Effect of high-pressure processing in improving the quality of phosphate-reduced Irish breakfast sausages formulated with ultrasound-treated phosphate alternatives

Karthikeyan Palanisamy Thangavelu[1, 2]*, Brijesh Tiwari[1], Joseph P Kerry[2], Carlos Álvarez[1]

[1]Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland.

[2]School of Food and Nutritional Sciences, University College Cork, Cork, Ireland.

*Corresponding author email: carlos.alvarez@teagasc.ie

Abstract

This work examined the effects of High-pressure processing (HPP) treatment on pork meat subsequently used to generate three phosphate-reduced sausage formulations (1-3) containing ultrasound (US) treated apple pomace (AP) and coffee silverskin (CSS) ingredients as phosphate replacers and compared against control (traditional) sausage formulations. Results showed that HPP and formulations produced significant interactive ($P<0.05$) positive changes in the water holding capacity (WHC), cook loss, emulsion stability values. Texture, colour, TBARS, and emulsion stability values for sausage formulations showed no significant interactive impacts. Overall comparison of treatment sausage formulations against control formulations with non-HPP treated meat showed that HPP improved overall sausage quality attributes, where sausage formulation 2 employing HPP-treated meat and US-treated AP and CSS was regarded as the optimal sausage formulation. In conclusion, there is potential to manufacture sausages with reduced-phosphate concentration using combined novel processing technologies and clean label ingredients such as AP and CSS.

Keywords

Functional ingredients, Non-thermal processing, Dietary fibres, physicochemical properties

1. Introduction

Global food processing industries have undergone tremendous changes in recent years concerning approaches employed in processing food products to meet the ever-changing requirements of consumers. Of particular note, consumer realisation of the association between food and health has resulted in a rapid increase in consumer demand for natural, higher quality, nutritious, and healthy food products that are free from any added preservatives or additives (Barbut, Wood, & Marangoni, 2016; Hygreeva & Pandey, 2016) and all delivered sustainably. The meat industry is one of the major food processing sectors and has been hugely affected by this consumer demand (Soladoye, Pietrasik, Hrynets, & Betti, 2021).

For centuries, the consumption of meat and processed meat products has been a part of human evolution (Moreira et al., 2022). However, in recent years, and due to the growing consumer trends described above, there are predominant health concerns over the general consumption of processed meat products. Consumption of traditional processed meat products has contributed to the higher intake of fat, salt, and synthetic additives, primarily phosphates, resulting in various associated health issues such as high blood pressure, cardiovascular and heart diseases (Conroy, O'Sullivan, Hamill, & Kerry, 2018). It was reported in the National adult nutrition survey that sausage was consumed by 39% of the Irish population of age 18-64 years and 31% of those of age ≥65 years (Irish Universities Nutrition Alliance IUNA, 2011; Conroy et al., 2018). This higher consumption has resulted in reducing and/or replacing various additives added to breakfast sausage by meat industries. Phosphate reduction in breakfast sausages is one such consumer-driven challenge currently faced by meat industries (O'Flynn, Cruz-Romero, Troy, Mullen, & Kerry, 2014). Phosphates are synthetic additives, generally added to processed meat such as sausages to bind added water molecules, elevate pH, stabilise meat emulsions, reduce oxidative rancidity, improve juiciness, tenderness, appearance, firmness and maintenance of product flavour (Long, Gál, & Buňka, 2011; O'Flynn et al., 2014; Thangavelu, Kerry, Tiwari, & McDonnell, 2019)**.** Higher consumption of phosphates can cause Hyperphosphatemia (higher accumulated blood phosphates) in people with chronic kidney diseases, thereby increasing their rate of mortality up to 40%, and can also reduce the absorption of calcium into bones within healthy individuals (Pinton et al., 2021; Takeda, Yamamoto, Yamanaka-Okumura, & Taketani, 2014). This signifies the importance of phosphate removal or control from sausages for the benefit of the general population, but specifically for population groups impacted by the presence of phosphates in the diet. However, alteration or removal of phosphate from processed meat products is a massive challenge for processed meat industries because of the critical roles played by phosphates in meat processing. Attempting to remove phosphates without compromising the eating quality of processed meat products is complex and very easily detected by consumers. This change in quality can be counteracted by the introduction of natural functional food ingredients as phosphate alternatives or by using advanced novel, non-thermal food processing technologies such as; power ultrasound (US), high-pressure processing (HPP), oscillating magnetic fields and cold plasma technologies to produce high quality and minimally-processed food (Khouryieh, 2021). Combining both approaches opens up the number of possibilities that can be employed to replace, reduce or control phosphate levels in relevant processed meat systems and products. In this work, US and HPP were combined at different points of the sausage manufacturing process, the objective being to improve final product quality. US application was employed to treat phosphate-replacing ingredients. HPP was used to treat the fresh pork meat following mincing because of the technology's proven ability to enhance ingredient performance and modify muscle properties, discussed in more detail below.

US is a green, non-thermal food processing technology that functions by creating vapour bubbles in liquid, solid, dispersion or gaseous media produced by exposing them to sound waves of higher frequencies (> 20kHz)(Arzeni et al., 2012; Cichoski et al., 2019). The US can be used to effectively modify the structure and improve ingredient functionalities (Pinton et al., 2019). More specifically, apple pomace (AP), obtained from the apple juice industry, and Coffee silver skin (CSS), obtained from the coffee powder industry, were the two functional food co-products or ingredients of interest used as phosphate alternatives in this study. These ingredients are two dietary, fibre-rich, techno-functional ingredients which contain natural phosphorous levels of 1.4g/kg (AP) and 1.5g/kg (CSS), respectively (Martuscelli, Esposito, Di Mattia, Ricci, & Mastrocola, 2021; Thangavelu, Tiwari, Kerry, McDonnell, & Álvarez, 2022). The presence of higher contents of total dietary fibre (TDF), mainly insoluble fibre, in AP (78-90%) and CSS (86%) can be used to improve the WHC, emulsion stability, cook loss and textural characteristics when introduced into meat products (Illippangama, Jayasena, Jo, & Mudannayake, 2022). In a previous study by our research team, Thangavelu, Tiwari, Kerry, McDonnell, & Álvarez (2022) demonstrated the phosphate-replacing/reducing capabilities of CSS and AP using a mixture design approach which resulted in the development of three optimised phosphate-reduced sausage formulations. These three formulations were later assessed following US treatment to ascertain if AP and CSS could be further improved concerning their functionalities and product quality improvements. Data generated from this study showed that the inclusion of US-treated (250W, 20 kHz for 30 min) AP and CSS in phosphate-reduced Irish breakfast sausages formulations improved their physicochemical properties, such as; WHC, cook loss and emulsion stability (Thangavelu, Tiwari, Kerry, & Álvarez, 2021). However, all three formulations detected no significant improvements in textural product attributes nor oxidative inhibition values. Hence, in this study, we explored the possibilities of further improving the quality of these optimised sausage formulations using HPP.

HPP is the process of applying high hydrostatic pressure (100-800 MPa) evenly on packaged liquid or solid food surfaces for milliseconds to several minutes at refrigeration or mild temperatures (<45^{o}C) using a liquid medium for pressure transfer (Hernández-Hernández, Moreno-Vilet, & Villanueva-Rodríguez, 2019; Khouryieh, 2021; Muntean et al., 2016). HPP food application modifies food structure, denatures proteins, and inactivates microorganisms and enzymes. These structural changes assist in increasing mass transfer rates, solvent permeability and secondary metabolite diffusion within the food (Andreou et al., 2017). HPP has emerged over the past two decades as a fast-growing eco-friendly, non-thermal food processing technology that offers adequate safety and quality advantages to meat and processed meat products (Baptista, Rocha, Cunha, Saraiva, & Almeida, 2016;

Xue et al., 2017). Grossi et al. (2016) reported that HPP, when employed at moderate pressures (100-300 MPa), can effectively modify meat protein without excessive denaturation, resulting in improved physicochemical properties like water holding capacity (WHC), texture and rheological properties. They can be used to produce minimally processed, additive-free meat products (O'Neill et al., 2018). For example, a study by O'Neill et al. (2019) to develop low-salt, shelf-stable frankfurters using the response surface methodology approach showed that a significant salt reduction could be achieved with salt replacer (0–100%), high-pressure processing (HPP) (0.1–600 MPa) and a mix of organic acids (0.2–0.4%). Similarly, O'Flynn et al. (2014) showed that HPP employed at 150 MPa (5 min) successfully reduced phosphate concentration in breakfast sausages to 0.25%, without any significant changes in their physicochemical properties.

Although individual reports for the US and HPP application to reduce phosphate in meat products have been published, no information on the combined effect of US and HPP on phosphate-reduced sausage manufacture employing AP and CSS as phosphate replacing ingredients could be determined from an extensive review of scientific literature. Thus, the study's main objective was to treat the alternative ingredients AP and CSS with the US and to study its combined effect with HPP-treated pork in improving the physicochemical properties of phosphate-reduced sausage formulations.

2. Methods and Materials

2.1 Pre-treatment of ingredients and meat

The alternative natural ingredients CSS and AP were supplied by Illy S.P. A. (Trieste, Italy) and Muns Agroindustrial S. LO. (Lleida, Spain), respectively. The individual aqueous solution (10% w/v) of the oven-dried (40°C) and finely-powdered ingredients were treated with high power (250W, 20kHz) US probe (UIP1000hDT, Hielscher Ultrasound Technology, Germany) for 30 min in temperature-controlled (≤20°C) jacketed glass beakers. Treated solutions were dried to a powdered form using a freeze drier (Cuddon Freeze Dry, New Zealand) and stored (4°C) in airtight containers until further use. The above-chosen parameters for US treatment were based on results from our previous research study (Thangavelu, Tiwari, Kerry, & Álvarez, 2022), which showed that ingredients treated with the US for 30 min had improved characteristics compared to those treated for 15 min.

Four fresh pork loins (four days from kill date) were used for each experimental repetition (Table 1) (pH 5.3–6.0), and all pork loins were derived from the same production batch and were purchased from a local butcher (Gleeson Butchers, Dublin, Ireland), all used in for sausage production. On the same day of purchase, back-fat was trimmed from the loins, and the lean meat loins were cut into two halves. A randomly selected loin half was minced together using a meat mincer (Meat Grinder MG510, Kenwood, UK) and vacuum packaged before treating them with HPP. The remaining untreated loin halves were minced together and used to manufacture control sausages.

The packaged minced meat was treated with HPP at 150 MPa for 5 min on the same day of purchase using HPP technology (Stansted Fluid Power Ltd., Essex, UK) containing a mixture of water-oil (90:10) as transmitting fluid (600 MPa as maximum working pressure capacity). The above pressure parameters were chosen based on results reported by O'Flynn et al. (2014). They showed that treating pork meat at 150 MPa for 5 min improved textural properties and did not negatively affect other properties of breakfast sausages with reduced phosphate (0.25%), whereas 300 MPa (5 min) negatively affected sausage quality. Pre- and post-treatment temperatures for samples were maintained between 6.5-8.5°C. HPP-treated meats were immediately used for sausage production.

2.2 Sample preparation

Phosphate-reduced sausages were produced by adding US-treated AP and CSS in three optimised mixture formulations obtained from the validation study of Thangavelu, Tiwari, Kerry, McDonnell, & Álvarez (2022), and a control formulation with 0.5% w/w phosphate. The seasoning mix, containing no phosphates, was purchased from Redbrook ingredients (Dublin, Ireland), while rusk and STPP required for sausage manufacture were bought from All in All ingredients (Dublin, Ireland). Control sausage formulations were produced containing untreated/HPP-treated pork meat (58.00%), fat (20.85%), water/ice (13.45%), rusk (5.75%), seasoning (1.45%), and STPP (0.50%). In contrast, the other three mixture formulations containing fat (20.35%) and ingredient mixture formulations of STPP, US-treated AP and CSS (1.00%) were produced. This adjustment in fat content in sausage formulations is to include 1% of ingredient mixture, since 0.5% of ingredient mixtures proved to be ineffective in phosphate reduction in preliminary studies. The mixture formulations were as follows,

(i) Formulation 1 - 0.20% STPP + 0.22% AP + 0.58% CSS
(ii) Formulation 2 - 0.20% STPP + 0.00% AP + 0.80% CSS
(iii) Formulation 3 - 0.06% STPP + 0.94% AP + 0.00% CSS

The ingredients were mixed together in a bowl using hands for 10 min and stuffed into the collagen casing (Select Collagen Casings, Glasgow, Scotland) using the meat mincer fitted with sausage filler (Meat Grinder MG510, Kenwood, UK). A total of 20 sausages (~10cm in length; 23 mm diameter) per formulation and replication were prepared, and sausages were chosen randomly for further physicochemical analyses. The prepared sausages were packed in a padded black food packaging tray (h 197mm x w 155mm x d 30mm; Silverstream packaging Ltd, Cork, Ireland) overwrapped using polyvinylidene chloride (PVDC) wraps (gas permeability−2.5 [g 100μm]/[m2d]; 300mm x 300m, Prowarp, Bristol, UK), and stored under simulated retail chilled conditions (EXPO PT, glass door upright display cooler, Framec, Italy) at 3-5°C throughout the analysis. The effect of HPP-treated meat on physicochemical properties from all three sausage formulations and the control formulation was studied by comparing them with those sausage formulations containing non-HPP treated meat. The physicochemical properties of breakfast sausages were WHC, cook loss, emulsion stability, texture profile analysis (TPA), lipid oxidation, colour, and proximate composition. Two independent trials were carried out on two different occasions.

2.3 WHC, water mobility, cook loss & emulsion stability

Three prepared sausage batters per formulation were assessed for quality alterations: WHC, cook loss and emulsion stability following HPP treatment. WHC and cook loss for sausages were evaluated using the method of Lianji & Chen (1989) with some minor modifications as described by Thangavelu, Tiwari, Kerry, McDonnell, & Álvarez (2022). The alteration in sausage WHC produced by HPP application was extensively studied using LF-NMR analysis. The activity of bound, myofibrillar and free water within the meat matrix was analysed based on the method described by McDonnell et al. (2013) using an LF-NMR Ultra instrument (Oxford instruments, Abington, Oxfordshire, UK) at a resonating frequency of 23.2 MHz.

Sausage batter stability was evaluated by centrifuging raw batter (~25g; exact weight recorded) at 2958 g (1 min) in a 50ml centrifuge tube followed by heating at 70°C (30min) in a water bath. Tubes were centrifuged again at 2958 g (3 min), and the supernatants were poured into pre-weighed crucibles for overnight drying at 100°C. The dried pellets were weighted to measure the volume of total expressible fluids (TEF (%)) and fat exudate (%) using the formula reported by Hughes, Mullen, and Troy (1998) as follows:

TEF = Weight of sample – weight of pellet (1)

TEF (%) = (TEF/ sample weight) x 100 (2)

Fat Exudate (%) = (Dried supernatant/ TEF) x 100 (3)

2.4 Lipid oxidation measurement

Lipid oxidation levels in sausages, as determined over storage time, were measured by the TBARS analysis method (Botsoglou et al., 1994), with some modifications. Raw blended sausage of 1.5g was mixed with 20 mL of milliQ water and homogenized with an Ultraturrax homogeniser (Labortechnik, Staufen, Germany) at 13500 rpm for 30s. Cold trichloroacetic acid (25% TCA) 5 mL was added followed by gentle stirring at 4 °C for 15 min and centrifuged at 3500 rpm for 15 min (4 °C). A 3.5 mL of the supernatant was mixed with 1.5 mL of 0.6% 2-thiobarbituric acid with the reaction performed in the water bath at 70 °C for 30 min. The tubes were cooled and TBARS were measured at 532 nm using UV–Vis Spectrophotometer (Shimadzu UV – 1700, Columbia, USA). Results were expressed as milligrams of malondialdehyde produced per kg of sausage (mg MDA/kg). Three sausage samples from day 0 and day 9 per formulation were analysed.

2.5 Colour analysis

Sausages covered in transparent PVDC cling film were analysed for changes in colour parameters impacted by HPP treatment. The three colour parameters L* (lightness), a* (redness) and b* (yellowness), were measured using the dual xenon flash UltraScan Pro spectrometer (Hunterlab, Reston, VA, USA), calibrated by a light trap (L=0) and a transparent cling film covered standard white tile (L=100; X=88.69; Y=93.58; Z=100.45). The viewing port was 25.54mm, and the standard illuminant D65 was used with an observer angle of ten degrees. Sausages were measured in triplicates per formulation and averaged for statistical analysis. The total colour difference between formulations was calculated using the equation presented by Salgado, Fernández, Drago, and Mauri (2011).

$$\Delta E_{ab}^{*} = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \quad (4)$$

2.6 Textural properties

The major textural parameters for sausages, such as; hardness (N), chewiness (N), gumminess (N), springiness (mm) and cohesion force ratio, were measured based on the methods of Bourne (1978). Five sausages per formulation and per replication were cooked all together in a single batch in a water bath (73±1°C) for 20-30 min until a sausage core of 70^{o}C was achieved and then cooled overnight in a refrigerator at 4^{o}C. Cooked sausage TPA values were calculated using the two-cycle compression test applied by an Instron universal testing machine, model 5534 (Instron Ltd., High Wycombe, UK). Sausage dimension cores of 14mm diam. X 20mm ht. was axially compressed at 70% of their original height at a crosshead moving speed of 100mm/min and a 500N load cell, and this was used to determine the force-time deformation curves. Average values of five cores per formulation were recorded.

2.7 Compositional analysis

The proximate composition of the homogenised sausages per formulation was measured using their respective AOAC methods, such as; protein (AOAC 992.15, 1992), moisture (AOAC 985.14, 1990), fat (AOAC 2008.06, 2008), ash (AOAC 920.153, 1920), salt (AOAC 935.47, 1987) and TDF (AOAC 991.43, 1995). The values were measured in triplicate per independent trial and averaged for statistical analysis.

2.8 Formulation Grading system

To determine the best overall formulation that can replicate the control formulation, considering all important parameters, a grading system was developed based on the study of Álvarez, Drummond, & Mullen (2018). Each parameter was standardised using the equations,

$$z = X - \mu/\sigma \tag{5}$$

$$z = -(X - \mu/\sigma) \tag{6}$$

where z is the score value, X – value of the parameter, μ - mean of all samples; σ – standard deviation. Equation (5) was used for parameters where higher values are desirable (e.g. WHC), and equation (6) was used for parameters where lower values were desired (e.g. Cook loss). A radar chart was prepared using the Microsoft Excel Sheet (Microsoft Inc.) to represent the overall grading system using the above equations pictorially.

2.9 Statistical evaluation

This randomised study was built using a split-plot experimental design, with HPP/Non-HPP treatment as the main effect to be compared within each formulation on the whole plot and one of the four formulations (including control formulation) on the subplot in Table 1 and the frequency distribution in Table 2. The experiment was replicated twice on two different occasions, and the replications were treated as the blocks to account for any difference between replications. The mean values for the responses represented were the average values of triplicates obtained from both independent study replications (n=6). The single factor impact and the interaction effect between the HPP treatment and different formulations was analysed by two–way analysis of variance (ANOVA) with HPP treatment, formulations and HPP treatment*formulations as the factors using Minitab®17.0 statistical software package. Tukey's multiple comparisons were used to compare the means of data with a confidence interval of 95%. In addition, one-way ANOVA, along with Tukey's multiple comparison, was carried out between control (traditional sausage formulation) with non-HPP treated meat and sausage formulations (1-3) with HPP-treated meat to compare their mean values.

3. Results & Discussion

3.1 Compositional analyses of sausages

The results of various parameters determining the proximate composition of the raw sausages are presented in Table 3. The results of 2-way ANOVA showed that HPP application did not produce any significant interaction in compositional changes of sausage formulations concerning moisture, protein, fat, ash, and salt percentages. Additionally, from the results of one-way ANOVA, it was observed that phosphate-reduced formulations with HPP-treated meat did not affect proximate composition compared to the control formulation with non-HPP treated meat containing 0.50% STPP. For instance, moisture, fat, protein, and salt content of formulations 1, 2 and 3 with HPP-treated meat were almost the same as control formulations (moisture 61.75–62.84%; fat–14.46-15.13%; protein-16.12–16.58%; salt–0.66-0.81%). However, a significant difference in ash and TDF values was observed for the sausage formulations with HPP-treated meat compared with the control with non-HPP treated meat. Sausage formulations 1, 2 and 3 had increased (P<0.05) TDF values due to AP and CSS, which are both rich in fibre content. The presence of inorganic STPP in the control (0.50%) and formulations 1 (0.20%) and 2 (0.20%) resulted in higher ash contents (P<0.05) when compared to formulation 3 (0.06%). The slightly higher ash values of sausage formulations 1 and 2 compared to formulation 3 were also due to higher mineral compositions present in CSS (Ballesteros, Teixeira, & Mussatto, 2014).

3.2 Emulsion stability

Emulsion stability results expressed as TEF (%) and fat exudate (%) are presented in Table 3. Results of 2-way ANOVA showed that the application of HPP to sausage formulations produced a desirable interactive effect on emulsion stability i.e. inclusion of HPP treated meat produced different effect on (P<0.05) different formulations. It was noted that HPP-treated meat produced larger impact, which reduced TEF (%) values for sausage formulations 1 and 3 (with AP), whereas in sausage formulation 2 (without AP), the value was insignificantly reduced when compared within the individual formulations. This result indicated that AP played a role in lowering TEF (%); however, no such effects were observed for fat exudate (%) values. A decreasing trend was observed in fat exudate (%) values for phosphate-reduced sausage formulations. HPP application reduced (P<0.05) fat exudate (%) values for formulation 3, whereas the reduction was insignificant in sausage formulations 1 and 2 when compared with their respective formulations with non-HPP treated meat. This reduction in TEF (%) and fat exudate (%) values indicate improved emulsion stability, which can be attributed to the structural changes produced in the myofibrillar proteins by HPP and which subsequently resulted in the increased binding of water and fat within the meat matrix (Furlán, Padilla, & Campderrós, 2014; Yang et al., 2021). Conversely, HPP produced the opposite trend in TEF (%) and fat exudate (%) values for control sausage formulations. The controls' TEF (%) and fat exudate (%) values increased when HPP-treated meat was used to formulate sausages; however, this increase was insignificant. This reverse in trend between the control and the phosphate-reduced sausage formulations could be due to the presence of techno-functionality improved US-treated AP and CSS in the sausage formulation, which along with the HPP-treatment synergistically improved the emulsion stability values. In addition, this insignificance in the emulsion stability values of control formulations can be explained by the saturated highest level of emulsion stability produced by STPP (0.50%) in the control formulations.

Results of a one-way ANOVA comparing HPP-treated meat, phosphate-reduced sausage formulations with control formulations (traditional sausage) with non-HPP treated meat showed that TEF (%) values and fat exudate (%) values significantly differed among the formulations. The TEF (%) and fat exudate (%) values analysis showed that reducing the phosphate concentration increased the TEF (%) and fat exudate (%) values. This concurs with O'Flynn et al. (2014), who showed that emulsion stability decreased with decreased phosphate concentration. However, HPP treatment at 150 MPa for 5 min improved the emulsion stability of phosphate-reduced sausages but could not match that achieved in the control formulation.

3.3 WHC

Results of sausage formulation WHC are presented in Table 3. It was evident from 2-way ANOVA results that HPP and sausage formulations had a significant interactive ($P<0.05$) effect on the WHC values. It was observed that the WHC values increased significantly for phosphate-reduced sausage formulations 3 with HPP-treated meat. There was an insignificant increase in WHC in formulations 1 and 2 when compared with their respective formulations with non-HPP treated meat; such effect was also observed in the control sausage formulation. This concurs with the increased emulsion stability values discussed above for the same samples, where the control formulation had reached its saturated WHC level that could not be increased further. The increase in WHC of phosphate-reduced sausage formulations was due to the increased emulsion stability created by HPP treatment Studies by Grossi, Søltoft-Jensen, Knudsen, Christensen, and Orlien (2012) reported the increase in WHC by HPP treatment was due to the disruptions of electrostatic and hydrophobic interactions producing the increased myofibrillar solubilisation.

Results of one-way ANOVA (Control non-HPP treated vs HPP-treated formulations) showed that control performed equal to formulations 1 and 2. However, a significant difference was observed in HPP-treated formulation 3 compared to control values. This difference was due to the low phosphate concentration (0.06%) in formulation 3. As an overall discussion, HPP treatment increased the WHC of the phosphate-reduced sausage formulations to the level observed for control (traditional Sausage formulations) WHC values, except for formulation 3.

3.4 Cook loss

Cook loss values for sausage formulations are presented in Table 3. Data from 2-way ANOVA showed that, similar to WHC, HPP treatment and sausage formulations produced significant (P<0.05) interactive impact on cook loss values (specifically in formulations 1 and 3). HPP reduced cook loss values for all three phosphate-reduced sausage formulations, but it was only significant for formulation 3. For the control formulation no significant modification was observed. This decrease in cook loss can be attributed to the improved emulsion stability of the sausage formulations. HPP application aided the meat protein depolymerisation and increased the solubility of myofibrillar proteins that formed a stable gel matrix, thereby reducing cook loss values (O'Flynn, Cruz-Romero, Troy, Mullen, & Kerry, 2014).

Similar to WHC, results of one-way ANOVA analysis of cook loss values has shown that sausage formulations 1 and 2 with HPP-treated meat had cook loss values similar to control sausage formulations, with non-HPP treated meat, containing 0.5% STPP. Also, the cook loss value for sausage formulation 3 was much higher (P<0.05) than the control. This was due to the low concentration of STPP in formulation 3 (0.06%). In general, HPP decreased the cook loss values of phosphate-reduced sausage formulations.

3.5 Water mobility analysis using LF – NMR

LF-NMR technique is widely used to study water mobility and distribution within meat matrices (Han, Wang, Xu, & Zhou, 2014). There are three components in the distribution curve, and each component represents each water type in the meat matrix: bound water, myofibrillar/immobilised (or entrapped) water and free water. The relaxation time (T) and correlated water proportion percentage (P) of the three components, bound water (T_{2b}, P_{2b}); myofibrillar/immobilised water (T_{21}, P_{21}) and free water (T_{22}, P_{22}), were assessed to measure the water mobility within the meat matrix before cooking. The analysis of the factors interaction on the relaxation time distribution data showed that HPP treatment and sausage formulations produced significant interaction effect on T_{2b} and T_{22} values; however the values of this parameter were not significantly affected by the treatments. Only value affected was T_{22} in formulation 3. Similarly, In terms of population distribution, HPP did not produce any significant changes in the population (P_{2b}) of inner bound water of control or sausage formulations 1 and 2. The significant increase in P_{2b} values for formulation 3 was unexpected since the internal water is primarily unaffected by any mechanical disturbance (McDonnell et al., 2013). HPP increased (P<0.05) P_{21} values for sausage formulations 1 and 2, whereas the increasing effect was insignificant in control formulations. Similarly, the application of HPP decreased (P<0.05) P_{22} values for sausage formulations 1 and 2, whereas the decreasing effect was insignificant in control formulations. This decrease in values of P_{22} can be attributed to decreased cook losses and increased WHC since the free water in

$P_{22,}$ responsible for cook and drip loss, had migrated to the myofibrillar/immobilised population matrix P_{21}. Concerning formulation 3, HPP application was ineffectual in causing P21 or P22 values changes.

Results of one-way ANOVA and comparison of the population distribution values in Figure 1 and Supplementary 1, showed that the phosphate-reduced sausage formulations with HPP-treated meat significantly differed from control formulations with non-HPP treated meat. All three phosphate-reduced sausage formulations had increased P_{21} (only significant for formulation 2) and decreased P_{22} values (significant for all formulations) compared with control formulations with non-HPP treated meat. The increase in P_{21} indicates the increase in myofibrillar/immobilised water, thus explaining the rise in WHC. Therefore, the application of HPP positively influenced the water mobility of sausage formulations.

3.6 Effect of HPP treatment on textural properties

The effect of HPP treatment on the hardness, chewiness, gumminess, springiness, and cohesiveness of breakfast sausages was measured and the results of Table 4 showed that the HPP and sausage formulations did not produce any significant interactive effect on these textural properties. The results observed that HPP treatment at 150 MPa for 5 min did not produce significant impact on sausage hardness values for control and all three sausage formulations. This contrasts with the O'Flynn et al. (2014) study, which showed that an HPP-treated meat (150 MPa for 5 min) improved hardness values for phosphate-reduced sausages (0.25%). Likewise, Yang et al. (2015) showed that HPP application improved sausage hardness values up to 200MPa. In our study, although insignificant, it was observed that the hardness values followed a reducing trend in the sausage formulations with HPP-treated meat, except sausage formulation 3. This decrease in sausage hardness can be attributed to the deterioration of functional proteins when treated with HPP (O'Flynn et al., 2014; Zhu, Yan, Yu, Wu, & Bennett, 2022). Similarly, the application of HPP in all sausage formulations produced a decreased effect in gumminess; however, these decreases were insignificant, except for the treated control (P<0.05). A similar trend was observed for sausage chewiness and springiness values for all sausage formulations, where HPP effects were insignificant. This decrease in the textural values for sausage formulations is not desirable since it affects the quality by reducing product firmness.

The results of one-way ANOVA showed that HPP did not improve sausage textural properties, so phosphate-reduced formulations cannot be enhanced under the research conditions employed in this study to equal the performance of control (0.5% STPP) sausage formulations with non-HPP treated meat. It was observed that formulation 2 had the highest chewiness, gumminess and springiness values among formulations assessed, with formulation 3 having the lowest values for the same parameters. The cohesiveness values for all sausage formulations, including controls, were almost the same (0.70-0.80). However, hardness, chewiness, gumminess, and springiness values of control formulation with non-HPP treated meat were higher (*P*<0.05) than all other phosphate-reduced sausage formulations with HPP-treated and US-treated ingredients. This overall observation shows why phosphates play a significant role in determining sausage texture characteristics. Although the review by Thangavelu et al. (2019) states that HPP improves the texture characteristics of meat products, the applied HPP treatment in this research to sausage formulations employed did not positively influence the texture properties of phosphate-reduced sausage formulations when compared to control formulation.

3.7 Effect of HPP treatment on colour properties

The colour of meat products plays a significant role in determining consumer purchasing decisions. Thus, meat colour is an important quality attribute that requires attention when altering processed meat formulations (Tomasevic, Djekic, Font-i-Furnols, Terjung, & Lorenzo, 2021). Results of 2-way ANOVA showed that HPP treatment and sausage formulations did not form any significant interactive effect on colour parameters except for a* where a significant interactive effect was produced meaning that HPP treatment produced different effects on different formulations; more evident for formulation 3. It was observed from the results (Table 4) that the L* values for HPP treatment showed a very slight but insignificant increase in sausage formulations, including controls. A study by Zhu et al. (2022) showed that HPP application (100-400 MPa for 15 min) increased L* values and decreased a* values in beef sausages. These changes in colour values were due to the denaturation of muscle proteins (Grossi, Søltoft-Jensen, Knudsen, Christensen, & Orlien, 2011). However, in contrast with this study, no a* value modification was observed in sausage formulations following HPP treatment of meat employed in these formulations. Similarly, after HPP treatment, no significant changes were observed in b* values within the individual formulations.

Overall, from the results of one-way ANOVA, phosphate-reduced sausage formulations (1-3) containing HPP-treated meat had significantly lower L* values (60.6-61.7) compared with the control formulation (L*-65.3) containing 0.5% of STPP and non-HPP treated meat. This increase in the darkness of the sausage formulations can be attributed to the addition of AP and CSS to the sausage formulations. The phosphate-reduced sausage formulations observed no significant differences in a* or b* values. However, the results of perceptual colour difference (ΔE) showed a clear colour difference (ΔE>2.5) in the phosphate-reduced sausage formulations when compared to the control (with non-HPP treated meat) since Mokrzycki & Tatol (2011) highlighted that a visible difference is observed if the colour difference (ΔE) is above 2.5. The observed colour difference was mainly due to the change in luminosity after US-treated AP and CSS inclusion in the phosphate-reduced sausage formulations. In contrast, HPP did not affect the sausages' colour properties.

3.8 Effect of HPP on Lipid Oxidation (TBARS value)

Lipid oxidation measures the oxidative degradation of lipids produced by the highly complex free radical reaction between fatty acids and oxygen, resulting in discolouration, drip losses, off-odour and off-flavour production, and the formation of potentially toxic compounds (Morrissey et al., 1998). This will result in the emergence of off-flavours and off-aromas in meat products as this value increases over time. In our previous research that analysed sausages from days 0, 3, 6 and 9, the TBARS values for all three phosphate-reduced sausage formulations containing US-treated AP and CSS were found to be similar to each other on day 3 (0.20 – 0.26mg MDA/kg) and day 6 (0.29-0.39mg MDA/kg) (Thangavelu et al., 2021). The main differences in TBARs were observed only on day 9, and hence, in this study, day 9 values were used to assess the effect of HPP on lipid oxidation.

Results of 2-way ANOVA showed that HPP treatment and sausage formulations produced significant interactive impact on day 9 TBARS values. In general, application of HPP increased TBARS values for most of the different pressure/time combination treatments, as previously reported (Omana, Plastow, & Betti, 2011). Studies have shown that HPP treatment applied between 300-600MPa induces lipid oxidation faster (Beltran, Pla, Yuste, & Mor-Mur, 2003; Cava, Higuero, & Ladero, 2021). However, from lipid oxidation values obtained on storage days 0 and 9 (Table 4), it was evident that HPP did not produce significant changes in TBARS values for sausage formulations 1 and 3, along with the control formulations when compared with their respective formulations with non-HPP treated meat. HPP treatment did not change TBARS values for either control or phosphate-reduced sausage formulations. Additionally, final TBARS values were within the detectable highest upper range threshold limit of 2.0-2.5mg MDA/kg, above which rancidity occurs with off-flavours and off-aromas and the formation of potentially dangerous free radicals (Zhang et al., 2019).

In addition, one-way ANOVA results showed TBARS values observed for control formulations (non-HPP treated meat) did not vary ($P>0.05$) with the three phosphate-reduced sausage formulations with HPP-treated meat. On further analysis, phosphate-reduced sausage formulation 3 (0.06% STPP) had a higher TBARS value, which might be due to its lower concentration of phosphate and lack of CSS since phosphates (Long et al., 2011) and CSS (Iriondo-DeHond et al., 2019) are excellent antioxidants. The values showed that HPP does not produce any induced oxidation, consequently playing no role in the quality degradation of phosphate-reduced sausage formulations.

3.9 Formulation grading

The formulation grading system indicated the overall performance of treatment on different formulations compared to whole sample populations. The total scores for formulations using Non-HPP treated meat were as follows, (i) Control: 1.96; (ii) Formulation 1: - 0.20; (iii) Formulation 2: - 0.16; (iv) Formulation 3: -3.19. Similarly, the total scores for formulations using HPP-treated meat were as follows, (i) Control: 1.41; (ii) Formulation 1: 0.17; (iii) Formulation 2: 0.87; (iv) Formulation 3: -1.08. It was evident that the control formulations possessed higher scores for non-HPP and HPP treatment, even after HPP treatment had reduced its overall score. It was also evident from Figure 2 that HPP treatment improved overall scores for phosphate-reduced sausage formulations to a greater extent. Sausage qualities, such as fat exudate values and lipid oxidation values, greatly influenced overall scores, besides those obtained for WHC and cook loss. It was also noted that STPP content influenced the scores. Although AP and CSS have almost similar fibre content, a difference in their STPP interaction was observed. Thus, HPP treatment in formulation 2, containing only CSS (0.80%), improved the overall score, thereby making it the best phosphate-reduced sausage formulation for those studied. Conversely, formulation 3, containing only AP (0.94%), had the lowest total score. A more targeted grading system can be developed using the weighted arithmetic mean from a commercial perspective.

4. Conclusion

The present study revealed that HPP treatment positively affected most qualitative properties for phosphate-reduced breakfast sausage formulations containing US-treated AP and CSS. In addition, HPP treatment produced significant interaction effect with sausage formulations on important properties like WHC, cook loss and emulsion stability, thus proving that different formulations reacted differently with the addition of HPP-treated meat. This interactive effect seems to be related to the AP content in each of the formulations. This explains the improvement in WHC, cook loss, and emulsion stability values for these novel sausage formulations when HPP was applied at 150MPa for 5 min. The lower values achieved for textural parameters could prove helpful in formulating processed meat products for the aged population, who have difficulty chewing meat. Further analysis of the results obtained in this study showed that sausage formulation 2, containing 0.20% STPP (60% phosphate reduction) and 0.80% CSS, produced the best quality results when compared to the other experimental formulations, proving that CSS has much better scope for replacing the phosphates in sausages than AP. Future studies examining the effect of different range of HPP pressure level at different time intervals could be explored since the pressure level used in this study is derived from previous literatures. In conclusion, HPP application and inclusion of US-treated AP and CSS in phosphate-reduced sausage formulations could successfully be used in commercial Irish breakfast sausage manufacture with minimal perceived loss in colour and other quality parameters.

Acknowledgements

This research work was aided by the Walsh Scholar research grant (No. 0100, 2017) provided by Teagasc – The Agricultural and Food Development Authority of Ireland.

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Table 1. The split-plot experimental design of independent trials in terms of HPP treatment and formulations

Repetition	HPP treatment	Formulations
1	HPP	Control
1	Non-HPP	F1
1	HPP	F1
1	Non-HPP	F3
1	Non-HPP	Control
1	HPP	F2
1	HPP	F3
1	Non-HPP	F2
2	HPP	F3
2	Non-HPP	F2
2	Non-HPP	Control
2	HPP	Control
2	HPP	F2
2	Non-HPP	F1
2	Non-HPP	F3
2	HPP	F1

Where Control – 0.50% STPP only; Formulation 1 (F1) - 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2 (F2) - 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3 (F3) - 0.06% STPP+0.94% AP+0.00% CSS

Table 2. The frequency distribution of the two independent trials of the study

<table>
<tr><td rowspan="6">Frequency</td><td colspan="6">Table of HPP by Formulations</td></tr>
<tr><td rowspan="2">HPP Treatment</td><td colspan="5">Formulations</td></tr>
<tr><td>Control</td><td>F1</td><td>F2</td><td>F3</td><td>Total</td></tr>
<tr><td>HPP</td><td>2</td><td>2</td><td>2</td><td>2</td><td>8</td></tr>
<tr><td>Non-HPP</td><td>2</td><td>2</td><td>2</td><td>2</td><td>8</td></tr>
<tr><td>Total</td><td>4</td><td>4</td><td>4</td><td>4</td><td>16</td></tr>
</table>

Where Control – 0.50% STPP only; Formulation 1 (F1) - 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2 (F2) - 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3 (F3) - 0.06% STPP+0.94% AP+0.00% CSS

Table 3. Mean and standard error mean values of compositional analysis, emulsion stability (as TEF% and fat exudate %), cook loss and NMR population distribution percentage of sausage formulations

	Control		Formulation 1		Formulation 2		Formulation 3		Interaction Sig.	SEM
	Non-HPP	HPP	Non-HPP	HPP	Non-HPP	HPP	Non-HPP	HPP		
Moisture (%)	62.5	61.9	61.9	61.7	62.1	61.9	62.1	62.8	ns	0.1
Fat (%)	14.8	15.3	15.0	15.0	15.1	15.1	15.0	14.4	ns	0.1
Protein (%)	16.1	15.7	16.3	16.3	16.5	16.5	16.1	16.4	ns	0.1
Ash (%)	2.1	2.1	1.9	1.9	1.9	1.9	1.9	1.8	ns	0.0
TDF (%)	0.8	1.5	4.1	3.6	3.5	4.2	4.4	4.6	ns	0.4
Salt (%)	0.6	0.6	0.7	0.7	0.7	0.7	0.7	0.7	ns	0.0
WHC (%)	89.1^{a}	88.7 ^{a}	85.4^{b}	87.5^{a,b}	85.9^{b}	87.9^{a,b}	78.3^{d}	82.7^{c}	*	0.7
Cook loss (%)	5.7^{d}	5.9^{d}	7.4^{b,c}	6.1^{c,d}	6.9^{c,d}	6.2^{c,d}	9.7^{a}	8.3^{b}	*	0.3
TEF (%)	6.4^{e}	6.8^{e}	10.7^{b,c}	8.8^{d}	9.8^{c,d}	9.7^{c,d}	13.0^{a}	11.5^{b}	*	0.4
Fat Exudate (%)	6.5^{c}	7.6^{b,c}	9.1^{a,b}	8.7^{a,b}	8.1^{a,b,c}	7.9^{a,b,c}	9.2^{a}	7.7^{a,b,c}	*	0.2
T_{2b} (ms)	3.8^{a,b}	3.1^{a,b}	3.5^{a,b}	3.2^{a,b}	2.6^{b}	3.4^{a,b}	2.9^{a,b}	4.1^{a}	*	0.1
T_{21} (ms)	38.9	38.9	37.7	38.9	36.5	38.9	38.9	38.8	ns	0.2
T_{22} (ms)	250.3^{a,b}	265.8^{a,b}	234.0^{b}	249.5^{a,b}	265.8^{a,b}	257.6^{a,b}	283.6^{a}	241.4^{b}	*	3.9
P_{2b} (%)	3.5	3.4	3.4	3.4	3.2	3.3	2.4	3.5	ns	0.1
P_{21} (%)	86.9	90.0	87.9	90.0	88.8	90.7	90.9	90.0	ns	0.3
P_{22} (%)	9.7	6.6	8.8	6.6	8.0	6.1	6.6	6.5	ns	0.3

Where Control – 0.50% STPP only; Formulation 1- 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2- 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3- 0.06% STPP+0.94% AP+0.00% CSS

*-Significance levels at P<0.05 of the interaction between HPP treatment and formulations using 2-way ANOVA, ns- not significant

SEM – Standard error mean of the sample population

$^{a-e}$ –Mean values with different superscripts within a row are statistically different (P<0.05) from each other using Tukey's comparison

Table 4. Mean and standard error mean values of colour, texture, and TBARS analysis of sausage formulations

		Control		**Formulation 1**		**Formulation 2**		**Formulation 3**		**Interaction Sig.**	**SEM**
		Non-HPP	HPP	Non-HPP	HPP	Non-HPP	HPP	Non-HPP	HPP		
L*		65.3	66.7	60.0	61.0	59.8	60.6	62.0	61.7	ns	
a*		$5.8^{a,b}$	5.7^{b}	$6.2^{a,b}$	$5.9^{a,b}$	$5.9^{a,b}$	$5.9^{a,b}$	$5.8^{a,b}$	6.3^{a}	*	0.1
b*		19.7	19.9	19.1	19.4	19.3	19.1	19.9	19.6	ns	0.1
Hardness (N)		48.2	39.3	30.9	29.1	28.0	27.3	21.7	22.4	ns	2.2
Chewiness (J)		109.9	81.3	31.4	25.9	38.4	35.1	15.2	14.7	ns	8.3
Cohesive force		0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.8	ns	0.0
Gumminess (N)		13.3	10.4	5.0	4.1	6.0	5.2	3.2	3.1	ns	0.9
Springiness (mm)		8.2	7.6	6.4	5.7	6.3	6.5	4.7	4.7	ns	0.3
TBARS (mg MDA/kg)	**Day 0**	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	ns	0.0
	Day 9	$0.6^{b,c}$	0.4^{c}	$0.5^{b,c}$	$0.6^{b,c}$	0.7^{b}	$0.5^{b,c}$	1.0^{a}	0.8^{a}	*	0.0

Where Control – 0.50% STPP only; Formulation 1- 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2- 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3- 0.06% STPP+0.94% AP+0.00% CSS

*-Significance levels at P<0.05 of the interaction between HPP treatment and formulations using 2-way ANOVA, ns- not significant

SEM – Standard error mean of the sample population

$^{a\text{-}e}$ –Mean values with different superscripts within a row are statistically different (P<0.05) from each other using Tukey's comparison

Supplementary Table 1. Mean values comparing the physicochemical properties Control (non-HPP treated meat) with phosphate-reduced sausages formulations with HPP-treated meat

		Non-HPP treated Control	HPP treated formulations			Sig.	SEM
			Formulation 1	Formulation 2	Formulation 3		
WHC		89.1^{a}	87.5^{a}	87.9^{a}	82.7^{b}	*	0.7
Cook loss		5.7^{a}	6.1^{a}	6.2^{a}	8.3^{b}	*	0.3
TEF (%)		6.4^{a}	8.8^{b}	9.7^{b}	11.5^{c}	*	0.4
Fat exudate (%)		6.5^{a}	8.7^{b}	7.9^{ab}	7.7^{ab}	*	0.2
$\mathbf{P_{2b}}$ (%)		3.5	3.4	3.3	3.5	ns	0.1
$\mathbf{P_{21}}$ (%)		86.9^{a}	$90.0^{a,b}$	90.7^{b}	$90.0^{a,b}$	*	0.3
$\mathbf{P_{22}}$ (%)		9.7^{a}	6.6^{b}	6.1^{b}	6.5^{b}	*	0.3
L*		65.3^{a}	61.0^{b}	60.6^{b}	61.6^{b}	*	0.4
a*		5.8	5.9	5.9	6.3	ns	0.1
b*		19.7	19.4	19.1	19.6	ns	0.1
ΔE			4.3	4.7	3.7		
Hardness (N)		48.2^{a}	29.1^{b}	27.3^{b}	22.4^{b}	*	2.2
Chewiness (J)		109.9^{a}	25.9^{b}	35.1^{b}	14.7^{b}	*	8.4
Cohesive force		0.7	0.7	0.7	0.8	ns	0.0
Gumminess (N)		13.3^{a}	4.1^{b}	5.2^{b}	3.1^{b}	*	0.9
Springiness (mm)		8.2^{a}	5.7^{bc}	6.5^{abc}	4.7^{c}	*	0.3
TDF (%)		0.8^{a}	3.6^{b}	4.2^{b}	4.6^{b}	*	0.4
Ash (%)		2.1^{a}	1.9^{ab}	1.9^{ab}	1.8^{b}	*	0.0
Salt (%)		0.6	0.7	0.7	0.7	ns	0.0
TBARS (mg MDA/kg)	Day 0	0.2	0.2	0.3	0.3	ns	0.0
	Day 9	0.6	0.6	0.5	0.8	ns	0.0

Where Control – 0.50% STPP only; Formulation 1- 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2- 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3- 0.06% STPP+0.94% AP+0.00% CSS

*-Significance levels at $P<0.05$ between formulations with HPP-treated meat and control formulations with non-HPP treated meat using one-way ANOVA, ns-not significant

SEM – Standard error mean of the sample population

$^{a\text{-}c}$ –Mean values with different superscripts within a row are statistically different ($P<0.05$) from each other using Tukey's comparisons.

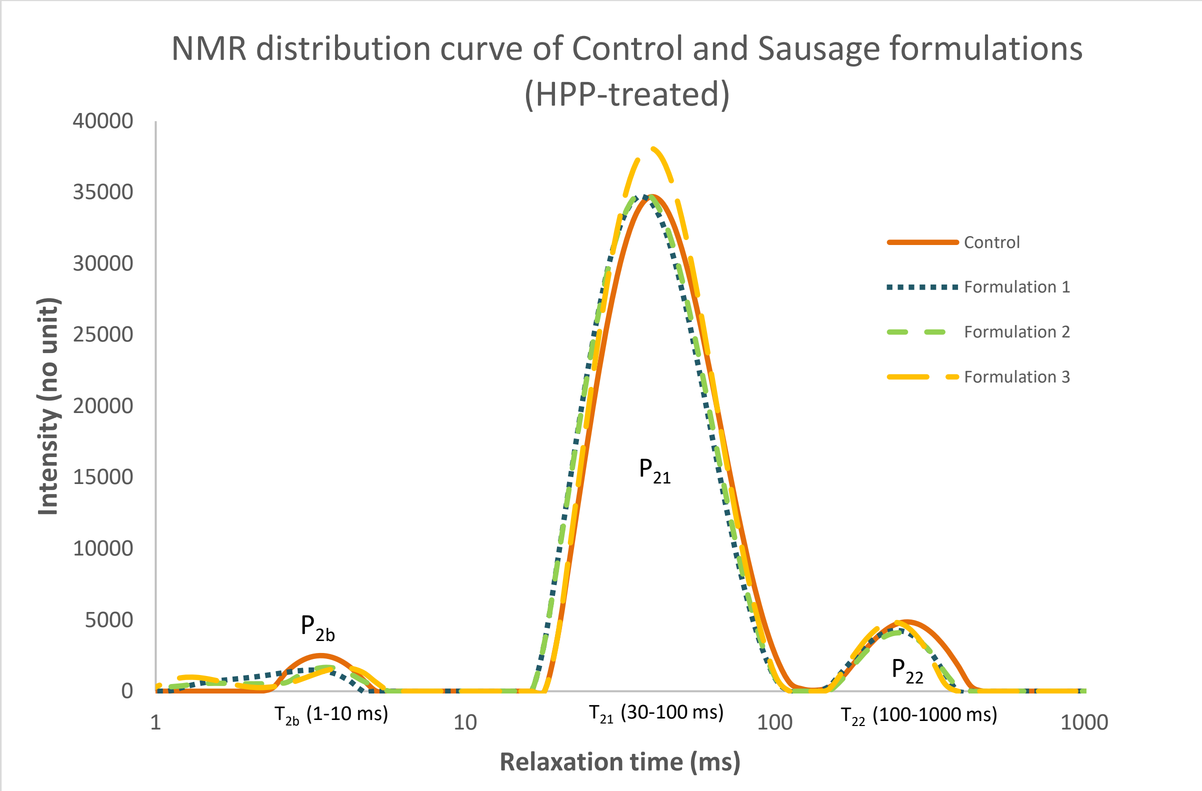

Figure 1. NMR distribution curves of control formulations and HPP treated phosphate reduced sausage formulations.

Where Control – 0.50% STPP only; Formulation 1- 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2- 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3- 0.06% STPP+0.94% AP+0.00% CSS

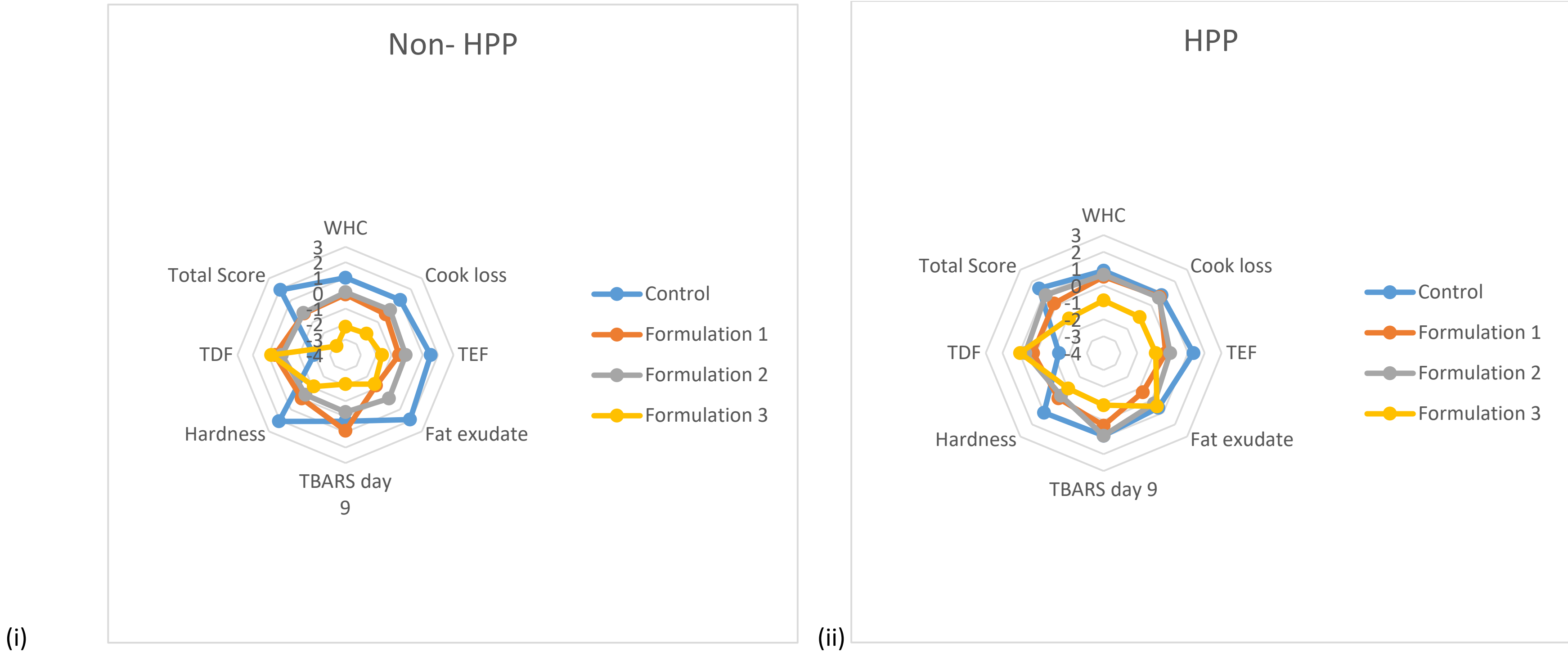

Figure 2. Scores for each of the main physicochemical properties analysed are reported for control and sausage formulations 1-3. (i) Sausage formulations with non-HPP treated meat; (ii) Sausage formulations with HPP-treated meat. The total score for each treatment (divided by 3) is presented.

Where Control – 0.50% STPP only; Formulation 1- 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2- 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3- 0.06% STPP+0.94% AP+0.00% CSS