Phosphate replacing potential of apple pomace and coffee silver skin in Irish breakfast sausage using a mixture design approach

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Abstract

The ability of apple pomace (AP) and coffee silver skin (CSS) powders to replace the techno-functional properties of synthetic phosphates used in Irish breakfast sausages was evaluated using a specialised response surface-mixture design approach. Sausages of 18 different formulations of sodium tripolyphosphate (STPP), AP and CSS mixtures were made and the results of water holding capacity (WHC), cook loss, colour, textural properties, low-field nuclear magnetic resonance (NMR), compositional analysis and lipid oxidation values were analysed. Addition of ingredients to the sausage formulations significantly improved the WHC ($P < 0.001$) and decreased the cook loss ($P < 0.001$) of the products. Interestingly, addition of AP and CSS decreased the hardness ($P < 0.001$), chewiness ($P < 0.001$), gumminess ($P < 0.004$) and springiness ($P < 0.001$). TBARS analysis showed that the addition of ingredients AP and CSS decreased the MDA content on day 9 ($P < 0.018$). Analysing these obtained results, the software has predicted three optimised sausage formulations based on the desirability response method. These formulations help in reducing the phosphate level in sausages with accepted desirability, thereby maintaining the overall quality of the product.

Keywords

Clean label; food by-products; phosphate replacing ability; breakfast sausages

1. Introduction

Consumers' demand for high quality and healthy food products has resulted in various innovations and the emergence of several processing technologies within the food industry. In particular, and in recent years, consumers are demanding more minimally processed and 'healthier' meat products (Shan et al., 2017). This has resulted in the introduction of clean-label trends in meat processing, where the usage of artificial additives in the product formulation is avoided. One such recent industrial trend is the removal of phosphate from processed meat products (Thangavelu, Kerry, Tiwari, & McDonnell, 2019).

Phosphates are natural inorganic elements found in many food products and are essential for human health, as they are required for the growth, maintenance and repair of cells and tissues, signalling, energy transfer and other important functions (Kalantar-Zadeh et al., 2010). Generally, phosphates act as emulsifiers, stabilisers, sequestrants and thickeners in food products. In restructured meat products, phosphates help in stabilising meat pH, decreasing cook loss and help in improving WHC, emulsion stability, texture and sensory qualities, etc. (Nguyen Huynh Bach Son, 2011). However, usage of phosphates in meat products is perceived as unhealthy as they are labelled on meat product

packaging as E-number additives (European Union approved artificial food additives) and there is a misconception, among general consumers that E-number additives have negative health implications. However, there is a clear direction that phosphates are not recommended for people who have kidney issues, such as Chronic Kidney Diseases (CDK), as it can cause hyperphosphatemia (Hruska, Mathew, Lund, Qiu, & Pratt, 2008; Ritz, Hahn, Ketteler, Kuhlmann, & Mann, 2012). Thus, replacing/reducing phosphates in processed meat products should be seen as an essential requirement for meat industries in a bid to improve processed meat composition. From an Irish perspective, breakfast-type sausages are one of the main products currently employing phosphates as a functional ingredient; thus, this product was selected as our experimental model food product.

Phosphates work in synergy with salt to improve myofibrillar protein extraction, thereby improving the WHC, textural properties and shelf-life stability of processed meats. Although it is a very big challenge to remove phosphates from processed meat products, studies have been made to replace phosphates with natural ingredient sources like starch, proteins and/or fibres (Casco, Veluz, & Alvarado, 2013; Choe et al., 2018; Resconi et al., 2016). However, the complete replacement of phosphates with other natural ingredients may result in negatively impacting product characteristics and emulsion stability (Resconi et al., 2016). Therefore, finding a better alternative source that could mimic phosphate characteristics is of relevance for current and future meat processing needs.

One such promising natural ingredient that could replace added phosphates is fibre. Consumption of dietary fibres can reduce the incidence of colon cancer, diabetes, obesity and cardiovascular diseases (DeVries et al., 2001). Dietary fibres are used to enhance WHC, modify texture, stabilize fat, improve emulsification properties and as a nutritional enhancer in processed meat products (Petracci, Bianchi, Mudalal, & Cavani, 2013). Fibres obtained from wheat, oat, rice bran, bamboo, peach, pea, grape, apple, orange, carrot, citrus, potato, soy and lupin-kernel are commercially available and are commonly used in meat products. A review by Thangavelu et al. (2019), has shown that fibres obtained from vegetable sources, like pea and carrot, are used in comminuted meat products to increase the WHC in both cold- and warm-processing conditions. Several studies have assessed the usage of fibres in marinated products for reduced cook loss and improved juiciness, tenderness and WHC (Choi et al., 2013; Petersson, Godard, Eliasson, & Tornberg, 2014; Zhuang et al., 2016). Many food by-products have been identified as a source of dietary fibre and they can be processed with emerging technologies to produce new commercial food products (Mullen, Álvarez, Pojić, Hadnadev, & Papageorgiou, 2015). Therefore, revalorising food by-products as a source of dietary fibre and phosphate replacers is a realistic approach, able to minimise food waste and to generate healthier and more natural meat products.

Apple pomace (AP) powder and coffee silver skin (CSS) powder are fibre-rich food by-product substances that have the potential of being used as phosphate replacers in sausage batters. AP is a by-product obtained from apple juice production and contains high dietary fibre, polyphenols, vitamins and organic acids. In powdered form, AP from different apple sources is reported to contain about 78.2 to 89.8% of total dietary fibre with IDF/SDF ratio varying from 4.5:1 to 12.9:1 (Figuerola, Hurtado, Estévez, Chiffelle, & Asenjo, 2005). A study made by *Choi et al. (2013)*, showed that AP could contribute to the improvement of WHC and emulsion stability of chicken sausage emulsions. Similarly, CSS is the fibre-rich outermost layer of the green coffee beans obtained as a co-product from the roasting process. Like AP, CSS has a high amount of soluble dietary fibre (~86% of total dietary fibre) and low-fat content, which could act as a suitable phosphate replacing/reducing agent and fibre

content enhancer in sausages emulsions. Studies of the mineral composition of both the ingredients showed that both AP (1.4 g/kg) (Feedinamics) and CSS (1.462 g/kg) (Martuscelli, Esposito, Di Mattia, Ricci & Mastrocola, 2021a) contains a significant amount of phosphorus in their structure. Using AP, CSS as phosphate replacers in breakfast sausages has not been reported yet, and it can prove economical, and the best solution to food by-products management issues.

In the present study, the replacement strategy was investigated using a mixture design to optimise a meat product formulation, using breakfast-type sausages as a model product. Mixture design is an advanced form of response surface methodology (RSM) in which factors are the ingredient mixtures and the responses are their functions in the product. It is a useful statistical method for multiple regression analysis using measurable results (Yolmeh, Khomeiri, & Ahmadi, 2017). The optimal condition(s) or formulation(s) are predicted using the linear, quadratic, special cubic or cubic model methods. The factors used are proportional to each other and help to identify/analyse the important factors (Keenan, Resconi, Kerry, & Hamill, 2014). Therefore, the main objective of the study was to investigate the impact of AP powder and CSS powder as phosphate replacers, using mixture design software approach, on technological properties such as emulsion stability, cook loss, colour, texture profile, WHC, lipid oxidation or water mobility.

2. Methods and Materials

2.1 Sausage Preparation

Fresh pork loins (6.0 < pH >5.4) were purchased from a local butcher (Gleeson Butchers, Dublin, Ireland). The samples were kept under refrigeration at 4 ^{o}C at all stages. The back fat and other visible fats were trimmed off from the lean meat and were minced individually using a meat mincer (Meat Grinder MG510, Kenwood, UK). The seasoning mix (salt (49.36%), white pepper (7.15%), preservative (sodium metabisulphite, E223, 4.35%), ground mace (6.49%), ground nutmeg (5.85%), chilli powder (3.25%), yeast extract(9.28%), ground sage (5.19%), ground marjoram (5.19%), ground ginger (2.6%), antioxidant (Sodium ascorbate, E301, 1.29%)) was purchased from Redbrook Ingredient service (Dublin, Ireland). Sodium tripolyphosphate (STPP) and rusk were purchased from All in All Ingredients (Dublin, Ireland). All breakfast sausage formulations were made containing pork lean meat (58%), pork back fat (20.35%), water/ice (13.45%), rusk (5.75%) and seasoning (1.45%), along with the designed mixture of ingredients (AP, CSS or TPP to a constant level of 1%) according to experimental design. Sausage batters of each formulation were prepared in three separate batches of each 1kg and the sausages were prepared from those batters to obtain the replication. Table 1 describes the experimental design developed by mixture design software (Design Expert v. 10, Stat-Ease Inc., Minneapolis, MN, USA) for the three variable ingredients (X_1 = STPP, X_2 = AP, X_3 = CSS) used in sausage formulations. The ingredients were mixed together in a bowl using hands for about 10 minutes and then the sausage batter was stuffed inside the collagen casing of 23 mm diameter (Select Collagen Casings, Glasgow, Scotland) using the meat mincer with sausage filler (Meat Grinder MG510, Kenwood, UK). The sausages (~10 cm in length) were then kept in tray of height 197 mm x width 155 mm x depth 30 mm (Silverstream packaging Ltd, Cork, Ireland), wrapped using the cling film (gas permeability -2.5 [g 100µm]/ [m^2d], 300 mm x 300 M, Prowrap, Bristol, UK). The trays were assigned based on analysis performed and the number of sausages in the individual tray was dependent on its assigned techno-functional analysis. The packed trays were stored in the retail storage refrigeration unit (EXPO PT, glass door upright display cooler, Framec, Italy) at 3 – 5 °C on the day of manufacture

(Day 0) for further analysis. Trays assigned for WHC, cook loss, NMR analysis, texture and compositional analysis were taken out the next day (Day 1) and respective analyses were performed. Trays assigned for respective time period of TBARS (Day 0, 3, 6 & 9) analysis were taken out on the respective days and analysis was performed.

2.2 WHC and Cook Loss

Three samples from each batch per treatment were taken from the retail unit on the day after the sausage production (day 1) and analysed for WHC as described by Lianji & Chen (1989) with some modifications**.** Approximately 10 g of raw sample (weight B) were weighed in the 50 ml centrifuge tube and heated in a 90 °C water bath for 10 minutes. After heating, the samples were removed from the tubes and cooled to room temperature. The samples were then weighed (weight C) and wrapped with a cheese cloth which was then placed in a 50 ml centrifuge tube filled with absorbent cotton wool (filled up to 1/3 part of the tube). Then the samples were centrifuged for 10 min at 204 x g (4°C) in a Sorvall Lynx 6000 centrifuge (Fischer Scientific Ireland, Dublin, Ireland) and the samples in the tube were weighed (weight A). The WHC and cook loss were then calculated by the following equations:

$$\text{WHC } (\%) = 1 - \frac{(B-A)}{M}\ x\ 100 \qquad \text{Eq (1)}$$

Where M was the total water content in sample meat calculated from the moisture values determined using the SmartTrac rapid fat/moisture analyser (SmartTrac 6, CEM Corporation, NC, USA) based on the AOAC methods 985.14 (1990) by microwave method.

$$\text{Cook Loss } (\%) = \frac{Initial\ weight\ (B) - Cooked\ weight\ (C)}{Initial\ weight\ (B)} x\ 100 \qquad \text{Eq (2)}$$

2.3 Texture Analysis

Five raw sausages (day 1) from each treatment were taken and cooked in a water bath for 20-30 minutes at 73±1 °C until the core of the sausages was cooked at 70 °C temperature. The sausages were then cooled and analysed for texture profile analysis (TPA). The sausages were cored (diam. 14 mm x ht. 20 mm) and axially compressed to 70% of their original height at a crosshead speed of 100mm/min in a two-cycle compression test using an Instron universal testing machine model 5543 (Instron (UK) Ltd., High Wycombe, UK) attached with compression anvil. Force time deformation curves were obtained using a 500 N load cell. The following attributes were recorded using the TPA: hardness (N), gumminess (N), Chewiness (N), springiness (mm) and Cohesion force ratio as described by (Bourne, 1978). The average values for five core per treatment were recorded.

2.4 Colour Measurement

Instrumental Colour measurements of three sausages (day 1) per treatment were recorded using an UltraScan Pro (Hunterlab, Reston, VA, USA) dual beam xenon flash spectrometer, with a viewing port of 25.54 mm and illuminant D65, 10^{o}. The specular component was included (RSEX included) and the size of the trap was 9 mm. Calibration was carried out using a light trap (L = 0) and a white standard tile (L = 100; X = 88.69; Y = 93.58; Z = 100.45), covered in the same material as the sample (transparent cling film) to eliminate any colour readings effect. The sausages were packaged into a PVC film for

measuring colour to maintain uniformity in the reading method. The colour measurements were expressed in L* (Lightness/ darkness), a* (redness/greenness) and b* (yellowness/blueness) units. The values were measured in triplicates and averaged for statistical analysis. The total colour difference (ΔE) was calculated using the formula obtained from Salgado, Fernández, Drago, & Mauri (2011).

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2} \quad \text{Eq (3)}$$

2.5 Lipid Oxidation

This analysis was performed based on the method described by Botsoglou et al. (1994) with some modifications. MilliQ water of 20 mL was added to 1.5 g of raw blended sausage and homogenised with an Ultraturrax homogeniser (Labortechnik, Staufen, Germany) at 13500 rpm for 30s. Cold trichloroacetic acid (25% TCA) of 5 mL was added followed by gentle stirring at 4 ^{o}C for 15 min. Supernatant was obtained by centrifugation at 3500 rpm for 15 min (4 ^{o}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 ^{o}C for 30 min. The tubes were cooled and TBARS were measured at 532 nm using UV-Vis Spectrophotometer (Shimadzu UV – 1700, Columbia, USA). A standard curve was prepared as per the procedure of (Maraschiello, Sárraga, & García Regueiro, 1999) and the results were expressed as milligrams of malondialdehyde per kilogram of sausage (mg MDA/kg sausage). Three sausage samples per batch of each formulation from days 0, 3, 6 and 9 of storage were analysed for the TBARS method.

2.6 Low- field NMR transverse relaxation measurements

The NMR experiment was performed exactly as described by McDonnell et al. (2013) using a Maran Ultra instrument (Oxford Instruments, Abington, Oxfordshire, UK) at a resonating frequency of 23.2 MHz. Three measurements were performed on each sample and averaged for statistical analysis.

2.7 Compositional Analysis

Sausage batters from all three batches of each treatment run were homogenized using the blender (Blender CH180A, Kenwood, China) for 5 minutes and triplicate sub-samples were analysed. Moisture and fat content were determined by SmartTrac5 rapid fat/moisture analyser (SmartTrac 6, CEM Corporation, North Carolina, USA) using AOAC 985.14 (1990) and AOAC 2008.06 (2008) respectively. Protein content was analysed using LECO Nitrogen content determiner (LECO FP628, LECO Corporation, Michigan, USA) using 6.25 as nitrogen to protein conversion factor. Protein analysis was based on the Dumas method and according to AOAC 992.15, (1990). Ash content was determined using a 550 °C Gellenkamp heating furnace using AOAC 920.153 (1920) and salt content (NaCl) was measured from the ash using the Bohr titration method. The total crude fibre content was measured using the ANKOM^{TDF} Dietary Fibre Analyser (ANKOM technology, NY, USA) according to AOAC 991.43 (1995) enzymatic- gravimetric method**.** The values were averaged for statistical analyses.

2.8 Statistical Analysis

The Mixture design experiment was designed and analysed using the statistical software Design Expert (v. 10, Stat- Ease Inc., Minneapolis, MN, USA). The factor response characteristics of the ingredients were studied using I-optimal design type with a quadratic design model for the three ingredient mixture systems with three meat blocks. Experimental design was divided into three meat blocks and for each block, separate batches of pork loins were employed, to consider potential variation derived

from the different raw materials employed in each block. As per the requirement for each meat block, different numbers of pork loins were purchased. The four constraints used were (i) Phosphate STTP (0% ≤ STTP ≥ 0.5%); (ii) AP (X_2) (0%≤ X_2 ≥ 1%); (iii) CSS (X_3) (0%≤ X_3 ≥ 1%); (iv) total ingredients (CSS + AP + STPP = 1%). This design was developed to study the phosphate replacing abilities of AP and CSS. The experiment consisted of 18 runs each representing the different substitution levels for STPP (eg. 100%, 50%, 40% substitution) by the natural ingredients. To create a strong design similar to the works of Keenan et al. (2014) & Baugreet et al. (2017), the experimental design contains, with few modifications, duplicates of maxima of the replacers without any phosphate percent (run 1 & 2, 3 & 7), maxima of ingredients with reduced phosphate percent (run 9 & 12, 16 & 17) and equal distribution of both ingredients with reduced phosphate percentage (run 5 & 6). The table was completed with the responses obtained after the analysis of the sausages and the experiments were carried out in random order to eliminate the extraneous factors on the observed responses. Parameters such as WHC, cook loss, texture, proximate composition (fat, moisture, protein and ash) were assessed as responses. After the results collection, the responses were calculated using the linear (Eq. (4)), quadratic (Eq. (5)) or Scheffe's special cubic models (eq. (6)) depending on the degree of fit, predictive power and robustness of the model.

$$Y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 \quad \text{Eq (4)}$$

$$Y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad \text{Eq (5)}$$

$$Y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3 \quad \text{Eq (6)}$$

Where Y is the predicted dependent variable; β is the equation coefficient and x is the proportion of pseudo components. The dependent variables were analysed, and model subjected to analysis of variance (ANOVA) to determine the significance ($P < 0.05$), determination coefficient (R^2) and lack of fit. Significant dependent variables were subsequently analysed using the software's optimisation tool and used to predict optimal sausage formulations.

3 Results & Discussions

3.1 Compositional Analysis

Mean values of moisture, fat, protein, ash, fibre and salt content of the sausages were given in Table 2. Each parameter was thoroughly analysed and fitted with an appropriate significant response surface model. The model of compositional analysis of moisture, protein, fat and salt (sodium chloride) contents were found to be not significantly different, which was expected since the formulations were designed to be identical (approximate target values: Moisture ~ Moisture ~ 65%; Fat ~ 12.4%; Ash ~ 2%; Protein ~ 15.14%; Salt ~ 0.8%) but for the 1% corresponding to functional ingredients. The ash content of the sausages was fitted with a linear equation. The model was significant with the experimental data fit ($R^2 = 0.72$). From Fig. 1d, the ash content gradually increased with an increased concentration of phosphorus. It can be explained by the definition that ash content represents inorganic mineral residue remaining after the ignition and complete oxidation of organic matter (Nunes, De Oliveira Matias, & Da Silva Catalao, 2017). Thus, phosphorus, an inorganic mineral, when added increased the ash content of the sausages.

It was found that a special cubic equation can fit the total dietary fibre content of the sausages (R^2= 0.74) and the model was significant proving the fibre analysis model is valid. In general, meat is

deficient in any dietary fibre content. However, the total dietary fibre analysis showed that there was little amount of dietary fibres present in sausages. It was highly due to the addition of AP and CSS to the formulations since both AP (78-89%) and CSS (86%) are rich in their dietary fibre content (Gemechu, 2020; Iriondo-DeHond et al., 2019). The average total fibre content of most of the sausages lies in the range of 1- 3%; thus, justifying the addition of AP and CSS as potential phosphate replacers and fibre enhancers in the sausage formulations.

3.2 WHC & cook loss

Results of WHC and cook loss of different sausage formulations are given in Table 3. WHC values were fitted with a quadratic model and the cook loss data was fitted with a linear model. The predicted model for both the WHC and cook loss was significant with a fit of experimental data (R^2 = 0.92 for WHC; R^2 = 0.74 for cook loss). The RSM model for prediction of WHC and cook loss in terms of the actual components was determined as follows:

$$WHC = 74.18\ x_1 + 72.72\ x_2 + 73.82\ x_3 + 55.23\ x_1x_2 + 46.66\ x_1x_3 + 10.37\ x_2x_3 \quad \text{Eq (7)}$$

$$\text{Cook loss} = -0.48\ x_1 + 12.37\ x_2 + 13.03\ x_3 \quad \text{Eq (8)}$$

The results of ANOVA and post-result analysis of WHC showed that linear and quadratic effects of phosphates were the most affecting factors on the WHC of sausages. It was observed that the values of WHC were low (68-72%) for the sausages containing no STPP in them when compared to the sausages containing different concentrations of the STPP (Fig. 2a). The higher values of WHC (85-88%) were observed in the sausage formulations containing 0.5% of added STPP (run 4, 13, 14). From Table 3, it was observed that the WHC of sausages decreased with the reduction in STPP concentration when compared to the one with 0.5% STPP added in them. This can be attributed to phosphates being very good meat water binders and help in improving the WHC of the meat products (Nguyen Huynh Bach Son, 2011; Thangavelu et al., 2019). However, the difference in reduction of WHC of sausages with reduced STPP (run 5, 6 - 0.202%, run 9 - 0.256%, run 12, 16, 17 - 0.257%, run 18 - 0.259%) was very small when compared with the runs 4, 13 and 14. It was also noted that run 11 with 0.329% of STPP recorded higher WHC (88.6%) along with AP and CSS mixtures. It was evident from the results that the STPP has a positive interaction with the alternative ingredients and the addition of AP and CSS to the sausage formulations has a significant positive impact on the WHC. This could be explained by the synergistic contribution of different concentrations of AP and CSS mixtures in their formulations. Despite the reduction in STPP concentration, the addition of AP and CSS improved the WHC (Ballesteros, Teixeira, & Mussatto, 2014; Younis & Ahmad, 2015) because of their high dietary fibre content.

Similar trend as of WHC values, although in the opposite direction, was observed in cook loss data of the sausages. Sausages containing reduced levels of STPP recorded lower cook loss values (5.7-13.4%) than the ones without any STPP (9.3 -18%) added in them. This proved that both the ingredients AP and CSS were unable to fully counteract the absence of STPP in terms of WHC and cook loss. Thus, proving the complete replacement of STPP in sausages is a challenging task.

However, lower cook loss value (5.6%) was recorded for the sausage containing 0.329% of STPP with 0.484% AP & 0.187% CSS in them. It was also noted that the cook loss values of formulations containing higher STPP level (0.5%) were nearly the same as that of ones containing lower level STPP

thus implying the addition of AP and CSS powders to the formulations compensated for the increased cook loss due to the reduction in STPP concentration. This can be explained by their ability to decrease the cooking loss in the meat products (Younis & Ahmad, 2015; Martuscelli, Esposito, and Mastrocola, 2021b) thus making both these ingredients as potential phosphate replacers in meat products.

3.3 NMR Measurements

The increase in WHC of sausages due to the addition of AP and CSS can be explained in detail using the low field NMR T_2 relaxation data of the samples. The relaxation data can be used to understand the water mobility and its distribution in the meat matrix. It was reported that there is a high correlation between the relaxation data T_2 and WHC traits of the meat matrix (Bertram, Purslow, & Andersen, 2002).

It was reported in the literature (Bertram, Andersen, & Karlsson, 2001), that the NMR relaxation time distribution graph of meat samples contain three components distributed at different time intervals when fitted with exponential fitting. The first component that is a minor one is distributed between 1- 10 ms, the second one that is a major component is distributed between 10 -100ms and the final component is distributed between 100 – 1000 ms. It was understood that the relaxation time obtained through the low field NMR analysis on meat samples can be used to demonstrate the water mobility and the area under the curve can indicate the amount of water present within the meat matrix. Thus, the T_{2b} relaxation time (1 -10 ms peak value) corresponds to the bound water that is bound to proteins, the T_{21} relaxation time (40 -60 ms peak value) corresponds to the myofibrillar water, and the relaxation time T_{22} relaxation time (150 – 400 ms peak value) corresponds to the free water present outside the myofibril lattice and muscle cell which can be considered as a potential drip loss (Bertram et al., 2001).

In this study, the three peaks obtained from the multi-exponential fitting of T_2 relaxation data resembled the observations mentioned by Bertram et al. (2001). Table 2 contains the percentage population distribution $T_{2b,}$ T_{21}, T_{22} of different sausage formulations. Response surface method analysis shows that there was a significant change in the $T_{2b,}$ T_{21} values and there were not any significant changes in T_{22} relaxation data values implying that the addition of ingredients AP and CSS have much positive effect on controlling the drip loss despite the reduction in phosphate concentration. The T_{2b} and T_{21} values were fitted with cubic and special cubic equations (R^2 = 0.96 and R^2 = 0.87) respectively. In general, the protein bound water molecules (T_{2b}) do not vary with any mechanical stress and changes in the meat matrix (Bertram et al., 2001; McDonnell et al., 2013). However, in this study, there was a significant change in the T_{2b} values due to the addition of AP and CSS. It was observed from the results, the values of T_{21} and T_{2b} of all the sausages were very much in range to each other within the blocks, which may be due to the different meat used in each one of them. It was observed that the ingredients did not affect the T_{22} distribution time and its population percentages within the meat blocks. The population percentages data of all three curves of all 18 treatments showed that the distribution of water was almost similar to one another within the meat blocks and little variation in the area and population distribution was observed in terms of the concentration of the ingredients. It was observed from the study, the NMR curve area and water distribution of the treatment run 1, 2, 3, 7, 10 and 15 (runs without STPP) had almost similar or higher T_{21} water distribution, when compared with the other treatment runs with STPP in them. Similar trend in opposite was observed with T_{22} values where the treatment runs without STPP (1, 2, 3, 7, 10 and

15) had lesser water distribution and lesser T_{22} area when compared with the ones with STPP in them, proving the above statement that the addition of AP and CSS has a positive effect on controlling the drip loss despite the reduction in phosphate concentration.

3.4 Instrumental Colour

Experimental data for colour of the sausages is shown in Table 3. The responses observed for all three parameters of colour measurements L*, a* and b* were significantly different in the response surface method. Linear model was predicted for L* to be significant with a reasonable experimental fit (R^2 = 0.85). It was noted from Fig. 2c, the increased addition of CSS to formulations decreased the L* values, which was in agreement with the experimental analysis of Martuscelli, Esposito, and Mastrocola (2021b) where the L* of CSS decreased with increased concentrations. It was also seen that formulations runs 9, 12 and 14 recorded higher L* values than the other formulations indicating the interaction effect of STPP with AP in increasing the L* values. In regards to a* values of the sausages, it is clear from Fig. 2d that increased STPP concentration increased a* values up to a maximum of 7.30 and in other terms increased addition of AP and CSS to the sausages reduced a* values of the meat. The values of a* were fitted with a linear model with significant data of R^2 = 0.46. The inclusion of CSS in the formulations decreased the b* values when compared with sausages containing AP and STPP (Fig. 2e). The b* values decreased from a high of 19.67 and 20.13 (full STPP and full AP respectively) to a low of 17.8 (full CSS) with an increased concentration of CSS. The model for b* values was fitted with a significant linear model with an experimental fit of only R^2 = 0.53 between the predicted and adjusted values. In general, Younis and Ahmad (2015), reported that the overall increased values of L*, a* and b* could be observed due to the inclusion of AP in buffalo meat sausages. This is in contradiction with the data obtained, which may effectively be due to the addition of CSS alongside AP that caused a reduction in the colour parameters. On observing the ΔE (measured using Eq. 3) of the sausages compared with the one from run 4 (containing maximum STPP and equal AP: CSS) in Table 3, it was observed that there were not much visible differences (1< ΔE <2) for most of sausages trial runs (1, 2, 5, 10, 11, 13, 15 and 17). However, a clear difference in colour (ΔE >2.5) was observed in trial runs (3, 6, 7, 8, 9, 12, 14, 16 and 18). It is understood that no differences in colour between the samples would be observed if 1< ΔE <2 and a clear difference in colour could be observed if ΔE > 2.5 between two samples (Alvarez, Drummond, & Mullen, 2018). On comparing the above results of ΔE, the same trial run combination 5 and 6 does not fall under the same category. A similar trend was observed for the other trial run combinations 16 and 17 where the trial run 5 and 17 had no visible difference when compared with trial run 4 whereas the results of trial run 6 and 16 showed a clear colour difference when compared with trial run 4. The colour difference values for 16 and 17 are negligible and there was very little observed visual difference between the sausage formulations disregarding the experimental data. It was also observed from Table 3, that the formulation run 3 and 7 with higher CSS concentration (CSS -1 %) showed greater colour difference (ΔE > 4) when compared with the trial run 1 and 2 where the AP concentration is higher (AP= 1%; ΔE <2). Thus, the complete replacement of STPP with AP and CSS could present a challenge since the change in colour difference could affect the consumer preference on the products.

3.5 Textural Properties

The texture of processed meat products is an important parameter from the customer's perspective. On post research analysis, similar to the observations of Keenan et al. (2014) in fat replaced sausages

with added inulin, four of the five textural properties were significantly different except cohesive force. From the results, the reduction of STPP in sausages composition significantly reduced the hardness, chewiness, gumminess and springiness values when compared with the sausages containing higher STPP content. This stable textural property with increased addition of STPP could be explained by its ability to increase the ion strength of meat to extract the salt-soluble proteins (Choe et al., 2018). Mean values of the textural parameters were found in Table 3. Hardness of sausages fitted with a linear model was found to be significant with an experimental data fit (R^2 = 0.64). Fig. 3a shows that there was a significant decrease in the hardness of sausages with an increase in AP and CSS (Hardness range: 13-24 N) concentrations when compared with the sausages with high STPP content (Hardness range (37-54 N). Also, from the observed data of Table 3, three different meat blocks seemed to have an effect on the overall textural data of sausages especially block 3. Hence, an increase in hardness values can be attributed to the different meat blocks used and the different inherent textural properties of the meat samples employed, rather than an impact for the different formulations (Wang, Xu, & Zhou, 2009). Similar trend was noticed in the chewiness of the sausages. Chewiness was found to be significant when fitted with a linear model with R^2 = 0.68. It was observed from Fig. 3b, chewiness decreased with reduced STPP levels. Gumminess, in turn, was fitted with a cubic model and was found to be significant with a good fit of R^2 = 0.94. From the experimental data (Table 3), it was evident that chewiness and gumminess values were higher for sausages with higher STPP content and reduced values were recorded for sausages without any added STPP but with added ingredients in them. Similarly, springiness was found to be significant when fitted with a quadratic model with the agreement of experimental data (R^2 = 0.93). As with other textural parameters, springiness values of the sausages were reduced with the addition of AP and CSS. This reduction in springiness could be explained by the formation of the protein-water or protein-protein gelation networks formed due to the addition of fibres to the meat products (Han and Bertram, 2017). Thus, the complete replacement of STPP with AP and CSS could be challenging since they could result in the softer texture of the product, thereby influencing the consumer preference over the product.

3.6 Lipid Oxidation

Lipid oxidation analysis is based on the detection of malondialdehyde (MDA) formed as a by-product of peroxidation of polyunsaturated fatty acids and esters, associated with off-flavours and off-aromas in meat products (Maraschiello, Sárraga, & García Regueiro, 1999; Pérez-Andrés et al., 2020). Table 2 shows the TBARS results of day 0, 3, 6 and 9 sausage samples that were stored at 4 ^{o}C. Although not considered as antioxidants, phosphates added in meat products inhibit lipid oxidation by chelating the metal ions and removing the catalysts responsible for lipid oxidation (Choe et al., 2018). It can be observed that decreasing the concentration of STPP in the sausages resulted in a slight increase in TBARS values. However, sausage formulations containing no phosphates recorded TBARS value (day 9) very much in range to those containing different levels of phosphates.

All the lipid oxidation values were analysed using Mixture design software. Day 0 and 6 analysis was fitted with linear equations (R^2 =0.47 and R^2= 0.46 respectively) and were highly significant. Similarly, day 9 was fitted with a special cubic model (R^2 = 0.76) and was statistically significant with their experimental data. The values of day 3 were not significantly different from each other, which implies none of the ingredients have any impact on TBARs value for day 3. From the results of Table 2, it was observed that the TBARS MDA values of day 0 and 3 are very much similar to each other and falls within the range of 0.15 – 0.30 mg MDA/kg. The real difference in lipid oxidation values can be

observed from the TBARS analysis of days 6 and 9. However, the day 9 values of all the sausages lie within the threshold limit of 2-2.5 mg MDA/ kg where no rancid flavours were observed in meat and meat products (Campo et al., 2006; Zhang et al., 2019).

On observation, samples containing higher concentrations of AP (run 1, 2, 8, 9, 11, 12, 14 and 15) recorded comparatively higher MDA content on day 9 when compared with the other products. Considering the application of CSS, the sausages containing a high concentration of CSS (run 3, 7, 10, 13, 16 and 18) showed a positive response against the MDA content. Their low values can be attributed to the fact that CSS is very good antioxidant (Iriondo-DeHond et al., 2019). This may indicate that the effect of reducing the phosphates on TBARS values are negligible and they are well counter- interacted by the addition of AP and CSS to the mixture. The antioxidant property of the AP and CSS can inhibit the lipid oxidation in phosphate-replaced sausages.

3.7 Optimisation of sausage formulation mixtures

Numerical optimisation was performed using the Design expert optimisation tool to obtain the satisfactory optimum level of independent variables that help to improve the overall quality of the products. In this study, the desirability response method was used to obtain the optimised concentration of independent variables (Keenan et al., 2014). The desirability function suggests the desired value range for each response, which varied from 0 to 1. The goal objective was to maximise or minimise particular response(s) to obtain the desired product range. From the results, it was observed, the complete replacement of STPP in Irish breakfast sausages was difficult since the complete replacement could reduce the product quality. Thus, in this study, the addition of phosphates level was minimised to the range of 0 to 0.2% with AP and CSS addition maximised from 0 to 1% with the total amount of all three ingredients employed was set at 1% goal. The dependent response WHC was maximised whereas cook loss and TBARS values of days 0, 3, 6 and 9 were minimised. These specific constraints were applied to highlight the main functionality of phosphates in meat products, so that, the solutions obtained will focus on reducing the phosphate content without any quality reduction. In other words, it was because of their closer association with the phosphates. The software predicted three possible optimised solutions with reduced phosphate levels that could improve the desired product quality. The first and second formulations had 0.2% of phosphates and the third one had 0.06 % of added phosphates. The three formulations mixture contents (STPP: AP: CSS) were (i) 0.2:0.22:0.58%, (ii) 0.2:0:0.8%, and (iii) 0.06:0.94:0%. The overall desirability of the three formulations was 0.67, 0.64 and 0.45 respectively. The predicted values of the dependent variables for the three formulations can be found in Table 4, which contained maximum WHC and less cook loss values. The experimental validation of the three formulation mixtures was conducted in two trials to confirm the reliability of the model. The obtained experimental WHC and cook loss values of the three experimental models were as follows: (i) 89.5±1.1 and 4.7±0.7, (ii) 90.2±0.5 and 4.5±0.4 and (iii) 87.3±2.5 and 5.7±0.3. It was noted that the obtained experimental values for WHC for three formulations were higher than the expected values and similarly the obtained cook loss values were lower than the expected values. This difference in the expected and obtained values can be explained by the property of meat samples purchased, which could have affected the WHC properties of the final sausage product. However, it was found that there was a constantly increasing trend in obtained experimental WHC values when compared with the expected values of the three mixture formulations. All other measured parameter values were very much in order with the expected values.

4 Conclusions

The response surface methodology study of phosphate replacing ability of AP and CSS was well established using a mixed design approach using the Design expert software. The method helped to reduce the number of experiments, studying several sausage formulations with reduced phosphate concentration and studying the impact of different ingredient combinations on the various physicochemical properties of the sausage products. Analysis of WHC and cook loss, major functionalities influenced by STPP, showed that including AP and CSS enhanced the WHC (88.6%) and reduced the cook loss (5.6%) in the formulation with reduced STPP concentration (0.33%) when compared with the likes of formulation with higher STPP concentration (0.5%). However, as a limitation of the study, the complete replacement of STPP resulted in reduced WHC, increased cook loss values and decreased textural properties. Thus, the mathematical models generated to describe the impact of the different formulations were used for the formulation optimisation; optimised recipes significantly reduced the STPP concentration (down to 0.06%-0.2%) regarding the control (0.5%) with an acceptable desirability level (0.44-0.68) where the formulations either meet or exceed the reporting requirements in reducing the phosphates. This addition of AP and CSS to Irish breakfast sausages could attract health minded consumers as they can reduce phosphate related health problems.

Acknowledgments

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Literature

Álvarez, C., Drummond, L., & Mullen, A. M. (2018). Protein recovered from meat co-products and processing streams as pork meat replacers in Irish breakfast sausages formulations. *LWT, 96,*679-685. doi: 10.1016/j.lwt.2018.06.020

AOAC 920.153 (1920). Association of Official Analytical Chemists (AOAC) official method 920.153. Ash of meat. Official methods of analysis of the AOAC International, Gaithersburg, Maryland: AOAC International.

AOAC 985.14 (1990). Association of Official Analytical Chemists (AOAC) official method 985.14. Moisture in Meat and Poultry Products – Rapid. By Microwave drying. Official methods of analysis of the AOAC International, Gaithersburg, Maryland: AOAC International.

AOAC 992.15 (1992). Association of Official Analytical Chemists (AOAC) official method 992.15. Crude protein in meat and meat products. Combustion method. Official methods of analysis of the AOAC International, Gaithersburg, Maryland: AOAC International.

AOAC 991.43 (1995). Association of Official Analytical Chemists (AOAC) official method 991.43. Total, Soluble and Insoluble dietary fiber in foods. By Enzymatic-Gravimetric method, MES –TRIS buffer. Official methods of analysis of the AOAC International, Gaithersburg, Maryland: AOAC International.

AOAC 2008.06 (2008). Association of Official Analytical Chemists (AOAC) official method 2008.06. Moisture and fat in meats. Microwave and nuclear magnetic resonance analysis.Official methods of analysis of the AOAC International, Gaithersburg, Maryland: AOAC International.

Ballesteros, L. F., Teixeira, J. A., & Mussatto, S. I. (2014). Chemical, functional, and structural properties of spent coffee grounds and coffee silverskin. *Food and bioprocess technology, 7*(12), 3493-3503. doi: 10.1007/s11947-014-1349-z

Baugreet, S., Kerry, J. P., Allen, P., & Hamill, R. M. (2017). Optimisation of protein- fortified beef patties targeted to the needs of older adults: a mixture design approach. *Meat Science, 134,* 111-118. doi: 10.1016/j.meatsci.2017.07.023

Bertram, H. C., Andersen, H. J., & Karlsson, A. H. (2001). Comparative study of low-field NMR relaxation measurements and two traditional methods in the determination of water holding capacity of pork. *Meat science, 57*(2), 125-132. doi: 10.1016/S0309-1740(00)00080-2

Bertram, H. C., Purslow, P. P., & Andersen, H. J. (2002). Relationship between meat structure, water mobility, and distribution: a low-field nuclear magnetic resonance study. *Journal of agricultural and food chemistry, 50*(4), 824-829. doi: 10.1021/jf010738f

Botsoglou, N. A., Fletouris, D. J., Papageorgiou, G. E., Vassilopoulos, V, N., Mantis, A. J., & Trakatellis, A. G. (1994). Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. *Journal of Agricultural and Food Chemistry, 42*(9), 1931-1937. doi:10.1021/jf00045a019

Bourne, M. C. (1978). Texture profile analysis. *Food Technology 33*(7), 62-66.

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., & Richardson, R. I. (2006). Flavour perception of oxidation in beef. *Meat Science, 72*(2), 303-311. doi:10.1016/j.meatsci.2005.07.015

Casco, G., Veluz, G. A., & Alvarado, C. Z. (2013). SavorPhos as an all-natural phosphate replacer in water- and oil-based marinades for rotisserie birds and boneless-skinless breast. *Poultry science, 92*(12), 3236-3243. doi: 10.3382/ps.2013-03142

Chemist, A. A. o. C. (2001). The definition of dietary fiber. *Cereal Foods World* (Vol. 46, pp. 112-129).

Choe, J., Lee, J., Jo, K., Jo, C., Song, M., & Jung, S. (2018). Application of winter mushroom powder as an alternative to phosphates in emulsion-type sausages. *Meat science, 143*, 114-118. doi: 10.1016/j.meatsci.2018.04.038

Choi, Y.-S., Park, K.-S., Kim, H.-W., Hwang, K.-E., Song, D.-H., Choi, M.-S., Lee, S.-Y., Paik, H.-D., Kim, C.-J. (2013). Quality characteristics of reduced-fat frankfurters with pork fat replaced by sunflower seed oils and dietary fiber extracted from makgeolli lees. *Meat science, 93*(3), 652-658. doi: 10.1016/j.meatsci.2012.11.025

DeVries, J., Camire, M., Cho, S., Craig, S., Gordon, D., Jones, J., Li, B., Lineback, D., Prosky, L., Tungland, B. (2001). The definition of dietary fiber. *Cereal Foods World, 46*(3), 112-129.

Figuerola, F., Hurtado, M. a. L., Estévez, A. M. a., Chiffelle, I., & Asenjo, F. (2005). Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. *Food chemistry, 91*(3), 395-401. doi: 10.1016/j.foodchem.2004.04.036

Gemechu, F. G. (2020). Embracing nutritional qualities, biological activities and technological properties of coffee byproducts in functional food formulation. *Trends in food science & technology, 104*, 235-261. doi: 10.1016/j.tifs.2020.08.005

Han, M. & Bertram, H. C. (2017). Designing healthier comminuted meat products: Effect of dietary fibers on water distribution and texture of a fat-reduced meat model system. *Meat Science*, 133, 159-165. doi:10.1016/j.meatsci.2017.07.001

Hruska, K. A., Mathew, S., Lund, R., Qiu, P., & Pratt, R. (2008). Hyperphosphatemia of chronic kidney disease. *Kidney International, 74*(2), 148-157. doi: 10.1038/ki.2008.130

Iriondo-DeHond, A., Aparicio García, N., Fernandez-Gomez, B., Guisantes-Batan, E., Velázquez Escobar, F., Blanch, G. P., San Andres, M. I., Sanchen-Fortun, S., del Castillo, M. D. (2019). Validation of coffee by-products as novel food ingredients. *Innovative food science & emerging technologies, 51*, 194-204. doi: 10.1016/j.ifset.2018.06.010

Kalantar-Zadeh, K., Gutekunst, L., Mehrotra, R., Kovesdy, C. P., Bross, R., Shinaberger, C. S., Noori, N., Hirschberg, R., Benner, D., Nissenson, A. R., Kopple, J. D. (2010). Understanding sources of dietary phosphorus in the treatment of patients with chronic kidney disease. *Clinical journal of the American Society of Nephrology, 5*(3), 519-530. doi: 10.2215/CJN.06080809

Keenan, D. F., Resconi, V. C., Kerry, J. P., & Hamill, R. M. (2014). Modelling the influence of inulin as a fat substitute in comminuted meat products on their physico-chemical characteristics and

eating quality using a mixture design approach. *Meat Science, 96*(3), 1384-1394. doi: 10.1016/j.meatsci.2013.11.025

Lianji, M., & Chen, N. (1989). *Research in improving the water holding capacity (WHC) of meat in sausage products.* Paper presented at the ICoMST.

Maraschiello, C., Sárraga, C., & García Regueiro, J. A. (1999). Glutathione peroxidase activity, TBARS, and α-tocopherol in meat from chickens fed different diets. *Journal of agricultural and food chemistry, 47*(3), 867-872. doi: 10.1021/jf980824o

Martuscelli, M., Esposito, L., Di Mattia, C. D., Ricci, A., & Mastrocola, D. (2021a). Characterization of coffee silver skin as potential food-safe ingredient. *Foods*, 10, 1367. doi:10.3390/foods10061367

Martuscelli, M., Esposito, L., & Mastrocola, D. (2021b). The role of coffee silver against oxidative phenomena in newly formulated chicken meat burgers after cooking. *Foods*, 10, 1833. doi:10.3390/foods10081833

McDonnell, C. K., Allen, P., Duggan, E., Arimi, J. M., Casey, E., Duane, G., & Lyng, J. G. (2013). The effect of salt and fibre direction on water dynamics, distribution and mobility in pork muscle: a low field NMR study. *Meat Sci, 95*(1), 51-58. doi: 10.1016/j.meatsci.2013.04.012

Mullen, A. M., Álvarez, C., Pojić, M., Hadnadev, T. D., & Papageorgiou, M. (2015). Chapter 2 - Classification and target compounds (pp. 25-57): Elsevier Inc.

Nguyen Huynh Bach Son, L. (2011). Use of phosphates in meat products. *African journal of biotechnology, 10*(86). doi: 10.5897/AJBX11.023

Nunes, L., Jr., De Oliveira Matias, J. C., & Da Silva Catalao, J. P. (2017). Torrefaction of biomass for energy applications: from fundamentals to industrial scale. Saint Louis: Elsevier Science & Technology.

Pérez-Andrés, J. M., de Alba, M., Harrison, S. M., Brunton, N. P., Cullen, P. J., & Tiwari, B. K. (2020). Effects of cold atmospheric plasma on mackerel lipid and protein oxidation during storage. *Food science & technology, 118*, 108697. doi: 10.1016/j.lwt.2019.108697

Petersson, K., Godard, O., Eliasson, A.-C., & Tornberg, E. (2014). The effects of cereal additives in low-fat sausages and meatballs. Part 2: Rye bran, oat bran and barley fibre. *Meat science, 96*(1), 503-508. doi: 10.1016/j.meatsci.2013.08.019

Petracci, M., Bianchi, M., Mudalal, S., & Cavani, C. (2013). Functional ingredients for poultry meat products. *Trends in food science & technology, 33*(1), 27-39. doi: 10.1016/j.tifs.2013.06.004

Resconi, V. C., Keenan, D. F., Barahona, M., Guerrero, L., Kerry, J. P., & Hamill, R. M. (2016). Rice starch and fructo-oligosaccharides as substitutes for phosphate and dextrose in whole muscle cooked hams: Sensory analysis and consumer preferences. *Food science & technology, 66*, 284-292. doi: 10.1016/j.lwt.2015.10.048

Ritz, E., Hahn, K., Ketteler, M., Kuhlmann, M. K., & Mann, J. (2012). Phosphate additives in food--a health risk. *Deutsches Ärzteblatt international U6 - Journal Article, 109*(4), 49-55. doi: 10.3238/arztebl.2012.0049

Salgado, P. R., Fernández, G. B., Drago, S. R., & Mauri, A. N. (2011). Addition of bovine plasma hydrolysates improves the antioxidant properties of soybean and sunflower protein-based films. *Food hydrocolloids, 25*(6), 1433-1440.

Shan, L. C., De Brún, A., Henchion, M., Li, C., Murrin, C., Wall, P. G., & Monahan, F. J. (2017). Consumer evaluations of processed meat products reformulated to be healthier – a conjoint analysis study. *Meat science, 131*, 82-89. doi: 10.1016/j.meatsci.2017.04.239

Thangavelu, K. P., Kerry, J. P., Tiwari, B. K., & McDonnell, C. K. (2019). Novel processing technologies and ingredient strategies for the reduction of phosphate additives in processed meat. *Trends in food science & technology, 94*, 43-53. doi: 10.1016/j.tifs.2019.10.001

Wang, P., Xu, X.-l., & Zhou, G.-h. (2009). Effects of meat and phosphate level on water-holding capacity and texture of emulsion-type sausage during storage. *Agricultural sciences in China, 8*(12), 1475-1481. doi: 10.1016/S1671-2927(08)60361-2

Yolmeh, M., Khomeiri, M., & Ahmadi, Z. (2017). Application of mixture design to introduce an optimum cell-free supernatant of multiple-strain mixture (MSM) for lactobacillus against food-borne pathogens. *Lwt-Food Science and Technology, 83*, 298-304. doi: 10.1016/j.lwt.2017.05.035

Younis, K., & Ahmad, S. (2015). Waste utilization of apple pomace as a source of functional ingredient in buffalo meat sausage. *Cogent food & agriculture, 1*(1), 1119397. doi: 10.1080/23311932.2015.1119397

Zhang, Y., Holman, B. W. B., Ponnampalam, E. N., Kerr, M. G., Bailes, K. L., Kilgannon, A. K., Collins, D., & Hopkins, D. L. (2019). Understanding beef flavour and overall liking traits using two different methods for determination of thiobarbituric acid reactive substance (TBARS). *Meat Science, 149*, 114-119. doi: 10.1016/j.meatsci.2018.11.018

Zhuang, X., Han, M., Kang, Z.-l., Wang, K., Bai, Y., Xu, X.-l., & Zhou, G.-h. (2016). Effects of the sugarcane dietary fiber and pre-emulsified sesame oil on low-fat meat batter physicochemical property, texture, and microstructure. *Meat science, 113*, 107-115. doi: 10.1016/j.meatsci.2015.11.007

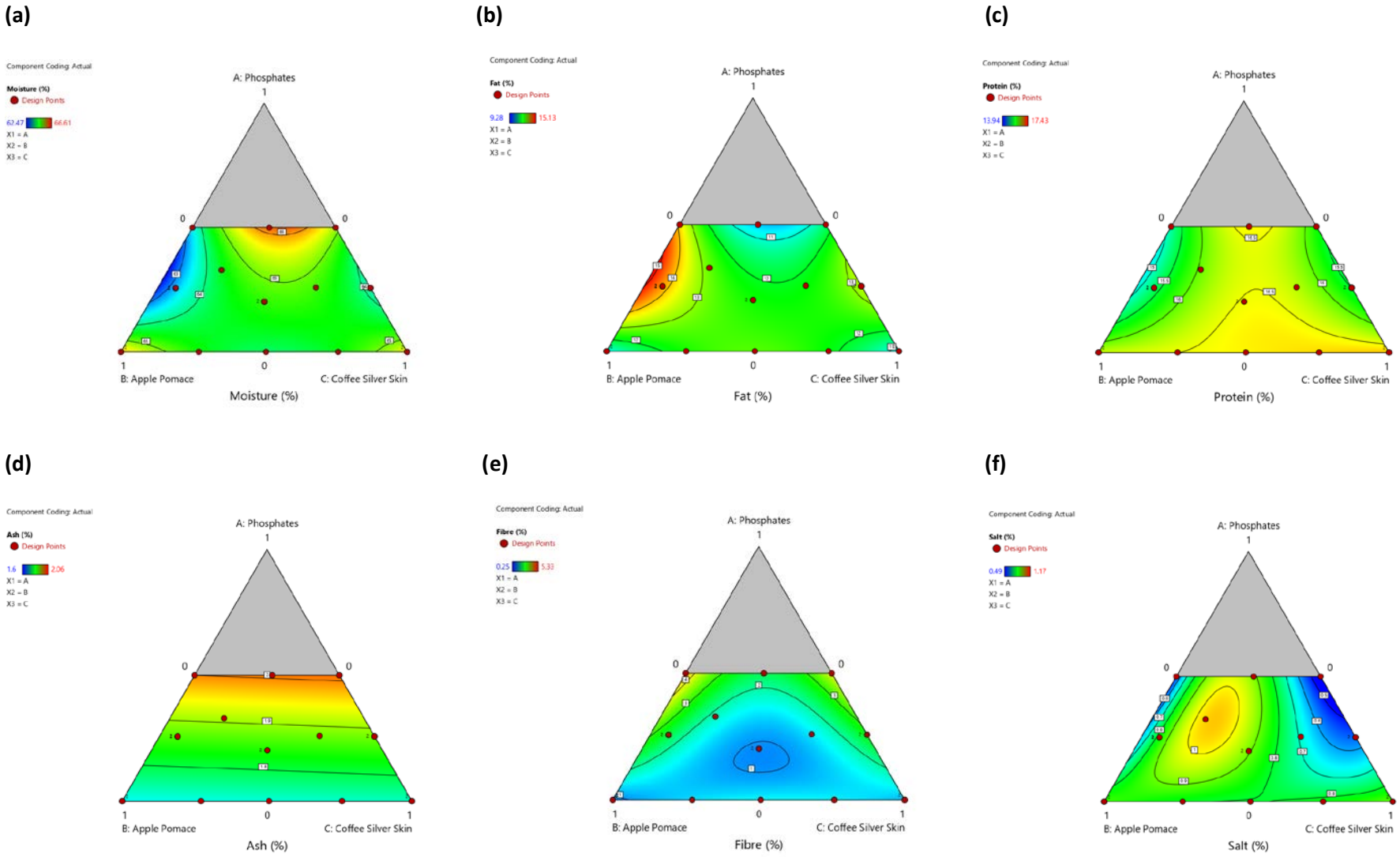


Fig. 1. Contour plots of different proximate compositional parameters of sausages (1a) Moisture, (1b) Fat, (1c) Protein, (1d) Ash, (1e) Fibre and (1f) salt.

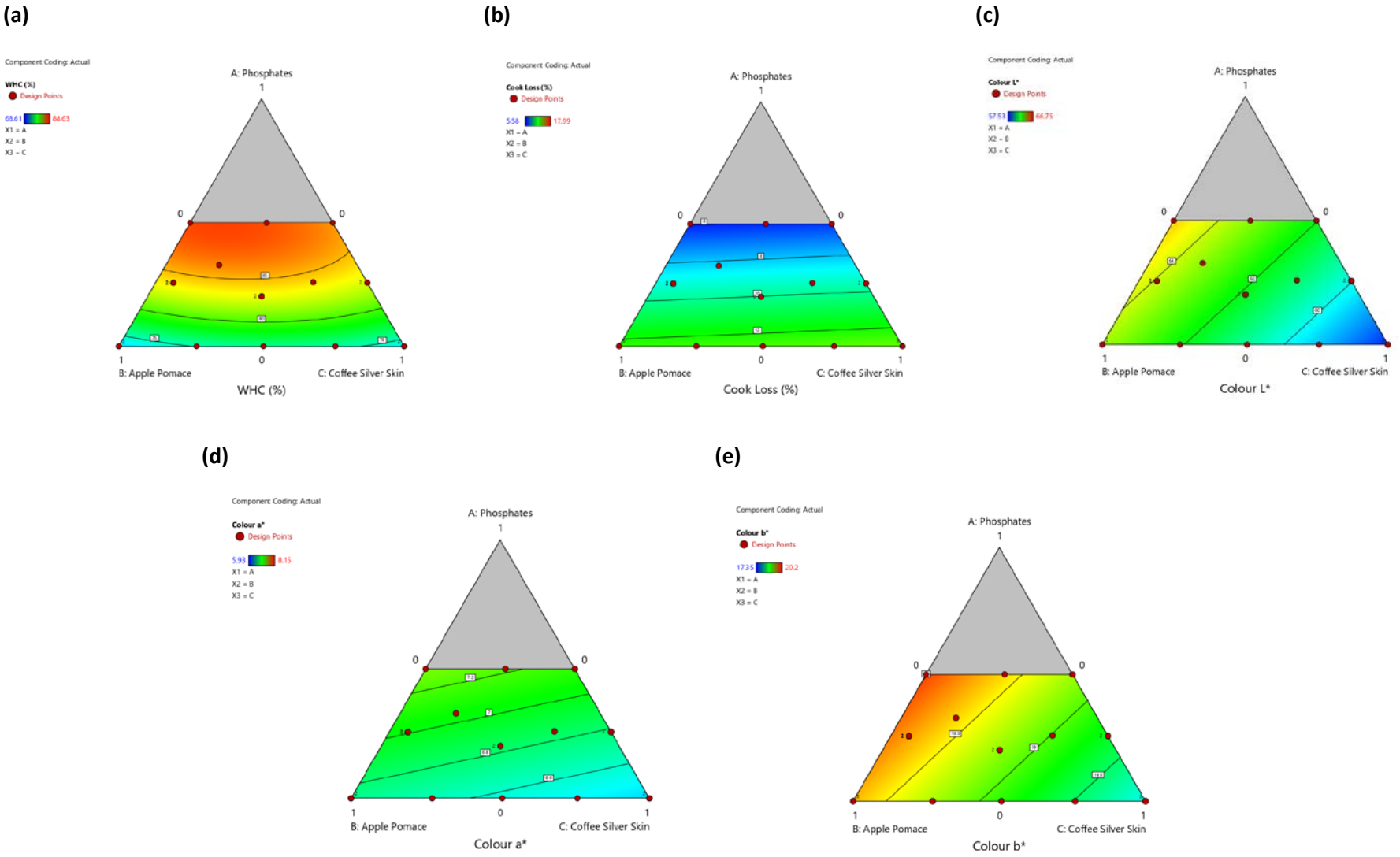


Fig. 2. Contour plots of water holding capacity (2a), cook loss (2b) and instrumental colour parameters L* (2c), a* (2d) & b* (2e).

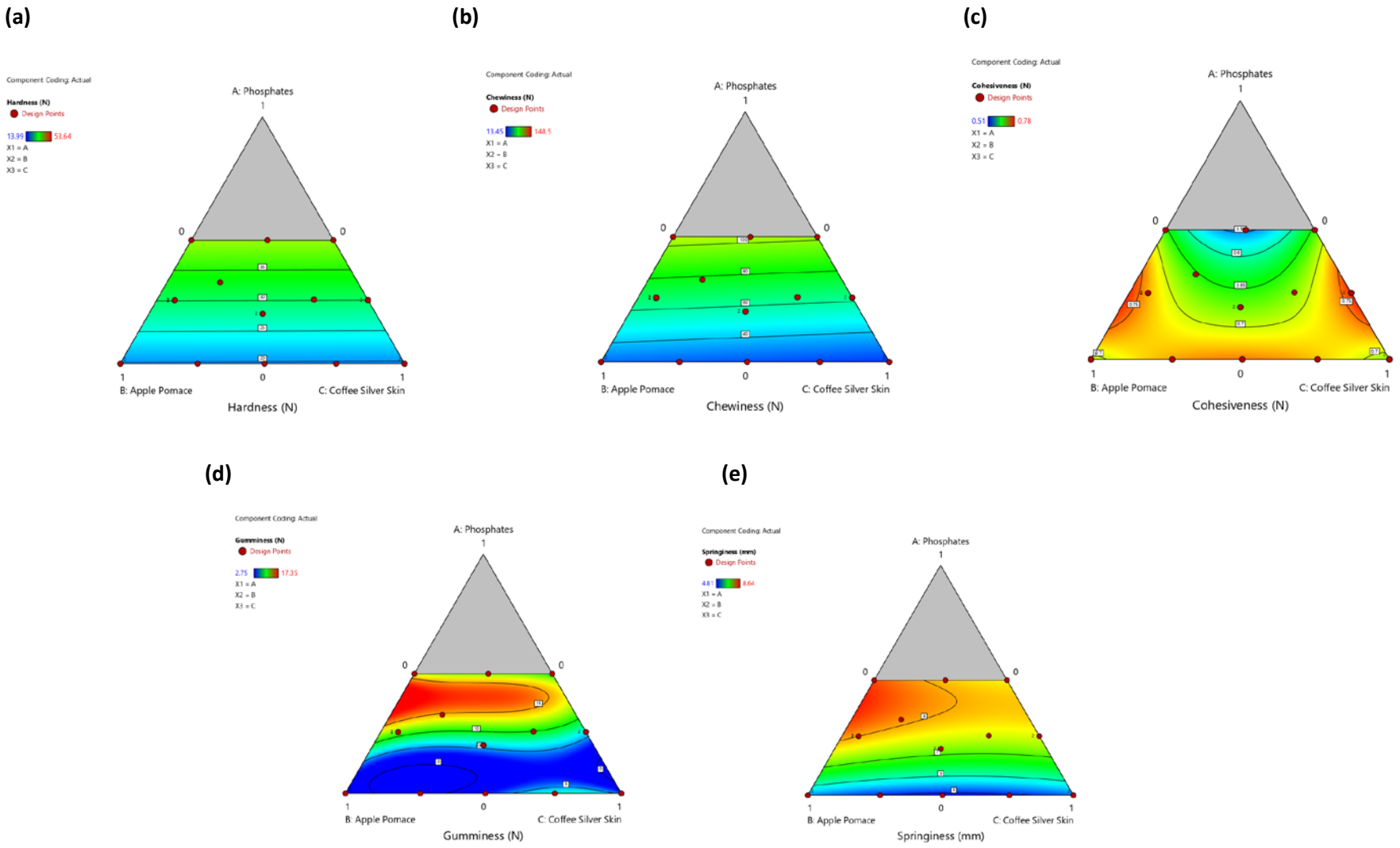
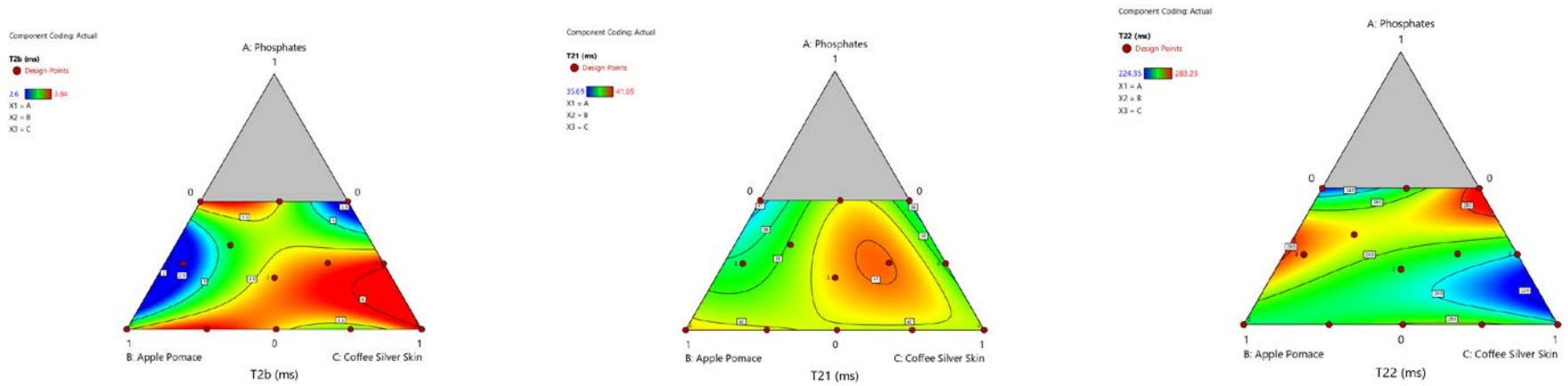


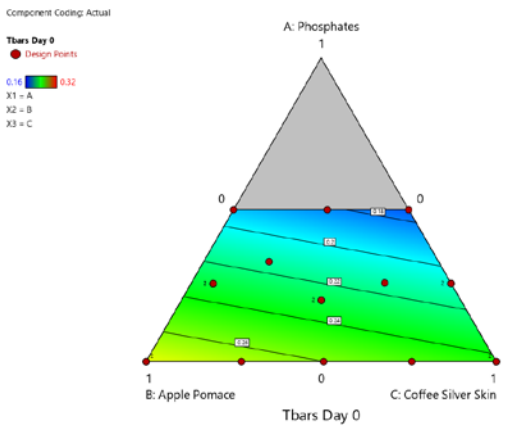
Fig. 3. Contour plots for the textural parameters of sausage formulations where 4a. Hardness, 4b. Chewiness, 4c. Cohesiveness, 4d. Gumminess and 4e. Springiness.

(a) **(b)** **(c)**

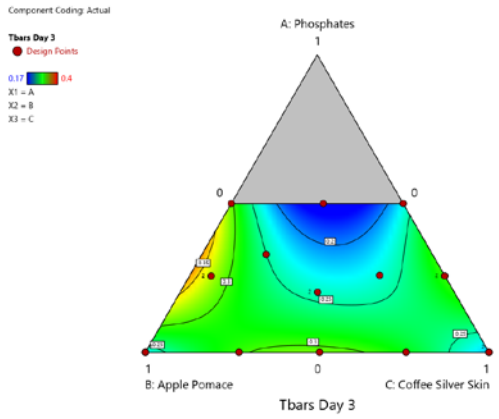


Supplement Fig. 1. Contour plots of Low field NMR values of sausages where (a) T_{2b}, (b) T_{21} and (c) T_{22}.

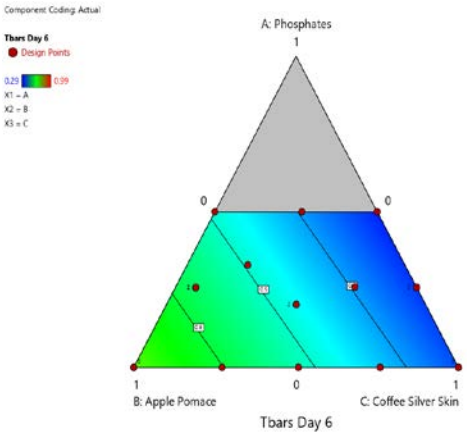
(a)



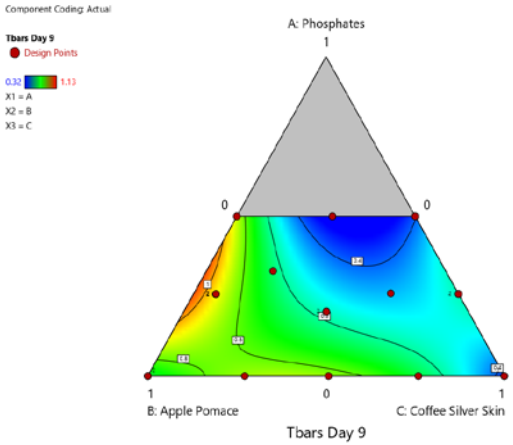
(b)



(c)



(d)



Supplement Fig. 2. Contour plots of TBARS values of day 0 (a), 3 (b), 6 (c) and 9 (d).

Table 1 Experimental design of three components in sausage formulations

Meat Blocks	Treatment run	STTP(%)(X_1)	Apple Pomace(%)(X_2)	CSS(%)(X_3)
1	**1**	0	1	0
	2	0	1	0
	3	0	0	1
	4	0.5	0.232	0.268
	5	0.202	0.398	0.4
	6	0.202	0.398	0.4
	7	0	0	1
	8	0	0.493	0.507
2	**9**	0.256	0.681	0.063
	10	0	0.241	0.759
	11	0.329	0.484	0.187
	12	0.257	0.68	0.063
	13	0.5	0	0.5
3	**14**	0.5	0.5	0
	15	0	0.728	0.272
	16	0.257	0	0.743
	17	0.257	0	0.743
	18	0.259	0.189	0.552

Where $X_1 + X_2 + X_3 = 1\%$, $(0 \leq X_1 \geq 0.5)$;$(0 \leq X_2$ and $X_3 \geq 1)$

Table 2 Mean and standard deviation values of proximate compositions, NMR percentage population and TBARS values of day 0, 3, 6 and 9.

Treatment	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Fibre (%)	Salt (%)	T_{2b}% Population	T_{21}% Population	T_{22}% Population	TBARS mg MDA/kg			
										Day 0	Day 3	Day 6	Day 9
1	66.3	9.9	17.2	1.7	1.6	0.8	2.41	91.62	5.96	0.27	0.28	0.55	0.67
2	66.4	10.0	16.7	1.6	0.7	0.7	2.15	92.72	5.12	0.22	0.26	0.99	0.79
3	66.6	9.3	16.9	1.7	1.9	0.7	2.76	92.28	4.95	0.25	0.26	0.32	0.32
4	65.6	10.8	17.0	2.0	1.8	0.7	2.48	91.74	5.76	0.18	0.22	0.39	0.38
5	64.5	12.7	16.5	1.8	1.7	0.7	1.93	91.58	6.48	0.21	0.25	0.66	0.67
6	65.5	11.0	17.2	1.8	1.3	0.7	1.98	92.55	5.46	0.2	0.32	0.43	0.72
7	65.3	10.8	17.4	1.8	1.3	0.8	1.70	93.15	5.13	0.26	0.23	0.44	0.61
8	65.1	11.1	17.4	1.7	1.8	0.7	1.46	93.43	5.09	0.27	0.33	0.48	0.85
9	62.9	15.1	13.9	1.8	2.4	0.6	1.90	90.59	7.50	0.32	0.26	0.54	0.70
10	64.4	12.8	15.6	1.7	2.0	0.8	2.29	90.86	6.84	0.25	0.26	0.29	0.60
11	65.7	11.3	15.6	2.0	2.8	1.0	2.11	91.93	5.95	0.19	0.22	0.39	0.53
12	63.5	15.1	14.0	2.0	4.0	1.0	1.99	92.54	5.46	0.21	0.31	0.79	1.13
13	66.0	11.5	14.4	2.0	5.3	0.5	2.46	91.40	6.13	0.16	0.17	0.32	0.37
14	63.2	13.9	15.9	2.0	3.6	0.6	2.84	88.41	8.74	0.23	0.30	0.33	0.82
15	63.3	13.6	16.8	1.8	0.3	0.9	2.09	90.82	7.08	0.28	0.31	0.53	0.92
16	63.3	13.3	16.2	1.9	1.7	0.7	3.24	88.70	8.05	0.19	0.23	0.36	0.48
17	62.5	14.3	16.4	1.9	1.5	0.6	3.77	87.53	8.68	0.21	0.40	0.46	0.59
18	64.7	12.3	16.9	1.9	0.7	0.9	2.92	90.03	7.04	0.20	0.19	0.31	0.36
Model Significance	ns	ns	ns	**	*	ns	***	*	ns	*	ns	*	*
SEM*	0.31	0.42	0.27	0.03	0.29	0.03	0.14	0.39	0.29	0.01	0.01	0.04	0.05

Where *, ** and *** are model significance levels at $P < 0.05$; $P < 0.01$; $P < 0.001$ respectively, ns- not significant

SEM* - Standard Error of Mean

Table 3 Mean and standard deviations values of water holding capacity, cook loss, colour, and textural parameters of sausages.

Treatment	Water holding capacity (%)	Cook Loss (%)	Colour				Hardness(N)	Chewiness(J)	Cohesive Force	Gumminess(N)	Springiness(mm)
			L*	a*	b*	ΔE					
1	69.6	16.1	63.4	6.6	20.1	1.74	21.6	23.1	0.7	3.8	6.1
2	68.6	18.0	61.9	6.9	20.1	0.68	19.0	18.4	0.7	3.4	5.3
3	69.2	17.9	57.6	6.6	18.6	4.48	24.5	25.9	0.7	4.7	5.5
4	85.2	8.3	61.9	7.4	19.6	Control	37.4	95.5	0.6	11.4	8.3
5	80.9	11.0	61.1	7.0	19.1	1.01	29.1	46.2	0.8	6.4	7.9
6	77.7	13.4	59.2	7.3	19.2	2.73	24.2	45.4	0.8	5.9	7.6
7	72.7	15.9	57.5	6.5	17.8	4.81	19.2	18.5	0.7	3.6	5.1
8	71.6	16.7	58.9	6.1	17.4	3.97	16.9	14.5	0.7	2.9	5.0
9	87.2	5.7	64.5	7.7	19.7	2.66	23.0	57.8	0.8	7.8	7.1
10	77.5	9.3	60.3	7.0	19.0	1.74	14.0	13.5	0.7	2.8	4.8
11	88.6	5.6	63.8	8.2	19.6	2.09	31.8	77.9	0.5	10.7	7.4
12	87.5	5.9	65.6	7.5	20.2	3.71	20.7	51.8	0.8	6.5	7.9
13	88.2	6.3	62.9	7.4	20.2	1.18	22.7	43.3	0.7	6.1	7.0
14	86.5	5.8	66.7	6.3	19.5	4.97	53.6	148.5	0.6	17.3	8.6
15	76.9	11.3	62.2	6.1	19.2	1.36	18.2	15.2	0.8	3.1	4.9
16	84.2	8.1	59.7	6.1	18.1	2.91	41.5	104.0	0.6	12.6	8.3
17	81.1	10.0	61.0	6.4	19.1	1.40	31.3	60.7	0.8	8.3	7.4
18	86.7	7.3	59.8	5.9	18.1	2.93	46.3	118.4	0.6	14.3	8.3
Model Significance	***	***	***	**	***		***	**	ns	**	***
SEM*	1.70	1.07	0.62	0.15	0.2		2.58	9.42	0.02	1.02	0.32

Where *, ** and *** are model significance levels at $P < 0.05$; $P < 0.01$; $P < 0.001$ respectively, ns- not significant

SEM* - Standard Error of Mean

Table 4. Optimised solutions and their predicted response obtained from Mixture design for the applied conditions

Variables	Mixture 1	Mixture 2	Mixture 3
Independent factors			
STPP(x_1)	0.2	0.2	0.06
AP(x_2)	0.22	0	0.94
CSS(x_3)	0.58	0.8	0
Total($x_1 + x_2 + x_3$)	1	1	1
Predicted optimised values with standard errors			
WHC	82.8±0.7	81.4±0.9	75.5±1.0
Cooking loss	10.2±0.4	10.3±0.6	11.7±0.7
Hardness	27.7±1.6	27.7±2.2	21.8±2.9
Chewiness	47.5±14.6	10.1±26.7	17.1±17.6
Gumminess	6.3±1.6	2.5±2.9	2.4±1.9
Springiness	7.2±0.2	7.3±0.2	6.1±0.3
L*	60.8±1.0	59.6±0.4	63.8±0.5
a*	7.3±0.2	7.9±0.4	6.7±0.2
b*	18.9±0.6	18.6±0.2	19.7±0.3
TBARS day 3	0.24±0.02	0.29±0.03	0.27±0.03
TBARS day 9	0.51±0.07	0.56±0.09	0.80±0.07
Protein	16.4±0.2	15.6±0.3	15.9±0.3
Fat	12.3±0.5	13.1±0.7	12.2±0.7
Ash	1.8±0.0	1.9±0.03	1.7±0.0
Moisture	64.7±0.4	63.9±0.5	64.9±0.5
Salt	0.7±0.1	0.6±0.1	0.8±0.1
Fibre	1.2±0.3	2.4±0.4	1.4±0.4
Desirability	**0.68**	**0.64**	**0.44**

Supplementary table 1. Significant factors affecting responses, R^2, R^2 adjusted, R^2 predicted and Lack of fitness of the model

	Significant Factors	**R^2**	**R^2 adjusted**	**R^2 predicted**	**Lack of fitness**
WHC	A, B, C, AB, AC	0.9278	0.8917	0.6709	0.745
Cooking loss	B, C	0.7492	0.7106	0.4884	0.522
L*	A, B, C	0.8531	0.8305	0.7228	0.534
a*	B, C, BC, BC (B-C)	0.9172	0.7931	-2.9503	0.701
b*	A, B, C	0.5302	0.4579	0.1987	0.218
Hardness	A, B, C	0.6430	0.5881	0.2316	0.266
Chewiness	BC(B-C)	0.9448	0.8620	0.5299	0.948
Gumminess	B, C, AB (A-B)	0.9441	0.8602	-0.7433	0.744
Springiness	B, C, AB, AC	0.9332	0.8998	0.7845	0.644
Cohesive force	A, B, C, AB, AC, ABC. AB (A-B), AC (A-C)	0.8541	0.6354	-6.7272	0.803
TBARS day 0	A, B, C	0.4718	0.3905	0.0098	0.101
TBARS day 3	B, C, AB, ABC	0.5044	0.1741	-0.7724	0.900
TBARS day 6	B, C	0.4690	0.3873	-0.0320	0.849
TBARS day 9	B, C, AB, BC, ABC	0.7634	0.6057	0.1555	0.287
Protein	A, B, C, AB, AC, ABC	0.6350	0.3917	-0.5806	0.333
Fat	B, C, AB, AC	0.5673	0.2788	-1.1346	0.315
Ash	A, B, C	0.7217	0.6788	0.4715	0.248
Moisture	A, B, C, AB, AC, ABC	0.5771	0.2952	-1.5288	0.283
Salt	B, C	0.6569	0.2648	-2.2967	0.004
Fibre	ABC	0.7467	0.5778	-0.6015	0.125
T_{2b}	B, C, BC (B-C)	0.9695	0.9239	-5.9470	0.279
T_{21}	A, B, C	0.8754	0.7330	-0.2840	0.061
T_{22}	B, C	0.7151	0.2879	-5.3354	0.168

A: Phosphate STPP; B: Apple Pomace; C: Coffee Silver skin.

Supplementary table 2. Regression Coefficient for the studied responses with respect to the constraints

	A	B	C	AB	AC	BC	ABC	AB (A-B)	AC (A-C)	BC (B-C)
WHC	74.19	72.73	73.82	55.23	46.67					
Cooking loss		12.37	13.03							
L*	66.15	63.65	57.92							
a*		6.97	6.72	-2.06						8.78
b*	20.35	19.68	18.11							
Hardness	60.12	19.7	19.55							
Chewiness										-426.23
Gumminess		3.52	4.04					409.5		
Springiness		5.38	5.22	19.65	17.41					
Cohesive force	14.94	0.74	0.76	-28.92	-28.57		37.42	-20.75	-18.70	
TBARS day 0	0.10	0.28	0.24							
TBARS day 3		0.23	0.21	1.51			-4.27			
TBARS day 6		0.70	0.34							
TBARS day 9		0.67	0.36	4.58		1.32	-13.68			
Protein	20.61	16.37	16.76	-13.40	-11.82		35.78			
Fat		10.85	10.75	42.14	33.32					
Ash	2.28	1.72	1.74							
Moisture	77.17	65.88	65.57	-32.25	-24.73		70.73			
Salt		0.88	0.90							
Fibre							-59.80			
T_{2b}		3.45	3.95							4.47
T_{21}	35.62	40.48	39.85							
T_{22}		247.53	245.92							

A: Phosphate STPP; B: Apple Pomace; C: Coffee Silver skin.

Supplementary table 3. NMR time delays and relation areas of the relaxation curves

Treatment	T_{2b}	T_{21}	T_{22}	T_{2b} area	T_{21} area	T_{22} area
1	3.18	39.07	252.33	9149.777	347365	22600.76
2	3.26	39.60	252.08	8127.477	349984.9	19330.16
3	3.78	39.09	242.72	10721.59	358305.9	19242.1
4	3.39	39.07	252.20	8923.517	328944.6	20688.02
5	3.32	39.58	252.08	7482.2	353895.4	25053.31
6	3.41	39.58	258.69	7415.32	346055.9	20438.82
7	3.65	38.57	258.69	6356.55	346654.7	19120.04
8	3.34	38.57	265.43	5555.103	353433.1	19261.04
9	2.86	39.58	258.69	5445.8	258682.6	21421.21
10	3.84	41.69	256.82	9950.953	393424.4	29617.62
11	3.62	40.62	265.56	8747.687	380868.8	24679.89
12	2.88	41.14	279.58	7961.397	369942.1	21849.88
13	2.60	39.58	283.23	10014.44	371791	24938.03
14	3.52	35.69	224.35	10985.71	341537.1	33774.83
15	3.66	39.58	252.08	8605.783	372283.5	29023.46
16	3.62	37.58	236.24	12621.27	344789.5	31297.41
17	3.75	38.07	242.47	14691.22	340406.5	33793.38
18	3.43	40.10	252.08	11772.14	362955.9	28419.53
Model Significance	***	*	ns			
SEM*	0.08	0.31	3.29			

Where *, ** and *** are model significance levels at $P < 0.05$; $P < 0.01$; $P < 0.001$ respectively, ns- not significant

SEM* - Standard Error of Mean