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RESEARCH NOTE

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# Effects of Combination Treatments of Nisin and High-intensity Ultrasound with High Pressure on the Functional Properties of Liquid Whole Egg

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**Abstract** Liquid whole egg (LWE) was subjected to high hydrostatic pressure (HHP), a consecutive combination of nisin and HHP (nisin-HHP), or a consecutive combination of ultrasound and HHP (ultrasound-HHP), and functional properties of processed LWE were compared to those of raw LWE. Little changes in foaming and emulsifying properties were observed by the application of HHP alone and the combined process of nisin and HHP. In contrast, ultrasound-HHP combination resulted in significant changes in color, foaming, and emulsifying properties. The maintenance of functional properties after HHP treatment agreed with expectation, because the HHP processing condition had been selected where minimal rheological changes had occurred.

**Keywords:** high hydrostatic pressure, ultrasound, nisin, liquid whole egg, combination treatment

## Introduction

The ultimate objective of liquid egg processing would be to ensure the safety of the consumer and provide sufficiently long shelf life for the manufacturer while the functional properties of fresh liquid eggs are retained. Conventional heat processes used to pasteurize liquid whole egg (LWE), for examples, 60°C for 3.5 min in the US or 64°C for 2.5 min in the UK, ensure food safety by eliminating pathogenic bacteria such as *Salmonella*, but these pasteurization requirements could achieve only one or two log reductions of viable cell counts, and pasteurized liquid egg products often contain $10^2$ or more than $10^3$ microbial cells/g (1). Although conventional heat treatments of LWE are carefully conducted on the critical temperature-time conditions where the egg protein denaturation is minimized, parts of LWEs are frequently over-processed during thermal treatments, and changes in functional properties due to pasteurization or subsequent frozen storage have been reported (2-4).

To overcome the limitations of conventional heat treatment an attempt was made to employ the non-thermal treatments in LWE processing. In previous works, the high hydrostatic pressure (HHP) processing conditions were optimized for LWE treatment considering egg protein coagulation and microbial inactivation kinetics (5,6). The selected HHP processing conditions were 250 MPa for 886 sec or 300 MPa for 200 sec at the treatment temperature of 5°C.

However, the selected HHP processing conditions could not inactivate every microbial strain as thermal pasteurization, and more resistant strain like *Listeria seeligeri* was not affected by such processing conditions. Therefore, combined processes of HHP with other non-thermal treatments, such as nisin and the high-intensity US, were explored to achieve further microbial inactivation. The ultrasound-HHP combination caused a slightly increased extent of *Escherichia coli* inactivation, which is attributed to the additional effect of ultrasound (7). More promising results were obtained by the combined treatment of nisin and HHP. The addition of nisin increased the lethal effects of HHP against Gram-positive *Listeria* up to 5 log cycles (7).

Because LWEs are mainly used as an ingredient in food industry, each end-product requires different demands on the functional property of LWE (8). The objective of this research was to verify the effects of combined non-thermal processes on the functional properties of LWE.

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## Materials and Methods

**Preparation of liquid whole egg** Fresh shell eggs, no older than 10 days after laid, were purchased from a local supermarket and were held overnight under refrigeration (4°C). The eggs were broken by hand, transferred into a sterile stomacher bag, and homogenized for 1 min in a stomacher laboratory blender 400 (Seward Medical, London, UK). The liquid whole egg (LWE) was kept in refrigerator until use.

**Combined treatments of nisin and high-intensity ultrasound with high hydrostatic pressure** Following combined processes were employed to determine the changes occurring in the functional properties of LWE after treatments: (i) untreated control; (ii) high hydrostatic pressure (HHP); (iii) nisin in combination with HHP (nisin-HHP); and (iv) ultrasound followed by HHP (ultrasound-HHP). The addition of nisin (10 mg/L) and the radiation of ultrasound (34.6 W for 30 sec) were conducted as described previously (7). HHP treatments (300 MPa for 200 sec at 5°C) were performed in a laboratory scale high pressure system (Engineered Pressure Systems International, Temse, Belgium) with a filling volume of 0.6 L.

**Color measurement** The Hunter L-, a-, and b-values were measured using a Minolta Colorimeter (CR-300;

Minolta, Osaka, Japan). The L-value denotes lightness, a-value denotes redness (or greenness), and the b-value denotes yellowness (or blueness). Each measurement was repeated more than 3 times and average values were reported.

**Foaming property** Foams of LWE were prepared by beating 100 mL of LWE using a cook mixer equipped with a double whipping beater (K1000; Braun, Kronberg, Germany). LWEs were added into the bowl of the mixer and beaten for 5 min at the speed of '5' which was specified as an 'egg whipping speed'. Then the produced foams were gently filled into the weighing boats (25-mL). The foaming power was expressed in terms of %overrun (9):

$$\%\text{Overrun} = \frac{(\text{wt 25 mL LWE}) - (\text{wt 25 mL foam})}{(\text{wt 25 mL foam})} \times 100 \quad (1)$$

Foam stability was determined by monitoring the drainage of foam at ambient temperature. The foams were filled into a 5-mL pipette with a tip diameter of 5.0 mm, and the weight of drained foams after 20 min was measured. The foam stability was calculated as %stability:

$$\%\text{Stability} = \frac{(\text{wt of foam}) - (\text{wt of drained foam})}{(\text{wt of foam})} \times 100 \quad (2)$$

Each measurement was repeated more than 3 times and average values were reported.

**Emulsifying property** The emulsifying property of LWE was examined by preparing an oil-in-water emulsion. Initially, 1 mL of LWE and 5 mL of commercial sunflower oil were blended at 8,000 rpm for 20 sec using a laboratory homogenizer (T25; IKA, Staufen, Germany). Then 5 mL of deionized water was added, and the obtained suspension was homogenized at 800 rpm for 1 min. This 2-step preparation of emulsion prevented the foaming of LWE during homogenization. Immediately after preparation, the oil droplets of emulsions were observed with 250 magnifications under a phase contrast microscope (Orthoplan, Leitz, Germany) connected with a color video camera head (TK 1070E; JVC, Yokohama, Japan).

**Statistical analysis** All experiments were performed in triplicate, and the resulting data were analyzed using a statistical analysis program (SPSS for windows, SPSS Inc., Chicago, IL, USA). All data were expressed as mean± standard deviation (SD). The differences between means were compared using Duncan's multiple range test with the significance of $p<0.05$.

## Results and Discussion

**Foaming property** The effects of combined processes on the foaming property of LWE are shown in Table 1. It can be seen that the application of HHP at the optimized process condition does not affect the foaming property of LWE. HHP alone and nisin-HHP induced slight increases both in foaming power and foaming stability, but they were not statistically significant. This maintenance of functional properties after HHP treatment was not unexpected, because the HHP processing condition had been selected where minimal rheological changes had occurred (5).

No publication was found concerning the foaming property of LWE treated by HHP. However, studies on egg white showed that the foaming power can be either decreased or increased by the extent of applied pressure (10,11). These contrary effects on foaming property were observed not only by HHP treatment but also by thermal treatment. The foaming property of LWE was either decreased (1,2) or not affected or even increased (3,12) depending on the pasteurization temperature and treatment time.

The larger increases of foaming power observed in the case of ultrasound-HHP combination might be explained by the homogenization effect of ultrasound. A mechanical homogenization process tended to increase the foaming power of LWE. The homogenization effect of ultrasound usually disperses the protein and fat particles in LWE more evenly, which may improve the foaming property of LWE. However, the physical property of ultrasound-HHP treated LWE showed larger differences from that of fresh LWE as indicated by significantly lowered viscosity of LWE (about 6.0 mPa·sec instead of 10.0 mPa·sec; from preliminary experiment).

**Emulsion property** The principal problem of food emulsions is the instability of emulsion which is caused by coalescence of droplets. Because coalescence is affected by the properties of the interfacial film and the droplet size of emulsion (13), the average droplet diameter and the size distribution provide useful information for the emulsifying property. Generally, smaller droplets are favored regarding emulsion stability.

Representative microphotographs of emulsions which were made with combined processes and control LWE are shown in Fig. 1. The average droplet size of an emulsion made with untreated LWE was 12.3±3.9 μm. The average droplet sizes of an emulsion made with HHP treated LWE and nisin-HHP treated LWE were statistically not different to that with untreated LWE ($p>0.05$), and the mean values were 13.5±6.3 and 13.6±4.6 μm, respectively. These results verify again that the application of HHP at the optimized process condition does not affect the functional properties of LWE. In contrast, the emulsion made with ultrasound-HHP treated LWE showed a significant increase in droplet size (16.9±5.1 μm). This increase in droplet size of an emulsion can be explained by the lowered viscosity of ultrasound-HHP treated LWE. Sala *et al.* (14) explained that US may induce the reduction of viscosity in complex food matrix due to depolymerization of high molecular weight macromolecules. The reduction of viscosity could increase the droplet diameter of an emulsion (13).

**Color** The effects of combined processes on the color of LWE are shown in Table 2. The L-values were almost constant among control, HHP, and nisin-HHP samples. However, the ultrasound-HHP resulted in a slight increase in L-value. In the case of thermal treatments, the increase in L-value, i.e., the whitening process was observed in the very early stage of thermal treatments and considered to be due to the denaturation of heat labile proteins (15). It has

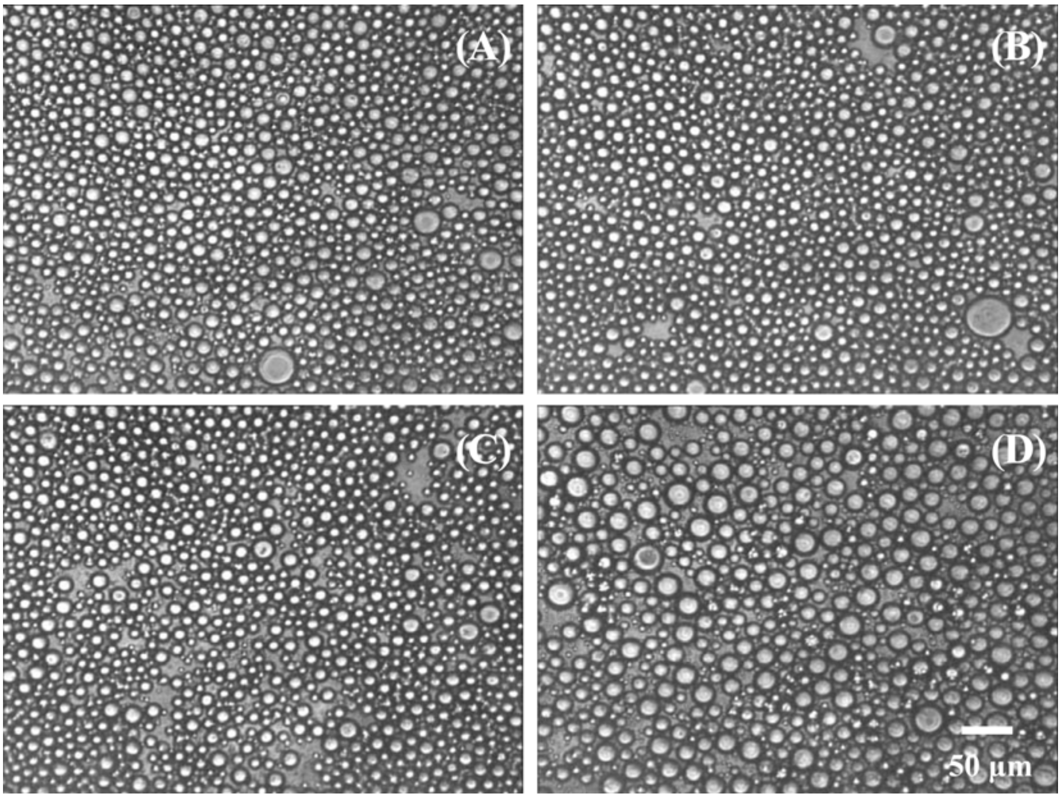


**Fig. 1. Effects of combined treatments on the emulsifying property of liquid whole egg (LWE).** Emulsions are made with untreated LWE (A); HHP treated LWE (B); nisin-HHP treated LWE (C); ultrasound-HHP treated LWE (D). Detailed process conditions are described in caption in Table 1.

**Table 1. Effects of combined processes on the foaming property of liquid whole egg**

| Process[1)] | Foaming power [% overrun] | SD[2)] | Foaming stability [% stability] | SD |
|---|---|---|---|---|
| Control | 479[a3)] | 13.7 | 52[a] | 5.1 |
| HHP | 490[a] | 10.0 | 56[a] | 3.1 |
| Nisin-HHP | 484[a] | 12.3 | 55[a] | 1.9 |
| Ultrasound-HHP | 638[b] | 14.4 | 50[a] | 2.6 |

[1)]HHP, 300 MPa for 200 sec at 5°C; nisin-HHP, combined process of nisin (10 mg/L) and HHP; ultrasound-HHP, combined process of ultrasound (34.6 W for 30 sec at 5°C) and HHP.
[2)]Standard deviation.
[3)]Columns with different superscript differ significantly ($p$<0.05).

**Table 2. Color changes of liquid whole egg by combined processes**

| Process[1)] | L (Whiteness) | a (Redness) | b (Yellowness) |
|---|---|---|---|
| Control | 60.88[a2)] | +5.90[a] | +33.06[c] |
| HHP | 60.92[a] | +8.32[b] | +30.10[ab] |
| Nisin-HHP | 61.04[a] | +8.32[b] | +30.45[b] |
| Ultrasound-HHP | 61.73[b] | +8.25[b] | +29.32[a] |

[1)]HHP, 300 MPa for 200 sec at 5°C; nisin-HHP, combined process of nisin (10 mg/L) and HHP; ultrasound-HHP, combined process of ultrasound (34.6 W for 30 sec at 5°C) and HHP.
[2)]Columns with different superscript differ significantly ($p$<0.05).

been reported that HHP also induces the increases of L-values especially in protein-rich food samples (16,17). However, this whitening process of HHP was not followed by a rapid shift of color toward the yellow and brown due to the Maillard reaction as reported by thermal treatments (18).

A more significant change in color was observed in a- and b-values of LWE. The increase in a value might be interpreted by the formation of Fe-conalbumin complex. The conalbumin yields a red color when it forms a complex with $Fe^{3+}$ ions (19). Despite the egg white in which conalbumin is suspended contains no $Fe^{3+}$ ions, but the $Fe^{3+}$ ions can be supplied from egg yolk during the preparation of LWE. However, it is not known whether HHP enhances the formation of Fe-conalbumin complex.

In conclusion, insignificant changes were observed in functional properties of LWE after HHP and nisin-HHP treatments. This maintenance of functional properties after HHP or nisin-HHP treatment agreed with our expectation, because the HHP processing condition had been selected where minimal rheological changes had occurred (5,6). In contrast, the ultrasound-HHP treated LWE showed a significant change in foaming and emulsion property indicating that some structural changes might have occurred during this treatment. The initial increase of L-value by ultrasound-HHP combination suggested denaturation of heat labile proteins. Combining previous microbiological inactivation data with the same processing condition (7) and present functional property data, a consecutive combination of nisin and HHP is regarded as more proper combined process of LWE in terms of microbial reduction and food quality.

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