

Effect of high hydrostatic pressure treatment on rehydrated egg white powder

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ABSTRACT

In the present study, egg white suspensions (12 w/v%) were HHP-treated at 300, 400, 450, 500, 550 and 600 MPa for 5 min. The effects of HHP treatment on the colour, rheological properties, microbiological status and digestibility of the samples was investigated. Colorimetric analysis revealed increased lightness (L^*) and yellowness (b^*), with decreased redness (a^*), indicating enhanced visual appeal. The rheological evaluation at 500 MPa and above showed a transition from dilatant to pseudoplastic behaviour, which is favourable for food processing due to improved flow under shear stress. SDS-PAGE and in vitro digestion confirmed extensive protein denaturation and enhanced digestibility—especially the complete hydrolysis of ovotransferrin—potentially reducing allergenicity and increasing nutrient bioavailability. Microbiologically, treatments at 500–600 MPa significantly reduced mesophilic aerobic bacteria and extended shelf-life, maintaining safety over 44 days. Overall, HHP-treated egg white demonstrates promising potential as a functional ingredient in safe, nutritious, and technologically adaptable food products.

KEYWORDS

HHP treatment, egg white powder, quality parameters, digestibility

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Eggs are high in nutrients and relatively affordable and are consumed globally, irrespective of age, religion, or ethnicity. In addition to being a premium source of protein, lipids, vitamins, and minerals, eggs are some of nature's best preserved biological elements (Abeyrathne et al., 2013). The whole egg is recognized as an excellent source of protein, particularly due to its established biological value of 100%. This biological value allows the egg to act as a benchmark for other foods and shows how effectively dietary protein can be converted into the body's own protein (Pirkwieser et al., 2022).

The egg can be used in a variety of dishes and is a key ingredient. However, the seasonal nature of egg production poses a challenge in ensuring year-round availability and accessibility as environmental temperatures below 16 °C negatively affect egg production (Kim et al., 2023). To address this, egg powdering has gained ground as a viable preservation technique with several advantages (Mnyandu et al., 2014). By drying eggs to powder, the product can be stored for longer (Mendes De Souza et al., 2019), reducing the impact of seasonal fluctuations in supply. Removing water from egg whites reduces their moisture content and their compact nature allows for more efficient storage and transportation, optimizing the use of available space (Sze et al., 2018).

Various food applications of HHP-treated egg whites have been reported (Hoppe et al., 2013; Singh and Ramaswamy, 2015; Tóth et al., 2017, 2020) however, to our knowledge, there are no reports available on the investigation of HHP-treated rehydrated egg whites. Therefore, the aim of this study was to determine an optimal pressure value/pressure range according to the desired food industry purpose. To achieve this, the changes in colour, rheological properties and protein structure of egg white suspensions and the effect on the digestibility of egg white were investigated. In addition, the development of mesophilic aerobic bacterial counts of HHP-treated egg white suspensions during the 44-day storage experiment was investigated.

MATERIALS AND METHODS

Preparation of the samples and the settings of HHP treatment

Egg white powder (Capriovus Ltd., Hungary) was used in this study. Three 350 mL suspensions with a concentration of 12 w/v% were prepared from the egg white powder samples intended for High Hydrostatic Pressure (HHP) treatment. The samples were then packaged in 200 mL PA-PE (polyamide-polyethylene) bags (AMCO Kft, Budapest, Hungary). For microbiological analysis, 20 mL samples were placed in the packaging bags. The samples were stored in a refrigerator at $4 \pm 1^\circ\text{C}$ until further use. The samples underwent pressure treatment using a Hiperbaric 135 (Hiperbaric, Burgos, Spain) system. All samples were subjected to 5 min of HHP treatment at the following pressures: 0 MPa (control), 300 MPa, 400 MPa, 450 MPa, 500 MPa, 550 MPa, and 600 MPa.

Colour measurement

A Konica Minolta CR-400 colourimeter (Konica Minolta Sensing Inc., Osaka, Japan) was used to perform the colour measurements. With a white tile, the instrument was calibrated before each measurement. The samples were placed in a Petri dish for colour measurement, and each sample was measured three times. The parameters determined were L^* (lightness), a^* (redness), and b^* (yellowness).

Examination of the rheological properties

The rheological behaviours of HHP-treated egg white suspensions were investigated using MCR 92 rheometer (Anton Paar, France) in rotational mode. The properties of the samples under varying shear stress were determined using a concentric cylinder (CC27-SN16145) measuring system. The Anton Paar RheoCompass software (version 1.21.852) was used to control the instrument and thus record the curves. Measurements were performed in triplicates for each sample at 20°C . The shear stress analysis was performed at decreasing shear rates between 1,000 and 1 s^{-1} , where 31 data points were recorded. The Herschel-Bulkley model (Equation (1)) was used to describe the rheological properties of the samples (Magnon and Cayeux, 2021; Penna et al., 2001). R^2 values of the fitted model exceeded 0.95 in all cases.

$$\tau = \tau_0 + K\dot{\gamma}^n \quad (1)$$

where: τ – shear stress (Pa); τ_0 – yield stress (Pa); $\dot{\gamma}$ – shear rate (s^{-1}), K – consistency coefficient (Pa s^n); n – flow behaviour index (dimensionless).

SDS-PAGE analysis

The protein analysis of HHP-treated egg white suspensions was performed by SDS-PAGE (Laemmli, 1970). Handcast gels with a concentration range of 6–15% (w/v) were used for electrophoresis. $5\text{ }\mu\text{L}$ of each sample was mixed with $150\text{ }\mu\text{L}$ of $2\times$ Laemmli sample buffer (Bio-Rad). Two minutes of 100°C water bath heating was used to warm up the mixture. For the analysis, in the wells $7\text{ }\mu\text{L}$ of molecular weight standard and $4\text{ }\mu\text{L}$ of protein solution were loaded. The electrophoresis was run at 200 V for 45 min. The molecular weight range of the standard (Precision Plus Protein All Blue Standards, Bio-Rad, Hercules, CA, USA) was

250–10 kDa. For half an hour, the gels were stained using 0.2% Coomassie brilliant blue (R-250, Bio-Rad). Gel images were scanned using the Gel Doc XR+ System (Bio-Rad), and the samples were analysed using the Quantity One and Image Lab 6.1 software programs (Bio-Rad, Hercules, CA, USA).

In vitro digestion

The in vitro digestion experiment was conducted following the method developed by [Minekus et al. \(2014\)](#). The simulated digestive fluids used included simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF). These digestive fluids contained the appropriate amounts and proportions of electrolyte solution, digestive enzymes, calcium chloride, and water, corresponding to those found in the relevant sections of the alimentary canal. As the primary focus was on protein breakdown, the SSF did not contain amylase enzyme. The initial weight of the samples to be digested was 1 g, with a food-to-artificial saliva ratio of 50:50 w/v. The activity of the pepsin and pancreatin used was determined based on the methods of [Anson \(1938\)](#) and [Hummel \(1959\)](#). The pepsin activity was 1124.7 U mg^{-1} , while the pancreatin activity was 5.08 U mg^{-1} . The bile acid concentration was 2.05 mM mg^{-1} as determined by the Bile Acids Enzymatic Cycling Kit (Dialab, 903120). The gastric phase digestion was carried out at 37°C for 2 h. The gastric phase was repeated during the simulated small intestinal digestion, and then the sample was mixed with simulated small intestinal fluid. The small intestinal digestion was carried out for 2 h at 37°C in a shaking thermostat, just like the gastric phase. The enzymatic reaction was halted by immediately cooling the sample to -70°C . SDS-PAGE gel electrophoresis was used to analyse the digested samples.

Microbiological analysis

The mesophilic aerobic bacterial count was determined using TGE agar. To culture the microbes, a mixture of the solid substrate and inoculum was incubated in an inverted position at 37°C for 24 h. Following the HHP treatment, the samples underwent a 44-day storage experiment, stored at $5 \pm 1^\circ\text{C}$ until measurement. The sampling times were as follows: 0, 7, 14, 24, 34, and 44 days. The growth of microbial populations during the storage experiment was modelled using a linear regression equation. The observed data were used to fit a regression model of the form $y = mx + b$, where the slope (m) represents the growth rate of the microorganisms, and the constant (b) gives the y -axis intercept. The R^2 value obtained provides evidence of the adequacy of the fitted regression model ([Badpa and Ahmad, 2015](#); [Maćkiw et al., 2024](#)).

Statistical analysis

IBM SPSS Statistics 22 software was used to conduct the statistical analysis. A one-way analysis of variance (ANOVA) was applied to identify significant effects of colour and rheological parameters at 5% significance level ($P < 0.05$). Levene's test was used to test for homogeneity of variances, and the Kolmogorov-Smirnov test was used to evaluate the normality of error terms. Post hoc tests were performed to further analyse the results when the ANOVA indicated significance. For homogeneous variances, the Tukey HSD post hoc test was used, while the Games-Howell test was applied for inhomogeneous variances.

RESULTS AND DISCUSSION

Changes in colour

The changes in the colour of HHP-treated egg white suspensions are presented in Table 1. HHP treatment is responsible for the denaturation and aggregation of egg white (Monfort et al., 2012), which in turn causes changes in the structure and colour of egg components. During HHP treatment, egg white changed from a liquid structure to a partial gel, and then to a completely denatured and aggregated product. The result of this was that the colour of egg white changed.

The lightness factor (L^*) significantly increased, showing that brightness increases with greater pressure treatment intensity. A minor reduction in L^* was noted at 450 MPa. This is probably because the pressure treatment at this pressure level caused the partial coagulation of the protein, resulting in gelation of the sample, and at higher pressure levels the protein gel network was formed, leading to a change in the colour of the egg white. The value of redness (a^*) follows a decreasing trend with increasing pressure values, indicating a deepening of the green colour. The value of yellowness (b^*) increases with increasing pressure intensity, indicating a deepening of the yellow colour, however, the change at pressure values above 400 MPa is not significant. HHP treatment may influence structural integrity, which impacts the characteristics and degree of scattered light as well as the surface reflection distribution, resulting in colour alterations (Macdougall, 2010). Singh and Ramaswamy (2015) treated fresh egg whites at 350, 450 and 550 MPa and investigated the effect of pressure treatment on the colour properties of the samples. In the case of b^* and L^* , like our results, an increasing trend was observed with increasing HHP intensity. In the case of a^* , a decrease was observed, unlike our results.

Rheological properties

Figure 1 shows the changes in the flow curves of egg white suspensions caused by HHP treatment. This figure shows that the HHP treatment caused a significant change in the rheological properties of the egg white suspensions.

The shape of the curves in Fig. 1 clearly shows that the samples treated at 550 and 600 MPa can be considered pseudoplastic fluids. The n values of the samples treated at 0, 300, 400 and 450 MPa are all above 1, indicating that these samples exhibit dilatational rheological behaviour. Although the sample treated at 500 MPa is quite close to the characteristics of a Newtonian fluid,

Table 1. The changes in the average values of the colour (L^* : lightness; a^* : redness; b^* : yellowness) of the HHP treated egg white suspensions

Pressure levels [MPa]	L^*	a^*	b^*
0	33.96 ± 1.15^a	-0.99 ± 0.12^a	0.17 ± 0.09^a
300	38.02 ± 1.09^{bc}	-1.05 ± 0.17^a	0.28 ± 0.07^b
400	40.60 ± 0.23^c	-1.59 ± 0.04^b	0.52 ± 1.11^c
450	37.32 ± 0.31^b	-1.37 ± 0.08^c	0.52 ± 0.03^c
500	39.29 ± 0.69^{bc}	-1.53 ± 0.06^{bc}	0.56 ± 1.17^c
550	45.08 ± 0.25^d	-1.54 ± 0.14^{bc}	0.54 ± 0.06^c
600	47.89 ± 1.51^d	-2.69 ± 0.03^d	0.55 ± 0.08^c

^{a-d} Different letters show significant differences ($P < 0.05$).

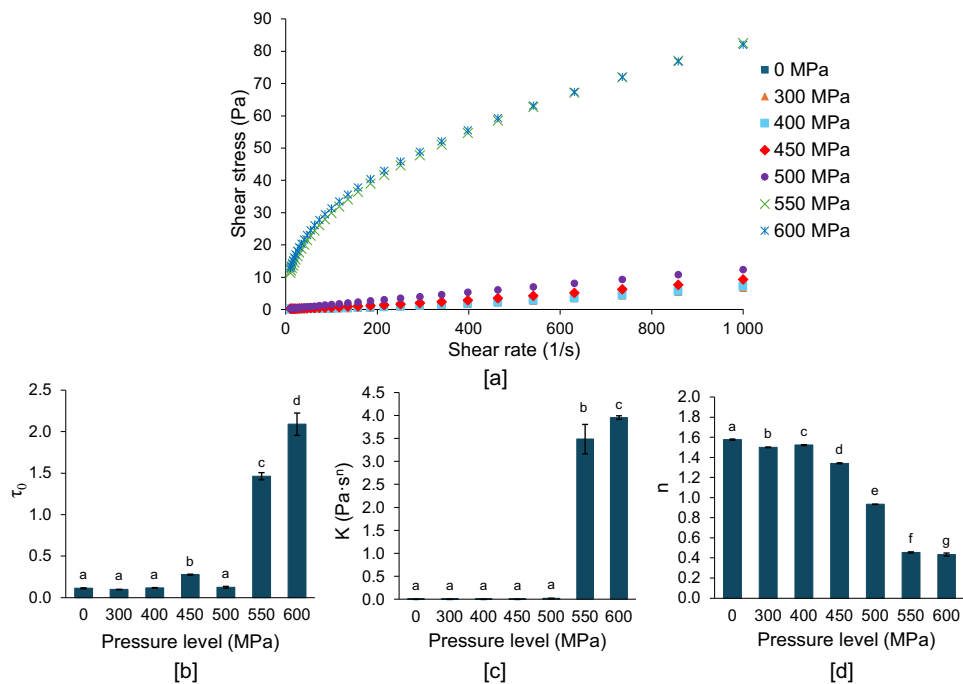


Fig. 1. Results of the rheological analysis; (a) changes in the flow curves of egg white suspensions due to HHP treatment, (b, c, d) effect of HHP treatment on the parameters of the Herschel-Bulkley model fitted to the flow curves of egg white suspensions

^{a-g} Different letters show significant differences ($P < 0.05$)

based on the value of n , it can be considered a pseudoplastic fluid, similar to the samples treated at 550 and 600 MPa. These samples were gelatinised, and their rheological behaviour was clearly changed. Samples showing pseudoplastic rheological behaviour need much higher shear stress values to start flowing. The τ_0 value of the sample treated at 550 and 600 MPa is significantly different from the sample treated at lower pressure levels and the control sample. The rheological properties of the egg white samples coagulated at 550 and 600 MPa behaved differently from the other samples, as there was already a large change in their structure. In the case of these samples, the coagulated protein probably increased the consistency coefficient values (Ahmed et al., 2003), which suggests that their apparent viscosity also increased compared to the other samples.

SDS-PAGE

The SDS-PAGE separation image of the egg white suspension (Fig. 2) shows the molecular weight distribution of the egg proteins.

Four main protein bands were identified for the globular proteins that constitute egg white. Ovalbumin (54%), the major constituent of the egg white, was identified at 45 kDa, ovotransferrin (12%) at 76 kDa, ovomucoid (11%) at 28 kDa and lysozyme (3.4%) at 14.3 kDa

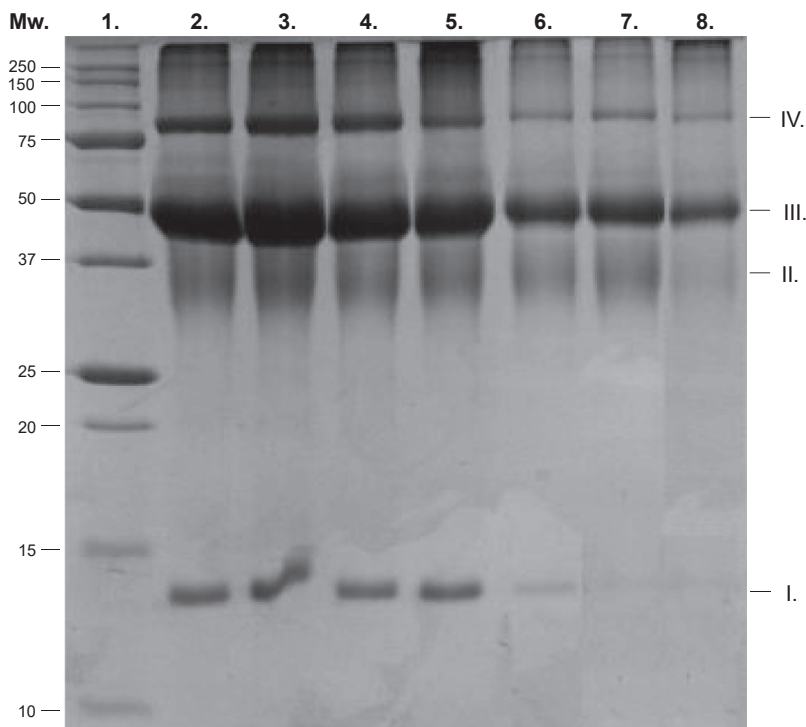


Fig. 2. SDS-PAGE patterns of HHP treated egg white suspensions (Mw = molecular weight; 1. standard; 2. 0 MPa; 3. 300 MPa; 4. 400 MPa; 5. 450 MPa; 6. 500 MPa; 7. 550 MPa; 8. 600 MPa; I. lysozyme; II. ovomucoid; III. ovalbumin; IV. ovotransferrin)

(Farjami et al., 2021; Guha et al., 2019). HHP treatment induced changes in the egg white pattern by increasing pressure values. This may result from protein denaturation and aggregation, leading to the creation of insoluble substances. The lysozyme remained stable at low and medium pressures, but at 500 MPa a significant denaturation was observed which reached 100% at 550 and 600 MPa, meaning that lysozyme was no longer detectable at these pressure values. In the case of ovalbumin, a minor protein denaturation occurred at 400 MPa, which did not change at 450 MPa. At pressures of 500 MPa, 550 and 600 MPa, the significant decrease in ovalbumin is indicated by the low colour intensity and the narrow band that appears. For the ovotransferrin fraction of egg white, partial protein denaturation was observed at higher pressure values (500, 550 and 600 MPa). Andrásy et al. (2006) provided evidence that at a pressure of 250 MPa, insoluble aggregates formed in egg white depending on pressure, and aggregation increased with increasing pressure value. The development of protein aggregates or the denaturation of food proteins under pressure is not invariably negative, as allergy-inducing substances have been identified in both egg yolk and egg white. Ovomucoid, ovalbumin, lysozyme and ovotransferrin found in egg white are allergenic proteins (Benedé et al., 2015). The effect of HHP on the structural modification of these proteins and other allergens in eggs could provide a solution to reduce the allergenicity of these types of ingredients and improve the safety of the final consumable product.

In vitro digestion

Egg white suspensions treated with HHP (450 MPa, 600 MPa) underwent in vitro digestion, where the effect of digestion on the proteins of the sample was monitored by SDS-PAGE (Fig. 3).

Compared to the gel image representing samples not exposed to the digestive enzyme (Fig. 3(A)), the gel image representing the gastric phase (Fig. 3(B)) shows more protein bands, which are the result of extensive hydrolysis caused by pepsin. Many peptides were released from the high and medium molecular weight proteins and migrated to the lower molecular weight range. Among the main egg protein fractions identified in Fig. 3(A), the ovotransferrin band completely disappeared, indicating that pepsin caused complete hydrolysis of this protein. The bands of ovalbumin, ovomucoid and lysozyme remained identifiable after pepsin digestion, the difference being in the intensity of the bands, indicating protein degradation. In the control sample, a continuous diffuse band appears between 25 and 37 kDa, presumably derived from degraded proteins of ovalbumin/ovotransferrin and makes it difficult to differentiate ovomucoid. However, it was found that the digestibility of this protein fraction was also improved by HHP treatment, as the sample treated at 450 MPa showed a more intense band than the sample treated at 600 MPa. These results are consistent with those those of Liu et al. (2018), who reported complete hydrolysis of ovotransferrin by pepsin and partial hydrolysis of ovalbumin and lysozyme in heat- and PEF-treated egg white samples. Hoppe et al. (2013) reached a different conclusion. Egg whites were HHP treated at 400, 600 and 800 MPa for 5 min. Research

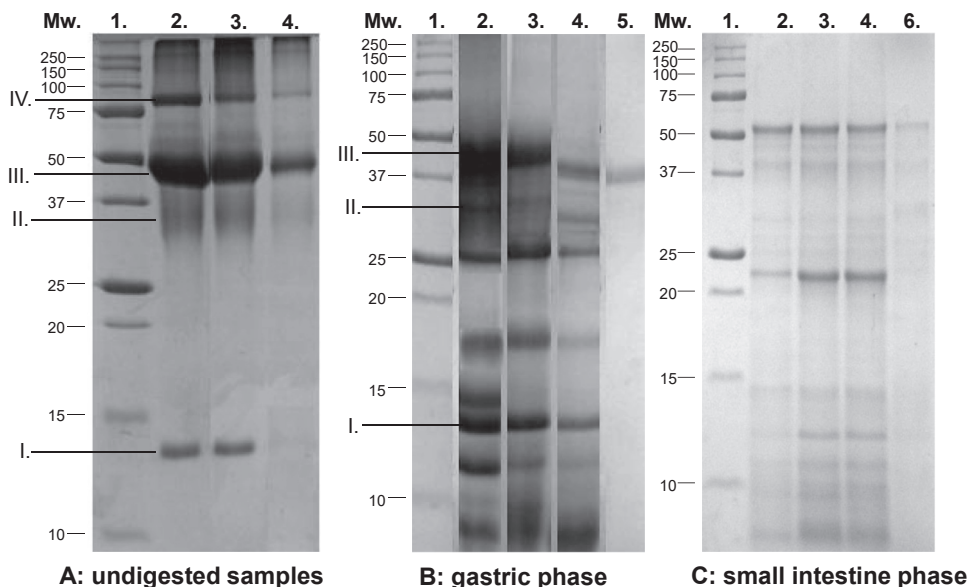


Fig. 3. Protein pattern of undigested samples of HHP treated (450 MPa, 600 MPa) egg white suspensions (A) and monitoring of changes in HHP treated (450 MPa, 600 MPa) egg white suspensions after in vitro gastric (B) and small intestinal digestion (C) by SDS-PAGE. (Mw = molecular weight; 1. standard; 2. 0 MPa; 3. 450 MPa; 4. 600 MPa; 5. pepsin; 6. pancreatin; I. lysozyme; II. ovomucoid; III. ovalbumin; IV. ovotransferrin)

revealed that the primary egg white components (ovotransferrin, ovalbumin, ovomucoid, lysozyme) were broken down by pepsin in under 2 min. In the small intestine phase (Fig. 3(C)), no protein bands derived from egg white were found and only protein bands of the digestive enzyme pancreatin were visible.

Microbiological evaluation

The microbiological results of the HHP-treated egg white suspensions were evaluated according to the Hungarian Decree 4/1998. (XI. 11.) (1998). In general, two limit values are considered for the evaluation of the number of microorganisms, which are marked in Fig. 4. The value “C” represents the compliance limit, and the value “R” indicates the rejection limit. Figure 4 shows the development of the mesophilic aerobic bacterial count of control and HHP treated egg white suspensions during the 44-day storage period.

The mesophilic aerobic bacterial count of the control sample measured on day 0 is 3.55 log CFU/mL, which is reduced to 1.80 log CFU/mL by the end of the 600 MPa pressure treatment. This represents a reduction of about 1,75 logarithmic units. These results were contradicted by Tóth et al. (2017), who pressure-treated liquid egg white at 300 MPa for 5 min. They found that HHP had a 3 log units microbiological inactivation effect. The rejection limit was exceeded only on day 44 of the control sample by the number of mesophilic aerobic bacteria. Samples treated at 500, 550 and 600 MPa remained below the limit of 4 log CFU/mL indicating the compliance level during the 44-day storage experiment. Based on the slopes of the equations, it was determined that there was minimal difference in the growth rate of cell numbers between samples treated at 400 and 450 MPa. For microbial growth rates, the largest decreasing trend was

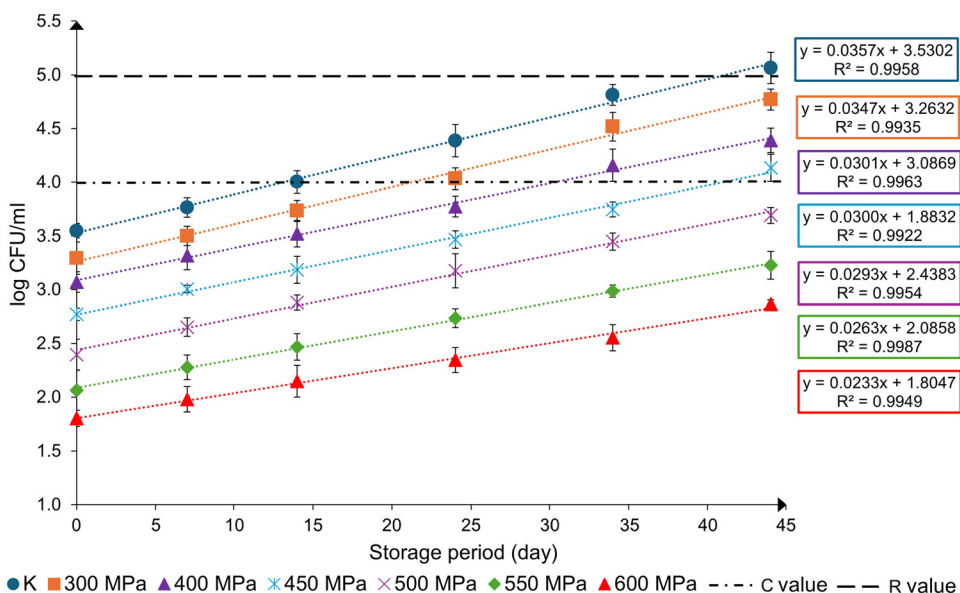


Fig. 4. Changes in the number of mesophilic aerobic bacteria in control and HHP-treated egg white suspensions during 44 days of storage (C = compliance limit; R = rejection limit)

observed between treatments at 300 MPa and subsequent treatments at 400 MPa, meaning that the application of 400 MPa pressure treatment induced a 13% slowdown in microbial growth compared to 300 MPa. Compared to the control sample, HHP treatment at 600 MPa induced a 35% reduction in cell number growth. Based on the results obtained, it was determined that the number of mesophilic aerobic bacteria in egg white suspensions can be effectively reduced by applying HHP treatment. By increasing the pressure values, the growth rate of microorganisms that survive HHP treatment can be reduced.

CONCLUSION

Based on the research findings, it can be concluded that high hydrostatic pressure (HHP) treatment significantly alters the physicochemical, rheological, and microbiological properties of egg white suspensions. The HHP process enhanced colour attributes, resulting in greater lightness and a more stable yellow hue, which may be visually appealing. Rheologically, samples treated up to 450 MPa exhibit dilatational behaviour, which is advantageous from a food technology perspective as it enhances stability and suspension consistency. At higher pressures (500, 550, 600 MPa), this behaviour transitions to pseudoplastic flow, ideal for improving processability and ease of use in food manufacturing due to reduced viscosity under shear. SDS-PAGE analysis revealed pressure-induced denaturation and aggregation of allergenic proteins, such as lysozyme and ovalbumin, potentially reducing allergenicity and improving food safety. From a nutritional perspective, the enhanced digestibility – especially at higher pressure levels (450–600 MPa) – is advantageous, as it facilitates more efficient protein hydrolysis during gastric and intestinal digestion. This leads to better nutrient bioavailability, which is crucial for populations with increased protein needs. The microbiological evaluation showed that HHP treatment was effective in reducing mesophilic aerobic bacterial counts and that increasing the pressure values slowed the growth rate of microbial populations that survived HHP treatment. Overall, these results highlight the dual advantages of HHP: enhancing nutritional efficiency and optimizing food technological performance, which make rehydrated egg white an excellent alternative to fresh counterparts for both consumers and manufacturers. Furthermore, the potential of this non-thermal technology to improve the quality and safety of egg-based products is emphasized.

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