Liquid egg white pasteurization using a centrifugal UV irradiator

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A B S T R A C T

Studies are limited on UV nonthermal pasteurization of liquid egg white (LEW). The objective of this study was to inactivate Escherichia coli using a UV irradiator that centrifugally formed a thin film of LEW on the inside of a rotating cylinder. The LEW was inoculated with E. coli K12 to approximately 8 log cfu/ml and was processed at the following conditions: UV intensity 1.5 to 9.0 mW/cm²; cylinder rotational speed 450 to 750 RPM, cylinder inclination angle 15° to 45°, and flow rate 300 to 900 ml/min, and treatment time 1.1 to 3.2 s. Appropriate dilutions of the samples were pouredplated with tryptic soy agar (TSA). Sublethal injury was determined using TSA + 4% NaCl. The regrowth of surviving E. coli during refrigerated storage for 28 days was investigated. The electrical energy of the UV process was also determined. The results demonstrated that UV processing of LEW at a dose of 29 mJ/cm² at 10 °C reduced E. coli by 5 log cfu/ml. Inactivation significantly increased with increasing UV dose and decreasing flow rate. The results at cylinder inclination angles of 30° and 45° were similar and were significantly better than those at 15°. The cylinder rotational speed had no significant effect on inactivation. The occurrence of sublethal injury was detected. Storage of UV processed LEW at 4° and 10 °C for 21 days further reduced the population of E. coli to approximately 1 log cfu/ml where it remained for an additional 7 days. The UV energy applied to the LEW to obtain a 5 log reduction of E. coli was 3.9 J/ml. These results suggest that LEW may be efficiently pasteurized, albeit at low flow rates, using a nonthermal UV device that centrifugally forms a thin film.

1. Introduction

Pasteurization of liquid egg white (LEW) is commercially performed in high-temperature-short-time equipment. In the U.S., pasteurization requirements for LEW are 55.6 °C for a minimum holding time of 6.2 min, or 56.7 °C for 3.5 min (Code of Federal Regulations, 2010). This heat treatment damages the functional properties of the LEW (Cunningham, 1995).

Ultraviolet light (UV) processing is a nonthermal alternative to heat pasteurization of liquid foods. While much UV research has been reported on juices, very little has been published on LEW. Unluturk et al. (2008) placed LEW in 6 cm diameter Petri dishes and UV irradiated the samples for up to 20 min. The population of Escherichia coli (strain ATCC 8739) was reduced more than 2 log. Processing LEW in a Petri dish with UV for 20 min inactivated E. coli K-12 (ATCC 25253), E. coli O157:H7 (NCTC 12900) and Listeria innocua (NRRL B33314) by 0.9, 1.4 and 1.0 log, respectively (Unluturk et al., 2010), de Souza and Fernandez (2011) reported that a reduction in Salmonella Enteritidis of 5.3 log was achieved after UV irradiating LEW in a Petri dish for 39 min. Ngadi et al. (2003) processed LEW in a Petri dish with UV and reduced E. coli O157:H7 by 4.2 log.

Besides the batch studies in Petri dishes, several continuous UV studies have been performed. At a LEW feed rate of 100 ml/min, UV was applied using a Centrifilmer irradiator (developed by General Motors), and S. Typhimurium and S. Senftenberg were reduced by 6 to 7 logs within 1 s (Ijichi et al., 1964). The Centrifilmer uses centrifugal force to form a thin liquid film that can easily be penetrated by UV. In addition to the Centrifilmer centrifugal system, a tubular system has also been used to continuously apply UV to LEW (Geveke, 2008). In this system, LEW is pumped through small diameter UV transparent tubing wrapped around UV lamps. At a flow rate of 330 ml/min, E. coli K12 was reduced by 4.3 log after being exposed to UV at 50 °C for 160 s. The electrical energy of the process was calculated to be 44 J/ml.

The Centrifilmer is no longer commercially available; however, Dill Instruments, Inc. (Kalamazoo, MI) currently manufactures a UV irradiator that similarly uses centrifugal force to form a thin liquid film. Using such a device, E. coli and Saccharomyces cerevisiae were inactivated by 5.8 and 6.1 log, respectively, in a 0.0076 cm thin film of grapefruit juice flowing at 300 ml/min (Geveke and Torres, 2012).

No studies exist on the incidence of sublethal cellular injury from UV processing of LEW, or on the fate of surviving bacteria after processing.
Also, the energy of processing LEW using a centrifugal UV system has yet to be determined. The overall goal of the present study was to determine the applicability of inactivating E. coli in LEW using a UV irradiator that centrifugally forms a thin film. Specific objectives were: (1) to determine the effect of the UV irradiator operating parameters (intensity, rotational speed, and angle) and LEW flow rate on the inactivation of E. coli, (2) to determine the occurrence of sublethal injury, (3) to determine if regrowth occurs during extended storage, and (4) to determine the operating energy of the UV process.

2. Materials and methods

2.1. Materials

Pasteurized LEW in 13.6 kg bags was obtained from Papetti’s (Easy Eggs® Extended Shelf Life, Minnetonka, MN). The UV processing was performed in a food pilot plant, so pathogenic bacteria could not be used. Nonpathogenic E. coli K12 (ATCC 23716) was maintained on tryptic soy agar (TSA; Becton, Dickinson and Company, Sparks, MD) at 4 °C. Prior to inoculation of product, E. coli was cultured in tryptic soy broth (Becton, Dickinson and Company) with shaking at 37 °C for 16–18 h. The LEW was inoculated from the stationary phase culture of E. coli to give an approximately 8 log cfu/ml population.

2.2. Equipment

The experimental system has previously been described (Geveke and Torres, 2012) and included a feed tank, pump, and UV irradiator that centrifugally formed a thin film on the inner wall of a rotating cylinder (Fig. 1). A peristaltic pump (Masterflex L/S driver model 7523-40 and head model 77200-62, Cole-Parmer, Vernon Hills, IL) delivered the LEW to the top of the UV irradiator (Model UV3-C, Dill Instruments, Kalamazoo, MI) through norprene tubing (Masterflex number 06402-15, Cole-Parmer) attached to a stainless steel nozzle having an internal diameter of 0.3 cm. The Dill UV irradiator consisted of a hollow stainless steel cylinder mounted for rotation about an inclined axis (Fig. 2). LEW flowed by gravity downward along the internal peripheral wall of the cylinder in a thin film. An assembly of four stationary UV 254 nm lamps (Model G36T6L, GoodMart, New York, NY) extended along the interior axis of the cylinder. Each lamp was rated at 39 W. The LEW and UV lamps were separated by a 2.5 cm air space. The lamps’ length and diameter were 84.3 cm and 1.5 cm, respectively. The cylinder’s internal diameter, length, and internal surface area were 10 cm, 70 cm, and 2200 cm², respectively. The cylinder’s angle of inclination and rotational speed, as well as the UV intensity and LEW flow rate, were varied. A collection container at the discharge end of the cylinder received the treated LEW. Process sensors (No. 89790, Dill Instruments, Kalamazoo, MI) monitored the intensity of the UV light at two locations along the cylinder. The sensors were calibrated using a NIST certified sensor.

2.3. UV processing

The feed rate of LEW was varied between 300 and 900 ml/min with corresponding UV treatment times of 3.2 to 1.1 s. The temperature of the LEW entering and exiting the UV irradiator was approximately 10 °C. Three cylinder inclination angles (15°, 30° and 45°) were tested. Angles less than 15° do not readily permit LEW to flow by gravity down the length of the cylinder. The tested rotational speeds of the cylinder were 450, 600, and 750 RPM. Rotational speeds of less than 450 RPM may not yield uniformly thin films. UV intensities were varied between 1.5 and 9.0 mW/cm². The UV doses (in mJ/cm²) were calculated using the following equation where intensity and treatment time have units of mW/cm² and s, respectively:

\[
\text{Dose} = \text{Intensity} \times \text{Treatment time.}
\]

2.4. Energy calculation

A common method of comparing efficiencies of various technologies is to calculate the energy per volume processed. The energy (in J/ml) to process LEW using the UV system in this study was calculated by the

![Fig. 1. UV irradiator system showing the feed tank, inclined stainless steel cylinder, and collection container, as well as the controller for the UV intensity and cylinder rotational speed.](image-url)
2.5. Film thickness calculation

The average thickness (in cm) of the LEW film was calculated using the following equation where treatment time, flow rate and area have units of s, ml/s and cm², respectively:

\[ \text{Film thickness} = \frac{\text{Treatment time} \times \text{Flow rate}}{\text{Area}} \]  

2.6. Microbial enumeration

Product samples were collected in polypropylene tubes. Appropriate dilutions of the product samples were made in buffered peptone water (Becton, Dickinson and Company) with a minimum 1 ml transfer. Inactivation of E. coli was determined by pour plating duplicate samples with TSA and incubating the plates at 37 °C for 24 h. Plates with 30 to 300 colonies were enumerated using a manual colony counter (model 920, Bantex, Burlingame, CA). Sublethal injury of E. coli was determined by pour-plating treated samples with TSA+4% sodium chloride (4% NaCl), incubating at 37 °C for 72 h, and enumerating visible colonies. Preliminary studies determined that healthy stationary phase E. coli cells were recovered in equivalent numbers on TSA and TSA + 4% NaCl. Microbial inactivation was defined as the difference in log cfu/ml of microorganisms between untreated and treated samples on nonselective media. Injury was defined as the difference in log cfu/ml of treated microorganisms between nonselective and selective media.

2.7. Post UV treatment storage

To evaluate the long term effect of UV processing on E. coli in LEW, UV treated (at a flow rate of 300 ml/min, dose of 14 mJ/cm², cylinder rotational speed of 750 RPM, and cylinder inclination angle of 30°) and untreated (control) samples were packaged in sterile 10-ml test tubes inside a sanitary laminar flow hood equipped with a HEPA air filter (Forma Scientific Inc., Marietta, OH). The closed tubes were stored at 4° and 10 °C for 28 days. Viable cells were determined from colony counts on TSA at selected days throughout this period as described above.

2.8. Statistics

Each UV experiment was performed in duplicate. The standard deviation and the significance of differences in results obtained (Student’s t test) were calculated using Microsoft Excel statistical analysis algorithms.

3. Results and discussion

3.1. UV dose

The effect of UV dose on the inactivation of E. coli in LEW is shown in Fig. 3. The dose was varied from 0 to 24 mJ/cm² (calculated using Eq. (1)) while the flow rate, cylinder inclination angle and rotational speed were kept constant at 300 ml/min, 30° and 450 RPM, respectively. The population of E. coli was significantly (P<0.05) affected by the dose. At a dose of 24 mJ/cm², a 5 log reduction was obtained. The data for inactivation (in log cfu/ml) versus UV dose (in mJ/cm²) were regressed (Eq. (4)) and the results are plotted in Fig. 3.

\[ \text{Inactivation} = 13.0 \times \left(1 - e^{-0.0172 \cdot \text{Dose}}\right) \]  

The results indicate that increasing the dose yields a diminishing return in terms of additional inactivation.

Ijichi et al. (1964) also obtained excellent inactivation of bacteria in LEW using a UV irradiator that centrifugally formed a thin film. Counts of S. Typhimurium and S. Senftenberg were reduced by 6 to 7 logs at a flow rate of 100 ml/min and a UV intensity of 7.22 mJ/cm². Irradiation at lower intensities decreased the microbial inactivation. Recently, Geveke and Torres (2012) used a Dill UV centrifugal irradiator to inactivate E. coli K12 (ATCC 23716) in grapefruit juice. Whereas in the present study a dose of 29 mJ/cm² produced a 5 log reduction of E. coli K12 in LEW, a UV dose of only 19 mJ/cm² was required to obtain the same reduction in grapefruit juice. The cause for this difference is the greater absorption coefficient at 254 nm for LEW, 118 cm⁻¹ (Geveke et al., 2011), versus that of grapefruit juice, 51.5 cm⁻¹ (Geveke and Torres, 2012). Absorption coefficient is inversely related to penetration depth. Therefore, UV can penetrate deeper into grapefruit juice, and inactivate more bacteria for a given dose, then it can for LEW.

Some research areas require follow-up studies. For example, E. coli K12 (ATCC 23716) (synonym EMG2) is an ancestral K12 wild-type strain which carries phage lambda in its chromosome as a prophage. UV treatment may cause induction of the prophage in the surviving bacteria. As a result, the phage may propagate and new phage particles may be released which can subsequently infect and lyse the remaining surviving
**3.2. Operating energy**

The UV processing energy for the operating condition (dose of 29 mJ/cm²) that resulted in a 5 log inactivation of E. coli in LEW at 10 °C, was 3.9 J/ml (from Eq. (2)). This value is much lower than that obtained in a previous study on LEW using a tubular UV system (Geveke, 2008). In that investigation, the UV processing energy that resulted in a 5 log inactivation of E. coli at 50 °C was 44 J/ml. The lower energy required for a 5 log inactivation in the present study can be attributed to the difference in the thicknesses of the LEW in the UV systems. The tubular UV system contained tubing with an internal radius of 0.16 cm (Geveke, 2008). The thickness of the LEW film in the centrifugal UV system was 0.0076 cm (from Eq. (3)). The penetration depth, where the intensity of the UV decreases to 37% of the value at the surface, is 0.0066 cm for LEW (Geveke et al., 2011). The thickness of the LEW in the centrifugal system was roughly the same as the UV penetration depth, whereas the LEW thickness in the tubular system was over 20 times greater than the penetration depth.

The energy for conventional thermal pasteurization of juice, for comparison, is approximately 35 J/ml (Kozempel et al., 1998). The thermal energy for liquid egg white is estimated to be 33 J/ml, based on the fact that the specific heat for egg white is approximately 6% less than that of juice. Thus, the centrifugal UV system is not only effective at inactivating E. coli in LEW, it also is energy efficient.

In the aforementioned study using a tubular system, the samples of UV-processed LEW were sniffed and an off-odor was observed (Geveke, 2008). By contrast, in the present study using the centrifugal system, the UV-processed LEW samples smelled the same as the untreated control samples. This advantageous result may be attributable to the lower energy applied to the LEW using the centrifugal system. Another reason for the beneficial result may be the lower processing temperature of the LEW with the centrifugal system. de Souza and Fernandez (2012) processed LEW with UV at 20 °C in a Petri dish. No off-flavors due to UV treatments were reported.

**3.3. Rotational speed**

The effect of varying the rotational speed of the UV irradiator’s stainless steel cylinder on the inactivation of E. coli in LEW was investigated. Keeping constant the flow rate at 300 ml/min, cylinder inclination angle at 45° and the UV dose at 19 mJ/cm², the cylinder rotational speed was set to 450, 600 or 750 RPM. The inactivation did not vary significantly (P>0.05) with rotational speed and averaged 3.8 log.

**3.4. Inclination angle**

The effect of the inclination angle of the UV irradiator’s stainless steel cylinder on the inactivation of E. coli in LEW was determined. A series of experiments was performed at a constant flow rate of 300 ml/min, UV dose at 19 mJ/cm² and cylinder rotational speed of 600 RPM. The cylinder inclination angle was varied between 15° and 45°, and the effect on the inactivation of E. coli was determined (Table 1). The inactivation increased significantly (P<0.05) from 15° to 30°. The inactivation did not significantly change between 30° and 45° (P>0.05). Based on these results, the recommended range of cylinder inclination angles is 30° to 45°. At an angle of 0°, the egg will not flow from one end of the cylinder to the other. At an angle of 15°, the egg will flow down the cylinder; however, a non-uniform film may be generated which could result in lower microbial inactivation. Although angles greater than 45° probably would be acceptable, these were not tested due to the physical limitations of the experimental system.

**3.5. Flow rate**

Although the centrifugal UV system effectively inactivated E. coli in LEW at a flow rate of 300 ml/min, it was not as successful at higher flow rates as seen in Fig. 4. The inactivation linearly declined from approximately 3.5 log at 300 ml/min to 1.1 log at 900 ml/min. As the flow rate increased, the UV dose decreased, which resulted in significantly lower inactivation (P<0.05). Ijichi et al. (1964) also observed substantially lower inactivation of S. Typhimurium in LEW as flow rate was increased from 100 to 300 ml/min to a centrifugal UV irradiator. While longer and larger diameter rotating cylinders could be assembled to increase the treatment time at higher flow rates, and more UV lamps could be used to provide greater doses, it is highly unlikely that a centrifugal UV system could process 100 l/min, a flow rate typically encountered in commercial LEW processing plants. However, at intermediate flow rates, a centrifugal UV system may provide a good nonthermal alternative to thermal pasteurization.

**3.6. Sublethal cellular injury**

The UV treatment produced sublethal injury of E. coli in LEW. There was a significant (P<0.05) difference in microbiological counts on selective (TSA + 4% NaCl) and nonselective media (TSA). At a dose of 14 mJ/cm² the inactivation of E. coli was 3.1 ± 0.5 log cfu/ml, and the surviving population was composed of 3.9 ± 1.7 log cfu/ml injured cells. This is in general agreement with Ukuku and Geveke (2010) who observed substantial injury of E. coli at moderate levels of UV inactivation. Apple juice inoculated with E. coli K-12 (ATCC 23716) was UV treated at 25°, 30°, and 40 °C, and the populations of E. coli were reduced by 4.0, 4.8, and 5.8 log, respectively (Ukuku and Geveke, 2010). Among the respective surviving populations, 2 log (99%), 1 log (90%), and less than 0.1 log (5%) were injured. Thus, at lower levels of UV inactivation, considerable injury was obtained.

**3.7. Post UV treatment storage studies**

The effect of UV processing on microbial populations during storage was investigated. LEW containing E. coli was UV processed and was stored at 4 °C for 28 days (Fig. 5). Similarly treated samples were also stored at 10 °C to determine the effect of mild temperature abuse. In addition, inoculated and unprocessed (control) samples were also kept at the identical temperatures. The populations of E. coli cells in the LEW processed with UV resulted in an approximate 3 log inactivation as indicated in Fig. 5 (Day 0). The population of UV processed E. coli cells stored at both 4° and 10 °C significantly (P<0.05) declined during the first 21 days to approximately 1 log cfu/ml and remained there for the duration of the study (28 days). While the populations of the untreated (control) E. coli cells in LEW stored at both 4 and 10 °C also declined.

<table>
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<tr>
<th>Inclination angle, °</th>
<th>Inactivation of E. coli, log cfu/ml*</th>
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<tbody>
<tr>
<td>15</td>
<td>2.0 ± 0.1 a</td>
</tr>
<tr>
<td>30</td>
<td>4.1 ± 0.4 b</td>
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<tr>
<td>45</td>
<td>3.7 ± 0.3 b</td>
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*Values are means of two independent trials of processing with duplicate sampling. Different letters (abcd) indicate a significant difference in mean values (P<0.05).
during storage, both populations were still greater than 2.7 log cfu/ml after 28 days.

4. Conclusions

LEW was pasteurized using a nonthermal UV device that centrifugally forms a thin film. The population of E. coli K12 (ATCC 23716) was reduced by more than 5 log cfu/ml at a UV dose of 24 mJ/cm². Increasing UV dose improved inactivation. For LEW, the recommended cylinder rotational speed and cylinder angle are 450 RPM and greater than or equal to 30°, respectively. At an intermediate level of E. coli inactivation (3.1 ± 0.5 log), the surviving population was composed of 3.9 ± 1.7 log cfu/ml injured cells. There was no regrowth of E. coli during 28 day storage at 4° and 10 °C. The electrical energy necessary to obtain a 5 log reduction of was 3.9 J/ml, which is substantially less than that for a tubular UV system. Inactivation decreased linearly with increasing flow rate limitation. These results suggest that a nonthermal UV irradiator that centrifugally forms a thin film can pasteurize LEW at moderate flow rates. While the energy consumption is minimal compared to conventional thermal pasteurization and the functional quality of the UV processed LEW should be excellent, it is questionable whether UV processing of LEW will be commercialized due to the flow rate limitation.

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