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Changes in microbial, chemical, physical and techno-functional properties of liquid egg yolk during hyperbaric storage

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ABSTRACT

The effects of hyperbaric storage (HS) at 200 MPa on the microbial, chemical, physical and techno-functional properties of liquid egg yolk were investigated and compared to refrigeration. Inoculated *Salmonella enterica* (3.35 ± 0.12 logCFU g^{-1}) and *Staphylococcus aureus* (2.78 ± 0.19 logCFU g^{-1}) resulted below the detection limit after 24 and 48 h HS, respectively. Liquid egg yolk oxidative status remained unaffected for up to 28 days under pressure, probably due to the presence of egg yolk antioxidants. The decrease in egg yolk denaturation enthalpy and the increase in aromatic amino acid exposure indicated that egg yolk proteins unfolded according to structural changes other than those observed during refrigeration. Liquid egg yolk viscosity progressively increased during HS, eventually leading to gelling. Protein modification did not affect liquid egg yolk foaming and emulsifying properties but impaired its solubility and thermally-induced gelling. HS could be used for microbial decontamination of liquid egg yolk while maintaining oxidative stability and the typical capacity to stabilize foams and emulsions.

1. Introduction

The possibility of storing foodstuff under pressure has attracted increasing interest during the last decade. This technological approach, also known as hyperbaric storage (HS), consists in preserving foods by applying moderate hydrostatic pressure ($P < 250$ MPa) (Santos, Fidalgo, et al., 2021). To apply HS, foods are packaged inside flexible plastic pouches, inserted inside a pressure-holding steel vessel, and pressurized by pumping a liquid (*e.g.*, water, propylene glycol) until the desired pressure is reached. At this point, these conditions are maintained for storage, which can last for up to several months (Santos, Fidalgo, et al., 2021). When storage temperature is not controlled, no further energy is needed for pressure maintenance during HS. For this reason, the technology has been proposed in the literature as a low-carbon footprint alternative to refrigerated storage (Bermejo-Prada, Colmant, Otero, & Guignon, 2017).

HS has been primarily applied to fresh foods (*i.e.*, meat, fish, fruit juices, cheese, raw skim milk, egg white), allowing irreversible inactivation of naturally present microorganisms as well as inoculated pathogenic, surrogate, and spore-forming bacteria (Basso, Manzocco, Maifreni, & Nicoli, 2021; Duarte et al., 2017; Pinto et al., 2017; Santos, Castro, Delgadillo, & Saraiva, 2019; Santos et al., 2018; Santos, Matos, et al., 2021). The technology is claimed to inactivate microorganisms at levels on par with those of thermal pasteurization (up to 5 log reductions) while preserving the typical properties of fresh foods (Basso, Maifreni, Innocente, Manzocco, & Nicoli, 2022; Duarte, Pinto, Gomes, Delgadillo, & Saraiva, 2022; Pinto et al., 2017). Literature data indicate that, depending on the nature of food, HS can also exert different effects on lipids stability and protein structure, ultimately affecting the chemical, physical and techno-functional properties of food (Basso, Manzocco, & Nicoli, 2022).

Oxidative reactions are favored under pressure due to their negative activation volume (Medina-Meza, Barnaba, & Barbosa-Cánovas, 2014). In this regard, different authors have reported that HS significantly increased the number of peroxides and thiobarbituric acid-reactive species in foods containing a significant amount of lipids in close contact with muscle tissues (*e.g.*, beef, pork, salmon) (Fidalgo, Lemos, Delgadillo, & Saraiva, 2018; Fidalgo, Simões, et al., 2020; Santos et al., 2019). By contrast, information relevant to the effect of HS (50–100 MPa) on the oxidative status of food dispersions containing a lipid phase is limited to full-fat raw milk, which did not show any change in thiobarbituric acid-reactive species for up to 60 days under pressure (Duarte, Casal, et al., 2022).

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Abbreviation

HS	Hyperbaric storage
EAI	Emulsifying activity index
LDL	Low density-lipoproteins
HDL	High density-lipoproteins

As regards the effects of HS on protein structure, apparently contradicting results are reported in the literature. In the case of meat and fish, HS induced denaturation of myofibrillar and sarcoplasmic proteins, with limited effects on color, texture, and techno-functional properties (*e.g.*, water-holding capacity) (Fidalgo, Delgadillo, & Saraiva, 2020; Fidalgo, Simões, et al., 2020; Santos, Matos, et al., 2021). A completely different scenario was evidenced by applying HS to raw skim milk, in which proteins occur in solvated and micellar states. In particular, 150 MPa-HS applied for 6 days promoted the denaturation of β-lactoglobulin and the destabilization of casein micelles (Basso, Maifreni, et al., 2022). These effects, along with the concomitant enhancement of proteose-peptones formation, substantially improved (~4-fold) milk foaming ability. A similar outcome was obtained in the case of raw egg white stored at 200 MPa for 28 days. Under such conditions, globular egg white proteins appeared compressed and electrically stabilized, resulting in a significant increase in viscosity and better foaming properties (Basso et al., 2021).

Based on the available evidence, it can be inferred that HS could be employed not only as a sustainable alternative to refrigeration but also to improve the microbiological stability and the technological functionality of perishable, protein-rich foods. In this framework, the pro-oxidant effect of hydrostatic pressure should be taken into careful consideration if the matrix undergoing HS contains a significant amount of oxidizable lipids. Furthermore, the highly food-specific structural organization of proteins is expected to influence the effect of HS on physical and techno-functional properties.

Egg yolk is a perishable, protein-rich food, characterized by a unique protein structure and organization. In particular, egg yolk proteins occur either as solvated in the matrix water phase (*e.g.*, livetins), complexed in poorly hydrated structures called granules (*e.g.*, phosvitin) or embedded in the membrane of lipid-filled spherical assemblies called lipoproteins (*e.g.*, apoproteins) (Anton, 2013). At the industrial level, egg yolk is widely employed as an ingredient, to confer remarkable gelling, foaming, and emulsifying properties to several food formulations (*e.g.*, mayonnaise, spreads, baked goods) (Anton, 2013). However, due to its perishability, egg yolk is mostly commercialized as a liquid derivative, which requires thermal stabilization and/or refrigeration (European Community, 2004). Based on these specific needs, liquid egg yolk could largely benefit from the antimicrobial efficacy and protein-modifying ability of HS. In other words, this matrix could represent a food on which HS could concomitantly exert all its main purposes (*i.e.*, storage, microbiological stabilization, techno-functionality improvement). Circumstantial literature evidence substantiating this hypothesis was recently published by Ribeiro, Casal, da Silva, and Saraiva (2023), studying moderate pressure pretreatment as assisting technology to reduce thermal pasteurization time of liquid egg yolk. These Authors observed up to 6 log reductions of *Salmonella* Seftenberg and *Listeria innocua*, along with a slight improvement of the emulsifying properties.

The major potential drawback of HS applied to liquid egg yolk is likely represented by the pro-oxidant effect of hydrostatic pressure, which could become particularly critical over the remarkably long pressurization times typical of the technology. To this regard, evidence relevant to brief (5–30 min) high-hydrostatic-pressure processing (500 MPa) applied to liquid egg yolk indicated that pressure boosted hydroperoxide formation (Andrássy et al., 2006). However, to the best of our knowledge, the effect of HS on liquid egg yolk has never been investigated.

The present study aimed to demonstrate the efficacy of HS in extending the microbiological stability and improving the technological functionality of liquid egg yolk without impairing its oxidative status. To this aim, liquid egg yolk was subjected to hyperbaric storage at 200 MPa at room temperature (20 ± 1 °C). At increasing time for up to 28 days, samples were withdrawn from hyperbaric storage and analyzed. The microbiological stability and oxidative status of liquid egg yolk were first evaluated to validate the use of HS on the matrix from food safety and quality standpoints, respectively. Following, the capability of HS to steer the structure and techno-functionality of liquid egg yolk proteins was studied. Refrigeration was specifically selected as reference storage condition based on its application being currently mandatory for the storage of liquid egg yolk (European Community, 2004).

2. Materials and methods

2.1. Materials

Two hundred and ten medium-sized shell eggs (maximum age: 7 days) and sunflower oil were purchased at a local retailer. Polyethylene/ethylene-vinyl-alcohol/poly-propylene pouches (PP/EVOH/PE; 15 × 30 cm; 80 μm thickness, water vapor permeability $<1\ g \cdot m^{-2} \cdot 24\ h^{-1}$) were purchased from Niederwieser Group S.p.A. (Campogalliano, Italy). Maximum recovery diluent and plate count agar were purchased from Oxoid (Milan, Italy). Chloroform (≥99.0%), potassium iodide (KI, >99.0%), sodium thiosulphate ($Na_2S_2O_3$, 99.0%), potato starch, Tris-HCl (≥99.0%), sodium chloride (NaCl, ≥99.0%), sodium-dodecyl sulfate (SDS, ≥99.0%), Tris (≥99.0%), glycine (≥98.5%), ethylenediaminetetraacetic acid (EDTA, ≥98.5%), and 5′,5-dithiobis-(2-nitrobenzoic acid) (≥99.0%) were purchased from Sigma Aldrich (Milan, Italy). Acetic acid (99.8–100.5%) was purchased from VWR International Srl (Milan, Italy). Aluminum pans and lids for differential scanning calorimetry were purchased from Mettler-Toledo (Greifensee, Switzerland).

2.2. Sample preparation

Preparation of liquid egg yolk (dry matter: 47.5 ± 0.9% w/w) samples was performed within 48 h from egg purchase as previously described by Basso et al. (2021) with minimal adaptations. For microbiological analyses, 50 eggs were thoroughly wiped with a cloth soaked with 70 mL/dL ethanol and broken under aseptic conditions. Egg yolks were then separated from whites and chalazae, and homogenously pooled by gentle manual stirring (2 min) until adequate mixing of light- and dark-colored fractions of egg yolk was visually achieved. The absence of *S. enterica* and *S. aureus* in liquid egg yolk was preliminarily verified before inoculations as previously described by Basso et al. (2021). Liquid egg yolk was then inoculated with about 3 logCFU g^{-1} *Salmonella enterica* subsp. *enterica* 9898 DSMZ or *Staphylococcus aureus* 226 belonging to the collection of the Department of Agricultural, Food, Animal and Environmental Sciences of the University of Udine (Italy). The inoculum concentration was specifically selected to enable detection of either bacteria growth or inactivation (Basso et al., 2021). Ten 50 g-aliquots of inoculated liquid egg yolk were then packaged in PP/EVOH/PE pouches with minimal headspace. Liquid egg yolk samples for analyses relevant to chemical, physical and techno-functional properties were obtained from the remaining 160 eggs. In particular, eggs were manually cleaned with a wet towel, shelled, and separated. Egg yolks were then pooled by manual stirring, divided into thirteen 200 g-aliquots, and packaged inside PP/EVOH/PE pouches as previously described.

Packaged liquid egg yolk samples were subsequently stored under hyperbaric conditions (200 MPa) using the HS pilot plant previously described by Basso et al. (2021). The working unit had a maximum operating pressure of 200 MPa and a maximum vessel capacity of 2 L.

Samples were pressurized at the maximum rate allowed by the equipment (50 MPa/min) and storage temperature was kept at 20 ± 1 °C by placing the HS plant in a thermostatic room. Reference samples were stored under refrigerated conditions (0.1 MPa, 4 °C). At increasing time for up to 28 days, samples were removed from the hyperbaric vessel and from the refrigerator. Microbial counts were performed immediately after sample decompression or removal from the refrigerator. Other samples were split into two aliquots that were either analyzed within 24 h or freeze-dried (Mini-Fast Edwards, mod. 1700, Edwards Alto Vuoto, Milan, Italy). Freeze-dried samples were vacuum-sealed in PP/EVOH/PE pouches and stored at −20 °C in the dark until further analyses.

2.3. Microbial counts

S. enterica and *S. aureus* were quantified in HS-stored and refrigerated samples by suspending 20 g of liquid egg yolk into 80 mL maximum recovery diluent (1:5, g/mL), and plating 0.1 mL aliquots of appropriately diluted egg yolk suspension onto plate count agar. Counts were performed after incubation at 37 °C for 24 h and for 36–48 h, for *S. enterica* and *S. aureus*, respectively.

Microbiological trials were interrupted when microbial counts were below the detection limit for two consecutive sampling times. This criterion was established based on the incapability of foodborne vegetative pathogens to grow under the conditions described in paragraph 2.2 (Abe, 2007; Lima & Saraiva, 2023).

The results were expressed as the decimal logarithm of colony-forming units per gram of sample (logCFU g^{-1}).

2.4. Fourier transform infrared spectroscopy

The oxidation state of liquid egg yolk was evaluated by performing Fourier transform infrared spectroscopy (FT-IR) at 25 ± 1 °C on freeze-dried samples finely ground with a domestic blender. An Alpha-P (Bruker Optics, Milan, Italy) infrared spectrometer, equipped with a Zn–Se crystal accessory, was used to record samples attenuated total IR reflection, as previously described by Melchior, Calligaris, Bisson, and Manzocco (2020). Spectra from 4000 to 400 cm^{-1} were acquired in absorbance mode by performing 32 scans per measurement with a resolution of 4 cm^{-1}. After normalizing, baselining and smoothing the recorded spectra, peak height of the bands associated with lipid oxidation was determined (Origin Pro 9, OriginLab, Northampton, MA, USA).

2.5. Peroxide value

Peroxide value analysis was conducted according to the method described by the International Olive Council (2017). Briefly, approximately 2 g of freeze-dried egg yolk sample was dissolved in 25 mL of chloroform in acetic acid (2:3 mL/mL) solution. A 1 mL aliquot of supersaturated KI solution in MilliQ water was added and the mix was vigorously stirred with a magnetic stirrer. After 5 min incubation at room temperature in the dark to develop color, 75 mL of MilliQ water was added to stop the reaction and the free iodine was titrated with 0.01 mol equi/L $Na_2S_2O_3$ aqueous solution, using potato starch as indicator. The peroxide value was determined according to Eq. (1):

$$PV = \frac{V \times T \times 1,000}{m} \quad \text{(Eq.1)}$$

where V (mL) is the volume of 0.01 mol equi/L $Na_2S_2O_3$ added to induce color change of the mix, T (mol/L) is the exact molarity of the $Na_2S_2O_3$ solution, and m is the mass (g) of the tested egg yolk sample.

2.6. Color

A tristimulus colorimeter (Chromameter-2 Reflectance, Minolta, Osaka, Japan), equipped with a CR-300 measuring head and a D65 standard illuminant, was calibrated against a standardized white tile and used to determine sample color in the L*a*b* (CIELAB) color space. Approximately 10 mL aliquots of samples were poured into Petri dishes and positioned over the calibration tile for analysis.

2.7. UV–Vis absorbance

Freeze-dried egg yolk samples (0.15 g) were diluted in 10 mL 0.05 mol/L Tris-HCl buffer, pH 9.0, containing 0.04 mol/L NaCl. This buffer was specifically selected to optimize protein stability during analyses (Ohba, Teramoto, & Ueda, 1993). To ensure complete solubilization, egg yolk suspensions were gently stirred at 4 °C overnight. Samples were further diluted 1:200 (mL/mL) for absorbance readings at 280 nm, and 1:60 (mL/mL) for absorbance readings at 680 nm. Absorbance values at these wavelengths were selected as indicators of the exposure of cysteine, phenylalanine and tryptophan on samples proteins, and of samples turbidity, respectively (Biter et al., 2019; Nakano et al., 2017). Absorbance was read at 4 °C using a UV–Vis spectrophotometer (UV-2501 PC, Shimadzu Kyoto, Japan), equipped with a 6-cell remotely controlled sample holder thermoregulated by a Peltier system, using 1 cm path-length quartz cuvettes.

2.8. Flow rheology

A RS6000 Rheometer (ThermoScientific Rheo Stress, Haake, Germany) equipped with a Peltier temperature control system, was used to determine liquid egg yolk apparent viscosity at 20 °C. Flow curves were obtained in the 0.1–200 s^{-1} shear rate range by using a bob-cup geometry with a gap of 27.2 mm (bob: CC25 DIN Ti; cup: CCB25 DIN/SS; ThermoScientific, Haake, Germany). The apparent viscosity of the samples was compared at 4.472 s^{-1} shear rate. This value was specifically selected since it allowed to best highlight differences occurring between the samples.

Flow curves, expressed as the dependence of the decimal logarithm of the shear stress ($\log \sigma$) on the decimal logarithm of the shear rate ($\log\dot{\gamma}$), were fitted with the Power Law model (Eq. (2)) to obtain samples flow parameters, namely the flow index (n, dimensionless) and the consistency coefficient (k, Pa · s^n).

$$\log \sigma = n * \log\dot{\gamma} + \log k \quad \text{(Eq.2)}$$

2.9. Differential scanning calorimetry

Liquid egg yolk thermograms were obtained by heating samples (15 mg of sample into 40 μL sealed aluminum pans) from 45 to 95 °C at a 5 °C min^{-1} rate in a DSC 3 Stare System differential scanning calorimeter, equipped with an FRS-5+ sensor (56 thermocouples) (Mettler-Toledo, Greifensee, Switzerland). An empty sealed pan was used as a reference. Peak temperature and enthalpy were calculated using the program STARe ver. 16.10 (Mettler-Toledo, Greifensee, Switzerland).

2.10. Particle size

Samples were prepared as previously described for UV–Vis absorbance, diluted 1:1000 (mL/mL) with 0.1 g/dL SDS in Tris-HCl buffer and filtered through Whatman n °1 paper. A dynamic light scattering system (NanoSizer 3000, Malvern Instruments, Malvern, UK) equipped with a Peltier temperature control system was used to determine sample particle size at 4 °C. The refractive index was set at 1.333 and the viscosity was approximated to that of pure water at 4 °C.

2.11. Free sulfhydryl groups

Free sulfhydryl group content was determined by adapting the method of Manzocco, Panozzo, and Nicoli (2013). Freeze-dried samples were diluted 1:150 (g:mL) in Tris–glycine buffer (TGE; 10.4 g L^{-1} Tris,

6.9 g L-1 glycine, 1.2 g L^{-1} EDTA; pH 8.0) added with 1 g/dL NaCl and gently stirred overnight at 4 °C to ensure complete solubilization.

A 1.67 mL aliquot of 0.5 g/dL SDS in TGE was added to 0.116 mL of diluted sample and 0.018 mL of Ellman's reagent (4 mg mL^{-1} 5′,5-dithiobis-(2-nitrobenzoic acid) in TGE) to develop color. The SDS-TGE solution was sonicated for 30 min (Ultrasonic Cleaner, VWR, Leuven, Belgium) and bubbled with pure nitrogen for 15 min under gentle stirring to purge solubilized oxygen. Samples were incubated for 15 min at 20 °C in the dark and centrifuged at 12,700×*g* at 4 °C for 15 min (Mikro 120, Hettich Zentrifugen, Tuttlingen, Germany). Absorbance of the supernatant was read at 412 nm by using a UV–Vis spectrophotometer (UV-2501 PC, Shimadzu Kyoto, Japan). Free sulfhydryl group concentration (μM g^{-1}) was calculated using Eq. (3):

$$SH = \frac{73.53 \bullet A \bullet D}{C} \quad \text{(Eq.3)}$$

where A is the absorbance; C is egg yolk concentration (mg/mL); D is the dilution factor; and 73.53 is derived from $\frac{10^6}{1.36 \bullet 10^4}$; $1.36 \cdot 10^4$ is the molar absorptivity.

2.12. Solubility

The solubility of liquid egg yolk was assessed using the method described by Melchior et al. (2020) with adaptations. Freeze-dried samples were suspended (10 g L^{-1}) in 0.05 mol/L Tris-HCl buffer pH 9.0 containing 0.04 mol/L NaCl. Maximum solubilization was achieved by gentle overnight stirring at 4 °C. Samples were centrifuged (Mikro 120, Hettich Zentrifugen, Tuttlingen, Germania) at 13,500×*g* for 5 min at 20 °C. The residual pellet (insoluble fraction) was dried at 75 °C in a vacuum oven (Vuotomatic 50, Bicasa, Milan, Italy) overnight and weighed to ± 0.0001 g precision. Samples solubility was calculated by using Eq. (4).

$$\text{Solubility } (\%) = \frac{S - I}{S} \bullet 100 \quad \text{(Eq.4)}$$

where S (mg) is the initial sample weight, and I (mg) is the weight of the dried insoluble fraction.

2.13. Gelling properties

Liquid egg yolk aliquots (50 mL) were poured in plastic Falcon tubes (50 mL capacity) and heated at 90 °C for 15 min to induce gelling. Gelled samples were then rapidly cooled in an ice bath and stored at 4 °C for 12 h to allow setting. Samples were gently extracted from the Falcon tubes and manually cut into 1.5 ± 0.1 mm thick slices using a sharp knife. Mechanical spectra of the heat-set gels were obtained using a parallel plate geometry (40 mm diameter, 1 mm gap) mounted on an RS6000 Rheometer. Linear viscoelastic stress domain was determined by stress sweep analysis (stress ramp at 1 Hz frequency: 1–200 Pa). Frequency sweep analysis was performed in the 0.1–16 Hz frequency range, applying stress within the linear viscoelastic stress domain. Liquid egg yolk gelling capacity was expressed as the elastic modulus (G', Pa) of the gelled sample at a frequency of 1 Hz.

2.14. Foaming properties

Foaming properties were determined by adapting the method from Melchior et al. (2020). Briefly, 10 mL of liquid egg yolk samples were diluted 1:10 (g/g) with MilliQ water and homogenized (Polytron DI 25 basic, IKA Werke GmbH & Co., Germany) for 3 min at 9500 rpm in a graduated cylinder. The total volume of the foamed samples was measured immediately and after 15 min. Foaming ability and foam stability were calculated using Eq. (5) and Eq. (6), respectively:

$$\text{Foaming ability } (\%) = \frac{V_0 - 10\ mL}{10\ mL} \bullet 100 \quad \text{(Eq.5)}$$

$$\text{Foam stability } (\%) = 100 - \left(\frac{V_0 - V_{15}}{V_0} \bullet 100\right) \quad \text{(Eq.6)}$$

Where V_0 (mL) is the sample volume after homogenization, 10 mL is the initial sample volume, and V_{15} (mL) is the sample volume after 15 min from homogenization.

2.15. Emulsifying properties

Emulsifying properties were evaluated by determining the emulsifying activity index (EAI) of freeze-dried samples adapting the methods of Anton and Gandemer (1997) and Yan et al. (2010). Briefly, 60 mL egg yolk solutions (3 g/dL) were prepared in 0.05 mol/L Tris-HCl buffer (pH 9) containing 0.04 mol/L NaCl. Sunflower oil (17 mL) was subsequently added and pre-emulsified by applying a high-speed blender (Polytron DI 25 basic, IKA Werke GmbH & Co., Germany) at 8000 rpm for 1 min. Stable emulsions were obtained by passing the pre-emulsion through a lab-scale two-stage (first stage valve: 50 MPa; second stage valve: 5 MPa) high-pressure homogenizer (Panda Plus 2000; GEA Niro Soavi, Parma, Italy). Exactly 7 mL of the obtained emulsion were transferred into 10 mL glass vials. Aliquots of 10 μL were then quickly withdrawn from the center of the vials, 10 mm above the bottom, using an automatic pipette, and diluted 1:6000 (mL/mL) in MilliQ water. Absorbance at 500 nm of diluted emulsions was measured with a UV–Vis spectrophotometer (UV-2501 PC, Shimadzu Kyoto, Japan) and EAI was calculated using Eq. (7):

$$EAI = \frac{2 \times 2.303 \times A_0 \times DF}{C \times \varphi \times (1 - \theta) \times 1,000} \quad \text{(Eq.7)}$$

Where 2 × 2.303 is the conversion factor of samples light absorption value to turbidity, A_0 is the absorbance of the diluted samples, DF is the dilution factor, C is the initial concentration of the sample, φ is the cuvette optical path, θ is the oil fraction in the emulsion (0.283).

2.16. Statistical analysis

Experiments were designed to take into account the concomitant effects of two independent factors, namely "storage condition" and "storage time". Storage condition was assigned with two categorical levels, namely "hyperbaric" (200 MPa, 20 ± 1 °C) and "refrigerated" (0.1 MPa, 4.0 °C). Storage time was assigned with up to 7 levels, corresponding 0, 1, 3, 5, 7, 14 and 28 days for indices relevant to chemical, physical, structural and techno-functional properties. In the case of microbiological trials, sampling was made more frequent in the 0–72 h range (0, 1, 3, 6, 24, 48, and 72 h) to better assess count reduction, which is known to occur within a shorter time scale under HS (Basso et al., 2021; Basso, Maifreni, et al., 2022).

Data were subjected to two-way analysis of variance (ANOVA, $p < 0.05$), considering storage condition and storage time as factors. Tukey's Honest Significant Differences test ($p < 0.05$) was used for post-hoc testing of the data with equal sample size. The Tukey-Kramer method was applied to run post-hoc tests of data with unequal sample size. Compact letter display was assigned to data not containing missing values to clarify the output of post-hoc tests. All statistical analyses were performed using R v. 4.2.3 for Windows (The R foundation for statistical computing).

3. Results and discussion

3.1. Effect on microbial counts

Microbiological tests were first performed to validate the application

of HS (200 MPa) on liquid egg yolk from a microbial safety standpoint. The attention was focused on the ability of the technology to inactivate inoculated *S. enterica* (3.35 ± 0.12 logCFU g^{-1}) and *S. aureus* (2.78 ± 0.19 logCFU g^{-1}). Inoculated samples stored at 0.1 MPa and 4 °C were kept as control. Results are shown in Table 1.

As expected, microbial counts of both *S. enterica* and *S. aureus* did not change in control refrigerated samples for up to three days ($p > 0.05$; two-way ANOVA), due to the well-known bacteriostatic effect of storage at low temperature. By contrast, in liquid egg yolk stored under hyperbaric conditions, the concentration of *S. enterica* significantly decreased after just 1 h ($p < 0.05$; two-way ANOVA) and reached values below the detection limit after just 24 h. Differently from *S. enterica*, counts of *S. aureus* in liquid egg yolk were not affected by HS for up to 6 h ($p > 0.05$; two-way ANOVA), and reached values below the detection limit only by prolonging HS for up to 48 h. The higher resistance to HS of *S. aureus* as compared to *S. enterica* was likely due to the higher pressure-stability of Gram (+) bacteria, which are better protected from physical stresses by their thicker peptidoglycan cell wall layer (Wuytack, Diels, & Michiels, 2002). Values below the detection limit were also recorded for both *S. enterica* and *S. aureus* upon further storage at 200 MPa for up to 72 h, confirming the complete inactivation of the microorganisms. Based on these results and on abundant literature reporting the incapability of foodborne vegetative pathogens to grow under this pressure (Abe, 2007; Lima & Saraiva, 2023), it was inferred that HS could easily maintain liquid egg yolk microbiological stability during further storage. For this reason, microbiological trials were interrupted.

It is worth noting that data shown in Table 1 agree with literature indicating the interesting capacity of HS to effectively decontaminate highly perishable foods. In particular, these results are similar to those obtained by applying 200 MPa-HS to egg white inoculated with approximately 4 logCFU g^{-1} of *S. enterica* and *S. aureus*. In that case, both microorganisms resulted below the detection limit after just 24 h under pressure (Basso et al., 2021). However, HS inactivation of these pathogens was much slower in liquid egg yolk. This was probably due to the high concentration of nutrients (*e.g.*, proteins, lipids, minerals) in egg yolk as compared to egg white. In other words, the presence of readily available substrates might have boosted the viability of bacterial cells, increasing their resistance to physical stresses (*e.g.*, pressure) (Bahrami, Moaddabdoost Baboli, Schimmel, Jafari, & Williams, 2020).

3.2. Effect on oxidative status

Further analyses were performed to evaluate if the advantages obtained in terms of hygienic properties were achieved to the detriment of the product oxidative status. The IR spectrum of fresh (*i.e.*, not stored) freeze-dried egg yolk displayed the presence of twelve well-defined bands that are ascribable to bonds of lipid compounds (Fig. 1). The attention was focused on specific bands, which, according to the literature, can indicate the occurrence of lipid oxidation and lipolysis. In particular, a decrease in egg yolk absorbance at 720 and 3008 cm^{-1} in concomitance to an increase at 1744 cm^{-1} specifically indicates the simultaneous loss of acyl chain *cis* double bonds and formation of carbonylated oxidation products, respectively. Furthermore, a decrease in absorbance at 1067, 1087, 1161 and 1234 cm^{-1} was reported to indicate disruption of ester and phosphodiester bonds due to triglyceride and phospholipid lipolysis (Araújo et al., 2011; Liu, Shaw, Man, Dembinski, & Mantsch, 2002; Muik, Lendl, Molina-Diaz, Valcarcel, & Ayora-Cañada, 2007). Data reported in Fig. 1 and Table 2 clearly indicate negligible changes in the absorbance of liquid egg yolk at the specific wavelengths associated with oxidation even after 28 days of either refrigerated or pressurized storage. This result suggests that storage under hyperbaric conditions for up to 28 days did not alter egg yolk oxidative status, similarly to refrigeration. The oxidative stability of egg yolk under hyperbaric conditions was further confirmed by the absence of changes in egg yolk peroxide value, which was always below the repeatability limit of the method (0.19 meqO_2 kg^{-1}) (data not shown). The abundance of chain-breaking and oxygen-quenching antioxidants (*i.e.*, tocopherol, retinol, carotenoids) in egg yolk likely played a crucial role in withstanding the harsh pro-oxidant conditions occurring during pressurization, even when it is maintained for days/weeks as during HS (Nimalaratne & Wu, 2015).

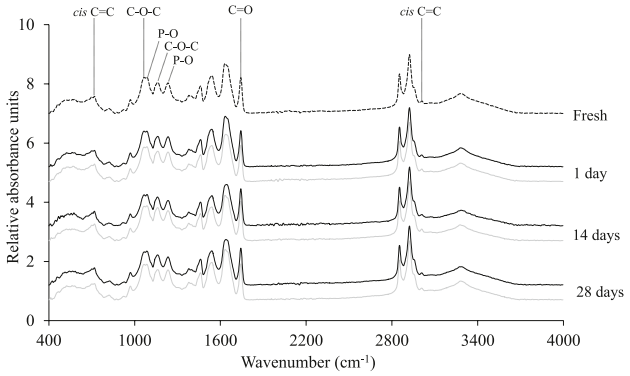

Fig. 1. Absorbance in IR spectrum of freeze-dried liquid egg yolk stored for increasing time under refrigerated (—) or hyperbaric (—) conditions. The spectrum of fresh (----) freeze-dried liquid egg yolk is also shown, and wavelengths associated to specific chemical groups of lipids and relevant oxidation products are highlighted.

Table 1
Salmonella enterica and *Staphylococcus aureus* counts in liquid egg yolk stored for increasing time under refrigerated or hyperbaric conditions. Data were obtained in single from two independent experiments and expressed as mean ± standard deviation.

Storage	Time (hours)	*S. enterica* (logCFU g^{-1})	*S. aureus* (logCFU g^{-1})
Fresh	0	3.35 ± 0.12	2.78 ± 0.19
Refrigerated	24	3.40 ± 0.04	2.90 ± 0.06
	48	3.30 ± 0.05	2.84 ± 0.06
	72	3.28 ± 0.01	2.98 ± 0.08
Hyperbaric	1	3.04 ± 0.10	2.84 ± 0.04
	3	2.54 ± 0.04	2.88 ± 0.02
	6	2.18 ± 0.04	2.78 ± 0.02
	24	< L.o.D.	2.00 ± 0.07
	48	< L.o.D.	< L.o.D.
	72	< L.o.D.	< L.o.D.

L.o.D. = 1.7 logCFU g^{-1}

3.3. Effect on physical properties

Despite the negligible effect of HS on oxidation, pressurized storage was associated with a significant modification of liquid egg yolk visual appearance. In particular, HS-stored samples appeared darker than refrigerated ones. Colorimetric analyses were thus performed (Fig. 2), confirming that HS caused a remarkable decrease in egg yolk luminosity (L*) and yellowness (b*) within 5 days, with limited changes upon further storage for up to 28 days. Measurements of absorbance at 680 nm also revealed a significant increase in turbidity of pressurized samples (Table 3). In protein-rich food matrices such as egg yolk, these concomitant effects could be associated to protein denaturation and networking, which are known to modify light scattering properties (Manzocco et al., 2013; Smith, Fiebig, Schwalbe, & Dobson, 1996). Based on these considerations, even color fading can be mainly attributed to light scattering effects rather than carotenoid oxidation (Nys, 2018).

When liquid egg yolk was removed from the HS vessel, the most evident change induced by hyperbaric storage was a remarkable thickening of the samples. In agreement with the literature, rheological analyses actually revealed that HS for up to 14 days significantly ($p < 0.05$;

Table 2
Absorbance (optical density) in IR spectrum at wavelengths relevant to specific chemical groups of lipids and relevant oxidation products in freeze-dried liquid egg yolk stored for increasing time under refrigerated or hyperbaric conditions. Data were obtained by at least triplicate measurements and are reported as mean with standard deviation in brackets.

Wavelength	Assignment	Fresh	Refrigerated		Hyperbaric	
(cm^{-1})		0 days	14 days	28 days	14 days	28 days
720	*cis* C═C	0.583^{a} (0.032)	0.589^{a} (0.020)	0.581^{a} (0.014)	0.588^{a} (0.024)	0.588^{a} (0.009)
1067	C-O-C	1.220^{a} (0.029)	1.158^{a} (0.044)	1.187^{a} (0.039)	1.159^{a} (0.063)	1.152^{a} (0.017)
1087	P-O	1.236^{a} (0.031)	1.183^{ab} (0.035)	1.199^{ab} (0.030)	1.163^{b} (0.058)	1.176^{ab} (0.016)
1161	C-O-C	1.069^{ab} (0.032)	1.090^{a} (0.020)	1.038^{bc} (0.025)	1.016^{c} (0.023)	1.028^{bc} (0.008)
1234	P-O	1.030^{a} (0.025)	0.996^{a} (0.030)	0.999^{a} (0.026)	0.932^{b} (0.055)	0.979^{ab} (0.017)
1744	C═O	1.250^{a} (0.033)	1.299^{a} (0.032)	1.250^{a} (0.043)	1.246^{a} (0.027)	1.236^{a} (0.011)
3008	*cis* C═C	0.423^{a} (0.006)	0.404^{b} (0.014)	0.408^{ab} (0.009)	0.406^{ab} (0.006)	0.418^{ab} (0.005)

[a] Different letters in the same row indicate significantly different means (two-way ANOVA; $p < 0.05$).

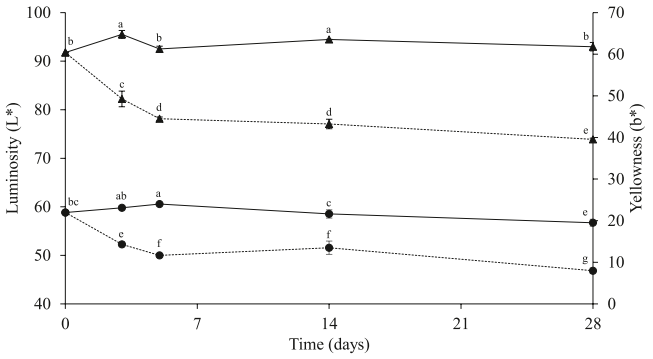

Fig. 2. Luminosity (●) and yellowness (▲) of liquid egg yolk stored for increasing time under refrigerated (—) or hyperbaric (····) conditions. Data were obtained by triplicate measurements, and are reported as mean ± standard deviation.
[a] Different letters for the same color parameter indicate significantly different means (two-way ANOVA; $p < 0.05$).

two-way ANOVA) enhanced sample apparent viscosity (Table 3) and modified liquid egg yolk pseudoplastic behavior (Fig. 3) (Odani, Miyamoto, Kasai, Hatae, & Shimada, 2000; Yan et al., 2010). Fitting samples flow curves with the Power Law equation (Eq. (2)) actually revealed that HS significantly ($p < 0.05$; two-way ANOVA) decreased liquid egg yolk flow index (n) (Table 4). This indicates that pressurized storage caused egg yolk to increasingly deviate from Newtonian behavior at increasing storage times, likely due to the onset of novel interparticle interactions in the matrix (Yan et al., 2010). At the same time, HS caused samples consistency coefficient (k) to significantly ($p < 0.05$; two-way ANOVA) increase (Table 4), indicating that hyperbaric conditions enhanced egg yolk viscosity in all the tested shear rate spectrum. By further prolonging HS to 28 days, a fully gelled system was obtained (Table 3). Structuring of this egg yolk sample into a weak gel was confirmed by the strong positive linear dependence ($R^2 > 0.99$) of elastic and viscous moduli logarithms on the logarithm of the oscillatory frequency (Fig. 4). It is noteworthy that, although brief (*i.e.*, 10 min) high-hydrostatic pressure (up to 400 MPa) treatments have already been observed to cause egg yolk thickening, changes in viscosity as intense as those reported in Tables 3 and 4 have never been detected (Aguilar, Cordobés, Jerez, & Guerrero, 2007; Ahmed, Ramaswamy, Alli, & Ngadi, 2003; Lai et al., 2010; Yan et al., 2010). These results suggest that the capacity of egg yolk to form a network under hyperbaric conditions does not depend on pressure value solely but would be strongly time-dependent, becoming evident on time scales typical of HS.

3.4. Effect on protein structure

In all samples, calorimetric analyses revealed the presence of a broad endothermic peak at 83 °C (Fig. 5), primarily ascribable to the thermal

Table 3
Turbidity, apparent viscosity and denaturation enthalpy of liquid egg yolk stored for increasing time under refrigerated or hyperbaric conditions. Turbidity measurements were performed at least in triplicate, whereas apparent viscosity and denaturation enthalpy data were obtained by duplicate measurements. All data are reported as mean ± standard deviation.

Storage	Time (days)	Turbidity (optical density at 680 nm)	Apparent viscosity (Pa · s) · 10^{-1}	Denaturation enthalpy (J g^{-1})
Fresh	0	0.204 ± 0.020^{ef}	5.04 ± 0.01	1.19 ± 0.08
Refrigerated	1	0.167 ± 0.026^{f}	3.11 ± 0.15	1.17 ± 0.04
	3	0.176 ± 0.008^{f}	2.94 ± 0.02	1.08 ± 0.01
	7	0.244 ± 0.005^{cde}	4.75 ± 0.01	1.28 ± 0.01
	14	0.258 ± 0.018^{cd}	5.67 ± 0.60	N.D.
	28	0.230 ± 0.011^{de}	6.14 ± 0.26	1.36 ± 0.01
Hyperbaric	1	0.278 ± 0.004^{abc}	28.11 ± 0.20	0.93 ± 0.04
	3	0.266 ± 0.002^{bcd}	32.44 ± 3.80	0.81 ± 0.01
	7	0.319 ± 0.016^{a}	61.23 ± 4.91	0.65 ± 0.01
	14	0.308 ± 0.015^{ab}	101.98 ± 8.94	0.47 ± 0.01
	28	0.289 ± 0.006^{abc}	Gelled	0.25 ± 0.02

N.D. Not determined.
[a] Different letters indicate significantly different means (two-way ANOVA; $p < 0.05$).

denaturation of low-density lipoproteins (LDL) and high-density lipoproteins (HDL) protein fractions (Yan et al., 2010). Upon storage for up to 28 days under refrigerated or hyperbaric conditions, no significant ($p > 0.05$; two-way ANOVA) change in lipoproteins denaturation temperature was observed (data not shown). Conversely, a slight but significant ($p < 0.05$; two-way ANOVA) increase in denaturation enthalpy was progressively detected when liquid egg yolk was stored under refrigerated conditions for up to 28 days (Table 3). Such an increase could be associated to the rearrangement of protein structures towards more thermally stable conformations, which were probably favored by the action of endogenous egg yolk proteases at low temperature (Arntfield & Murray, 1981; Gao, Qiu, Liu, & Ma, 2016; Li-Chan & Ma, 2002). By contrast, an opposite and much more significant ($p < 0.01$; two-way ANOVA) change in peak enthalpy was detected during HS (Fig. 5B), so that after 28 days it was about 20% of the one detected in the untreated sample (Table 3). Such a different trend suggests that HS promoted extensive denaturation of egg yolk proteins by modifying their structure towards highly disordered random coil conformation (Seelig &

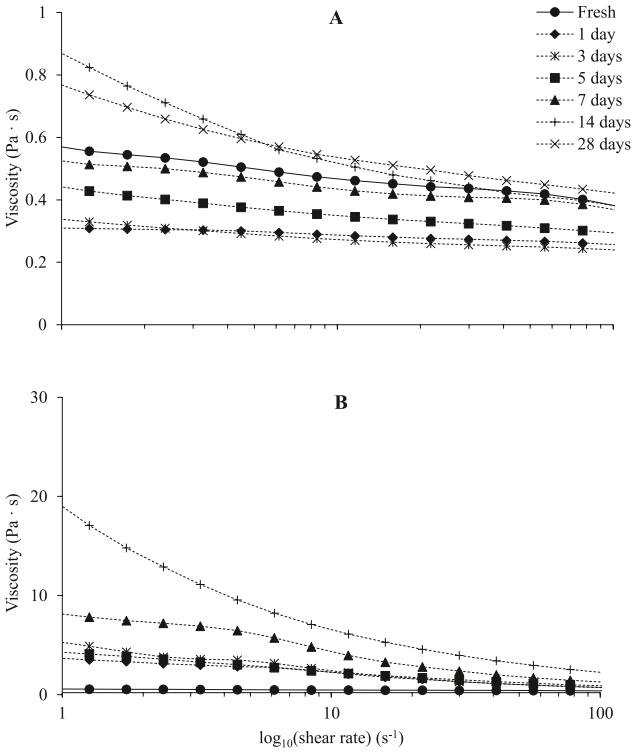

Fig. 3. Viscosity curves of liquid egg yolk stored for increasing time under refrigerated (A) or hyperbaric (B) conditions.

Table 4
Power Law model parameters (flow index, *n*; consistency index, *k*) relevant to flow curves of liquid egg yolk stored for increasing time under refrigerated or hyperbaric conditions. Flow curves were measured in duplicate.

Storage	Time (days)	*n* (–)	*k* (Pa · s^n)	R^2 (–)
Fresh	0	0.934 ± 0.009	0.540 ± 0.007	>0.99
Refrigerated	1	0.953 ± 0.038	0.328 ± 0.052	>0.99
	3	0.938 ± 0.001	0.320 ± 0.003	>0.99
	7	0.927 ± 0.003	0.519 ± 0.003	>0.99
	14	0.847 ± 0.050	0.750 ± 0.141	>0.99
	28	0.854 ± 0.021	0.805 ± 0.074	>0.99
Hyperbaric	1	0.743 ± 0.014	3.128 ± 0.106	>0.98
	3	0.618 ± 0.012	4.654 ± 0.431	>0.98
	7	0.628 ± 0.025	8.147 ± 0.870	>0.98
	14	0.541 ± 0.003	19.826 ± 1.673	>0.99
	28	N.D.	N.D.	N.D.

N.D. Not determined.

Schönfeld, 2016).

It is noteworthy that a strong negative correlation (r = −0.851) was found between protein denaturation enthalpy and viscosity (Table 3) of pressurized liquid egg yolk, further supporting the hypothesis that matrix thickening was mainly accounted for by protein structural changes (Lai et al., 2010; Yan et al., 2010).

To better understand the effects of HS on egg yolk proteins, samples were further analyzed for particle size, free SH groups, and absorbance at 280 nm (Table 5).

Before storage, egg yolk showed the presence of a single particle family with ~134 nm diameter, ascribable to LDL and HDL particle aggregates (Speroni et al., 2005). During storage under both pressurized and refrigerated conditions, size, SH groups exposure, and absorbance at 280 nm of these particles progressively increased (Table 5), indicating that, regardless of the applied conditions, egg yolk proteins swelled and exposed sulphurated and aromatic aminoacidic residues (Biter et al., 2019; Nakano et al., 2017). In the refrigerated samples, these changes

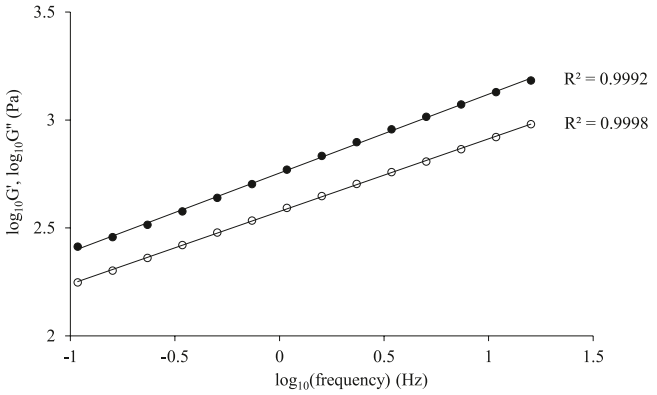

Fig. 4. Logarithmic dependence of storage (G′, ●) and loss (G″, ○) moduli on oscillatory frequency of liquid egg yolk subjected to 200 MPa-HS for 28 days. Linear models fitting the data (——) are also shown.

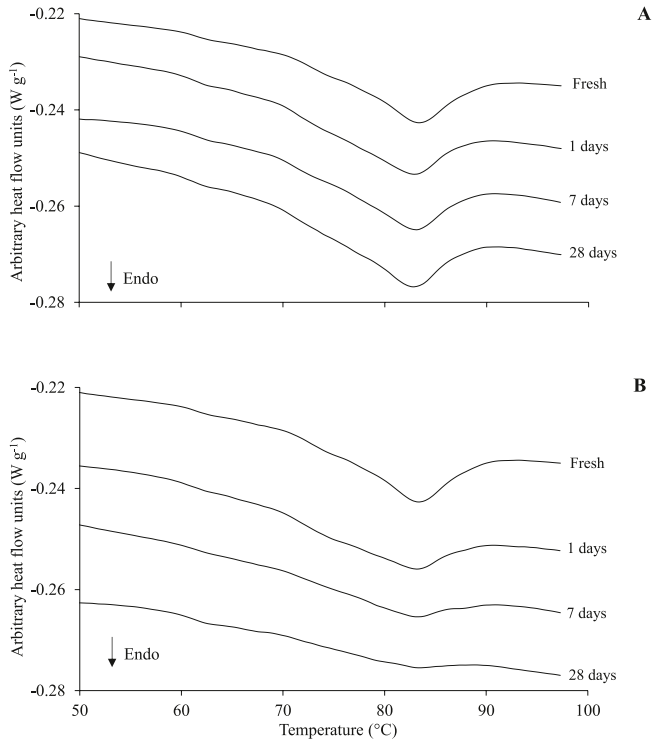

Fig. 5. Differential scanning calorimetry thermograms of liquid egg yolk stored for increasing time under refrigerated (A) or hyperbaric conditions (B).

might be associated with the activity of egg yolk endogenous proteases (*e.g.*, aspartic proteases, matrix metalloproteinase), which are known to modify protein structure during prolonged storage (*e.g.*, 20–40 days) (Gao et al., 2016). When samples were stored by HS, changes in particle size, free SH groups and absorbance at 280 nm occurred in shorter times. Such an effect was probably due to the action of pressure on the highly barosensitive structure of egg yolk proteins (Yan et al., 2010). In particular, absorbance at 280 nm of liquid egg yolk increased by 70% after just 1 day and remained significantly higher than that of refrigerated samples for up to 28 days. These results indicate that HS promoted a higher exposure of hydrophobic aromatic amino acids. In light of this evidence, it is reasonable that egg yolk protein denaturation (Fig. 5) occurred based on intense hydrophobic interaction between exposed aromatic amino acids (Lai et al., 2010; Yan et al., 2010).

Table 5
Particle size, free SH groups and absorbance at 280 nm of liquid egg yolk stored for increasing time under refrigerated or hyperbaric conditions. Data were obtained by at least triplicate measurements, and are reported as mean ± standard deviation.

Storage	Time (days)	Particle size (nm)	Free SH groups (μM g^{-1})	Absorbance at 280 nm
Fresh	0	134.38 ± 4.82^{f}	34.24 ± 1.18^{cd}	0.223 ± 0.006^{f}
Refrigerated	1	138.40 ± 0.40^{ef}	28.73 ± 1.21^{e}	0.281 ± 0.002^{d}
	3	142.70 ± 3.73^{def}	33.05 ± 0.92^{de}	0.283 ± 0.008^{d}
	7	138.44 ± 6.22^{ef}	35.48 ± 2.22^{cd}	0.274 ± 0.011^{de}
	14	133.34 ± 6.14^{f}	38.75 ± 1.60^{bc}	0.264 ± 0.006^{e}
	28	157.96 ± 7.16^{bc}	48.79 ± 1.63^{a}	0.308 ± 0.001^{c}
Hyperbaric	1	155.57 ± 2.17^{bcd}	36.57 ± 1.18^{cd}	0.376 ± 0.003^{a}
	3	155.90 ± 3.60^{bcd}	42.10 ± 1.24^{b}	0.366 ± 0.005^{a}
	7	148.16 ± 7.39^{cde}	43.53 ± 1.85^{b}	0.361 ± 0.006^{ab}
	14	181.17 ± 5.52^{a}	48.25 ± 2.15^{a}	0.316 ± 0.005^{c}
	28	165.48 ± 5.90^{b}	49.45 ± 1.85^{a}	0.345 ± 0.004^{b}

[a] Different letters in the same column indicate significantly different means (two-way ANOVA; $p < 0.05$).

3.5. Effects on techno-functional properties

Based on the changes induced by hyperbaric storage on liquid egg yolk physical properties and protein structure, solubility, foaming ability, foam stability, thermal gelling capacity and emulsifying activity were assessed, and results are shown in Table 6.

Table 6
Solubility, foaming capacity, foam stability, thermally-obtained gel elastic modulus (G′) and emulsifying activity index (EAI) of liquid egg yolk stored for increasing time under refrigerated or hyperbaric conditions. Data were obtained by triplicate measurements except G' (duplicate), and are reported as mean ± standard deviation.

Storage	Time (days)	Solubility (%)	Foaming capacity (%)	Foam stability (%)	G' (Pa · 1000)	EAI (m^2 g^{-1})
Fresh	0	95.7 ± 1.2^{a}	113.3 ± 15.3^{bc}	88.8 ± 6.0^{ab}	33.95 ± 1.02^{a}	9.71 ± 0.43^{ab}
Refrigerated	7	N.D.	140.0 ± 10.0^{abc}	97.2 ± 2.5^{a}	33.75 ± 4.62^{a}	9.58 ± 0.68^{ab}
	14	92.0 ± 1.0^{a}	156.7 ± 5.8^{a}	92.3 ± 3.8^{ab}	25.42 ± 1.20^{b}	7.95 ± 0.65^{b}
	28	92.7 ± 1.5^{a}	150.0 ± 10.0^{ab}	97.3 ± 2.4^{a}	22.82 ± 2.51^{b}	9.54 ± 0.19^{ab}
Hyperbaric	7	N.D.	146.7 ± 5.8^{ab}	97.3 ± 2.3^{a}	16.85 ± 0.57^{c}	11.07 ± 0.68^{a}
	14	23.0 ± 4.5^{c}	133.3 ± 15.3^{abc}	95.7 ± 0.3^{ab}	15.54 ± 1.48^{c}	9.25 ± 0.87^{ab}
	28	45.3 ± 0.6^{b}	106.7 ± 20.8^{c}	85.6 ± 3.9^{b}	17.16 ± 0.56^{c}	9.88 ± 0.78^{ab}

N.D. Not determined.
[a] Different letters in the same column indicate significantly different means (two-way ANOVA; $p < 0.05$).

No changes in solubility were observed under refrigerated storage for up to 28 days, whereas liquid egg yolk was substantially less soluble after just 14 days under HS. Such a trend has been frequently associated to protein unfolding and often observed in concomitance to thickening in pressurized liquid egg yolk (Naderi, House, Pouliot, & Doyen, 2017).

A slightly increasing trend in foaming capacity (Table 6) was observed after 14 days under refrigeration ($p < 0.05$; two-way ANOVA), suggesting storage at low temperature to promote a more efficient displacement of proteins at solvent-air interfaces during storage of liquid egg yolk. On the other hand, HS did not induce any significant ($p > 0.05$; two-way ANOVA) change for up to 28 days. Differently, foam stability seemed not to be affected by storage at any condition for up to 28 days ($p > 0.05$; two-way ANOVA) (Table 4). The capability of liquid egg yolk samples to self-structure in a gelled network upon heating was thus evaluated by mechanical spectrum analysis (Fig. 6, Table 6). Heat-gelled fresh samples showed a typical strong-gel behavior (Fig. 6), with G′ values coherent with those reported in the literature (Aguilar et al., 2019). The application of both refrigerated and hyperbaric storage did not change the rheological behavior of heat-gelled samples (Fig. 6), but caused a progressive decrease in G' at 1 Hz, indicating a loss of gelling capacity (Table 6). In particular, this effect occurred significantly ($p < 0.05$; two-way ANOVA) faster under pressure. This is likely because pressurized proteins would be already interconnected in a network (Fig. 4), and thus less prone to interconnect upon further heating (Kioseoglou, 2003). In agreement with the literature, the capacity of liquid egg yolk to form stable emulsions (EAI) remained unaffected throughout pressurized storage and was not different from that of the fresh sample (Speroni et al., 2005; Yan et al., 2010). This result indicates that even prolonged pressurization at 200 MPa did not significantly affect the capability of proteins to position at water-oil interfaces despite their extensive unfolding (Fig. 5). It is however noteworthy that the maintenance of egg yolk emulsifying activity could be also due to the high concentration of amphiphilic phospholipids (Anton, 2013), which could have made negligible the effects of protein structural modifications.

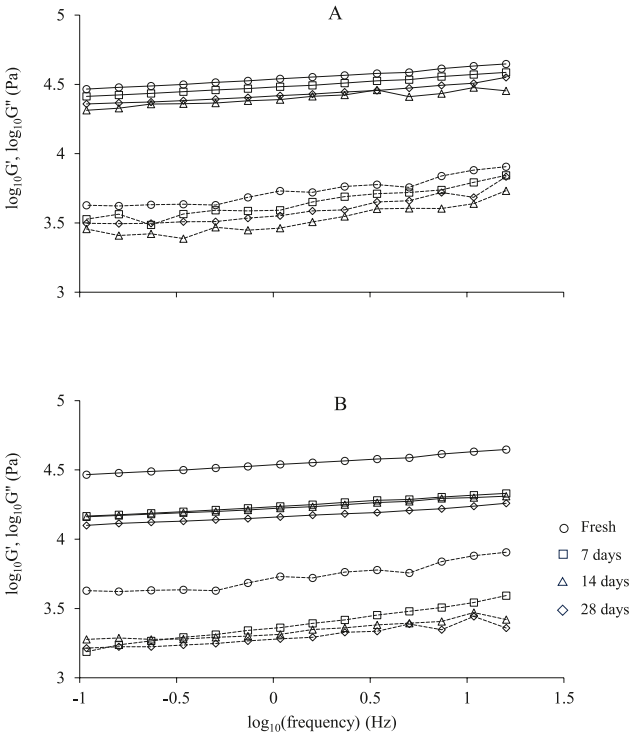

Fig. 6. Logarithmic dependence of storage (G′, ▬) and loss (G″, – – –) moduli on oscillatory frequency of liquid egg yolk stored for increasing time under refrigerated (A) or hyperbaric (B) conditions and thermally gelled.

4. Conclusions

This work demonstrated that HS at 200 MPa guarantees liquid egg yolk microbial stability to be achieved within 48 h and does not affect its oxidative stability for up to 28 days. In addition, HS induces extensive structural modification of egg yolk proteins, changing the matrix physical and techno-functional properties. These modifications might represent an interesting opportunity for specific liquid egg yolk applications. HS could be successfully applied to improve the performance of liquid egg yolk when used in formulations requiring a thick texture (*e.g.*, dips and sauces) and a high hydrophobicity (*e.g.*, fat-rich spreads). On the other hand, the use of hyperbarically-stored liquid egg yolk would not be optimal when the matrix is used as an ingredient expected to undergo thermal gelling during food processing (e.g. bakery products).

Based on these considerations, liquid egg yolk HS could be regarded as a "four-in-one" technological approach. In particular, pressurized storage would contemporarily allow: (*i*) microbial inactivation; (*ii*) maintenance of microbial properties; (*iii*) oxidative stability; (*iv*) development of specific techno-functionalities. The decontamination capability of HS could be even extended to perform non-thermal pasteurization treatments on liquid egg yolk during its storage. However, at the present moment, such wide potential of HS cannot be harnessed in the food industry context. The technology is still characterized by a low TRL (about 3), because safe, easy-to-operate, and economically feasible HS plants have not been designed yet. The main hurdle is represented by the technology being far more expensive and time-consuming than the storage (*i.e.*, refrigeration) and pasteurization (*e.g.*, heat treatments, HHP) processes currently applied by the industry. Nevertheless, the results of this study indicate that HS could be proposed as an alternative to the combination of these processes, also enabling the tailoring of techno-functional properties for specific applications. To the best of our knowledge, a cost analysis of HS within such expanded boundaries has never been performed but could represent the missing tile in making the technology appealing within the food industry framework.

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CRediT authorship contribution statement

Federico Basso: Investigation, Formal analysis, Data curation, Writing – original draft, Visualization. **Lara Manzocco:** Conceptualization, Validation, Resources, Writing – original draft, Writing – review & editing, Supervision. **Michela Maifreni:** Investigation, Formal analysis, Data curation, Writing – original draft. **Marilisa Alongi:** Writing – review & editing. **Maria Cristina Nicoli:** Conceptualization, Resources, Writing – review & editing, Supervision.

Declaration of competing interest

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.

Data availability

Data will be made available on request.

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References

Abe, F. (2007). Exploration of the effects of high hydrostatic pressure on microbial growth, physiology and survival: Perspectives from piezophysiology. *Bioscience Biotechnology & Biochemistry, 71*, 2347–2357. https://doi.org/10.1271/bbb.70015

Aguilar, J. M., Cordobés, F., Bengoechea, C., & Guerrero, A. (2019). Heat-induced gelation of egg yolk as a function of pH. Does the type of acid make any difference? *Food Hydrocolloids, 87*, 142–148. https://doi.org/10.1016/j.foodhyd.2018.07.045

Aguilar, J. M., Cordobés, F., Jerez, A., & Guerrero, A. (2007). Influence of high pressure processing on the linear viscoelastic properties of egg yolk dispersions. *Rheologica Acta, 46*, 731–740. https://doi.org/10.1007/s00397-007-0170-2

Ahmed, J., Ramaswamy, H. S., Alli, I., & Ngadi, M. (2003). Effect of high pressure on rheological characteristics of liquid egg. *LWT - Food Science and Technology, 36*, 517–524. https://doi.org/10.1016/S0023-6438(03)00050-1

Andrássy, É., Farkas, J., Seregély, Z., Dalmadi, I., Tuboly, E., & Lebovics, V. (2006). Changes of hen eggs and their components caused by non-thermal pasteurizing treatments II. Some non-microbiological effects of gamma irradiation or hydrostatic pressure processing on liquid egg white and egg yolk. *Acta Alimentaria, 35*, 305–318. https://doi.org/10.1556/AAlim.35.2006.3.8

Anton, M. (2013). Egg yolk: Structures, functionalities and processes. *Journal of the Science of Food and Agriculture, 93*, 2871–2880. https://doi.org/10.1002/jsfa.6247

Anton, M., & Gandemer, G. (1997). Composition, solubility and emulsifying properties of granules and plasma of egg yolk. *Journal of Food Science, 62*, 484–487. https://doi.org/10.1111/j.1365-2621.1997.tb04411.x

Araújo, S. V., Rocha, B. S., Luna, F. M. T., Rola, E. M., Jr., Azevedo, D. C. S., & Cavalcante, C. L., Jr. (2011). FTIR assessment of the oxidation process of castor oil FAME submitted to PetroOXY and Rancimat methods. *Fuel Processing Technology, 92*, 1152–1155. https://doi.org/10.1016/j.fuproc.2010.12.026

Arntfield, S. D., & Murray, E. D. (1981). The influence of processing parameters on food protein functionality I. Differential scanning calorimetry as an indicator of protein denaturation. *Canadian Institute of Food Science and Technology, 14*, 289–294. https://doi.org/10.1016/S0315-5463(81)72929-8

Bahrami, A., Moaddabdoost Baboli, Z., Schimmel, K., Jafari, S. M., & Williams, L. (2020). Efficiency of novel processing technologies for the control of *Listeria monocytogenes* in food products. *Trends in Food Science and Technology, 96*, 61–78. https://doi.org/10.1016/j.tifs.2019.12.009

Basso, F., Maifreni, M., Innocente, N., Manzocco, L., & Nicoli, M. C. (2022). Raw milk preservation by hyperbaric storage: Effect on microbial counts, protein structure and technological functionality. *Food Research International, 156*. https://doi.org/10.1016/j.foodres.2022.111090. Article 111090.

Basso, F., Manzocco, L., Maifreni, M., & Nicoli, M. C. (2021). Hyperbaric storage of egg white at room temperature: Effects on hygienic properties, protein structure and technological functionality. *Innovative Food Science and Emerging Technologies, 74*. https://doi.org/10.1016/j.ifset.2021.102847. Article 102847.

Basso, F., Manzocco, L., & Nicoli, M. C. (2022). Hyperbaric storage of food: Applications, challenges, and perspectives. *Food Engineering Reviews, 14*, 20–30. https://doi.org/10.1007/s12393-021-09296-7

Bermejo-Prada, A., Colmant, A., Otero, L., & Guignon, B. (2017). Industrial viability of the hyperbaric method to store perishable foods at room temperature. *Journal of Food Engineering, 193*, 76–85. https://doi.org/10.1016/j.jfoodeng.2016.08.014

Biter, A. B., Pollet, J., Chen, W. H., Strych, U., Hotez, P. J., & Bottazzi, M. E. (2019). A method to probe protein structure from UV absorbance spectra. *Analytical Biochemistry, 587*. https://doi.org/10.1016/j.ab.2019.113450

Duarte, R. V., Casal, S., Da Silva, J. A. L., Gomes, A., Delgadillo, I., & Saraiva, J. A. (2022). Nutritional, physicochemical, and endogenous enzyme assessment of raw milk preserved under hyperbaric storage at variable room temperature. *ACS Food Science and Technology, 2*, 961–974. https://doi.org/10.1021/acsfoodscitech.2c00027

Duarte, R. V., Moreira, S. A., Fernandes, P. A. R., Santos, D. I., Inácio, R. S., Alves, S. P., et al. (2017). Whey cheese longer shelf-life achievement at variable uncontrolled room temperature and comparison to refrigeration. *Journal of Food Processing and Preservation, 41*. https://doi.org/10.1111/jfpp.13307. Article e13307.

Duarte, R. V., Pinto, C. A., Gomes, A. M., Delgadillo, I., & Saraiva, J. A. (2022). A microbiological perspective of raw milk preserved at room temperature using hyperbaric storage compared to refrigerated storage. *Innovative Food Science & Emerging Technologies, 78*. https://doi.org/10.1016/j.ifset.2022.103019. Article 103019.

European Community. (2004). *EC commission regulation No 853/2004 of 30 april 2004*. Official Journal of the European Union. L139/55.

Fidalgo, L. G., Delgadillo, I., & Saraiva, J. A. (2020). Autolytic changes involving proteolytic enzymes on Atlantic salmon (*Salmo salar*) preserved by hyperbaric storage. *LWT - Food Science and Technology, 118*. https://doi.org/10.1016/j.lwt.2019.108755. Article 108755.

Fidalgo, L. G., Lemos, Á. T., Delgadillo, I., & Saraiva, J. A. (2018). Microbial and physicochemical evolution during hyperbaric storage at room temperature of fresh Atlantic salmon (*Salmo salar*). *Innovative Food Science and Emerging Technologies, 45*, 264–272. https://doi.org/10.1016/j.ifset.2017.11.003

Fidalgo, L. G., Simões, M. M. Q., Casal, S., Lopes-da-Silva, J. A., Carta, A. M. S., Delgadillo, I., et al. (2020). Physicochemical parameters, lipids stability, and volatiles profile of vacuum-packaged fresh Atlantic salmon (*Salmo salar*) loins preserved by hyperbaric storage at 10 °C. *Food Research International, 127*. https://doi.org/10.1016/j.foodres.2019.108740. Article 108740.

Gao, D., Qiu, N., Liu, Y., & Ma, M. (2016). Comparative proteome analysis of egg yolk plasma proteins during storage. *Journal of the Science of Food and Agriculture, 97*, 2392–2400. https://doi.org/10.1002/jsfa.8052

International Olive Council IOC/T. (2017). *Determination of peroxide value*, 20/Doc No 35/Rev 1.

Kiosseoglou, V. (2003). Egg yolk protein gels and emulsions. *Current Opinion in Colloid & Interface Science, 8*, 365–370. https://doi.org/10.1016/S1359-0294(03)00094-3

Lai, K. M., Chuang, Y. S., Chou, Y. C., Hsu, Y. C., Cheng, Y. C., Shi, C. Y., et al. (2010). Changes in physicochemical properties of egg white and yolk proteins from duck shell eggs due to hydrostatic pressure treatment. *Poultry Science, 89*, 729–737. https://doi.org/10.3382/ps.2009-00244

Li-Chan, E. C. Y., & Ma, C.-Y. (2002). Thermal analysis of flaxseed (*Linum usitatissimum*) proteins by differential scanning calorimetry. *Food Chemistry, 77*, 495–502. https://doi.org/10.1016/S0308-8146(01)00365-X

Lima, V., & Saraiva, J. A. (2023). Microbial inactivation during hyperbaric storage of food: Current data, impacting factors and the potential of a novel pasteurization methodology. *Trends in Food Science and Technology, 139*, Article 104126. https://doi.org/10.1016/j.tifs.2023.104126

Liu, K., Shaw, R. A., Man, A., Dembinski, T. C., & Mantsch, H. H. (2002). Reagent-free, simultaneous determination of serum cholesterol in HDL and LDL by infrared spectroscopy. *Clinical Chemistry, 48*, 499–506. https://doi.org/10.1093/clinchem/48.3.499

Manzocco, L., Panozzo, A., & Nicoli, M. C. (2013). Effect of pulsed light on selected properties of egg white. *Innovative Food Science and Emerging Technologies, 18*, 183–189. https://doi.org/10.1016/j.ifset.2013.02.008

Medina-Meza, I. G., Barnaba, C., & Barbosa-Cánovas, G. V. (2014). Effects of high pressure processing on lipid oxidation: A review. *Innovative Food Science and Emerging Technologies, 22*, 1–10. https://doi.org/10.1016/j.ifset.2013.10.012

Melchior, S., Calligaris, S., Bisson, G., & Manzocco, L. (2020). Understanding the impact of moderate-intensity pulsed electric fields (MIPEF) on structural and functional characteristics of pea, rice and gluten concentrates. *Food and Bioprocess Technology, 13*, 2145–2155. https://doi.org/10.1007/s11947-020-02554-2

Muik, B., Lendl, B., Molina-Diaz, A., Valcarcel, M., & Ayora-Cañada, M. J. (2007). Two-dimensional correlation spectroscopy and multivariate curve resolution for the study of lipid oxidation in edible oils monitored by FTIR and FT-Raman spectroscopy. *Analytica Chimica Acta, 593*, 54–67. https://doi.org/10.1016/j.aca.2007.04.050

Naderi, N., House, J. D., Pouliot, Y., & Doyen, A. (2017). Effects of high hydrostatic pressure processing on hen egg compounds and egg products. *Comprehensive Reviews in Food Science and Food Safety, 16*, 707–720. https://doi.org/10.1111/1541-4337.12273

Nakano, T., Ueki, M., Mizoguchi, R., Takeshita, M., Arima, Y., & Aoki, T. (2017). Heat-induced aggregation of casein in reconstituted skimmed milk at pH 3.30 - 3.55. *Food Science and Technology Research, 23*, 249–254. https://doi.org/10.3136/fstr.23.249

Nimalaratne, C., & Wu, J. (2015). Hen egg as an antioxidant food commodity: A review. *Nutrients, 7*, 8274–8293. https://doi.org/10.3390/nu7105394

Nys, Y. (2018). Dietary carotenoids and egg yolk coloration - a review. *European Poultry Science, 64*, 45–54.

Odani, S., Miyamoto, I., Kasai, M., Hatae, K., & Shimada, A. (2000). Rheological properties of egg yolk subjected to high hydrostatic pressure. *Journal of Home Economics of Japan, 51*, 905–912. https://doi.org/10.11428/jhej1987.51.905

Ohba, R., Teramoto, Y., & Ueda, S. (1993). Clarification of spray-dried egg yolk suspensions and solubilization of proteins from lipoproteins. *Journal of Food Science, 58*, 307–309. https://doi.org/10.1111/j.1365-2621.1993.tb04262.x

Pinto, C., Moreira, S. A., Fidalgo, L. G., Santos, M. D., Vidal, M., Delgadillo, I., et al. (2017). Impact of different hyperbaric storage conditions on microbial, physicochemical and enzymatic parameters of watermelon juice. *Food Research International, 99*, 123–132. https://doi.org/10.1016/j.foodres.2017.05.010

Ribeiro, A. C., Casal, S., da Silva, J. A. L., & Saraiva, J. A. (2023). Improved egg yolk pasteurization using sublethal moderate pressure pre-treatments. *Food Chemistry Advances, 2*. https://doi.org/10.1016/j.focha.2022.100166. Article 100166.

Santos, M. D., Castro, R., Delgadillo, I., & Saraiva, J. A. (2019). Improvement of the refrigerated preservation technology by hyperbaric storage for raw fresh meat. *Journal of the Science of Food and Agriculture, 100*, 969–977. https://doi.org/10.1002/jsfa.10083

Santos, M. D., Fidalgo, L. G., Inácio, R. S., Duarte, R. V., Delgadillo, I., Roohinejad, S., et al. (2018). Hyperbaric storage of fruit juice and impact on composition. In G. Rajauria, & B. K. Tiwari (Eds.), *Fruit juices: Extraction, composition, quality and analysis* (pp. 607–619). Elsevier Inc. https://doi.org/10.1016/B978-0-12-802230-6.00030-8.

Santos, M. D., Fidalgo, L. G., Pinto, C. A., Duarte, R. V., Lemos, Á. T., Delgadillo, I., et al. (2021). Hyperbaric storage at room like temperatures as a possible alternative to refrigeration: Evolution and recent advances. *Critical Reviews in Food Science and Nutrition, 61*, 2078–2089. https://doi.org/10.1080/10408398.2020.1770687

Santos, M. D., Matos, G., Pinto, C. A., Carta, A. S., Lopes-da-Silva, J. A., Delgadillo, I., et al. (2021). Hyperbaric storage effect on enzyme activity and texture characteristics of raw meat. *Food Engineering Reviews, 13*, 642–650. https://doi.org/10.1007/s12393-020-09261-w

Seelig, J., & Schönfeld, H. (2016). Thermal protein unfolding by differential scanning calorimetry and circular dichroism spectroscopy: Two-state model versus sequential unfolding. *Quarterly Reviews of Biophysics, 49*, 1–24. https://doi.org/10.1017/S0033583516000044

Smith, L. J., Fiebig, K. M., Schwalbe, H., & Dobson, C. M. (1996). The concept of a random coil: Residual structure in peptides and denatured proteins. *Folding & Design, 1*, 95–106. https://doi.org/10.1016/S1359-0278(96)00046-6

Speroni, F., Puppo, M. C., Chapleau, N., de Lamballerie, M., Castellani, O., Añón, M. C., et al. (2005). High-pressure induced physicochemical and functional modifications of low-density lipoproteins from hen egg yolk. *Journal of Agricultural and Food Chemistry, 53*, 5719–5725. https://doi.org/10.1021/jf0502808

Wuytack, E. Y., Diels, A. M. J., & Michiels, C. W. (2002). Bacterial inactivation by high-pressure homogenisation and high hydrostatic pressure. *International Journal of Food Microbiology, 77*, 205–212. https://doi.org/10.1016/S0168-1605(02)00054-5

Yan, W., Qiao, L., Gu, X., Li, J., Xu, R., Wang, M., et al. (2010). Effect of high pressure treatment on the physicochemical and functional properties of egg yolk. *European Food Research and Technology, 231*, 371–377. https://doi.org/10.1007/s00217-010-1286-7