J Food Sci Technol (May 2019) 56(5):2660–2669
https://doi.org/10.1007/s13197-019-03754-1

ORIGINAL ARTICLE

Partial and total replacement of meat by plant-based proteins in chicken sausage: evaluation of mechanical, physico-chemical and sensory characteristics

Mohammad Hassan Kamani[1] · Manchanahally Shivanna Meera[1] · Narayan Bhaskar[2] · Vinod Kumar Modi[2]

Revised: 22 March 2019 / Accepted: 26 March 2019 / Published online: 6 April 2019

Abstract The processed meats are classified in the first category of carcinogenic compounds due to its numerous health issues. For this reason, there is a growing interest to utilize healthy ingredients for formulation of meat-based products. The objective of this study was to replace completely and partially meat by plant proteins in sausage formulation and compare the characteristics of these novel formulae with full meat sample. The results showed that the plant proteins minimized the cooking loss and shrinkage and improved emulsion stability by creating a strong structural network in cooked emulsion. In contrast, the full meat samples had better strength/elasticity in terms of folding score (4.67 out of 5) and gel strength (2553.68 g mm) when compared to meat-reduced and meat-free samples. The sensory assessment showed that replacement of chicken meat by plant proteins was highly acceptable in terms of texture, odor, color and overall acceptance. Overall, it is concluded that plant proteins can be regarded as promising ingredients to replace 80–100% meat in sausage.

Keywords Meat alternative · Sausage · Emulsified meat · Plant proteins · Textural properties

✉ Manchanahally Shivanna Meera
meera@cftri.res.in

[1] Department of Grain Science and Technology, CSIR-Central Food Technological Research Institute, Mysore, Karnataka 570020, India

[2] Department of Meat and Marine Sciences, CSIR-Central Food Technological Research Institute, Mysore, Karnataka 570020, India

Introduction

Meat products are considered as one of the commonly consumed food items in family's food basket. Several epidemiological studies have been reported that consumption of processed meat is associated with several health issues such as cardiovascular disease, diabetes and various types of cancers (Arihara 2006; Schulze et al. 2003). There are potential explanations for these health issues. The existence of high amounts of saturated fatty acids (40–50%) namely, Myristic and Palmitic acids and their detrimental effects, oxidation of polyunsaturated fatty acids (PUFAs) during frying as well as using synthetic preservatives have been documented as the major reasons for these adverse health effects (Schulze et al. 2003; FAO 1992). Thus far, numerous attempts have been made in order to optimize the composition of meat products and reduce the health issues using plant extracts, fat and nitrite replacer (Arihara 2006; Yıldız-Turp and Serdaroğlu 2008; Choi et al. 2009; Kurćubić et al. 2014; Amani et al. 2017). Nevertheless, it seems that these efforts have not been fully satisfactory, and as a result, a decreased consumption of processed meats has strongly been suggested by the WHO's International Agency for Research on Cancer (IARC 2015). Therefore, utilizing a healthier resource to replace partially or totally meat seems to be necessary.

According to the FAO's report (1992), meat is not an essential item in the diet and the witness of this fact is the large number of vegetarians who have a nutritionally adequate diet (FAO 1992). The food technologists have been used this reference point to tackle the health issues of processed meats and has recently focused to develop meat-reduced products using alternative proteinous ingredients (Asgar et al. 2010; Danowska-Oziewicz 2014). The strategy of partially replacing meat was somewhat effective;

however, the results have not been still satisfactorily from at least two perspectives: (i) the existence of a high portion of meat in formulation; and (ii) a reduction in the textural and sensory qualities, which is a key criterion for today's consumers (Danowska-Oziewicz 2014; Elzerman et al. 2011; Youssef and Barbut 2011). Hence, it can be inferred that the meat must be completely substituted by plant-based ingredients in such a way that the resultant mimic product possesses the highest similarity to the original product with respect to textural and sensory acceptability and with adequate nutritional value.

However, retaining the sensory and textural quality are accounted as big challenges in production of meat analog products. The texture and taste (in particular the juiciness and tenderness) of meat are highly valued by consumers, while the non-meat ingredients may not possess the same sensory attributes (Elzerman et al. 2011). To overcome the mentioned issue, various plant proteins such as legume, cereal, oilseed and soy proteins group (textured, flour, concentrate and isolate) can be used (Malav et al. 2015). These ingredients have appropriate functional properties (e.g., emulsification, water and oil absorption capacity) as well as high nutritional values (e.g., well-balanced amino acids, essential fatty acids, vitamins and easy digestibility), which make them as unique meat substitutes (Malav et al. 2015; Asgar et al. 2010). To date, limited research works have been carried out to develop meat analog-based products. Majzoobi et al. (2017) formulated the meat-free sausages using textured soy protein, corn starch, vegetable oil, salt and soy protein isolate. They reported substantial quality loss such as poor sliceability, high frying loss and low water-holding capacity when the meat was replaced by alternative ingredients. However, some parameters such as cooking loss, water-holding capacity and general acceptability could be improved using konjac mannan and K-carrageenan. Another formulation recorded by Savadkoohi et al. (2014), who attempted to improve the quality of meat-free sausage using bleached tomato pomace. Their results showed that the meat-free sausage has a weaker textural (hardness, cohesiveness, springiness and chewiness) attributes compared to the beef frankfurter. However, they considerably improved all the mentioned parameters by incorporation of bleached tomato pomace. To our knowledge, few studies have been reported on formulation of meat analog products, and there is lack of knowledge in this regard, while it has an excellent scope for food processors as well as health-conscious consumers.

This study is part of an effort to introduce a new approach for manufacturing meat-minimized as well as meat-free sausages. Therefore, the specific aims of the present study were (1) to develop formulations with minimized meat and meatless sausages; (2) to measure the mechanical, physico-chemical and sensory characteristics of the developed sausages; and (3) to comparatively assess the quality and desirability of new formulae with the full meat sausage.

Materials and methods

Ingredients

The frozen chicken breasts were purchased from a local retailer in Mysore, India. The various ingredients used like soy protein isolate 90% (SPI) (Sonic Biochem Co., Indore, India), soy flour (SF) (Sevenhills Co., Mysore, India), gluten (Royal Ingredients Co., Navi Mumbai, India), albumen (Ovobel Foods Co., Bangalore, India), dalda oil (Bunge India Private Co., Bangalore, India), sodium tripolyphosphate (STPP) (Loba Chemical Co., Mumbai, India), carboxymethyl cellulose (CMC) (S. D. Fine-Chem Private Co., Mumbai, India), salt, chickpea flour, corn starch and seasoning blend (a mix of pure spices from Everest Spices Co., Mumbai, India) were procured for the sausage formulations.

Preparation of sausages

The sausages samples were prepared in the pilot plant of Central Food Technological Research Institute (CFTRI), Mysore, India. The frozen chickens were thawed at 4 °C for 16 h prior to use and the excess visible fat and connective tissues were trimmed. The lean chickens were then cut into cubes and minced twice through a 6–10 mm steel plates using a laboratory mincer (Model 82-Classic, Dadaux SAS Co., France). The SPI and gluten were properly hydrated by soaking in cold water for 2 h with a ratio of (1:3) and (1:2), respectively. Three different sausages namely, meat-free (sample A), reduced-meat (sample B) and full meat sausages (sample C) were formulated for 4 kg batter. The meat-free sausages were produced by mixing SPI (15.2%), gluten (6.1%), water (50.4%), chickpea flour (10.7%), oil (8%), albumen (0.5%), STPP (0.4%), salt (1.5%), CMC (0.9%), starch (3%) and mixed spices (3.3%). The same recipe used for formulation of sample B with the difference that 20% chickens were added and the water and SPI reduced to 36% and 9.6%, respectively. To compare the quality of meat-free and reduced-meat samples with full meat sausages, the sausages C were prepared according to the formulation of Hidayat et al. (2017) with modifications using chicken (60%), water (18.25%), oil (15.4%), STPP (0.4%), salt (1.22%), starch (0.5%), soy flour (2.43%) and spices (1.8%). To manufacture sausage, the minced chickens were placed in a bowl chopper (Model R-10, Robot Coupe, France) and dry ingredients (salt, corn starch, STPP, SF, etc.) were slowly added to the ground chicken while processing. Cold water was also added continuously during chopping to control temperature rise. Afterwards, oil, CMC and spices were incorporated, respectively. Total mixing time was 10 min and the final temperature of batters was kept below 12 °C. The batters were transferred into a hydraulic piston-type stuffer (Model EC-12, Mainca Co., Spain) and stuffed into impermeable cellulose casings. The sausage links were twisted and tied manually and kept at 4 °C for 30 min. One set was kept as uncooked and another set were cooked in a steam chamber to an internal temperature of 76 °C. The cooking was completed in 70 min, and the core temperature was monitored continuously using a digital probe thermometer (Model Center-309, HTA Instrumentation Co., Bangalore, India). Finally, the sausages were cooled by immersion in an ice-water bath to reach a final temperature below 10 °C and stored overnight at 4 °C. Each type of sausage was produced in two different batches and three sausages per batch were selected for the subsequent analyses.

Mechanical properties

The texture profile analysis (TPA) of cooked sausages were carried out using Universal Texture Measuring Instrument (Model LR-5 K, Lloyd Instruments Ltd, Hampshire, UK) equipped with a 1 KN load cell. The samples were cut into 25 mm pieces in diameter and were axially placed on a platform. A two-cycle compression test was performed up to 50% strain compression of the original portion height using a steel probe. A time of 5 s was allowed to elapse between the two compression cycles. Force–time deformation curves were recorded at a crosshead speed of 50 mm/min and attributes were calculated as follows: hardness (N), resistance force required at first compression (first peak); cohesiveness (dimensionless), the ratio of the positive force area during second compression to that during the first compression; springiness (mm), distance sample spring back after deformation during the first compression; adhesiveness (kgf mm), the negative force area for the first compression; gumminess (N) the result of hardness multiplied by the cohesiveness; and chewiness (kgf mm), the result of hardness multiplied by cohesiveness and springiness (Jiménez-Colmenero et al. 2012).

Warner–Bratzler Shear Force (WBSF) test was also performed using the same texture analyzer, while equipped with a load cell capacity of 100 kg and a shearing V-shaped blade with 1 mm of thickness. The sausage samples (5 × 2.2 cm in length × diameter) were sheared at a crosshead speed of 50 mm/min to 50% of their original height and force–time curves were recorded. WBSF values were measured as the maximum peak force of shearing in the deformation curve and expressed in N.

To determine penetration force, a cylindrical punch probe was used to penetrate 15 mm of sample at 50 mm/min crosshead speed and 1 KN load-cell. The corresponding force-distance curves were recorded using the Nexygen software version 4.0 (Lloyd Instruments Co., Hampshire, UK). Penetration force was calculated as the maximum force during penetration at the first peak of curve. Gel strength (g mm) was then calculated by multiplying the penetration force (g) with distance of the penetration (penetration depth) (mm).

Physico-chemical analyses

Emulsion stability and cooking loss

Raw batter (10 g) was weighed in a tube and centrifuged at $2500 \times g$ for 5 min at 4 °C using a tabletop centrifuge (Model 5804-R, Eppendorf Co., Hamburg, Germany). Subsequently, the tube was heated in a water bath at 80 °C for 60 min. The tube was then left to stand upside-down for 45 min to release the exudates. The supernatant was decanted and the total fluid released (TFR) was expressed as a percentage of the sample weight using the Eq. 1. Water released was determined after evaporating TFR in an oven at 100 °C for 16 h. Released fat (%) was calculated as the difference between TFR and released water (Cofrades et al. 2008).

$$TFR(\%) = \frac{(W_t + W_s) - (W_t + W_p)}{W_s} \times 100 \quad (1)$$

where W_t is the weight of centrifuge tube; W_s is the weight of sample; and W_p is the weight of pellet.

The cooking loss of the samples was determined according to the method of Choi et al. (2009) with slight modification. Thirty grams raw emulsion were stuffed into screw top test tubes and were heated in a steam bath at 70 °C for 30 min. The cooked samples quickly immersed in cool water for 10 min. Cooking loss were determined by weighing individual sample before and after cooking, and difference expressed as a percentage of the original weight.

Jelly and fat separation

To measure jelly and fat separation, three pre-weighed glass jars were filled with raw batter and then heated in boiling water for 35 min (with core temperature about 90 °C). The jars were then cooled by running tap water and stored at 4 °C for 24 h. Afterwards, the jars were re-heated in a water bath at 45 °C for 1 h and the fluid from each sample was collected in a volumetric cylinder. The Jelly and fat separation calculated as a percentage of each original batter weight as described by Luruena-Martınez et al. (2004).

Folding test

The folding test was performed using a five-point grade system according to the method of Cardoso et al. (2008). Sausages were sliced into 3 mm thick pieces. The slices were folded slowly in half to observe the way in which they broke. They were graded as follows: (1) breaks by finger pressure, (2) cracks immediately when folded in half, (3) cracks gradually when folded in half, 4) no cracks showing after folding in half, and (5) no cracks showing after folding twice.

Instrumental color measurement

Color measurement was performed using a Minolta colorimeter (Model CM-5, Konica Minolta Optics Co., Japan), calibrated to white standards tiles prior to measuring. The samples were sliced into 3 × 2 cm (length × diameter) pieces and then placed in a circular template. Afterwards, the cap was locked and the data of CIE L^*, a^*, b^* processed using Spectra Magic^{TM} NX software under illuminant D65.

Frying loss

Sliced sausages with 1 cm thick, were deep-fried in sunflower oil for 2 min and then cooled down to room temperature. The frying loss was calculated by weighing the slices before and after frying and expressed as a percentage (Hwang et al. 2011).

pH and shrinkage

Ten grams of each sample was weighed and homogenized with 90 ml of distilled water for 5 min. The pH of prepared sample was measured using a pH meter (Model HI 84530, Hanna Instruments Co., USA). The effect of cooking treatment on shrinkage of sausages was also determined as changes in diameter after cooking by an induction cooker and measured using Eq. 2.

$$\text{Shrinkage }(\%) = \frac{\text{Diameter before cooking} - \text{Diameter after cooking}}{\text{Diameter before cooking}} \times 100 \quad (2)$$

Sensory evaluation

Sensory evaluation was performed on pan-fried sausages according to the method of Savadkoohi et al. (2014). The sausages were assessed by eight trained panelists, comprising of post-graduate students (4 males, 4 females; aged between 22 and 29 years) at CFTRI (Mysore, India), under identical light (daylight) at room temperature. The samples were prepared for each panelist and were asked for their opinions on color and appearance, odor, taste, texture and overall acceptability. Scores were assigned using a nine-point hedonic scale as follows: Like extremely—9; Like very much—8; Like moderately—7; Like slightly—6; Neither like nor dislike—5; Dislike slightly—4; Dislike moderately—3; Dislike very much—2; Dislike extremely—1.

Statistical analysis

A completely randomized design was applied for data collocation. Data were subjected to analysis of variance (ANOVA) using the general linear models procedure of the SPSS^{TM} software (version 16, SPSS Inc., Chicago, USA). Differences among the means values were analysed by Duncan's Multiple Range Test (DMRT), and the significance level was defined at $p < 0.05$. The experiments were conducted twice (two different production batches) with tree replications and the average data were expressed as mean ± SD.

Results and discussion

Mechanical properties

Tables 1 is represented the mechanical properties of the cooked samples. The hardness in sample A (meat-free sausage) was found to be significantly lower than the sausages containing meat, i.e., samples B and C ($p < 0.05$). A similar decreasing trend observed for cohesiveness when the meat completely replaced by the mixture of plant proteins. It was found that the samples containing meat (samples B and C) required a greater force to chew than the non-meat sample. This is probably due to the existence of stronger network in myofibril proteins, which consequently increased the product's resistance to compression. Feng and Xiong (2002) provided strong evidence regarding interactions between soy and myofibrillar proteins, which cause a lesser stable conformation for both proteins. Similarly, Youssef and Barbut (2011) reported that the SPI interferes in making gel-network in meat emulsion, which may result a softer texture. The sample A showed the lowest values for gumminess (0.12 kgf) and springiness

Table 1 The mean (± SD) of hardness, cohesiveness, chewiness, stiffness, springiness, adhesiveness and gumminess of three different combinations of sausage

Sample	A	B	C
Hardness (N)	13.39 ± 1.01[a]	29.16 ± 0.24[b]	34.56 ± 1.74[c]
Cohesiveness	0.09 ± 0.003[a]	0.13 ± 0.00[b]	0.13 ± 0.01[b]
Chewiness (kgf mm)	0.24 ± 0.03[a]	1.51 ± 0.03[b]	1.51 ± 0.42[b]
Stiffness (kgf/mm)	0.47 ± 0.14[a]	0.68 ± 0.21[a]	0.80 ± 0.05[a]
Springiness (mm)	1.99 ± 0.09[a]	3.89 ± 0.02[c]	3.19 ± 0.36[b]
Adhesiveness (kgf mm)	2.26 ± 0.03[a]	4.28 ± 0.86[b]	4.51 ± 0.79[b]
Gumminess (kgf)	0.12 ± 0.01[a]	0.38 ± 0.007[b]	0.46 ± 0.08[b]

A: Meat-free sausage; B: Sausage with 20% meat; C: Chicken meat sausage (60% meat). Different letters in each row indicate significant difference ($p < 0.05$)

(1.99 mm), while these parameters were significantly higher in sample B and C ($p < 0.05$). Similarly, a lower springiness values was found by Youssef and Barbut (2011) when SPI was used as meat substitute in emulsified meat batter. They stated that non-meat proteins could hold more water and fat contents, which may fill the interstitial spaces within the protein matrix and reduces springiness. The textural analysis showed that the presence of meat in sample is associated with higher values for stiffness and adhesiveness; however this effect was not significant in terms of stiffness ($p > 0.05$). Overall, it can be interpreted that reduction in meat amount may result in a remarkable decrease in consistency of final cooked emulsion.

The results of WBSF test on the cooked sausages are given in Table 2. The shear forces values reduced from 6.12 N in sample C to 5.67 N and 3.93 N in samples B and A, respectively; however, these changes were not significant ($p > 0.05$). Similarly, a decline in shear force value was reported by Das et al. (2008a), who incorporated soy paste in comminuted nugget system. The present results also confirmed the findings of Danowska-Oziewicz (2014), who observed lesser value for Warner–Bratzler force for the samples containing SPI as compared to control. The results of present study imply that the addition of mixture of soy protein and gluten affects the mechanical properties associated with peak load and causes softness in the sausage. Stromal and myofibrillar proteins are responsible for firmness in ground meat and addition of other proteins ingredients may dilute the effect of these proteins and results in softness of product (Das et al. 2008b).

Table 2 shows the results of gel strength and penetration test for all samples. The gel strength and penetration force for the chicken meat sausages were found to be 2553.68 g mm and 3.12 N, respectively. These parameters did not change significantly when meat content reduced to 20% in sample B ($p > 0.05$), which indicates the feasibility of meat replacement (up to 40%) by plant proteins in sausage. Su et al. (2000) stated that fat globules can physically entrap within SPI and contributes in improving the firmness in batter system. On the contrary, Feng and Xiong (2002) reported that the presence of β—conglycinin in soybean diminishes the self-aggregations of myosin heavy chains during heating (from 50 to 100 °C), which may reduce gel strength in meat emulsion system. The reason for this inconstancy may probably be the processing factors; since gel strength can be affected by temperature, pH and ionic strength of the system (Jang et al. 2015), As shown in Table 2, the meat-free sausages showed the lowest gel strength (1700.58 g mm) and penetration force (2.08 N), indicating that, in absence of meat, lesser breaking force is required to penetrate the outer skin of sample. This could be due high amount of water required in the formulation of sample A, which notably increases the softness in texture of sausage.

Table 2 The mean (± SD) of Warner–Bratzler Shear Force (WBSF), gel strength and penetration force of three different combinations of sausage

Sample	WBSF (N)	Gel strength (g*mm)	Penetration force (N)
A	3.93 ± 0.14[a]	1700.58 ± 280.76[a]	2.08 ± 0.34[a]
B	5.67 ± 0.44[a]	2804.72 ± 489.99[b]	3.43 ± 0.59[b]
C	6.12 ± 2.55[a]	2553.68 ± 160.36[b]	3.12 ± 0.19[b]

A: Meat-free sausage; B: Sausage with 20% meat; C: Chicken meat sausage (60% meat); Different letters in each column indicate significant difference ($p < 0.05$)

Physico-chemical analyses

Emulsion stability and cooking loss

Emulsion stability indicates the ability of meat emulsion to retain moisture and fat during processing (Aktaş and Genccelep 2006). Emulsion stability specifically shows how well the juices are retained in system. As it is shown in Table 3, there was no total expressible fluid (TEF), in the sausages containing plant proteins (samples A and B). This result is in line with the findings of Serdaroglu and Ozsumer (2003), who reported improved emulsion stability in cooked beef sausages using soy, gluten and whey proteins. Soy proteins are hydrophilic which hold moisture and fat by creation of an advhesive gel matrix and consequently bring about stabilization for emulsion (Serdaroglu and Ozsumer 2003). No released fat or water observed in sample B (Table 3). It indicates that the non-meat ingredients (SPI, gluten) actively involved with meat proteins to hold fat and water. Siegel et al. (1979) stated that wheat gluten possesses high binding ability when interacting with myosin and make a uniform and fibrous structure in emulsion system. It has also been reported that soy protein acts as fat-encapsulating agent by supplementing myosin and acto-myosin and prevents fat separation during cooking (Das et al. 2008b). The chicken meat sausage showed a high TEF, indicating lesser stability of meat protein during cooking (Table 3). The majority of this TEF was released water (8.73 out of 9.67%). Other studies have been confirmed a similar trend in the ratio of released water/fat in meat sausages (Cofrades et al. 2008).

Cooking loss measures the ability of system to bind water and fat after protein denaturation and aggregation. The results of cooking loss are given in Table 4. Similar to emulsion stability, the cooking loss values were found to be zero in samples A and B, which is very promising. It implies that the network structure of emulsions in non-meat and meat-reduced sausages were very strong in retaining moisture during cooking. The value for cooking loss in chicken meat sausage was significantly higher (8.72%) ($p < 0.05$), which indicates a weaker ability of meat proteins in binding the water as compared to the plant proteins. The cooking loss can be dependent on the various factors such as temperature, time and method of cooking, additives used in emulsion, type and content of fat in formulation and casing material (Choi et al. 2009). The present findings are in consistent with the earlier evidence, which reported positive effects of plant ingredients on reduction of cooking loss in emulsified meat batters (Youssef and Barbut 2011).

Jelly and fat separation

Jelly deposit and fat separation are considered as undesirable factors in comminuted meat products. The results of jelly and fat separation are represented in Table 4. The negligible jelly and fat separation were found in the samples containing plant proteins (samples A and B), while the chicken meat sausage showed the highest value (12.42%) for this parameter. Therefore, the present finding suggests that the mixture of plant proteins (SPI and gluten) significantly decreased the amount of jelly and fat separation ($p < 0.05$). In this respect, Flores et al. (2007) reported a low amount of fat and jelly separation (around 2%) in pork emulsion system when SPI used as emulsifier. In addition, other literature has been shown that the addition of other ingredients such as locust bean (Luruena-Martınez et al. 2004) effectively reduced fat and jelly separation of meat-based products.

Folding test

Sensory analysis using folding test helps to determine the elasticity of texture. The results of folding test are provided in Table 4. There was no significant difference ($p > 0.05$) between meat-free sausage and meat-reduced sample. However, the full meat samples showed the highest elasticity with the score of 4.67. Folding ability is directly related to the formation of protein gel-network during cooking process. In meat emulsion, myofibrillar proteins are responsible for creation of this network (Jang et al. 2015; Akesowan 2010). According to the data shown in Table 4, the mixture of plant proteins had lesser ability to form this proteinus network as compared to myofibrillars

Table 3 The mean (± SD) of emulsion stability of three different combinations of sausage

Sample	Emulsion stability (TEF %)	Emulsion stability (Released fat %)	Emulsion stability (Released water %)
A	0 ± 0^a	0 ± 0^a	0 ± 0^a
B	0 ± 0^a	0 ± 0^a	0 ± 0^a
C	9.67 ± 1.32^b	0.94 ± 0.15^b	8.73 ± 1.17^b

A: Meat-free sausage; B: Sausage with 20% meat; C: Chicken meat sausage (60% meat); Different letters in each column indicate significant difference ($p < 0.05$)

Table 4 The mean (± SD) of jelly and fat separation, folding test and cooking loss of three different combinations of sausage

Sample	Jelly and fat separation (%)	Folding test	Cooking loss (%)
A	0.11 ± 0.19^{a}	2.33 ± 0.57^{a}	0.0 ± 0^{a}
B	0.57 ± 0.50^{a}	2.67 ± 0.57^{a}	0.0 ± 0^{a}
C	12.42 ± 0.84^{b}	4.67 ± 0.57^{b}	8.72 ± 2.1^{b}

A: Meat-free sausage; B: Sausage with 20% meat; C: Chicken meat sausage (60% meat); Different letters in each column indicate significant difference ($p < 0.05$)

and their presence markedly reduced the gel strength and folding ability in final product. A Similar report by Elang et al. (2018) showed that incorporation of Mocaf flour with fish mince decreased the folding test value in fish sausage. Overall, the folding test results revealed that replacement of meat by plant proteins reduce the gel formation/elasticity in the cooked emulsion. Further research works are needed in order to improve this elasticity deficiency using various gelling agents such as hydrocolloids, high-gel proteins and plant-based flours.

Surface color

Color is regarded as first qualitative criterion in meat products and plays a key role in consumer's perception and product acceptability (Kamani et al. 2017). The data of colorimetric evaluation are given in Table 5. According to these results, the addition of plant proteins led to decrease in lightness (L*) and increase of yellowness (b*). The highest yellowish (31.05) observed in the meat-free sausages, while the minimum value of b* was found to be 24.64 for full meat sample (sample C). The highest lightness value also observed for sample C, which was 67.51. This value reduced to 61.1 and 58.01 when meat contents reduced to 20% and zero, respectively. The redness parameter (a*) did not show any significant changes amongst all samples ($p > 0.05$). A similar trend (reduction in L* and increase in b*) observed by Akesowan (2010), who fortified pork burgers with SPI. This change may possibly be due to the existence of light brown color in added SPI flour. Shahiri Tabarestani and Mazaheri Tehrani (2014) also reported that addition of soy flour led to reduction of lightness in burger. Apart from SPI, other factors such as types of spices and the contents of heme-proteins may highly affect the color indexes of end product. In conclusion, the colorimetric results revealed that partial and total replacement of meat by plant proteins may result in a more yellowish color in cooked sausage.

Frying loss

The ability of emulsion to bind water, fat, and/or other ingredients during frying is a key factor affecting product quality. The results of frying loss under deep-frying condition are shown in Table 5. Frying loss was found to be highest (11.24%) in sample C, imparting a high loss of fat and moisture during frying compared to other samples; however, this parameter was insignificantly lower in sample B with the value of 10.08%. A marked reduction was observed in sample A when meat was completely replaced by plant proteins. This strong resistance against frying loss might be due to high ability of SPI in moisture absorption and act as good barrier in emulsion system. Frying loss is linked with various processing factors such as frying time and temperature, fat content, and amounts of particular additives like dietary fibers or isolated proteins in emulsion system (Hwang et al. 2011). For instance, Kilincceker and Hepsag (2012) reported that coating potato balls with chickpea flour caused a significant decrease in frying loss. They observed that chickpea flour functions as good barrier, which inhibits the mass loss from the food matrix to outside and, as a result, increase the adhesion degree in texture of product. Overall, the obtained results are an indication that the mixed plant proteins used may develop barrier properties against percentage of loss at frying temperature.

Table 5 The mean (± SD) of shrinkage, frying loss (%), pH and colorimetric parameters (L*, a*, b*) of three different combinations of sausage

Sample	Shrinkage	Frying loss	pH	L*	a*	b*
A	0 ± 0^{a}	6.9 ± 1.01^{a}	7.01 ± 0.02^{b}	58.01 ± 0.78^{a}	5.11 ± 0.25^{a}	31.05 ± 1.01^{b}
B	0.92 ± 0.84^{a}	10.08 ± 2.084^{b}	6.95 ± 0.005^{b}	61.1 ± 1.56^{b}	4.07 ± 0.89^{a}	30.05 ± 1.94^{b}
C	10.93 ± 1.81^{b}	11.24 ± 0.95^{b}	6.6 ± 0.08^{a}	67.51 ± 1.63^{c}	4.17 ± 0.32^{a}	24.64 ± 0.18^{a}

A: Meat-free sausage; B: Sausage with 20% meat; C: Chicken meat sausage (60% meat); Different letters in each column indicate significant difference ($p < 0.05$)

Table 6 The mean (± SD) of sensory parameters (overall appearance, color, odor, taste, texture and overall acceptance) of three different combinations of sausage

Sample	Overall appearance	Color	Odor	Taste	Texture	Overall acceptance
A	7.43 ± 0.78^{b}	7.29 ± 1.25^{a}	6.57 ± 1.39^{a}	6.43 ± 0.97^{a}	7 ± 1^{ab}	7 ± 0.81^{a}
B	7.43 ± 1.13^{b}	7.14 ± 0.90^{a}	7 ± 0.57^{a}	6.14 ± 0.89^{a}	7.43 ± 0.78^{b}	7.29 ± 0.75^{a}
C	6 ± 0.63^{a}	6.17 ± 0.75^{a}	7 ± 0.89^{a}	7.5 ± 0.83^{b}	6.17 ± 0.98^{a}	6.83 ± 0.40^{a}

A: Meat-free sausage; B: Sausage with 20% meat; C: Chicken meat sausage (60% meat); Different letters in each column indicate significant difference ($p < 0.05$)

Shrinkage and pH

Cooking shrinkage is considered as important physical attribute from the consumer's viewpoint (Shahiri Tabarestani and Mazaheri Tehrani 2014). As shown in Table 5, the use of plant proteins as meat substitute significantly changed the shrinkage of samples ($p < 0.05$). No shrinkage observed in meat-free sausage (sample A) which indicates the excellent ability of SPI and gluten in retaining of network structure after cooking. The shrinkage was 0.92% when 20% meat was added in emulsion system and further increased to 10.93% as chicken content increased to 60%. It is obviously due to denaturation of proteins during cooking process, which causes release of fluid, change of structure and shrinking incident (Shahiri Tabarestani and Mazaheri Tehrani 2014; Serdaroğlu and Değırmencioğlu 2004). The results of present study are in constant with the previous report of Akesowan (2010), who reduced reduction in diameter of light pork burger using SPI incorporation. The results are also in agreement with Shahiri Tabarestani and Mazaheri Tehrani (2014), who utilized soy flour, split-pea flour and wheat starch to improve texture properties of low-fat hamburger by reducing shrinkage. Smith et al. (1976) also confirmed that presence of textured soy protein is associated with substantial reduction of shrinkage in blended ground beef patty. Cooking temperature and time are key effective factors in dimension changes of emulsion-type products (Das et al. 2009). Cooking at high temperature causes protein denaturation, which consequently reduces pore sizes in system and collapse of emulsion structure (Kassama et al. 2003). It has also been reported that the content (Serdaroğlu and Değırmencioğlu 2004) and type of fat (Das et al. 2009) in formulation can have remarkable effect on the diameter and thickness of cooked emulsion.

As shown in Table 5, the lowest pH was obtained 6.6 for chicken meat sausage. This obtained pH is close to the pH of fresh chicken, which is approximately between 5.9 to 6.3 (Swatland 2008). The pH of sample increased to 6.95 and 7.01 when chicken was replaced by plant proteins in sample B and A, respectively. Ahmad et al. (2010) reported that incorporation of SPI at 25% significantly increased the pH (from 5.7 to 6.8) in buffalo meat sausage, which is comparable to the findings of the current study.

Sensory evaluation

The results of sensory assessment are shown in Table 6. No significant change ($p > 0.05$) observed in the scores of appearance, color, odor, taste, texture and overall acceptance between samples A and B. However, the chicken meat sausage (sample C) showed significant differences in terms of overall appearance, taste and texture. The meat-free and meat-reduced sausages had better overall appearance and texture as compared to sample C, however, the chicken meat sausage showed superiority in terms of taste. No significant difference found in terms of color, odor and overall acceptance amongst all groups. Soy-based ingredients can affect the flavor of comminuted meat depending on its quantity used and type of product. Porcella et al. (2001) reported that incorporation of SPI at 7.5 and 10% in raw sausage was detectable by panelists, while at 5% SPI, no beany flavor was detected in the taste of sausages. Other studies also reported that incorporation of different types of soy family (soy paste, SPI or TSP) create a distinct beany flavor in meat products and reduced sensory scores (Danowska-Oziewicz 2014; Das et al. 2008a). Interestingly, in the present study, no beany flavor was detected by the panelists, and the taste and odor were highly acceptable. This might be due to the type and quantity of spices used which could successfully cover the beany flavor in the cooked sausages. Overall, the sensory evaluation revealed that using the mixed plant proteins, as a meat alternative in sausages is highly desirable with respect to sensory criteria.

Conclusion

This study was carried out to investigate the suitability of plant proteins to substitute completely and partially chicken meat in sausage. The results demonstrated that the mixed plant ingredients, namely SPI and gluten improved the emulsion stability and minimized the shrinkage, cooking and frying loss. However, the non-meat proteins in the emulsion system resulted in a poor folding/elasticity and gel quality. According to the results of sensory evaluation, the overall acceptability of the meat-free sausages was similar to the full-meat sausage. In conclusion, the findings of this study suggest that the replacement of 100% meat with plant proteins in sausage formulation is promising. Nevertheless further works are required to improve the gel-forming characteristics, which is the major obstacle in manufacturing meat-free sausage.

Acknowledgment The authors are grateful to the Director CSIR-CFTRI, Mysore for providing necessary facilities. The first author also wishes to thank the Council of Scientific and Industrial Research (CSIR), India and The Third World Academy of Sciences (TWAS), Italy for the award of research fellowship (FR No. 3240280457).

Compliance with ethical standards

Conflicts of interest The authors declare no conflict of interest.

References

Ahmad S, Rizawi JA, Srivastava PK (2010) Effect of soy protein isolate incorporation on quality characteristics and shelf-life of buffalo meat emulsion sausage. J Food Sci Technol 47:290–294

Akesowan A (2010) Quality characteristics of light pork burgers fortified with soy protein isolate. Food Sci Biotechnol 19:1143–1149

Aktaş N, Genccelep H (2006) Effect of starch type and its modifications on physicochemical properties of bologna-type sausage produced with sheep tail fat. Meat Sci 74:404–408

Amani H, Rigi S, Shahrokhisahne B (2017) Processed meat products: health issues and attempts toward healthier food. Int J Agric Sci Vet Med 5:1–9

Arihara K (2006) Strategies for designing novel functional meat products. Meat Sci 74:219–229

Asgar MA, Fazilah A, Huda N, Bhat R, Karim AA (2010) Nonmeat protein alternatives as meat extenders and meat analogs. Compr Rev Food Sci Food Saf 9:513–529

Cardoso C, Mendes R, Nunes ML (2008) Development of a healthy low-fat fish sausage containing dietary fibre. Int J Food Sci Technol 43:276–283

Choi YS, Choi JH, Han DJ, Kim HY, Lee MA, Kim HW et al (2009) Characteristics of low-fat meat emulsion systems with pork fat replaced by vegetable oils and rice bran fiber. Meat Sci 82:266–271

Cofrades S, López-López I, Solas MT, Bravo L, Jiménez-Colmenero F (2008) Influence of different types and proportions of added edible seaweeds on characteristics of low-salt gel/emulsion meat systems. Meat Sci 79:767–776

Danowska-Oziewicz M (2014) Effect of soy protein isolate on physicochemical properties, lipid oxidation and sensory quality of low-fat pork patties stored in vacuum, MAP and frozen state. J Food Process Preserv 38:641–654

Das AK, Anjaneyulu ASR, Gadekar YP, Singh RP, Pragati H (2008a) Effect of full-fat soy paste and textured soy granules on quality and shelf-life of goat meat nuggets in frozen storage. Meat Sci 80:607–614

Das AK, Anjaneyulu ASR, Verma AK, Kondaiah N (2008b) Physicochemical, textural, sensory characteristics and storage stability of goat meat patties extended with full-fat soy paste and soy granules. Int J Food Sci Technol 43:383–392

Das AK, Anjaneyulu ASR, Thomas R, Kondaiah N (2009) Effect of different fats on the quality of goat meat patties incorporated with full-fat soy paste. J Muscle Foods 20:37–53

Elang M, Liviawaty E, Junianto J, Rochima E (2018) The Effect of Addition Mocaf Flour to the Preference Level of Gray Eel Catfish Sausage. World Sci News 112:24–54

Elzerman JE, Hoek AC, Van Boekel MA, Luning PA (2011) Consumer acceptance and appropriateness of meat substitutes in a meal context. Food Qual Prefer 22:233–240

FAO (1992) Meat and meat products in human nutrition in developing countries. Food and Agriculture Organization of the United Nations. Bender A. ISBN: 92-5-103146-0

Feng J, Xiong YL (2002) Interaction of myofibrillar and preheated soy proteins. J Food Sci Technol 67:2851–2856

Flores M, Giner E, Fiszman SM, Salvador A, Flores J (2007) Effect of a new emulsifier containing sodium stearoyl-2-lactylate and carrageenan on the functionality of meat emulsion systems. Meat Sci 76:9–18

Hidayat BT, Wea A, Andriati N (2017) Physicochemical, sensory attributes and protein profile by SDS-PAGE of beef sausage substituted with texturized vegetable protein. Food Res 2:20–31

Hwang KE, Choi YS, Choi JH, Kim HY, Kim HW, Lee MA, Kim CJ (2011) Effect of ganghwayakssuk (*Artemisia princeps Pamp.*) on oxidative stability of deep fried chicken nuggets. Food Sci Biotechnol 20:1381–1388

IARC (2015) Monographs evaluate consumption of red meat and processed meat. International Agency for Research on Cancer. World Health Organization. https://www.iarc.fr/en/media-centre/pr/2015/. Accessed Mar 2018

Jang HS, Lee HC, Chin KB (2015) Evaluation of porcine myofibrillar protein gel functionality as affected by microbial transglutaminase and red bean [*Vignia Angularis*] protein isolate at various ph values. Korean J Food Sci An 35:841

Jiménez-Colmenero F, Cofrades S, Herrero AM, Fernández-Martín F, Rodríguez-Salas L, Ruiz-Capillas C (2012) Konjac gel fat analogue for use in meat products: comparison with pork fats. Food Hydrocoll 26:63–72

Kamani MH, Safari O, Mortazavi SA, Atash MMS, Azghadi NM (2017) Using an image processing based technique and predictive models for assessing lipid oxidation in rainbow trout fillet. Food Biosci 19:42–48

Kassama LS, Ngadi MO, Raghavan GSV (2003) Structural and instrumental textural properties of meat patties containing soy protein. Int J Food Prop 6:519–529

Kilincceker O, Hepsag F (2012) Edible coating effects on fried potato balls. Food Bioprocess Tech 5:1349–1354

Kurćubić VS, Mašković PZ, Vujić JM, Vranić DV, Vesković-Moračanin SM, Okanović ĐG, Lilić SV (2014) Antioxidant and antimicrobial activity of *Kitaibelia vitifolia* extract as alternative to the added nitrite in fermented dry sausage. Meat Sci 97:459–467

Luruena-Martınez MA, Vivar-Quintana AM, Revilla I (2004) Effect of locust bean/xanthan gum addition and replacement of pork fat with olive oil on the quality characteristics of low-fat frankfurters. Meat Sci 68:383–389

Majzoobi M, Talebanfar S, Eskandari MH, Farahnaky A (2017) Improving the quality of meat-free sausages using κ-carrageenan, konjac mannan and xanthan gum. Int J Food Sci Technol 52:1269–1275

Malav OP, Talukder S, Gokulakrishnan P, Chand S (2015) Meat analog: a review. Crit Rev Food Sci Nutr 55:1241–1245

Porcella MI, Sanchez G, Vaudagna SR, Zanelli ML, Descalzo AM, Meichtri LH, Lasta JA (2001) Soy protein isolate added to vacuum-packaged chorizos: effect on drip loss, quality characteristics and stability during refrigerated storage. Meat Sci 57:437–443

Savadkoohi S, Hoogenkamp H, Shamsi K, Farahnaky A (2014) Color, sensory and textural attributes of beef frankfurter, beef ham and meat-free sausage containing tomato pomace. Meat Sci 97:410–418

Schulze MB, Manson JE, Willett WC, Hu FB (2003) Processed meat intake and incidence of Type 2 diabetes in younger and middle-aged women. Diabetologia 46:1465–1473

Serdaroğlu M, Değırmencioğlu Ö (2004) Effects of fat level (5%, 10%, 20%) and corn flour (0%, 2%, 4%) on some properties of Turkish type meatballs (koefte). Meat Sci 68:291–296

Serdaroglu M, Ozsumer MS (2003) Effects of soy protein, whey powder and wheat gluten on quality characteristics of cooked beef sausages formulated with 5, 10 and 20% fat. EJPAU 6(2)

Shahiri Tabarestani H, Mazaheri Tehrani M (2014) Optimization of physicochemical properties of low-fat hamburger formulation using blend of soy flour, split-pea flour and wheat starch as part of fat replacer system. J Food Process Preserv 38:278–288

Siegel DG, Church KE, Schmidt GR (1979) Gel structure of nonmeat proteins as related to their ability to bind meat pieces. J Food Sci 44:1276–1279

Smith GC, Marshall WH, Carpenter ZL, Branson RE, Mnnke W (1976) Textured soy proteins for use in blended ground beef patties. J Food Sci 41:1148–1152

Su YK, Bowers JA, Zayas JF (2000) Physical characteristics and microstructure of reduced-fat frankfurters as affected by salt and emulsified fats stabilized with nonmeat proteins. J Food Sci 65:123–128

Swatland HJ (2008) How pH causes paleness or darkness in chicken breast meat. Meat Sci 80:396–400

Yıldız-Turp G, Serdaroğlu M (2008) Effect of replacing beef fat with hazelnut oil on quality characteristics of sucuk-A Turkish fermented sausage. Meat Sci 78:447–454

Youssef MK, Barbut S (2011) Effects of two types of soy protein isolates, native and preheated whey protein isolates on emulsified meat batters prepared at different protein levels. Meat Sci 87:54–60