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The effect of High Hydrostatic Pressure treatment on the physical, chemical, biological, and nutritional properties of rehydrated animal-derived food proteins

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1. INTRODUCTION AND OBJECTIVES

Animal-derived foods have been fundamental components of human nutrition for thousands of years, playing a crucial role in providing essential nutrients. Modern nutritional science also confirms that animal-based protein sources such as meat, blood, milk, eggs and fish provide a wide range of macro- and micronutrients. The consumption of these raw materials in appropriate amounts as part of a balanced diet is safe and significantly contributes to meeting daily nutritional requirements.

The present study focuses on powdered blood, egg, and whey raw materials, which have high water activity and nutrient-rich composition in their raw (liquid) state. These properties favour microbial growth, making them highly perishable. Their short shelf life is a technological challenge for the food industry. Drying (pulverisation), which involves significant reduction in water content, is a preservation method that not only improves handling, storage, and transport but also facilitates their widespread use in the food industry.

Eggs are highly versatile ingredients with an excellent nutritional profile and are applicable in a wide range of food industry products. However, the seasonal fluctuations in egg production hinder the consistent supply of raw materials, which limits their industrial applicability. Egg powder, produced through drying, offers an effective solution to overcome these limitations by providing extended shelf life while minimizing losses due to spoilage.

The two other raw materials included in the research are industrial by-products: blood is produced during the processing of meat in slaughterhouses, while whey is produced in the technological process of cheese, cottage cheese and casein production. These materials were previously treated primarily as waste, but they are increasingly recognized as valuable, recyclable protein sources. Efforts toward sustainability in the food industry encourage technological solutions that not only reduce environmental impact but also enhance the nutritional value of end products. The utilization of by-products thus offers a dual advantage: it contributes to waste reduction and provides opportunities for the development of functional food ingredients, promoting the implementation of a circular economy model within the food industry.

Consumer behaviour has undergone a major transformation in recent years. At the end of the 20th century, consumer expectations focused primarily on food safety and favourable cost–benefit ratios, to which the industry responded by producing highly processed products, often without regard to the nutritional and sensory quality of the final product. Today, however, consumer preferences have changed considerably and there is a growing demand for foods of natural origin, free of additives, fresh and mildly processed. This paradigm shift has spurred the development and widespread adoption of minimal processing technologies that aim to maintain

high quality and nutritional value during processing. One of the most prominent of these technologies is High Hydrostatic Pressure (HHP), a non-thermal preservation process. The application of HHP allows the inactivation of pathogenic and spoilage-causing microorganisms without adversely affecting the nutritional composition, sensory attributes or quality of the food (Serna-Hernandez et al., 2021). The concept of high-pressure processing was introduced in the late 19th century, but its industrial application only became widely accepted and available in the second half of the 20th century (Francisco Purroy Balda et al., 2012). The driving force behind this increased interest has been the rising consumer demand for minimally processed food products.

HHP treatment is also promising for rehydrated food powders, as it can enhance their microbiological safety and the bioavailability of the nutrients they contain. As a result, these ingredients may be suitable to produce nutritionally valuable and sustainable food products. This is particularly relevant as rehydration of food powders and incorporation of the resulting suspensions into food matrix is a widespread industrial practice. This process allows for homogeneous distribution of functional ingredients, nutrients, additives, accurate dosing, and improvement of product consistency and quality, especially in product categories such as yoghurts, beverages, bakery products, ice creams and frozen desserts.

Besides eggs and whey, which are commonly used in the food industry, blood of animal origin remains an under-utilised raw material, but research on all three raw materials focuses mainly on examining them in their raw state. Consequently, the application of high hydrostatic pressure (HHP) treatment to rehydrated, animal-derived powder products represent a relatively unexplored area with significant untapped potential for the food industry. The recognition of this scientific gap has determined the direction and objectives of my research.

In the first phase of my research, I focused on the following objectives, which were to understand the properties and behaviour of the basic, starting powders:

- What flow properties characterize the different samples based on bulk and tapped density measurements?
- What rehydration properties (wettability, dispersibility, and solubility) do the examined samples have, and how do these influence the instantizability of the powders?
- What amino acid composition characterizes the samples, and how does this affect their nutritional evaluation, with particular attention to the essential amino acid content?

In the subsequent phase of the research, I aimed to comprehensively investigate the effects of HHP treatment by treating rehydrated powders at pressures between 300 and 600 MPa for 5 minutes. The primary objective of the study is to address the following research questions,

thereby gaining deeper insights into the mechanisms underlying the changes induced by HHP treatment:

- To what extent do the colour parameters of various suspensions (blood plasma, haemoglobin, whole blood, egg white, whole egg, whey) change as a result of HHP treatment?
- What is the effect of HHP treatment on the rheological properties of blood plasma, haemoglobin, whole blood, egg white, whole egg and whey suspensions?
- What changes does HHP treatment induce in the molecular weight distribution of the above suspensions?
- To what extent does HHP treatment improve the digestibility of the protein content of these suspensions?
- What is the impact of HHP treatment on the shelf life of the different suspensions, with special attention to the changes in mesophilic aerobic colony counts over a 44-day cold storage period?

By achieving these research objectives, it becomes possible to determine an optimal pressure level/range for each sample that yields the most favourable effects on the quality and functional properties of the suspensions, thereby supporting the optimization of their application in the food industry.

2. MATERIALS AND METHODS

In my research, I investigated various animal-derived, spray-dried products. When selecting the raw materials, it was important to ensure that none of the samples contained additives, auxiliary agents, or processing aids so that the results of the measurements would not be affected by these substances. The experimental raw materials included the following powdered products: 1.) plasma powder 70 B (Sonac Burgum, The Netherlands), 2.) haemoglobin powder 92 B (Sonac Burgum, The Netherlands), 3.) whole blood powder Vepro 95 (Solvent Kereskedőház, Hungary), 4.) egg white powder (Capriovus Ltd., Hungary) 5.) whole egg powder (Capriovus Ltd., Hungary) 6.) WPI 90 natural whey protein isolate (Buda Family Ltd., Hungary).

During the planning of my research, I divided the measurements into two main phases. In the first step, I analysed the powder-form raw materials, and then prepared the suspensions, which were subjected to HHP treatment. During sample preparation, 3 x 350 mL of 12 m/v% suspensions of each sample were prepared. For colour measurement, rheological property analysis, SDS-PAGE gel electrophoresis, and the in vitro digestion model experiment, the samples were packaged in 200 mL PA-PE (polyamide–polyethylene) pouches (90 µm: 20 µm PA + 70 µm PE; AMCO Ltd., Budapest, Hungary) and sealed using a heat sealer, ensuring minimal air remained in the packaging. A similar procedure was applied for the samples intended for microbiological analysis, with the exception that 20 mL of sample was used per pouch. I stored the samples in a refrigerator at 4°C until use.

In my experiment, the HHP treatment was carried out at the premises of SKC-Consulting Ltd. in Lengyeltóti (Hungary). The pressure treatment of the samples was performed using a Hiperbaric 135 (Hiperbaric, Burgos, Spain) equipment. All suspensions were subjected to a 5 min pressure treatment. Pressure release occurred in less than 3 seconds. The applied pressure levels for all samples were as follows: 0 MPa (control), 300 MPa, 400 MPa, 450 MPa, 500 MPa, 550 MPa, and 600 MPa.

2.1. Measurement methods used for powder products

2.1.1. Determination of flowability

To determine the flowability of the powders, I first measured bulk density (Pugliese et al., 2017) and tapped density (Lebrun et al., 2012). The measurement was performed three times for each sample and the mean value of the obtained data was calculated. Based on the resulting data, I calculated the Carr index and Hausner ratio (Lebrun et al., 2012; Reddy et al., 2014). These indices reflect the flow properties of powders: the lower their values, the better the flowability. Using the obtained values, the flowability of the materials can be classified according to reference values from the literature (Reddy et al., 2014; Venkateswara Rao et al., 2021).

$$bulk\ density = \frac{mass\ of\ powder\ (g)}{volume\ of\ powder\ (cm^3)} \quad [1.]$$

$$tapped\ density = \frac{mass\ of\ powder\ (g)}{volume\ of\ tapped\ powder\ (cm^3)} \quad [2.]$$

$$Carr\ index\ (CI) = 100\ x\ \frac{tapped\ density\ \left(\frac{g}{cm^3}\right) - bulk\ density(\frac{g}{cm^3})}{tapped\ density(\frac{g}{cm^3})} \quad [3.]$$

$$Hausner\ ratio\ (HR) = \frac{tapped\ density(\frac{g}{cm^3})}{bulk\ density\ (\frac{g}{cm^3})} \quad [4.]$$

2.1.2. Determination of the rehydration properties

Wettability:

The wettability properties of the powder samples were determined based on the research of Fitzpatrick et al., (2017) and Szulc and Lenart (2016).

$$Wettability\ \% = \frac{mass\ of\ powder\ disappeared\ (g)}{mass\ of\ initial\ powder\ (g)}\ x\ 100 \quad [5.]$$

Dispersibility:

The dispersibility measurement was carried out according to the procedure described by Jinapong et al., (2008). The dispersibility of the powder samples was determined using the following formula (Schuck et al., 2012):

$$Dispersibility\ \% = \frac{(100 + w) * Xdm}{w * \frac{100 - Xrw}{100}} \quad [6.]$$

where, *w* = mass of powder used (g), *Xrw* = moisture content of powder (w/w%) and *Xdm* = dry matter content of reconstituted powder after sieve filtration (w/w%).

Solubility:

The solubility of the powders was determined at room temperature (24 ± 2 °C) using standard methods (GEA Niro, 2006; IDF, 1988) with minor modifications. The results obtained are expressed as a percentage of solubility. (Fournaise et al., 2020).

$$Solubility\ \% = \frac{(S - M)}{S} * 100 \quad [7.]$$

where, S: mass of sample (g) and M: mass of undissolved sediment (g) after drying.

2.1.3. Determination of amino acid composition

The amino acid analysis was performed by liquid chromatography using an AAA 400 automatic amino acid analyser (Ignos Ltd., Czech Republic). For separation, I used gradient elution with lithium-citrate-based buffers. From the homogenized samples, 0,3–0,4 g of protein was measured, followed by hydrolysis using 6 M HCl at 110 °C for 24 hours. After neutralization, the hydrolysed samples were diluted with distilled water and measured at 10-fold dilution after two filtrations. Tryptophan could not be detected using this method. The prepared samples were stored frozen. Chromatograms obtained from the measurements were evaluated using the program CHROMULAN V 0.82 (PIKRON, Czech Republic).

2.2. Measurement methods for high hydrostatic pressure treated suspensions

2.2.1. Colour measurements

For the colour measurements, I used a Konica Minolta CR-400 colorimeter, which measures the L*, a*, and b* values of colours according to the CIELAB colour space, simulating the human eye's colour perception. The L* value represents lightness, a* indicates red-green and b* indicates yellow-blue colour coordinates. Based on these values, I calculated the colour difference (ΔEab*) and the chroma (C*), which characterizes colour saturation. After calibration, the instrument was applied to samples placed in Petri dishes, with three replicates performed in each case.

2.2.2. Investigation of the rheological properties of the samples using a rotational rheometer

The rheological properties of the HHP treated samples were investigated using a MCR 92 rheometer in rotational mode with a concentric cylinder measuring system. Measurements were performed at 20 °C at decreasing shear rate, with three replicates for each sample. Based on the obtained data, I plotted the shear stress as a function of shear rate. To describe the rheological properties of the samples, I applied the Herschel-Bulkley model (Equation 8) (Magnon and Cayeux, 2021; Penna et al., 2001). To fit the model, I minimized and then aligned the sum of squared differences between the measured and calculated shear rates using the Microsoft Excel 365 Solver add-in. The adjustable parameters were $\tau_{0,}$ K, and n. The goodness of fit of the model was evaluated based on the coefficient of determination (R^2).

$$\tau = \tau_0 + K\gamma^{\,n} \qquad [8.]$$

τ= shear stress (Pa)

τ_0: yield stress (Pa)

K: consistency coefficient ($Pa \cdot s^n$)

γ: shear rate (s^{-1})

n: flow behaviour index (dimensionless)

2.2.3. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

The size separation of proteins was performed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970). The separation was carried out using a Bio-Rad apparatus (Bio-Rad Mini-PROTEAN Tetra System, Bio-Rad, USA). The gels were prepared in two steps: first by pouring a 15% resolving gel then a 6% stacking gel. The samples were diluted with Laemmli sample buffer (2×Laemmli sample buffer and 2-mercaptoethanol, Bio-Rad, USA) and mixed using a vortex mixer. The diluted samples were then boiled and allowed to cool. I loaded 4 µl of sample into the gel wells, while the first well was filled with 7 µl of molecular weight standard. Electrophoresis was carried out at 200 V for 45 min and the gels were fixed with 20% trichloroacetic acid (TCA). Staining was performed with Coomassie Brilliant Blue, and excess stain was removed with 10% acetic acid. I placed the gels on a shaker overnight and then placed them in distilled water to prevent them from drying out. I performed the evaluation of the gels using the Image Lab 6.1 software.

2.2.4. In vitro digestion model experiment

The *in vitro* digestion experiments were performed according to a standardized protocol developed by Minekus et al. (2014) using simulated digestive fluids (saliva, gastric and small intestinal fluid). The digestive fluids contained appropriate concentrations and ratios of electrolyte solutions, digestive enzymes, calcium chloride dihydrate, and water, reflecting the physiological conditions characteristic of the respective sections of the gastrointestinal tract. As the study was exclusively focused on protein digestion, the oral phase did not contain alpha-amylase enzyme. Enzyme activities were adjusted to match the physiological conditions presumed to exist in the human body. I used pepsin in the gastric phase and pancreatin and bile extract in the small intestine phase. The ratio of digestion to artificial digestive fluid was 50:50 w/v% in each phase. The digestion in the gastric and intestinal phases was performed at 37°C for 2 hours with continuous mixing. Enzymatic reactions were stopped by immediate cooling to -70°C. The efficiency of digestion was evaluated using SDS-PAGE gel electrophoresis. Digestibility analyses were performed only on control, 450 MPa and 600 MPa pressure treated samples. This allowed for information to be obtained from the beginning, middle, and end of the sample matrix, enabling the monitoring of the effects of pressure treatment.

2.2.5. Determination of the mesophilic aerobic microbial count of the samples - storage experiment

To determine the total microbial counts, the samples were subjected to a 44-day (~1.5 months) storage experiment following the HHP treatment and stored at 4-6 °C until measurement. The sampling time points were as follows: day 0, 7, 14, 24, 34, and 44. From the samples to be analysed, a tenfold serial dilution was prepared, and under sterile conditions, aliquots were plated onto TGE agar in Petri dishes. The inoculum was gently mixed into the medium, the samples were incubated at 37 °C for 24 hours and the number of colonies grown was determined using a colony counter.

The growth of microbial populations during storage was described using a linear regression model, where in the equation $y = mx + b$, the slope (m) represents the growth rate of the microorganisms, while (b) indicates the y-axis intercept. The accuracy of the fit of the regression model was assessed using R^2.

2.2.6. Statistical evaluation

Statistical analyses were performed using one-way analysis of variance (ANOVA) with IBM SPSS Statistics 22 at 5% significance level ($p < 0.05$). The normality of error terms was tested using the Kolmogorov-Smirnov test, while homogeneity of variances was assessed with Levene's test. In the case of significant ANOVA, Tukey HSD test was used to separate the different groups when the homogeneity of variance condition was met. If the homogeneity of variance condition was not met, I performed the Games-Howell test.

3. RESULTS

Results of measurements on powder products:

During the examination of the bulk and tapped density of the powder samples, I found that none of the samples were completely free flowing. Based on the flowability indicators (Carr-index, Hausner-ratio), the egg powders (egg white, whole egg) exhibited the weakest flow characteristics. In terms of this attribute, the blood powders, especially the haemoglobin powder, showed the most favourable flow properties.

In the study of rehydration properties, I concluded that whey powder exhibited the most favourable rehydration behaviour in all examined parameters (wettability, dispersibility, solubility), and it differed significantly from the other samples in each of these properties.

For the total protein content (%) of the tested animal protein powder products, the following results were obtained in decreasing order: whey powder (93%), haemoglobin powder (93%), whole blood powder (88%), egg white powder (86%), plasma powder (69%) and whole egg powder (48%). Haemoglobin had the highest content of essential amino acids, which was characterized by an exceptionally high leucine content. All the samples I examined contained all the essential amino acids and, in most cases, the amino acid content exceeded the reference value. Exceptions to this were the isoleucine content of all blood powder and the methionine and cysteine content of whole blood powder and haemoglobin powder, as indicated by the amino acid values obtained. It can therefore be concluded that none of the blood powder samples can be considered a complete protein source.

Measurement results of HHP treated suspensions:

Results of colour measurements:

For all tested samples, it was found that the application of HHP treatment caused significant changes in the colour parameters (L*, a*, b*) of the samples in addition to increasing pressure values. As a result of HHP treatment, blood plasma suspensions became lighter and shifted towards a blue tone. Haemoglobin suspensions darkened and shifted towards a red colour range with increasing pressure values. In the case of whole blood suspensions, I found that with increasing pressure values, the samples became lighter and increasingly shifted towards the red or yellow colour range. By increasing the intensity of the HHP treatment, the egg white suspensions became lighter, and the samples were increasingly characterized by green and yellow tones. The application of 500 MPa pressure induced significant changes in the colour parameters of whole egg suspensions. On this parameter, the sample was characterized by a darker, greener and more yellowish colour compared to the control and samples treated at lower

pressures. The colour parameters of whole egg suspensions were characterized by a lightening, a decrease in the green hue, and a shift towards a blue hue with increasing pressure values.

Measurement results of rheological properties:

During the investigation of the rheological properties of HHP treated suspensions, I found that the application of HHP treatment did not significantly affect the rheological behaviour of whey suspensions. In the case of blood plasma, egg white and whole egg suspensions, I found that samples characterized by dilatant rheological behaviour at lower pressures already show pseudoplastic rheological properties when higher pressures were applied (500, 550, 600 MPa). Regarding whole blood and haemoglobin suspensions, it could be established that the application of HHP treatment did not change the flow properties of the samples and all samples had dilatant rheological properties. In the case of whole blood suspension, there was a difference in yield point due to the effect of HHP treatment, as higher shear stress values were required to initiate the flow of whole blood by increasing the pressure values.

Results of polyacrylamide gel electrophoresis (SDS-PAGE):

Based on the electrophoretic separation images of the blood plasma and haemoglobin suspension, there was no difference between the control and the samples treated with HHP at 300-600 MPa, so the rehydrated haemoglobin and blood plasma resisted the high pressure intensely. In the electrophoretic pattern of whole blood, even the lowest pressure (300 MPa) induced a decrease in the colour intensity of the β- and γ-globulin and α- and β-globin protein bands, which did not change with increasing pressure. During the SDS-PAGE analysis of egg white, I found that the lysozyme bands disappeared at 550 and 600 MPa. HHP treatment at 500 MPa and above caused a large reduction in band intensity of ovalbumin. Application of the same pressures also resulted in a decrease in the band intensity of ovotransferrin. Based on the SDS-PAGE electrophoretic pattern of whole egg, the 550 and 600 MPa pressure resulted in a complete decrease in the lysozyme band intensity, as well as in egg whites. Regarding the ovalbumin fraction, it was found that the application of pressure up to 450 MPa did not affect on this protein, while the application of 500 MPa pressure has already caused a lower reduction in the colour intensity of the band, which has not changed at increasing pressure. Among the proteins of the egg yolk, the band intensity of the lower molecular weight α_2-phosvitin and β-livetin was completely reduced at 550 MPa. Based on the SDS-PAGE study of whey suspensions, I found that the β-lactoglobulin band intensity was slightly reduced at 300 MPa, but there was no significant change in the band intensity at the subsequent pressure values up to 550 MPa. In the case of α-lactalbumin, a slight decrease in band intensity can be observed at 300 MPa pressures, which did not change with further pressure values.

Results of in vitro digestibility:

Based on the changes in the gastric phase of *in vitro* digestion, it can be concluded that the application of HHP improved the digestibility of blood plasma proteins, however, increasing the pressure value (from 450 MPa to 600 MPa) did not further improve the digestibility of blood plasma. In the case of haemoglobin and whole blood suspensions, all proteins were degraded in the gastric phase, even in the control sample. It can be concluded that these samples are easily digestible protein sources and that the use of HHP treatment to enhance digestibility is not justified. During the *in vitro* digestion of egg white, I found that the bands of ovalbumin, ovomucoid and lysozyme remained identifiable after pepsin digestion, however, the intensities of the bands decreased, indicating the degradation of the proteins. The extent of this change was proportional to the increase in pressure, thus the use of HHP contributed to the improvement of egg white digestibility. The *in vitro* gastric phase of whole eggs and whey also showed that the digestibility properties of these samples could be improved by applying HHP treatment and increasing the pressure values.

Results of the mesophilic aerobic total plate count test:

During the examination of the microbiological condition of the samples, the germicidal effect of the HHP treatment was clearly determined. For all samples except the whole egg suspension, it was true that the application of 600 MPa pressure reduced the initial germ count by at least half compared to the control sample. None of the HHP treated samples of plasma, haemoglobin, egg white and whey exceeded the rejection limit on day 44 of the storage experiment. In contrast, samples from whole blood and whole egg suspensions pressure-treated at 300 MPa on day 44 already exceeded this critical limit from a food safety perspective. Furthermore, during the storage experiment, it was determined for each sample that the growth rate of the microorganisms surviving the HHP treatment showed a continuous slowing down as the pressure values increased.

4. CONCLUSIONS AND RECOMMENDATIONS

With global population growth, the food industry is increasingly challenged to provide adequate protein in sufficient quantity and quality. Current agricultural systems are unable to meet the rising demand for animal-derived proteins, highlighting the need for sustainable alternatives and advanced processing technologies. High Hydrostatic Pressure (HHP) technology offers a promising solution, as it can inactivate pathogens and extend shelf life without compromising the nutritional value or organoleptic properties of food products. This approach is playing an increasingly important role in ensuring a sustainable protein supply for the future.

In my thesis, I investigated rehydrated suspensions of various animal-derived protein powders after HHP treatment, as there is little comprehensive data available on the behaviour of these materials in this form. My research has provided answers to several open questions, thereby contributing to a deeper understanding of the application of HHP technology in the food industry. Based on the results of the conducted measurements, an optimal pressure value or pressure range can be determined for each sample, which aligns with the desired food industry objectives. Accordingly, I recommend the following maximum pressure values for the use of the investigated suspensions in liquid form or as liquid materials: whole blood 400 MPa, egg white 450 MPa, blood plasma and whole egg 550 MPa, haemoglobin and whey 600 MPa. Above these pressure levels, the suspensions turn into a gel state, enabling the production of more structured products, such as puddings or gels. HHP treatment at 550 and 600 MPa ensures adequate microbiological purity for all suspensions for 44 days following the treatment. HHP treatment improves the digestibility of the protein fractions in the suspensions, which may be a significant factor for the application of protein powders in the food industry, particularly in the development of infant formulas, functional foods, ready-to-eat meals for special dietary needs, or fortified yogurts, beverages, and bakery products. Based on the digestion studies, I concluded that all blood-derived samples can be considered easily digestible protein sources for the human body, therefore, the use of HHP technology for enhancing digestibility is not justified in the case of whole blood and haemoglobin samples. However, in the case of blood plasma and whole egg, I recommend applying 450 MPa pressure, while for egg whites and whey, a pressure of 600 MPa or above should be used to enhance the bioavailability of the proteins in the suspensions.

Consequently, the results suggest that HHP treatment of rehydrated protein suspensions offers new opportunities for the food industry, particularly in the development of functional and specialized nutritional products. However, the successful market introduction of such products requires proper scientific grounding as well as effective consumer communication to ensure that their technological and nutritional benefits are widely recognized and accepted. Therefore, HHP

technology may not only play a role in extending shelf life and enhancing food safety but also contribute to the development of a product portfolio aligned with modern, health-conscious nutritional trends.

Further investigations are necessary to understand the long-term storability, stability of functional properties, and sensory characteristics. In addition, special attention must be paid to the allergenic properties of individual protein sources and the changes that occur during HHP treatment. When introducing the industrial-scale implementation of the technology, economic aspects must also be considered, particularly in terms of energy consumption and costs of equipment. Based on all these factors, the application of HHP technology requires a complex approach that integrates microbiological safety, nutritional benefits, economic efficiency, and consumer expectations. The results presented in this dissertation aim to contribute to this complex perspective and provide guidance for future research and innovation developments. The processing of animal-derived proteins using HHP technology not only enables the development of novel products but can also contribute to the establishment of a more sustainable, safer and health-conscious food system.

5. NEW SCIENTIFIC RESULTS

1. I have determined that the rheological behaviour of blood plasma, egg white, and whole egg suspensions changed significantly ($p < 0.05$) by HHP treatment. At lower pressure levels (300–500 MPa), the samples exhibited dilatant behaviour, while at higher pressures (550–600 MPa), pseudoplastic properties were observed. My results represent a novelty for the HHP treatment of blood plasma, egg white and whole egg, as no previous studies have investigated the rheological characteristics of these samples in this manner.

2. I demonstrated that the rheological parameters of the haemoglobin suspensions did not change significantly ($p > 0.05$) within the 300–600 MPa pressure range, indicating that these samples can be considered rheologically stable regardless of the intensity of HHP treatment.

3. Based on the results of my SDS-PAGE analyses, I established that the application of 300 MPa pressure to whole blood suspensions led to a decrease in the band intensity and width of β- and γ-globulin, as well as α- and β-globin fractions. The extent of these changes did not increase with further pressure elevation, suggesting that the effect of HHP treatment on these fractions was independent of pressure.

4. Based on the SDS-PAGE separation image of egg white and whole egg suspensions, I found that the decrease in ovalbumin band intensity began at 400 MPa in egg white and at 500 MPa in whole egg. Furthermore, for both samples, I found that lysozyme remained stable and resistant to pressure up to 450 MPa, however, applying 550 and 600 MPa resulted in a complete reduction in lysozyme band intensity.

5. Based on the SDS-PAGE profile of whey protein suspensions, I determined that the intensity of β-lactoglobulin (β-LG) and α-lactalbumin (α-LA) bands began to decrease at 300 MPa. However, the extent of this reduction did not increase further up to 600 MPa for α-LA and up to 550 MPa for β-LG, indicating that within this range, these proteins remained stable against HHP treatment.

6. Based on the results of *in vitro* digestion experiments in the gastric phase, I found that HHP treatment at 450–600 MPa improved the digestibility of proteins in blood plasma, egg white, whole egg, and whey protein suspensions. In contrast, haemoglobin and whole blood proteins were completely degraded even without treatment, suggesting that HHP is not necessary to improve digestibility in these cases.

7. Based on studies conducted over a 44-day storage period, I established that the 600 MPa HHP treatment resulted in a significant reduction in the number of mesophilic aerobic microorganisms in all samples (exceeding 1.5 logarithmic units). The microbiological condition of the whey, egg white, haemoglobin, and blood plasma suspensions were found to be impeccable at the end of the storage period, indicating that HHP treatment proved to be an effective intervention in terms of microbiological shelf-life.

6. PUBLICATIONS

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