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A PILOT STUDY OF ULTRASONICATION PRE-TREATMENT AND HIGH PRESSURE PROCESSING AFFECTING MICROBIAL INACTIVATION AND COLOR ATTRIBUTES OF LIQUID WHOLE EGG

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Abstract

Ultrasonic processing of a variety of liquids, drinks and beverages has generated much interest with published papers increasing within this area in recent years. Combination of ultrasonication and high hydrostatic pressure could be used as a combined non-thermal minimal processing technology for fluids. This work investigated the impact of ultrasound (US) pre-treatment combined with high hydrostatic pressure (HHP) on color and microbial inactivation in liquid whole egg (LWE).

Homogenized LWE was pre-treated with ultrasound (US) (12.50 ± 0.31W and 55, 65 and 75% amplitude) for 30 min. prior to high hydrostatic pressure (HHP) treatment (300 and 350 MPa, 5 min.). Microbiological measurements were carried out by a usual plating method. For statistical examination one-way ANOVA ($\alpha = 0.05$) was employed.

Our results revealed that color of LWE was varied statistically significant (one-way ANOVA, $\alpha = 0.05$), but nevertheless color difference ΔEab showed maximum visible differences. Increasing treatment parameters caused decreasing microbial cell count, increased microbiological inactivation was significant caused by different treatment parameters.

Summarizing our results show that UH pre-treatment combined with HHP can provide a microbiological safe product while its color stays stable.

Key words*: High hídrostatic pressure, Ultrasonication, Food preservation, Food safety.*

1. Introduction

In recent years there has been much activity in the area of applying ultrasound to process and interact with liquid foods primarily with dairy and fruit juices. A literature search for novel research papers, written in 2015 - 2016 alone, resulted in over 100 papers being located thus indicating the large extent of work in this field. Several authors have also written book chapters and review articles covering the diverse areas of ultrasound use in the food industry namely that of non-thermal food processing of beverages (Paniwnyk [1]).

The main frequencies used for ultrasonic processing appear to be in the power ultrasound region with the range 20 - 25 kHz being the most effective. Ultrasound induces mechanical, chemical and biochemical effects in liquids via the production and subsequent collapse of cavitation bubbles (Mason and Peters, [2]).

Several studies have shown the ability of ultrasound to inactivate spoilage and pathogenic microorganisms and enzymes in several food products.

Sound is propagated through a liquid via a series of compression and refraction waves induced in the molecules of the medium through which it passes. If the frequency of sound applied is of sufficient intensity voids within the liquid are produced which are known as cavities. These cavities often grow as a result of a process known as rectified diffusion, whereupon volatile gases already present within the bulk medium enter the cavities however are not fully expelled during the subsequent compression phase according Mason *et al.*, [3]. As these cavities grow they eventually become unstable and collapse releasing high temperatures and pressures on a microscopic scale resulting in extreme shear forces causing mass transfer, highly efficient mixing and homogenization in addition to high temperature 'hotspots' within the bulk liquid being processed. It is this cavitational collapse that generates the energy for various effects observed during food processing such as reduced fat globule sizes, greater homogeneity of liquids and enhanced extraction (Tiwari and Mason, [2], and Paniwnyk [1]).

The food industry uses conventional thermal pasteurization and sterilization techniques in order to inactivate microorganisms and enzymes and to increase the shelf life of many products. While most vegetative microorganisms and some spores respond well to such techniques, others prove more resistant and this can result in spoilage. Thermal processes require high levels of energy and as a result impact, sometimes unfavourably, on the nutritional content, sensory properties and quality of the final product (Chandrapala *et al.*, [4]). In addition to disadvantageous effects on the products the economic costs of energy involved in these high temperature processes have a large impact on the food production process and ultimately on the manufacturer themselves. Additionally, there is always the risk of foodborne microbial infections associated with the consumption of inadequately sterilised/pasteurized dairy products and fruit juices (Paniwnyk, [1]).

An opportunity of replacement of heat treatment is High Hydrostatic Pressure (HHP) which is applied combined or single for preservation of several food products (Sanz-Pulg *et al.*, [5], Baptista *et al.*, [6]). HHP is a novel, non-thermal technology usually used for preservation of liquid products like beverages and dairy products, or meat products (Sampedro *et al.*, [7], Santhirasegaram *et al.*, [8]). In the industrial application HHP treatments are used mostly between 350 and 650 MPa and between 2 and 15 minutes holding time depending on among others microbiological spoilage and pH value of samples (Bates *et al.*, [9], Khan *et al.*, [10]).

Only a few studies are available in topic of combined effects of HHP and ultrasound (Bashari *et al.*, [11], Sanz-Plug *et al.*, [12]). Our study investigates the opportunities of combined HHP and ultrasound treatments of liquid whole egg. The combination of the two minimal processing technologies may decrease techno-functional changes of LWE while the microbiological aspects of food safety are fulfilled.

Egg products may cause many food infections and are established as media of foodborne microbes with *Salmonella* being responsible in most cases (EFSA, [13]). Several pasteurization methods are developed and used in food industry (Fellows, [14], Hogue *et al.*, [15], Keener [16] Hogue *at al.*, [15]), but microorganisms like *Alcaligenes, Bacillus, Pseudomonas, Proteus, Listeria*, including the pathogenic *Listeria monocytogenes*, or some species of *Escherichia*, like *Escherichia coli* have been isolated from pasteurized liquid whole eggs (LWE) (Rioval *et al.*, [17], Monfort *et al.*, [18]).

This work investigated the impact of ultrasound (US) pre-treatment combined with high hydrostatic pressure (HHP) on colour and microbial inactivation in LWE. Our results point out a special new opportunity for preservation of egg products.

2 Materials and Methods

Samples for our experiments were taken from the production lines of Capriovus Ltd. Liquid whole egg was homogenised. The ultrasound treatment was carried out in a beaker without any packaging at room temperature. The US treatment (12.50 ± 0.31W and 70% amplitude) was applied for 10, 40 and 60 minutes.

After US treatment samples were packaged for HHP treatment in polyethylene plastic bags. For microbiological examinations samples were separately packaged, from every sample 50 - 50 mL were taken in 3 - 3 bags for microbiological test, for colour measurements 200 - 200 mL was packed.

HHP treatments of samples was carried out in a Resato FPU 100 - 2000 HHP equipment. Applied pressure was 300 MPa, holding times were 3 and 10 minutes, increase of pressure was 100 MPa/min.

Treatment parameters and sample nominations are summarised in Table 1.

Table 1. Applied treatment times and nominations of LWE samples

Sample	US, min.	HHP, min.
Control	-	-
40 + 0	40	-
10 + 10	10	10
10 + 3	10	3
40 + 10	40	10
40 + 3	40	3
60 + 10	60	10
60 + 3	60	3

In microbiological tests of samples mesophilic aerobe cell counts were inspected. As usual, Tellurite Glycine Agar (TGA) agar and a usual spread plate method were used with an incubation of 30 ^{0}C and 48 hours. For every samples 3 - 3 repetitions were taken from decimal dilution series. Tests were performed in 24 hours cold storage (5 ^{0}C) after treatments.

Colours of samples were inspected by Konica Minolta CR 400. Colour differences to control sample were evaluated in Lab colour space according to CIE76. Average and standard deviation calculations for the ten repetitions and a one-way ANOVA analysis to test significant differences between samples were carried out.

The goodness of fit of the model was assessed by using the adjusted regression coefficient (adjusted-R^2). The statistical analysis was performed by SPSS 20.0 (IBM).

3. Results and Discussion

Our results show that colour of LWE is influenced by combined treatments. Figure 1 shows L* which became higher by longer treatments.

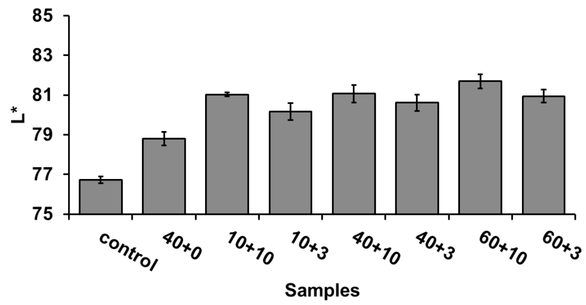

Figure 1. L* of LWE samples

Statistical analyse points out that changes in L* are significant. Less changes are perceptible in a* values.

Figure 2 shows that no essentially impact of treatments can be appointed in a* values.

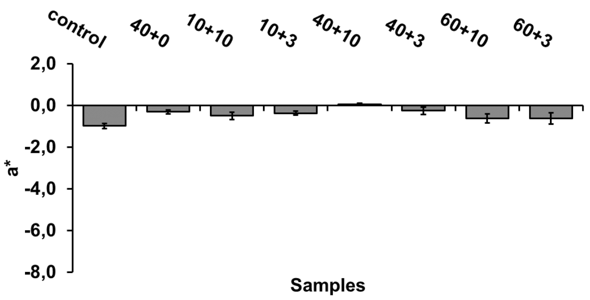

Figure 2. The a* values of LWE samples

Statistical evaluation confirms this finding.

On Figure 3, the b* values are presented.

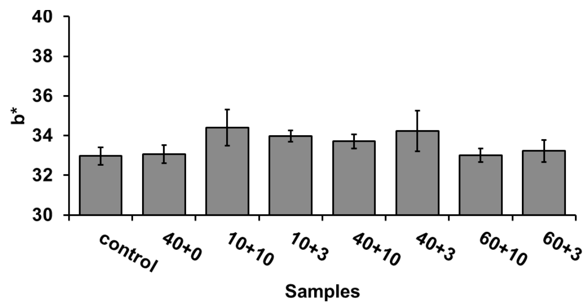

Figure 3. The b* values of LWE samples

Statistical analyse highlights that two samples (sample 10 + 10 and 40 + 3) have significant changes in b* values.

According to Figure 4 the colour difference is in case of sample 60 + 10 the highest.

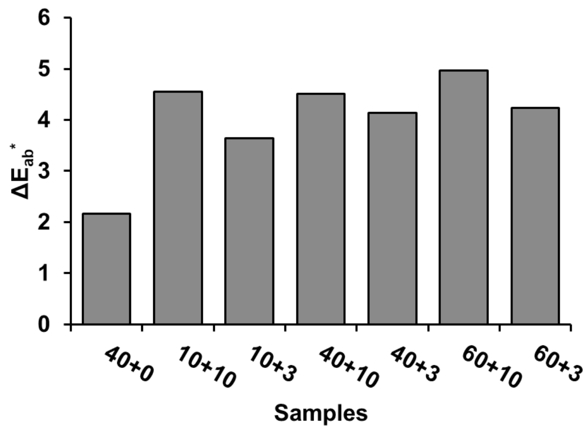

Figure 4. ΔE_{ab}^* values of treated samples compared to control sample

This result was expected then this sample has the longest US and HHP treatments. ΔE_{ab}^* points out that the holding time of HHP has a higher effect on colour than the treatment time of ultrasound pre-treatment.

Microbiological test highlights that longer holding time of HHP has no higher effectivity in inactivation of LWE microbiological spoilage. On Figure 5 is visible that just 2.5 magnitude decrease in mesophyll aerobe cell count was achieved using the longest holding time of HHP and longest treatment time of US.

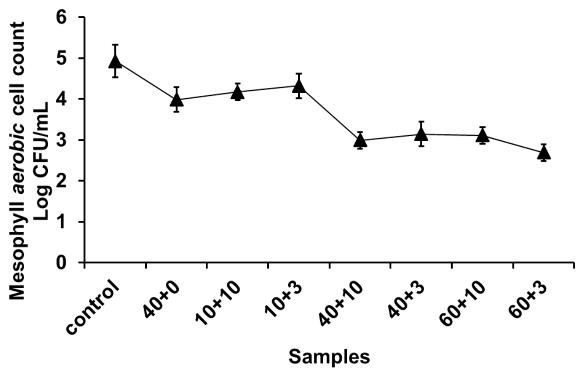

Figure 5. Mesophyll aerobic cell count of control and treated samples

4. Conclusions

- Our results show that combinations of US and HHP treatments are great opportunities to preserve LWE. In colour of samples (which is elemental consumer's choice) is inmost samples just slightly influenced.

- The microbiological spoilage of samples is decreased, highest decline is 2.5 magnitude. However, this decrease is not always enough for achieve microbiological food safety, for that reason other combinations of applied US and HHP parameters have to be investigated.

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