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Quality and stability of reduced-fat fermented pork sausage (Sai Krok E-san) with konjac gel during chilled storage

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Abstract The quality and shelflife of reduced-fat Thai fermented pork sausage (Sai Krok E-san) were evaluated and the effect of replacing pork backfat with konjac gel on the changes in qualities during chilled storage was measured. Weight loss, pH, color and protein degradation between control (30% pork back fat) and reduced-fat products (50% of substitution pork back fat with konjac gel) were not significantly different (p<0.05). However, reduced-fat product with konjac gel exhibited a superior texture, with higher values for hardness, cohesiveness, gumminess, springiness and chewiness (p<0.05). During the 2 weeks of storage, lipid oxidation was lower in the reduced-fat sample (p<0.05) when compared with the control, thereafter these values for both products were increased without significant differences (p>0.05). Although lactic acid bacteria (LAB) were not influenced by the reformulation (p<0.05), LAB gradually increased during storage and ranged from 8 log CFU/g at the begining to 9 log CFU/g after 4 weeks. The sensory panel stated that both products had acceptable sensory quality if the products were kept less than 3 weeks. Longer periods led to a more rancid flavor, especially in the control sample.

Keywords: Fermented meat products, Glucomannan konjac, Healthy meat products

Introduction

Sai Krok E-san is a fermented sausage originating from the Northeastern region of Thailand. It is widely consumed in Thailand and known as Sai Krok Preaw. It is made from minced pork, pork back fat, cooked rice, garlic and curing ingredients. The mixture is stuffed into natural pork casings followed by hanging and fermentation for 2-3 days at room temperature. This allows fermentation by naturally occurring lactic acid bacteria (LAB) in the meat to produce a sour taste. The final product has a protein content of ≥12% and relatively high amounts of fat of up to ~30% (Thai community product standard, 2012), which leads to a short shelflife even when refrigerated.

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Consumers and producers are becoming increasingly interested in the minimal use of fat in meat processing. The saturated fat from red meat is often implicated to some health problems such as cardiovascular disease, coronary heart disease and obesity (World Health Organization, 2003).

The minimal use of fat in a meat-based food is generally gained by fat replacer or fat analog formulated by hydrocolloid from plants. The desirable characteristics of hydrocolloid-based fat replacer are low in calories and similarity of product appearance to animal fat. Among them is konjac glucomanan-based fat analog which is usually selected because it can form a gel with starch, carrageenan and water. Many researchers have used this fat analog in various meat products such as frankfurters (Jiménez-Colmenero *et al.*, 2010), bologna sausage (Chin *et al.*, 2000), fresh sausages (Osburn and Keeton, 2004) and pork nuggets (Berry and Bigner, 1996). Recently, Sorapukdee *et al.* (2019) found that substitution of 50% of pork back fat with konjac gel in Sai Krok E-san was the optimum formulation, having similar characteristics to the control formulation (30% pork back fat). Additionally, this reformulated product showed 46% less fat and 32% less energy, compared to the control. However, no research has been done regarding the effect on shelflife of Sai Krok E as effected by the reformulation, that is fat reduction using konjac. The changes in quality characteristics and refrigerated storage stability of Sai Krok E-san after partial substitution of pork back fat with konjac gel were evaluated.

Materials and methods

Experimental treatment and product manufacturing

Thai fermented pork sausage and konjac glucomanan-based fat analog were processed as described by Sorapukdee *et al.* (2019). To evaluate the storage stablity, two different formulations were designed as shown in Table 1. A control with normal fat content (30% pork back fat) and a reduced-fat sample with 50% replacing pork back fat with konjac gel (15% konjac gel and 15% pork back fat) were prepared. The modified fat content formulation was selected from the most appropriate technological and eating qualities as well as energy value based from the previous work of Sorapukdee *et al.* (2019). The mixtures was stuffed into natural pork casings and fermented at ambient temperature (32 ± 2°C) for 3 days. Samples were collected at weeks 0 (at the end of fermentation), 1, 2, 3 and 4 for monitoring chemical characteristics (pH, trichloroacetic acid-soluble peptides, protein degradation and lipid oxidation), physical characteristics (weight loss, color, and texture profile analysis), microbiological analysis and sensory evaluation. Three independent batches (*n*=3) were made.

Table 1. Formulation of Thai fermented pork sausage

Composition	Control	Reduced-fat (Konjac)
Main ingredients (%)		
- Ground pork	55	55
- Ground back fat	30	15
- Ground konjac gel	-	15
- Cooked rice	15	15
Curing and seasoning ingredients (g/kg of main ingredient)		
- Curing salt (prague powder)	15	15
- Sodium tripolyphosphate	3	3
- Sodium erythorbate	2	2
- Monosodium glutamate	2.5	2.5
- Sugar	5	5
- Ground garlic	50	50
- Fine ground black pepper	4	4

Determination of pH

A 2 g sample was homogenized with 20 mL distilled water. The pH of the homogenate was determined using a pH meter (SevenEasy pH meter S20, Mettler Toledo, Schwerzenbach, Switzerland).

Determination of trichloroacetic acid (TCA)-soluble peptides

TCA-soluble peptide was measured following the method of Morrissey *et al.* (1993). Ground product (2 g) was placed in a 50mL centrifuge tube, added with 20 mL 5% (w/v) TCA and then homogenized. The homogenate was stored in ice for 30 min. After centrifugation (4 ℃) for 20 min at 5000×*g*, the supernatant was collected and soluble peptides were measured by the Lowry method. TCA-soluble peptide content was calulated as µmol tyrosine/g.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The degradation pattern of myofibrillar protein was determined by SDS-PAG using 10% running and 4% stacking gels. The solubilized proteins

(15 µg) were subjected to electrophoresis with constant current of 20 mA per gel (miniPAGE chamber AE-6530ATTO Corporation, Tokyo, Japan). The protein bands were stained with 0.125% (w/v) Coomassie Brilliant Blue R-250 in 45% (v/v) ethanol and 10% (v/v) acetic acid, followed by destaining using 30% (v/v) methanol and 10% (v/v) acetic acid.

Determination of thiobarbituricacid reactive substances (TBARS)

TBARS were determined following the method of Buege and Aust (1978). The fermented meat product (5g) was placed into a 50 mL centrifuge tube then added with 25 mL thiobarbituric acid (TBA) solution (0.0375% (w/v) TBA,15% (w/v) TCA and 0.25 M HCl). After homogenization for 1 min, the homogenate was boiled (100 ℃) for 10 min, then cooled and centrifuged. The absorbance of the supernatant was recorded at 532 nm. The TBARS amount was calculated against a standard solution of 0-10 ppm malondialdehyde (MDA). The TBARS concentration was expressed as mg MDA/kg sample.

Determinationof weight loss and instrumental color

For weight loss estimation, the mass of the products at each storage time was recored and expressed as a percent (%) of initial sample mass at the beginning of storage (week 0). Color of cross-sectional samples was measured by a Colorimeter MiniScanEZ 4000L, in the CIE L*, a*, b* space (Hunter Lab Inc, USA). Lightness (L*), redness (a*), and yellowness (b*) values were recorded. All measurements were taken in triplicate.

Determination of texture profile analysis (TPA)

For texture analysis, Instron universal testing machine (model 1011) with a compression plate surface was used. Samples, 25 mm diameter × 25 mm height, were placed on the instrument base. TPA parameters - hardness (N), cohesiveness (ratio), gumminess (N), springiness (ratio), and chewiness (N) - were derived from the force-time curves generated by the Bluehill 2 software.

Microbiological counts

Microbiological analyses, in triplicate, were made on the first four weeks of storage for lactic acid bacteria (LAB), yeast and molds, *Staphylococcus aureus*, *Salmonella spp.* and *Escherichia coli*. A 25 g sample was placed in a sterile stomacher bag, 225 mL sterile saline (0.85% w/v NaCl) was added and then homogenized with a Stomacher. The media used for microbial analysis were: (1) de Man Rogosa Sharpe (MRS) agar plate supplemented with 0.8% (w/v) calcium carbonate for LAB (anaerobic incubation, 24–48 h, 30 ℃) (Kheadr, 2006), (2) potato dextrose agar incubated (3–5 days, 25 ℃) for yeast and mold counts, (3) Baird-Parker agar incubated (24–48 h, 37 ℃) and the coagulase test was used to identify and count of *S. aureus* colonies (Bennett, 2001), and (4) Prevalence of *Salmonella* spp. was estimated in 25 g of each sample (ISO 6579: 2002, International Organization for Standardization, 2002). The microbal counts, except for *Salmonella* spp., were expressed as log colony-forming units per gram (CFU/g). For *E.coli*, Fluorocult® LMX Broth was used and incubated (24–48 hr, 37 ℃), then the IMViC test was used for *E. coli* to estimate the most probable numbers per gram (MPN/g) (Feng, 2002).

Sensory evaluation

Fermented pork sausages were grilled to reach a 75 ℃ core temperature. Twelve trained panelists were served with 2 cuts of cooked fermented pork sausages (20 mm thick) from each treatment. The appearance, color, flavor, sourness, texture, juiciness, and overall acceptability were evaluated using nine-point hedonic scale.

Statistical analysis

The effect of reformulation and storage time on the quality characteristics were analyzed using the Generalized Linear Model (GLM). The model used the reformulation (control and reducted-fat sample by konjac gel) and storage time (0, 1, 2, 3, and 4 weeks) as fixed factors. Duncan's Multiple Range Test (DMRT) was used to compare the mean storage times, when the main effect was found. Statistical analysis used the SPSS package.

Results

Chemical characteristics and degradation of myofibrillar protein

Both pH and TBARS values of fermented sausage were affected by product formulation (p<0.05) (Table 2). The reduced-fat sample, substituting pork back fat with konjac gel, showed lower pH and TBARS than the control (p<0.05). Additionally, TBARS and TCA-soluble peptides contents increased with storage time (p<0.05) but not for product formulation (p>0.05). Hydrolysis of myofibrillar protein by SDS-PAGE in both sausage formulations at various storage times is shown in Fig. 1. Similar degradation patterns of myofibrillar protein among the formulations were observed. However, predominant degradation was found during fermentation, where samples were fermented from D0 (begin of fermentation) to week 0 (or day 3 of fermentation). During these times, degradation of protein was largely observed in the bands corresponding to myosin (~200 kDa), α-actinin (~100 kDa) and actin (~45 kDa). Thereafter, degradation patterns were unchanged for 4 weeks of storage.

Table 2. Chemical and physical characteristics of Thai fermented pork sausage from different formulation and storage times

Charateristics	Formulation			Storage time (weeks)						P-Value		
	Control	Konjac	SE	0	1	2	3	4	SE	F	S	F×S
pH	$4.47^{a1/}$	4.32^{b}	0.01	4.41	4.39	4.40	4.40	4.41	0.01	< 0.001	ns	ns
TCA-soluble peptides (μmol tyrosine/g sample)	5.66	5.94	0.11	5.15^{c}	5.74^{b}	5.64^{b}	6.12^{ab}	6.35^{a}	0.17	ns	< 0.001	ns
TBARS (mg MDA/kg sample)	35.80^{a}	32.62^{b}	0.77	21.16^{c}	28.57^{b}	38.95^{a}	40.08^{a}	42.29^{a}	1.22	0.005	< 0.001	ns
Weight loss (%)	8.62	10.17	1.41	10.18	9.65	9.55	9.82	9.42	2.03	ns	ns	ns
Product color												
- Lightness (L*)	5.82	5.85	1.03	54.71	55.71	55.97	57.74	55.06	1.74	ns	ns	ns
- Redness (a*)	6.18	6.75	0.48	8.02^{a}	7.81^{a}	6.75^{ab}	5.35^{bc}	4.40^{c}	0.76	ns	0.01	ns
- Yellowness (b*)	8.47	8.93	0.02	8.74	8.91	8.18	8.72	8.95	0.32	ns	ns	ns
Texture profile analysis												
- Hardness (N)	4.09^{b}	10.06^{a}	0.48	14.76^{a}	6.35^{b}	5.90^{bc}	4.38^{bc}	3.98^{c}	0.76	< 0.001	< 0.001	0.001
-Cohesiveness (ratio)	0.39^{b}	0.55^{a}	0.01	0.52^{a}	0.49^{a}	0.46^{b}	0.45^{b}	0.43^{b}	0.01	< 0.001	< 0.001	ns
- Gumminess (N)	1.65^{b}	5.55^{a}	0.26	7.53^{a}	3.37^{b}	2.98^{bc}	2.15^{bc}	1.99^{c}	0.42	< 0.001	< 0.001	< 0.001
- Springiness (ratio)	0.45^{b}	0.65^{a}	0.01	0.61^{a}	0.59^{a}	0.54^{b}	0.52^{b}	0.51^{b}	0.02	< 0.001	< 0.001	ns
- Chewiness (N)	0.78^{b}	3.69^{a}	0.18	4.85^{a}	2.17^{b}	1.79^{bc}	1.24^{c}	1.14^{c}	0.29	< 0.001	< 0.001	< 0.001

SE:Standard error; F: Product formulation ; S: Storage time ; F×S : Interaction between product formulation and storage time; ns: non significant (P>0.05)

[1/]Different superscriptson Least Squared Mean values within the same row of each main effect indicate significant differences (P<0.05)

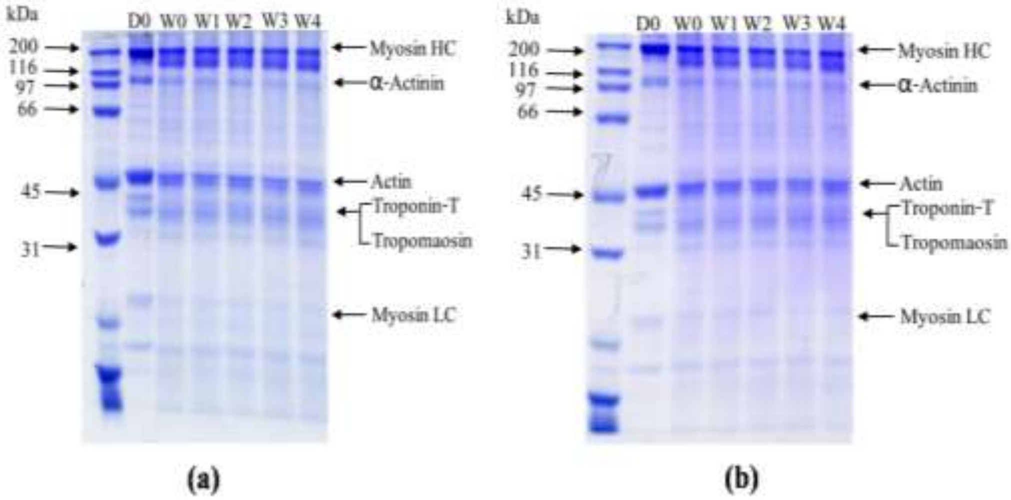

Figure 1. Degradation pattern of myofibrillar protein extracted from Thai fermented pork sausagefrom (a) control and (b) konjac formulation kept for 4 weeks in chilled storage. D0: sample before fermentation; W0: product after fermentation for 3 days; W1-W4: product stored for 1, 2, 3, and 4 weeks, respectively

Physical characteristics

There were no significant differences in weight loss, lightness and yellowness with product formulation and storage time ($p>0.05$) (Table 2). However, redness decreased with storage time ($p<0.05$), but not impacted by product formulation P>0.05). Texture was affected by product formulation and also storage time ($p<0.05$). Product with konjac gel showed higher values of hardness, cohesiveness, gumminess, springiness and chewiness than the control ($p<0.05$). Longer storage times led to lower values of the texture parameters ($p<0.05$). Moreover, interactions between product formulation and storage time were found in hardness, gumminess and chewiness values ($p<0.05$): the formulation containing konjac gel at the beginning of storage (W0) had the highest texture values.

Microbiology

LAB counts increased during chilled storage from 8.33 log CFU/g at week 0 to 9.57 log CFU/g at week 4 ($p<0.05$), but were not influenced by product formulation ($p>0.05$) (Table 3). Yeast counts were higher in konjac added product than the control (p<0.05) and the counts increased from 1.8 log CFU/g at week 0 to 3.06 log CFU/g at week 4 (p<0.05). Other counts (mold, *S. aureus*, *E. coli*, and *Samonella*) had values below detectable levels.

Table 3. Microbial counts of Thai fermented pork sausage from different formulation and storage times

Microbial counts	Formulation			Storage time (weeks)						P-Value		
	Control	Konjac	SE	0	1	2	3	4	SE	F	S	F×S
LAB (log CFU/g)	8.70	8.80	0.89	8.33^{c}	8.38^{bc}	8.69^{bc}	8.81^{b}	9.57^{a}	0.14	ns	< 0.001	ns
Yeast (log CFU/g)	2.38$^{b1/}$	2.60^{a}	0.06	1.80^{c}	2.38^{b}	2.45^{b}	2.78^{a}	3.06^{a}	0.09	0.021	< 0.001	ns
Mold (log CFU/g)	<1	<1	-	<1	<1	<1	<1	<1	-	-	-	-
S. aureus (log CFU/g)	<1	<1	-	<1	<1	<1	<1	<1	-	-	-	-
E.coli (MPN/g)	<3	<3	-	<3	<3	<3	<3	<3	-	-	-	-
Salmonella spp.	ND$^{2/}$	ND	-	ND	ND	ND	ND	ND	-	-	-	-

SE :Standard error; F: Product formulation ; S: Storage time ; F×S : Interaction between product formulation and storage time; LAB: lactic acid bacteria ; ns: non significant (P>0.05)

$^{1/}$Different superscripts on means within the same row indicate significant differences (***p***<0.05)

$^{2/}$ND: Not detected in 25 g

Table 4. Sensory tests on Thai fermented pork sausage from different formulations and storage times

Attributes	Formulation			Storage time (weeks)					P-Value		
	Control	Konjac	SE	0	1	2	3	SE	F	S	F×S
Appearance	6.92	7.58	0.25	7.95	7.00	7.05	7.00	0.36	ns	ns	ns
Color	7.00$^{b1/}$	7.77^{a}	0.26	8.05	7.20	7.20	7.10	0.37	0.045	ns	ns
Flavor	6.85^{b}	7.63^{a}	0.24	8.20^{a}	7.30^{bc}	7.15^{c}	6.30^{c}	0.35	0.032	0.005	ns
Sourness	7.60	8.15	0.27	8.10	7.95	7.80	7.65	0.36	ns	ns	ns
Texture	6.40^{b}	7.45^{a}	0.36	7.45	7.00	6.65	6.60	0.51	0.046	ns	ns
Juiciness	6.48^{b}	7.65^{a}	0.37	7.55	7.50	7.00	6.20	0.52	0.032	ns	ns
Overall acceptability	7.00^{b}	7.80^{a}	0.27	8.2^{a}	7.35^{ab}	7.05^{ab}	6.75^{b}	0.32	0.044	0.049	ns

SE : standard error; F: product formulation ; s: Storage time ; F×S : interaction between formulation and storage time; ns: not significant (p>0.05)

$^{1/}$Different superscripts on means in the same row indicate significant differences (p<0.05).

Sensory evaluation

Because rancid flavors of fermented sasuages at W4 were detected, sausages at W4 were not served in the sensory test. Sensory liking scores of products within W3 are shown in Table 4. Product appearance and sourness were not influenced by reformulation and storage time ($p>0.05$). Konjac replacing pork back fat led to higher color, flavor, texture juiciness, and overall acceptability scores than control ($p>0.05$). The longer period resulted in lower sensory liking scores for flavor and overall acceptability ($p>0.05$).

Discussion

In comparision with control or full-fat sausage (formulated with 30% pork back fat), the reduced-fat product with 50% replacing pork back fat (formulated by 15% back fat together with 15% konjac gel) showed a superior sensory scores in color, flavor, texture, juiciness and overall acceptability. A higher flavor score for the product containing konjac gel resulted from its lower detectable racid flavour as well as lower TBARS value. Similarly, Triki *et al.* (2013) found that the substitution of pork back fat with konjac gel resulted in a lower TBARS value when compared to a normal fat formulation. Besides saturated fatty acid, pork back fat is rich in unsaturated and polyunsaturated fatty acids which are susceptible to oxidation. Therefore, the reduced-fat formulation led to reduced lipid oxidation.

Konjac added sample exhibited a higher yeast counts than control. In fresh meat, there are several genera of yeasts that are naturally found namely: *Candida* spp., *Rhodotorula* spp., *Debaryomyces* spp. and *Trichosporum* spp. (Osei Abunyewa *et al.*, 2000). In some fermented meat products, e.g. commercial salami, an initial yeast concentration, 3 log CFU/g, was found at the beginning of process, which continuously increased to 5 log CFU/g after 20 days of maturation (Osei Abunyewa *et al.*, 2000). Yeasts were reported to enhance both flavor and aroma in fermented meat products (Selgas and Garcia, 2015), thus a higher number of yeasts in the reduced-fat formulation might be expected to lead to higher flavor scores.

Products, using konjac to replace fat provided better texture scores. The reduced-fat sausage with konjac gel had higher values in the texture profile analysis (hard and elastic texture). During fermentation of meat products, lactic acid accumulation induced the gelation of muscle protein, leading to better texture, as decribed as more firm and elastic textures (Visessanguan *et al.*, 2004). A previous research found that pH in the Thai fermented pork sausage containing 15% konjac gel had declined rapidly during 3 days of fermentation than the control (Sorapukdee *et al.*, 2019). This would accelerate acid-induced gelation and produce a superior texture in the konjac containing product. This agrees with Ruiz-Capillas *et al.* (2012), who reported that a reduced-fat fermented sausage with konjac gel showed a higher hardness and chewiness than a normal fat formulation.

During chilled storage, the degradation of protein could be detected at peptide level by increase in TCA-soluble peptides. However, the proteolysis of myofibrillar protein was not observed in SDS-PAGE, which might be due to low temperature limiting proteolytic enzyme activity. Additionally, TBARS values rapidly increased during storage, leading to a limit in shelflife. The rancid flavor of the product was the main problem: products kept for 4 weeks had an unacceptable flavour, leading to product rejection. However, in chilled storage for 3 weeks or less, product that replaced fat with konjac gel had a better flavor.

The 50% replacement of pork back fat by konjac gel (15% of pork back fat+15% of konjac gel) was found as the optimum reformulation for Thai fermented pork sausage. This reduced-fat product had no negative impact on the sensory qualities. Additionally, the shelflife of reduced-fat sausages showed a lower lipid oxidation rates.

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