Inactivation of *Lactobacillus brevis* in Liquid Egg White by Radio-Frequency Flash Heating

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An electric pasteurizer utilizing Radio-Frequency Flash Heating (RF-FH) was constructed in order to pasteurize liquid egg white. This pasteurizer inactivated *Lactobacillus brevis* in liquid egg white using a radio-frequency electric field. RF-FH enabled inactivation of *L. brevis* at 60°C in 2.4 s equally well as conventional heating at 54°C for 29.5 min. The shorter heating time yielded higher quality liquid egg white. A foam made from RF-FH treated egg white maintained its strength similarly to untreated egg white, but foam made from liquid egg white conventionally heated at 54°C for 29.5 min showed reduced strength.

Keywords: radio frequency, liquid egg white, inactivation, *Lactobacillus brevis*

Introduction

Liquid egg white is used extensively in the food industry because of its convenience and reduced shell waste, as well as its ability to produce mayonnaise, confectionery, bread, noodles, etc. Heat pasteurization is regularly used to protect against food poisoning agents such as *Salmonella* in liquid egg white (FSA, 2008; Little, 2007). However, heat treatment reduces foam stability and degrades proteins (Hamid-Samimi et al., 1987; Doyle and Mazzotta, 2000; Humphrey, 1990; Manas et al., 2003). Nonthermal technologies, such as pulsed-electric fields (PEF), are thus being researched for liquid egg white pasteurization (Amiali et al., 2007; Geveke, 2008; Manas et al., 2000; Ponce et al., 1999). PEF permits inactivation of up to only 3 log of various *Salmonella* serovars at temperatures below 35°C (Monfort et al., 2010). Therefore, PEF technology is not suitable for pasteurizing liquid egg white.

Ohmic heating is based on the passage of electrical current through a food product that serves as an electrical resistor (Reznick, 1996; Sastry and Salengke, 1998). It can be used as a continuous in-line heater for cooking and sterilizing liquid food products (Icier and Tavman, 2006; Ayadi et al., 2007; Bozkurt and Icier, 2009). There are commercial-scale plants producing liquid food products, particularly pasteurized liquid egg white (Zell et al., 2009). We developed an electrical inactivation method for liquid food called High Electric Field AC (HEF-AC) that uses an electric field 100 or more times greater than conventional ohmic heating, and we applied it to inactivate microorganisms in high-quality fruit juice in Japan (Uemura and Isobe, 2002; Uemura and Isobe, 2003; Uemura et al., 2010a).

When HEF-AC is applied to juice between closely spaced electrodes, vegetative cells such as *Escherichia coli* are killed immediately by the high electric field (Uemura and Isobe, 2002), and spores such as *Alicyclobacillus* are damaged by the HEF-AC and are inactivated in the next holding section 40 times faster than by conventional heating (Uemura et al., 2009). In addition, the processing time is shortened by 1/10 to 1/100 of the conventional heating sterilization time, and the shorter heating time of the liquid food mitigates the loss of flavor and nutrients. However, in protein-containing materials, including proteins such as liquid egg white, the protein exhibits thermo-coagulation at the electrode surface. There are also problems associated with burning on the electrode surface, so HEF-AC is unable to treat liquid egg white. Therefore, we developed radio-frequency flash heating (RF-FH), which uses a Teflon-coated electrode and radio-frequency alternating current. This system could be applied to soybean milk because of the insulation of the Teflon film, and RF-FH was able to inactivate *Bacillus subtilis* spores in soybean milk (Uemura and Kobayashi, 2010b). In this study, we developed an effective RF-FH pasteurizing technology for liquid egg white. Using the present experimental device,
we confirmed the sterilization of liquid egg white and examined the suitability of the device for processing, such as thermal denaturation of the protein in the liquid egg white.

Materials and Methods

Liquid egg white Eighty eggs purchased from a nearby supermarket were sanitized with ethanol and cracked open, the egg white was separated from the egg yolk, and the egg white was filtered to 1 mm with a 0.56 stainless-steel mesh. The pH of the egg white was 9.21. The viscosity was 3.2±0.1 mPa·s, as measured by a viscometer (SV-10; A&D Co., Tokyo, Japan).

Radio-Frequency Flash Heating (RF-FH) treatment A schematic diagram of the RF-FH setup is presented in Fig. 1. Raw liquid egg white in a stainless-steel tank was fed into heat exchanger 1 at a constant flow rate (6.5 ml/s) using a mohno pump (2NL10F; Heisin, Kobe, Japan). Temperature was increased from 5°C to 45°C for 30 s in heat exchanger 1, and the temperature of the liquid egg white was controlled from 45°C to 60°C for 0.45 s with output power of 500 W to 1000 W applied through an electrode unit. A pair of electrode plates (6 mm × 80 mm) were covered with a 50-μm layer of Teflon film and separated by a distance of 5 mm (Fig. 2 (a)). The RF power supply fed the electrode with a standing wave ratio (SWR) of less than 1.2 using an impedance-matching device (AT1500DT; Palstar Inc., Ohio, USA), with the output of the exciter (IC-7200; ICOM, Osaka, Japan) amplified to a maximum power of 1 kW using a linear amplifier (IC-PW1; ICOM, Osaka Japan). The temperature of the liquid 20 mm from the outlet for the electrode that produced the maximum temperature was measured using an optical-fiber thermometer (FL-2000, Anritsu, Japan) that was impervious to the radio-frequency electric field (Fig. 2 (b)). The liquid then passed through a temperature-holding pipe for 2.4 s at the maximum temperature. The cooling unit, consisting of heat exchanger 2 (8A double coaxial; Frontier Engineering, Tokyo, Japan), decreased the temperature to 30°C in 20 s.

Conventional heat treatment Capped test tubes containing 50 ml of liquid egg white were heated in a water bath controlled at 54°C for 20 to 30 min. The temperature measured by a thermocouple at the center of the liquid egg white was increased to 54°C for 9.5 min and was held at 54°C for 10.5 to 20.5 min.

Microorganisms Lactobacillus brevis (JCM1113) and Escherichia coli (JCM1649), which have greater heat resistance than Salmonella enteritidis, were used (Shokuhin Kagaku Binran, 1978). L. brevis were incubated in MRS broth at 30°C for 48 h in a 5% CO₂ incubator (4020; Asahi Life Science, Tokorozawa, Japan). E. coli were incubated in nutrient broth at 37°C for 24 h. Ten milliliters of L. brevis solution or E. coli solution was added to 2 l of liquid egg white. Each of the experiments was repeated three times. Initially, there were $N_0 = 5.0 \times 10^5$ cfu/ml L. brevis cells and $N_0 = 4.6 \times 10^7$ cfu/ml E. coli cells. After each treatment, 100 μl of the L. brevis sample was spread onto an MRS agar plate and incubated at 30°C for 48 h in a 5% CO₂ incubator; the colony number n was then counted. For E. coli, 1 ml of sample was mixed with nutrient agar and incubated at 37°C for 24 h; colony number n was then counted.

SDS polyacrylamide gel electrophoresis The protein

![Fig. 1. Schematic diagram of RF-FH setup.](image-url)
Results and Discussion

Inactivation of L. brevis and E. coli

Figure 3 plots the survival rates \( \frac{n}{N_0} \) of L. brevis and E. coli against heating time in a constant-temperature water bath controlled at 54°C. The survival rate decreased with increasing heating time and was reduced by four logarithmic orders after heating for 20 min. The D value for L. brevis (E. coli) was 7.0 min (6.0 min) at 54°C, exceeding the 1.51 min of Salmonella enteritidis at 54°C in liquid egg white (Jin, 2008). Figure 4 plots the survival rate \( \frac{n}{N_0} \) of L. brevis and E. coli against outlet temperature of the electrode with RF-FH treatment. Temperature was controlled by increasing the output power of the RF power supply. The inactivation of L. brevis (E. coli) increased above a threshold temperature of 55°C (60°C) with increasing temperature. The survival rate of L. brevis (E. coli) reached four orders of magnitude at 60°C (69°C). PEF treatment alone showed a 1.0 log reduction of Salmonella in liquid egg white, but a 4.3 log reduction of Salmonella was achieved by combined PEF thermal treatment at 55°C (Hermawan et al., 2004). The estimated electric field and current density of RF-FH were 450 V/cm and 3.0 A/cm², respectively. The influence of electric field effects on inactivation was found to be insignificant, but the dielectric current den-
above 65℃. Therefore, subsequent RF-FH treatments were performed at less than 63℃.

It was found that inactivation of microorganisms increased with RF-FH temperature. However, a coagulated protein attached to the electrode after RF-FH treatment above 65℃. Therefore, subsequent RF-FH treatments were performed at less than 63℃.

SDS electrophoresis of liquid egg white Figure 5 (a) ((b)) shows an SDS electrophoretogram of liquid egg white treated by conventional heating (RF-FH). The protein concentration after all heat treatment was unchanged at 62.9±2.2 mg/ml. The 77-kDa band representing ovotransferrin was reduced by conventional heating at 54℃ for 29.5 min. Ovotransferrin is
The viscosity after heat treatment slightly decreased to 2.60±0.04 after heating for 19.5 min, to 2.28±0.05 after heating for 24.5 min, and to 1.83±0.14 after heating for 29.5 min. The lower viscosity is one cause of the reduction in foam hardness. Ovotransferrin binding of metal ions can increase the stability of ovotransferrin. At pH7, Fe$^{3+}$, Cu$^{2+}$ and Al$^{3+}$ exhibit markedly superior binding when compared with all other ions tested, preventing coagulation and maintaining functionality such as foaming (Mine, 1995). Denatured ovotransferrin is thought to increase foaming capacity.

**Evaluation of foaming strength**  
Figure 6 (a) ((b)) illustrates the breaking strength of foam made by conventional heating (RF-FH treatment). In conventionally heated liquid egg white, the foam hardness decreases, increasing the temperature retention time. The foam hardness after RF-FH treatment decreased slightly with increasing process temperature. The pH after all heat treatment was unchanged at 9.14±0.03. The viscosity after heat treatment slightly decreased to 2.60±0.04 after heating for 19.5 min, to 2.28±0.05 after heating for 24.5 min, and to 1.83±0.14 after heating for 29.5 min. The lower viscosity is one cause of the reduction in foam hardness. Ovotransferrin binding of metal ions can increase the stability of ovotransferrin. At pH7, Fe$^{3+}$, Cu$^{2+}$ and Al$^{3+}$ exhibit markedly superior binding when compared with all other ions tested, preventing coagulation and maintaining functionality such as foaming (Mine, 1995). Denatured ovotransferrin is thought to increase foaming capacity.
Conclusions

The present results indicate that RF-FH treatment effectively inactivates \textit{L. brevis} in liquid egg white while leaving the protein components unaffected because the heating time of RF-FH was clearly shorter than that of conventional heating. To inactivate \textit{E. coli} by RF-FH, the outlet temperature should be 70°C. Coagulated protein was observed on the electrode after RF-FH treatment at 70°C. A hot spot of 10°C or more above the average temperature was calculated to form on an electrode with HEF-AC (Uemura et al., 2007). This issue requires more advanced electrode design to scale RF-FH up to industrial levels. RF-FH appeared to have a shorter process time and to produce better product quality when compared with conventional heating. RF-FH has the potential for pasteurizing liquid egg white.

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References


