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Effect of *Hibicus sabdariffa* on the Nutritional and Sensory Properties of Novel Naem Product

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Abstract

Naem is a traditional Asian fermented sausage made from the mixture of raw meat, cooked rice, garlic, salt, sugar, spices, and sodium nitrite. With consumers more concerned with healthy food choices, *Hibiscus sabdariffa* (HS) can be used as substitute for sodium nitrite in Naem preparation. Therefore, the objective of this study was to evaluate the effects of HS on the sensory, quality, and physicochemical analyses of Naem products. Four concentrations of HS were evaluated in this experiment: 1) 0%, 2) 1%, 3) 3% and 4) 5% HS. Treatments were analyzed for sensory evaluation by using a 9-point hedonic scale (trained panelists = 38). Physicochemical characteristics were evaluated for color (L*, a*, and b* values), pH, water activity, moisture (%), ash content, and lipid stability (TBARS). In addition, nutrition profiles, lactic acid bacteria, aerobic plate counts, *Escherichia coli*, and *Listeria* spp. were completed. SPSS with one-way ANOVA was used to evaluate any significant differences with $p<0.05$. The sensory evaluation revealed that Naem prepared with 3% HS had the highest overall acceptance scores (5.76), flavor (5.81), and taste (5.66). In addition, fermented Naem with 3% HS showed the highest scores of acceptability (92.2%), purchase intent (71.1%), and lactic acid bacteria counts (5.41 log CFU/g). The initial pH values, water activity, moisture (%), and ash content in this experiment ranged from 5.44-5.57, 0.93-0.94, 63.17-64.94%, and 1.51-1.74%, respectively. There was a significant ($p<0.05$) on color after 7 days storage at 3℃. Specifically, a* values were decreased in all treatments. The control treatment obtained the highest TBARS values (0.83 mg MDA/kg). No *E. coli* or *Listeria* spp. were detected. The results of this study indicate that *Hibiscus sabdariffa* can be used as a natural spice for Naem products which may help the meat industry increase market share through this innovative product.

Keywords: Naem, *Hibiscus sabdariffa*, fermentation

1. Introduction

Fermentation is a common method used to produce and preserve food that has been practiced for many years (Salampessy et al., 2010). Fermentation helps to enhance nutritive values, destroy undesirable factors, salvage raw materials that are not usable for human consumption, reduce energy used for cooking and improve appearance and taste (Hutkins, 2018). The global fermented foods and ingredient markets were valued at $39,180 million in 2020 and will increase to $59,980 million by the end of 2026 which is growing at a compound annual growth rate (CAGR) of 6.2% from 2022-2026 (More, 2021). In addition, fermented meat products contribute significantly to the World's population including the Asian meat market. Naem is an example of semi-dry fermented sausage which is in high demand due to a more modern lifestyle and increased interest in healthy and convenient food (Lücke, 2016). Previous studies concluded that *Hibiscus sabdariffa* (HS) can be used to substitute sodium nitrite in Naem production as a healthy alternative food choice for consumers and to reduce microorganism growth (Valenzuela, 2016). This is due to HS calyces which is high in vitamin C, β-carotene, thiamin, riboflavin, calcium, iron, and anthocyanins (Hopkins et al., 2013, Wu et al., 2018). Because of these nutritional benefits and antioxidant activity, HS extracts are being used in Naem production (Wu et al., 2018). Therefore, the objective of this study is to determine the effects of *Hibiscus sabdariffa* (HS) on the properties and consumer acceptability of novel Naem products.

2. Method

2.1 Preparation of Naem Samples

Hibiscus sabdariffa (HS) was collected from Lake Charles, Louisiana (30º9'45.8942' N, 93º 3'24.7191' W). The HS calyces were washed, chopped, blended, and pasteurized at 60 ℃ for 15 minutes (Paramee et al., 2008). The sample was filtered with a strainer to remove all pulps. The HS extraction was stored at 3 ℃ for Naem preparation.

Raw ground pork was obtained from the Center for Advancement of Meat Production and Processing (CAMPP), McNeese State University, Lake Charles, Louisiana. Meat samples (4 kg) were removed from the freezer and placed in a cold room (3 ℃) 1 day prior to preparing Naem products. Sweet rice (Wei-Chuan U.S.A., Inc., California) was rinsed, and mixed with drinkable water at a ratio 1:1 (wt/wt) and heated at 75 ℃ for 25 minutes until cooked. All ingredients were weighed accordingly to respective treatments (Table 1). Salt (Morton, Chicago, IL) was added as the first ingredient and mixed well with ground meat (3 ℃) to release the myofibrillar protein to improve the binding texture. The remaining ingredients: cooked rice, sugar (Imperial Savannah LP Sugar Land, Texas), chopped garlic (Kroger commercial), and HS extract (0, 1, 3, 5% wt/wt) were mixed and combined, respectively (Table 1). All mixed samples were stored at 3 ℃ for 30 min. Each treatment was weighed in 60 g samples and wrapped with polyvinyl chloride (PVC) material to eliminate air. All samples were transferred to anaerobic containers and fermented at 27 ℃ for 72 hours until pH reached 4.7.

Table 1. Experimental treatments for Naem preparation (%)

Ingredient	Control (0% HS)	1% HS	3% HS	5% HS
Ground pork	78.2	77.2	75.2	73.2
Hibiscus sabdariffa extract	0.0	1.0	3.0	5.0
Cooked sweet rice	14.8	14.8	14.8	14.8
Garlic	4.4	4.4	4.4	4.4
Sugar	1.5	1.5	1.5	1.5
Salt	1.1	1.1	1.1	1.1

2.2 Sensory Evaluation

The discriminative test with ranking technique was used to distinguish relative sourness and firmness of Naem products (Table 2). Consumers were asked to rank four treatment preparations in order of most to least sourness and firmness. Using a 9-point hedonic scale, 38 untrained panelists (9 males, 29 females) solicited from students and faculty in the McNeese State University School of Agricultural Sciences evaluated Naem products. Each treatment was unpacked and cooked at 177 ℃ for 25 minutes, and then cut into 2x2x0.5 cm slices. Each sample was evaluated on appearance, flavor, texture, color, taste, smell, overall acceptability, and overall liking (9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like nor dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, 1=dislike extremely) (Table 2). Panelists were required to cleanse their palates with water between tasting the samples. Panelists also completed an acceptability and purchase intent questionnaire (Table 3).

Table 2. Sourness and firmness evaluation

Rank Responses	1st Most sour	2nd	3rd	4th Least sour
Treatment (%)				

Part I. Sourness evaluation

Note: 1) You will be presented with the 4 labeled samples in random order.

2) Please evaluate them from left to right and rank the samples in order of sourness intensity with 1 = Most sour and 4 = Least sour

Rank Responses	1^{st} Most sour	2^{nd}	3^{rd}	4^{th} Least sour
Treatment (%)				

Part II. Firmness evaluation

Note: 1) You will be presented with the 4 labeled samples in random order.

2) Please evaluate them from left to right and rank the samples in order of firmness intensity with 1 = Most firm and 4 = Least firm

Table 3. Hedonic scale

Parameter	9-point hedonic scale								
Flavor	1	2	3	4	5	6	7	8	9
Texture	1	2	3	4	5	6	7	8	9
Taste	1	2	3	4	5	6	7	8	9
Sourness	1	2	3	4	5	6	7	8	9
Firmness	1	2	3	4	5	6	7	8	9
Liking	1	2	3	4	5	6	7	8	9

Table 4. Acceptability and purchase intent questionnaire of Naem treatments (n = 38)

	Control	1% HS	3% HS	5% HS
Acceptable				
Yes				
No				
Purchase				
Yes				
No				

2.3 Mineral Analysis

Mineral content was analyzed using ICP-OES techniques described by the Official Methods of Analysis (AOAC, 2000). For sample digestion, 0.5 g of each treatment was weighed and transferred to an acid washed volumetric flask. Twenty ml of 7:1 v/v nitric acid/perchloric acid was added to each flask and boiled until white fumes appeared. Samples were allowed to cool, 10 ml of deionized water was added, placed on hotplate, and heated until all samples turned to a liquid solution. All samples were filtered and analyzed by Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES).

2.4 Crude Protein Analysis

Protein content was analyzed using the total nitrogen combustion method following the AOAC (2000). Samples were weighed (15-20 mg) and placed in a tared tin capsule. All treatments were loaded to an autosampler of NA2100 Protein Nitrogen Analyzer for combustion analysis.

2.5 Crude Fat Analysis

Crude fat was analyzed by following the procedure of the 2050 Soxtec Avanti Automatic System User Manual 1000 7414, Rev 3.0. Each sample was weighed (3 g) into tared thimble and placed into the condenser of extraction unit. Fifty milliliters (50 ml) petroleum ether was added to each extraction cup. All extraction samples were analyzed with the 2050 Soxtec Avanti Auto Fat Extraction System.

2.6 pH Test

Naem products were analyzed for pH with an electrode portable meter probe (Model 2000 VWR Scientific). Calibration of the pH meter was accomplished using pH 7 and pH 4 standardization buffers before use.

2.7 Water Activity (Aw)

Naem samples were chopped until homogeneous and placed in a disposable cup until the bottom of the cup was covered. Samples were placed in the chamber and analysis was initiated. After approximately 40 seconds, the first Aw measurement was displayed on the LCD and recorded. Each Naem treatment was replicated twice and evaluated for water activity with Meter AquaLab Pawkit, Portable Water Activity Meter (Meter Group, Inc. USA).

2.8 Moisture Content

Moisture content was determined according to the method of the Association of Official Analytical Chemists (AOAC, 2000). Each 3-5 g sample was dried in a hot air oven (Model 26 Precision Thelco) at 60 ℃ for 24 hours. Samples were reweighed and recorded. Percent moisture was then calculated using the following formula:

$$\% \text{ Moisture content} = \left(1 - \frac{\text{dry basis}}{\text{wet basis}}\right) * 100 \quad (1)$$

2.9 Ash Content

Ash content was determined according to the method of the Association Official Analytical Chemists (AOAC, 2000). Samples were weighed (5 g) and placed into tared crucible. Each sample was dried, placed in crucibles, and transported to muffle furnace to burn samples for ash content. After 24 hours, crucibles were transferred to desiccator and allowed to cool. Samples were reweighed and recorded for ash content calculation.

2.10 Color Testing

Color was measured by using the Minolta Colorimeter (Model CR-10 portable). This is a reflectance spectrophotometer that calculates color appearance parameters from the reflectance spectrum by using an 8 mm aperture, 10 ºobserver angle, D65 illuminant source. The instrument reports color appearance in the *L, a*, b* scale (white=100, black=0), a* (+40=red, -40=green), b* (+40=yellow, -40=blue). Each treatment was measured at three different locations for determine L*, a*, and b* values.

2.11 Lipid Oxidation (TBARS Test)

The thiobarbituric acid-reactive substances (TBARS) method (Papastergiadis et al., 2012) was used to measure lipid oxidation. Fifteen grams of each treatment with two replications was blended with 30 mL of trichloroacetic acid solution. The sample solution was filtered through Whatman No. 1 filter paper. Five ml aliquots of the filtrate were transferred to separate test tubes (in duplicate) and mixed with 5 ml of 0.02 M TBA. The mixture was vigorously agitated in a vortex and heated in a boiling water bath (100°C) for 45 min to develop a pink color. After cooling the reaction mixture under running water, the absorbance was determined at 530 nm using a Beckman Du-640 spectrophotometer against a blank containing 5 mL of distilled water and 5 mL of TBA reagent. The TBA value used to express the results were calculated from standard curves and known dilutions of tetraethoxypropane (TEP) and the results were expressed as mg malondialdehyde (MDA)/kg sample.

2.12 Microbial Analysis

Microorganisms were determined by the standard of the AOAC (2000). Naem products were assayed for four microorganisms: Aerobic plate count (APC), *Listeria* spp., lactic acid bacteria, and *Escherichia coli.*

2.12.1 Buffered Peptone Water

Eight hundred ml of buffered peptone water was prepared by mixing 8 g peptone powder, 4 g sodium chloride, 2.8 g dipotassium phosphate, and 1.2 g mono-potassium phosphate. All mixtures were boiled until dissolved and autoclaved at 121oC for 15 minutes. After sterilizing, buffered peptone water was cooled and stored in refrigerator until further analyses.

2.12.2 Aerobic Plate Count (APC)

The following protocol was used for APC. Buffered peptone water was added as a diluent option for serial dilutions. The 3M^{TM} PetrifilmTM Aerobic Count Plate (3M^{TM}, United States) were used to determine the enumeration (Log CFU/g) of APC. All samples were mixed with a vortex for one minute to release bacteria. Each 1 ml of sample solution with two replications was aseptically transferred and plated on the 3M™ Petrifilm™. These films were incubated in a horizontal position, clear side up, in stacks of no more than twenty at 37oC for 24 hours.

2.12.3 *Listeria* spp.

Listeria selective agar base powder was weighed 25.5 g and dissolved with 500 ml distilled water. The solution was heated until dissolved completely and autoclaved at 121oC for 15 minutes. Twenty-five ml of solution was poured into sterile media agar plates and left idle until solidified. All plates were transferred to a metal container and stored in the refrigerator (3 ℃). Using the spread technique, each 0.1 ml of sample with two replications was aseptically transferred and plated on a *Listeria* selective agar plate to determine the enumeration (Log CFU/g) of *Listeria* spp.

2.12.4 Lactic Acid Bacteria

All-purpose tween (APT) agar base powder was weighed 29.75 g and dissolved with 500 ml of distilled water. The solution was heated until dissolved completely and autoclaved at 121oC for 15 minutes. Twenty-five ml of solution was poured into sterile media agar plates and left idle until solidified. All plates were transferred to a metal container and stored in the refrigerator (3 ℃). Using the spread technique, each 0.1 ml of sample with two replications was aseptically transferred and plated on APT plates to determine the enumeration (Log CFU/g) of lactic acid bacteria.

2.12.5 Escherichia coli (E. coli)

Buffered peptone water (BPW) was added as a diluent option for serial dilutions. Following 3M™ Petri film plating instructions, each 1.0 ml of sample with two replications was aseptically transferred and plated on 3M™ Petrifilm™ to determine the enumeration (Log CFU/g) of *E. coli*.

2.13 Statistical Analysis

Statistical Product and Service Solutions (SPSS) was used to analyze treatment means. One-way ANOVA was used to evaluate any significant differences. All data are presented as least squares means with standard error of the mean (SEM) and a significance level of $p<0.05$ was used for statistical analysis of means from four Naem treatments during 7-day storage at 3 ℃.

3. Results and Discussion

3.1 Nutrition Profile

A nutritional analysis was completed on each treatment to determine macro and micronutrients. Naem prepared with 5% HS obtained higher amount of boron (27.1 ppm) but lower in magnesium (0.035%), phosphorus (0.247%), sulphur (0.341%) and zinc (42.5 ppm). However, the control treatment provides higher levels of magnesium (0.037%), phosphorus (0.281%) and zinc (47.8 ppm) (Table 5).

Table 5. Nutrition profile for four Naem treatments

Nutrition profile	Control	1% HS	3% HS	5% HS
Protein (%)	44.6	41.3	42.4	44.6
Total fat (%)	19.4	18.0	18.8	15.1
Boron (ppm)	19.6	18.5	25.8	27.1
Calcium (%)	0.014	0.014	0.016	0.016
Copper (ppm)	<4	<4	<4	<4
Iron (ppm)	23.3	26.7	22	22.2
Magnesium (%)	0.037	0.036	0.036	0.035
Manganese (ppm)	<4	<4	<4	<4
Phosphorus (%)	0.281	0.267	0.261	0.247
Potassium (%)	0.561	0.591	0.544	0.488
Sodium (%)	0.834	0.865	0.811	0.753
Sulphur (%)	0.373	0.346	0.363	0.341
Zinc (ppm)	47.8	43.5	44.9	42.5

3.2 Demographic and Product Information

The two largest age groups are 18-24 year-old (n= 16) and 25-34 years old (n=17) accounted for 86.80% of the total panelist. Female participants (76.30%) exceeded males (23.70%). The discriminative test with ranking technique was used to distinguish relative sourness and firmness of Naem products. Consumers were asked to rank four treatment preparations in order of most to least sourness and firmness. There was no significant ($p>0.05$) difference in sourness evaluation among all treatments (Table 6). However, Naem with 3% HS received a plurality of 42% as the most sour. The control treatment was rated as the least sour at 12%. The 1% HS and 3% HS samples were rated at 28% and 18%, respectively (Table 6).

There was no significant difference ($p>0.05$) in firmness evaluation across all treatments (Table 7). However, samples prepared with 5% HS were rated the most firm by 39.7% of consumers. The control samples were rated the least firm at 14% (Table 7). These results suggest that consumers can distinguish the rates of sourness and firmness in Naem products with or without *Hibiscus sabdariffa*.

Table 6. Sourness evaluation (n=38)

Rank Responses	1st Most sour	2nd	3rd	4th Least sour
Treatment (%)	3% HS (42.0)	1% HS (28.0)	5% HS (18.0)	Control (12.0)

No difference ($p>0.05$)

Table 7. Firmness evaluation (n=38)

Rank Responses	1st Most firmness	2nd	3rd	4th Least firmness
Treatment (%)	5% HS (39.7)	1% HS (29.1)	3% HS (17.2)	Control (14.0)

No difference ($p>0.05$)

3.3 Consumer Acceptability

Using the 9-point hedonic scale, participants evaluated Naem products for flavor, texture, taste, sourness, firmness, and overall liking (Table 8). Flavor, texture, taste, sourness, and overall liking scores among all four treatments were not different ($p>0.05$). However, there was a significant ($p<0.05$) difference in firmness between the control and 5% HS treatment (Table 8). Specifically, the sensory evaluation revealed that Naem prepared with 3% HS had the highest scores in flavor (5.81), texture (5.63), taste (5.66), and overall liking (5.76) (Table 8). These results suggest that Naem with 3% HS was the most acceptable for consumers.

3.4 Acceptability and Purchase Intent

Each treatment was evaluated separately using a 2-point hedonic scale (yes/no). Using the acceptability and purchase intent questionnaire, consumers evaluated Naem products for acceptability, whether they would purchase the product and whether or not they would purchase the product if it claimed to be considered a probiotic meat product with beneficial effects. There was no significant ($p>0.05$) difference in acceptable and purchase intent among all treatments (Table 9). However, Naem with 3% HS showed the highest scores of acceptability (92.2%) and purchase intent (71.1%). Therefore, these results suggest that Naem prepared with 3% HS can be used as an alternative to original Naem products.

Table 8. Consumer acceptance scores for sensory attributes and overall liking of four Naem treatments (n=38)

Parameter	Control	1% HS	3% HS	5% HS	SEM
Flavor	5.24	5.16	5.81	5.05	0.46
Texture	5.39	5.13	5.63	5.03	0.46
Taste	5.45	5.13	5.66	4.76	0.45
Sourness	5.42	5.47	5.34	4.74	0.42
Firmness	6.24^{a}	5.42^{ab}	5.63^{ab}	4.97^{b}	0.45
Overall Liking	5.53	5.11	5.76	4.82	0.48

^{a,b} LSMeans with different superscripts within a row are significantly different ($p<0.05$).

Means are two replications per treatment for each day of storage.

Table 9. Acceptability and purchase intent questionnaire of four Naem treatments (n = 38)

	Control	1% HS	3% HS	5% HS
Acceptable	Number/%			
Yes	33/87.00	26/68.40	35/92.20	24/63.20
No	5/13.00	12/31.60	3/7.80	14/36.80
Purchase Intend	Number/%			
Yes	33/87.00	13/34.20	27/71.10	12/31.60
No	25/65.80	25/65.80	11/28.90	26/68.40

No difference ($p>0.05$)

3.5 pH

Overall, there was a significant difference ($p<0.05$) in all Naem treatments when stored at 3℃ for 7 days. The initial values of each individual Naem treatment ranged from 5.46 to 5.62 (Figure 1). The pH values of all treatments decreased over the experimental period. This was accelerated post-acidification resulting in reduced storage stability. Adding 5% HS in Naem provided the lowest ($p<0.05$) pH value at 5.26 after 7 days of storage (Figure 1). This could be due to the composition of *Hibicus sabdariffa*, which contains a high level of acidic compounds (Bozkurt & Belibagli, 2009). Therefore, this result suggests that *Hibicus sabdariffa* had the general effect to reduce the acidity of Naem products. The Food and Agriculture Organization of the United Nations (2021) reports semi-dry fermented sausage, possess a pH value ranging between 4.8-5.4 which is similar to our

study. Therefore, Naem is considered as ready-to-eat food and microbiological safe because of a lower numerical pH that reduces most pathogen growth (Terefe, 2016).

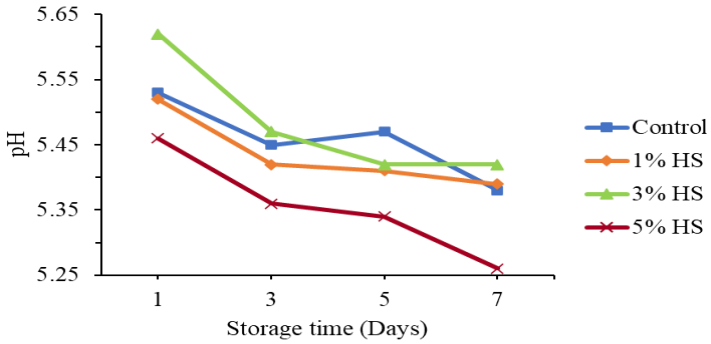

Figure 1. Least-squares means for pH values of four Naem treatments stored at 3 ℃ for 7 days. SEM: day 1 = 0.02, day 3 = 0.03, day 5 = 0.04, day 7 = 0.02. Means are two replications per treatment for each day of storage

3.6 Water Activity (Aw)

During the 7-day storage, there was no difference ($p>0.05$) in water activity of Naem products (Table 10). Specifically, Naem prepared with and without HS had the same value of Aw at 0.94 on day 7. Adding a higher concentrate of HS (5%) did not affect water activity. Thus, our study supported Holck et al. (2017) who stated that water activity of semi-dry sausage should provide the range of 0.90 to 0.95. Due to this range, Naem products can prolong the shelf-life for 7 days stored in the refrigerator which limits microorganism growth (Lücke, 2000).

3.7 Moisture Content

There was no significant difference ($p>0.05$) among treatments in moisture content of Naem products over the experimental period. The initial moisture content in this experiment ranged from 63.17-64.94% (Table 11). Moisture content in Naem products decreased for all treatments during 7-day storage at 3 ℃. Specifically, Naem prepared with 5% HS had the lowest moisture content at 61.16% on day 7 (Table 11). This is due to the nature composition of HS extract in the Naem product and the loss of water holding capacity of this sausage during 7-day storage. Gibis and Weiss (2010) reported that using a marinade of RS extract on beef patties decreased weight loss 31% after 120s of broiling time.

Table 10. Water activity of four Naem treatments stored at 3 ℃ for 7 days

Storage time (Days)	Control	1% HS	3% HS	5% HS	SEM
1	0.93	0.94	0.94	0.94	0.002
3	0.94	0.93	0.93	0.94	0.003
5	0.92	0.92	0.93	0.93	0.001
7	0.94	0.94	0.94	0.94	0.002

No difference ($p>0.05$)

Table 11. Moisture content (%) of four Naem treatments stored at 3 ℃ for 7 days

Storage time (Days)	Control	1% HS	3% HS	5% HS	SEM
1	64.06	64.94	63.46	63.17	0.004
3	60.29	63.75	62.91	60.70	0.009
5	59.20	63.19	60.52	62.20	0.008
7	62.43	61.73	62.95	61.16	0.010

No difference ($p>0.05$)

3.8 Ash Content

Ash content tended to increase in all treatments from days 1 to 3. There were no significant differences ($p>0.05$) in ash content of Naem treatments over the 7-day storage (Table 12). Our results are similar to the study of

Perez-Baez et al. (2021) who found that adding either 4% or 8% of HS in frankfurter sausages had no effect on ash content. However, Naem with 5% HS had the highest ash content at 1.83% as compared to other treatments on day 7. This is due to *Hibicus sabdariffa* containing 14.5% ash content (Balarabe, 2019). Therefore, the higher concentration of HS used, the higher ash content obtained.

3.9 Color Testing

The lightness L* value increased from 29.87 to 39.43 and 31.30 to 35.70, respectively in the control treatment and 1% HS during 7-day of storage but decreased in 3% HS from 33.33 to 32.63 and 5% HS from 30.27 to 32.40, respectively (Table 13). There was no difference ($p>0.05$) among treatments on days 3 and 5. However, adding HS affected the lightness (L*) color on days 1 and 7 in Naem products stored at 3 ℃.

Naem prepared with 5% HS had a lower a*value (2.13) compared to the control treatment (2.70) on day 3 (Table 13). On day 7, there was no effect ($p>0.05$) among treatments. Redness a* values decreased in all treatments when stored at 3 ℃ for 7 days. This is due to a reduction in the color values a* over time, indicating a change of the meat color, from red (oxymyoglobin) to brown (metmyoglobin) (Cooper et al., 2016). In addition, the high content of fiber in HS composition affected the a* values due to a gel formation between the fibers of the shell and the meat proteins, with a reduction of red coloration (Angiolillo et al., 2014). Furthermore, Bozkurt and Belibagli (2009) explained that the HS has acidic components which cause browning reactions in meat products.

Yellowness is measured in terms of positive b* values. Naem prepared with 3% HS had the lowest yellowness b* value at 8.97 on day 7 (Table 13). No significance effect ($p>0.05$) was found among treatments on days 3, 5 and 7. Therefore, adding *Hibiscus sabdariffa* extract had no effect yellowness b* value in Naem products.

Table 12. Ash content (%) of four Naem treatments stored at 3 ℃ for 7 days

Storage time (Days)	Control	1% HS	3% HS	5% HS	SEM
1	1.65	1.51	1.74	1.71	0.001
3	1.75	1.79	1.83	1.84	0.002
5	1.81	1.85	1.74	1.85	0.003
7	1.74	1.79	1.79	1.83	0.002

No difference ($p>0.05$)

Table 13. HunterLab L*, a*, and b* values of four Naem treatments stored at 3 ℃ for 7 days

Colorimeter	Days	Control	1% HS	3% HS	5% HS	SEM
L*	1	29.87^{a}	31.30^{ab}	33.33^{b}	30.27^{a}	0.59
	3	31.97	29.77	34.17	34.30	1.22
	5	33.57	36.23	37.43	39.10	0.97
	7	39.43^{a}	35.70^{ab}	32.63^{b}	32.40^{b}	1.53
a*	1	3.40	3.83	2.97	3.50	0.17
	3	2.70^{ab}	3.03^{a}	2.33^{ab}	2.13^{b}	0.15
	5	3.13	2.37	2.47	2.60	0.21
	7	3.33	3.03	2.30	2.63	0.20
b*	1	13.60^{a}	11.17^{abc}	9.73^{bc}	10.93^{bc}	0.51
	3	12.40	10.13	13.77	10.43	0.93
	5	10.63	10.73	12.43	10.63	0.41
	7	12.13	11.57	8.97	13.47	0.99

^{a,b,c} LSMeans with different superscripts within a row are significantly different ($p<0.05$).

Means are two replications per treatment for each day of storage.

3.10 Lipid Oxidation (TBARS test)

Lipid oxidation of all treatments increased during 7-day storage (Figure 2). Overall, there were no differences ($p>0.05$) among treatments over the experimental period. Specifically, Naem prepared with 5% HS had the lowest TBARS at 0.6 mg MDA/kg per sample on day 7 (Figure 2). This is due to the natural antioxidants of HS reducing potential for lipid oxidation (Bozkurt & Belibagli, 2009). According to Villasante et al. (2018), the combination of pecans with Hibiscus sabdariffa was effective in extending the freshness and food safety in sardine burgers. Therefore, our study suggested that adding *Hibiscus sabdariffa* can be used as an antioxidant agent to reduce TBARs values in Naem products stored at 3 ℃ for 7 days.

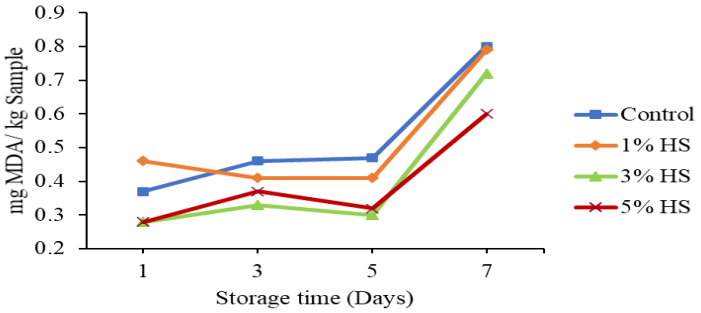

Figure 2. Least-squares means for lipid oxidation (TBARS) (mg MDA/kg sample) of four Naem treatments stored at 3 ℃ for 7 days. SEM: day 1 = 0.04, day 3 = 0.04, day 5 = 0.05, day 7 = 0.05. Means are two replications per treatment for each day of storage

3.11 Microbiological Analysis

3.11.1 Aerobic Plate Counts (APC)

On day 7, Neam prepared with 3% and 5% HS had APC of 2.6 and 1.0 Log CFU/g, respectively (Table 14). This differed ($p<0.05$) compared to control and 1% HS. This study showed that using *Hibiscus sabdariffa* in Naem products can reduce the counts of aerobic bacteria which is similar to the study of Mansour (2018) who reported that HS can be used as an antibacterial agent. *Hibiscus sabdariffa* provides high contents of anthocyanins and flavonoids, including quercetin, delphinidin-3-sambubioside, delphinidin-3-glucoside, cyanidin-3-glucoside, cyanidin-3-sambubioside and kaempferol which inhibit undesirable microorganisms' growth (López et al., 2004).

3.11.2 *Listeria* spp.

There was no significant difference ($p<0.05$) across treatments of *Listeria* spp. counts in Naem products over the 7-day experimental period (Table 15). Therefore, *Hibiscus sabdariffa* had no effect in the counts of *Listeria* spp. during the process of Naem fermentation and these bacteria can be contaminated during the early phase of the fermentation process (Chokesajjawatee et al., 2009; Holck et al., 2017).

Table 14 Aerobic plate counts (APC) (Log CFU/g)

Storage time (Days)	Control	1% HS	3% HS	5% HS	SEM
1	2.00^{a}	3.20^{b}	2.39^{a}	2.48^{a}	0.51
3	3.75^{a}	3.50^{a}	2.00^{b}	1.24^{c}	0.56
5	3.59^{a}	3.79^{a}	3.38^{ab}	2.84^{b}	0.13
7	3.84^{a}	3.76^{a}	2.60^{b}	1.00^{c}	0.49

^{a,b,c} LSMeans with different superscripts within a row are significantly different ($p<0.05$).

Means are two replications per treatment for each day of storage.

Table 15 *Listeria* spp. (Log CFU/g)

Storage time (Days)	Control	1% HS	3% HS	5% HS	SEM
1	5.34	5.31	5.43	5.38	0.01
3	5.50	5.55	5.57	5.53	0.01
5	5.53	5.50	5.51	5.51	0.01
7	5.79	5.79	5.82	5.70	0.02

No difference ($p>0.05$)

3.11.3 Lactic Acid Bacteria

The initial lactic acid bacteria count for the four treatments ranged 5.03-5.19 Log CFU/g (Table 16). Specifically, Naem prepared with higher percent of HS had the higher counts of lactic acid bacteria on day 1 stored at 3 ℃ (Table 16). This is due to *Hibiscus sabdariffa* which provided high vitamin C and anthocyanins (Hopkins et al.,

2013; Wu et al., 2018) in the preparation of Naem. Therefore, *Hibiscus sabdariffa* could be used to modify the quality and safety characteristics which prolong shelf-life longer than 7 days.

Table 16. Lactic acid bacteria (Log CFU/g)

Storage time (Days)	Control	1% HS	3% HS	5% HS	SEM
1	5.03[a]	5.13[a]	5.18[a]	5.19[a]	0.03
3	5.36[a]	4.63[b]	4.53[b]	3.92[c]	0.22
5	5.51[a]	5.35[a]	5.14[a]	4.27[b]	0.19
7	5.26[a]	4.83[b]	5.41[a]	4.86[b]	0.09

[a,b,c] LSMeans with different superscripts within a row is significantly different ($p<0.05$).

Means are two replications per treatment for each day of storage.

3.11.4 *Escherichia coli* (*E. coli*)

The initial *E. coli* counts in this experiment ranged from 2.55-4.04 Log CFU/g (Table 17). Using *Hibiscus sabdariffa* in Naem products can decrease the number of *E. coli* as compared to the control treatment. Specifically, Naem prepared with 5% HS had the lowest counts of *E. coli* at 2.3 Log CFU/g on day 7. This is due to the higher concentration of HS which contain higher antimicrobial agents and higher anthocyanins which can inhibit *E. coli* in Naem products (Mansour, 2018; Chao & Yin, 2009).

Table 17. *Escherichia coli* (Log CFU/g)

Storage time (Days)	Control	1% HS	3% HS	5% HS	SEM
1	4.04[a]	4.09[a]	3.66[a]	2.55[b]	0.26
3	4.21[a]	3.66[b]	3.20[b]	2.85[c]	0.23
5	4.20[a]	3.63[b]	3.42[b]	3.46[b]	0.13
7	4.60[a]	3.62[b]	3.27[b]	2.30[c]	0.31

[a,b,c] LSMeans with different superscripts within a row are significantly different ($p<0.05$).

Means are two replications per treatment for each day of storage.

4. Conclusions

The results of this study indicate that *Hibiscus sabdariffa* can be used as an alternative ingredient for Naem products which may help the meat industry increase market share through this innovative product. Specifically, participants rated 3% HS treatments the highest overall acceptance scores, flavor, and taste. In addition, fermented Naem with 3% HS showed the highest scores of acceptability, purchase intent, and lactic acid bacteria counts (6.25 log CFU/g). Therefore, novel Naem prepared with HS is an excellent choice marketable alternative to traditional Naem.

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