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Study on the Development and Functional Characteristics of Salted Egg with Liquid Smoke

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Abstract   In this study, the duck eggs were salted with none or 2.5% and 5.0% (v/v) of liquid smoke (LS), respectively. As a control, samples salted without LS were used. The 2-thiobarbituric acid (TBA) values, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability, and reducing power of the three groups were tested at 0, 7, 14, and 21 and 28 days to determine the effects of LS on the antioxidant activity of treated eggs. In addition, gas chromatography-mass spectrometry (GC-MS) and electronic nose (E-Nose) were used to analyze the volatile flavor components of fresh duck eggs, LS, control, and salted duck eggs enriched with 2.5% (v/v) LS after 28 days of salting. The TBA value considerably increased with an increase in salting period, and the treated egg's TBA value significantly associated with LS concentration. The TBA value decreased as the LS concentration increased. The amount of LS present was highly associated with their capacity to scavenge DPPH radicals. The reducing power of the samples was substantially correlated with the LS concentration, and the reducing power increased with increasing LS concentration. The GC-MS data revealed that phenols and ketones were the predominant chemicals present in the LS, and they were also found in the eggs added to the LS even though they were absent in the fresh eggs and control. The flavor of the control group and treated eggs with LS differed significantly, according to the principal component analysis and radar map of the E-nose. The texture study results revealed that the LS significantly impacted the hardness, cohesiveness, and chewiness of eggs.

Keywords   liquid smoke, salted egg, antioxidant activity, gas chromatography-mass spectrometry (GC-MS), electronic nose (E-nose)

Introduction

Salted egg is a common preserved egg product, also known as salted duck egg. It is usually pickled in brine or mixed with yellow mud, plant ash and salt and is a popular traditional pickled egg product in Indonesia. During pickling, the egg yolks gradually

become solidified and hardened. Egg whites lose viscosity and become diluted (Li et al., 2022). The rate of salt penetration of the egg whites and yolks may have an effect on changes in the composition and characteristics of eggs, especially yolks, during pickling. In addition, the marinating time also plays an important role in the formation of salted eggs desired by consumers (Kaewmanee et al., 2011).

During storage, there are many factors that reduce the quality of salted eggs. The most important factor is the oxidation of oil, which not only affects the flavor of salted eggs, but also affects the nutritional value of salted eggs. The oil in salted egg mainly exists in the egg yolk; the main reason for the reduction of the shelf life of salted egg is the oxidation of oil in the egg yolk and the overall quality of the salted egg (Ahn et al., 1995; Harlina et al., 2015). Therefore, it is necessary to reduce the oxidation of oil to prolong the storage time of salted eggs. The oxidation of oil can be reduced by adding synthetic or natural additives (Hayat et al., 2010), and the liquid smoke (LS) with good antioxidant properties can be used as an additive for pickling salted duck eggs.

Many natural antioxidants are based on phenolic compounds. LS contains a lot of phenolic compounds. The constituents in LS include many different compounds such as phenols, ketones, alcohols, esters, aldehydes, acids, furan and pyran derivatives (Montazeri et al., 2013). Among them, phenolic derivatives probably represent the most important one in terms of quality, and phenolic compounds are used to optimize antimicrobial performance, antioxidant potential and sensory properties (Milly et al., 2005). The antioxidant properties of phenols can reduce chronic diseases, degenerative diseases, heart disease, eye diseases, etc. (Hasler, 2000). Adding LS to the product can delay the lipid oxidation process (Estrada-Muñoz et al., 1998). They can act as hydrogen donors, reducing agents, singlet oxygen quenchers and metal atoms and free radicals, thereby neutralizing their toxic effects (Rice-Evans et al., 1996). In particular, methoxyphenols among phenolic compounds and their derivatives have been considered as major contributors to aroma in LS. In addition, they are also the main substances responsible for the antibacterial and antioxidant effects of smoked foods (Guillén and Ibargoitia, 1998; Suñen et al., 2003).

At present, in Indonesia, people's living standards are gradually improving, and the requirements for consumer goods are getting higher and higher, and at the same time, the processing industry of egg products is gradually becoming more advanced and modern. Therefore, innovation in the processing of salted duck eggs, increasing the shelf life of salted duck eggs and developing salted egg products with more functional properties can be achieved and need to be achieved. At present, domestic scholars have developed many new pickling techniques, which have improved the flavor and storage time of salted eggs. In recent years, developed countries have been very advanced in food processing, and LS technology is basically used for popular smoked products such as bacon. Little research has been done on egg products, especially as food additives or new processing methods for preserved egg products.

The purpose of this study was to investigate the effects of LS as an additive for pickled salted duck eggs on the antioxidant properties, volatile flavor compounds, and texture of salted duck eggs. Studying the antioxidant properties of LS and the influence of its unique flavor in salted eggs is not only beneficial to provide new ideas for the study of extending the shelf life of salted duck eggs, but also to develop new types of salted duck eggs with smoked flavors to increase consumers' choices and developed the new markets.

Materials and Methods

Materials

Methanol, ethanol, NaOH, NaCl, potassium ferricyanide, $FeCl_3$, 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), 1-

diphenyl-2-picrylhydrazyl (DPPH) were all Pro Analysis quality chemical reagents. LS were bought from Lubna Company (Semarang, Indonesia). LS was extracted from coconut shell pyrolysis that produces LS containing 4.13% phenolic compounds, 11.3% carbonyl and 10.2% organic acids.

Sample preparation

Fresh duck eggs were harvested within three days of laying were purchased from local producers. After thoroughly cleaning numerous duck eggs with water, place the eggs in a solution of salt that is 18% (w/w; salt solution:duck eggs=1:1, w/w), the egg samples were then treated with LS (Liquid Smoke Food Grade A, Lubna Company) at concentrations of 2.5% and 5% (v/v) and curing them for 28 days.

Determination of lipid oxidation

According to Harlina et al. (2018), oxidative rancidity of fat was assessed using a thiobarbituric acid reactive substances (TBARS) assay of malondialdehyde (MDA). Briefly, 5 g of the sample was combined with 25 mL of TCA 7.5%, homogenized, and left at room temperature for 1 hour. The solution was centrifuged (model 3,740, Kubota, Osaka, Japan) at 2,000×g for 10 min. The filtrate was then added 50 mL of purified water. 5 mL of the prepared solution and 5 mL of 0.02 M TBA were combined. A UV-vis spectrophotometer was used to test the reaction mixture's absorbance at 532 nm after being maintained in a water bath at 100°C for 15 min. In a 50-mL volumetric flask, distilled water was added to 25 mL of 7.5% TCA, and the resulting solution (5 mL) was combined with 5 mL of 0.02 M TBA to create the blank sample. Malondialdehyde standard curve data was used to determine TBARS value, which was then represented as mg MDA/g sample.

Sample preparation for antioxidant activity assay

Combining egg white and yolk with 95% ethanol at a ratio of 1:10 (w/v), the mixture was extracted for 2 h at 60°C while being continuously shaken at a speed of 170×g. The resulting slurry was centrifuged for 10 min at 10,000×g. The antioxidant activity of the supernatant was examined. Salted duck eggs treated with LS were examined *in vitro* for their antioxidant properties, including DPPH radical scavenging activity and reducing power assay.

Determination of the ability to scavenge 1-diphenyl-2-picrylhydrazyl (DPPH) free radicals

Free radical scavenging activity was used to measure the antioxidant activity (DPPH method). It was made with a 0.002% concentration of DPPH. Supernatant samples were obtained at various concentrations and formed up to 2 mL quantities in each test tube. Each test tube was then filled with 2 mL of the DPPH solution, which was then left in the dark for 30 min. Each sample was examined three times. Using a UV-Visible spectrophotometer, optical density was later measured at 517 nm. As a control, ethanol combined with DPPH was employed. With a small modification, the procedure was the same as that employed by Harlina et al. (2019). The formula was:

$$\% \ \text{Inhibition of DPPH activity} = [(A - B)] \div A \times 100 \tag{1}$$

Where: A=Optical density of control; B=Optical density of sample.

Determination of reducing power

The reducing power assay was evaluated described by Harlina et al. (2018). In a summary, 2.0 mL of sample was combined with 2.0 mL of 1% (w/v) potassium ferricyanide and 2.0 mL of 0.2 mol/L sodium phosphate buffer (pH 6.6). The aforementioned combination was incubated for 20 min at 50℃. Two milliliters of 10% TCA was then added after cooling. The supernatant was recovered after centrifugation at 3,000×g for 10 min, and 2.0 mL was combined with 2.0 mL of distilled water and 0.4 mL of 0.1% (w/v) $FeCl_3$. After 10 min of mixtures standing at room temperature, the absorbance at 700 nm was measured. As a blank, the same amount of distilled water was used.

Determination of volatile flavor compounds using gas chromatography-mass spectrometry (GC-MS)

The control and treated eggs were put in 20 mL headspace extraction bottles. CAR/PDMS Fused Silica 24 Ga (Manual Holder, 3pk; Supelco, Bellefonte, PA, USA) extracted for 2 h, desorbed in the GC-MS inlet for 5 min, and determined volatile flavor compounds. GC-MS conditions: Agilent 7890B/5977A (Agilent, Santa Clara, CA, USA) GC-MS, HP-5 (30 m ×0.25 mm×0.25 mm) column, inlet temperature 230℃, split less injection. Heating program: the initial temperature was 40℃, maintained for 6 min, increased to 135℃ at a heating rate of 3℃/min, and then increased to 230℃ at a heating rate of 6℃/min and held for 10 min. Mass spectrometry conditions: EI ion source, ion source temperature 200℃, electron capacity 70 ev, filament current 150 μA, scanning mass range 33–450 u.

Determination of volatile flavor substances using an electronic nose

FOX-3000 electronic nose (E-nose; Alpha MOS, Toulouse, France) was used to collect and evaluate data. It contains an HS-100 autosampler, sensor array, air generator tool, sampling instrument, and software called Alpha Soft V11. Eighteen metal oxide sensors are put in the sensor array, which is separated into three series chambers called T, P, and LY (Li et al., 2012). Take 2–3 g of the egg yolk sample into a 10 mL headspace vial, seal and equilibrate for 30 min. Using pure air as the carrier gas, the vial was put into an autosampler and equilibrated at 50℃ for 10 min at a flow rate of 200 mL/min. The stirring speed was 250×g, the injection volume was 2,500 mL, and the injection time was 3 s. The injection delay time was 180 s, while the acquisition time was 50 s. Analysis was conducted using the electronic nasal maximum response point that was automatically recorded with each of the 18 sensors (Song et al., 2013). Three different evaluation samples were used.

Texture profile analysis (TPA) of egg yolk

Texture analysis can be used to determine a product's texture. A TA.XT Plus texture analyzer was used to determine the texture of salted egg yolk. Samples of egg yolks were divided in half before texture analysis with a compressed 36 mm diameter cylindrical probe. Each sample is compressed after being sandwiched between specific stainless steel plates (using a texture analyzer at room temperature). Prior to and during the test, the texture analyzer's speed was 2 and 5 mm/s, respectively. At an intermediate speed of 3 mm/s, the torque-deformation curves were captured. Evaluations included hardness, cohesiveness, elasticity, tackiness, chewiness, and recovery. The software Micro Stable was used to obtain these parameters (Stable Micro System, Godalming, UK). Three replicates were used on average.

Data processing

Experiments were performed in triplicate. Data are presented as SD. One-way analysis of variance (ANOVA) was

performed using SPSS 20.0 software (SPSS, Chicago, IL, USA). Means were compared using Duncan's multiple range test to determine the least significant difference between methods at a significance level of 0.05.

Results

The effect of liquid smoke (LS) on the antioxidant activity of salted duck eggs

The effect of liquid smoke (LS) on lipid oxidation

Malondialdehyde, a result of lipid peroxidation that can be represented by TBA values, can be created from polyunsaturated fatty acids with at least three double bonds (Ayala et al., 2014). The concentration of the reaction product, TBARS was calculated by reacting the peroxidation product in the sample with thiobarbituric acid. Due to the presence of TBA reactive substance resulting from the oxidation of peroxides to aldehydes and ketones during the second stage of autoxidation, the TBA value has been commonly employed to quantify the extent of lipid oxidation (Song et al., 2011). The equation derived from the standard curve was used to compute the TBA value, which was then represented as mg (MDA)/kg. Fig. 1 displays the findings of the TBA values for the control group and the LS-treated salted eggs during the duration of the 28 day curing period.

During the 28 day marinating period, TBA value of the salted egg yolk considerably increased over time ($p<0.05$). The steady increase in the degree of partial oxidation and dehydration of unsaturated fatty acids may be responsible for the rise in TBA value. The TBA value of the control sample was considerably different from the samples that had been treated with LS ($p<0.05$). The TBA value of the control sample was higher than that of the salted egg treated with LS, and the TBA value decreased with increasing LS concentration. The oxidation reaction is inhibited by the high LS concentration of phenolic compounds and their derivatives, which have good antioxidant activity and can be employed as antioxidants. According to Estrada-Muñoz et al. (1998), the instability of the generated MDA or the oxidation of various lipid populations at various intervals could both account for the rise and fall in TBA values at different storage times.
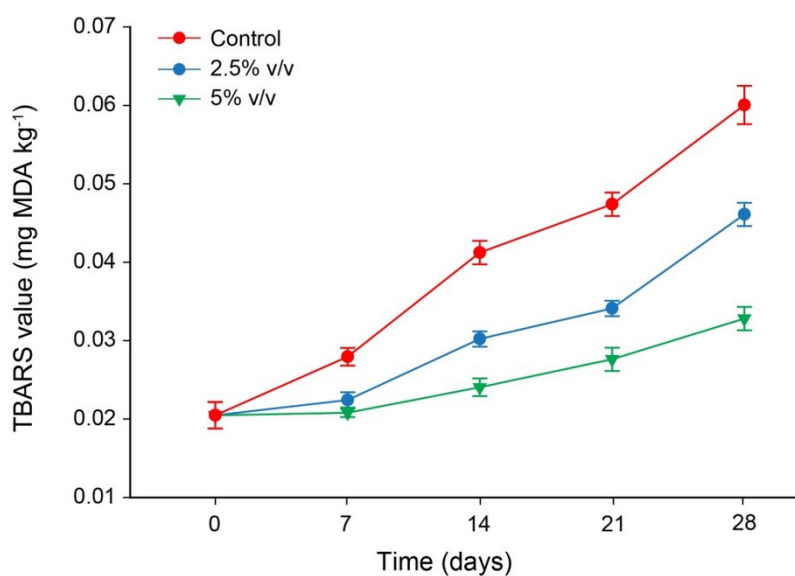
Fig. 1. TBARS value of egg yolk of salted duck egg during salting. TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde.

Effects of liquid smoke (LS) on the ability of scavenging 1-diphenyl-2-picrylhydrazyl (DPPH) free radicals

Fig. 2 displays the changes in the free radical scavenging rate of the salted eggs treated with the LS and the control group throughout the duration of the 28 day curing period. According to the findings, during the first 14 days of curing, salted duck eggs treated with LS considerably improved over time at scavenging DPPH free radicals in the yolk and protein ($p<0.05$). After the 14th day, the amount of the increased protein capacity to scavenge free radicals reduced with time and tended to stabilize in the salted egg yolk treated with LS. During the 28-day curing period, free radical scavenging rate of the control group did not rise significantly ($p>0.05$).

Salted eggs supplemented with LS 2.5% and 5.0%, (v/v) considerably outperformed the control sample in terms of their ability to scavenge free radicals ($p<0.05$), and it was found that the concentration of LS was inversely correlated with the rate of free radical scavenging. Egg yolks often scavenge free radicals at a higher rate than egg whites.

The effect of liquid smoke (LS) on reducing power

Fig. 3 illustrates changes in the reduction power of the salted eggs in the control group and the salted eggs treated with LS over the duration of the 28-day curing period. The results indicated that reduction power of salted egg yolk significantly increased over the duration of the 28-day pickling period ($p< 0.05$). After the first seven days ($p<0.05$), the reducing power of the control of salted egg white and the 2.5% (v/v) LS increased dramatically with time and then progressively stabilized. With the exception of the second week during the 28 curing cycles, the reducing power of salted egg white in 5.0% (v/v) LS increased significantly over time ($p<0.05$). The reducing power of salted eggs added with LS (2.5% and 5.0%, v/v) was significantly higher than that of the control samples ($p<0.05$). The reducing power of egg yolk is overall higher than that of egg white.

Phenolic compounds, which compose the majority of LS, act as reducing and antioxidant agents by chelating metal ions or providing hydrogen to prevent the oxidation chain reaction (Bursal and Köksal, 2011). The electrons provided by natural antioxidants are frequently calculated using the reducing force (Dorman et al., 2003). According to You et al. (2010), antioxidants cause Fe^{3+}/FeSCN complexes to degrade into the Fe^{2+} form, which results in the formation of reducing power in
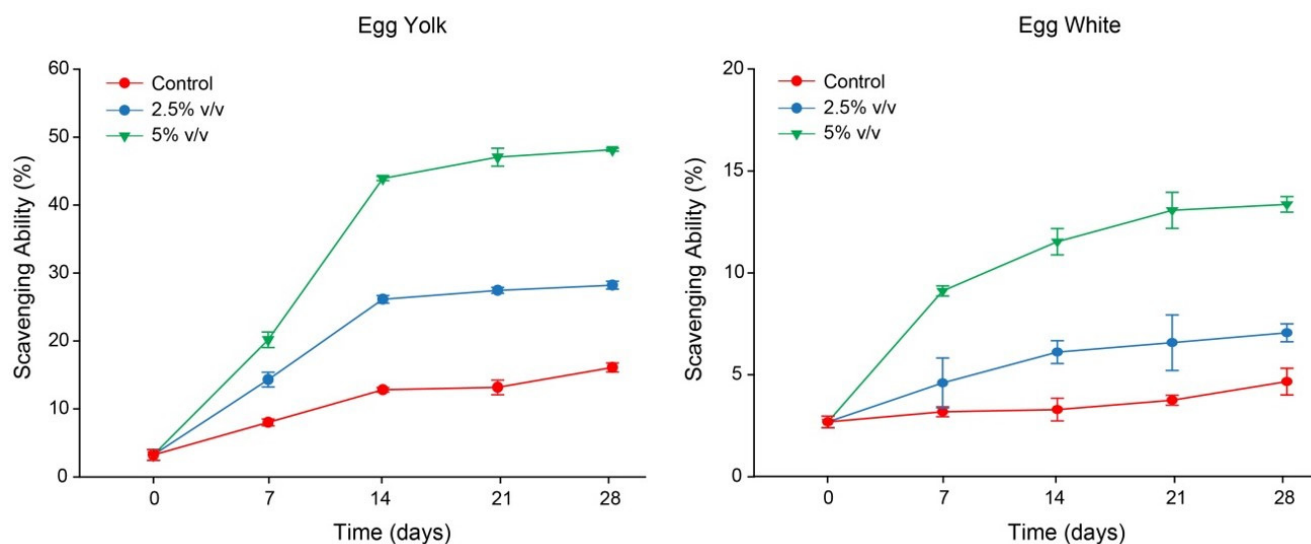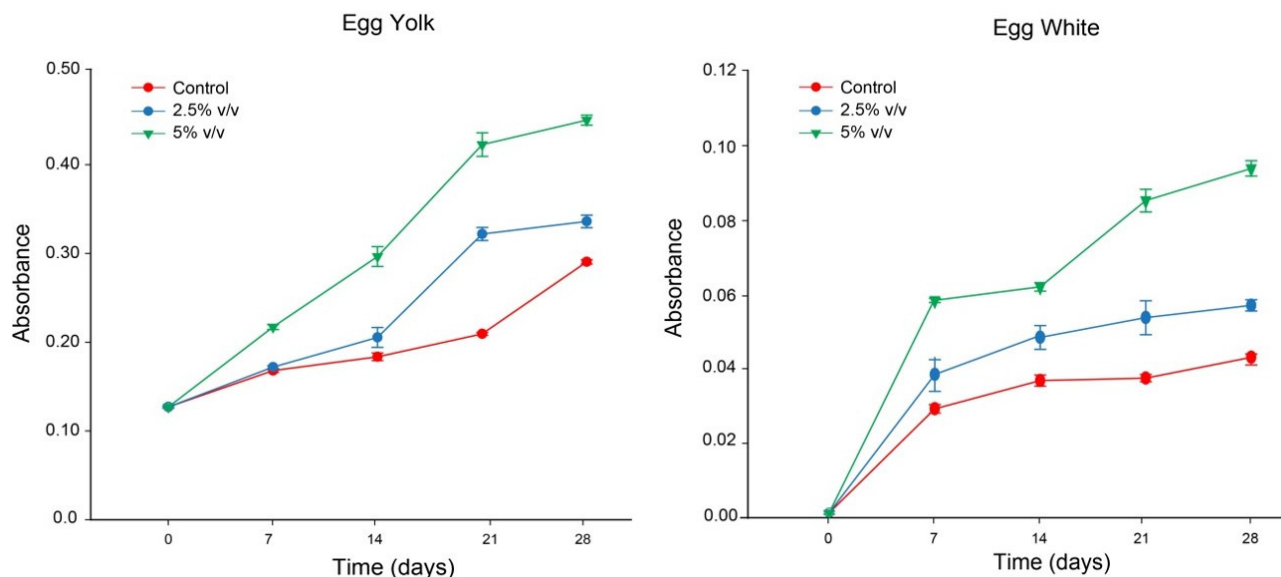

Fig. 2. Radical scavenging ability of egg yolk and egg white of salted duck egg during salting.

Fig. 3. Reducing power of egg yolk and egg white of salted duck egg during salting.

the samples. The strongest reducing power is demonstrated by the absorbance at 700 nm (Duh, 1998; Memarpoor-Yazdi et al., 2013). Yellow color of sample reagent turns green or blue-green during the test. Antioxidants that act as a reducing agent transform ferricyanide from its complicated state to its simple ferrous form. As a result, Fe^{2+} content can be determined by observing Prussian blue absorption at 700 nm. The ability of a phenolic antioxidant to provide electrons is key, and its indication, the valence reduction of Fe(III), is frequently used (Dorman et al., 2003).

Effect of liquid smoke (LS) on volatile flavor compounds of salted duck eggs

Volatile flavor compounds identified by gas chromatography-mass spectrometry (GC-MS)

Lipid oxidation is the main reaction that produces volatile flavor compounds, which can improve flavor. The volatile substances found on fresh duck eggs and control salted duck eggs by GC-MS are listed in Table 1. There are 3 esters, 5 acids, 1 ketone, 3 aldehydes, 5 nitrogenous compounds, 1 ether, 2 hybrid cyclic compounds, 3 hydrocarbons, and 1 other substance among the 24 volatile compounds found in fresh duck eggs, which are classified into 9 groups. The salted duck eggs of the control group included 21 volatile components that could be categorized into 10 different groups. These included 1 ester, 5 acids, 3 alcohols, 1 ketone, 4 aldehydes, 5 nitrogenous compounds, 1 ether, 1 heterocyclic compound, 3 hydrocarbons, and 1 other substance.

The volatile flavor components found on salted duck eggs that were cured in LS 2.5% (v/v) that analyzed by GC-MS are listed in Table 2. 53 phenols, 8 esters, 3 acids, 4 alcohols, 32 ketones, 11 aldehydes, 6 nitrogen-containing compounds, 3 ethers, 7 heterocyclic compounds, 18 hydrocarbons, and 2 others are among the 147 volatile substances in LS, which are subdivided into 11 categories. A total of 63 volatile compounds, subdivided into 8 categories, were found in salted duck eggs that were cured in LS at a 2.5% (v/v) concentration. These included 23 phenols, 2 esters, 1 acid, 2 alcohols, 13 types of ketones, 4 types of aldehydes, 2 types of heterocyclic compounds, and 16 types of hydrocarbons. For total ion chromatogram of volatile flavor component from these samples can be seen in Supplementary Fig. S1.

Antibacterial, antioxidant, and organoleptic qualities are connected to phenols and their derivatives. Numerous phenolic substances, primarily phenol, guaiacol, eugenol, etc., are present in LS. When phenolic compounds are thermally degraded, they

Table 1. Analysis of volatile flavor substances from egg yolk of fresh egg and salted duck egg by GC-MS

Fresh duck egg yolk			Control group salted duck egg yolk		
	Retention time (min)	Compound name		Retention time (min)	Compound name
Esters			Esters		
1	20.937	Methyl benzoate	1	20.960	Methyl benzoate
2	17.872	Succinic acid, decyl tetrahydrofurfuryl ester			
3	22.708	1-Alanine, N-pentanoyl, myristyl ester			
Acids			Acids		
1	2.476	Acetic acid	1	2.720	Acetic acid
2	22.255	5-(1,1-Dimethylethoxy)-2-thiophenecarboxylic acid	2	21.477	4-(Methylamino)butanoic acid
3	25.123	Propyl-malonic acid	3	33.831	Pterin-6-carboxylic acid
4	36.937	Octadec-9-enoic acid	4	36.930	6-Octadecenoic acid
5	44.514	n-Hexadecanoic acid	5	44.507	n-Hexadecanoic acid
Alcohols			Alcohols		
	nd	nd	1	3.993	Ethanol
			2	7.138	2-Methyl-1-propanol
			3	10.103	3-Methyl-1-butanol
Ketones			Ketones		
1	33.783	2',4'-Dihydroxypropiophenone	1	23.324	(+)-2-Furanone
Aldehydes			Aldehydes		
1	7.514	Furfural	1	7.554	3-Furfural
2	7.631	3-Furfural	2	7.590	Furfural
3	14.240	5-Methyl-2-furfuraldehyde	3	9.104	3-Methyl-butyraldehyde
			4	17.773	Benzaldehyde
Nitrogen compounds			Nitrogen		
1	20.387	Methylpent-4-enylamine	1	35.336	Dimethylamine
2	23.118	2,2-Dinitro-N-methylbutylamine			
3	24.376	Propionamide			
4	27.359	2-(Adamantan-1-yl)-1-methyl-ethylethylamine			
5	28.982	N-Formyl DL-alanine			
Ethers			Ethers		
1	41.176	Polyethylene glycol monolauryl ether	1	38.329	Octaethylene glycol monolauryl ether
Heterocyclic			Heterocyclic		
1	7.469	3,5-Dimethylpyrazole	1	7.554	3,5-Dimethylpyrazole
2	7.609	1,4-Dimethyl-pyrazole			

Table 1. Analysis of volatile flavor substances from egg yolk of fresh egg and salted duck egg by GC-MS (continued)

Fresh duck egg yolk			Control group salted duck egg yolk		
	Retention time (min)	Compound name		Retention time (min)	Compound name
Hydrocarbons			Hydrocarbons		
1	25.632	3-Azabicyclo[3.2.2]nonane	1	30.798	2-Methylaminomethyl-1,3-dioxolane
2	29.477	2-Methylaminomethyl-1,3-dioxolane	2	31.007	3-Azabicyclo[3.2.2]nonane
3	28.071	2-Methylbicyclo[4.3.0]non-1(6)-ene	3	35.189	4-Methyl-1,3-dioxolane
Others			Others		
1	8.702	Methyl cyanogen chloride	1	22.852	Naphthalene

GC-MS, gas chromatography-mass spectrometry.

sometimes contain dihydroxybenzene derivatives, which have a high antioxidant activity (Guillén and Manzanos, 2002). Macromolecular polymers that are randomly cross-linked make up lignin. It is composed of a disorganized collection of hydroxyl groups with various linkages and methoxy-substituted phenylpropane units. Pyrolysis involves competing thermal degradation reactions that, depending on the bond energies involved, lead to various bond breakage. The considerable structural variety of lignin allows for a wide range of compounds to be produced during pyrolysis (Guillén and Ibargoitia, 1998).

Salted duck eggs marinated in 2.5% (v/v) LS contained a significant amount of phenolic chemicals. Fresh duck egg and salted duck egg without LS did not contain phenolic substances. Additionally, only a very little number of ketones were identified in fresh duck eggs and salted duck eggs without LS but there were 32 and 13 ketones in the LS and treated eggs with LS 2.5% (v/v), respectively. The primary element responsible for the color and flavor of smoked goods is ketone. Carbonyl groups found in ketones can interact with amino groups in protein molecules to produce a distinctive smoky color.

Fresh duck eggs, salted duck eggs in the control group, and salted egg yolks cured in 2.5% (v/v) LS, they all contained trace amounts of esters, alcohols, and aldehydes. Esterification is the process by which fatty acids and alcohols are converted into esters. Alcohols often make minor contributions to food taste (Barclay et al., 1997). Aldehydes are mostly produced as a result of the oxidation of fats and oils during heating (Mensink et al., 2003). Therefore, fresh duck eggs, the control group, and salted egg yolks treated with LS did not contain a significant amount of aldehydes. Salted duck eggs were cured in 2.5% (v/v) LS, and significant amounts of hydrocarbons were found in both. Alkenes dominate the liquid-cured, salted duck egg yolks.

Discrimination of volatile flavor substances by electronic nose

The electronic nose (E-nose) imitates the olfactory system of mammals by employing a number of sensors that react to volatile substances (Cramp et al., 2009). The E-nose is a device that combines a gas sensor structure and an air sample tool with a personal computer. The ability of sensor array function of the E-nose device to react differently to distinct flavors sets it apart from other devices used for flavor characterization. Hundreds or even thousands of distinct volatile flavor components can be found in each flavor. The identification and measurement of numerous flavor constituents in a single flavor sample is accomplished by widely used analytical techniques like GC-MS. However, unlike the human nose, which analyzes flavors as a whole and detects them by our brains (for example, by identifying patterns of flavors in memory), the E-nose can react to a sample of total flavor compounds. This is because the human olfactory system does not require isolation of individual compounds as part of the sample analysis process (Cramp et al., 2009).

Table 2. Analysis of volatile flavor substances from liquid smoke and egg yolk of salted duck egg supplemented with LS by GC-MS

LS		Salted duck egg yolk supplemented with 2.5% (v/v) LS		
	Retention/min	Compound name		Retention/min	Compound name
Esters			Esters		
1	12.192	Methyl caproate	1	16.512	Glycine, N-(2-furylcarbonyl)-methyl ester
2	14.811	Methyl 2-furoate	2	20.937	Methyl benzoate
3	21.286	Methyl benzoate			
4	22.704	Methyl octanoate			
5	26.845	4-Methyl-benzoic acid methyl ester			
6	32.130	2-Sec-butylphenylmethylcarbamate			
7	32.584	3-Methoxy-benzoic acid methyl ester			
8	35.098	2'-Hydroxy-5'-methoxyacetophenone, acetate			
Acids			Acids		
1	2.674	Acetic acid	1	2.291	Acetic acid
2	6.688	Butyric acid			
3	6.884	(E)-2-Butenoic acid			
Alcohols			Alcohols		
1	29.098	O-Methoxy-α-methylbenzyl alcohol	1	8.597	3-Furanmethanol
2	35.098	6-Methyl-5-(1-methylethyl)-5-hepten-3-yn-2-ol	2	8.597	2-Furanmethanol
3	39.710	Dihydroartemisinin			
4	40.082	5-Tert-butylpyrrolidinol			
Ketones			Ketones		
1	10.968	2-Methyl-2-cyclopent-1-one	1	10.972	2-Methyl-2-cyclopent-1-one
2	10.968	3-Methyl-2-cyclopent-1-one	2	10.972	3-Methyl-2-cyclopent-1-one
3	11.225	1-(2-Furyl)-ethanone	3	11.280	1-(2-Furyl)-ethanone
4	13.213	2,3-Dimethyl-2-cyclopent-1-one	4	11.362	1-(1H-pyrazol-4-yl)-ethanone
5	15.941	3,4-Dimethyl-2-cyclopent-1-one	5	12.688	3,4-Dimethyl-2-cyclopent-1-one
6	16.585	1-(2-Furyl)-1-propanone	6	15.920	2,3-Dimethyl-2-cyclopent-1-ene
7	18.351	2,3,5-Trimethyl-2-cyclopent-1-one	7	17.133	4,4-Dimethyl-2-cyclopenten-1-one
8	18.351	3,4,4-Trimethyl-2-cyclopent-1-one	8	17.366	2,3,4-Trimethyl-2-cyclopent-1-one
9	19.565	Acetophenone	9	19.385	Acetophenone
10	21.408	1-(2-Furyl)-ethanone	10	19.951	3-Ethyl-2-cyclopent-1-one
11	23.084	1-(2-Methylphenyl)-ethanone	11	23.578	2,3,4,5-Tetramethyl-2-cyclopent-1-one
12	23.172	2,3,4-Trimethyl-2-cyclopent-1-one	12	33.778	2',4'-Dihydroxypropiophenone
13	24.144	2,3-Dimethylhydroquinone	13	33.778	2,5-Dihydroxypropiophenone

Table 2. Analysis of volatile flavor substances from liquid smoke and egg yolk of salted duck egg supplemented with LS by GC-MS (continued)

	LS			Salted duck egg yolk supplemented with 2.5% (v/v) LS	
	Retention/ min	Compound name		Retention/ min	Compound name
14	24.293	4'-Hydroxy-acetophenone			
15	24.293	1-(2-Hydroxyphenyl)-ethanone			
16	26.598	Spiro[4.5]decan-2-one			
17	27.139	4-Hydroxy-2,4,5-trimethyl-2,5-cyclohexadien-1-one			
18	28.460	4-Hydroxy-3-methylacetophenone			
19	30.818	1-(2,5-Dihydroxyphenyl)-ethanone			
20	31.014	1-Methylinden-2-one			
21	31.014	2,3-Dihydro-2-methyl-1H-inden-1-one			
22	32.008	2',6'-Dihydroxy-3'-methylacetophenone			
23	32.008	1-(2-Hydroxy-4-methoxyphenyl)-ethanone			
24	32.200	1-(2-Hydroxy-6-methoxyphenyl)-ethanone			
25	33.552	1-(4-Hydroxy-3-methoxyphenyl)ethanone			
26	33.552	Tert-butyl hydroquinone			
27	34.470	2,3-Dihydro-3,3-dimethyl-1H-inden-1-one			
28	34.470	1-(2,3-Dihydro-1H-inden-5-yl)-ethanone			
29	34.649	7-Methylinden-1-one			
30	36.606	1-(3,4-Dimethoxyphenyl)-ethanone			
31	37.243	4-(4-Methoxyphenyl)3-buten-2-one			
32	38.685	6,7-Dimethoxy-1(3H)-benzofuranone			
Aldehydes			Aldehydes		
1	7.630	Furfural	1	7.405	Furfural
2	7.630	3-Furfural	2	7.438	3-Furfural
3	13.794	Benzaldehyde	3	13.765	Benzaldehyde
4	14.080	5-Methyl-2-furfuraldehyde	4	14.063	5-Methyl-2-furfuraldehyde
5	18.138	2-Hydroxy-benzaldehyde			
6	18.138	3-Hydroxy-benzaldehyde			
7	22.801	2-Hydroxy-4-methylbenzaldehyde			
8	22.801	2-Hydroxy-3-methylbenzaldehyde			
9	22.801	2-Hydroxy-6-methyl-benzaldehyde			
10	35.667	2-Ethoxy-4-anisaldehyde			

Table 2. Analysis of volatile flavor substances from liquid smoke and egg yolk of salted duck egg supplemented with LS by GC-MS (continued)

	LS		Salted duck egg yolk supplemented with 2.5% (v/v) LS		
	Retention/min	Compound name		Retention/min	Compound name
11	36.889	3-Ethoxy-4-methoxybenzaldehyde			
Nitrogenous			**Nitrogenous**		
1	22.050	4-Methoxy-1,3-phenylenediamine		nd	nd
2	34.368	N-(Adamantan-1-yl)methyl-2,5-dichloro-benzenesulfonamide			
3	38.000	1-Methyl-N-vanillyl-(+)-s-2-phenethylamine			
4	41.291	1-Methyl-N-vanillyl-(+)-S-2-phenethylamine			
5	41.291	1-Methyl-N-(-)-R-phenethylamine			
6	41.291	1-Methyl-N-vanillyl-(+-)-2-phenylethylamine			
Ethers			**Ethers**		
1	29.098	3,4-Methylenedioxyanisole		nd	nd
2	31.489	O-Isopropyl anisole			
3	36.606	3-Tert-butyl-4-hydroxyanisole			
Heterocyclic			**Heterocyclic**		
1	8.920	2,4-Dimethylfuran	1	7.438	3,5-Dimethylpyrazole
2	9.741	3,5-Dimethylpyrazole	2	15.689	2-Ethyl-5-methyl-furan
3	20.044	2,3-Dihydro-benzofuran			
4	24.197	1,3,5-Trimethyl-1H-pyrazole			
5	36.167	1,3-Dihydro-5,6-dimethyl-2-benzothiophene			
6	37.243	2-Ethyl-7-methyl-benzo[b]thiophene			
7	38.000	2,5-Dibutyl-furan			
Hydrocarbons			**Hydrocarbons**		
1	9.439	1-Cyclohexyl-2,5-diene	1	12.688	1,3-Dimethyl-1-cyclohexene
2	12.682	1-Methylcycloheptene	2	12.756	Tetramethylmethylenecyclopropane
3	12.703	1,2-Dimethyl-cyclohexene	3	12.792	5,5-Dimethyl-1,3-hexadiene
4	17.022	1-Methoxy-3-methyl-benzene	4	12.792	2,3-Dimethyl-2,4-hexadiene
5	18.351	3-Fluoro-o-xylene	5	12.792	2,5-Dimethyl-2,4-hexadiene
6	22.487	1,1,2-Trimethyl-3-(2-methyl-1-propenyl)-cyclopropane	6	13.113	1-Methylcycloheptene
7	28.849	2-Methoxy-1,3,5-trimethylbenzene	7	13.195	1,6-Dimethyl-cyclohexene
8	29.332	2,6-Dimethoxytoluene	8	17.133	3,4-Dimethyl-(Z,Z)-2,4-hexadiene
9	29.332	3,5-Dimethoxytoluene	9	17.709	1-Methylcyclooctene

Table 2. Analysis of volatile flavor substances from liquid smoke and egg yolk of salted duck egg supplemented with LS by GC-MS (continued)

	LS			Salted duck egg yolk supplemented with 2.5% (v/v) LS	
	Retention/min	Compound name		Retention/min	Compound name
10	30.541	1-Methoxy-4-(1-methylethyl)-benzene	10	21.336	1-Isopropylcyclohex-1-ene
11	30.818	1,4-Dimethoxy-2-methyl-benzene	11	22.284	1,1,2-Trimethyl-3-(2-methyl-1-propenyl)-cyclopropane
12	31.567	1,2,3-Trimethoxybenzene	12	22.808	2,6-Dimethyl-2,4-heptadiene
13	32.008	4-Ethyl-1,2-dimethoxy-benzene	13	27.597	1,2,3,4,5-Pentamethyl-1,3-cyclopentadiene
14	34.368	1-Ethyl-1(1-cyclobutylethylene)cyclobutane	14	28.125	2,3-Dimethoxytoluene
15	34.368	1-Methoxy-4-(1-methylpropyl)-benzene	15	28.125	3,4-Dimethoxytoluene
16	35.481	1,2,3-Trimethoxy-5-methylbenzene	16	31.525	1,2,3-Trimethoxybenzene
17	35.667	1,2-Dimethoxy-4-n-propylbenzene			
18	39.710	9-Ethylfluorene			
Phenolics			Phenolics		
1	15.570	Phenol	1	15.325	Phenol
2	19.409	p-Cresol	2	19.091	2-Methyl-phenol
3	19.409	2-Methyl-phenol	3	20.198	p-Cresol
4	20.703	3-Methyl-phenol	4	20.198	3-Methyl-phenol
5	20.782	Methoxyphene	5	20.666	2-Methoxy-phenol
6	20.782	2-Methoxy-phenol	6	20.666	Methoxyphene
7	21.730	2,6-Dimethyl-phenol	7	21.536	2,6-Dimethyl-phenol
8	22.050	Methoxycresol	8	22.284	2,6-Dimethyl-1,4-benzenediol
9	23.439	2-Ethyl-phenol	9	23.319	2-Ethyl-phenol
10	23.973	2,3-Dimethyl-phenol	10	23.755	2,3-Dimethyl-phenol
11	23.973	3,5-Dimethyl-phenol	11	23.755	2,4-Dimethyl-phenol
12	23.973	2,4-Dimethyl-phenol	12	23.755	3,5-Dimethyl-phenol
13	24.931	3-Ethyl-phenol	13	25.104	2-Methoxy-6-methylphenol
14	24.931	4-Ethyl-phenol	14	25.104	2-Methoxy-3-methyl-phenol
15	25.248	2-Methoxy-6-methylphenol	15	25.104	4-Methoxy-3-methyl-phenol
16	25.248	2-Methoxy-3-methyl-phenol	16	25.472	2-Methoxy-5-methylphenol
17	25.248	4-Methoxy-3-methyl-phenol	17	25.472	Cresol
18	26.456	2,4,5-Trimethyl-phenol	18	25.665	2,5-Dimethyl-1,4-benzenediol
19	26.456	2,4,7-Trimethyl-phenol	19	26.297	2,4,6-Trimethyl-phenol
20	27.021	m-Tert-butyl-phenol	20	27.597	2-Ethyl-4-methyl-phenol
21	27.021	p-Tert-butyl-phenol	21	27.597	3-Ethyl-5-methyl-phenol

Table 2. Analysis of volatile flavor substances from liquid smoke and egg yolk of salted duck egg supplemented with LS by GC-MS (continued)

	LS			Salted duck egg yolk supplemented with 2.5% (v/v) LS	
	Retention/ min	Compound name		Retention/ min	Compound name
22	27.392	2-Propyl-phenol	22	29.844	4-Ethyl-2-methoxy-phenol
23	27.695	2-Ethyl-4-methyl-phenol	23	33.778	2-Methoxy-4-propyl-phenol
24	27.695	2-Ethyl-6-methyl-phenol			
25	27.695	3-Ethyl-5-methyl-phenol			
26	28.200	2-(1-Methylethyl)-phenol			
27	28.200	3-(1-Methylethyl)-phenol			
28	28.460	3,4-Diethylphenol			
29	28.460	3-Methyl-5-(1-methylethyl)-methylcarbamatephenol			
30	29.503	2,3,5-Trimethyl-phenol			
31	29.747	2,4,6-Trimethyl-phenol			
32	30.090	4-Ethyl-2-methoxy-phenol			
33	30.541	3-Methyl-4-isopropylphenol			
34	30.541	Thymol			
35	30.750	2-Methyl-5-(1-methylethyl)phenol			
36	31.170	2,3,4,6-Tetramethyl-phenol			
37	31.170	2-Ethyl-4,5-dimethyl-phenol			
38	31.356	2,3,5-Trimethyl-1,4-benzenediol			
39	31.356	3-Isopropylthiophenol			
40	31.489	2-Methyl-5-(1-methylethyl)phenol			
41	32.130	2-(1-Methylpropyl)-phenol			
42	32.200	P-Tert-butylcatechol			
43	33.149	2,6-Dimethoxy-phenol			
44	33.392	3-Allyl-6-methoxyphenol			
45	33.392	Eugenol			
46	33.552	4-(1,1-Dimethylethyl)-thiophenol			
47	33.685	2,3,5,6-Tetramethyl-phenol			
48	33.883	2-Methoxy-4-propyl-phenol			
49	37.328	2-Ethyl-5-n-propylphenol			
50	39.635	2,5-Bis(1,1-dimethylethyl)phenol			
51	39.635	2,4-Bis(1,1-dimethylethyl)phenol			
52	39.710	4,6-Dimethoxy-phthalide			
53	43.170	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol			

Table 2. Analysis of volatile flavor substances from liquid smoke and egg yolk of salted duck egg supplemented with LS by GC-MS (continued)

	LS		Salted duck egg yolk supplemented with 2.5% (v/v) LS	
	Retention/min	Compound name	Retention/min	Compound name
Others			Others	
1	25.561	Dimethylphenylphosphine	nd	nd
2	32.584	M-Phenylhydrazide		

LS, liquid smoke; GC-MS, gas chromatography-mass spectrometry.

Figs. 4 and 5 show the PCA analysis and radar chart of the volatile flavor E-nose in fresh duck eggs, the control group, and the eggs treated with LS (2.5% and 5.0%, v/v), respectively. In the PCA principal component analysis graph, the abscissa represents the first principal component and the ordinate represents the second principal component, both of which show that salted duck eggs differ significantly from fresh duck eggs and those treated with LS. The radar chart demonstrates that fresh duck eggs had considerably lower relative values of T40/2, P30/2, P40/2, P30/1, PA/2, T70/2, P40/1, P10/2, and P10/1 than a sample of treated eggs. Salted eggs supplemented with LS (2.5% and 5.0%, v/v) had significantly lower PA/2, T70/2, P40/1, P10/2, P10/1, and T30/1 values than the control samples. E-nose sensor intensity for volatile compounds of these samples is shown in Supplementary Fig. S2.

Effects of liquid smoke (LS) on the texture of salted duck eggs

Salted egg quality is correlated with the product texture. Table 3 summarizes the texture of salted eggs enriched with LS (2.5% and 5.0%, v/v) and the control group after 28 days of curing. Significant changes in hardness may be seen between
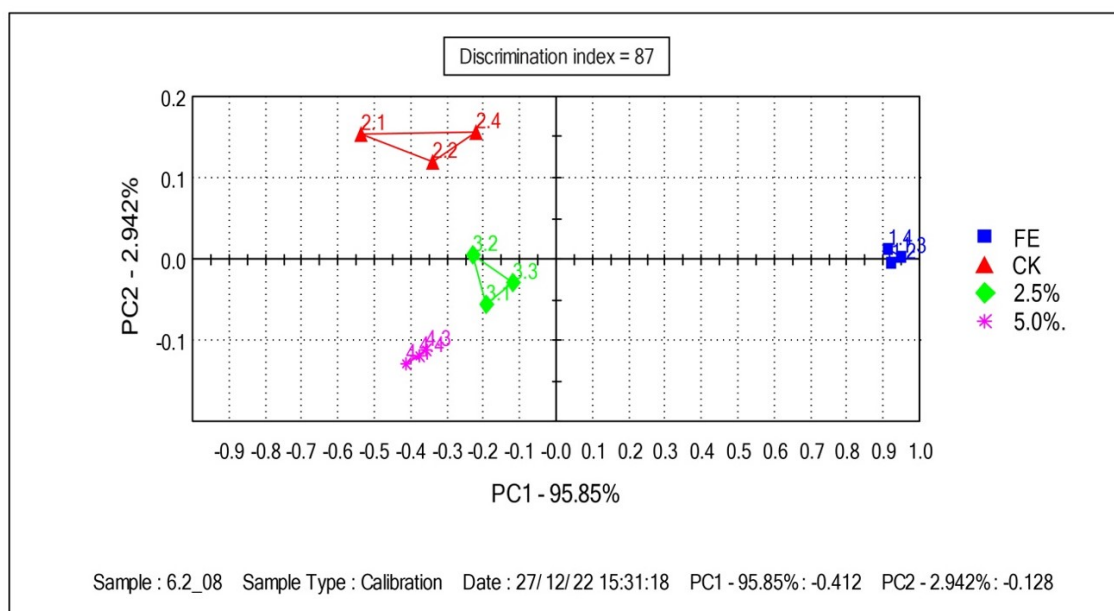
Fig. 4. PCA analysis for egg yolk of duck egg. FE, fresh duck eggs; CK, salted duck eggs of the control group after 28 days of curing; 2.5%, salted duck eggs with 2.5% (v/v) liquid smoke added after 28 days of curing; 5.0%, salted duck eggs after 28 days of curing salted duck eggs with 5.0% (v/v) liquid smoke.
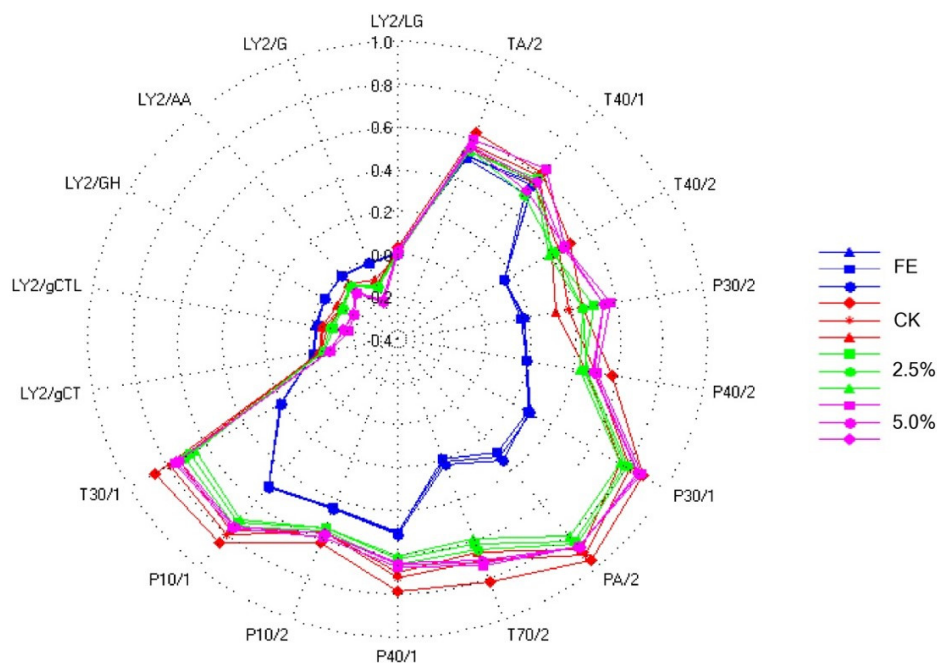
Fig. 5. The electronic-nose radar profile of egg yolk of duck egg. FE, fresh duck eggs; CK, salted duck eggs of the control group after 28 days of curing; 2.5%, salted duck eggs with 2.5% (v/v) liquid smoke added after 28 days of curing; 5.0%, salted duck eggs after 28 days of curing salted duck eggs with 5.0% (v/v) liquid smoke.

Table 3. Texture profile analysis of egg yolk of salted duck egg

Variable	Control	2.5%	5.0%
Hardness (g)	3,342.97±154.82[b]	3,659.51±99.87[a]	1,874.17±111.10[c]
Springiness (mm)	0.338±0.043[a]	0.269±0.027[a]	0.240±0.081[a]
Cohesiveness	0.411±0.012[a]	0.363±0.021[b]	0.251±0.009[c]
Gumminess (N)	1,375.32±84.60[a]	1,328.52±90.58[a]	469.19±34.62[b]
Chewiness	462.53±40.05[a]	359.14±57.50[b]	114.27±46.10[c]
Resilience	0.180±0.011[a]	0.172±0.008[a]	0.109±0.006[b]

[a-c] Different lowercase letters indicate significant differences between salted duck eggs with different treatments ($p<0.05$).

control samples and salted egg yolks treated with various concentrations of LS ($p<0.05$). The results showed that the structure of the salted duck egg yolk became more solidified in the eggs treated with LS, but the hardness of the egg yolk was reduced if the concentration of the LS was too high. Kaewmanee et al. (2009a) and Kaewmanee et al. (2009b) have reported that the hardness of salted duck egg yolk is associated with an increase in the hardening rate. The hardening rate of the yolk is defined as the ratio of the hard outer yolk to the weight and is used as an indicator of the completion of the pickling. The more dehydrated the marinated egg yolks are, the rougher the texture will be.

Between the control group and the LS-treated salted yolk, there was no noticeable change in terms of elasticity ($p>0.05$). The three groups of salted duck eggs varied significantly ($p<0.05$) in terms of cohesion and chewiness. In addition, the control and 2.5% (v/v) LS-treated eggs were significantly different from the 5.0% (v/v) LS-treated egg for gumminess and resilience ($p<0.05$), the gumminess and resilience of eggs control and 2.5% (v/v) LS were higher than those of egg treated with 5.0% (v/v) LS. It is possible that the excessively high concentration of LS causes the protein in the egg yolk to denature

and alter its structure, making it flakier and lowering its viscosity and recovery. The picture of control and treated of salted duck eggs after 28 days of salting can be seen in Fig. 6.

Discussion

In recent years, the use of LS as a flavor enhancer and preservative in food products has increased. Salted duck eggs, in particular, are a popular traditional food product in many cultures, and the addition of LS to this product has intrigued the interest of researchers due to its potential effect on the antioxidant activity and lipid oxidation of the eggs. LS contain a variety of compounds with antioxidant properties, including phenols and polyphenols (Montazeri et al., 2013). These compounds may contribute to the overall antioxidant capacity of salted duck eggs, preventing lipid oxidation and maintaining egg quality (Li et al., 2022). LS can improve the antioxidant properties of salted duck eggs by increasing their DPPH scavenging activity and reducing power (Soldera et al., 2008). This is most likely because LS contains phenols and polyphenols, both of which have been shown to have powerful antioxidant properties.

The fact that the hydroxyl group of the phenolic component in LS can provide stable free radicals and reduce fat oxidation caused by the interaction of free radicals and fatty acids may explain its high antioxidant activity. Phenols and their derivatives are most likely the primary class of chemicals in LS that act as antioxidants and free radical scavengers (Montazeri et al., 2013). Polyphenolic substances such as flavonoids and phenols have also been studied and found to have a variety of biological effects, including antioxidant activity (Kumar and Pandey, 2013; Shahidi and Ambigaipalan, 2015; Tungmunnithum et al., 2018).

According to Barclay et al. (1997), the antioxidant properties of phenolics and their derivatives are derived from lignin (4-propylguaiacol, eugenol, isoeugenol, and 4-allyl-2,6-dimethoxyphenol). These substances, according to reports, have greater antioxidant potential than commercially available 2,6-di-tert-butyl-4-methylphenol (BHT). The primary goal of anti-oxidation is to delay oxidation by avoiding and limiting free radical production. Antioxidants can be formed from phenolic compounds and their derivatives. As a result, using LS as a good source of phenolic chemicals can benefit functional foods. Antioxidant properties can also be used to inhibit oxidative food reactions (Albishi et al., 2013).

The addition of LS to salted duck eggs can alter their volatile flavor profile significantly. LS can improve the overall flavor of the eggs while also adding a smoky, woody aroma. This is most likely because the LS contain volatile flavor compounds
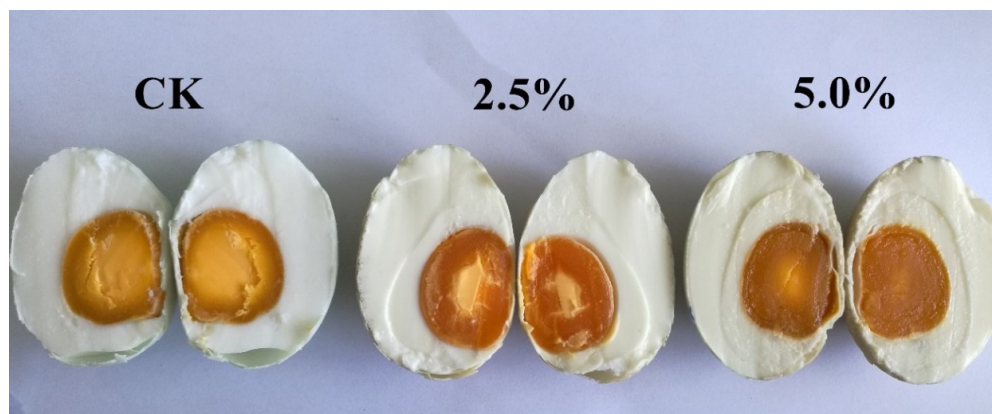
Fig. 6. The picture of salted duck egg control and salted duck egg supplemented with liquid smoke (2.5% and 5.0%, v/v) after 28 days of salting.

such as phenols, aldehydes, and ketones (Guillén and Ibargoitia, 1998; Suñen et al., 2003). It can make the eggs more tender and juicy by improving their texture. Because the presence of volatile flavor compounds in the LS is likely, this can improve the sensory properties of the eggs and increase their overall appeal.

Conclusion

Comprehensive analysis of the experimental findings revealed that the LS did have a significant impact on the antioxidant properties of salted duck eggs, which may have the potential to enhance the antioxidant capacity of these eggs to some extent and alleviate the oxidation of the lipids in the egg yolk. In addition, GC-MS detected several phenolic compounds in both the LS and the salted duck eggs treated with LS groups. These compounds may be the primary factors contributing to the eggs treated with LS having increased antioxidant activity. Additionally, LS can alter the physical characteristics of the salted duck eggs, such as hardness, cohesiveness, chewiness, oil exudation, color, etc., for the egg yolks. Therefore, LS may be beneficial to increase the sensory quality of salted duck eggs.

Supplementary Materials

Supplementary materials are only available online from: https://doi.org/10.5851/kosfa.2023.e10.

Conflicts of Interest

The authors declare no potential conflicts of interest.

Acknowledgements

Author Contributions

Conceptualization: Harlina PW, Ma M. Data curation: Yuliana T, Fetriyuna. Methodology: Harlina PW, Yuliana T, Fetriyuna, Shahzad R. Validation: Harlina PW, Ma M. Investigation: Harlina PW. Writing - original draft: Harlina PW, Ma M. Writing - review & editing: Harlina PW, Yuliana T, Fetriyuna, Shahzad R, Ma M.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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