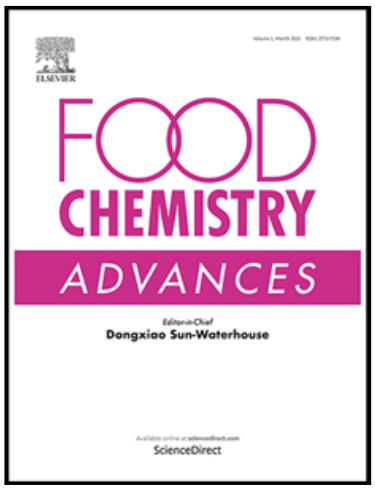

Influence of pressure pre-treatments on liquid whole egg thermal pasteurization – microbiological, physicochemical and functional properties

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Highlights

- Moderate pressure (MP) pre-treatments studied for egg thermal pasteurization (TP)
- MP-TP could allow shorter than commercial TP of liquid whole egg (LWE)
- MP-TP (200 MPa) reduced *S.* Senftenberg 775/W equally level *vs* commercial TP
- MP-TP (200 MPa) had higher emulsifying/gelling properties and protein solubility
- MP before a shorter TP is a viable alternative to commercial TP for LWE

Influence of pressure pre-treatments on liquid whole egg thermal pasteurization – microbiological, physicochemical and functional properties

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Abstract:

As a possible alternative to commercial thermal pasteurization[1] (TP, 60 ºC/3.5 min) of liquid whole egg[2] (LWE), a pasteurization based on the application of pressure pre-treatments (50 – 250 MPa/5 min) before a shorter TP (60 ºC/1.75 min) was investigated. These combined

[1] Thermal pasteurization (TP)
[2] Liquid whole egg (LWE)

treatments inactivated 3.35 to at least 6.09 $\log_{10}$ cycles of *S.* Senftenberg 775/W, achieving comparable to greater inactivation as commercial TP (at pressures ≥ 200 MPa).

The treated samples presented lower soluble protein and emulsifying activity than non-treated LWE, yet higher viscosity and improved foaming capacity. In contrast to commercial TP, the LWE treated by moderate pressure[3] (MP) before a shorter TP (MP-TP) showed higher soluble protein (7 %) and viscosity (49 %), better emulsifying properties (27 – 67 %), and lower total carotenoids content (9 %). Concerning sensory analysis, egg tarts prepared with non-treated and treated LWE obtained similar sensory acceptability.

Therefore, the heat sensitization effect induced by pressure pre-treatments on the *S.* Senftenberg 775/W population allows the reduction of TP time, a lethality effect equivalent to commercial TP and similar or improved functionality, being a very promising alternative to produce safe and better functional quality LWE.

Keywords: Liquid whole egg, pressure treatment, pasteurization, *Salmonella* Senftenberg 775/W, functionality

1. Introduction

Eggs are considered by World Health Organization as a standard food protein of high biological value (one egg provides ≈ 14 % of the recommended protein daily intake) (Food and Agriculture Organization, 2015; Franco et al., 2020). Yet, eggs are an excellent substrate for spoilage food-borne pathogens, and even stored under refrigeration are a highly perishable product (De Souza et al., 2015). *Salmonell*a spp. and *Listeria monocytogenes* are pathogens of public health concern in liquid whole egg (LWE), hence thermal pasteurization (TP) is necessary to maintain product safety (European Commission, 2007). Commercially, LWE is

[3] Moderate pressure (MP)

pasteurized at 60 – 70 ºC for 1.5 – 4.5 min (Food and Drug Administration, 2002; Monfort, Ramos, et al., 2012), nevertheless, this has a negative impact on egg products characteristics (De Souza et al., 2015; De Souza & Fernández, 2013; Herald & Smith, 1989).

In response to consumer demands for natural and minimally processed food products, and in an attempt to overcome the detrimental effects on eggs quality of conventional TP, the combination of non-thermal technologies such as high pressure (HP), pulsed electric fields (PEF), or ultrasound with a less intense TP have attracted increasing interest, as they may improve food safety and reduce losses of nutritional and sensory quality (Rahman, 2015). This possibility has been confirmed by the few published studies, in which microbial cells underwent sublethal damage during HP (200 – 300 MPa/3 – 30 min) or PEF (25 kV/cm/48 – 250 µs) treatments, thereby becoming more sensitive to subsequent TP (52 ºC/3.5 min and 55 ºC/2 min), with observed synergistic effects. However, these treatments only reduced about 1.5 – 3.5 $\log_{10}$ cycles of *Salmonella* spp. and *Escherichia coli* K12 DH 5α (*Salmonella* Enteritidis surrogate) in LWE, which is comparatively lower than that achieved by commercial TP at 60 ºC/3.5 min and 64 ºC/2.5 min (5 – 9 $\log_{10}$ cycles reductions of the most frequent *Salmonella* serotypes) (Jin et al., 2009; Monfort et al., 2011; Monfort, Ramos, et al., 2012). So, in an effort to increase microbial lethality, Monfort et al. (2011) and Monfort, Ramos, et al. (2012) combined the previous treatments with additives (triethyl citrate (TC) and EDTA), resulting in a reduction more than 5 $\log_{10}$ cycles of *S.* Enteritidis and *E. coli* K12 DH 5α. Nevertheless, changes observed in the treated samples (reduced soluble protein, increased viscosity, and improved foaming properties) were mainly attributed to the presence of the additives (Monfort, Mañas, et al., 2012; Monfort, Ramos, et al., 2012).

Therefore, in the search for processing methodologies that could lead to improved pasteurized LWE, a combined process using a pressure pre-treatment at 50 – 250 MPa/5 min before a TP at the commercial pasteurization temperature (60 ºC), but with a shorter pasteurization

time (3 min) was investigated. The purpose of this work was to optimize the conditions of pressure pre-treatments followed by a shorter TP in LWE, using *Salmonella enterica serovar* Senftenberg (ATCC 43845) (referred throughout this paper as *S.* Senftenberg 775/W) as a reference, due to its greater heat resistance compared to the serovars associated with most human infections (*S.* Typhimurium and *S.* Enteritidis) (European Food Safety Authority & European Centre for Disease Prevention and Control, 2021). In order to study this possibility in more detail, the impact on LWE quality properties, as well as the development of two microorganisms of interest in LWE (gram-positive and gram-negative bacteria), under post-TP refrigeration storage were also assessed.

2. Materials and Methods

2.1. Sample preparation

Fresh shell eggs were acquired from a local supermarket the day before they were used and held overnight under refrigeration. The eggshells were cleaned completely with 70 % ethanol, left to dry at room temperature inside a laminar flow cabinet, then cracked aseptically and the whole egg was carefully passed through a colander, while the vitellin membrane was disrupted with a scalpel blade. The whole egg was collected in a sterile cup and gently homogenized for 20 min at 1250 rpm using a magnetic stirrer (MS-3000, Biosan, Latvia).

2.2. Microorganisms

The strains used in this study were *S.* Senftenberg 775/W (ATCC 43845) and *Listeria innocua* (ATCC 33090). One glass bead from a deep-frozen culture was transferred to 250 mL of sterile Tryptic Soy Broth (TSB, Merck, Germany) and incubated at 37 ºC with rotational stirring (150 rpm) overnight (Incubating orbital shaker, VWR, Portugal). Thereafter, 1 mL of this culture was transferred to 250 mL of sterile TSB and incubated again at 37 ºC with

rotational stirring (150 rpm) for 12 h (until the stationary growth phase was reached). The liquid culture was allowed to settle at room temperature for 15 min, and glycerol was added to a final 10 % (v/v) concentration. Then, the samples were manually homogenized, transferred to Eppendorf tubes, immediately frozen under liquid nitrogen and stored at – 80 °C for later use.

2.3. Samples inoculation and packaging

The microorganism suspension prepared before (section 2.2) was thawed at room temperature and used immediately afterwards. This suspension was added to LWE and stirred for 5 min at 1250 rpm with magnetic stirring (MS-3000, Biosan, Latvia). Then, non-inoculated and inoculated samples were packed under aseptic conditions in polyamide-polyethylene bags (Plásticos Macar Lda., Portugal), which had been previously sterilized with UV radiation, and manually thermally sealed to minimize the amount of air inside the bags. The initial concentrations of *S.* Senftenberg 775/W and *L. innocua* were approximately 10^6 – 10^7 CFU/mL.

2.4. Pressure treatments and thermal pasteurization

The pressure treatments were performed in a High-Pressure industrial equipment (Modelo 55, Hyperbaric, Spain), with a 55 L pressure vessel at 50 – 250 MPa for 5 min at 20 ºC, using water as the pressure-transmitting medium. For TP, the samples were immersed in a circulating water bath (Circulator Bath, FALC, Italy) at 60 ºC/1.75 min (shorter TP) and 60 ºC/3.5 min (commercial TP), with the temperature monitored by using a *K*-type thermocouple (Thermometer 305, Roline, Switzerland). The time started to count after a come-up time of 40 seconds (previously verified at the geometric centre of the bags (4x3x0.5 cm)). For the sequentially combined treatments, samples that had been submitted to pressure treatments

were subsequently thermally treated, as described above. The maximum time between pressure treatments and TP was approximately 20 min. After treatments, the sample bags were immediately placed on ice and stored at 4 °C until analysis.

2.5. Microbiological analyses

The samples were analysed in triplicated for *S.* Senftenberg 775/W and *L. innocua* counts, and the appropriate serial dilutions were prepared in Ringer's solution. The microorganisms were determined by the spread plate method, by plating 100 µL of the appropriate dilution, in duplicate, onto Xylose Lysine Deoxycholate Agar (XLD Agar, Himedia, India) (ISO 6579:2017) and Listeria Palcam Agar (Liofilchem, Italy) (ISO 11290:2017). The plates were incubated at 37 ºC for 24 h and 48 h, for *S.* Senftenberg 775/W and *L. innocua*, respectively. Plates with 10 to 300 colonies were selected for counting and the results were expressed as log CFU/mL (microbial load). The results are presented as microbial log load variation (log (N/N_0)), which is calculated by the log load difference between the microbial load at each treatment (N) and the initial microbial load (without any treatment) (N_0).

2.6. Post thermal pasteurization refrigeration storage

LWE pasteurized by the optimized combined treatment was stored for 61 days at 4 ºC and compared with commercial TP, to study the microbial development of *S.* Senftenberg 775/W and *L. innocua*. For this purpose, microbiological analysis was conducted before and after treatments, as well as on days 2, 8, 14, 22, 30, 42 and 61 of storage. The experiment was carried out in 3 replicates at each time point.

2.7. Physicochemical and functional analyses

2.7.1. pH and colour

The pH was measured at 20 ºC by directly submerging a calibrated glass electrode (pH electrode 50 14, Crison Instruments, S. A., Spain) in homogenized egg samples.

Colour of LWE samples was analysed using a Konica Minolta CM 2300d spectrophotometer (Konica Minolta, Japan). The colour parameters L* - lightness (0, dark; 100, light), a* – redness (+, red; – green) and b* – yellowness (+, yellow; – blue) were obtaining at room temperature with the CIELab system. The CIELab parameters were determined using the original SpectraMagicTM NX Software (Konica Minolta, Japan), according to the International Commission on Illumination regulations. For measurements, 6 mL of sample was homogenized and placed in a sample container. The total colour difference (*ΔE**) was calculated by **Equation 1** (Koç et al., 2011):

$$\Delta E^* = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2} \quad (1)$$

where Δ*E** is the total colour difference between the sample and the control (raw sample), L* and L_0*are the lightness of treated and raw samples, respectively; a* and a_0*are the redness of treated and raw samples, respectively; and b* and b_0* are the yellowness of treated and raw samples, respectively.

2.7.2. Soluble protein content

The LWE samples were diluted at 10 % (m/v) in distilled water, and then centrifuged at 10,000 g for 15 min at 4 °C (Heraeus Biofuge Stratos, Thermo Electron Corporation, USA) (Sheng et al., 2018). The microassay Bradford method was used to determine the soluble protein content in the supernatant (Kruger, 2002). A standard curve was obtained using bovine serum albumin (Sigma-Aldrich, Portugal) in the range of 0.02 – 0.40 g/L.

Measurements were performed in triplicate, and the results are expressed in wt. % (g of protein/100 g of whole egg).

2.7.3. Viscosity

Viscosity was measured using a rotational rheometer (Kinexus PRO, Malvern Panalytical, Malvern, United Kingdom) with an attached cone-and-plate geometry (stainless steel cone, 4 º and 40 mm diameter). The experimental temperature was controlled at 20.0 ± 0.1 °C by a Peltier system at the bottom plate. After transferring the sample to the rheometer bottom plate and allowing for a 5 min equilibration time, the sample was subjected to a 3 min increasing shear rate ramp from 0.1 to 100 s^{-1}, followed by a 3 min decreasing ramp, from 100 to 0.1 s^{-1}. Determination of the apparent viscosity and analysis of the flow curves were performed using the rSpace software (Malvern Panalytical version 1.76s). Apparent viscosity measured at an intermediate shear rate of 50 s^{-1} was used for comparison among samples. Measurements were performed in triplicate of sample and duplicate of analysis.

2.7.4. Secondary lipid oxidation

Lipid oxidation was evaluated by quantification of secondary lipid oxidation products using the thiobarbituric acid-reactive substances (TBARS) method, as described by Vyncke (1970) with slight modifications. Two grams of egg were homogenized with 2 mL of 7.5 % trichloroacetic acid, and the resulting suspension was centrifuged at 6000 rpm during 15 min at 4 ºC (Centurion Scientific Ltd, Scansci, Portugal). 1 mL of the supernatant was added to 1 mL of 2-thiobarbituric acid (20 mM TBA in 99 % acetic acid glacial) and the mixture was gently shaken. The mixture was immersed in a 100 ºC water bath for 40 min before being cooled on ice. The absorbance was measured at 538 nm (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific Inc., USA). A standard curve of

malondialdehyde (MDA) was obtained with 1,1,3,3-tetramethoxypropane, in the range of 0.5 – 10 µM MDA. The measurements were carried out in triplicate, and the results were expressed as µg MDA/100 g of whole egg.

2.7.5. Total carotenoids content

Total carotenoids were quantified based on the method described by Nagata and Yamashita (Nagata & Yamashita, 1992). Briefly, 1.0 g of LWE was dissolved in 20 mL of a (4:6, v/v) acetone-hexane mixture, and the absorbance was measured at 453, 505, 645 and 663 nm (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific Inc., USA). The calculations were conducted using the formulas given by the mentioned authors [24]. The measurements were performed in triplicate of sample and duplicate of analysis, and the results were expressed in mg/100 g of whole egg.

2.7.6. Foaming properties

Foaming properties were measured according to the method used by Sheng et al. (2018). Foams were produced using 7 mL whole egg sample by whipping for 2.5 min with a laboratory homogenizer (Ultra-Turrax, T25 basic, IKA®-Werke, Germany) at a speed of 9500 rpm and room temperature in a 20 mL graduated cylinder. Foaming capacity (FC) was calculated as percentage of increase in foam volume using the **Equation 2**:

$$\mathrm{FC}\ (\%) = {}^{V_1}\!/_{V_0} \times 100 \qquad (2)$$

The foaming stability (FS) was measured by loss of foam volume after 30 min of standing at room temperature. FS was calculated by **Equation 3**:

$$\text{FS}\ (\%) = {}^{V_2}/_{V_1} \times 100 \quad (3)$$

where V_0 is the initial volume of egg (mL); V_1 is the foam volume after whipping (mL); V_2 is the foam volume after 30 min (mL). The foaming properties was evaluated in triplicate of sample and duplicate of analysis.

2.7.7. Emulsifying properties

As previously described by Zhang et al. (2019), the emulsifying activity index (EAI) and emulsion stability index (ESI) were measured at room temperature. To prepare emulsions, 3 mL of LWE were stirred by a homogenizer (Ultra-Turrax, T25 basic, IKA®-Werke, Germany) with 2 mL of sunflower oil, at 9500 rpm for 90 sec. An aliquot of emulsion was collected from the bottom of the homogenized emulsion immediately (0 min) or 10 min after homogenization and diluted 300 times in a 0.1 % (w/v) SDS solution. After dilution, the emulsion was shaken and the absorbance was read at 500 nm using a UV-Vis spectrophotometer (UVmini-1240, Shimadzu, Japan). Measurements were performed in triplicate of sample and duplicate of analysis and the EAI and ESI were calculated by **Equation 4** and **5**, respectively (Zhang et al., 2019):

$$\text{EAI}\ (m^2/\text{g}) = \frac{2 \times 2.303 \times A_0 \times \text{DF}}{c \times \Phi \times 10^4} \quad (4)$$

$$\text{ESI}\ (\text{min}) = \frac{A_0}{(A_0 - A_{10}) \times \Delta t} \quad (5)$$

where A_0 and A_{10} are the absorbance of the diluted emulsions at 0 and 10 min, respectively, DF is the dilution factor, *c* is the initial concentration of protein (g/mL); Φ is the fraction of oil used to form the emulsion (0.4), and Δt is the time interval between taking the first and second aliquots of emulsion.

2.7.8. Gelling properties

Heating set gels were prepared by pouring each LWE sample into a plastic casing, sealed at the both ends and heated at 85 ºC for 35 min in a programmable circulating water bath (FALC, Italy). The LWE gels were quickly cooled in a water-ice bath and stored at 4 ºC for 18 h. Before texture analysis, the gels were brought to room temperature, removed from the plastic and cut with a wire knife into cylinders of 22 mm diameter × 19 mm height. Textural analysis was conducted using a TA.XT.plusC Texture Analyzer (Stable Micro Systems, United Kingdom) equipped with a 30 kg load cell and a 50 mm diameter cylinder aluminium probe (P/50). The uniaxial compression tests up to rupture were performed at 2.00 mm/s. The Exponent Connect software (version 8.0.3.0, Stable Micro Systems, United Kingdom) was used to construct force-time curves and to obtain the following mechanical parameters: maximum force (N), area under the curve (N.sec) and slope at 10 % height. For the analysis, at least six cylindrical gels of each treatment were used.

The water holding capacity (WHC) of the LWE gels was determined as reported by Marco-Molés et al. (2011), with some modifications. Centrifuge tubes filled with LWE (2.0 mL) were placed in a water bath (Circulator Bath, FALC, Italy) at 85 ºC for 35 min for gel formation. After heating, the samples were immediately placed on ice during 15 min and then, kept at room temperature (20 ± 1 ºC) for 1 h. The tubes were then centrifuged at 740 *g* for 10 min at 20 ºC (Centurion Scientific Ltd, Scansci, Portugal). The WHC was given by **Equation 6**:

$$\text{WHC}\ (\%) = {}^{\text{WAC}}/_{\text{WBC}} \times 100 \quad (6)$$

where WAC is the weight of gel after centrifugation and WBC is the weight of gel before centrifugation.

2.8. Sensory analyses

Sensory analysis was carried out for egg tarts prepared with non-treated and treated LWE. The ingredients (LWE and salt) were mixed with a hand mixer for 30 seconds, and then poured into 8-mold silicone pie trays (each one with 7 cm in diameter at the top and 5 cm in diameter at the bottom). The samples were baked at 190 ºC for 12 minutes in a pre-heated oven (Flama, Cesar, Portugal). The egg tarts were stored at ambient temperature for cooling before the sensory evaluation. A panel of 13 untrained volunteers from the Chemistry Department at the University of Aveiro, Aveiro, Portugal (5 females and 8 males aged between 24 and 54 years) evaluated the sensory characteristics of egg tart. Each egg tart sample was randomly presented to the panellists in plastic dishes labelled with randomized three-digit codes. The panellists were served with water and crackers and instructed to cleanse their palate between tastings. Panellists were asked to evaluate each sample regarding acceptability based on 8 criteria (visual appearance, surface colour, interior colour, aroma, texture when cutting, texture when cracking, overall sensation when chewing, flavour) using a 9-point hedonic scale (1 = ‘dislike extremely’, 9 = ‘like extremely’). In addition, panellists were asked to order the samples according to their preference, from the one they liked least to the one they liked the most.

2.9. Statistical analyses

The data were tested at a 0.05 probability level ($p<0.05$) and the effect of each condition was tested with a one-way analysis of variance (ANOVA), followed by a multiple comparisons test (Tukey's Honestly Significant Difference, HSD) to identify statistically significant differences between treatments (SPSS Statistics v.28).

3. Results and Discussion

3.1. *Microbial analyses:* Pressure treatments

Figure 1 presents the evaluation of the inactivation of *S.* Senftenberg 775/W using moderate pressure (MP, 50 – 250 MPa/5 min) and high pressure (225 – 250 MPa/5 min) treatments. In general, as expected, an improvement in *S.* Senftenberg 775/W inactivation was obtained (the initial load was 6.94 ± 0.08 $\log_{10}$ CFU/mL) with increasing pressure, consistent with what other authors have reported (Bari et al., 2008; Monfort, Ramos, et al., 2012). MP treatments in the range of 50 – 90 MPa induced a decrease from 0.17 ± 0.11 to 0.39 ± 0.16 $\log_{10}$ cycles ($p\geq0.05$), but at pressure ≥ 125 MPa a significantly higher inactivation was achieved (0.53 ± 0.09 – 2.46 ± 0.18 $\log_{10}$ cycles, $p<0.05$). Nevertheless, other studies, despite applying similar or stronger conditions (200 – 250 MPa/5 – 30 min), obtained a lower inactivation (about 1 $\log_{10}$ cycles) for *E. coli* K12 (*S.* Enteritidis surrogate in liquid egg) and *S.* Enteritidis PT4 E10 compared to our study, suggesting a greater resistance to pressure of these two microorganisms (Işiker et al., 2003; Monfort, Ramos, et al., 2012). Therefore, the pressure conditions tested were not sufficient to ensure the LWE safety, although, higher pressures were not considered, since treatments over 300 MPa may have a drastic impact on the physical properties of LWE (Monfort, Ramos, et al., 2012).

3.2. *Microbial analyses:* Pressure treatment followed by thermal pasteurization

In this study, the use of pressure pre-treatments (50 – 250 MPa/5 min, at room temperature), aiming to cause sublethal damages in microorganisms and reduce their resistance to heat, before a shorter TP (60 ºC/1.75 min) was evaluated to determine the lethal effect on *S.* Senftenberg 775/W, and compared to commercial TP (60 ºC/3.5 min) (**Figure 2**). When the MP pre-treatments were conducted at 50 and 70 MPa, about 3.4 $\log_{10}$ cycles of the *S.* Senftenberg 775/W population were inactivated ($p \geq 0.05$), whereas at 90 MPa lethality improved slightly (3.8 ± 0.1 $\log_{10}$ cycles, $p < 0.05$), and a similar lethal effect ($p \geq 0.05$) was obtained with increasing pressure up to 125 MPa. Moreover, the treatments carried out at pressures ≥ 200 MPa caused even greater inactivation ($p < 0.05$), resulting in reductions of at least 4.95 $\log_{10}$ cycles, with counts reaching below the quantification limit (≤ 2.00 log CFU/mL) for 200 MPa and below the detection limit (≤ 1.00 log CFU/mL) for 225 and 250 MPa. These findings are more encouraging than those of previously published works, which reported reductions of 1.5 to 3.1 $\log_{10}$ cycles for *E. coli* K12 DH 5α (*S.* Enteritidis surrogate in liquid egg) in LWE treated at 200 – 300 MPa/3 – 30 min followed by TP at 52 – 55 ºC/2 – 3.5 min (Monfort, Ramos, et al., 2012).

Otherwise, the shorter TP alone reduced *S.* Senftenberg 775/W counts by 3.01 ± 0.12 $\log_{10}$ cycles, but the commercial TP achieved a greater decrease of at least 4.95 $\log_{10}$ cycles (counts below the quantification limit, ≤ 2.00 log CFU/mL). Compared to the latter, the combined treatments induced a comparable (at 200 MPa, $p \geq 0.05$) or greater (at pressures ≥ 225 MPa, $p < 0.05$) inactivation. The synergistic effect observed at pressures ≥ 200 MPa suggest that *S.* Senftenberg 775/W was sublethally injured by pressure pre-treatments, possibly reducing their thermal resistance, and leading to the subsequent TP being more effective than when applied alone. In addition, the effects induced by pressure pre-treatments may be due to damage of the cell membrane and its structure, changes in mechanisms of nutrient absorption and release of metabolites; changes in proteins structure (denaturation, aggregation or gel

formation) (Thakur & Nelson, 1998; Yaldagard et al., 2008), disruption of non-covalent bonds (mainly affecting tertiary and quaternary structures) (Knorr, 1999); and multimeric disintegration ribosomes (Niven et al., 1999). Thus, when this technology is applied at sublethal intensities, the damage caused will likely be to the same structures but to a lesser extent.

According to the results discussed previously, two combined treatments were chosen for further investigation: 200 MPa/5 min – 60 ºC/1.75 min, which corresponded to a processing condition that accomplished a similar inactivation level to commercial TP, and did not cause a large increase in viscosity (as confirmed by a simple test of flow time along a glass surface at a 45 º angle), and 50 MPa/5 min – 60 ºC/1.75 min. Due to the intention of the authors to conduct additional studies involving the sequential combination of two nonthermal pre-treatments (pressure and ultrasound), in order to enhance microbial inactivation, the latter case was evaluate to determine if it would result in lower quality changes, despite having caused a lower microbial reduction. In addition, a post-TP refrigeration storage evolution of *S.* Senftenberg 775/W and *L. innocua* was examined, although only for the 200 MPa /5 min – 60 ºC/1.75 min treatment, which achieved microbial inactivation comparable to that of commercial TP.

3.3. *Microbial analyses:* Assessment of post thermal pasteurization refrigeration storage

The impact of MP-TP (200 MPa/5 min followed by 60 ºC/1.75 min), shorter TP and commercial TP on *S.* Senftenberg 775/W and *L. innocua* (*L. monocytogenes* surrogate) counts during storage at 4 ºC, was evaluated (**Figure 3**). LWE was inoculated with an initial load of 6.62 ± 0.11 log CFU/mL of *S.* Senftenberg 775/W (**Figure 3a**), and immediately after treatments (day 0), a reduction of 4.15 $\log_{10}$ cycles was observed for shorter TP, and at least

4.62 log_{10} cycles were obtained for MP-TP and commercial TP ($p<0.05$). During storage, *S.* Senftenberg 775/W counts decreased in all samples, with a faster reduction in commercial TP- followed by MP-TP- and shorter TP-treated samples, with counts reaching below the detection limit (≤ 1.00 log CFU/mL) after 3, 8 and 42 days of storage, respectively. No further variation until the end of storage was observed.

Concerning *L. innocua* (**Figure 3b)**, the initial load was 6.99 ± 0.03 log CFU/mL, and the three treatments (day 0) reduced the counts by at least 4.99 log_{10} cycles (below the quantification limit, ≤ 2.00 log CFU/mL). Regarding to commercial TP- and MP-TP-treated LWE, the loads did not change during 23 and 14 days (≤ 2.00 log CFU/mL), respectively, but then increased until day 42, with the increase being slightly more pronounced in MP-TP-treated samples (up to 4.92 ± 0.26 log CFU/mL) and remaining similar until the end of the experiment. A greater and faster *L. innocua* growth was observed in shorter TP-treated LWE, with levels of 5.5 ± 0.21 log CFU/mL after 30 days of storage and no significant variations until day 61. In addition, the prevalence and growth of *L. innocua*, during storage, suggests that, despite this microorganism counts being below the quantification limit after treatments, the remaining cells were able to multiply under refrigerated conditions, although to a less extent and later for commercial TP, followed by MP-TP and shorter TP.

3.4. pH and colour

The pH values measured range from 7.72 ± 0.01 (raw LWE) to 7.68 ± 0.01 for treated samples (**Table 1**). Regarding LWE colour, in general, treated samples showed similar lightness (L*), redness (a*) and yellowness (b*) ($p\geq0.05$) to non-treated sample. However, the MP-TP (200 MPa)-treated LWE exhibited a decrease in yellow colour (lower b* value, $p<0.05$), resulting in a significantly higher ΔE^*, which might be indicate a lower carotenoids content (Monfort, Mañas, et al., 2012). Nonetheless, the colour variation produced by the four

treatments would be not detected by the naked eye (ΔE^*<3) (De Souza & Fernández, 2011). Similar results were reported by Monfort, Mañas, et al. (2012) for LWE treated with a PEF pre-treatment (25 kV/cm, 75 kJ/kg) followed by a TP (60 ºC/1 min) in the presence of TC.

3.5. Soluble protein content

The soluble protein content represents the most practical criteria for predicting the overall functionality of LWE, and a reduction often reflects a decrease in egg proteins functionality (Herald & Smith, 1989; Monfort, Ramos, et al., 2012). As can be seen in **Table 1**, all treatments reduced significantly the soluble protein content of non-treated LWE (3.16 ± 0.11 to 2.78 ± 0.10 g/100 g, $p<0.05$), showing a decrement of about 7 % for MP-TP (50 and 200 MPa)- and shorter TP-treated LWE, while a slightly higher loss (12 %, $p<0.05$) was caused by commercial TP. Heat may tigger the unfolding of egg proteins by exposing the hydrophobic groups buried inside, hence promoting aggregation by hydrophobic interactions, and reducing soluble protein content (Chang et al., 2022). Moreover, the negative impact on MP-TP-treated samples might be attributed to the themal effects, that resulted in a less pronounced unfolding compared to commercial TP. Our results are supported by the findings of Souza and Fernández (De Souza & Fernández, 2013), who observed that a mild LWE pasteurization (60 ºC/3.5 min) can cause proteins unfolding and aggregation, possibly affecting ovomucoid, livetins, and some LDL apoproteins. Likewise with MP-TP-treated LWE, previous works indicated a soluble protein decreased (about 12 %) after TP (52 ºC/3.5 min) preceded by a pressure treatment (300 MPa/5 min) in presence of TC (Monfort, Ramos, et al., 2012).

3.6. Apparent viscosity

In accordance with previous authors (Monfort, Ramos, et al., 2012), the apparent viscosity of raw LWE was 13.0 ± 1.0 mPa.s (**Table 1**), but all treated LWE samples showed higher

apparent viscosity (20.53 ± 1.41 – 30.64 ± 4.17 mPa.s, $p<0.05$). The increment found for TP-treated LWE (about 59 %) is in line with the soluble protein decrease (**Table 1**), probably due to protein unfolding and consequent aggregation of unfolded proteins (Jaekel & Ternes, 2009). MP-TP (50 and 200 MPa)-treated LWE showed an increase up to 49 % and 136 % higher ($p<0.05$) than TP- and non-treated LWE, respectively. However, despite the soluble protein data did not show a significant difference between shorter TP- and MP-TP-treated samples, the higher impact caused by MP-TP on viscosity, may indicate more marked protein aggregation under these conditions. Consequently, the results suggest that, in addition to thermal effects, the MP pre-treatment might also have an impact on viscosity *per se*. In fact, according to previous studies, increasing pressure and treatment time resulted in a LWE viscosity increment, possibly due to a greater protein-protein interaction under pressure (Anson & Mirsky, 1931; Monfort, Ramos, et al., 2012).

3.7. Secondary lipid oxidation

Secondary lipid oxidation (TBARS, expressed as MDA content) was assessed (**Table 1**). The MDA content of raw LWE was 13.29 ± 0.56 µg /100 g, being comparable to values reported by other authors (Hur et al., 2003). No significant differences were noticed among raw and treated LWE, with values ranging from 12.92 ± 0.58 to 13.36 ± 0.57 µg MDA/100 g ($p\geq0.05$). These findings are consistent with what was reported in a previous study (De Souza & Fernández, 2011), with no significant effect on TBARS values being observed for pasteurized LWE (60 ºC/3.5 min).

3.8. Total carotenoids content

The non-treated LWE presented a total carotenoids content of 0.92 ± 0.02 mg/100 g (**Table 1**), which is consistent with values reported by Fredriksson et al. (2006). As shown in **Table**

1, for all treatments, no significant differences ($p \geq 0.05$) were observed compared to raw LWE, with exception of MP-TP (200 MPa) (a reduction of about 12 %). The lack of effect noted for the TP is corroborated by published data when egg yolk (EY) was heated at 61.5 °C for 3.5 min (Wenzel et al., 2010). On the other hand, the significant loss observed for MP-TP-treated LWE at the highest pressure (~12 %), may be attributed to degradation reactions that occur during processing (Cano et al., 2019), suggesting that the decrease was likely a result of the pressure pre-treatment. Indeed, published studies reported that MP treatment (200 MPa/6 min) reduced the total carotenoid content of persimmon fruit (Cano et al., 2019). Furthermore, the carotenoids reduction resulted in a less yellow colour and a greater ΔE compared to the other treated LWE, however, these differences are not considered noticeable by the consumer as $\Delta E^* < 3$ (De Souza & Fernández, 2011).

3.9. Foaming properties

The foaming properties of LWE is attributed to the egg white (EW) proteins, while EY components are considered as an inhibitor due to the competition by yolk compounds (proteins and lipids) with EW proteins in the air bubble interfaces (Li et al., 2019). **Table 2** summarizes the influence of the studied treatments on foaming properties of LWE. All treatments improved the FC of non-treated LWE (47 – 67 %, $p < 0.05$), but had no impact on FS. Additionally, the treated LWE samples exhibited comparable foaming properties to each other ($p \geq 0.05$), and so, suggesting that the impact on MP-TP-treated LWE was related with the thermal effects. The improvement of FC could probably be associated with the conditions used for foam production. As reported above, the treatments led to the formation of aggregates by hydrophobic interactions, which are quite weak and can probably be dissociated during the homogenization used for foaming (9500 rpm/2.5 min), resulting in the formation of smaller aggregates. Consequently, these aggregates can likely move faster to the foam

interface, than their corresponding native proteins, thus improving FC (Guilmineau & Kulozik, 2006; Liang & Kristinsson, 2007). Similar findings were described by Van der Plancken et al. (2007), when heated EW solutions (pH 7.6) at 60 ºC. Actually, other works also reported no significant changes on FC of EW treated at 200 MPa (Yang et al., 2009) or EW solutions (pH 7.6) pressure-treated (0.1 – 700 MPa) at 60 ºC (Van der Plancken et al., 2007).

3.10. Emulsifying properties

Egg emulsifying properties are mainly associated to yolk compounds, however the whole egg is also a good emulsifier (Hatta et al., 1997). As indicated in **Table 2**, the parameters used to describe emulsifying properties of LWE were EAI and ESI. For all treatments, in general, it was observed a significant reduction ($p<0.05$) of EAI (11 – 38 %) and ESI (22 – 53 %) compared to non-treated LWE, however, a less pronounced effect was found for MP-TP-treated samples. This decrease might be attributed to the proteins unfolding, which promotes aggregation by hydrophobic interactions, and consequently, these aggregated proteins could not adsorb at the interface (Chang et al., 2022; Le Denmat et al., 1999). In opposition to what was suggested for foaming properties, the homogenization conditions used for emulsions production were less intense (9500 rpm/1.5 min) and were likely insufficient to allow the dissociation of aggregates formed with the treatments. Also, when less aggregates adsorb at the interface, the stability of the formed emulsions reduce, since this property is related to the interfacial area that can be coated by proteins (Pearce & Kinsella, 1978).

Further, LWE pasteurized at commercial conditions presented an ESI 1.6-fold lower than shorter TP-treated LWE ($p<0.05$), showing that the pasteurization time had a significant effect on this parameter. On the contrary, Lechevalier et al. (2017) highlighted that pasteurization at 60 ºC (maximum) optimized the emulsifying properties of LWE, and only at 66 ºC the

pasteurization time has a significant effect. Regarding MP-TP-treated samples, similar or higher emulsifying properties than shorter-TP-treated LWE was detected, consistently with Chen et al. (2019) work, who noticed an increase in EAI and ESI when using pressures up to 200 MPa, possibly indicating that in our work the thermal effects counteract the positive effect of MP pre-treatments. Additionally, enhanced emulsifying properties (1.3 – 1.7-fold higher) was found for MP-TP (200 MPa)-treated LWE compared to commercial TP LWE.

3.11. Texture and water holding capacity of LWE gels

The texture properties (maximum force, area under the curve, and slope at 10 % of height) of thermal-induced LWE gels were evaluated by uniaxial compresion tests. The typical force-time curves were characterized by a rise in force until the end of the test, and the obtained results are shown in **Table 2**.

The gels formed from raw LWE presented a significantly lower maximum force ($p<0.05$) than those obtained from commercial TP- and MP-TP (200 MPa)-treated LWE (25 – 43 % higher), and an increment of about 20 % was found with the treatment time increase (1.75 to 3.5 min). As presented in **Table 2**, the area under the curve calculated for gels produced from raw LWE showed non-significant differences among samples ($p\geq0.05$), with the exception of gels produced with MP-TP (200 MPa)-TP-treated LWE, which presented a higher value ($p<0.05$). Moreover, these last gels also demonstrated a significant increment ($p<0.05$) in slope value relative to the other gels, although no significant changes ($p\geq0.05$) were found between TP treated samples and raw LWE. Furthermore, the higher hardness and rigidity (15 – 43 %) of gels from MP-TP (200 MPa)-treated LWE as compared to the gels treated by only temperature, suggest that the pre-treatment at the highest pressure was responsible for the observed increase. For MP-TP (50 MPa)-treated gels, although there were no significant differences compared to shorter TP and non-treated gels, an increasing trend was observed.

Therefore, the findings indicate that gels produced from commercial TP more closely resembled gels produced from raw LWE than those produced from MP-TP (200 MPa)-treated LWE.

Concerning WHC, as can be seen in **Table 2**, non-significant differences ($p \geq 0.05$) were found among the WHC of gels produced from raw and treated LWE, as also observed by Monfort, Mañas, et al. (2012) and Monfort, Ramos, et al. (2012) in LWE supplemented with 2 % TC and treated by HP (300 MPa/5 min) or PEF (25 kV/cm) followed by TP (52 – 55 ºC/2 - 3.5 min).

3.12. Sensory analysis

Sensory analysis of an egg-derived product (egg tart) prepared with non-treated and treated LWE (200 MPa/5 min followed by 60 ºC/1.75 min, 60 ºC/3.5 min) was assessed, and the results are shown in **Figure 4**. On a 9-point hedonic scale, the range of attributes were from 5.2 to 7.3, which is equivalent to "neither like nor dislike slightly" and "like moderately", respectively. The sensory characteristics scores (interior colour, texture when cracking, overall feeling when chewing, flavour and global acceptability) were highest for non-treated samples, while the scores of visual appearances, surface colour, aroma and texture when cutting were higher for the LWE thermally treated. Besides, the egg tarts prepared with MP-TP-treated LWE received the lowest scores for all sensory characteristics. For this sample, the lower score for texture when cracking is consistent with the highest texture properties observed for thermal-induced gels. Yet, there were no significant differences ($p \geq 0.05$) between the three egg tarts. Egg tarts obtained a similar global acceptability by the panellists, although they were ordered by preference as follows: egg tart produced from MP-TP-treated LWE, raw LWE and commercial TP-treated LWE (from the one panellist liked least to the one they liked the most).

4. Conclusions

The application of pressure treatments alone (50 – 250 MPa/5 min) was ineffective in controlling *S.* Senftenberg 775/W, but when followed by a shorter TP, inactivation was significantly increased (at least 6.09 $\log_{10}$ cycles), obtaining similar or even higher lethality than commercial TP, using a pressure pre-treatment ≥ 200 MPa. On the other hand, all the treatments caused protein unfolding and aggregation, resulting in a drop in soluble protein content and an increase in viscosity. Consequently, these changes in treated LWE resulted in enhanced FC (47 – 88 %), higher texture properties (13 – 43 %) and reduced emulsifying properties (18 – 53 %) in comparison to non-treated LWE. Compared to commercial TP, the MP-TP treatment presented higher viscosity, emulsifying and gelling properties, equivalent foaming properties, and a reduced total carotenoids content. Additionally, egg tarts produced with non-treated and treated LWE had a similar global acceptability. Hence, the findings hint the possibility to use sequentially combined treatments to enhance inactivation of *S.* Senftenberg 775/W, using a shorter TP and maintaining/improving some properties of pasteurized LWE.

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Tables

Properties	Raw	Thermal Pasteurization		Combined Treatments	
		60 ºC/3.5 min	60 ºC/1.75 min	50 MPa/5 min – 60 ºC/1.75 min	200 MPa/5 min – 60 ºC/1.75 min
pH	7.72 ± 0.01 [a]	7.69 ± 0.01 [bc]	7.70 ± 0.01 [b]	7.68 ± 0.01 [c]	7.69 ± 0.01 [c]
L*	47.16 ± 0.16 [ab]	47.24 ± 0.07 [ab]	46.89 ± 0.67 [bc]	46.65 ± 0.72 [c]	47.35 ± 0.13 [a]
a*	7.37 ± 0.12 [ab]	7.40 ± 0.06 [a]	7.13 ± 0.39 [b]	7.19 ± 0.45 [ab]	7.41 ± 0.09 [a]
b*	11.30 ± 0.16 [a]	10.96 ± 0.22 [ab]	10.57 ± 0.73 [b]	10.55 ± 0.74 [b]	10.62 ± 0.16 [b]
ΔE^*	-	0.46 ± 0.16 [b]	0.38 ± 0.09 [b]	0.40 ± 0.11 [b]	0.64 ± 0.04 [a]
Soluble Protein (g/100 g whole egg)	3.16 ± 0.11 [a]	2.78 ± 0.09 [c]	2.93 ± 0.10 [b]	2.94 ± 0.17 [b]	2.95 ± 0.19 [b]
Viscosity (mPa.s, at shear rate of 50 s^{-1})	12.98 ± 1.03 [c]	20.62 ± 1.33 [b]	20.53 ± 1.41 [b]	28.64 ± 2.26 [a]	30.64 ± 4.17 [a]
Lipid Oxidation (TBARS, µg/100 g whole egg)	13.29 ± 0.56 [a]	13.11 ± 0.51 [a]	12.92 ± 0.58 [a]	13.15 ± 0.73 [a]	13.36 ± 0.57 [a]
Total Carotenoids (mg/100 g whole egg)	0.92 ± 0.02 [a]	0.90 ± 0.03 [a]	0.94 ± 0.01 [a]	0.93 ± 0.02 [a]	0.81 ± 0.04 [b]

Table 1. Physicochemical properties, lipid oxidation and total carotenoids of raw and treated liquid whole egg (mean ± standard deviation). Different letters along each row denote significant differences ($p<0.05$) between processing conditions.

Properties	Raw	Thermal Pasteurization		Combined Treatments	
		60 ºC/3.5 min	60 ºC/1.75 min	50 MPa/5 min – 60 ºC/1.75 min	200 MPa/5 min – 60 ºC/1.75 min
Foaming Capacity (%)	39.3 ± 7.3 [b]	60.5 ± 7.9 [a]	58.6 ± 6.5 [a]	65.5 ± 2.8 [a]	57.6 ± 9.5 [a]
Foaming Stability (%)	82.9 ± 8.1 [a]	70.3 ± 6.2 [a]	72.1 ± 5.7 [a]	73.7 ± 10.6 [a]	72.0 ± 9.6 [a]
Emulsifying Activity Index (m^2/g)	65.8 ± 4.2 [a]	44.2 ± 4.8 [c]	40.8 ± 1.0 [c]	45.1 ± 1.6 [c]	58.5 ± 1.9 [b]
Emulsifying Stability Index (min)	1.3 ± 0.3 [b]	0.6 ± 0.0 [d]	1.0 ± 0.2 [c]	1.5 ± 0.2 [a]	1.0 ± 0.0 [bc]
Maximum Force (N)	79.0 ± 11.2 [b]	98.6 ± 9.7 [a]	79.1 ± 7.9 [b]	80.9 ± 12.1 [b]	113.2 ± 9.7 [a]
Area under the curve (N.sec)	639 ± 31 [b]	689 ± 51 [b]	631 ± 32 [b]	688 ± 51 [b]	800 ± 37 [a]
Slope at 10% Height	0.62 ± 0.01 [bc]	0.63 ± 0.04 [bc]	0.61 ± 0.01 [c]	0.66 ± 0.02 [b]	0.70 ± 0.03 [a]
Water Holding Capacity (%)	99.0 ± 0.6 [a]	99.0 ± 0.9 [a]	99.1 ± 0.7 [a]	99.4 ± 0.4 [a]	98.6 ± 0.7 [a]

Table 2. Functional properties of raw and treated liquid whole egg (mean ± standard deviation). Different letters along each row denote significant differences ($p<0.05$) between processing conditions.

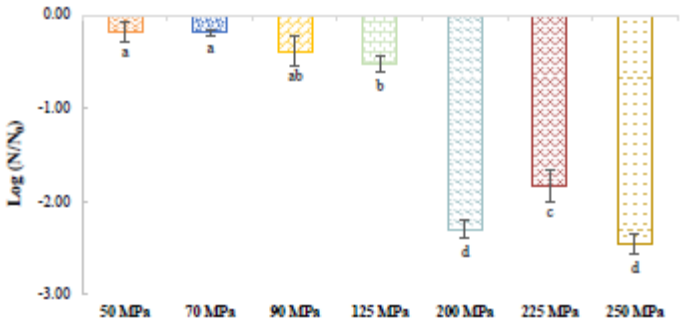

Figure 1. Log_{10} cycles reductions of *Salmonella* Senftenberg 775/W (ATCC 43845) in liquid whole egg treated by pressure treatments at 50 – 250 MPa during 5 min. N denotes the microbial load measured for each treatment and N_0 the initial microbial load (without any treatment). Different letters indicate significant differences ($p<0.05$) between treatments.

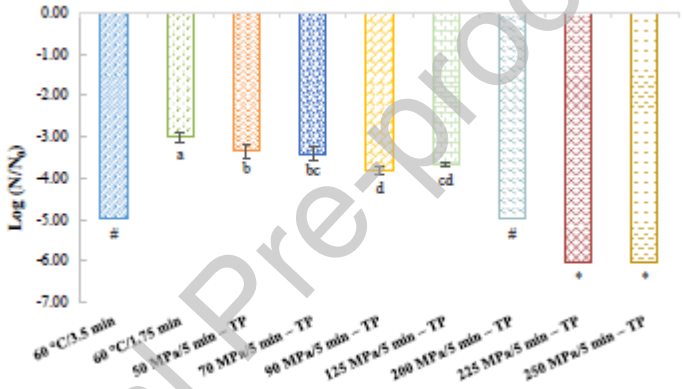

Figure 2. Log_{10} cycles reductions of *Salmonella* Senftenberg 775/W (ATCC 43845) in liquid whole egg treated by pressure pre-treatments (50 – 250 MPa/5 min) before a shorter thermal pasteurization (TP, at 60 ºC/1.75 min) or TP only (60 ºC/1.75 min and 60 ºC/3.5 min). N denotes the microbial load measured for each treatment and N_0 the initial microbial load (without any treatment). The symbols # and * means that microbial counts were below the quantification limit (≤ 2.00 log CFU/mL) or detection limit (≤1.00 log CFU/mL), respectively. Different letters indicate significant differences ($p<0.05$) between treatments.

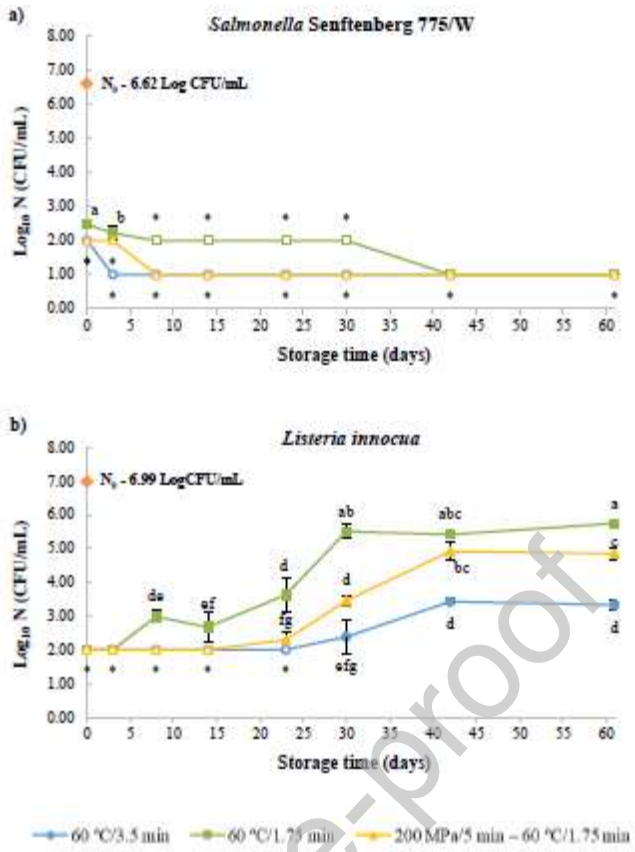

Figure 3. Development of a) *Salmonella* Senftenberg 775/W (ATCC 43845) and b) *Listeria innocua* (ATCC 33090) up to 61 days of storage after moderate pressure followed by a shorter thermal pasteurization (TP) (200 MPa/5 min – 60 ºC/1.75 min) or TP only (60 ºC/1.75 min and 60 ºC/3.5 min). Unfilled symbols on graphics and # or * mean that microbial counts were below the quantification limit (≤ 2.00 log CFU/mL) or detection limit (≤1.00 log CFU/mL), respectively, and N_0 corresponds to the initial load before each treatment. Different letters denote significant differences ($p<0.05$) between each processing condition and storage days.

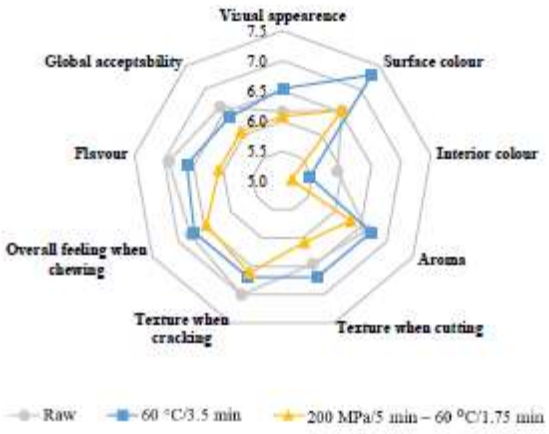

Figure 4. Comparative diagram of sensory attributes of the egg tart prepared from raw and treated liquid whole egg (9-point hedonic scale: 1-disliked extremely, 9-liked extremely).

CRediT

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Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: