

Effects of Ya-nang leaves (*Tiliacora triandra*) powder on properties and oxidative stability of tilapia emulsion sausage during storage

¹Sriket, P. and ^{2*}Sriket, C. and ³Nalinanon, S.

¹Program in Food Business and Nutrition, Faculty of Agriculture, Ubon Ratchathani Rajabhat University, Ubon Ratchathani, 34000, Thailand

²Program in Food Science and Technology, Faculty of Agriculture, Ubon Ratchathani Rajabhat University, Ubon Ratchathani, 34000, Thailand

³Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Ladkrabang, Bangkok 10520, Thailand

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Abstract

Effect of Ya-nang leaves (*Tiliacora triandra*) powder (YLP) (0.5-2.0%) on qualities and lipid oxidation of tilapia emulsion sausages during 21 days of refrigerated storage was investigated. Control sample had lower fiber content than samples added with YLP ($p < 0.05$) at day 0 of storage. YLP treated samples (1.0-2.0%) revealed lower L^* value but higher b^* and ΔE^* values, compared to the control samples ($p < 0.05$). With the addition of YLP, peroxide value (PV) and thiobarbituric acid-reactive substances (TBARS) value in the sausages were retarded effectively, compared to control sample ($p < 0.05$), especially when the YLP at high contents were used. Addition of YLP were also effective in retarding the formation of fishy odor in the samples, compared to control sample ($p < 0.05$). Samples treated with YLP had higher hardness and springiness, compared with control sample during storage ($p < 0.05$). YLP had no detrimental effect on the sensory attributes of sausages. This study pointed out that YLP can be utilized in sausages to enhance quality.

Keywords

Antioxidant

Ya-nang

Tiliacora triandra

Fish sausage

Tilapia

Oxidative stability

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Introduction

Sausage is typically made of beef, pork, or chicken. Fish sausages can also be made by similar methods with those used for making meat sausages (Dincer and Cakli, 2010). Fish flesh have recently been used as a raw material for emulsion sausage production, particularly in Asian countries (Konno and Park, 2005). However, fish sausages, especially prepared from freshwater fish such as tilapia (*Oreochromis niloticus*) meat can rapidly develop hydrolytic rancidity or oxidative rancidity flavors under chilled or frozen storage conditions (Oliveira Filho *et al.*, 2010; Amano, 2012). Fish meat contains high content of unsaturated fatty acids and is susceptible to lipid oxidation, thereby negatively affecting flavor, odor, color, texture and the nutritional value of fish products (Tang *et al.*, 2001). Textural properties changed by biochemical degradation leading to softening of fish meat also considered as an important factor determined fish quality. Normally, fish sausages have a short shelf life at both refrigerated and frozen storage in the absence of preservatives. To retard such a quality loss, synthetic antioxidants have been used to decrease lipid oxidation during the processing and storage of fish and fish products (Boyd *et al.*, 1993). However, the use of synthetic antioxidants has raised

questions regarding safety and toxicity (Chang *et al.*, 1977). The applications of natural antioxidants from plants in meat and fish products have been reported (Karre *et al.*, 2013). Phytochemicals in food materials and their effects on health, especially the suppression of active oxygen species by natural antioxidants from spices, herbs and local plants have been extensively studied (Aksoy *et al.*, 2013). Due to their high content of phenolic compounds, fruits and other plant materials are a good source of natural antioxidants and provide an alternative to currently used conventional antioxidants (Nuñez de Gonzalez *et al.*, 2008).

Recently, Ya-nang (*Tiliacora triandra*) leaves extracts contain specific phenolic compounds such as ferulic acid, *p*-coumaric acid, sinapic acid and syringic acid that are able to stabilize free radicals and break the oxidation chain have been reported (Sriket, 2014). Ya-nang is a native plant of Southeast Asia and is widespread in the northeastern Thailand (Singthong *et al.*, 2009). It is used particularly in many cuisines of the northeast of Thailand, especially in bamboo shoot soup. Nevertheless, no information related with the use of natural antioxidants, especially Ya-nang leaves in preventing the lipid oxidation and quality maintaining of fish sausages has been reported. Therefore, this research was aimed to

*Corresponding author.

Email: psriket@hotmail.com

Tel: +66-45-352000 Ext. 1600; Fax: +66-45-352088

evaluate the effect of Ya-nang leaves powder (YLP) on the properties and oxidative stability of emulsion sausages prepared from the meat of tilapia (*O. niloticus*) during refrigerated storage.

Materials and Methods

Chemicals

Menhaden oil was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium chloride, sodium bicarbonate, potassium iodide and trichloroacetic acid were obtained from Merck (Damstadt, Germany). Disodium hydrogen phosphate, sodium tripolyphosphate, soy protein isolate, 2-thiobarbituric acid, ammonium thiocyanate and ferrous chloride were purchased from Fluka Chemical Co. (Buchs, Switzerland). All chemicals used were of analytical grade.

Collection of Ya-nang leaves

Ya-nang (*T. triandra*) can be grown throughout the year. A single leaf was selected with width of 3–4 cm and length of 5–7 cm. The fresh leaves were obtained from three representative markets in the Ubon Ratchathani province during September to November, 2013. At each market, 3 kg samples were sampled from three representative outlets. Single composite samples for each representative market, were prepared by combining about 500 g of homogenized single sample of the same leaves variety from three representative outlets and then homogenizing again to obtain a uniform single composite sample.

Preparation of YLP

All samples were cleaned and dried under hot air oven (Memmert Model UN30, Schwabach, Germany). The samples were placed in sample tray (25x40 cm) and placed between and parallel to the top and bottom heaters and the distance between each set of heaters and a tray was fixed at 15 cm. Drying temperature was set at 50°C and velocity at 1.5 m/s (Raksakantong *et al.*, 2011) until the moisture content reached 4–5% (wet weight basis). The dried samples were cut into small pieces and ground using a blender (National Model MKK77, Tokyo, Japan) and finally sieved using a stainless steel sieve of 80 mesh size (with the diameter of 0.177 mm). The obtained powder was placed in a polythene bag, sealed and kept at room temperature until use.

Preparation of tilapia emulsion sausages containing YLP

Tilapia (*O. niloticus*) with a size of 1 fish/kg, off-loaded 24 h after capture and stored in ice, were purchased from the fish market in Ubon Ratchathani, Thailand. The fish were kept in ice during transportation to the Program in Food Science and Technology, Faculty of Agriculture, Ubon Ratchathani Rajabhat University. Upon arrival, fish were washed with tap water, filleted, deskinning and minced using a grinder with a hole diameter of 5 mm. Moisture content of the minced fish was adjusted to 75%. Fish emulsion sausages were prepared following the method described by Panpipat and Yongsawatdigul (2008) with slight modifications. Fish mince (85 g) was added with sodium chloride (2 g), sodium tripolyphosphate (1.5 g), soy protein isolate (1.5 g) and menhaden oil (10 g). The mixture was ground for 3 min using a Panasonic Food Processor (MK, 5087M, Selangor Darul Ehsan, Malaysia).

To study the effect of YLP on lipid oxidation and properties of emulsion sausages, YLP containing 10 mg of phenolic content (gallic acid equivalent/g dry powder) was added to obtain the designated final concentration. YLP (0.5, 1.0, 1.5 and 2.0 % w/w) dissolved in distilled water were added to the mixture along with menhaden oil (10% v/w). Subsequently, the mixture was further ground thoroughly for 5 min in order to obtain a homogenous paste. The paste was stuffed into a cellophane casing (diameter of 22 mm) and pre-incubated at 40°C for 40 min prior to cooking at 80°C for 15 min (Panpipat and Yongsawatdigul, 2008) in a temperature controlled water bath (Memmert, D-91126, Schwabach, Germany). The control samples were prepared in the similar manner but distilled water was added instead of YLP. Samples were cooled for about 30 min in iced water. Samples were cut into cylinders (30 mm height×20 mm diameter) and place in polythene bags and further stored at 4°C. Samples were randomly taken at day 0, 7, 14 and 21 of storage for analysis of lipid oxidation products. Textural and sensory analyses were conducted at day 0 and 21.

Determination of proximate composition

The proximate compositions of sausage samples, including moisture, ash, fat, fiber and protein contents were determined according to the methods of AOAC 1999 (AOAC, 1999). Moisture content was determined by drying to a constant weight at 105°C. The crude lipid content was determined by extracting the sample with petroleum ether with a Soxhlet apparatus. The protein content was determined by the micro-Kjeldahl method. The carbohydrate content

was calculated by subtracting the contents of ash, fat, fiber and protein.

Color determination

Color value of the sausage samples was measured using a colorimeter (Hunter Lab, Model color Flex, Reston, VIRG, USA) with the port size of 0.50 inch. The determination of color was done on ten different samples. Standardization of the instrument was done using a black and white Minolta calibration plate. The values were reported in the CIE color profile system as L^* -value (lightness), a^* -value (redness/greeness), and b^* -value (yellowness/blueness). Total difference in color (ΔE^*) was calculated according to the following equation (Gennadios *et al.*, 1996):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Where ΔL^* , Δa^* and Δb^* are the differences between the corresponding color parameter of the sample and that of white standard ($L^* = 93.63$, $a^* = -0.92$ and $b^* = 0.42$).

Determination of peroxide value

Peroxide value (PV) was determined as per the method of Richards and Hultin (2002) with a slight modification. Samples (1 g) were mixed with 11 mL of chloroform/methanol (2:1, v/v). The mixtures were homogenized at a speed of 13,500 rpm for 2 min, using an IKA homogenizer (Salangor Malaysia). Homogenates were then filtered using a Whatman No. 1 filter paper. Two milliliters of 0.5% NaCl were then added to 7 mL of the filtrate. The mixtures were vortexed at a moderate speed for 30 s using a Vortex-Genie2 mixer 4 (Bohemia, NY, USA) and then centrifuged at 3,000 x g for 3 min to separate the sample into two phases. Two millilitres of cold chloroform/methanol (2:1, v/v) were added to 3 mL of the lower phase. Twenty-five microliters of 30 % ammonium thiocyanate and 25 μ L of 20 mM iron (II) chloride were added to the mixture (Shantha and Decker, 1993). Reaction mixtures were allowed to stand for 20 min at room temperature and the absorbance was read at 500 nm. A standard curve was prepared using cumene hydroperoxide with the concentration range of 0.5-2 ppm.

Determination of thiobarbituric acid-reactive substances (TBARS)

Thiobarbituric acid-reactive substances (TBARS) were determined as described by Buege and Aust (1978). Samples (0.5 g) were mixed with 2.5 mL of a TBA solution containing 0.375 % thiobarbituric acid, 15 % trichloroacetic acid and 0.25 N HCl. The

mixtures were heated in a boiling water for 10 min to develop a pink color, cooled with running tap water and then sonicated for 30 min using an Elma (S 30 H) sonicator (Kolpingstr, Singen, Germany). The mixture was then centrifuged at $5,000 \times g$ at 25 °C for 10 min. The absorbance of the supernatant was measured at 532 nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane (MDA) at the concentration ranging from 0 to 10 ppm and TBARS were expressed as mg of MDA equivalents/kg sample.

Textural profile analysis (TPA)

TPA was performed using a TA-XT2i texture analyzer (Stable Micro Systems, Surrey, England) with cylindrical aluminum probe (50 mm diameter). The samples were cut into cylinders (30 mm height \times 20 mm diameter) and placed on the instrument's base. The tests were performed with two compression cycles. TPA textural parameters were measured at room temperature with the following testing conditions: crosshead speed 5.0 mm/s, 50% strain, surface sensing force 99.0 g, threshold 30.0 g, and time interval between the first and the second compressions was 1 s. The Texture Expert version 1.0 software (Stable Micro Systems, Surrey, England) was used to collect and process the data. Hardness, springiness, cohesiveness, gumminess and chewiness were calculated from the force-time curves generated for each sample (Bourne, 1978).

Sensory analysis

The sensory evaluation of tilapia emulsion sausages added with and without YLP (0.5, 1.0, 1.5 and 2.0%) at day 0 and 21 of storage was performed by 50 untrained panelists, who were the students in Food Science and Technology Program with the age of 20-22 years and were familiar with sausage consumption. The sausage samples were placed in the polythene bags and were dipped in the boiling water for 15 min (Masniyom *et al.*, 2002). Stick water was drained and samples were allowed to cool to room temperature (25–28 °C) prior to evaluation. Panelists were asked to evaluate for color, odor, taste, texture, appearance and overall likeness of sausage samples using a 9-point hedonic scale (Mailgaard *et al.*, 1999): 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. Samples were also evaluated for fishy odor at day 0, 7, 14 and 21 by 50 panelists using a scale 0 to 10, where 0 represented no fishy odor and 10 represented the strongest fishy odor as described by Thiansilakul *et al.* (2010).

Statistical analysis

All experiments were run in triplicate using three different lots of samples. Completely randomized design (CRD) was used for this study. The experimental data were subjected to Analysis of Variance (ANOVA) and the differences between means were evaluated by Duncan's New Multiple Range Test (Steel and Torrie, 1980). Data analysis was performed using a SPSS package (SPSS 11.0 for Windows, SPSS Inc, Chicago, IL, USA).

Results and Discussion

Proximate composition of tilapia emulsion sausages added with and without YLP

Proximate compositions of the fish sausages with and without YLP addition at day 0 of refrigerated storage are shown in Table 1. Fish sausages contained moisture, protein, lipid, carbohydrate, ash and fiber contents ranging from 71.33-72.40%, 20.12-20.76%, 1.62-1.74%, 1.97-3.44%, 1.21-2.18% and 0.45-2.60%, respectively. Generally, the addition of YLP did not significantly affect ($p > 0.05$) the moisture, protein and carbohydrate contents. However, fish sausages with YLP addition (0.5-2.0%) have the higher ash content and lower carbohydrate content than control sample. It was noted that fiber content (1.97-2.60%) of fish sausages increased with increasing YLP content (0.5-2.0%) ($p < 0.05$). High fiber and ash contents in samples added with YLP might be due to the high contents of minerals and fiber in Ya-nang leaves. Ya-nang leaves contain high levels of beta-carotene and minerals, such as calcium and iron (Singthong *et al.*, 2009). High fiber content in Ya-nang leaves was also reported (Sriket, 2014). Some fibers obtained from algae such as carrageenans (Ortiz and Aguilera, 2004) or seeds such as Guar gum (Montero *et al.*, 2000) have been used for technological purposes in fish products. The importance of dietary fiber in nutrition and health is well established (Kritchevsky, 2001). This result suggested that Ya-nang leaves can be used to promote the nutritional value of tilapia emulsion sausages.

Color values of tilapia emulsion sausages added with and without YLP

Color values expressed as L^* , a^* , b^* and ΔE^* of tilapia sausages added with and without YLP (0.5-2.0%) at day 0 of refrigerated storage is shown in Table 2. After addition of YLP at all levels, the changes in L^* (lightness), a^* (redness) and b^* (yellowness) values of fish sausages, compared to the control ($p < 0.05$) were observed. Control sample (without YLP addition) has higher L^* and lower b^* and ΔE^* values

Table 1. Proximate compositions of tilapia emulsion sausages added with and without Ya-nang leaves powder (YLP) at different levels at day 0 of refrigerated storage

YLP (%)	Content (%) [*]					
	Moisture ^(ns)	Protein ^(ns)	Lipid ^(ns)	Carbohydrate	Ash	Fiber
Control	72.40±0.86	20.76±0.35	1.74±0.12	3.44±0.21 ^{***}	1.21±0.06 ^b	0.45±0.01 ^e
0.5	72.02±0.79	20.74±0.38	1.71±0.08	2.28±0.07 ^b	1.28±0.05 ^b	1.97±0.05 ^d
1.0	71.61±0.76	20.16±0.41	1.67±0.15	2.23±0.17 ^b	2.13±0.05 ^a	2.20±0.03 ^c
1.5	71.65±0.84	20.19±0.33	1.66±0.03	1.97±0.23 ^b	2.15±0.04 ^a	2.38±0.04 ^b
2.0	71.33±0.78	20.12±0.31	1.62±0.02	2.15±0.06 ^b	2.18±0.02 ^a	2.60±0.05 ^a

^{*}Mean ± SD ($n = 3$).

^{**}Different superscripts in the same column indicate the significant differences ($p < 0.05$)

Table 2. Color values of tilapia emulsion sausages added with and without Ya-nang leaves powder (YLP) at different levels at day 0 of refrigerated storage

YLP (%)	Color values [*]			
	L^*	a^*	b^*	ΔE^*
Control	71.59±1.07 ^a	1.89±0.21 ^a	14.73±0.04 ^c	2.24 ^c
0.5	70.74±0.06 ^a	2.05±0.12 ^a	15.55±0.07 ^c	1.50 ^d
1.0	69.78±0.07 ^b	2.50±0.64 ^a	18.16±0.08 ^b	3.15 ^b
1.5	69.50±0.08 ^b	-1.74±0.02 ^c	21.35±0.55 ^a	7.04 ^a
2.0	66.75±0.08 ^c	-1.08±0.04 ^b	19.99±0.88 ^a	7.27 ^a

^{*}Mean ± SD ($n = 3$).

^{**}Different superscripts in the same column indicate the significant differences ($p < 0.05$)

than samples added with YLP ($p < 0.05$). However, the lower a^* value (-1.74 and -1.08) of YLP samples (1.5 and 2.0%) compared with control sample was observed ($p < 0.05$). The decreased lightness and redness values in YLP samples might be due to the slightly increase in green color of YLP at high concentration (1.5-2.0%) used. The negative effect of natural extract on color of fish product has been reported (Balange and Benjakul, 2009). Therefore, the use of YLP at low content had no impact on color of the resulting sausages, while high YLP addition (1.5-2.0%) showed the detrimental effect on color to some degree.

Effect of added YLP on lipid oxidation of tilapia emulsion sausages during refrigerated storage

Changes in peroxide values (PV)

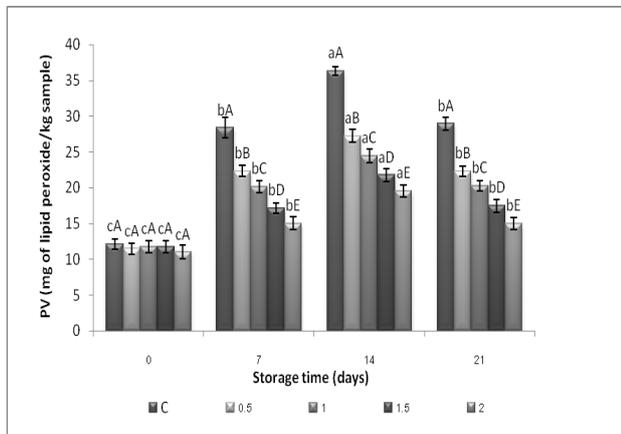
Effect of YLP (0.5, 1.0, 1.5 and 2.0%) addition on lipid oxidation of tilapia emulsion sausages during refrigerated storage for 21 days is depicted in Figure 1a. Generally, no difference in PV among all samples at day 0 of refrigerated storage was observed

Table 3. Textural properties of tilapia emulsion sausages added with and without Ya-nang leaves powder (YLP) at different levels during refrigerated storage for 21 days

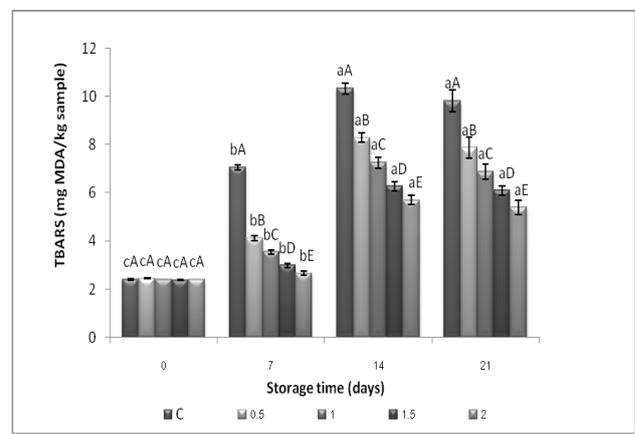
Storage time (day)	YLP (%)	Texture profile analysis (TPA)*				
		Hardness (N)	Springiness	Cohesiveness	Gumminess	Chewiness (N)
0	Control	34.37±0.14 ^{bb**}	0.77±0.01 ^{ba}	0.49±0.01 ^{aa}	19.35±0.36 ^{ab}	17.44±0.86 ^{ab}
	0.5	35.68±0.27 ^{ab}	0.81±0.02 ^{aa}	0.50±0.07 ^{aa}	20.17±0.38 ^{ab}	18.22±0.38 ^{ab}
	1.0	35.85±0.18 ^{ab}	0.81±0.01 ^{aa}	0.49±0.06 ^{aa}	19.78±0.33 ^{ab}	18.21±0.35 ^{ab}
	1.5	35.94±0.21 ^{ab}	0.82±0.03 ^{aa}	0.52±0.08 ^{aa}	20.65±0.37 ^{ab}	18.47±0.39 ^{ab}
	2.0	36.27±0.25 ^{ab}	0.82±0.02 ^{aa}	0.51±0.01 ^{aa}	20.42±0.34 ^{ab}	18.61±0.51 ^{ab}
21	Control	40.24±1.15 ^{ba}	0.76±0.02 ^{ba}	0.50±0.01 ^{aa}	23.65±0.99 ^{aa}	20.08±0.01 ^{aa}
	0.5	45.91±2.82 ^{aa}	0.80±0.01 ^{aa}	0.51±0.02 ^{aa}	24.23±0.60 ^{aa}	20.12±0.03 ^{aa}
	1.0	45.99±1.73 ^{aa}	0.80±0.01 ^{aa}	0.50±0.03 ^{aa}	24.58±0.44 ^{aa}	20.14±0.01 ^{aa}
	1.5	46.14±2.64 ^{aa}	0.81±0.01 ^{aa}	0.52±0.01 ^{aa}	24.51±2.40 ^{aa}	21.20±0.02 ^{aa}
	2.0	46.54±1.44 ^{aa}	0.81±0.02 ^{aa}	0.52±0.01 ^{aa}	24.60±2.40 ^{aa}	21.10±0.02 ^{aa}

*Mean ± SD (n = 3).

**Different lowercase letters within the same column in the same storage time indicate the significant differences (p < 0.05). Different uppercase letters within the same column in the same YLP level indicate the significant differences (p < 0.05)



(a)



(b)

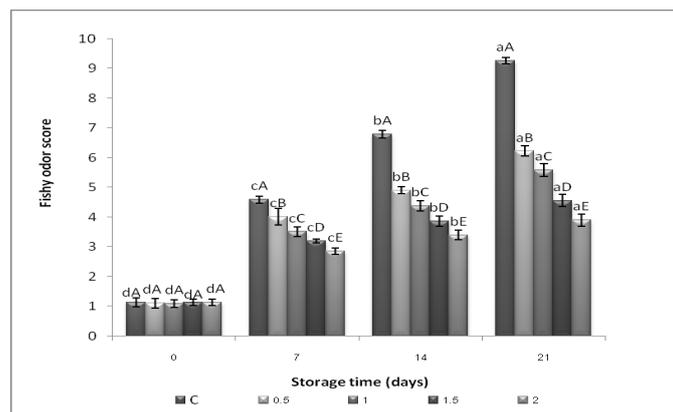


Figure 1. Peroxide value (PV) (a), thiobarbituric acid-reactive substance (TBARS) (b) and fishy odor (c) of tilapia emulsion sausages during 21 days of refrigerated storage. Bars represents the standard deviation ($n = 3$). Different lowercase letters within the same treatment (same Ya-nang leaves powder (YLP) level) indicate the significant differences ($p < 0.05$). Different uppercase letters within the same storage time indicate the significant differences ($p < 0.05$). C: control (sausage without addition of YLP); 0.5: sausage added with 0.5% YLP; 1: sausage added with 1% YLP; 1.5: sausage added with 1.5% YLP; 2: sausage added with 2% YLP.

(Figure 1a). A sharp increase in PV was found in all samples up to 14 days of refrigerated storage ($p < 0.05$) (Figure 1a). However, the decrease in PV was observed in all samples at the end of storage

($p < 0.05$). The increase in PV of samples added with and without YLP indicated that the samples were in propagation stage of lipid oxidation with the lower rate of decomposition of hydroperoxide formed

Table 4. Likeness score of tilapia emulsion sausages added with and without Ya-nang leaves powder (YLP) at different levels during refrigerated storage for 21 days

Storage time (day)	YLP levels (%)	Likeness score*					
		Color	Odor	Taste	Texture	Appearance	Overall
0	Control	7.19±0.74 ^{aA**}	7.09±0.10 ^{aA}	7.47±0.04 ^{aA}	7.02±0.64 ^{aA}	7.18±0.38 ^{aA}	7.06±0.71 ^{aA}
	0.5	7.20±0.89 ^{aA}	7.09±0.10 ^{aA}	7.51±0.09 ^{aA}	7.12±0.71 ^{aA}	7.28±0.60 ^{aA}	7.16±0.72 ^{aA}
	1.0	7.11±0.78 ^{aA}	7.10±0.19 ^{aA}	7.49±0.06 ^{aA}	7.06±0.83 ^{aA}	7.18±0.52 ^{aA}	7.08±0.69 ^{aA}
	1.5	7.07±0.87 ^{aA}	7.08±0.17 ^{aA}	7.57±0.09 ^{aA}	7.10±0.86 ^{aA}	7.10±0.48 ^{aA}	7.12±0.74 ^{aA}
	2.0	7.12±0.84 ^{aA}	7.10±0.14 ^{aA}	7.55±0.06 ^{aA}	7.03±0.84 ^{aA}	7.14±0.40 ^{aA}	7.14±0.71 ^{aA}
21	Control	7.00±0.77 ^{aA}	5.09±0.50 ^{bB}	5.12±0.04 ^{bB}	6.77±0.52 ^{aA}	6.78±0.48 ^{aA}	6.54±0.54 ^{aA}
	0.5	7.14±0.27 ^{aA}	6.59±0.20 ^{ab}	6.44±0.08 ^{ab}	6.88±0.62 ^{aA}	6.88±0.35 ^{aA}	6.84±0.55 ^{aA}
	1.0	7.10±0.41 ^{aA}	6.60±0.39 ^{ab}	6.54±0.06 ^{ab}	6.80±0.51 ^{aA}	6.77±0.45 ^{aA}	6.71±0.74 ^{aA}
	1.5	7.02±0.88 ^{aA}	6.70±0.37 ^{ab}	6.53±0.55 ^{ab}	6.74±0.44 ^{aA}	6.68±0.68 ^{aA}	6.82±0.34 ^{aA}
	2.0	7.05±0.54 ^{aA}	6.81±0.64 ^{ab}	6.62±0.05 ^{ab}	6.87±0.49 ^{aA}	6.70±0.60 ^{aA}	6.88±0.42 ^{aA}

*Mean ± SD (n = 3)

**Different lowercase letters within the same column in the same storage time indicate the significant differences (p < 0.05).

Different uppercase letters within the same column in the same YLP level indicate the significant differences (p < 0.05)

(Maqsood *et al.*, 2012). The decrease in PV with extended storage time was presumed to be due to the decomposition of hydroperoxide formed into the secondary oxidation products. Hydroperoxides underwent decomposition, yielding a wide variety of decomposition products, including aldehydes, ketone, acid, etc. (Chaijan *et al.*, 2006). Nevertheless, sample added with YLP showed the lower rate of increase in PV, compared with control sample, especially when high contents were used (p<0.05). It was noted that efficacy in preventing lipid oxidation of YLP was dose-independent. The results indicated that YLP samples were more effective in retarding the formation of hydroperoxide, compared with control sample. YLP showed the radical scavenging activity via hydrogen donating and reducing power, thereby terminating the propagation. Therefore, YLP can be used as antioxidant in tilapia emulsion sausage. The result confirmed the *in vitro* antioxidative activities of YLP as reported by Sriket (2014).

Changes in TBARS values

Effect of various contents of YLP (0.5, 1.0, 1.5 and 2.0%) on TBARS values of tilapia emulsion sausages is shown in Figure 1b. No marked differences in TBARS value of emulsion sausages were observed in all samples at day 0 of storage (p>0.05) (Figure 1b). Thereafter, TBARS values in all samples sharply increased up to day 14 of storage (p<0.05) and remained unchanged at the end of storage (Figure 1b). No changes in TBARS at the end of storage was probably due to their reaction with free amino acids, proteins and peptides present in the sausages to form Schiff's base (Dillard and Tappel, 1973). In addition, volatile oxidation products with low molecular weight could be lost during extended storage. At day 0 of storage, TBARS values of all sample ranged from 2.3 to 2.5 mg MDA/kg of sample, indicating that the

lipid oxidation occurred during the processing and cooking of the sausages. Control samples showed the higher formation of TBARS throughout the storage of 21 days, compared with other samples (p<0.05). With the addition of YLP (0.5-2%), the formation of TBARS in tilapia emulsion sausage was retarded effectively. Among all samples, sample added with 2% YLP had the lower formation of TBARS, compared to other samples (p<0.05). It was noted that efficacy in retardation of TBARS by YLP was achieved in dose dependent manner. This result was well correlated with PV of YLP samples. Thus, YLP (0.5-2%) was effective in retarding lipid oxidation in tilapia emulsion sausage during the refrigerated storage for 21 days. Furthermore, delaying lipid oxidation can have a significant contribution towards prevention of nutritional loss and lowering the risk of health problem (Hayes *et al.*, 2011).

Changes in fishy odor

Changes in fishy odor in the emulsion sausages added with and without YLP (0.5-2%) during 21 days of refrigerated storage are depicted in Figure 1c. At day 0 of storage, no marked difference in fishy odor among all samples was observed (p>0.05) (Figure 1c). Fishy odor intensity in all samples increased continuously with increasing storage time (p<0.05). At the same storage time, control sample showed higher fishy odor, compared with other samples (p<0.05). The retarded development of fishy odor in the YLP sample correlated well with the lower rate of lipid oxidation (Figure 1 a and b). Lipid oxidation occurred in fish muscle was considered as a main factor causing fishy odor (Fu *et al.*, 2009). Tilapia muscle is susceptible to lipid oxidation and subsequent development of rancid and fishy odor during process and storage (Yarnpakdee *et al.*, 2012). Lipids of fish muscle are prone to oxidation due to

the higher content of unsaturated fatty acids than those of mammals and birds (Sohn *et al.*, 2005). Yarnpakdee *et al.* (2012) reported that the addition of Trolox and EDTA could inhibit lipid oxidation as well as fishy odor in protein hydrolysate from tilapia muscle. This result indicated that YLP can be used to prevent fishy odor associated with lipid oxidation in tilapia emulsion sausages during refrigerated storage. However, efficacy in retardation of fishy odor by YLP was achieved in dose dependent manner.

Changes in textural properties of tilapia emulsion sausages during refrigerated storage

Texture profile analysis of the tilapia emulsion sausages added with and without YLP (0.5, 1.0, 1.5 and 2.0%) at day 0 and 21 of refrigerated storage is shown in Table 3. At day 0 of storage, there was no difference in all textural parameters among all samples ($p>0.05$) except hardness and springiness of sausages added with YLP (0.5-2%) were higher than control sample ($p<0.05$). Among all samples containing YLP at various contents (0.5-2%), there was no difference in force required to compress sample to attain a given deformation (hardness), capability in breaking down the internal structure (cohesiveness) and the required energy to chew the sample to the point required for swallowing it (chewiness). Hardness, gumminess and chewiness values of all samples increased at day 21 of storage ($p<0.05$), while there was no change in springiness and cohesiveness for all samples ($p>0.05$). The results indicated that hardening texture occurred at day 21 of storage, which was probably due to the decrease in moisture content of sausage samples (data not shown). However, at the end of storage (day 21), samples added with YLP showed the higher hardness and springiness values, compared to control sample ($p<0.05$). The higher hardness in the samples containing YLP might be due to the higher fiber content in the samples compared with control sample. Higher hardness value of South African hake (*Merluccius capensis*) sausage added with chicory root inulin (dietary fiber) than control sample was reported (Cardoso *et al.*, 2008). The higher hardness values of YLP samples were in agreement with higher fiber content, compared to control sample (Table 1). The result suggested that textural properties of tilapia emulsion sausage could be stabilized over the time of storage with the addition of YLP.

Effect of added YLP on sensory properties of tilapia emulsion sausages during refrigerated storage

Color, odor, taste, texture, appearance and overall likeness of the tilapia sausages added with and without YLP (0.5-2%) at day 0 and 21 of storage

were scored by 50-untrained panelists as shown in Table 4. Generally, no difference in all attributes among all samples ($p>0.05$) was observed at day 0 of storage. Thus, the addition of YLP at various levels to tilapia emulsion sausages had no impact on sensory properties of all samples. The incorporation of other natural antioxidants such as kiam wood extract (0.04 and 0.08%) and tannic acid (0.02 and 0.04%) (Maqsood *et al.*, 2012), lutein, ellagic acid and sesamol (0.20, 0.30 and 0.25%) (Hayes *et al.*, 2011) into emulsion sausages also had no impact on consumer likeness. At the end of storage, color, texture, appearance and overall likeness of all samples remained unchanged (Table 4). However, the decrease in odor and taste likeness for the all samples were found ($p<0.05$). The decreased odor and taste likeness scores of all sample at day 21 of storage was probably due to the presence of some lipid oxidation products in the sausages, which caused the off odor and taste in the resulting sausages. Nevertheless, control sample had lower odor (5.09) and taste (5.12) likeness scores than did YLP (6.44-6.81) sample ($p<0.05$). This was in accordance with the intensive oxidation taken place in the control sample at extended storage (Figure 1a and b). YLP samples containing phenolic compound might prevent the coalescence of emulsion through its protective role in retardation of the oxidative damage to the protein, which act as an emulsifier. Proteins have emulsifying properties, yielding the stable meat emulsion. The oxidative damage of proteins has an impact on protein solubility, leading to aggregation and complex formation due to cross links, thus impairing their emulsifying property (Karel *et al.*, 1975). Phytochemicals in plant extracts might have increased the emulsion stability in the sausage through their protective role on proteins against oxidation (Hayes *et al.*, 2011; Maqsood, 2012). Thus, the addition of YLP was able to maintain sensory property of fish emulsion sausage during refrigerated storage for 21 days. Therefore, YLP can be incorporated into tilapia emulsion sausages without having any detrimental effect on the organoleptic quality of products.

Conclusion

Tilapia emulsion sausages added with YLP had higher fiber content compared to control sample. Samples treated with YLP (0.5-2%) had lower L^* and higher b^* values, however, panelist could not detect any difference in the color of sausages treated with YLP, compared to the control. YLP was able to prevent lipid oxidation of emulsion sausages and to lower rancidity in dose-dependent manner. Addition

of YLP had no detrimental effect on the organoleptic properties. Thus, YLP at least 0.5% can be used as an effective natural antioxidant in the fish emulsion sausages during refrigerated storage for 21 days.

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