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Impact of high-pressure processing on technological properties of hybrid plant-beef patties

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ABSTRACT

Consumer interest in hybrid meat/plant-based products is growing. Optimizing technological properties of these novel alternatives is an essential step to ensure products meet expectation to closely match the attributes of meat-based counterparts. This study investigated technological properties of four hybrid beef-plant-based patty (HBP) formulations at 30% plant-protein substitution for beef; faba bean protein/blend of pea and wheat protein (each in concentrate and texturized formats) modulated with high-pressure processing (HPP) treatment at 200 MPa or 500 MPa. Significant interactions between HPP treatment and plant-based formulation were observed for a range of product attributes including pH, water-holding capacity (WHC), colour, chewiness, springiness, and lipid oxidation. HBP exhibited higher lightness (L^*) and yellowness (b^*) compared to beef patties. Redness (a^*) increased at 200 MPa but decreased at 500 MPa. Compared to beef patties, HBP showed reduced cooking loss and improved WHC, although WHC decreased at higher pressure. Both plant protein incorporation and HPP treatment reduced lipid oxidation in most formulations. Among all HBP, patties containing 30% faba bean protein 2 (F2) treated at 200 MPa had desirably technological functionality, while F2 treated at 500 MPa most closely matched the control – untreated beef patties. Overall, moderate HPP (200 MPa) enhanced the redness and textural properties of HBP, offering potential in new product development in this format.

1. Introduction

Protein is an essential nutrient required for human growth, maintenance, and overall health. Meat is a primary source of high-quality protein, comprising essential amino acids in desirable proportions, but also bioavailable iron, and numerous micronutrients relevant in the human diet. Meat demand in western countries is projected to increase by between 15% and 71% between 2020 and 2100 (Jia et al., 2023). The projected growth in meat consumption (Alexandratos & Bruinsma, 2012), particularly in emerging economies, raises concerns regarding the sustainability of meeting the forecast volume. Although technical solutions are becoming increasingly available to support reductions in methane emissions from animal-based agriculture (Morsy et al., 2025; Palangi et al., 2022), alternative protein sources are likely to play an important role to support the demand for protein associated with a global population forecast to reach 9.8 billion by 2050, and an increase in middle class consumers, that is expected in emerging economies

(Grasso & Jaworska, 2020; Rubio et al., 2020; Santo et al., 2020; Song et al., 2026). Consequently, the food industry has made forays into developing alternative applications for plant proteins, including expansion of research into the development of plant-based meat analogues, which aim to provide alternative protein sources for vegetarians, vegans and flexitarians and associated growing consumer demand for alternative protein sources (Mulla et al., 2022).

Plant-based meat analogues, most commonly derived from soy, pea proteins and wheat gluten, have been widely promoted as substitutes for conventional meat products, especially in value-added products such as burgers, sausages and ready to eat formats (Broucke et al., 2022). However, despite substantial investment in formulation and processing optimisation, such plant-based meat alternatives continue to exhibit significant limitations and struggle to successfully match conventional meat products in terms of not only nutritional value, but consumer appeal in terms of texture, flavour, and sensory quality (Song et al., 2026; Villacis-Chiriboga et al., 2025). These shortcomings have negatively

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affected consumer acceptance and market penetration (Malila et al., 2024; Song et al., 2025). For example, sunflower protein concentrate extrudates have been characterised by bitterness, burnt flavours, dark colour, and low perceived fibril formation during mastication, whereas pea protein isolate extrudates have exhibited a pronounced pea-like odour and flavour (Pöri et al., 2023). In addition, the absence of certain essential amino acids and micronutrients in some meat analogues necessitate further formulation improvements (Jang & Lee, 2024). Consequently, plant-based meat analogues currently occupy a relatively small niche market, and their growth has been lower than predicted in recent years, with a retrenchment of expected growth forecasts for 100% plant-based meat analogues since 2021 (Siegrist et al., 2024; Siegrist & Hartmann, 2023). For example, in the U.S. retail market, plant-based unit sales declined by 5% in 2024, compared with 2023 (Chalmers et al., 2025).

In order to bridge this gap and promote sustainable plant protein consumption, hybrid plant and meat (HPM) products have emerged as an innovative product typology (Grasso & Goksen, 2023; Song et al., 2026; Villacís-Chiriboga et al., 2025). HPM products partially substitute traditional meat with plant-based ingredients, thereby combining the strengths of both conventional meat and alternative proteins while compensating for their individual limitations (Song et al., 2025; Villacís-Chiriboga et al., 2025). HPM products provide a balanced approach, allowing consumers to increase plant protein consumption while maintaining the familiar sensory attributes of traditional meat products. Baune et al. (2023) demonstrated that a meat hybrid containing 30% plant-based protein substitution can remain attractive to consumers. A range of plant-based ingredients, including chopped vegetables or fruits, powdered plant proteins (flours, concentrates, or isolates), and texturized plant proteins produced by either low- or high-moisture extrusion, have been used to partially substitute meat in HPM products (Song et al., 2026). Karwowska et al. (2025) reported that a hybrid formulation consisting of turkey meat and beans at a 60:40 ratio could meet consumer expectations while maintaining product safety, with juiciness, hardness, meat flavour and odour intensity, and overall quality compared to those of a 100% meat product. Similarly, Bakhsht et al. (2021) demonstrated that textured vegetable protein can replace 10–40% of beef in patties without compromising overall quality compared with beef patties. Major retailers such as Tesco, Lidl, and ICL Food, have actively launched HPM products such as meatballs, burgers, sausages, and mince (ICL, 2026; Informa, 2024; Song et al., 2026).

However, despite their promise, a critical challenge in developing HPM products is that their textural attributes often remain somewhat inferior to those of 100% meat counterparts. This probably at least in part due to the fundamental structural and functional differences between plant and meat proteins, particularly in their gelation behaviour during cooking. During heating, sarcoplasmic and myofibrillar proteins denature and aggregate, forming a three-dimensional gel network that contributes to gel formation and the development of meat texture (Chen & Jaczynski, 2007). This process begins with the disruption of hydrogen bonds, leading to unfolding of protein structures and subsequent aggregation. At the same time, connective tissue proteins undergo structural changes, becoming granular around 60 °C and gelatinizing near 80 °C (Khalid et al., 2022). The combined effects of protein denaturation, aggregation, and collagen transformations lead to the formation of a stable gel matrix that determines the characteristic consistency of cooked meat, a structure that plant proteins often struggle to replicate due to differences in their structural and functional properties. Plant proteins are predominantly globular in nature and stabilised by hydrophobic interactions (dos Santos et al., 2023), therefore compared to fibrous meat proteins, such as myosin and actin, higher temperatures are required to unfold plant protein chains, and gelation that is comparable with meat remains elusive. The softer texture observed in many HPM products may also be partly attributed to the higher moisture and fat retention properties of the added plant-based ingredients, but also to an increased fibre content, both of which features disrupt the formation of a

continuous protein gel network (Song et al., 2025; Wan Ishak et al., 2011). Among plant-based ingredients incorporated into HPM products, legume-derived proteins such as soy, pea, and faba bean offer potential for partial substitution of animal protein due to their nutritional composition and techno-functional properties (Carbonaro & Nucara, 2022; Goldstein & Reifen, 2022; Mulla et al., 2022). The functional characteristics of legume-derived proteins, including, hydration, gelation, and emulsification make them suitable for a wide range of food applications (Goldstein & Reifen, 2022; Zhang et al., 2024). Faba bean (*Vicia faba* L.) is a cool-season legume well adapted to European agroclimatic conditions and characterised by a protein-rich composition (Martineau-Côté et al., 2022; Sengar et al., 2025). In this study, proteins extracted from Irish-grown faba beans, together with commercially sourced faba bean protein isolates, were selected for incorporation into meat products. A pea-wheat protein blend was also included, as wheat gluten exhibits superior water-holding capacity and can enhance product juiciness and tenderness (Jang & Lee, 2024). In addition, to examine a range of plant-based ingredient formats for their functional performance in HPM products, a texturized pea-wheat blend was also assessed.

Emerging non-thermal processing techniques, such as pulsed light, high pressure processing (HPP), irradiation, ultrasound, supercritical carbon dioxide, plasma technology, and pulsed electric field, have been used to modify protein structure and characteristics in many food products (Arteaga-Marin et al., 2025; Mulla et al., 2022; Rostamabadi et al., 2023). Among these technologies, HPP has emerged as a promising, energy-efficient, and environmentally friendly method for modifying the functional properties of animal- and plant-derived proteins, as high pressure can alter protein conformation and induce protein denaturation, aggregation or gelation (Júnior et al., 2020; Messens et al., 1997; Sosa et al., 2020). Application of HPP enables the development of diverse textures in foods and on the whole there are minimal impacts on flavour, colour, and nutrient stability (Mulla et al., 2022; Pathare & Roskilly, 2016; Sun & Holley, 2010). In meat systems, high pressure treatment induces enzymatic, protein (particularly myofibrils), and structural modifications in muscle foods (Chéret et al., 2005). Pressure can alter myofibrillar proteins by promoting depolymerization and structural rearrangement, which increases protein solubility and enhances gel formation (Pathare & Roskilly, 2016; Sun & Holley, 2010). For example, HPP has been shown to induce protein gelation, particularly myosin gelation, in raw sea bass, resulting a more compact myofibrillar structure as pressure increased up to 500 MPa (Chéret et al., 2005). The pressure induced gelation, together with inactivation of endogenous proteases, contributed to increased hardness compared to untreated samples (Chéret et al., 2005). In plant-based ingredients, the techno-functional and bio-functional properties of legume-derived protein concentrates and isolates can significantly modified by high pressure treatment. HPP mainly affects the quaternary and tertiary structures of proteins and, at higher pressures, can also alter secondary structures, whereas the primary structure is generally pressure-insensitive (He et al., 2014). These conformational changes promote protein unfolding, subunit dissociation and reassociation, and modifications in intra- and intermolecular interactions, thereby affecting key techno-functional properties such as gelation, texture, and water-holding capacity. (Hall & Moraru, 2021; He et al., 2014; Queirós et al., 2018). In legume proteins, such structural modifications may also enhance enzymatic hydrolysis and promote the release of bioactive peptides with antioxidant potential (Gharibzadeh & Smith, 2021). For example, He et al. (2014) reported that HPP improved the gelation and textural properties of plant proteins by promoting unfolding, hydrophobic interactions, and the formation of stronger gel networks. Similarly, Hall and Moraru (2021) showed that HPP can facilitate the formation of protein gel networks with high water-holding capacity. These effects are particularly relevant in HPM products, where pressure-induced gelation of plant proteins may contribute to water retention, texture development, and overall product structure (Hall & Moraru, 2021). However, the effects of HPP are pressure-dependent and the

extent of impact can differ depending on the quality attribute assessed: moderate pressures (150–200 MPa) induce structural modifications with minimal denaturation, whereas higher pressures (300–700 MPa) cause irreversible protein changes (Mulla et al., 2022).

Despite increasing research on HPM products, there is limited information available regarding the effects of HPP on hybrid formulations, particularly those containing texturized plant proteins and using beef from mature cattle (Janardhanan et al., 2023). Therefore, our study objective was to investigate the effects of HPP (200 and 500 MPa) on the technological properties of beef and HBP formulated with powdered and texturized plant proteins, and to identify hybrid formulations that most closely matched the conventional beef patty.

2. Materials and methods

2.1. Plant protein ingredients and meat

Alternative plant-based ingredients used in this study are listed, along with their composition, in Table 1. Fab bean protein 1 (FP1) was prepared using alkaline solubilisation and isoelectric precipitation technique at Moorepark Technology (MTL), Fermoy, Ireland according to Kamani et al. (2024). Fab bean protein 2 (FP2), pea/wheat protein blend (PWPB, Trilisse MVP 1201), and pea/wheat textured protein (PWTP, Cargill™ plant protein TEX PW80 M) were kindly provided by Eurosoy GmbH, Cargill, and Brenntag, respectively. Lean beef and beef suet were purchased from KEPAK (Dublin, Ireland). Lean beef, trimmed of connective tissue and visible fat and beef fat were diced, vacuum-packed, and stored at -20°C until use.

2.2. Sample preparation

All beef and hybrid beef formulations were prepared according to the procedure outlined in Fig 1. Before processing, lean beef and fat were thawed overnight at 4°C and minced separately using a mincer with a 9mm plate (MAINCA, Barcelona, Spain). The coarse-minced lean beef was then blended for 3 min using a kitchen blender (Kenwood Limited, Hampshire, UK). To ensure that all formulations contained approximately 10% fat, the control sample (beef patty) was prepared by mixing 94% coarse-minced lean beef, 5% fat, and 1% salt for 2 min. Based on the formulation design, 30% of the meat protein was replaced with plant-based protein ingredients in the hybrid formulations. Prior to patty preparation, each plant protein ingredient was hydrated in distilled water at a ratio of 1:2 (w/v) and stored overnight at 4°C. Four hybrid formulations were prepared by mixing the hydrated plant protein slurry, coarse-minced lean beef, fat, 1% salt for 2 min. The amount of plant protein slurry added was calculated on a total formulation weight basis and included both the plant protein and hydration water (Fig 1).

Table 1
Proximate compositions of plant-based ingredients.

Plant ingredients	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	Carbohydrate (%)	pH
Faba bean protein 1 (FP1)	84.62 ± 0.47	5.97 ± 0.10	3.77 ± 0.23	4.11 ± 0.11	1.53 ± 0.18	6.29 ± 0.01
Faba bean protein 2 (FP2)	86.31 ± 0.07	6.15 ± 0.05	2.71 ± 0.10	3.79 ± 0.06	1.04 ± 0.06	5.79 ± 0.02
Pea/wheat protein blend (PWPB)	72.01 ± 0.04	8.45 ± 0.01	3.51 ± 0.09	4.96 ± 0.03	11.07 ± 0.08	6.02 ± 0.01
Pea/wheat textured protein (PWTP)	76.28 ± 0.17	7.72 ± 0.04	5.58 ± 0.73	2.74 ± 0.04	7.68 ± 0.90	7.55 ± 0.01

Note: Data are reported as mean ± standard deviation of replicate samples.

After mixing, each formulation was minced again through a 5mm plate to ensure uniform consistency.

All mixtures were vacuum-packed using polyamide (PA) and polyethylene (PE) bags (Versatile Packaging, Monaghan, Ireland). Packaged samples from each formulation were randomly assigned either to HPP treatment or to the untreated group after 24h storage at 4°C (Day 1). Packaged samples were subjected to HPP at two pressure levels: 200 MPa and 500 MPa for 5 min using a commercial HPP system (High Pressure Process Tolling Ltd., Dublin, Ireland). The pressure parameters above were chosen based on results reported by previous study Zhu, Tan, et al. (2022), which showed that HPP treatment at 200 MPa produced sausages with properties preferred by consumers. A higher pressure of 500 MPa was selected to evaluate the effects of intensive processing conditions within the operational capacity of the equipment (maximum 600 MPa) and to assess potential technological modifications at elevated pressure (Thangavelu et al., 2022). Sample temperatures, both before and after HPP treatment were 4.5°C and 5.4°C, respectively. Treated formulations were immediately used for patty formation on the same day (Day 1). Approximately 100g of each mixture was shaped into a patty with a diameter of 10cm and a height of 1cm using a burger former. Patties were vacuum-packed and stored at 4°C for further analysis. A total of 15 formulations (including control and hybrid samples) were prepared in triplicate (Table 2), and all samples were vacuum-packed and randomly selected for physicochemical analyses. Freshly opened patties were tested for pH and thiobarbituric acid reactive substances (TBARS) at each point during storage.

2.3. pH measurement

pH of each formulation was determined over the storage period (Days 1, 6, and 11), under vacuum storage at 4°C. A total of 5g of raw sample were homogenized with 45mL of distilled water using a homogenizer (T 25 digital ULTRA-TURRAX®, IKA, Germany) for 2 min. The pH of each sample homogenate was measured by pH meter probe insertion (Hanna Instruments Ltd., Leighton Buzzard, UK) into the sample. Readings were taken in triplicate.

2.4. Cooking loss and WHC

Raw patties were cooked in a pre-heated oven at 160°C for 6 min, flipped, and then cooked for a further 5 min until the internal core temperature reached 72 ± 2°C. Cooking loss was determined according to equation 1 (Akwetey & Knipe, 2012).

$$\text{Cooking loss (\%)} = (\text{Initial weight} - \text{Final weight}) / \text{Initial weight} \times 100 \quad (1)$$

WHC was determined according to the method of Thangavelu et al. (2022), with modification to centrifugation conditions. Here, samples were centrifuged at 9000 × g for 10 min at 4°C using a Sorvall Lynx 6000 centrifuge (Fischer Scientific Ireland, Dublin, Ireland).

2.5. Colour analysis

Colour changes for both raw and cooked patties on day 1 was evaluated using a dual xenon flash UltraScan Pro spectrometer (Hunterlab, Reston, VA, USA). Patties were covered with transparent PVdC cling film during measurement. Colour parameters including lightness (L^*), redness (a^*), and yellowness (b^*) were recorded. The instrument was calibrated using a light trap ($L=0$) and a cling film-covered standard white tile ($L=100$; $X=88.69$; $Y=93.58$; $Z=100.45$). Measurements were taken using a 19mm viewing port, standard illuminant D65, and a 10° observer angle. The average of nine readings per sample was recorded.

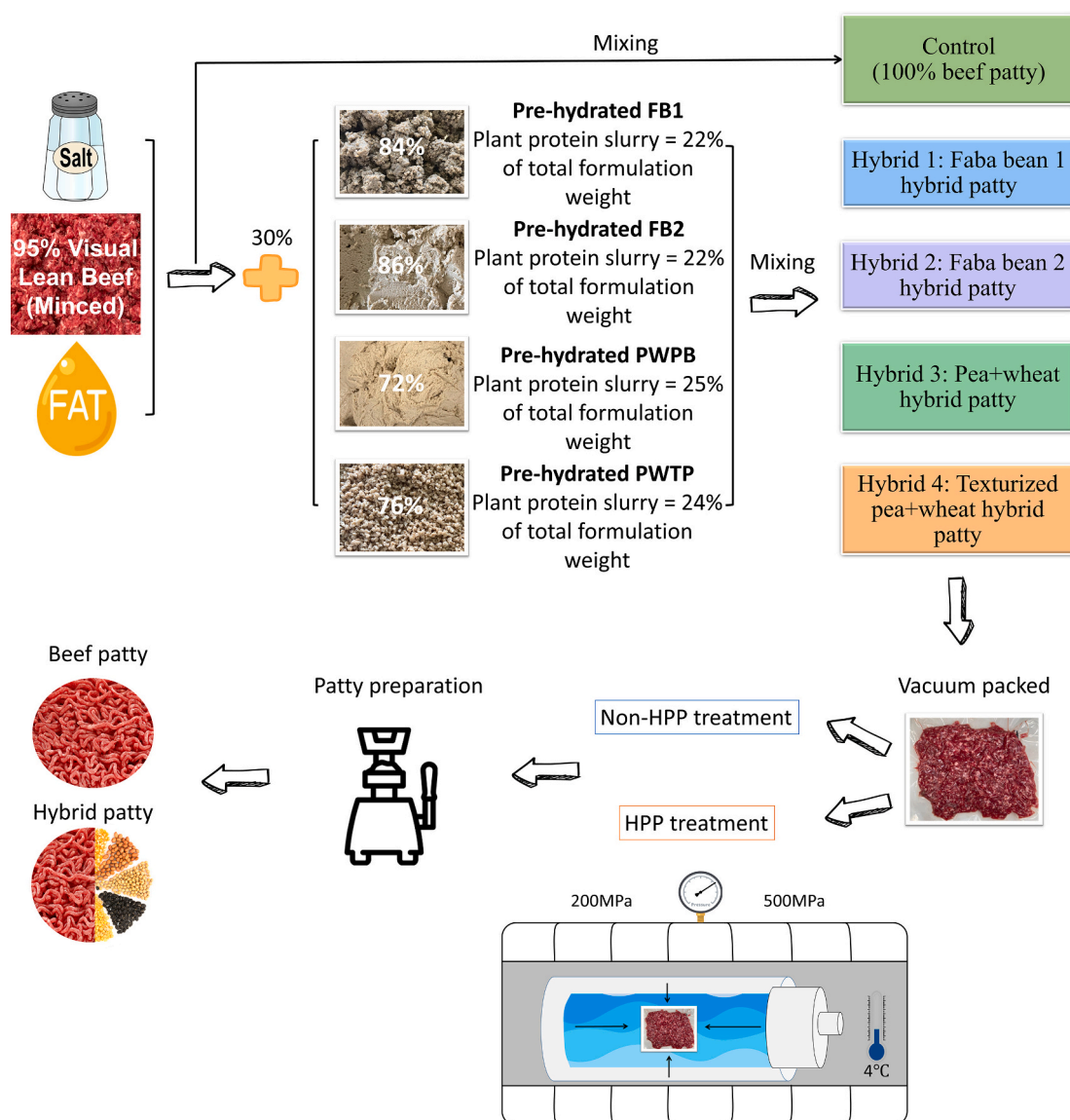


Fig 1. Workflow of the experiment.

Table 2

Experimental formulations of control and hybrid patties with various plant ingredients and HPP treatments.

Formulation No.	Formulation	Plant ingredient	HPP treatment	Formulation code
1	Control (Beef)	NA	Non-HPP	C
2			200MPa	C_200
3			500MPa	C_500
4	Hybrid formulation 1	Faba bean protein 1	Non-HPP	F1
5			200MPa	F1_200
6			500MPa	F1_500
7	Hybrid formulation 2	Faba bean protein 2	Non-HPP	F2
8			200MPa	F2_200
9			500MPa	F2_500
10	Hybrid formulation 3	Pea/wheat protein blend	Non-HPP	PWB
11			200MPa	PWB_200
12			500MPa	PWB_500
13	Hybrid formulation 4	Pea/wheat texturized protein	Non-HPP	PWT
14			200MPa	PWT_200
15			500MPa	PWT_500

2.6. Proximate composition

Raw and cooked samples were homogenised using a grinder (Cookworks, Argos Limited, Milton Keynes, UK) prior to analysis. Moisture content of raw patties was determined using TGM800 thermogravimetric moisture determinator (LECO, St. Joseph, MI, USA) according to AOAC method 925.10 (International, 1925). Moisture content of cooked samples was determined by oven drying at 105 °C overnight in a drying oven (Gallenkamp Labs, Cambridge, UK), and expressed as percentage weight loss. Fat content was evaluated using an NMR Smart Trac rapid analyser (CEM Corporation, Matthews, NC, USA) according to AOAC method 2008.06 (Leffler et al., 2008). Protein content was measured using a LECO FP628 (LECO Corporation, St. Joseph, MI, USA) according to AOAC method 992.15 (King-Brink & Sebranek, 1993). Ash content was analysed using an muffle furnace (Carbolite, Sheffield, UK) at 550 °C according to AOAC method 920.153 (International, 1920).

2.7. TPA analysis

Texture Profile Analysis of cooked patties was performed based on the method described by Bourne (2002). Parameters including hardness

(N), cohesiveness, springiness (mm), and chewiness (N·mm) were measured using an Instron universal testing machine (model 5543, Instron Ltd., High Wycombe, UK) (Song et al., 2025). Cylindrical samples were taken from random locations in each patty using a No. 18 cork-borer. Each sample underwent two-cycle axial compression to 90% of its original height, with a crosshead speed of 100 mm/min under a 500N load.

2.8. Lipid oxidation (TBARS)

Lipid oxidation in beef and HBP was measured using the 2-thiobarbituric acid reactive substances (TBARS) method (Botsoglou et al., 1994), with slight modifications. Formulations were assessed on storage days 1, 6, and 11. A total of 3g of raw patty was mixed with 20mL of Milli-Q water and homogenized at 13500rpm for 30s in a 50mL centrifuge tube. A total of 5ml of cold trichloroacetic acid (25% TCA) were added, and the mixture was vortexed and chilled at 4°C for 15 min. Samples were then centrifuged at 3500 rpm for 30 min. A 3.5mL aliquot of the supernatant was filtered through Whatman Grade 52 filter paper (Scientific Laboratory Supplies Ltd., Dublin, Ireland) to obtain a clarified extract. The filtrate was then mixed with 1.5mL of 0.6 % 2-thiobarbituric acid solution and incubated in a water bath at 70°C for 30 min. Following incubation, tubes were cooled inside an ice bath for 5 min to stop the reaction. The absorbance was measured at 532nm using a UV-Vis Spectrophotometer (Shimadzu UV-1700, Columbia, USA). A standard curve relating the absorbance of the standards to their concentration was prepared. Results were expressed as milligrams of malondialdehyde (MDA) equivalents per kilogram of patty (mg MDA/kg).

2.9. Statistical evaluation

Three independent sample batches of each beef and HBP formulation were prepared. Within each batch, all analyses were conducted at least in triplicate and mean and standard deviation values were reported. The effects of HPP treatment, formulation type, and their interaction were analysed using two-way analysis of variance (ANOVA) with treatment, formulation, and treatment * formulation as factors, performed in SPSS 29.0 (SPSS Inc., Chicago, IL, USA). Duncan's post-hoc test was used to compare least squares means, with a confidence interval of 95%.

2.10. Formulation grading system

To provide a common basis to report the overall performance of different treatments and formulations, a grading system was developed based on the study of Álvarez et al. (2018). This system aimed to integrate the main technological parameters and thereby identify the most suitable combination of plant-based formulation and HPP treatment based on the target product characteristics. The parameters considered were redness of raw patties, cooking loss, WHC, hardness, and liquid oxidation at day 11, as these represent important indicators of product quality. The following equations were applied:

For parameters where higher values are considered desirable (for example WHC):

$$z = X - \mu / \sigma \quad (2)$$

For parameters where lower values are considered desirable (for example cooking loss):

$$z = -(X - \mu / \sigma) \quad (3)$$

where Z is the standardised score, X is the observed value of the parameter, μ is the mean of all samples, and σ is the standard deviation. A radar chart was prepared to compare the scores and demonstrate the relative performance of each formulation and pressure combination.

To quantify the overall similarity of hybrid formulations to the beef

control, the multivariate deviation of each formulation from the beef control was calculated using standardised Euclidean distance as follows:

$$D = \sqrt{\sum (z - z_b)^2} \quad (4)$$

where D value indicates greater similarity to the beef control. z_b represents the corresponding standardised value of each analysed parameter for the standardised beef control.

3. Results and discussion

3.1. pH measurement

pH is an informative indicator of meat quality, influencing eating quality, colour, WHC, tenderness, and microbial stability. Lower pH generally improves shelf stability, while higher pH produces darker meat with higher WHC (Ming-Min & Ismail-Fitry, 2023; Santos et al., 2022; Young et al., 2004). A significant interactive effect ($P < 0.05$) was found between HPP treatment and patty formulation on pH values across the storage period (days 1, 6, and 11). Beef patties showed an increase in pH at the higher pressurisation level (Fig 2), which is consistent with previous studies (Kent et al., 2025; Ma & Ledward, 2004; Omana et al., 2011). For example, it has been suggested that the effect of HPP in elevating pH in meat products is due to the protein denaturation and reduced exposure of acidic groups under pressurisation (Omana et al., 2011). Similarly, an increase of 0.14-0.17 pH units in the initial pH of raw beef has been attributed to a decreased availability of acidic groups in meat proteins after pressurization (Ma & Ledward, 2004). HBP exhibited higher pH values than the 100% beef patties on day 1, which may be attributed to the naturally higher pH of plant-based ingredients compared with fresh beef (Song et al., 2025). All plant proteins used in this study had pH values above 5.79 (Table 1), consistent with previous studies that incorporation of plant-based ingredients increases the pH of HPM products (Bakhsh et al., 2021; Ming-Min & Ismail-Fitry, 2023). For example, Chandler and McSweeney (2022) noted the incorporation the pulses into chicken burgers increased pH values due to the pulse flours (with an average pH of 6.68) being more alkaline in comparison to the chicken (with a pH of 6.52). Kamani et al. (2019) demonstrated that chicken sausages exhibited increased pH values when partially substituted with plant-based ingredients, which aligns with our findings. Although the pH of all patties increased when formed as hybrids, a reduction in pH was observed in HBP treated at 500 MPa compared to those treated at 200 MPa on day 1 (except hybrid formulation F1). The compression effect during high-pressure processing may increase the concentration of free H^+ ions (Zhang et al., 2022). Among all HBP, hybrid formulation F2 treated at 500 MPa exhibited the lowest pH values. The observed differences among hybrid patties may be influenced by redistribution of ions because of the changes in plant protein conformation (Janardhanan et al., 2023). Variations in protein extraction method, degree of denaturation, or compositional characteristics among plant protein ingredients could further explain their distinct responses to high-pressure treatment. Throughout storage, pH values in all formulations decreased, however, HBP treated with HPP consistently maintained higher pH levels compared to their untreated counterparts on days 6 and 11. The decrease in pH observed in untreated beef patties during storage may be attributed to the growth of lactic acid bacteria, (Chmiel et al., 2025). While beef patties treated with HPP at 500 MPa exhibited higher pH values on day 11. This observation is consistent with previous study reporting that HPP, particularly at higher pressure such as 400 and 500 MPa, is highly effective in inhibiting the growth of lactic acid bacteria in skin-packed, thereby limiting acid production during storage (Chmiel et al., 2023). On both day 6 and 11, HBP without HPP treatment exhibited significantly lower pH values compared to untreated beef patties. This may be attributed to the higher water availability in hybrid patties, which may promote microbial spoilage due to their high protein content, additional nutrients from plant protein

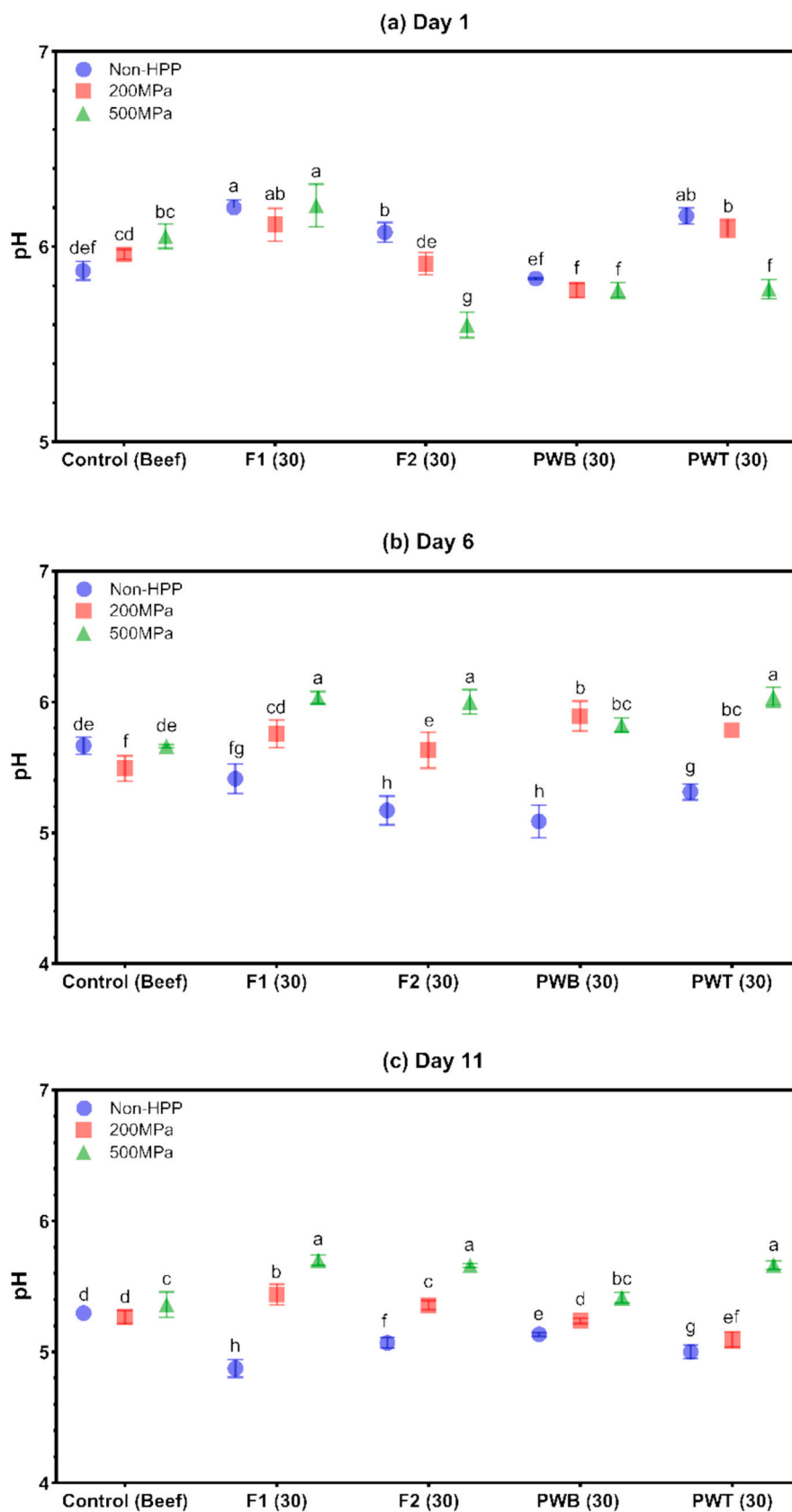


Fig 2. pH values of beef/hybrid patties during storage.

^{a-h} Means that do not share a common superscript are significantly different at $p < 0.05$. Data are presented as mean $\pm$ standard deviation.

ingredients, and near-neutral pH, as similarly observed in plant-based meat analogues (Toth et al., 2021). While greater pH stability observed in HPP-treated HBP, particularly at 500 MPa, further supports the pressure-dependent effectiveness of microbial inactivation. The observed pH changes during storage may be attributed to multiple factors, including formulation differences, the effects of HPP treatment, and possible variations in the initial microbial load of the samples. Further shelf-life studies, including microbiological analyses, are needed to better clarify these effects.

3.2. Colour analysis

Colour is a key quality attribute influencing consumer purchasing decisions for meat products (Thangavelu et al., 2022). For instance, a bright red colour is desirable and is widely perceived as an indicator of freshness of beef meat (Bak et al., 2019). Therefore, any modifications in formulation or processing technology, such as inclusion of plant-based proteins or application of HPP, necessitate thorough evaluation of colour characteristics on the resultant product. Significant interactions between HPP treatment and patty formulation were observed for all colour parameters (L^* , a^* , and b^*) in both raw and cooked samples, indicating that HPP effects varied across different formulations.

In raw patties (Fig 3), the incorporation of plant-based ingredients increased L^* compared to the control, except in hybrid formulation F1,

which exhibited lower L^* values. This reduction observed in F1 is likely due to the darker colour of the faba bean protein, which may result from the high pH protein extraction process used (Kamani et al., 2024). Previous studies have suggested that colour darkening in faba bean proteins is associated with oxidative alteration of phenolic compounds, and proteins extracted at high pH are generally more prone to oxidative reactions. HPP treatment increased lightness in most formulations, particularly at 500 MPa. This increase in L^* values is consistent with the findings of Carlez et al. (1995), who reported increased lightness in fresh minced beef treated at pressures ranging from 200 to 350 MPa at 10°C. The increase in L^* values following HPP has been attributed to structural modifications of myoglobin, including globin denaturation, displacement or release of heme, and ferrous ion oxidation (Cheftel & Culioli, 1997), which result in a paler appearance. In addition to its effects on meat pigments, high pressure treatment increased L^* values in chickpeas, which was linked to pressure-induced enzymatic reactions (Alsaman & Ramaswamy, 2020). Regarding redness, hybrid formulation PWT without HPP treatment exhibited higher a^* values than the control under non-HPP condition, while hybrid formulations F1 and F2 reduced a^* values. Hybrid formulation PWB showed no significant difference compared to the untreated control. At 200 MPa, a^* values slightly increased across all formulations, however, a reduction in a^* value was observed at 500 MPa. The variation in a^* values of patties under different high pressure treatments exhibited a trend consistent

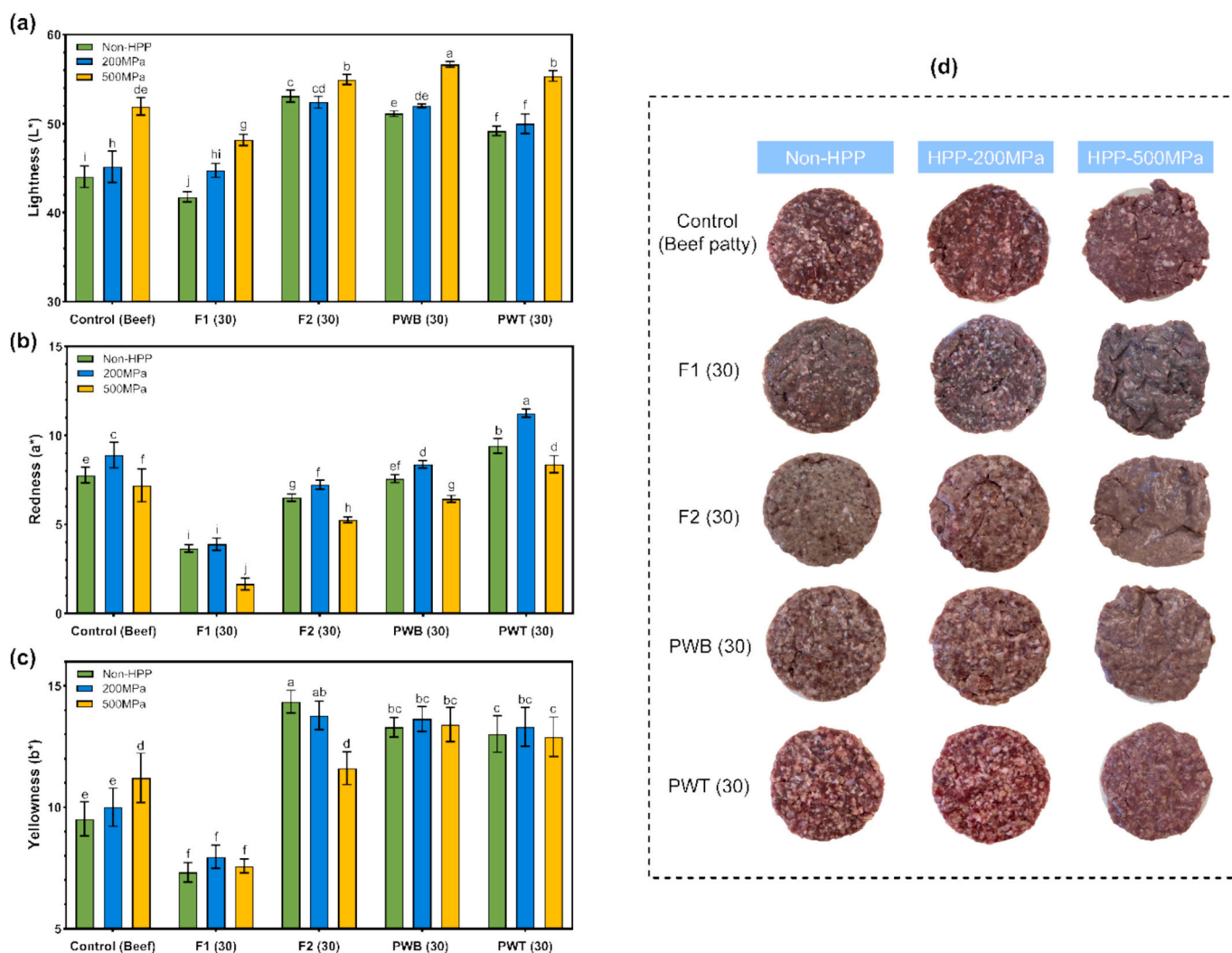


Fig 3. Colour parameters (a) L^* (lightness), (b) a^* (redness), and (c) b^* (yellowness), and visual appearance (d) of raw beef and hybrid patties. ^{a-j} Means that do not share a common superscript are significantly different at $p < 0.05$. Data are presented as mean $\pm$ standard deviation.

with previous findings (Ohnuma et al., 2013). Beef samples treated at 100 and 200 MPa exhibited higher a^* values than untreated samples, whereas higher pressures (400–500 MPa) reduced a^* values relative to those observed at 300 MPa (Ohnuma et al., 2013). These results are consistent with Zhu, Tan, et al. (2022), who reported that HPP (100–400 MPa for 15 min) increased L^* and decreased a^* in beef sausages. The decrease in redness at higher pressures was primarily attributed to the loss of active pigment due to the oxidation of ferrous myoglobin (Mb^{2+}) to brown metmyoglobin (Mb^{3+}), as well as pressure induced denaturation of myoglobin, which further promotes the formation of the ferric Mb-form (Bak et al., 2019; Zhu, Tan, et al., 2022). As for yellowness, all hybrid formulations, with the exception of HBP formulation F1 displayed increased b^* values compared to the control under non-HPP conditions. Following HPP treatment, b^* values varied by formulations, suggesting formulation-specific responses to pressure. Previous studies (Bak et al., 2019) have reported that yellowness in meat is generally less affected by HPP and remains relatively stable.

Similar to the raw samples, a significant interaction between HPP treatment and formulation type on colour parameters in cooked patties was observed (Fig 4). HPP did not affect cooked L^* values, whereas plant protein type was significant. Under non-HPP conditions, cooked HBP F2 (46.4), PWB (49.7), and PWT (49.2) showed higher L^* values than the control (45.5), whereas F1 exhibited the lowest L^* value (43.6),

following a similar pattern to that observed in the raw state. For redness, most hybrid formulations were comparable to the control, apart from formulation F1, which consistently showed lower a^* values. A similar trend was observed for b^* values under non-HPP conditions: formulation F1 exhibited the lowest b^* value, followed by the control, with formulations PWB, PWT, and F2 showing progressively higher values. After HPP treatment, no significant differences in b^* values were observed for the control, F1, or PWT compared to their respective non-HPP counterparts.

In summary, under non-HPP conditions, the incorporation of plant proteins increased the L^* values of HBP, except for F1, whereas the effects on a^* and b^* values were formulation-dependent. HPP treatment significantly increased lightness, primarily due to pressure-induced denaturation of muscle proteins, which leads to protein aggregation and alterations in surface structure. These structural modifications affect light absorption, scattering, and reflection properties, thereby enhancing perceived lightness (Bak et al., 2019). Enzymatic modifications within the plant protein matrix may have further contributed to the observed lightness increase. All patties exhibited increased a^* at 200 MPa, whereas a marked reduction was observed at 500 MPa. The pressure-induced decline in redness at higher pressure is primarily associated with enhanced oxidation, leading to heme displacement, myoglobin denaturation, and the formation of brown metmyoglobin.

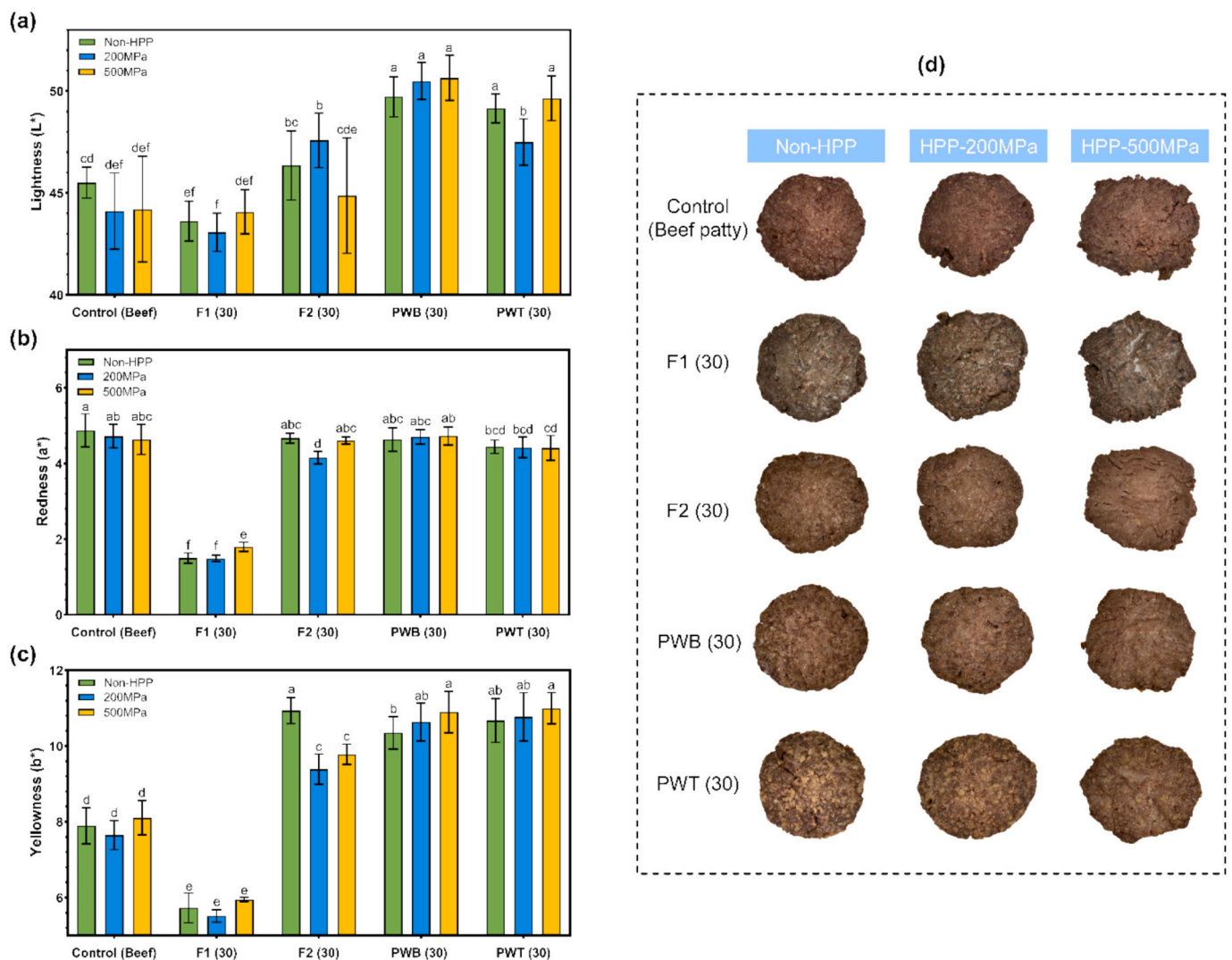


Fig 4. Colour parameters (a) L^* (lightness), (b) a^* (redness), and (c) b^* (yellowness), and visual appearance (d) of cooked beef and hybrid patties. ^{a-f} Means that do not share a common superscript are significantly different at $p < 0.05$. Data are presented as mean $\pm$ standard deviation.

3.3. Compositional analysis

The proximate composition of raw and cooked patties is presented in Table 3 and Table 4, respectively. HPP treatment did not have a significant interactive effect on moisture, protein, or fat content in raw patties, nor on protein and ash content in cooked patties. However, HPP-treated raw patties exhibited a slight reduction in moisture content compared to their respective non-HPP counterparts. This trend may be attributed to pressure-induced structural modifications in muscle proteins, which can alter the water-binding capacity and distribution within the matrix. The fat content was maintained at approximately 10% across all formulations, as per the formulation design. In raw patties, protein content ranged from 20% to 22%, consistent with the formulation design, in which 30% of the beef protein (approximately 20% of total patty weight in the control) was substituted with various plant protein ingredients. The ash content of raw patties ranged from 1.70% to 1.98%, and the minor variation observed may be attributed to fibre content present in plant-based substitutes (Table 1) (Beño et al., 2025; Ebert et al., 2021). After cooking, patties treated with HPP exhibited higher moisture, fat, and protein content compared to non-HPP counterparts. This observation is likely due to the reduced WHC of HPP-treated formulations, leading to increased cooking loss and a lower final product weight. The higher post-cooking nutrient concentrations in HPP-treated samples can be explained by the relative concentration of solids as moisture was lost during cooking.

Table 3
Mean and standard error values of compositional analysis of raw patty formulations.

	Treatment	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
Control	Non-HPP	67.04 ± 0.64 ^a	10.47 ± 0.81 ^{abcd}	20.14 ± 0.67 ^d	1.79 ± 0.07 ^{cdef}
	200 MPa	66.39 ± 1.13 ^{ab}	10.60 ± 1.20 ^{abcd}	20.63 ± 0.30 ^{cd}	1.78 ± 0.04 ^{cdefg}
	500 MPa	65.42 ± 0.31 ^{cd}	10.46 ± 1.66 ^{abcd}	20.42 ± 0.76 ^d	1.74 ± 0.08 ^{efg}
F1 (30)	Non-HPP	66.00 ± 0.48 ^{bc}	10.34 ± 0.66 ^{bcd}	21.74 ± 0.39 ^{ab}	1.83 ± 0.06 ^{bcd}
	200 MPa	65.91 ± 0.32 ^{bcd}	9.96 ± 0.56 ^{cd}	22.00 ± 0.30 ^a	1.85 ± 0.09 ^{bc}
	500 MPa	65.65 ± 0.71 ^{bcd}	10.70 ± 1.14 ^{abcd}	22.16 ± 0.83 ^a	1.89 ± 0.08 ^b
F2 (30)	Non-HPP	65.62 ± 0.88 ^{cd}	10.86 ± 1.23 ^{abc}	22.00 ± 0.31 ^a	1.75 ± 0.04 ^{defg}
	200 MPa	65.47 ± 0.79 ^{cd}	10.65 ± 0.98 ^{abcd}	21.94 ± 0.23 ^a	1.78 ± 0.10 ^{cdefg}
	500 MPa	65.22 ± 0.62 ^{cd}	10.53 ± 0.71 ^{abcd}	22.30 ± 0.31 ^a	1.70 ± 0.05 ^{fg}
PWB (30)	Non-HPP	65.16 ± 0.15 ^d	11.51 ± 0.52 ^a	20.76 ± 0.37 ^{cd}	1.89 ± 0.08 ^b
	200 MPa	65.20 ± 0.49 ^d	11.22 ± 0.60 ^{ab}	20.68 ± 0.42 ^{cd}	1.83 ± 0.06 ^{bcd}
	500 MPa	65.21 ± 0.39 ^d	10.86 ± 0.90 ^{abc}	21.16 ± 0.27 ^{bc}	1.98 ± 0.09 ^a
PWT (30)	Non-HPP	65.89 ± 0.21 ^{bcd}	9.65 ± 0.59 ^d	21.85 ± 0.65 ^a	1.79 ± 0.03 ^{cdef}
	200 MPa	65.48 ± 0.37 ^{cd}	10.66 ± 0.70 ^{abcd}	21.99 ± 0.68 ^a	1.70 ± 0.07 ^g
	500 MPa	65.56 ± 0.60 ^{cd}	10.02 ± 0.86 ^{cd}	21.75 ± 0.74 ^{ab}	1.81 ± 0.09 ^{bcd}
Interaction					
Sig.	ns	ns	ns	ns	0.002
SEM	0.08	0.09	0.09	0.09	0.01

Note: Data are reported as mean ± standard deviation of replicate samples. ^{a-g} Means in the same column that do not share a common superscript are significantly different ($p < 0.05$) from each other. ns, not significant. SEM, Standard error of the mean of the sample population.

Table 4
Mean and standard error values of compositional analysis of cooked patty formulations.

	Treatment	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
Control	Non-HPP	38.81 ± 1.64 ^{efg}	9.93 ± 0.67 ^{cd}	27.31 ± 0.95 ^{cd}	2.00 ± 0.05 ^{def}
	200 MPa	40.33 ± 1.63 ^{cd}	10.90 ± 0.65 ^b	28.01 ± 1.27 ^b	1.96 ± 0.07 ^{efg}
	500 MPa	42.85 ± 1.47 ^a	11.98 ± 1.02 ^a	29.28 ± 1.05 ^a	1.94 ± 0.05 ^{efg}
F1 (30)	Non-HPP	37.19 ± 0.67 ^h	9.43 ± 0.51 ^{de}	25.99 ± 1.13 ^e	2.09 ± 0.06 ^{bc}
	200 MPa	37.69 ± 0.48 ^{gh}	10.01 ± 0.40 ^{cd}	25.84 ± 0.91 ^e	2.08 ± 0.07 ^{bcd}
	500 MPa	39.15 ± 0.68 ^{def}	10.49 ± 0.84 ^{bc}	26.89 ± 1.00 ^{cde}	2.00 ± 0.07 ^{cde}
F2 (30)	Non-HPP	39.63 ± 0.82 ^{cde}	10.95 ± 0.74 ^b	27.30 ± 0.60 ^{cd}	1.91 ± 0.06 ^{fgh}
	200 MPa	40.62 ± 0.83 ^{bc}	10.90 ± 0.50 ^b	28.52 ± 0.68 ^{ab}	1.88 ± 0.07 ^{gh}
	500 MPa	41.61 ± 0.61 ^b	11.14 ± 0.48 ^b	29.14 ± 0.41 ^{ab}	1.83 ± 0.12 ^h
PWB (30)	Non-HPP	39.55 ± 0.95 ^{cdef}	9.58 ± 0.61 ^{de}	27.01 ± 1.18 ^{cde}	2.22 ± 0.06 ^a
	200 MPa	39.53 ± 0.68 ^{cdef}	10.10 ± 0.29 ^{cd}	26.53 ± 0.38 ^{de}	2.15 ± 0.05 ^{ab}
	500 MPa	40.58 ± 0.93 ^{bc}	9.88 ± 0.74 ^{cd}	28.06 ± 0.95 ^{bc}	2.06 ± 0.07 ^{cd}
PWT (30)	Non-HPP	38.28 ± 0.81 ^{fgh}	8.96 ± 0.30 ^e	27.30 ± 1.02 ^{cd}	2.01 ± 0.09 ^{cde}
	200 MPa	39.44 ± 0.62 ^{cdef}	9.31 ± 0.63 ^{de}	26.91 ± 0.91 ^{cde}	1.94 ± 0.06 ^{efg}
	500 MPa	39.84 ± 0.63 ^{cde}	9.02 ± 0.32 ^e	28.85 ± 0.85 ^{ab}	1.97 ± 0.07 ^{efg}
Interaction					
Sig.	0.03	0.01	ns	ns	
SEM	0.18	0.11	0.14	0.01	

Note: Data are reported as mean ± standard deviation of replicate samples. ^{a-h} Means in the same column that do not share a common superscript are significantly different ($p < 0.05$). ns, not significant. SEM, Standard error of the mean of the sample population.

3.4. Cooking loss

Cooking loss values for the various formulations are presented in Fig 5 (a). A significant interaction between HPP treatment and formulation type on cooking loss was observed. All HBP formulations exhibited lower cooking losses compared to the untreated beef control. The reduced cooking loss in HBP can be explained by starches, dietary fibre, and protein matrix present in plant ingredients, which enhance water retention during heat processing (Argel et al., 2020; Baugreet et al., 2016; Chandler & McSweeney, 2022). In addition, legumes can bind water through interactions with polar amino acids, further contributing to improved water retention (Ma et al., 2011). These findings are consistent with previous studies reporting reduced cooking loss following the incorporation of plant proteins (Argel et al., 2020; Baugreet et al., 2016; Chandler & McSweeney, 2022; Janardhanan et al., 2023; Rocha-Garza & Zayas, 1995; Sulaiman et al., 2018; Zhu, Tan, et al., 2022). For example, Zhu, Tan, et al. (2022) reported that the addition of 9% pea protein isolate reduced cooking loss in duck meat batters by promoting protein deformation and aggregation, which enhanced protein-protein interactions and formed a stable network capable of binding water and improving WHC.

Cooking loss increased in both beef and HBP as HPP pressure levels rose from 200 MPa to 500 MPa. This effect likely reflects HPP-induced protein denaturation and structural changes in the meat matrix, which facilitate the release of water and soluble proteins (Zhu, Tan, et al., 2022). Under pressure and heat, meat releases both water and melted fat. Additionally, HPP induces protein dissociation and aggregation,

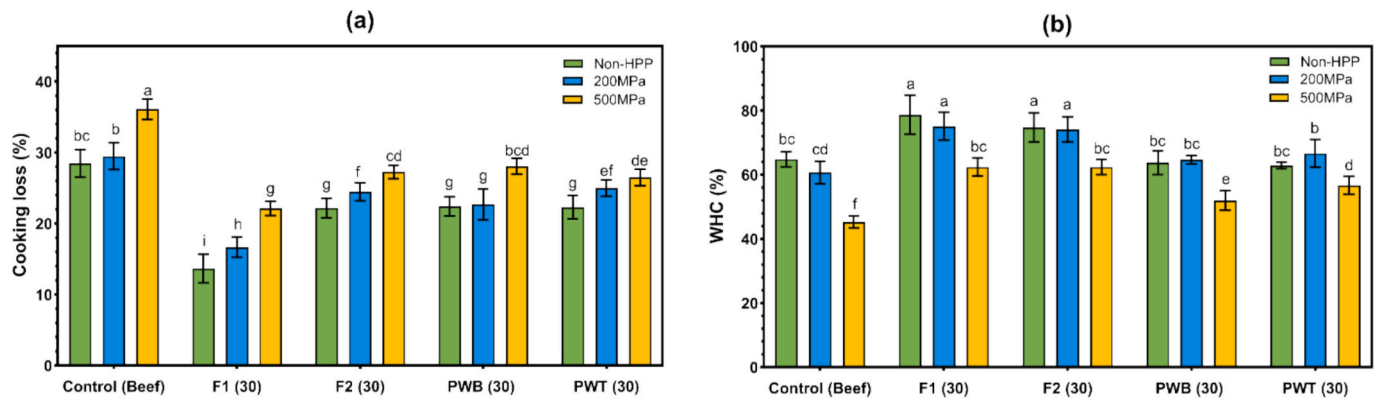


Fig 5. Cooking loss (a) and WHC (b) of beef/hybrid beef patties.

^{a-f} Means that do not share a common superscript are significantly different at $p < 0.05$. Data are presented as mean $\pm$ standard deviation.

consequently allowing aggregates to reduce the surface area available for water binding and hence, further increasing fluid loss (Bernasconi et al., 2020). At higher pressure level (500 MPa), extensive unfolding, depolymerisation, and aggregation of proteins further compromise the sample's water-holding capacity, resulting in higher cooking losses. Beef control treated at 500 MPa had the highest cooking loss among all samples, highlighting the limited WHC under high-pressure treatment. In contrast, hybrid formulation F1 without HPP treatment had the lowest cooking loss, likely due to the superior WHC of faba bean protein 1. Under moderate pressure, meat protein undergo mild unfolding while largely retaining their native configurations. This partial unfolding can promote protein reassociation and gel network formation, improving water immobilisation. However, higher pressure (500 MPa) causes extensive denaturation and potentially excessive aggregation, disrupting the microstructural, reducing WHC, and facilitating fluid migration during cooking processing (Zhao et al., 2022).

3.5. WHC of patties

The result demonstrated significant effects of formulation and pressure level on WHC (Fig 5 (b)), with a significant formulation $\times$ pressure interaction, indicating that the response to HPP was formulation-dependent. At 200 MPa, WHC did not differ. In contrast, treatment at 500 MPa resulted in a significant reduction in WHC across all formulations. Similar to cooking loss, the beef control treated at 500 MPa exhibited the lowest WHC among all samples. At moderate pressure (200 MPa), partial dissociation of non-covalent interactions may induce limited protein unfolding of muscle proteins while allowing re-association upon decompression, resulting in minimal disruption to the water-binding network. As a result, only minor changes in WHC were observed at this pressure level. In contrast, higher pressure (500 MPa) likely induces extensive denaturation of both myofibrillar and plant proteins. These structural alterations promote excessive protein aggregation and construction of the gel matrix, weakening the integrity of the protein network and reducing its capacity to immobilise water (Zhao et al., 2022). Therefore, water becomes less tightly bound within the matrix and more susceptible to migration during thermal processing, resulting in reduced WHC. Similar pressure-induced reductions WHC have been reported in surimi gels, where pressure exceeding 500 MPa disrupt protein cross-linking and partially fracture the gel network structure. This structural damage promotes the migration of free water from the internal gel matrix to the external phase, resulting in decreased WHC (Ma et al., 2015). In addition, Zhang et al. (2025) also reported that increased HPP induced water loss by damage cell membrane. Despite the pressure-induced decline at 500 MPa, HBP generally exhibited higher WHC than the beef control. This observation supports the role of plant protein isolates in reinforcing the composite gel network and enhancing water retention within hybrid matrix.

Nevertheless, the decline in WHC at 500 MPa indicates that excessive pressurisation may disrupt the integrated protein network, thereby reducing its capacity to retain water. Similar pressure-induced reductions in WHC have been reported cowpea protein (extracted at pH 8), where treatment at 600 MPa promoted protein aggregation and precipitation, leading to decreased WHC (Mulla et al., 2022).

3.6. Textural properties

The effect of HPP treatment and patty formulation on texture parameters, including hardness, cohesiveness, springiness, and chewiness are presented in Fig 6. There was no significant interaction between HPP and formulation for hardness and cohesiveness, but a significant interaction was observed for springiness and chewiness. Both HPP treatment and formulation had significant effect on these textural properties.

Hardness, defined as the maximum force required to compress a sample, quantifies the resistance of patties to being bitten (Sun et al., 2022). As discussed previously, the thermal shrinkage of connective tissue and the denaturation of actin during heating may contribute to the increased hardness observed in the beef control (Yoon et al., 2025). The incorporation of plant-based ingredients generally reduced the hardness of HBP, with F1 exhibiting the lowest values compared to other patties without HPP treatment. The reduction in hardness is likely attributed to the increased moisture and fat retention, as well as disruptions in the protein matrix caused by plant proteins and carbohydrates, which interfere with gel network formation (Baugreet et al., 2016; Leonard et al., 2019; Sun et al., 2022). Hybrid formulations F2 and PWT without HPP treatment showed no significant difference in hardness compared to the untreated beef control, while hybrid formulation PWB treated at 500 MPa also exhibited comparable hardness. Hardness values increased with pressure across all formulations except for hybrid formulation F1. The observed increase in hardness is likely due to protein denaturation and cellular damage during HPP, which enhances protein aggregation and strengthens the product structure (Yagiz et al., 2009). Similar findings were reported by Omana et al. (2011), who observed increased gel hardness in chicken breast protein with elevated pressure, due to alterations in water distribution, the formation of new protein networks, and solubilisation or denaturation of myofibrillar proteins. Zhu, Yuwen, et al. (2022) also reported that beef sausages treated with HPP at 200 MPa exhibited textural attributes preferred by consumers, whereas the textural quality decreased at pressure ≥ 300 MPa. This effect was attributed to the reduction in myofibrillar protein particle size at 200 MPa, which facilitated uniform alignment during subsequent heating and promoted the formation of a dense and homogeneous gel network. In contrast, higher pressures (300–400 MPa) resulted in a gel structure with lower density, increased porosity, and a coarser texture.

Cohesiveness indicates the ability of the samples to maintain their

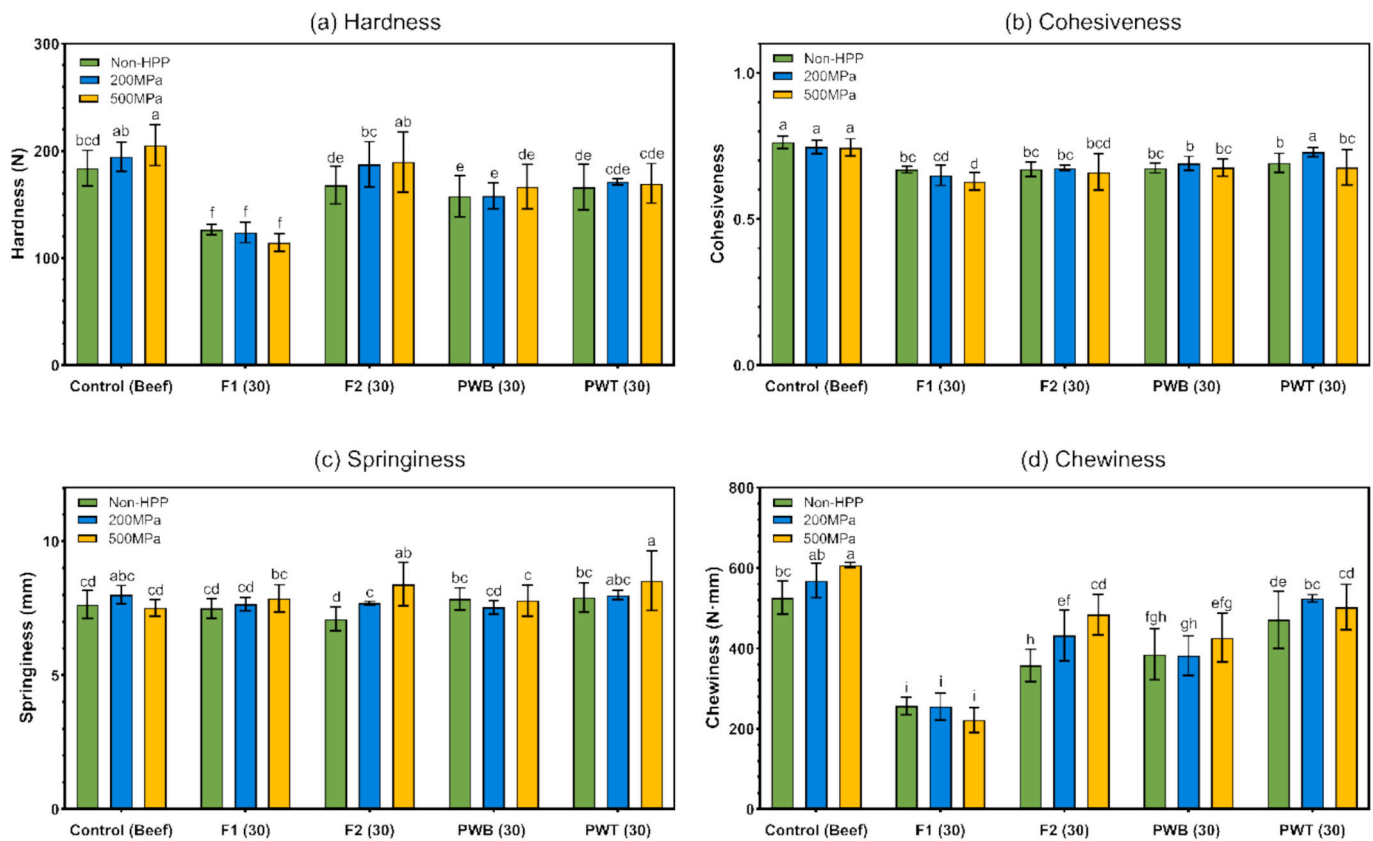


Fig 6. Hardness (a), cohesiveness (b), springiness (c), and chewiness (d) analysis of beef/hybrid beef patties.

^{a-i} Means that do not share a common superscript are significantly different at $p < 0.05$. Data are presented as mean $\pm$ standard deviation.

structural cohesion (Sun et al., 2022), during the texture profiling process which replicates the act of chewing during eating (Ming-Min & Ismail-Fitry, 2023). Cohesiveness values across all samples ranged from 0.7 to 0.8. Although HBP showed lower cohesiveness than the beef control ($P < 0.05$), except for hybrid formulation PWT at 200 MPa, hybrid formulations PWB and PWT exhibited increased cohesiveness at 200 MPa, followed by a reduction at 500 MPa. This trend may reflect differing degrees of protein restructuring under varying pressure conditions. The improvement in cohesiveness at moderate pressure could be attributed to enhanced solubility and binding of myofibrillar proteins (Yang et al., 2015). The reduced cohesiveness in hybrid meat products was also noted by Leonard et al. (2019) who incorporated roasted lupin flour in hybrid meat formulations, which subsequently led to a reduction in intermolecular forces and a consequent decrease in cohesiveness.

Springiness characterizes the capacity of the samples to revert to their original form or size after undergoing compression-induced deformation, which reflects the elastic properties of the samples (Ming-Min & Ismail-Fitry, 2023; Talens et al., 2022). There was no significant difference observed among all non-HPP treated samples. However, most HBP showed increased springiness, to different extents, following HPP treatment, suggesting improved matrix elasticity. This effect aligns with previous studies indicating pressure-induced restructuring of protein matrices (Janardhanan et al., 2023).

Chewiness, which integrates hardness, cohesiveness, and springiness, followed a similar trend to hardness. All HBP had lower chewiness values than the beef control without HPP treatment. HPP treatment increased chewiness values compared to each non-HPP treated samples across formulations. HBP formulation F2 at 500 MPa and HBP formulation PWT at both 200 MPa and 500 MPa showed no significant difference in chewiness when compared to the non-HPP beef patty control.

Under non-HPP conditions, the hardness values of F2 and PWT were comparable to those of the beef control. At 200 MPa, the cohesiveness of

PWT did not differ significantly from the non-HPP beef control. Regarding chewiness, only F2 and PWT treated at 500 MPa exhibited values comparable to the untreated beef control. In general, the inclusion of plant-based ingredients in beef patties lead to softer textured properties compared to the beef patty control, while HPP treatment can compensate for the softness induced by plant protein inclusion, increased hardness and chewiness are likely attributable to protein unfolding and aggregation, thereby leading to stronger gel networks. At 500 MPa, structural changes became more visually and texturally pronounced, possibly due to compaction, which ultimately alter the textural profile. These results suggest that while HPP can enhance the textural integrity of hybrid meat products, its effectiveness is formulation dependent. Optimising pressure level and protein source is crucial for achieving desirable texture comparable to traditional beef patties.

3.7. Lipid oxidation

Lipid oxidation is a complex free radical process in which unsaturated fatty acids react with molecular oxygen radicals via a free radical mechanism, forming hydroperoxides as primary oxidation products. Their instability leads to rapid decomposition into secondary compounds such as hydrocarbons, aldehydes, ketones, alcohols, esters and acids. This process negatively affects the quality of meat, thereby leading to colour deterioration, texture changes, off-flavour via rancidity development, nutrient loss, the formation of toxic and peroxidative compounds (Domínguez et al., 2019; Thangavelu et al., 2022). The end-products of the chain reaction are generally associated with off-flavours and off-odours in meat (Domínguez et al., 2019; Mozuraityte et al., 2016). The thiobarbituric acid reactive substances (TBARS) (Bernardez-Morales et al., 2024; Medina-Meza et al., 2014) results (Table 5) shows that lipid oxidation in patties was significantly affected by both HPP treatment and patty formulation, as well as their interaction, throughout

Table 5

TBARS values of beef/hybrid patties during storage.

Formulation	Treatment	Day 1	Day 6	Day 11
Control (Beef)	Non-HPP	0.90 ± 0.02 ^{aC}	1.54 ± 0.03 ^{bB}	2.54 ± 0.02 ^{aA}
	200 MPa	0.67 ± 0.02 ^{dC}	1.33 ± 0.00 ^{eB}	1.96 ± 0.00 ^{cA}
	500 MPa	0.64 ± 0.01 ^{dC}	1.85 ± 0.02 ^{aB}	2.41 ± 0.03 ^{bA}
F1 (30)	Non-HPP	0.39 ± 0.01 ^{fC}	0.60 ± 0.03 ^{fB}	0.65 ± 0.03 ^{hA}
	200 MPa	0.39 ± 0.01 ^{fC}	0.54 ± 0.01 ^{gB}	0.66 ± 0.02 ^{hA}
	500 MPa	0.54 ± 0.04 ^{eB}	0.53 ± 0.01 ^{gB}	0.62 ± 0.02 ^{iA}
F2 (30)	Non-HPP	0.26 ± 0.04 ^{gB}	0.27 ± 0.01 ^{iB}	0.33 ± 0.01 ^{kA}
	200 MPa	0.27 ± 0.03 ^{gB}	0.27 ± 0.01 ^{iB}	0.31 ± 0.02 ^{kA}
	500 MPa	0.27 ± 0.02 ^g	0.26 ± 0.01 ⁱ	0.26 ± 0.02 ^j
PWB (30)	Non-HPP	0.39 ± 0.01 ^{fC}	0.61 ± 0.04 ^{fB}	1.02 ± 0.04 ^{dA}
	200 MPa	0.38 ± 0.05 ^{fC}	0.53 ± 0.01 ^{gB}	0.72 ± 0.07 ^{8A}
	500 MPa	0.40 ± 0.01 ^{fC}	0.47 ± 0.01 ^{hB}	0.52 ± 0.02 ^{jA}
PWT (30)	Non-HPP	0.86 ± 0.01 ^{bC}	0.98 ± 0.01 ^{dB}	1.02 ± 0.01 ^{dA}
	200 MPa	0.75 ± 0.03 ^{eB}	0.76 ± 0.02 ^{eB}	0.89 ± 0.03 ^{eA}
	500 MPa	0.52 ± 0.01 ^{eB}	0.54 ± 0.02 ^{gB}	0.81 ± 0.04 ^{iA}
Interaction Sig.		0.00	0.00	0.00
SEM		0.02	0.05	0.07

Note: Data are reported as mean ± standard deviation of replicate samples. Different lowercase letters in the same column of the same parameter indicate significant difference ($p < 0.05$); Different uppercase letters in the same row indicate significant differences ($p < 0.05$). SEM means Standard error of the mean of the sample population.

the shelf life. In beef patties, TBARS values increased during storage regardless of HPP treatment. On day 11, both HPP-treated samples showed lower TBARS values than the untreated beef control, with values of 1.96 mg MDA/kg at 200 MPa and 2.41 mg MDA/kg at 500 MPa, compared with 2.54 mg MDA/kg in the untreated sample. The 200 MPa treatment consistently produced lower TBARS values throughout storage, whereas the 500 MPa treatment showed a less consistent pattern, with a higher TBARS value than the untreated beef control on day 6. The increase observed at 500 MPa on day 6 may be related to conformational changes in haemoproteins under high pressurisation, which expose the catalytic heme group to unsaturated fatty acids, thereby accelerating oxidation (Bou et al., 2008). In addition, the release of heme molecules from disrupted membranes and 'free' iron from iron complexes further promotes oxidation under high pressure (Ma et al., 2007; Orlén et al., 2000). Nevertheless, the lower TBARS values observed in HPP-treated samples, particularly at 200 MPa, may be related to pressure-induced changes in cellular structure that affect enzyme activity and reduce the progression of lipid oxidation during storage compared with the untreated beef control (Dave & Ghaly, 2011; Lee et al., 2023). These results indicate that the effect of HPP on TBARS development is pressure-dependent, with moderate or high pressures potentially limiting TBARS development during storage.

Interestingly, HBP had lower TBARS values than beef patties on days 6 and 11. Specifically, hybrid formulation F2, which incorporated faba bean protein 2, had the lowest oxidation levels among the hybrid formulations. This may be attributed to the antioxidant activity of plant proteins, which correlates with total phenolic content (Millar et al., 2019). Wen et al. (2020) reported that plant-derived proteins can generate antioxidant peptides, and Millar et al. (2019) showed that higher antioxidant activity and phenolic content in faba bean flour compared to pea and wheat flours, supporting our findings. No significant difference in TBARS values was observed across storage days in hybrid formulation F2 under 500 MPa treatment. Furthermore, TBARS values in some hybrid patties decreased at 500 MPa treatment. This may be due to HPP-induced increases in phenolic compound availability. Structural modifications in legumes under pressure expose protein cleavage sites, enhancing the release of amino acids and small antioxidant peptides with hydrogen-donating capacity. These peptide fractions, combined with liberated phenolic compounds, contribute to the enhanced antioxidant potential of HPP-treated hybrid formulations (Garcia-Mora et al., 2015). Previous studies showed that the effect of HPP on the antioxidant activity of legumes appears to be ascribed to the

synergistic or antagonistic effect of various aspect, including oxidation reactions, leaching of antioxidant compounds, breakdown or formation of new antioxidant compounds, and losses during HPP (Garcia-Mora et al., 2015; Mulla et al., 2022).

Overall, these results suggest that incorporating plant proteins, particularly faba bean protein 2, into meat products, combined with HPP processing, can effectively reduce lipid oxidation and improve product stability. The lower TBARS values observed in HBP during storage may be explained by two possible mechanisms. On the one hand, antioxidant compounds present in plant ingredients, including phenolics and bioactive peptides, may reduce oxidative development, potentially enhanced by pressure-induced structural modifications in legume proteins. On the other hand, carbohydrate fractions derived from plant-based ingredients may interfere with TBARS reaction and affect spectrophotometric readings. However, TBARS reflects only part of the overall lipid oxidation process. In the present study, the observed TBARS responses may have been influenced by the complex formulation composition, including differences in oxidation-related components derived from both meat and plant ingredients, as well as possible HPP-induced changes in oxidation pathways and enzyme activity. Therefore, these results should be interpreted with caution. Further studies are needed to better understand the differences in oxidation-related components between meat and HPM products.

3.8. Formulation grading

To comprehensively evaluate the impact of HPP and inclusion of specific plant-proteins and their combinations on the technological properties of patties, a formulation grading system was applied. This system integrates multiple quality factors (e.g., cooking loss, WHC, hardness, lipid oxidation at day 11, and redness of raw patties) to generate an overall performance score for each formulation. The grading provides a comparative overview of how each formulation and pressure level performs relative to the entire sample population. Two approaches were used, firstly an overall score, and secondly a closest match approach, to identify the closeness of the overall match to the properties of the 100% beef control, not subject to HPP.

Under the overall scoring system, (Fig 7 (a)), hybrid formulation F2 treated at 200 MPa achieved the highest total formulation grade score (2.96), followed by hybrid formulation F2 without HPP treatment (2.40), and hybrid formulation PWT at 200 MPa (2.27). In contrast, beef control treated at 500 MPa (-4.63) and hybrid formulation F1 at 500 MPa (-3.33) received the lowest scores, indicating the poorest overall technological performance. The low score of the beef control at 500 MPa was primarily attributed to higher cooking loss and TBARS values, and lower WHC, all of which comprise product quality. For hybrid formulation F1 at 500 MPa, its poor performance was mainly due to excessively soft texture and low redness values, which are undesirable in consumer perception of meat products (Grasso et al., 2022; Zhang et al., 2025). All HBP formulations, regardless of pressure level, scored higher for technological attributes than the beef control, probably because of the greatly improved performance in relation to particular technological attributes such as WHC and cooking loss, which are greatly enhanced in HBP. Moreover, for each formulation, except F1, treatment at 200 MPa consistently outperformed the non-HPP counterpart, indicating that moderate pressure levels may improve functional properties such as texture and oxidation stability. Conversely, 500 MPa treatments resulted in the lowest scores, likely due to protein degradation and structural damage induced by high pressure.

To identify the formulation most similar to untreated beef control, standardised Euclidean distance was calculated based on the same criteria and the results are presented in Fig 7 (b). Lower distance values indicate greater similarity to the beef control, whereas higher values reflect greater deviation under the technological attributes measured. The beef control subjected to 200 MPa (1.07) showed the smallest deviation from the untreated beef control, indicating minimal structural

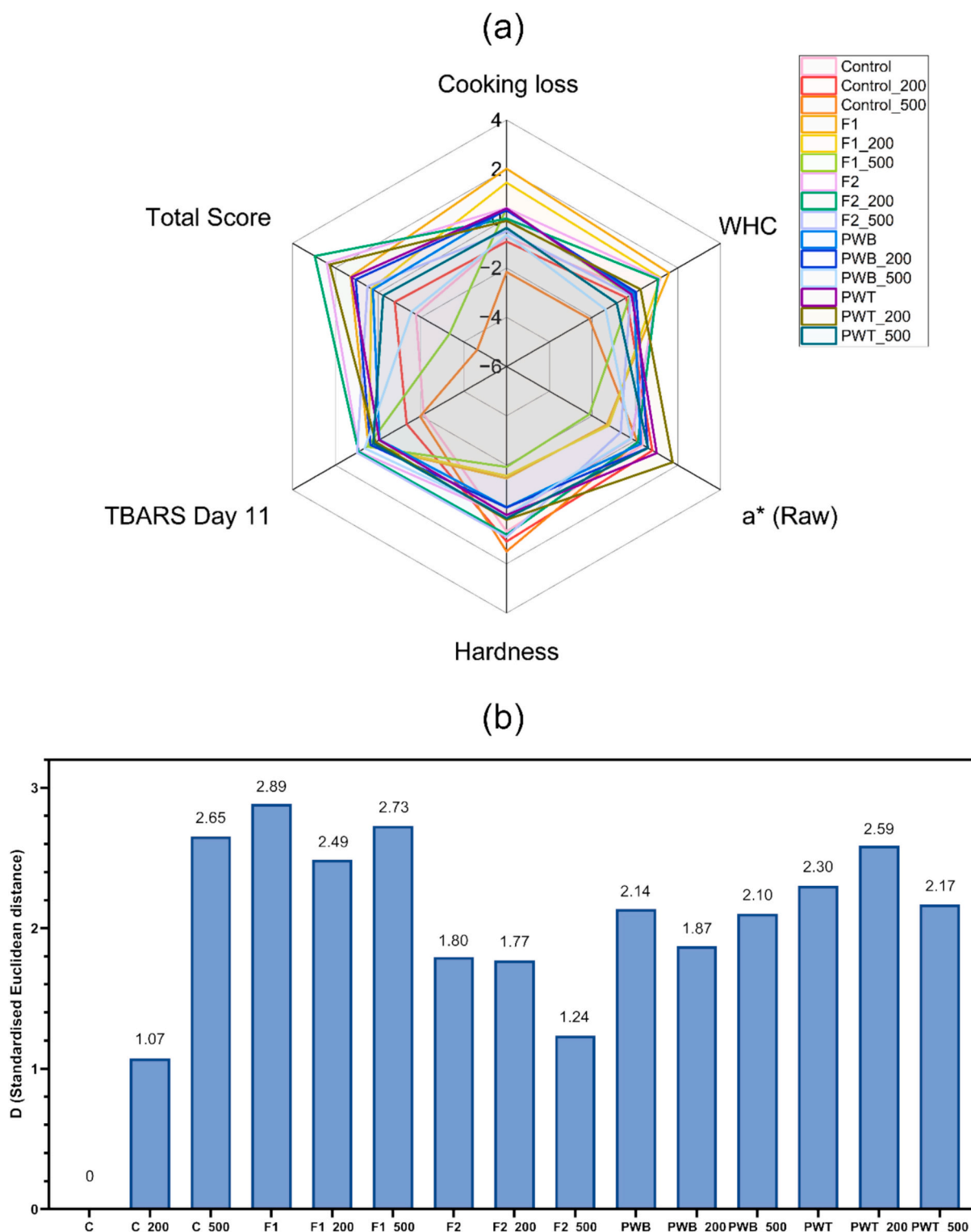


Fig 7. (a) Standardised scores of key physicochemical properties for the beef control and hybrid formulations under different HPP treatments. (b) Standardised Euclidean distance between the untreated beef control and other formulations, where lower distance values indicate greater overall similarity to the control.

and physicochemical changes under moderate pressure. Among the HBP formulations, F2 formulation (30% faba bean protein 2) treated at 500 MPa (1.24) demonstrated the closest similarity to the beef control, followed by F2 treated at 200 MPa (1.77), F2 without HPP treatment (1.80), and PWB (30% texturized pea and wheat protein) treated at 200 MPa (1.87). Overall, the Euclidean distance analysis indicates that hybrid F2 with 30% faba bean protein 2, most closely resembled the

technological profile of conventional beef patties, particularly under 500 MPa treatment. This suggests that incorporation of faba bean protein 2 may better preserve structural and functional properties compared to other plant-based ingredients used in this study. However, not all quality attributes hold the same importance from a commercial perspective. For retail consumers, visual appearance, colour stability, flavour, texture, and overall sensory acceptance are key determinants of

purchase and repeat consumption. In contrast, for food service, functional performance parameters such as WHC, cooking loss, texture, and consistency during reheating are often more critical. Therefore, a weighted grading model could be developed in future work to prioritise attributes according to the intended commercial application.

4. Conclusion

This study evaluated the effects of HPP and plant-based protein incorporation on the quality attributes of hybrid meat patties. The results showed that both HPP treatment and formulation type significantly influenced the quality characteristics of patties. Compared with the beef control, HBP generally exhibited improved moisture retention, softer texture, and lower TBARS values, which likely reflect the WHC and antioxidant and antioxidant potential of plant-derived compounds. Under non-HPP conditions, the addition of plant proteins generally increased L^* values, apart from formulation F1, while the effects on a^* and b^* values were formulation dependent. Moderate HPP treatment (200 MPa) enhanced textural properties and redness while maintaining acceptable WHC, whereas higher pressure (500 MPa) negatively affected WHC and redness due to excessive protein denaturation and pigment oxidation. Formulation grading results further identified hybrid formulation F2 at 200 MPa as achieving the highest overall technological performance. Regarding identifying a hybrid plant/meat product that most closely matched the properties of the conventional beef control, F2 treated at 500 MPa exhibited the closest similarity.

Overall, these findings indicate that the strategic integration of functional plant proteins with optimised HPP conditions represents a promising approach for developing HPM products with technological properties comparable to conventional meat. From an industrial perspective, the integration of functional plant proteins with moderate HPP treatment offers a promising approach for the HPM products that meet both sustainability goals and consumer expectations for meat-like quality. Future work should focus on optimising plant protein functionality and conducting sensory and shelf-life evaluations, including colour and microbiological analyses, to better assess consumer acceptance and product stability. In addition, the use of complementary lipid oxidation markers, such as peroxide values, would help clarify the underlying oxidation mechanisms in hybrid systems. Finally, the development of weighted grading models in the industry may help prioritise product attributes according to specific commercial applications.

CRediT authorship contribution statement

Zuo Song: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Joseph P. Kerry:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Mohammad Hassan Kamani:** Investigation, Formal analysis. **Ahmed Rady:** Writing – review & editing, Methodology, Investigation. **Eoin G. Murphy:** Writing – review & editing, Investigation, Formal analysis. **Ruth M. Hamill:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare no conflict of interest.

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None.

Data availability

Data is contained within the article.

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