Poultry Science®

Hybrid meat batter system: effects of plant proteins (pea, brown rice, faba bean) and concentrations (3−12%) on texture, microstructure, rheology, water binding, and color

Weilun Lin [*], and Shai Barbut [*,†,1]

[*]Department of Food Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada; and [†]Adaptation Physiology Group, Wageningen University, Netherlands

ABSTRACT A lean meat batter system was mixed with four plant proteins at 3, 6, 9, and 12% (w/w): pea protein A (**PA**), pea protein B (**PB**), brown rice protein (**BR**) and faba bean protein (**FB**). Texture profile analysis (**TPA**) revealed that increasing plant protein levels hardened the hybrid meat batters, with PA and PB leading to the hardest gels. TPA results were supported by micrographs, demonstrating that the two pea proteins formed large aggregates, contributing to a firmer hybrid meat gel. Dynamic rheology showed that the incorporation of plant proteins lowered the storage modulus (G') during the heating stage (20 to 72°C), yet the 6% PA treatment produced a final G' (after cooling) closest to the control (**CL**). Nuclear Magnetic Resonance (**NMR**) T_2 relaxometry also demonstrated that plant proteins reduced the water mobility in hybrid meat batters. Results were in line with the cooking loss, except for a higher cooking loss in the BR formulation compared to the CL. Color measurement showed that increasing plant protein levels led to darker and yellower meat batters; however, the effect on redness varied among treatments. Overall, the findings suggest that pea proteins have superior functionality and compatibility within a lean poultry meat protein system, compared to BR and FB tested here.

Key words: gel structure, hybrid meat, nuclear magnetic resonance, plant protein, texture

2024 Poultry Science 103:103822
https://doi.org/10.1016/j.psj.2024.103822

INTRODUCTION

Due to environmental concerns of conventional meat production and an increasing global demand for meat consumption, interest in plant-based meat analogues and alternative proteins have sprung up over the last decade. The surge of alternative protein industries has posed a challenge for the meat industry: meeting demand sustainably. As the plant-forward trend has plateaued over the past two years, current meat alternatives seem less convincing and encouraging to reduce meat consumption among meat-eaters. Intrinsic differences between plant and meat proteins have impeded the development of plant-based meat analogues that closely mimic muscle fiber structure and texture (Xiong, 2023). Such technological difficulties, as well as premium pricing and consumer perceptions of ultra-processed food, contradict the high expectations that came along with the plant-forward movement. For example, the iconic fast-food chain, McDonald's, has recently discontinued plant-based burgers in the U.S. market due to low demand (Lucas, 2022).

It seems unrealistic to expect a shift from a carnivorous or omnivorous diet to plant-based ones in the near future, especially considering cultural, societal, and sensorial attachments to meat. An 11,399-respondent survey in the United States revealed that 84% of former vegetarians and vegans went back to eating meat within less than one year (Asher et al., 2014). Therefore, it is more practical to reduce rather than eliminate meat consumption, especially given the growing popularity of the flexitarian diet, which allows for occasional consumption of meat. Hybrid meat products, in which meat proteins are partially substituted with non-meat proteins, is deemed an appropriate mean of reducing meat consumption without sacrificing the sensory and nutritional aspects of meat products (Grasso and Goksen, 2023). Recent studies showed positive consumer acceptability toward hybrid meat products (Tarrega et al., 2020; Profeta et al., 2020,2021; Grasso et al., 2022).

Plant proteins have long been used, in small amounts (2−3%), as meat extenders in reformed meat products to reduce cost and improve functionality (Barbut,

Received February 15, 2024.
Accepted May 2, 2024.

[1]Corresponding author: sbarbut@uoguelph.ca

2015). Most prior research on meat extenders has primarily focused on soybeans and their derivatives. The rising incidence of soybean-induced allergies has led to a growing interest in pulse and rice proteins within the alternative protein sector, particularly owing to their fewer reported intolerance and allergy cases (Boye et al., 2010; Amagliani et al., 2017). Previously, different types of pulse flours (typically 30% protein content) have been investigated for use in meat patties and sausages (Dzudie et al., 2002; Akwetey et al., 2012; Baugreet et al., 2016; Argel et al., 2020,2022; Chandler and McSweeney, 2022). In recent years, the promising functionalities of high-protein (70−90% protein content) meat extenders derived from pea and rice proteins have drawn great interest from researchers (Shoaib et al., 2018; Broucke et al., 2022; Revilla et al., 2022; Santos et al., 2022; Shen et al., 2022; Zhu et al., 2022). Nevertheless, few studies have investigated the interactions between plant and meat proteins within a lean meat system or investigated the optimal non-soy plant protein for the formulation of hybrid meat products. Therefore, this research aimed to identify the most suitable low-allergenic plant protein concentrate(s) for hybrid meat production and to expand existing knowledge of the cogelation between plant and meat proteins.

MATERIALS AND METHODS

Materials

Fresh, skinless and boneless chicken breast fillets (22.46% protein, determined by the Dumas method according to AOAC 992.15, using a N conversion factor of 6.25) were obtained from a local retailer (Costco Wholesale Corporation, Ontario, Canada). Visible fat and connective tissues were trimmed off, and the meat was cut into 2 × 2 cm pieces, that were minced in a bowl chopper (Schneidmeister SMK 40, Berlin, Germany) at the low-speed setting for 40 s. The meat was vacuum-packed in individual polyethylene bags (0.5 kg per bag) and kept frozen (−20°C) prior to use. Pea protein concentrate A (**PA**; 80% protein), brown rice protein concentrate (**BR**; 80% protein), and faba bean protein concentrate (**FB**; 60% protein) were supplied by Hela Spice Company, Ontario, Canada; pea protein concentrates B (**PB**; 80% protein) was obtained from Grand River Foods, Ontario, Canada.

Meat Batter Preparation

Frozen meat was thawed overnight (4°C) before use, and any remaining visible connective tissue was removed. Five different formulations were prepared in three separate trials. The control formulation (**CL**) contains 69.8% meat, 27.8% distilled water, and 2.4% salt (sodium chloride). The other four formulations consisted of a control formulation supplemented with increasing amounts of added plant proteins (based on adding 3, 6, 9, and 12% total protein w/w). A higher amount of faba bean protein was incorporated into its corresponding treatment to compensate for its lower protein content (60%) compared to the other three plant proteins (80%). Batters were prepared by hand mixing the meat and water for 1 min, then adding the plant protein and salt. The batters were then manually mixed for another min before resting at 4°C for 1 h to allow sufficient meat's salt soluble protein extraction. This was followed by 30 s of manual mixing (at approximately 60 rpm) and then stuffing into three 50-mL polyethylene test tubes (30 g each) and centrifuging (Fisher Scientific, Model 225, Pittsburgh, PA) for 30 s at the low-speed setting to remove any remaining small air bubbles.

Cooking and Cooking Loss

Test tubes were heated in a circulating water bath (Haake, SC 100, Newington) from 20°C to an internal temperature of 72°C, monitored by a thermocouple (Omega, Model HH23, Stamford). Once 72°C reached, the tubes were immediately cooled in cold water until reaching 30°C. The samples were then stored at 4°C overnight. Cooking loss was determined as percentage loss on the following day, after decanting the fluid and pat drying the meat cylinder with a paper towel.

Texture Profile Analysis

Chilled, cooked samples were brought to room temperature, and texture parameters were measured using six pucks (19 mm diameter and 10 mm height) taken from the core of cooked batters. The measurement of hardness, springiness, cohesiveness, gumminess, chewiness, and resilience parameters was performed by twice compressing pucks to a height of 5 mm, by a cylindrical flat probe (diameter: 100 mm) at a speed of 1.5 mm/s (for both compression and retrieval), using a texture analyzer (Texture Technologies Corp., Model: TA-XTPlusC, New York, NY) equipped with a 50 kg load cell (Barbut, 2023a).

Microstructure

Following Youssef and Barbut (2009), 3 mm-thick discs were cut from the center of cooked batter cores, fixed, embedded in parafin, sectioned, and stained with hematoxylin-eosin and Periodic acid−Schiff. Specimens were imaged using a light microscope (Model BX60, Olympus Optical Ltd., Tokyo, Japan) with a digital camera (Model DP71, Olympus Optical Ltd.).

Dynamic Rheology

Following a procedure by Saengsuk et al. (2024) with slight modifications, transitions of the meat batters containing 6% added plant proteins and the control were measured by an oscillating rheometer (Anton Paar, Model Physica MCR 301, Graz, Austria) using a 50 mm parallel plate geometry (with a 1.0 mm gap) at a shear strain of 0.2% and a frequency of 1.0 Hz. Amplitude and

frequency sweeps were initially performed on raw and cooked meat batters to ensure the 0.2% strain used was within the linear viscoelastic region. Samples were heated from 20 to 72°C, and then cooled down to 20°C at a rate of 2°C/min. Mineral oil was used to coat the rim of the meat sample to minimize moisture loss and drying. Storage modulus (**G'**) and loss modulus (**G''**) measurements were determined in triplicate.

Pulsed NMR T_2 Relaxometry

T_2 relaxation profiles of the raw and cooked meat batters containing 6% added plant proteins were determined by a 20 MHz (0.47 T) bench-top NMR spectrometer (Bruker Canada, Model: mq 20 series, Milton, ON, Canada) maintained at 5°C. The procedure used was adapted from (Gravelle et al., 2020). Free induction decay (**FID**) was collected from a Carr-Purcell-Meiboom-Gill spin echo pulse train employing 16 scans and a 10 s recycle delay between scans. The 90° and 180° pulse lengths were optimized to 9.40 μs and 18.46 μs, respectively, using a raw control meat sample. The 90 to 180° pulse separation τ was set to 1.2 ms. Raw samples were filled into disposable glass NMR tubes (height: 180 mm; diameter: 10 mm; wall thickness: 0.6 mm) to an approximate height of 10 mm and stored at 5°C. Initial FID signals, from raw samples, were collected. For a second set of FID signals, these samples were cooked in a 72°C water bath for 10 mins, then immediately cooled in cold water and kept at 5°C. The CONTIN algorithm (Bruker Corp.) was used to analyze the FID data to obtain T_2 relaxation profiles and peak areas. The relaxation times were calculated based on the peak position, and the proportion of water molecules at each relaxation time was determined from the area under the peak.

Color Evaluation

Three freshly cut samples (10 mm-thick cross-sections) from each cooked treatment were evaluated. A colorimeter (Konica-Minolta CR-400 Chroma Meter, Osaka, Japan) with a 2° viewing angle was used under the D65 illuminant setting to measure the lightness (**L***), redness (**a***), and yellowness (**b***), according to the Commission International de l'Eclairage system.

Statistical Analysis

The experiment followed a completely randomized design with three independent replications. ANOVA was performed with a Tukey's multiple comparison test to determine statistical differences between formulations ($P < 0.05$) using the GraphPad Prism 9.3.1 software.

RESULTS AND DISCUSSIONS

Cooking Loss

All plant proteins except for BR improved the water holding capacity (**WHC**) of the meat batters (**Figure 1**). The cooking loss of PA, PB, and FB treatments was significant lower compared to the CL, starting at an inclusion level of 3%. This agrees with Barbut (2023a), who reported a similar result when integrating 2% of pea and faba bean proteins into a lean turkey meat batter. In the current study, PA, PB, and FB treatments displayed descending trends in cooking loss when the inclusion level increased. Our findings are also consistent with Zhu et al. (2022), who reported that adding 0-9% pea protein isolate resulted in a gradual reduction in cooking loss in duck meat batters, and Vaisey et al. (1975), reporting that incorporating 10% of faba bean protein concentrate lowered cooking loss by about 50% in ground beef patties. The two pea proteins studied here exhibited the lowest cooking loss across all inclusion levels. On the other hand, BR showed inferior water holding compared to the other three plant proteins, especially at the 3 and 6% addition levels, where the cooking loss was approximately 70% higher compared to the CL. This can be explained by the generally high surface hydrophobicity and low solubility of rice

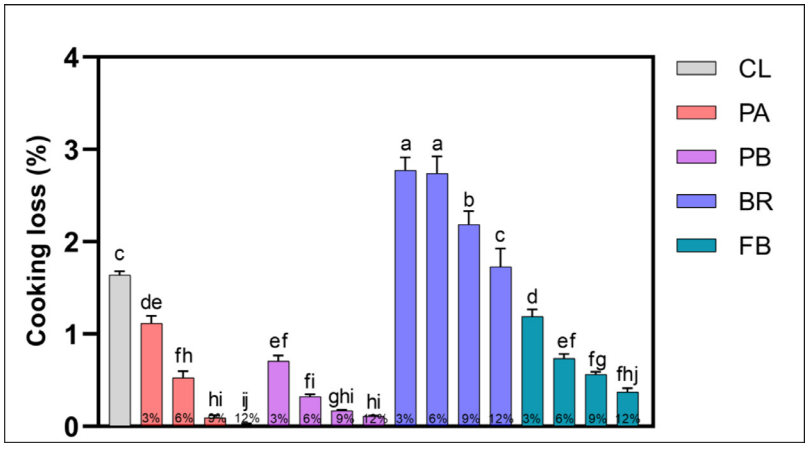

Figure 1. Cooking loss means (with standard error bars) of lean meat batters formulated with 0 to 12% plant proteins(indicated at the bottom of each bar), n = 9. CL: control; PA: pea protein A; PB: pea protein B; BR: brown rice protein; FB: faba bean protein. Treatments with a different superscript ($^{a\text{-}j}$) are statistically different ($P < 0.05$).

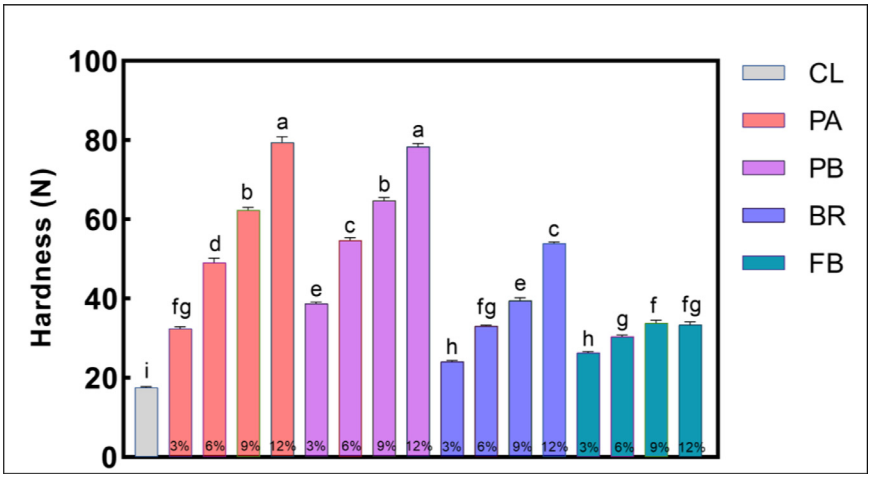

Figure 2. Hardness means (with standard error) of lean meat batters formulated with 0 to 12% plant proteins(indicated at the bottom of each bar), n = 18. CL: control; PA: pea protein A; PB: pea protein B; BR: brown rice protein; FB: faba bean protein. Treatments with a different superscript ($^{a\text{-}i}$) are statistically different ($P < 0.05$).

proteins (Felix et al., 2016; Amagliani et al., 2017). When the BR level was further raised, there was a significant decreasing trend in cooking loss. BR-treatment's lowest cooking loss was found at the 12% level, which was only comparable to the CL sample. In contrast, Shoaib et al. (2018) observed that 3% and 12% of added rice protein isolate led to a significantly lower cooking loss in chicken nuggets compared to pea protein isolate.

Texture Profile Analysis

Plant proteins significantly increased the hardness of the cooked meat batters (Figure 2). There were ascending trends in hardness at increasing plant protein levels. Overall, FB treatments exhibited the lowest hardness values across all inclusion levels; however, at 12%, the hardness was still twice as hard as the CL. The 12% PA and PB showed the highest hardness (>78 N), which increased more than 4-fold from that of the CL. These results align with previous studies (Baugreet et al., 2016; Broucke et al., 2022; Shen et al., 2022; Zhu et al., 2022; Barbut, 2023a), in which pea protein isolates (even at a 2% level) resulted in increased instrumental hardness of various cooked meat systems. On the contrary, Shoaib et al. (2018) reported that adding 0 to 12% pea protein and rice protein isolates had a minor (yet statistically significant) impact on the hardness of a chicken nugget system. It is speculated that the hard crust formed on chicken nuggets, during deep frying, helped alleviate the difference in hardness between samples with and without the added plant proteins. Gumminess and chewiness followed a similar pattern as hardness. The inclusion of PB, BR, and FB did not have a significant impact on springiness (Table 1), which is consistent with previous research conducted on plant proteins (Broucke et al., 2022; Shen et al., 2022; Zhu et al., 2022; Barbut, 2023a). However, PA significantly increased the springiness of the cooked hybrid meat batter across all concentrations. The aforementioned finding is supported by Barbut (2023a), who reported a notable increase in springiness with the inclusion of 2% pea protein isolate. In terms of cohesiveness, adding PA, PB, and FB (except for FB at 12%) increased the cohesiveness of the cooked batters, whereas BR showed no significant effect until inclusion level reached 12%. PA and PB had the greatest impacts on cohesiveness, with FB falling in between and BR having the least. This suggested that the two pea proteins interacted well with muscle proteins in such a hybrid meat system. Similar patterns were observed for resilience.

Microstructure

Micrographs of hybrid meat batters containing 0 to 12% added plant proteins are presented in Figure 3. PA formed rough-edged, uneven-shaped aggregates that trapped tiny air bubbles within their structure. The incorporation of higher levels of PA (9 and 12%) led to larger voids in the myofibrillar protein gel matrix. The entrapped voids and air cells can be attributed to the increased viscosity of the meat batter following the addition of PA, which competed with myofibrillar proteins for water. In contrast, Zhu et al. (2022) showed that the inclusion of the pea protein increased the density of the pea-duck meat batter by occupying voids within the matrix. The aggregates of PA and PB share some similarities, although the latter formed larger and denser aggregates that trapped fewer air bubbles than PA, especially at higher PB protein levels. BR, on the other hand, produced the smallest aggregates that were dispersed throughout the myofibrillar protein gel matrix. This can be attributed to BR's poor gelling ability, which also explains why BR had a lesser impact on hardness than the two pea proteins. Moreover, BR did not seem to bind well with the myofibrillar proteins, leading to small channels in the gel matrix. This is in agreement with Barbut (2023a). Channels were particularly noticeable at the 12% BR level. This finding aligns with a recent study by Santos et al. (2022), who observed large disruptions in the microstructure of a hybrid meat

Table 1. Texture profile analysis parameters (mean ±standard error) of cooked meat batters with 0 to 12% plant proteins. n = 18.

Treatment	Springiness (-)	Cohesiveness (-)	Gumminess (N)	Chewiness (N)	Resilience (-)
CL	0.34 ± 0.01^{bc}	0.24 ± 0.01^{g}	4.26 ± 0.08^{m}	1.44 ± 0.04^{i}	0.06 ± 0.01^{e}
3% PA	0.39 ± 0.01^{a}	0.34 ± 0.01^{cd}	11.08 ± 0.23^{h}	4.36 ± 0.14^{e}	0.10 ± 0.01^{c}
6% PA	0.39 ± 0.01^{a}	0.37 ± 0.01^{b}	18.32 ± 0.55^{e}	7.24 ± 0.32^{cd}	0.12 ± 0.01^{b}
9% PA	0.40 ± 0.01^{a}	0.40 ± 0.01^{a}	25.05 ± 0.46^{c}	9.96 ± 0.32^{b}	0.14 ± 0.01^{a}
12% PA	0.39 ± 0.01^{a}	0.41 ± 0.01^{a}	32.25 ± 0.78^{a}	12.69 ± 0.48^{a}	0.14 ± 0.01^{a}
3% PB	0.35 ± 0.01^{b}	0.33 ± 0.01^{d}	12.75 ± 0.20^{g}	4.49 ± 0.07^{e}	0.10 ± 0.01^{c}
6% PB	0.35 ± 0.01^{b}	0.35 ± 0.01^{cd}	19.10 ± 0.18^{e}	6.63 ± 0.09^{d}	0.12 ± 0.01^{b}
9% PB	0.34 ± 0.01^{bc}	0.36 ± 0.01^{bc}	23.13 ± 0.24^{d}	7.94 ± 0.09^{c}	0.12 ± 0.01^{b}
12% PB	0.34 ± 0.01^{bc}	0.36 ± 0.01^{bc}	28.34 ± 0.23^{b}	9.76 ± 0.10^{b}	0.12 ± 0.01^{b}
3% BR	0.34 ± 0.01^{bc}	0.24 ± 0.01^{g}	5.80 ± 0.09^{i}	1.97 ± 0.03^{hi}	0.06 ± 0.01^{e}
6% BR	0.34 ± 0.01^{bc}	0.25 ± 0.01^{g}	8.23 ± 0.12^{jk}	2.82 ± 0.05^{fg}	0.06 ± 0.01^{e}
9% BR	0.34 ± 0.01^{bc}	0.25 ± 0.01^{g}	10.03 ± 0.21^{hi}	3.43 ± 0.06^{f}	0.06 ± 0.01^{e}
12% BR	0.34 ± 0.01^{bc}	0.28 ± 0.01^{e}	14.88 ± 0.16^{f}	5.14 ± 0.06^{e}	0.08 ± 0.01^{d}
3% FB	0.33 ± 0.01^{bc}	0.27 ± 0.01^{ef}	7.18 ± 0.22^{kl}	2.41 ± 0.10^{gh}	0.08 ± 0.01^{d}
6% FB	0.33 ± 0.01^{bc}	0.27 ± 0.01^{ef}	8.20 ± 0.18^{jk}	2.70 ± 0.08^{fgh}	0.08 ± 0.01^{d}
9% FB	0.32 ± 0.01^{c}	0.27 ± 0.01^{ef}	9.13 ± 0.29^{ij}	2.95 ± 0.12^{fg}	0.07 ± 0.01^{de}
12% FB	0.32 ± 0.01^{c}	0.26 ± 0.01^{fg}	8.61 ± 0.38^{ijk}	2.78 ± 0.15^{fgh}	0.07 ± 0.01^{de}

CL: control; PA: pea protein A; PB: pea protein B; BR: brown rice protein; FB: faba bean protein. Significantly different values ($P < 0.05$) within each column are denoted with different superscripts ($^{a\text{-}m}$).

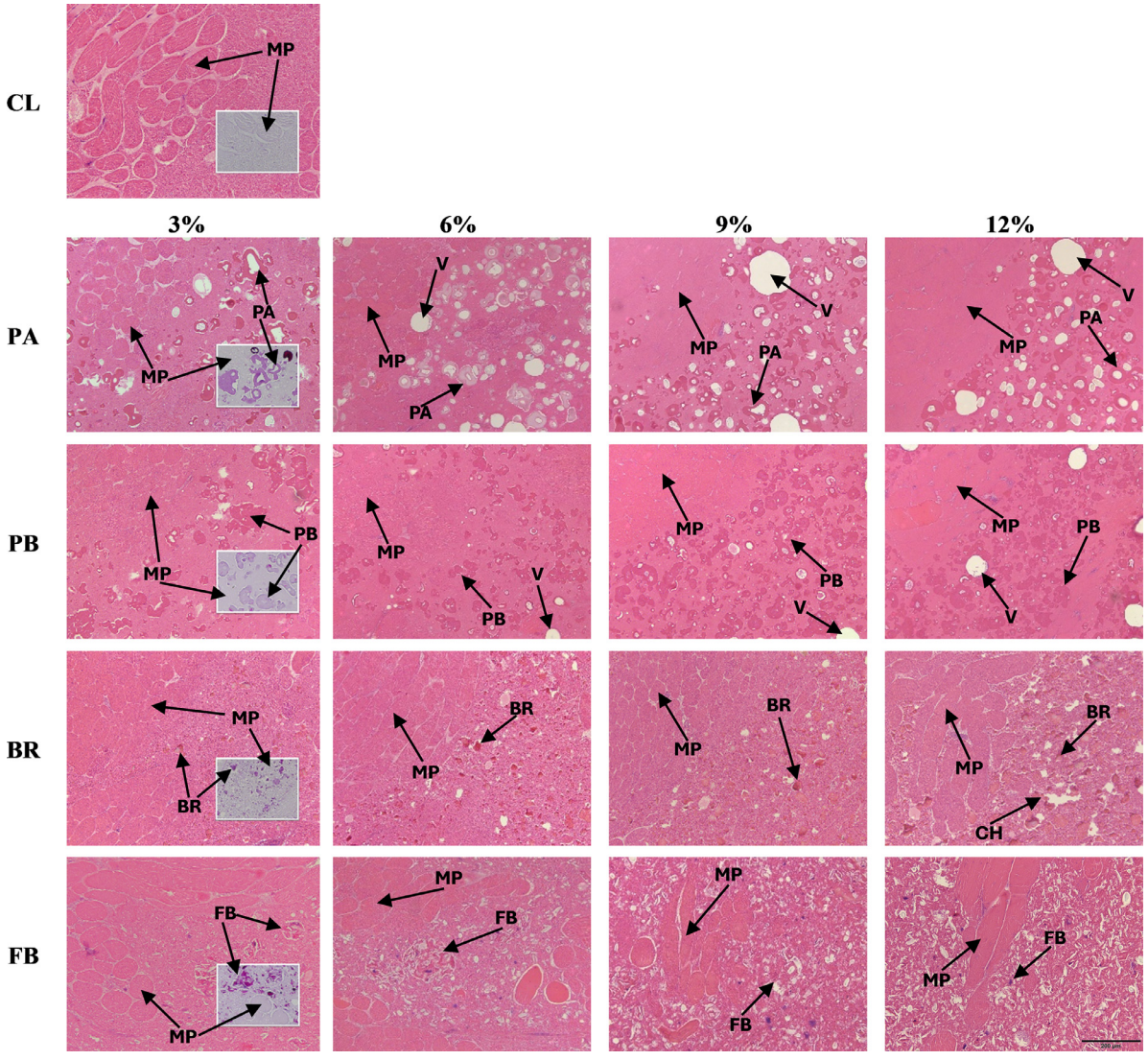

Figure 3. Light micrographs of hematoxylin-eosin-stained cooked hybrid meat batter with 0 to 12% plant proteins incorporation. Periodic acid −Schiff stained sections are embedded into hematoxylin-eosin images at 3% plant protein. CL: control; PA: pea protein A; PB: pea protein B; BR: brown rice protein; FB: faba bean protein; CH: channel; V: void; MP: meat protein. Bar = 200μm.

emulsion system with 50% hydrated rice protein. The FB treatment showed small, dispersed particles (as in the case of BR) but also showed some of the largest yet not so well-connected aggregates of the four plant proteins evaluated. It appears that because of its higher carbohydrate content, FB exhibited strong binding to meat proteins. FB's higher carbohydrate content and larger aggregate size could be why it had the least pronounced impact on enhancing the hardness of the hybrid meat batters compared to the other three proteins. This observation is consistent with a study by (Barbut, 2023b), suggesting that the presence of breadcrumbs in a turkey meat batter system, particularly when occupying relatively larger areas, contributes to a softer texture in comparison to breadcrumbs occupying smaller areas.

Dynamic Rheology

The storage modulus (G') exhibited a small gradual decrease during the initial heating phase (Figure 4). For the PA and PB samples, the decrease occurred between 20 to 47°C. For the BR and FB samples, the G' values decreased from 20 to 54°C. However, the CL exhibited a gradual continuous increase in G' values from 20 to 51°C. This means that adding plant proteins interfered with the meat protein gelation and delayed the rise of the meat batter's G' value. Also, these plant proteins formed aggregates within the hybrid meat batters (Figure 3), potentially acting as particle fillers, affecting the development of G' values compared to the CL. Above 54°C, the G' values of the PA, PB, and CL treatments started to rise rapidly and peaked at 68°C. This profound increase, seen in the CL and the other treatments, is typical and can be explained by the denaturation of myofibrillar proteins (Barbut, 2005; Zhu et al., 2022). Overall, the maximum G' values observed here indicate the formation of a stable protein gel matrix (Broucke et al., 2022; Zhu et al., 2022).

During the cooling phase, all treatments showed a further increase in G'. The CL had the highest final G' value, followed by PA, PB, BR, and FB. Unlike the TPA hardness results, the final G' values of all 4 plant protein treatments were lower than the CL, but their rank within protein treatments followed a similar pattern to that in TPA hardness values. This observed lower final G' value in hybrid meat batter is consistent with Broucke et al. (2022), who reported a comparable outcome when adding pea protein isolate to emulsified pork sausages. Moreover, Santos et al. (2022) reported a similar diminishing effect in the final G' values when 50% of beef in a meat emulsion system was replaced with soybean, pea, rice, faba bean, and sunflower proteins. Overall, the CL showed a higher G' value than the hybrid ones. Despite that the CL had a lower TPA hardness value compared to those of the plant protein treatments (Figure 2), the ranking of G' (small deformation testing) does not always correlate with that of large deformation instrumental texture analysis of protein gels (Montejano et al., 1984; Sadeghi-Mehr et al., 2018). It should also be pointed out that the denaturation temperature of the plant proteins examined in this study is reported to range from 70 to 97°C (Liang and Tang, 2013; Zhang et al., 2021; Ferawati et al., 2021; Kelemen et al., 2022). However, most fully cooked / ready-to-eat commercial sausages are heated to 72°C (Barbut, 2015). Therefore, these plant proteins were unlikely to undergo complete unfolding and potentially maximum gelation.

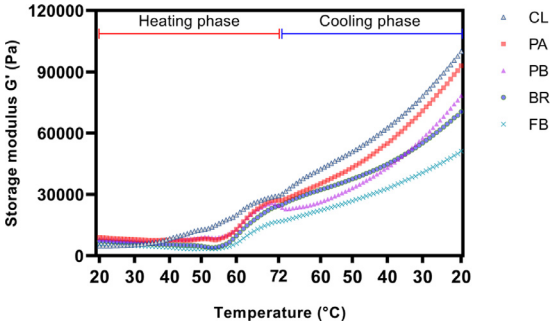

Figure 4. Storage modulus G' of cooked (20−72°C at 2°C / min) and cooled (72−20°C at 2°C / min) hybrid meat batters containing 6% plant proteins. CL: control; PA: pea protein A; PB: pea protein B; BR: brown rice protein; FB: faba bean protein. Each data point represents an average of 3 runs.

The effect of these plant proteins on the loss modulus (G'') (Figure S1) was similar to that on G', with minor exceptions. G" of the CL showed a steady increase from 20 to 72°C. Except for the PB treatment, all plant protein treatments showed an initial decrease in G'' from 20 to 54°C. The G" of the PB treatment initially dropped from 20 to 34°C, subsequently ascended from 34 to 48°C, and then decreased from 48 to 54°C. The observed change in the G'' of PB can be interpreted as an indication of the disruption and subsequent reestablishment of the gel structure. In all cases, G'' values further increased from 54 to 68°C, reaching their maximum in the heating phase (20−72°C), and subsequently continued to rise during the cooling phase (72−20°C). The final G'' value of PA was similar to that of CL, and the final G'' values of the other three plant proteins were lower than PA and CL.

Pulsed NMR T_2 Relaxometry

T_2 relaxometry can be used to provide insight into the mobility of water molecules in a gel matrix. In the muscle, three distinct peaks of T_2 relaxation time are typically observed: T_{2B}, T_{21}, and T_{22}, referring to water tightly bound to protein molecules, water residing in the protein matrix, and free water in the extra myofibrillar space, respectively (Bertram et al., 2001). The present study placed emphasis on the interpretation of T_{21} and T_{22} since they provide valuable information about the water-binding of hybrid meat matrices and the water existing outside of these matrices. Table 2 summarizes the T_2 relaxation times in the raw and cooked states. Prior to gelation, only the T_{2B} and T_{21} components were present, with the CL sample having the longest T_{21} time, followed by PA, PB, BR, and FB. The addition of plant proteins restricted intracellular water by

Table 2. Mean T2 relaxation times (± standard error) of raw and cooked meat batters containing 6% plant proteins.

Treatment	T_{2B} (ms)	T_{21} (ms)	T_{22} (ms)
CL (raw)	5.4 ± 0.96	80.1 ± 0.10	-
PA (raw)	9.0 ± 0.70	56.0 ± 0.30	-
PB (raw)	10.6 ± 0.43	53.2 ± 0.25	-
BR (raw)	7.2 ± 0.7	50.6 ± 0.2	-
FB (raw)	5.2 ± 0.65	46.1 ± 0.12	-
CL (cooked)	9.2 ± 0.86	60.5 ± 0.22	1013.3 ± 20.00[a]
PA (cooked)	8.1 ± 0.79	47.0 ± 0.25	423.7 ± 47.83
PB (cooked)	10.7 ± 0.63	44.6 ± 0.36	383.4 ± 33.81
BR (cooked)	9.2 ± 0.86	43.7 ±0.32	345.0 ± 14.14
FB (cooked)	9.1 ± 0.98	40.6 ± 0.15	145.6 ± 32.46

CL: control; PA: pea protein A; PB: pea protein B; BR: brown rice protein; FB: faba bean protein. The symbol (-) denotes no relaxation peak was detected for the sample. a denotes an additional, minor T_{22} peak was found at 266.8 ± 33.65 ms.

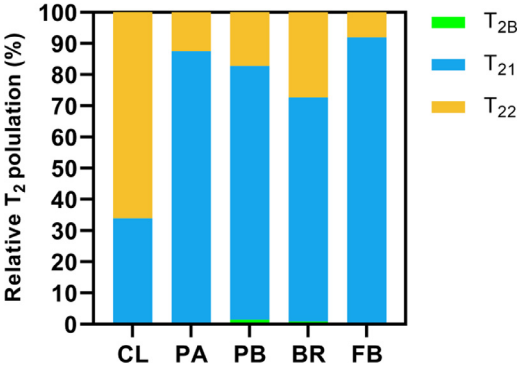

Figure 5. Relative proportion of T_2 populations (T_{2B}, T_{21}, and T_{22}) of cooked meat batters containing 6% plant proteins. CL: control; PA: pea protein A; PB: pea protein B; BR: brown rice protein; FB: faba bean protein.

competing with myofibrillar proteins for water. Post-cooking T_{21} times decreased in all cases and followed the same ranking order as observed in the raw state.

Typically, a protein gel that has a low cooking loss is expected to have a shorter T_{21} time than a protein gel that has a higher cooking loss. As discussed previously, the BR led to the highest cooking loss among all treatments at the 6% inclusion level. It was expected that the BR treatment would have a longer T_{21} time than the other treatments; however, this was not the case here. The BR treatment showed the second-shortest T_{21} time (Table 2). This discrepancy could be explained by its microstructure. The BR treatments had smaller voids than the PA and PB treatments (Figure 3). These smaller voids may have restricted the movement of water molecules within them more efficiently than larger voids, resulting in a shorter-than-expected T_{21} value for the BR treatment. This is supported by (Bertram et al., 2002), who showed that the T_{21} value was shorter in contracted muscles (decreased volume) compared to stretched (increased volume) muscles.

The decline in T_{21} values was accompanied by the appearance of T_{22} peaks, indicating the migration of a portion of the T_{21} water molecule population towards the extra myofibrillar region (T_{22}). This observation is consistent with previous studies (Shao et al., 2016; Gravelle et al., 2016,2020; Li et al., 2018; Cheng et al., 2020). The pattern of T_{22} times was similar to that of T_{2B} and T_{21}, with the exception of two T_{22} peaks in the CL. The lower T_{22} times in hybrid meat samples, compared to the CL, imply that plant proteins retained more water in the protein matrix.

A short post-gelation T_{22} time is usually correlated with a low cooking loss (Santos et al., 2022). However, this is not always the case. In the present investigation, BR treatments resulted in the highest cooking loss at all inclusion levels (Figure 1) but exhibited the second-shortest T_{22} time. A combination of two effects can possibly explain this unusual phenomenon. First, BR treatment resulted in a higher T_{22} population (Figure 5) compared to other plant proteins, suggesting that the BR sample released more fluid during the cooking process. Second, BR did not favour forming aggregates or much interaction with the meat gel matrix under the conditions applied here; therefore, we can speculate that a decent proportion of the BR protein did not integrate into the myofibrillar protein matrix but became suspended within the extra myofibrillar water. Anderssen and McCarney (2022) suggested that T_{22} times in meat can be impacted by the interaction of water with the protein dissolved in the sarcoplasm upon the damage of muscle cells. Hence, it can be that the BR protein, found in the extra myofibrillar space, interfered with the free water's relaxation time via a volume-sinks effect (Anderssen and McCarney, 2022).

Color Evaluation

The incorporation of plant proteins significantly diminished the lightness (L*) but increased the yellowness (b*) values of the cooked meat batters (Table 3). There were descending trends in L* values and ascending trends in b* values with increasing plant protein levels. At any

Table 3. Color parameters (L*=lightness; a*=redness; b*=yellowness) (mean ± standard error) of cooked meat batters formulated with 0 to 12% plant proteins, n = 9.

Treatment	L*	a*	b*
CL	81.61 ± 0.06^{a}	1.28 ± 0.02^{h}	12.18 ± 0.05^{j}
3% PA	78.52 ± 0.09^{c}	2.62 ± 0.02^{c}	13.01 ± 0.06^{i}
6% PA	75.94 ± 0.09^{f}	2.52 ± 0.02^{cd}	14.53 ± 0.09^{h}
9% PA	73.67 ± 0.12^{i}	2.35 ± 0.02^{e}	15.68 ± 0.17^{fg}
12% PA	71.36 ± 0.08^{j}	2.42 ± 0.03^{de}	17.13 ± 0.16^{e}
3% PB	79.76 ± 0.13^{b}	1.23 ± 0.02^{h}	16.34 ± 0.08^{f}
6% PB	78.55 ± 0.15^{c}	1.21 ± 0.02^{h}	19.61 ± 0.24^{c}
9% PB	76.92 ± 0.12^{e}	1.49 ± 0.04^{g}	22.01 ± 0.33^{b}
12% PB	76.07 ± 0.13^{f}	1.95 ± 0.02^{f}	23.90 ± 0.39^{s}
3% BR	79.28 ± 0.07^{b}	2.36 ± 0.01^{e}	16.06 ± 0.09^{f}
6% BR	77.66 ± 0.04^{d}	2.78 ± 0.02^{b}	18.36 ± 0.11^{d}
9% BR	75.75 ± 0.07^{fg}	3.08 ± 0.03^{a}	19.90 ± 0.08^{c}
12% BR	75.27 ± 0.07^{g}	3.12 ± 0.02^{a}	20.33 ± 0.12^{c}
3% FB	79.61 ± 0.12^{b}	0.71 ± 0.03^{i}	15.28 ± 0.05^{gh}
6% FB	77.91 ± 0.14^{d}	0.39 ± 0.02^{j}	17.72 ± 0.05^{de}
9% FB	76.27 ± 0.05^{f}	0.21 ± 0.02^{k}	20.00 ± 0.06^{c}
12% FB	74.53 ± 0.10^{h}	0.19 ± 0.02^{k}	21.55 ± 0.10^{b}

CL: control; PA: pea protein A; PB: pea protein B; BR: brown rice protein; FB: faba bean protein. Values, within a column, followed by a different superscript ($^{a\text{-}k}$) are statistically different ($P < 0.05$).

inclusion level, PA showed the lowest L* values (71.36to 78.52). This was due to the inherently darker color of the PA powder compared to other protein powders (Table S1). The plant proteins had varying effects on the redness (a*) of cooked hybrid meat formulations. PA treatments had a* values that were approximately twice that of the CL. The addition of BR also significantly raised the a* value compared to the CL, but the increase was less than that of PA at 3%. As the inclusion level increased, BR displayed greater a* values than PA. PB exhibited a notable increase in a* values relative to the CL only at 9% and above. FB, on the other hand, substantially reduced the a* value at all addition levels. The b* value exhibited significant increases compared to the CL in all cases. PA showed a lower b* value than the other three proteins at all inclusion levels. Incorporating PB into the meat batter produced the highest b* value, which was twice as high as the CL.

CONCLUSIONS

PA, PB, and FB reduced the cooking loss of the CL. The inclusion of 3 and 6% BR increased the cooking loss over the CL, however, this effect diminished as the protein level increased. As for texture, in all cases, increasing plant protein levels (3-12%) led to higher hardness values of the cooked products, particularly in PA and PB treatments, which were also more cohesive than the rest of the treatments. Micrographs showed that PA and PB formed larger protein aggregates compared to BR, which explains the firm texture of the pea protein treatments. FB formed the largest yet sparse aggregates but made the hybrid meat batter softer than most products, due to FB's higher carbohydrate content compared to the other plant proteins. Dynamic rheology showed that the CL had more resistance to small deformations and resulted in a higher G' than the plant protein treatments. However, it is worth noting that the PA treatment had a final G' value close to that of the CL. Pulsed NMR T_2 relaxometry indicated that adding plant proteins effectively confined the water (T_{21}) inside the hybrid meat matrix, resulting in a smaller percentage of free water in comparison to the CL. In terms of color, higher plant protein levels reduced the L* value and raised the b* value of the hybrid meat batter, while this effect varied for the a* value. Overall, the two pea proteins evaluated here produced robust and coherent composite systems when blended with meat proteins, making them more suitable for application in hybrid meat products.

ACKNOWLEDGMENTS

This study is funded by the Ontario Ministry of Agriculture, Food, and Rural Affairs (Grant No. 054855). The authors would like to express their gratitude to the Ontario Ministry of Agriculture, Food, and Rural Affairs for its continuous support, and to Dr. Dérick Rousseau's laboratory for generously allowing us to use their Nuclear Magnetic Resonance (NMR) equipment.

DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2024.103822.

REFERENCES

Akwetey, W. Y., W. O. Ellis, and I. N. Oduro. 2012. Using whole cowpea flour (WCPF) in frankfurter-type sausages. J. Anim. Prod. Adv. 2:450–455. Accessed Oct. 2023 www.grjournals.com verified 25 October 2022.

Amagliani, L., J. O'Regan, A. L. Kelly, and J. A. O'Mahony. 2017. The composition, extraction, functionality and applications of rice proteins: a review. Trends Food Sci. Technol. 64:1–12.

Anderssen, K. E., and E. R. McCarney. 2022. Mechanisms of transverse relaxation of water in muscle tissue. Food Control 132:108373.

Argel, N. S., G. Lorenzo, R. Domínguez, M. J. Fraqueza, J. Fernández-López, M. E. Sosa, P. Cesar, B. Campagnol, J. M. Lorenzo, and S. C. Andrés. 2022. Hybrid meat products: Incorporation of white bean flour in lean pork burgers. Appl. Sci. 2022 12:7571. Accessed Oct. 2023 https://www.mdpi.com/2076-3417/12/15/7571/htm verified 20 August 2022.

Argel, N. S., N. Ranalli, A. N. Califano, and S. C. Andrés. 2020. Influence of partial pork meat replacement by pulse flour on physicochemical and sensory characteristics of low-fat burgers. J. Sci. Food Agric. 100:3932–3941. Accessed Oct. 2023 https://onlinelibrary-wiley-com.subzero.lib.uoguelph.ca/doi/full/10.1002/jsfa.10436 verified 24 May 2022.

Asher, K., C. Green, H. Gutbrod, M. Jewell, G. Hale, and B. Bastian. 2014. Study of current and former vegetarians and vegans: initial findings. Faunalytics4. Accessed Oct. 2023 https://faunalytics.org/wp-content/uploads/2015/06/Faunalytics_Current-Former-Vegetarians_Full-Report.pdf verified 14 October 2022.

Barbut, S. 2005. Effects of chemical acidification and microbial fermentation on the rheological properties of meat products. Meat Sci. 71:397–401.

Barbut, S. 2015. Principles of meat processing. The Science of Poultry and Meat Processing. University of Guelph, Guelph, Ontario, Canada. Accessed Oct. 2023 www.poultryandmeatprocessing.com.

Barbut, S. 2023a. Effects of plant and dairy proteins on the texture and microstructure of lean turkey meat batters. Poult. Sci. 102. Available at https://pubmed.ncbi.nlm.nih.gov/37011466/ verified 18 August 2023. Accessed Oct. 2023.

Barbut, S. 2023b. Research note: producing lean poultry meat composite gels with different carbohydrate-based breadcrumbs. Poult. Sci. 102:102914.

Baugreet, S., J. P. Kerry, C. Botineştean, P. Allen, and R. M. Hamill. 2016. Development of novel fortified beef patties with added functional protein ingredients for the elderly. Meat Sci 122:40–47.

Bertram, H. C., A. H. Karlsson, M. Rasmussen, O. D. Pedersen, S. Dønstrup, and H. J. Andersen. 2001. Origin of multiexponential T_2 relaxation in muscle myowater. J Agric. Food Chem. 49:3092–3100. Accessed Oct. 2023 https://pubs.acs.org/doi/full/10.1021/jf001402t verified 17 September 2023.

Bertram, H. C., P. P. Purslow, and H. J. Andersen. 2002. Relationship between meat structure, water mobility, and distribution: a low-field nuclear magnetic resonance study. J. Agric. Food Chem. 50:824–829. Accessed Oct. 2023 https://pubmed.ncbi.nlm.nih.gov/11829651/ verified 3 October 2023.

Boye, J., F. Zare, and A. Pletch. 2010. Pulse proteins: Processing, characterization, functional properties and applications in food and feed. Food Res. Int. 43:414–431.

Broucke, K., C. Van Poucke, B. Duquenne, B. De Witte, M.-C. Baune, V. Lammers, N. Terjung, S. Ebert, M. Gibis, J. Weiss, and G. Van Royen. 2022. Ability of (extruded) pea protein products to partially replace pork meat in emulsified cooked sausages. Innovat. Food Sci. Emerg. Technol. 78:102992.

Chandler, S. L., and M. B. McSweeney. 2022. Characterizing the properties of hybrid meat burgers made with pulses and chicken. Int. J. Gastron. Food Sci. 27:100492.

Cheng, S., X. Wang, H. Yang, R. Lin, H. Wang, and M. Tan. 2020. Characterization of moisture migration of beef during refrigeration storage by low-field NMR and its relationship to beef quality. J. Sci. Food Agric. 100:1940–1948. Accessed Oct. 2023 https://onlinelibrary-wiley-com.subzero.lib.uoguelph.ca/doi/full/10.1002/jsfa.10206 verified 7 August 2023.

Dzudie, T., J. Scher, and J. Hardy. 2002. Common bean flour as an extender in beef sausages. J. Food Eng. 52:143–147.

Felix, M., A. Romero, and A. Guerrero. 2016. Development and evaluation of rheological and bioactive properties of rice protein-based gels. J. Cereal Sci. 72:91–100.

Ferawati, F., I. Zahari, M. Barman, M. Hefni, C. Ahlström, C. Witthöft, and K. Östbring. 2021. High-moisture meat analogues produced from yellow pea and faba fean protein isolates/concentrate: effect of raw material composition and extrusion parameters on texture properties. Foods 2021, Vol. 10:843. Accessed Oct. 2023 https://www.mdpi.com/2304-8158/10/4/843/htm verified 4 September 2023.

Grasso, S., D. Asioli, and R. Smith. 2022. Consumer co-creation of hybrid meat products: a cross-country European survey. Food Qual. Prefer. 100:104586.

Grasso, S., and G. Goksen. 2023. The best of both worlds? Challenges and opportunities in the development of hybrid meat products from the last 3 years. LWT 173:114235.

Gravelle, A. J., A. G. Marangoni, and S. Barbut. 2016. Insight into the mechanism of myofibrillar protein gel stability: Influencing texture and microstructure using a model hydrophilic filler. Food Hydrocoll 60:415–424.

Gravelle, A. J., A. G. Marangoni, and S. Barbut. 2020. Modulating water mobility in comminuted meat protein gels using model hydrophilic filler particles. LWT 129:109376.

Kelemen, V., A. Pichler, I. Ivić, I. Buljeta, J. Šimunović, and M. Kopjar. 2022. Brown rice proteins as delivery system of phenolic and volatile compounds of raspberry juice. Int. J. Food Sci. Technol. 57:1866–1874. Accessed Oct. 2023 https://onlinelibrary.wiley.com/doi/full/10.1111/ijfs.15023 verified 4 September 2023.

Li, X., X. Wei, H. Wang, C. hui Zhang, and W. Mehmood. 2018. Relationship between protein denaturation and water holding capacity of pork during postmortem ageing. Food Biophys 13:18–24. Accessed Oct. 2023 https://link.springer.com/article/10.1007/s11483-017-9507-2 verified 7 August 2023.

Liang, H. N., and C. H. Tang. 2013. pH-dependent emulsifying properties of pea [Pisum sativum (L.)] proteins. Food Hydrocoll 33:309–319.

Lucas, A. 2022. Beyond Meat Stock Falls After Conclusion of McDonald's McPlant Test. Consumer News and Business Channel. Accessed Oct. 2023 https://www.cnbc.com/2022/07/28/beyond-meat-stock-falls-after-conclusion-of-mcdonalds-mcplant-test.html verified 28 February 2023.

Montejano, J. G., D. D. Hamann, H. Fl Ball, and T. C. Lanier. 1984. Thermally induced gelation of native and modified egg white-Rheological changes during processing; final strengths and microstructures.

Profeta, A., M.-C. Baune, S. Smetana, S. Bornkessel, K. Broucke, G. Van Royen, U. Enneking, J. Weiss, V. Heinz, S. Hieke, and N. Terjung. 2021. Preferences of German consumers for meat products blended with plant-based proteins. Sustainability 13:650. Accessed Oct. 2023 https://www.mdpi.com/2071-1050/13/2/650.

Profeta, A., M. C. Baune, S. Smetana, K. Broucke, G. Van Royen, J. Weiss, V. Heinz, and N. Terjung. 2020. Discrete choice analysis of consumer preferences for meathybrids—findings from Germany and Belgium. Foods 10:71. Accessed Oct. 2023 https://www.mdpi.com/2304-8158/10/1/71/htm verified 24 May 2022.

Revilla, I., S. Santos, M. Hernández-Jiménez, and A. M. Vivar-Quintana. 2022. The effects of the progressive replacement of meat with texturized pea protein in low-fat frankfurters made with olive oil. Foods 11:923. Accessed Oct. 2023 https://www.mdpi.com/2304-8158/11/7/923/htm verified 26 June 2022.

Sadeghi-Mehr, A., P. Raudsepp, D. A. Brüggemann, R. Lautenschlaeger, and S. Drusch. 2018. Dynamic rheology, microstructure and texture properties of model porcine meat batter as affected by different cold-set binding systems. Food Hydrocoll 77:937–944.

Saengsuk, N., S. Barbut, and N. Laohakunjit. 2024. Texture modification of easily chewable pork meat batter for masticatory dysfunction people: effects and interactions of bromelain, κ-carrageenan, and plant protein hydrolysates. Int. J. Food Sci. Technol 59:197–207.

Santos, M., dosd. da Rocha D.A.V.F., O. D. Bernardinelli, F. D. Oliveira Júnior, D. G. de Sousa, E. Sabadini, R. L. da Cunha, M. A. Trindade, and M. A. R. Pollonio. 2022. Understanding the performance of plant protein concentrates as partial meat substitutes in hybrid meat emulsions. Foods 11:3311. Accessed Oct. 2023 https://www.mdpi.com/2304-8158/11/21/3311/htm verified 8 November 2022.

Shao, J. H., Y. M. Deng, N. Jia, R. R. Li, J. X. Cao, D. Y. Liu, and J. R. Li. 2016. Low-field NMR determination of water distribution in meat batters with NaCl and polyphosphate addition. Food Chem. 200:308–314.

Shen, Y., S. Hong, Z. Du, M. Chao, T. O'Quinn, and Y. Li. 2022. Effect of adding modified pea protein as functional extender on the physical and sensory properties of beef patties. LWT 154:112774.

Shoaib, A., A. Sahar, A. Sameen, A. Saleem, and A. T. Tahir. 2018. Use of pea and rice protein isolates as source of meat extenders in the development of chicken nuggets. J Food Process Preserv. 42:13763. Accessed Oct. 2023 https://onlinelibrary.wiley.com/doi/full/10.1111/jfpp.13763 verified 23 June 2022.

Tarrega, A., A. Rizo, A. Murciano, L. Laguna, and S. Fiszman. 2020. Are mixed meat and vegetable protein products good alternatives for reducing meat consumption? A case study with burgers. Curr. Res. Food Sci. 3:30–40.

Vaisey, M., L. Tassos, B. E. McDonald, and C. G. Youngs. 1975. Performance of fababean and field pea protein concentrates as ground beef extenders. Can. Inst. Food Sci. Technol. J. 8:74–78.

Xiong, Y. L. 2023. Meat and meat alternatives: where is the gap in scientific knowledge and technology? Ital. J. Anim. Sci. 22:482–496. Accessed Oct. 2023 https://www.tandfonline.com/action/journalInformation?journalCode=tjas20 verified 14 August 2023.

Youssef, M. K., and S. Barbut. 2009. Effects of protein level and fat/oil on emulsion stability, texture, microstructure and color of meat batters. Meat Sci. 82:228–233.

Zhang, S., W. Huang, E. Feizollahi, M. S. Roopesh, and L. Chen. 2021. Improvement of pea protein gelation at reduced temperature by atmospheric cold plasma and the gelling mechanism study. Innovat. Food Sci. Emerging Technol. 67:102567.

Zhu, X., B. Tan, K. Li, S. Liu, Y. Gu, T. Xia, Y. Bai, P. Wang, and R. Wang. 2022. The impacts of different pea protein isolate levels on functional, instrumental and textural quality parameters of duck meat batters. Foods 11:1620. Accessed Oct. 2023 https://www.mdpi.com/2304-8158/11/11/1620/htm verified 26 June 2022.