it uncooked. However, the mycotoxin that mold generates do not disappear even when heat-treated as in cooking, and the grain suffers quality damage when mold grows in grain warehouses and silos[7] and mycotoxin remains undetected. Food processing companies in particular want grain material with the fewest microorganisms possible, thus microorganism control for grain will become important in the future.

Microorganisms that adhere to dried grain are heat-resistant, and since grain has low thermal conductivity, it is necessary to heat sterilize it for a long time. This makes it difficult to sterilize grain by heating. Since γ-ray radiation sterilization does not reach the necessary temperature for grain, it is used for the sterilization of spices or dehydrated vegetables in many countries. However, γ-ray radiation is not legal in Japan, except for potatoes[3].

UV sterilization has been applied in many cases, and the sterilization mechanism is as follows. If the nucleic acid of a microorganism is irradiated with UV, thymine, which is a DNA base, will change to a thymine-dimer. Consequently, it will become impossible to read the genetic code and the microorganisms will die[8,9]. Therefore, UV can influence the nucleic acid directly so that it sterilizes almost all microorganisms regardless of type[2]. Moreover, there are no concerns about residues, as with sterilization by postharvest application[10].

Danno et al.[1] and Murata et al.[6] applied UV steriliza-

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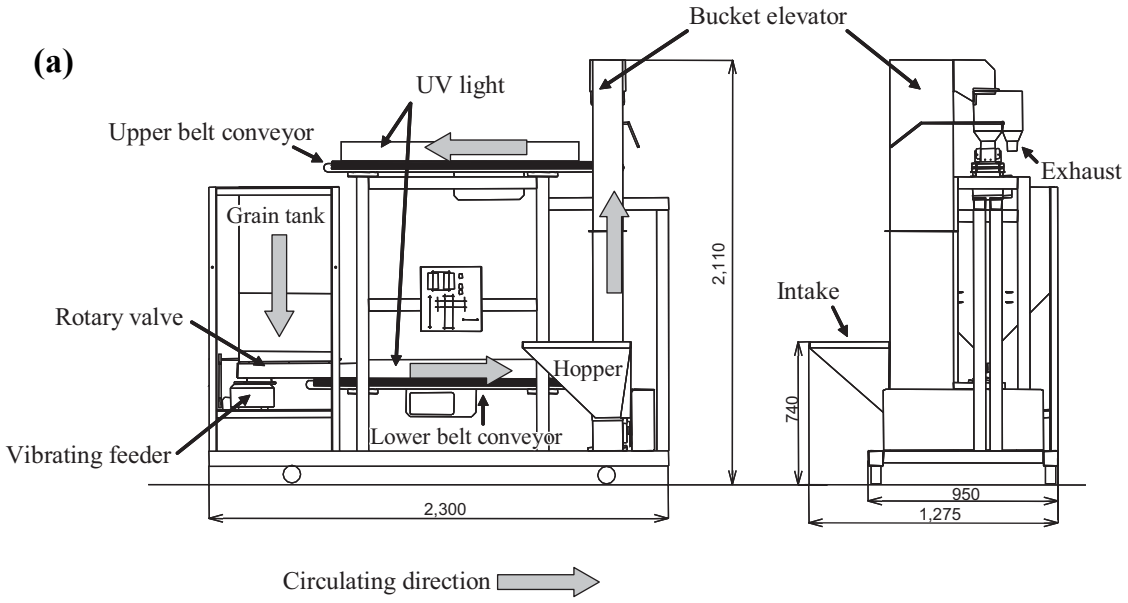
tion to grain microorganisms on a culture medium and suggested the possibility of using UV sterilization for agricultural products. We also examined the UV sterilization effect on the microorganisms that were separated from the grain on a culture medium[4]. However, there have been no studies that investigated the sterilization effects of direct UV irradiation on grain.

In this paper, wheat was directly UV irradiated using the experimental equipment, and we investigated the sterilization effect and its influence on grain quality.

Experimental equipment

The experimental equipment consisted of a UV light, grain tank and conveying equipment (a belt conveyor and a bucket elevator). The grain circulated in the equipment so that it became uniformly irradiated. The grain in the tank was discharged in a fixed quantity by a rotary valve. The grain was uniformly distributed by the vibrating feeder, sent to the lower belt conveyor, passed over the lower belt conveyor, lifted up to the upper belt



(b)

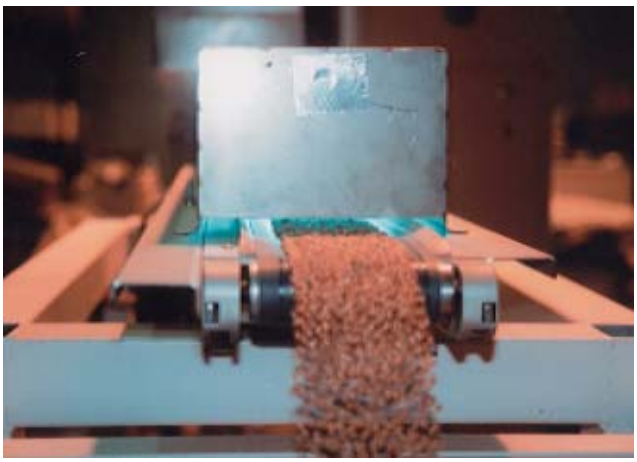


Fig. 1. Schematic diagram of recirculating grain sterilization equipment using ultraviolet irradiation
a: Recirculation grain sterilization equipment using ultraviolet irradiation.
b: View of UV irradiation for wheat.

conveyor by the bucket elevator, passed over the upper belt conveyor and came back to the grain tank. The UV lights (IWASAKI ELECTRIC Co. Ltd., QGL65; wavelength 254 nm) were set on the upper and lower belt conveyors. The grain tank capacity was 100 kg (Fig. 1 (a)). The distance between the UV light and the wheat layer was 2 cm, and UV irradiance (E) was 97 W/m^2. UV irradiation time (T_{UV}) was 4 s per circulation.

The permeability of ultraviolet is low so that the sterilizing effect influences only the surface. We have adjusted the amount of the grain supply and the speed of the belt conveyer to make a thin layer as effectively as we can. Then the grain circulation capacity was 500 kg/h. Moreover, thickness of the wheat layer (h) on a conveyor belt was calculated by the equation (1).

$$h = \frac{M_B}{A \times \sigma} \quad (1)$$

Here, σ is the wheat bulk density (836 kg/m^3 dense packing), M_B is the weight of grain on the belt conveyor (0.81 kg), and A is the irradiation area (0.24 m^2). Thickness of the wheat layer (h) under UV irradiation was set to 4.0 mm. If the thickness of one kernel of wheat is about 2.5 mm, a thin layer of two kernels or less of wheat will form (Fig. 1 (b)).

Materials and methods

Wheat harvested at the BRAIN experimental farm in Saitama Prefecture (variety NOURIN No. 61) was used for this experiment, and the moisture content was 12.1% w.b. We set the processing stages at 3, 6, 9, 12, and 15 h. The UV irradiated wheat at each experimental stage was immediately moved to a sterilized container and inspected for microorganisms and quality. The number of microorganisms was determined by the streak plate method. Namely, to separate grain microorganisms, 10 g of samples were put into a sterilized flask containing 90 mL of sterilized isotonic sodium chloride solution (0.85% NaCl) and shaken for 3 min. Next, 1 mL of suspension diluted for suitable magnification was applied uniformly on an agar culture medium with a Conradistick. We prepared five Petri dishes with 20 mL of agar culture media for every experiment stage. Each Petri dish was put into a 30ºC incubator, and the number of colonies was counted after two weeks. The average value of the five Petri dishes was converted into the number of microorganisms per gram of sample, and this value is represented as a Colony Forming Unit (CFU/g)[5]. The genus of the bacteria was distinguished using Gram-staining reagent, and the genus of the mold was distinguished by observing a SEM image (SHIMADZU SUPERSCAN model 220).

In order to investigate the quality of UV-irradiated wheat, germination and amylograph tests were performed using the Food Agency measuring method.

In addition, kabicidin was added to the nutrient agar medium to detect bacteria[5]. For mold detection, 0.02 g of tetracycline hydrochloride was added to the suspension as a bacteria antibiotic, and 25% of sucrose concentration and czapek dox agar medium at pH 5 was used for the culture medium[11].

Results and discussion

1. Sterilization of bacteria

The bacteria colonies appeared as yellow or red spheres, and we confirmed them to be *Bacillus* and *Pseudomonas*. The number of bacteria in the control stage was 18.7 × 10^4 CFU/g. Colony numbers decreased as UV irradiation time increased (Fig. 2). Then the survival curve was expressed by the following equation.

$$y = 100e^{-0.37x} \ (R^2 = 0.96) \quad (2)$$

The processing time required in order to obtain a sterilization rate of 90% was 6.3 h from equation (2).

2. Sterilization of mold

As in the case of bacteria, increased UV irradiation time decreased the number of mold colonies (Fig. 2). The number of the mold colonies in the control stage was 9.8 × 10^3 CFU/g. We obtained the following survival curve equation.

$$y = 100e^{-041x} \ (R^2 = 0.98) \quad (3)$$

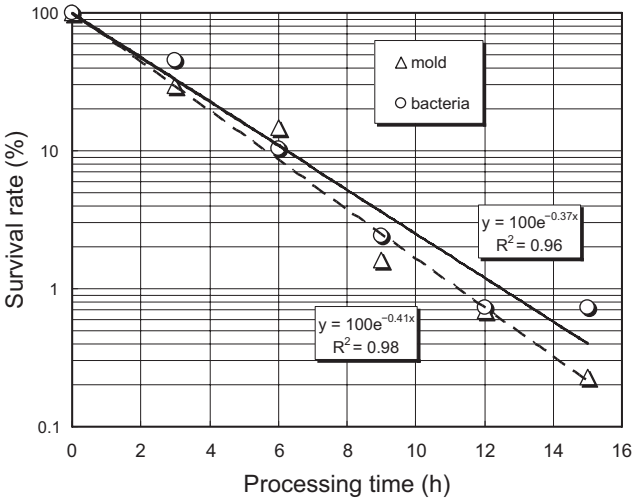
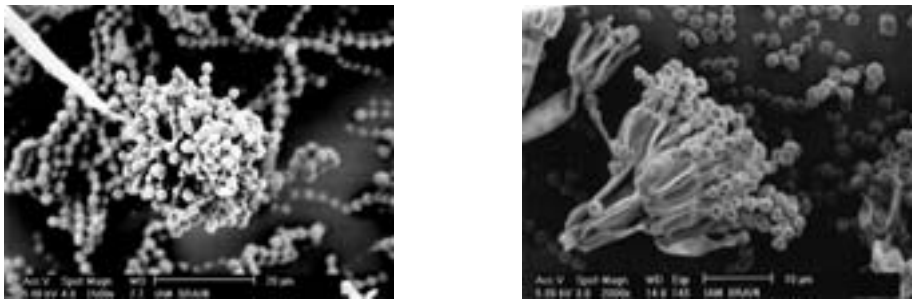


Fig. 2. Survival curve of microorganisms



① *Aspergillus* ② *Penicillium*

Fig. 3. SEM image of mold

The processing time required in order to obtain a sterilization rate of 90% was 5.6 h from equation (3). The inclination of the survival curve was a close value between mold and bacteria. In this experiment, our device demonstrated the bactericidal effect regardless of the kind of grain's microorganisms.

The mold colonies appeared as cotton- or disk-like and were white, black, and brown. The SEM image confirmed the molds to be *Aspergillus* and *Penicillium*, which are microorganisms that proliferate in storage (Fig. 3).

3. Quality

UV irradiation did not reduce germination (Fig. 4). In the amylograph test, there was no relationship between UV irradiation energy and viscosity (Table 1). It was thus confirmed that UV irradiation had little influence on quality.

4. Energy estimation

The processing time required to obtain 90% sterilization (T_D) was 6.3 h from the survival curve. The circulation time (T_C) was 0.2 h and UV irradiation time (T_{UV}) per circulation was 4 s. Therefore, the real UV irradiation time (T) was calculated from the equation (4).

$$T = \frac{T_D}{T_C} \times T_{uv} \tag{4}$$

The real UV irradiation time (T) was 126 s.

UV irradiant energy (Q_M) was calculated from the equation (5) to obtain the sterilization rate of 90% for 1 t grain.

$$Q_M = \frac{E \times A \times T}{M_B} \times 1,000 \tag{5}$$

Here, E is the UV irradiance (97 W/m^2), A is the irradiation area (0.24 m^2), T is the real UV irradiation time (126 s), and M_B is the grain weight on the belt conveyor (0.81 kg). The UV irradiant energy (Q_M) was calculated to be 3.6 MJ/t.

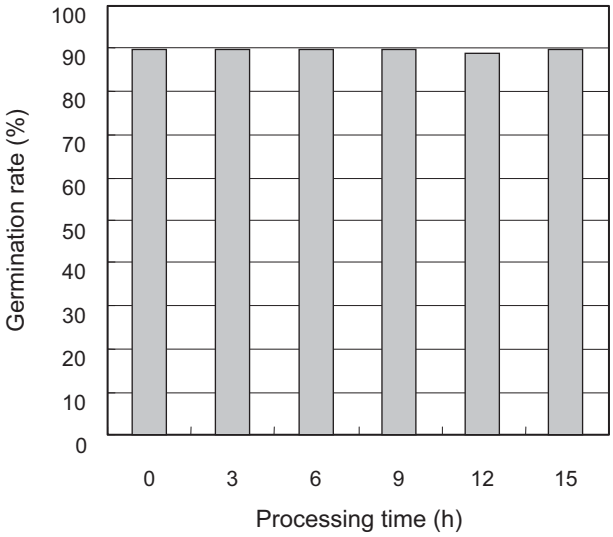


Fig. 4. Result of germination test

Table 1. Result of amylograph test

UV irradiation time (h)	Temperature of gelatinization (ºC)	Temperature of max viscosity (ºC)	Max viscosity (BU)
0	58.5	89.0	985
3	60.0	88.5	990
6	58.5	88.2	905
9	60.0	88.5	910
12	60.8	87.5	970
15	59.3	88.2	965

Conclusion

Using grain circulation UV irradiation equipment, we conducted a sterilization experiment on the grain

microorganisms that caused quality degradation and obtained the following results.

(1) UV was directly irradiated onto wheat, and its sterilization effect on microorganisms adhering to grain was confirmed. The amount of UV energy to reduce microorganisms by 90% was estimated to be 3.6 MJ/t.
(2) UV irradiation had little influence on wheat quality in this energy range.

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