Title of the manuscript: A healthier Northeastern Thai fermented sausage (Sai Krok E-san) by partial replacement of pork backfat with konjac gel.

ABSTRACT

Objective: In order to evaluate the feasibility of using konjac gel as fat analog in Northeastern Thai fermented sausage (Sai Krok E-san), the influence of konjac gel level on fermentation process and product qualities were assessed.

Methods: Five treatments of fermented sausages were formulated by replacing pork backfat with 0%, 7.5%, 22.5%, and 30% konjac gel. The changes in lactic acid bacteria (LAB) and important physicochemical properties of samples were assessed during 3 days of fermentation. After the end of fermentation at day 3, water activity (a_w), instrumental texture, color, microbial counts, and sensory evaluation were compared. The best product formulation using konjac for replacing pork back fat were selected and used to compare proximate composition and energy value with control sample (30% pork backfat).

Results: An increased in konjac gel resulted in higher values of LAB, total acidity, and proteolysis index with lower pH and lipid oxidation during 3 days of product fermentation (p<0.05). It was noted that larger weight loss and product shrinkage during fermentation was observed with higher levels of konjac gel added (p<0.05). The resulting sausage at day 3 with 15-30% konjac gel exhibited higher hardness, cohesiveness, gumminess, springiness, and chewiness than control (p<0.05). The external color of samples with 22.5-30% konjac gel were redder than others (p<0.05). Mold, Salmonella spp., S. aureus, and E. coli in all finished products were lower than detectable levels. Product with 15% konjac gel had the highest scores of sourness linking and overall acceptability (p<0.05).

Conclusion: The product with 15% of konjac gel was the optimum formulation for replacing pork backfat. It had higher sensorial scores of sourness and overall acceptability than control with less negative impact on external appearance (product shrinkage) and weight loss. Moreover, it provided 46% fat reduction and 32% energy reduction than control.

Keywords: Sai Krok E-san, Fermented sausage, Konjac, low-fat meat product, Healthy meat product, Functional meat
INTRODUCTION

Northeastern Thai fermented sausage, which is called Sai Kork E-san, is a traditional fermented sausage originating from the northeastern part of Thailand and nowadays it is widely consumed in various parts of Thailand. It is also called in Thai as Sai Krok Prew because of its sour taste resulting from meat fermentation by lactic acid bacteria (LAB). The product is roasted and consumed as a main dish together with cooked rice or steamed glutinous rice and also thinly sliced fresh or pickled ginger, whole bird chilli, and cabbage. Another similar product, called Nham, have been extensively studied providing the understand of acid meat fermentation [1-5]. These two types of Thai-fermented meat are also formulated from minced pork, cooked rice, garlic, and curing and seasoning ingredients, which are fermented under restrictive oxygen condition for 2–3 days at room temperature, allowing lactic acid fermentation and pH drop consequently producing the sour taste. However, there were some differences in recipes and manufacturing processes among products. Northeastern Thai fermented sausage contains a high level of fat and the product mixture is stuffed into natural hog casing prior to hanging for some water drain out, which the final product has a protein content of at least 12% and fat content of 30% or below [6]. On the contrary, Nham is produced from lean meat with pork rind and tightly packaged in plastic casing, where a protein content of at least 20% and fat content of 8% or less are contained in the final product [7]. Currently, consumer is more pay attention to the product with lower fat levels due to health concerns such as obesity, colon cancer, and cardiovascular diseases [8]. As reviewed by Grasso et al. [9], regarding fat issue, two important strategies to produce healthier meat products include the fat reduction and the modification of fatty acid profile with other healthier fat or oil. Among several ingredients that are incorporated into low-fat meat product, konjac-based fat analog are one interested strategy.

Konjac is a neutral polysaccharide produced by a root of native plant from East Asia, namely Amorphophallus konjac, which is utilized as a food additive in Europe with the number of E-425, and classified as GRAS by the FDA [10]. The konjac provide the numerous technological properties (water-holding capacity and gelling and thickening agents) as well as potential health implication (dietary fiber, prebiotic, reducing of cholesterol, insulin, and glucose levels or satiating and laxative effects), leading the widely utilization in food products [11]. However, its gelation with high ability to bind water seems to be the main advantage of konjac flour and often use to forms gel with other ingredients such as starch, carrageenan, and gellan. Moreover, its gel can be ground to a desired particle size giving the appearance of visible granulated fat and used as “fat analogs” [12]. To replace animal fat in low-fat meat products, the konjac gel with different forms at various levels has been used to reduce fat in products such as frankfurters [13-14], bologna [15], fresh sausages [16], pork nuggets
Spanish dry-fermented sausage [12], and merguez sausage [10]. However, no studies regarding konjac gel application for Northeastern Thai fermented sausage which possess up to 30% fat content have been reported as well as the changes in qualities during fermentation of both normal- and reduced-fat formulation of this fermented sausages have not been characterized. Since fat contribute positively to taste, texture, and mouth feeling, how much fat in this distinct sausage can be replaced by konjac gel with negatively impact to sensorial and technological qualities need to be clarified. Therefore, the objectives of this research was to elucidate the changes in microbiological and physicochemical properties of Northeastern Thai fermented sausage during fermentation when part of pork backfat was replaced with konjac gel, and also to evaluate the sensorial quality, proximate composition, and total energy among different levels of konjac gel replacing pork backfat in finished product.

MATERIALS AND METHODS

Raw material preparation

Fresh pork ham, pork backfat were purchased form a local market. Salted natural hog casing (diameter 28–30 mm, Van Hessen, Van Hessen B.V., Netherlands) was soaked in water prior to use. Lean ham meat and backfat were separately ground through a 6 mm plate. Konjac gel was prepared as the process described by Osburn and Keeton [14] and Jiménez-Colmenero et al. [15]. The ingredients of konjac fat analog were formulated as follows; 5% konjac flour, 1% k-carragenan, 3% corn starch, 0.1% Ca(OH)$_2$, and 81% water, which these ingredients were kindly donated by Thai Food and chemical Co., Ltd, Thailand. After forming of a konjac gel, it was ground through a 6 mm plate and resulting ground gel is shown in Figure 1a. The ground konjac gel, consisting of approximately 91.5% water, 0.65% ash, 0.39% protein, 0.11% fat, and 5.38% dietary fiber, were used for reformulation of fermented sausage. These raw materials were randomly assigned to five different treatments of fermented sausage.

Processing of Northeastern Thai fermented sausage and experimental design

Northeastern Thai fermented sausage was performed as described by Sethakul and Sivapirunthep [18] with slight modifications. Briefly, this sausage consisted of ground pork (55% w/w), ground backfat (30%), and cooked rice (15% w/w) with the following ingredients (in g/kg of meat mixture) were added: prague powder (15 g/kg, also known as curing salt for meat preservative that curing mixture composed of 0.90% sodium nitrite and 99.1% sodium chloride), sodium tripolyphosphate (STPP, 3.0 g/kg), sodium erythorbate (2.0 g/kg),...
monosodium glutamate (2.5 g/kg), sugar (5.0 g/kg), ground garlic (50 g/kg), and fine ground black pepper (4.0 g/kg). To determine the effect of konjac gel for use as pork backfat replacer on product quality, five formulations of mixture were performed with the following treatments: 1) a control treatment (30% backfat without konjac gel); 2) 7.5% konjac gel and 22.5% pork backfat; 3) 15% konjac gel and 15% pork backfat; 4) 22.5% konjac gel and 7.5% pork backfat; 5) 30% konjac gel without pork backfat. Each sausage treatment (3 kg/batch) was manufactured with the same process as the following details. Firstly, meat was mixed with prague powder, STPP, and erythrobate. Then, a backfat and/or a konjac gel were mixed followed by cooked rice. The remaining ingredients were added and manually mixed until homogenously. The prepared sausage mixture was stuffed into natural hog casing using hydraulic sausage stuffer and then hand-linked to sizes of 11–12 cm with approximately 65–75 g of stuffed samples, which a representative of sausage picture after stuffing is shown in Figure 1b. A total of 40 stuffed samples were prepared for each treatment. To start the fermentation process, the stuffed sample was hung at room temperature (32 ± 2°C) for 3 days. The manufacturing processes were done in triplicate with 2 weeks interval, resulting in three independent replication batches (n=3) for each of the 5 treatment groups (a total of 15 experimental units). Samples from each formulation were taken at day 0, 1, 2, and 3 for monitoring the changes in LAB and physicochemical properties. Moreover, after the end of the fermentation at day 3, water activity (a_w), instrumental texture, color, microbial counts, and sensory evaluation were compared among five formulated treatments. Then, the optimum reformulation (based on sensory quality together with physicochemical property) was selected and determined proximate composition and energy value as compared with control sample.

Changes in LAB and physicochemical properties during 3 days of fermentation

LAB analysis

The samples (25 g) from triplicate sampling were aseptically transferred into 225 mL of sterile saline (0.85% w/v NaCl) in a sterile plastic bag. Samples were then homogenized using the Stomacher BagMixers 400 VW (Interscience Co., France). One mL of appropriate dilutions (10^-2–10^-9) was dropped in duplicate on de Man Rogosa Sharpe (MRS) agar plate supplemented with 0.8% (w/v) calcium carbonate (10 mM CaCl_2 [19]) (Sigma-Aldrich, St. Louis MO). After anaerobic incubation at 30°C for 24–48 h, colonies with clear zones were counted and expressed as logarithms of colony forming units per gram (Log CFU/g).
Determination of total acidity

Total acidity of sample was performed according to the method of AOAC [19,20]. Sample (2 g) was homogenized in 20 mL of distilled water using homogenizer (Ultra-Turrax, IKA Labortechnik, Staufen, Germany). The homogenate was then centrifuged at 5000×g for 15 min and the supernatant was filtered through a filter paper (Whatman No.4). The filtrate was added with three drop of phenolphthalein solution (1% w/v) and titrated with a standardized 0.1 N NaOH until a light pink color persisted. Total acidity of the sample was expressed as percentage (%) lactic acid. Triplicate determinations on each treatment were performed.

Measurement of pH

The pH of sample was determined by homogenizing 2 g of samples with 20 mL of distilled water using a homogenizer. The pH of suspension was recorded using a combined glass electrode with a digital pH meter (SevenEasy pH meter S20, Mettler Toledo, Schwerzenbach, Switzerland). Triplicate determinations on each treatment were performed.

Determination of weight loss and moisture content

Weight loss during fermentation process was evaluated as % of initial weigh of sample (day 0). Moisture content was determined according to AOAC methods [19,20]. All determinations were performed in triplicate.

Determination of trichloroacetic acid (TCA)-soluble peptides

TCA-soluble peptide contents were determined using the method of Morrissey et al. [21]. Ground sample (2 g) was homogenized with 20 mL of 5% (w/v) TCA using an IKA labortechnik homogenizer. The homogenate was kept in ice for 30 min and then centrifuged at 5000×g for 20 min (Jouan CR3i, Saint-Herblain, France). Soluble peptides in the supernatant were measured by the Lowry method, using tyrosine as standard, and expressed as μmol tyrosine/g sample.

Determination of thiobarbituric acid reactive substance (TBARS)

The TBARS was determined to establish the extent of lipid oxidation and was performed according to the method of Buege and Aust [22]. Briefly, sample (5 g) was dispersed in 25 mL of TBA solution containing 0.0375% (w/v) TBA, 15% (w/v) TCA and 0.25 M HCl. The mixture was homogenized for 1 min, heating at
100°C for 10 min and cooled to room temperature with running water. The mixture was centrifuged at the speed of 3,600×g for 20 min. The absorbance of the supernatant was read at 532 nm using double beam UV–VIS spectrophotometer (UV–1601, Shimadzu Corporation, Japan). The TBARS value was calculated using a standard curve produced from malonaldehyde bis (dimethyl acetal) (MDA) at concentration ranging from 0 to 10 ppm, and the value was expressed as mg MDA/kg sample. Triplicate determinations were done on each treatment.

Physicochemical, microbiological, sensorial, and nutritional qualities of finished product

Measurement of aw

Regarding aw measurement, ground sample was put into water activity pan and aw value was determined using a Novasina® LabMaster-aw (Axair Ltd., Switzerland). Triplicate determinations were done on each treatment.

Measurement of texture profile analysis (TPA)

The sample was subjected to TPA using an Instron universal testing machine model 1011 with a compression plate surface. Six cylinder-shaped samples (~25 mm diameter × 25 mm height) were prepared and placed on the instrument's base. TPA textural parameters were measured at room temperature with the following testing conditions: crosshead speed was 60 mm/min and compressed twice to 40% of their original height. The Bluehill 2 software (Instron Engineering Corp., Canton, MA) was applied to collect and process the data. TPA analyses including hardness (N), cohesiveness (ratio), gumminess (N), springiness (ratio) and chewiness (N) were calculated from the force-time curves generated for each sample.

Measurement of instrumental color

The external and cross-sectional color of samples were measured by the CIE L*, a*, b* system using a Colorimeter MiniScan EZ 4000L (Hunter Lab Inc, USA) standardized with a white plate and black plate. The three replicates of each treatment were taken. Lightness (L*), redness (a*) and yellowness (b*) values were recorded.
Microbiological analysis

Microbiological analyses were triplicate sampling at day 0 and day 3 of fermentation for yeast and mold, *Staphylococcus aureus*, *Salmonella* spp., and *Escherichia coli*. The samples (25 g) were placed in 225 mL sterile saline solution (0.85% w/v NaCl) in a sterile stomacher bag and were then homogenized using the Stomacher. Serial dilutions were performed in duplicate and were grown in different culture media. The following media and incubated conditions were used: (1) potato dextrose agar (Merck, Darmstadt, Germany) incubated at 25°C for 3–5 days for yeast and mold counts, (2) Baird-Parker agar (Merck, Darmstadt, Germany) incubated at 37°C for 24–48 h and the coagulase test was used for the identification of *S. aureus* colonies for *S. aureus* count[^223]^, and (3) *Salmonella* spp. was estimated in 25 g according to ISO 6579: 2002[^224]^, these microbial counts were expressed as logarithms of colony forming units per gram (Log CFU/g). Regarding *E. coli*, Fluorocult® LMX Broth (Merck, Darmstadt, Germany) incubated at 37°C for 24–48 h and the IMViC test was used for the identification of *E. coli* for estimating as most probable numbers per gram (MPN/g)[^245].

Sensory evaluation

The five groups of fermented sausages were prepared for sensory evaluation. Samples were grilled in a pan until the core temperature (CT) reached 75°C, as monitored by probes of Type-K thermocouple from a digital thermometer (52 Series II, Fluke Corp., Everett, WA). Cooked samples were evaluated by 12 trained panelists from researchers and meat science graduate students of Department of Animal Production Technology and Fishery, KMITL. There are 3 sensory evaluation sessions in the present study corresponding to each processing batch and every treatment (5 treatments) was provided in a session. During each evaluation, panelists were served with a half string of sausage from each treatment for evaluating the external appearance, which determined by visual observation based on product shrinkage and wrinkles on the casing surface. Additionally, panelists were served with 2 cuts of sausages (20 mm thickness) from each treatment for evaluating color, flavor, sourness, texture, juiciness, and overall acceptability of samples. The following nine-point hedonic scale was carried out: 1 = Dislike Extremely 2 = Dislike very much 3 = Dislike moderately 4 = Dislike slightly 5 = Neither like nor dislike 6 = Like slightly 7 = Like moderately 8 = Like very much 9 = Like Extremely. Panelists were served with unsalted cracker and water for refreshing their palates before between samples.

Determination of proximate composition and energy value
The best formulation of fermented sausage using konjac for replacing pork back fat were selected and used to analyze proximate composition and energy value as compared with control sample. The proximate compositions (moisture, ash, crude protein, crude fat, dietary fiber, and carbohydrate) of samples were determined in triplicate using the methods described by the AOAC [19-20]. Total energy content of product was measured by automatic bomb calorimeter (Leco AC-350 calorimeter, Leco Corporation, St. Joseph, MI, USA), which expressed as kcal/100 g. Energy value from fat content (× 9 kcal/g) was calculated and reported as a percentage of calories from fat. Total fat and total energy reductions were also calculated.

**Statistical analysis**

Statistical analysis for fermented sausage quality during fermentation and finished product quality at the end of fermentation among five treatment of konjac gel were carried out by two-way ANOVA and one-way ANOVA, respectively. When the significant effect was found, mean values was compared by the Duncan's multiple range test (DMRT). Pearson’s correlation coefficients were evaluated to describe the correlation among parameters. Analysis was performed using the SPSS package (SPSS 16.0 for windows, SPSS Inc., Chicago, IL, USA).

**RESULTS AND DISCUSSION**

**Changes in LAB, total acidity, and pH during fermentation**

The numbers of LAB in fermented sausages processed by replacing pork backfat with different level of konjac gel are shown in Figure 2a. The initial levels of LAB in samples at day 0 were 2.91–4.07 Log CFU/g. After 3 days of fermentation, these microbial populations increased sharply to 9.42–11.38 Log CFU/g, which were higher in samples with higher level of konjac gel (p<0.05). In sausage formulated with normal fat or a control, the number of LAB were within the range of those found in Nham, where were the highest values of 8–9 Log CFU/g within the third days of fermentation [5]. Lactobacilli and pediococci are predominant LAB and important for acid production during Nham fermentation [5]. The significant greater LAB in samples with increased konjac gel (p<0.05) during 3 days of fermentation was probably due to the enhanced growth of bacteria by konjac gel. This evidence was also found in merguez sausage [10], which was discussed later in the results of total acidity and pH fact that the composition in konjac gel might enhance the proliferation of lactic acid producing bacteria.
Results of total acidity and pH are shown in Figure 2b and Figure 2c, respectively. The acidity of the samples increased during fermentation with being the highest values at day 3 (1.02–1.29%), concomitantly with a decrease in the pH approximately 4.36–4.89 within 3 days. These values were similar to another type of fermented meat, which Nham had a total acidity of 0.77–1.60% with pH values ranged from 4.4–4.8 within 3 days of fermentation [2]. Glucose and fructose mainly from cooked rice and garlic in Nham formulation were used as the major sources of fermentable carbohydrate by LAB for lactic acid production, resulting in the pH decrease [2526]. Visessanguan et al. [3] found that among the organic acids detected in Nham, lactic acid was dominantly found as 80–90% of the total, followed by acetic and oxalic acid. Furthermore, at the end of fermentation, total acidity was found to be the lowest in control (1.02 ± 0.02%) and the highest in 30% konjac (1.29 ± 0.09%) (p<0.05). These results were coincidental with the dramatic decrease of pH, where the largest pH drop was found in 30% konjac (p<0.05). Konjac is polysaccharide-based fiber and mainly composed of mannose separated by glucose units in the backbone, where the glucose to mannose ratio is 1 to 1.6 with one acetyl group per six glucose residues [2627]. Konjac provides a prebiotic potential because it shows very little degradation in the digestive tract and is fermented by beneficial human bacterial strains [2627-2728]. Williams [11] stated that konjac glucomannan provides a prebiotic addition to the diet, which acts to stimulation of the growth of lactic bacteria in the colon and improves fermentation in food processing. Triki et al. [10] also found that not only konjac flour but also the pre-gelatinized starch in konjac gel could serve as fermentable carbohydrate sources in a merguez sausage, resulting in lower pH values of formulations with higher levels of konjac gel. Since there were a strong significant correlation of LAB with total acidity (r = 0.820, p<0.01) and pH (r = −0.792, p<0.01) in the present study (data not shown), the increasing of fermentable carbohydrate content implied by replacing of pork backfat with a higher content of konjac gel could stimulate the LAB growth, provide the greater extent of lactic acid production and lead to the lower pH.

Changes in weight loss and moisture during fermentation

Weight loss of samples were increased during fermentation (p<0.05) as presented in Figure 3a. Consequently, the moisture content of samples decreased as the fermentation time increased (p<0.05) Figure 3b. The extent of water loss during fermentation was mainly dependent on the capacity of meat protein to retain water. Generally, when pH of product during fermentation decline closer to the isoelectric point (pl) of the major proteins (especially for myosin, pl=5.4 [2829]), the net charge of the protein was zero, leading to a reduction in the amount of water was attracted by those protein. Moreover, partial denaturation of the myosin.
head at low pH when the temperature is still high is also believed to be impacted for the shrinkage of myofibrillar lattice spacing \[267\]. These evidence led to an increase in weight loss and a decrease in moisture content of samples during fermentation. Furthermore, increasing proportion of konjac gel resulted in higher weight loss, but it provided higher moisture content throughout 3 days of fermentation \(p > 0.05\). These results were in agreement with the study of Ruiz-Capillas et al. \[12\], who reported that a dry fermented sausage with more konjac gel provided a higher weight loss, but exhibited a higher water content. Konjac gel used in the current study contained approximately 91% moisture, 0.1% fat, 0.4% protein, 0.6% ash and 5.4% dietary fiber, while Heinz and Hautzinger \[29\] reported that pork backfat composed of 7.7% water, 88.7% fat, 2.9% protein and 0.7% ash. The substitution of pork backfat with equal amount of konjac gel not only reduced the total fat content of fermented sausage but also increased water added to the product mixture. Moreover, there was a significant negative correlation between weight loss and pH \(r = -0.596, p < 0.01\) \(\text{data not shown}\). These observations pointed out that a greater water loss in a product with 30% konjac gel along fermentation presumably caused by a higher extent of meat protein denaturation due to rapid pH decline together with some syneresis water from konjac gel during processing. Although reformulation with increasing konjac gel could contribute the more water containing in product and resulted in high moisture content, the presence of higher weight loss with increasing of konjac gel led to a higher level of product shrinkage as shown in Figure 4a, which the highest level of shrinkage was found in 30% konjac gel added. Unlike water, fat slightly lost during fermentation process and could be retained in a finished product; therefore, it could retard the shrinkage of high-fat formulation.

**Changes in TCA-soluble peptides during fermentation**

TCA-soluble peptides, an indication of the extent of proteolysis among fermented meat, are shown in Figure 5a. It was found that these peptides increased from 2.6–2.8 μmol tyrosine/g sample at the beginning to 4.0–4.8 μmol tyrosine/g sample at the end of fermentation \(p < 0.05\). Endogenous muscle proteases is considered as a primary role in proteolysis, while exogenous microbial enzymes from lactic acid bacteria may play a minor role \[30\] \[31\], where the extent of these proteolysis depends on the product and conditions during ripening \[32\] \[33\] \[34\] \[35\]. The degradation of myofibrillar and sarcoplasmic proteins was mediated by endogenous cathepsin, followed by the action of bacterial enzymes degraded oligopeptides into small peptides and free amino acids, contributing the flavor and aroma of dry fermented sausages \[32\] \[33\] \[34\] \[35\] \[36\]. Moreover, samples with 15–30% konjac added had higher TCA-soluble peptides than control \(p < 0.05\). There was a positive correlation between LAB...
and these peptides contents ($r = 0.908$, $p<0.01$) (data not shown). The results confirmed that konjac formulation could accelerate the fermentation process via stimulating LAB growth as aforementioned detail, resulting in increasing of small peptides.

Changes in TBARS during fermentation

Increasing of TBARS value, indicating increased lipid oxidation during sausage fermentation, were observed in all sample ($p<0.05$) (Figure 5b). The LAB and other aerobic bacteria isolated from Thai-fermented meat product namely Nham were hydrogen peroxide ($H_2O_2$) producers which is a strong oxidizing agent to accelerate lipid oxidation and produce rancid flavor [1]. Visessanguan et al. [3-4] stated that although the oxidation level in Nham was relatively high, the levels would not sufficient to increase the detrimental impact on odor or tastes in Nham. Stahnke [334] reported that there are several ethyl esters occurred during fermentation of dried sausage which associated with fruity aromas can mask rancid odors in the resulting product. Additionally, the control sample showed a higher lipid oxidation than samples with 7.5–22.5% konjac, and 30% konjac, respectively ($p<0.05$). The significant reduction in TBARS value of low-fat merguez sausage by replacing pork backfat with konjac gel has been reported by Triki et al. [10]. When pork backfat that possesses high amounts of unsaturated and polyunsaturated fatty acids and susceptible to oxidation was replaced by konjac gel, the lipid oxidation would be normally minimized. Moreover, there is the strong possibility that the replacing pork backfat with konjac gel would contribute to the shelf-stability of the product.

Finished product characteristics

$a_w$ and texture

Values of $a_w$ among various formulation were approximately 0.97–0.98, which are presented in Table 1, where was shown no significant differences among samples at the end of fermentation. The $a_w$ of fermented meats varies depending on the size of the meat, length of ripening, salt and fat contents, casing permeability, temperature and the relative humidity of the air [3435]. An $a_w$ value of Nham after the end of fermentation ranged from 0.95–0.98 depending on the formulation [4536], while levels of $a_w$ for dry fermented sausage is around 0.81–0.83 [12].

At the end of fermentation, fermented sausages with 15–30% konjac gel exhibited higher hardness, cohesiveness, gumminess, springiness, and chewiness as compared to control ($p<0.05$) (Table 1). In fact, the acid-induced gelation of muscle protein during meat fermentation causes the formation of texture become
more rigid, elastic, cohesive, and less adhesive [3]. The more rapid pH decline with increased konjac gel in products might partly associate with the formation of larger aggregation of proteins and resulted in a markedly increase in all texture parameters evaluated by TPA. Similar results have been reported by Ruiz-Capillas et al. [12] for dry fermented sausage ripening for 17 days. They stated that the increasing of konjac gel for replacing fat in dry fermented sausage contributed an increase in hardness and chewiness. However, a decrease in cohesiveness was found as increased the proportion of konjac gel [12], which this detrimental effect was not observed in the current study. This is because the composition of the current product was largely different from another one. While a dry fermented sausage formulated by 74% pork and 18.5% pork backfat, Northeastern Thai fermented sausage produced by 55% pork, 30% pork backfat, and 15% cooked rice. The cooked rice could represent as a binder between meat and konjac parts, therefore, homogenous product was produced and showed a high cohesiveness even though it contained up to 30% konjac gel. Concomitantly with the greater acid-induced gelation with the increasing konjac gel, the higher cohesive texture, especially as compared with control, could observe in Figure 4b.

Color

The instrumental color parameters of samples at the end of fermentation are presented in Table 1. While cross-sectional color of samples among various konjac contents (in terms of lightness, redness, and yellowness) were not significant differences (p > 0.05), the significant differences in redness of external surface among samples were observed (p < 0.05). External parts of samples with 22.5–30% konjac gel had redder (higher a* value) than those with 0–15% konjac (p < 0.05). These results corresponded with product pictures as illustrated in Figure 4a, which external parts of sausages containing 22.5–30% konjac were redder than others, while cross-sectional colors among samples seem to be not differently observed (Figure 4b). The redder of external part of samples with increased konjac gel was related to higher weight loss during fermentation and consequently case hardening. However, the small differences in cross-sectional color might be related to the suppressible effect by added konjac gel, which is a white color and contained a high content of water in a gel. There are inconsistency results regarding effect of replacing fat with konjac gel among various types of meat products. In dry fermented meat product, the redness tended to decrease with sample adding with a higher proportion of konjac gel [12]. In frankfurter sausage, reduced-fat products tended to lower L* and higher a* with increasing konjac gel [13]. Liaros et al. [26,27] suggested that pH, weight loss, fat reduction, and product
composition contributed variations in the myoglobin concentration of fermented sausage and affected to product color.
Microbiological quality

As previously stated, the LAB was the predominate microorganism in fermented sausage with 9–11 Log CFU/g at the 3rd day of fermentation. There were no molds and *Salmonella* spp. detected both in initial and finished products (Table 2). *S. aureus* counts were 1–2 Log CFU/g at the beginning with the dramatic decrease as increased konjac gel (p<0.05), indicating more detection in pork backfat rather than konjac gel. However, the population of *S. aureus* reached to a non-detectable level after 3 days of fermentation. *E. coli* were detected only in the control group at the first day and were not found in all samples after the end of fermentation.

Enterobacteriaceae and *Staphylococcus aureus* decreased during 3 days of Nham fermentation also reported by Wiriyacharee [35]. The numbers of yeast were initially found as 3–4 Log CFU/g and then decrease to 2–3 Log CFU/g at day 3, which were not significant differences among treatment (p>0.05). As summarized by Selgas and Garcia [37], yeasts are most generally found in fresh meat including *Candida*, *Rhodotorula*, *Debaryomyces*, and *Trichosporum*. In fermented meats, although the lactic acid produced by bacteria modified environmental factors that hinder the growth of yeasts, many species can even grow at pH 4 [38]. Osei Abunyewa et al. [39] reported that yeasts were found with initial values of 3 Log CFU/g in commercial salami, but this number increased after 12 days of maturation, reaching a maximum of 5 Log CFU/g at day 20.

The presence of yeasts has also been considered to enhance the flavor and aroma of fermented sausage and the *Debaryomyces hansenii* was found to be the dominant yeast observed in fermented sausages with initially 3 Log CFU/g to 6 Log CFU/g along 60 days of fermentation, where the final pH value was about 5.7. However, the yeast biodiversity in different types of fermented sausages could be varied and also depend upon the pH of final product. In case of the fermented sausage in the present study, the rapid fermentation by LAB producing lactic acid caused a product attained to pH around 4.6 within 3 days of fermentation, leading to retard the growth of yeasts and also the development of pathogenic bacteria.

Sensory evaluation

The effect of replacing pork backfat with konjac gel in fermented sausage is given in Table 3. The sausage with 22.5–30% konjac gel had a lower sensory score of external appearance than the lower levels samples. This meant that the shrinkage of products and the presence of wrinkles on the surfaces of samples containing more than 15% of konjac gel were noticed, leading to the loss of the appearance liking score which is observed in Figure 4a. However, samples containing more konjac gel had similar sensory scores of cross-sectional color, texture, and juiciness as compared with control (p>0.05). In some case, a low-fat dry
fermented sausage with 80% replacing pork backfat with konjac gel and ripening for 17 days showed a lower juiciness than control [12]. Mendoza et al. [40] also reported that the adverse effects of fat reduction in dry fermented sausage were less tender and juicy. In the present study, samples were fermented with a shorter time (3 days) as compared with those reports. Thus, when they formulated with a higher proportion of konjac gel, they still contained higher water content. The release of water fluids from these products could lubricate the texture during chewing, thus the sensation of juiciness and tenderness were not different as compared with the full-fat formulation.

Adding konjac gel with 15–30% in product showed a higher flavor scores as compared with control (p>0.05). The favorable intensity of overall flavor of this kind of fermented sausage was combined by fermented, acidic, and garlic flavors. Due to increased konjac gel, the fermentation process was accelerated and those intensities were increased, giving a higher score of flavor liking. Product with 15% konjac gel had the highest score of sourness liking, while excessive sourness in those with 22.5–30% could be detected by panelists and resulted in lower sourness liking score. Furthermore, the highest overall acceptability was found in the sample with 15% konjac gel, representing the balance formulation for replacing pork back fat as evaluated by sensory.

Proximate analysis and energy value

From the previously result, the product with 15% of konjac gel was the optimum formulation for replacing pork backfat. It provided higher sensory attributes in terms of sourness and overall acceptability than control. It also less negative impact on external appearance (product shrinkage) and weight loss, as compared with higher levels of konjac gel. When 15% of the pork backfat was replaced with 15% of konjac gel, a low-calorie ingredient with a high content of dietary fiber, fat content in sausage was reduced normally reduced (Table 4). As the fat content was reduced, moisture, dietary fiber, and carbohydrate contents were greater increased in sausage formulated with 15% konjac gel than control. There were no significant differences in protein and ash contents among samples (p>0.05). These changes represented a fat reduction of around 46%.

Not only compositions but also total calorie content and the percentage of calorie from fat are a key consideration in designing the new composition of any food product [40]. The total energy content of control sample as measured by bomb calorimeter was 339.70 kcal/100 g (around 76% from fat), while the reformulated sample with 15% konjac gel was 228.91 kcal/100 g (around 61% from fat). These changes resulted in total energy reduction around 32.6% as compared with control sample. Similar energy reduction
levels were reported in dry fermented sausage [12], fresh pork sausage [14], and merguez sausage [10]. The regulation in Thailand state that the reduced-fat and reduced-total energy products define as a minimum of 25% reductions in fat and total energy as compared with a conventional product [42,43]. Thus, our developed Northeastern Thai fermented sausage products using 15% konjac gel as fat analog could be considered as the “reduced-fat and reduced-calorie” product, because reductions in fat and total energy were accounted for 46% and 33%, respectively, comparing to the conventional formulation.

CONCLUSION

The sensory evaluation demonstrated that the 15% konjac gel was the optimum content to replace pork backfat in Northeastern Thai fermented sausage, which provided the highest sourness and overall acceptability scores. This reformulation contained about 46% lower fat content and also superior texture and lactic acid production as well as less lipid oxidation than regular product together. It also exhibited less detrimental impact on product shrinkage and weight loss during fermentation process as compared to 22.5-30% konjac gel.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ACKNOWLEDGEMENTS

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REFERENCES


Figure 1. A ground konjac gel used for replacing pork backfat (a) and Northeastern Thai fermented sausage containing 15% pork backfat and 15% konjac gel (b).

Figure 2. Changes in LAB (a), total acidity (b), and pH (c) of Northeastern Thai fermented sausage with different levels of konjac gel during fermentation. Bars represent the standard deviation among triplicate manufacturing of sausages (3 batches, n=3). Significant differences between treatments and fermentation times were observed in all dependent parameters (p<0.05).

Figure 3. Changes in weight loss (a) and moisture content (b) of Northeastern Thai fermented sausage with different levels of konjac gel during fermentation. Bars represent the standard deviation among triplicate manufacturing of sausages (3 batches, n=3). Significant differences between treatments and fermentation times were observed in all dependent parameters (p<0.05).

Figure 4. Effect of konjac gel for replacing pork backfat on external (a) and cross-sectional (b) appearances of Northeastern Thai fermented sausage at day 3 of fermentation.

Figure 5. Changes in TCA-soluble peptides (a) and TBARS (b) of Northeastern Thai fermented sausage with different levels of konjac gel during fermentation. Bars represent the standard deviation among triplicate manufacturing of sausages (3 batches, n=3). Significant differences between treatments and fermentation times were observed in all dependent parameters (p<0.05).
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.
Table 1. Values of aw, instrumental texture, and color of Northeastern Thai fermented sausage with different levels of konjac gel.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0% Konjac (Control)</th>
<th>7.5% Konjac</th>
<th>15.0% Konjac</th>
<th>22.5% Konjac</th>
<th>30.0% Konjac</th>
</tr>
</thead>
<tbody>
<tr>
<td>aw</td>
<td>0.97 ± 0.00abc</td>
<td>0.97 ± 0.01a</td>
<td>0.98 ± 0.00a</td>
<td>0.97 ± 0.01a</td>
<td>0.98 ± 0.00a</td>
</tr>
<tr>
<td><strong>Instrumental texture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Hardness (N)</td>
<td>6.51 ± 1.08c</td>
<td>7.74 ± 1.13c</td>
<td>14.40 ± 1.77b</td>
<td>17.84 ± 2.64a</td>
<td>18.27 ± 1.21a</td>
</tr>
<tr>
<td>- Cohesiveness (ratio)</td>
<td>0.43 ± 0.01c</td>
<td>0.46 ± 0.01d</td>
<td>0.52 ± 0.03c</td>
<td>0.55 ± 0.01b</td>
<td>0.60 ± 0.01a</td>
</tr>
<tr>
<td>- Gumminess (N)</td>
<td>2.82 ± 0.46c</td>
<td>3.53 ± 0.58c</td>
<td>7.45 ± 0.87e</td>
<td>9.85 ± 1.48d</td>
<td>10.92 ± 0.79a</td>
</tr>
<tr>
<td>- Springiness (ratio)</td>
<td>0.51 ± 0.01c</td>
<td>0.54 ± 0.03d</td>
<td>0.67 ± 0.02d</td>
<td>0.69 ± 0.03d</td>
<td>0.77 ± 0.02a</td>
</tr>
<tr>
<td>- Chewiness (N)</td>
<td>1.44 ± 0.26d</td>
<td>1.91 ± 0.29d</td>
<td>4.99 ± 0.62a</td>
<td>6.81 ± 0.99b</td>
<td>8.43 ± 0.78a</td>
</tr>
<tr>
<td><strong>External color</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Lightness (L*)</td>
<td>45.04 ± 1.87c</td>
<td>42.29 ± 2.66b</td>
<td>41.29 ± 1.73c</td>
<td>42.84 ± 2.61a</td>
<td>44.40 ± 2.08c</td>
</tr>
<tr>
<td>- Redness (a*)</td>
<td>4.82 ± 0.64b</td>
<td>6.05 ± 0.80b</td>
<td>6.09 ± 0.43b</td>
<td>6.79 ± 0.67b</td>
<td>7.11 ± 0.95a</td>
</tr>
<tr>
<td>- Yellowness (b*)</td>
<td>9.17 ± 0.23a</td>
<td>8.83 ± 1.13a</td>
<td>9.78 ± 0.92a</td>
<td>9.25 ± 0.54b</td>
<td>8.93 ± 0.53a</td>
</tr>
<tr>
<td><strong>Cross-sectional color</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Lightness (L*)</td>
<td>46.92 ± 1.94c</td>
<td>45.17 ± 3.77c</td>
<td>46.14 ± 1.89c</td>
<td>46.67 ± 3.26c</td>
<td>49.62 ± 2.47c</td>
</tr>
<tr>
<td>- Redness (a*)</td>
<td>4.89 ± 0.40a</td>
<td>5.18 ± 1.48a</td>
<td>6.07 ± 0.32a</td>
<td>6.18 ± 1.37c</td>
<td>6.28 ± 0.85a</td>
</tr>
<tr>
<td>- Yellowness (b*)</td>
<td>6.02 ± 0.90a</td>
<td>6.49 ± 1.09a</td>
<td>6.53 ± 0.28a</td>
<td>6.61 ± 0.53a</td>
<td>6.87 ± 0.81a</td>
</tr>
</tbody>
</table>

Values are given as means ± SD of each of each processing batch (n=3).

Different superscripts in the same row indicate significant differences (p<0.05).
Table 2. Microbiological counts of Northeastern Thai fermented sausage with different levels of konjac gel.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Day</th>
<th>0% Konjac (Control)</th>
<th>7.5% Konjac</th>
<th>15.0% Konjac</th>
<th>22.5% Konjac</th>
<th>30.0% Konjac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeasts (Log CFU/g)</td>
<td>0</td>
<td>3.24±0.01*†,‡</td>
<td>3.29±0.04*‡</td>
<td>3.18±0.03*‡</td>
<td>3.20±0.04*‡</td>
<td>3.19±0.03*‡</td>
</tr>
<tr>
<td>Mold (Log CFU/g)</td>
<td>3</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>S. aureus (Log CFU/g)</td>
<td>3</td>
<td>2.25±0.03*</td>
<td>2.20±0.12*‡</td>
<td>1.97±0.10*‡</td>
<td>1.95±0.07*‡</td>
<td>1.54±0.09*‡</td>
</tr>
<tr>
<td>E. coli (MPN/g)</td>
<td>0</td>
<td>11</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Salmonella spp. (Log CFU/g)</td>
<td>3</td>
<td>ND&lt;4</td>
<td>ND&lt;4</td>
<td>ND&lt;4</td>
<td>ND&lt;4</td>
<td>ND&lt;4</td>
</tr>
</tbody>
</table>

*Values are given as means ± SD of each processing batch (n=3).
†Different superscripts in the same row indicate significant differences (p<0.05).
‡ND: Not detect in 25 g
Table 3. Sensorial scores of Northeastern Thai fermented sausage with different levels of konjac gel.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>0% Konjac (Control)</th>
<th>7.5 % Konjac</th>
<th>15.0 % Konjac</th>
<th>22.5 % Konjac</th>
<th>30.0 % Konjac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>6.54 ± 1.66a,b,c,d</td>
<td>6.32 ± 2.10a</td>
<td>6.30 ± 1.09a</td>
<td>5.16 ± 1.54a</td>
<td>4.92 ± 1.55a</td>
</tr>
<tr>
<td>Color</td>
<td>5.93 ± 1.49a</td>
<td>6.77 ± 1.87a</td>
<td>6.92 ± 1.25a</td>
<td>6.69 ± 1.60a</td>
<td>6.77 ± 1.69a</td>
</tr>
<tr>
<td>Flavor</td>
<td>5.58 ± 1.61c</td>
<td>5.96 ± 1.66a</td>
<td>6.88 ± 1.55ab</td>
<td>7.27 ± 1.09a</td>
<td>7.38 ± 1.12a</td>
</tr>
<tr>
<td>Sourness</td>
<td>5.81 ± 1.52b</td>
<td>6.31 ± 1.09ab</td>
<td>7.46 ± 1.19b</td>
<td>6.77 ± 1.69ab</td>
<td>6.69 ± 1.55ab</td>
</tr>
<tr>
<td>Texture</td>
<td>5.69 ± 1.89a</td>
<td>5.61 ± 1.85a</td>
<td>5.76 ± 1.78a</td>
<td>5.86 ± 1.71a</td>
<td>5.54 ± 1.81a</td>
</tr>
<tr>
<td>Juiciness</td>
<td>6.27 ± 1.59a</td>
<td>6.65 ± 1.49a</td>
<td>6.20 ± 1.19a</td>
<td>6.46 ± 1.88a</td>
<td>6.15 ± 1.22a</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>5.54 ± 1.45b</td>
<td>5.50 ± 1.87b</td>
<td>6.77 ± 1.06a</td>
<td>6.59 ± 1.74ab</td>
<td>6.46 ± 1.98ab</td>
</tr>
</tbody>
</table>


1 Values are given as means ± SD of each of each processing batch (n=3).
2 Different superscripts in the same row indicate significant differences (p<0.05).
Table 4. Proximate composition and energy values of the different formulations of Northeastern Thai fermented sausage.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0% Konjac (Control)</th>
<th>15.0% Konjac (gel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>47.59 ± 0.65 (^a, 1, 1)</td>
<td>59.43 ± 1.14 (^a)</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>28.72 ± 0.25 (^a)</td>
<td>15.53 ± 0.35 (^b)</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>13.06 ± 0.06 (^a)</td>
<td>13.46 ± 0.31 (^a)</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>2.35 ± 0.04 (^a)</td>
<td>2.58 ± 0.35 (^a)</td>
</tr>
<tr>
<td>Dietary fiber (%)</td>
<td>0.11 ± 0.00 (^b)</td>
<td>1.42 ± 0.00 (^a)</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>7.78 ± 0.08 (^a)</td>
<td>8.89 ± 0.10 (^a)</td>
</tr>
<tr>
<td>Energy value (kcal/100 g)</td>
<td>339.70 ± 3.02 (^a)</td>
<td>228.91 ± 2.22 (^b)</td>
</tr>
<tr>
<td>Energy from fat (kcal/100 g)</td>
<td>258.48 ± 2.25 (^a)</td>
<td>139.77 ± 3.15 (^b)</td>
</tr>
<tr>
<td>Energy from fat (%)</td>
<td>76.09 ± 0.66 (^a)</td>
<td>81.06 ± 1.38 (^b)</td>
</tr>
<tr>
<td>Fat reduction (%)</td>
<td>-</td>
<td>45.9</td>
</tr>
<tr>
<td>Energy value reduction (%)</td>
<td>-</td>
<td>32.6</td>
</tr>
</tbody>
</table>

\(^1\)Values are given as means ± SD of each of each processing batch (n=3).
\(^1\)Different superscripts in the same row indicate significant differences (p<0.05).