Effect of clove extract on lipid oxidation, antioxidant activity, volatile compounds and fatty acid composition of salted duck eggs

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Abstract Antioxidant activity, lipid oxidation, fatty acid composition and volatile compounds of duck eggs supplemented with clove extract were monitored over the salting period. The results indicated that application of clove extracts significantly reduced 2-thiobarbituric acid reactive substances and anisidine values as well as the conjugated dienes levels during curing. Clove extracts along with salting time had significant effects on the fatty acid composition. The predominant fatty acid in salted duck eggs supplemented with clove extracts for 14 day of the salting process was oleic acid, followed by palmitic acid and arachidonic acid. Additionally, treated eggs exhibited a higher docosahexaenoic acid content than that of control. The results of SPME GC–MS showed the presence of 46 and 37 volatiles in the treated eggs. Eugenol, as the primary bioactive component of clove, was detected in salted eggs supplemented with clove extracts. In addition, analyzing the results obtained through electronic nose showed that clove extract brought significant changes in salted eggs flavor components.

Keywords Clove extract · Salted duck eggs · Antioxidant · Lipid oxidation · Fatty acid · Volatile compounds

Abbreviation

TBARS	2-thiobarbituric acid reactive substances
DHA	Docosahexaenoic acid
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
PG	Propyl gallate
TBHQ	Tertiary butylhydroquinone
MDA	Malondialdehyde
PCA	Principal component analysis
E-nose	Electronic nose
RSA	Radical scavenging ability
DPPH	1,1-diphenyl-1-picrylhydrazyl
CD	Conjugated diene
p-AV	p-anisidine value
FA	Fatty acid
ARA	Arachidonic acid
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
UFA	Unsaturated fatty acid

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Introduction

Fresh egg is one of the perfect foods that supply best nutrition, although with highly perishable nature. Hence, unique approaches in the processing and preservation methods are needed to maintain quality and to lengthen egg shelf life. Conventionally, salted eggs are made from duck eggs because they attain more desirable characteristics than do hen eggs (Harlina et al. 2012). Preservation of duck eggs by salting could maintain quality and extend the

storage period. During storage, the desirable quality attributes of salted egg product decrease due to lipid oxidation (Harlina et al. 2015), the oxidation process may lead to chemical changes and sensory properties in food, as well as a decrement in nutritional value. In order to delay, decrease or inhibit this process, antioxidants are supplemented with food. Synthetic additives such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary butylhydroquinone (TBHQ) are not without side effects, so their application in food processing has been questioned (Gouda et al. 2017). In order to prolong shelf life and improve food quality and safety, it is of great importance to find additives from natural sources, as introduction of these natural antioxidant spices could hopefully inhibit oxidative deterioration without any side effects (Azizkhani and Tooryan 2015).

Spices are well recognized for their potential antioxidative ability against the oxidation of lipid and protein in various food systems (Ahmad Shah et al. 2014). Clove contains large amounts of phenolic components and antioxidant substances therefore can be potentially used in food products (Zengin and Baysal 2015). Antioxidant capacity of clove extract is principally because of the presence of high content of eugenol. In clove, the major constituent besides eugenol is eugenol acetate and β caryophyllene (Lee and Shibamoto 2001). Clove has been broadly applied in the flavoring, fragrance and cosmetics industries (Baskaran et al. 2010). The clove buds are mainly used in the manufacturing of clove extract and considered safe to be applied to food items during their processing. For example, a study on shelf life of chicken meat reported by Krishnan et al. (2014), demonstrated that supplementation of clove extract could successfully prevent bacterial growth, alleviate lipid oxidation and improve sensory properties of chicken meat during refrigerated storage. Shan et al. (2009) found that clove, oregano, cinnamon, pomegranate and seeds of grape used in pork meat could decrease the total microbial count and substantially delayed lipid oxidation process at a certain temperature until 9 days during storage, and clove extract showed the highest antioxidant activity. Additionally, Halder et al. (2011) successfully investigated that pretreatment with essential oil of clove could diminish the oxidative stress determined by malondialdehyde (MDA) level and decreased glutathione rates in the brain of mice. It was concluded that using clove essential oil could recover the memory loss and learning loss in the brain induced by scopolamine as an effect of the subtraction oxidative stress. Given that adding clove extract enhances various food products, however very little is known about its application to egg products. An attempt to add clove into salted duck eggs, which could possibly enhance the flavor of salted eggs as well as revealing its antioxidant functions and

ultimately increasing the nutritional value, was achieved in this study.

Previously, Harlina et al. (2015) reported that successful incorporation of garlic oil into salted duck egg showed significant effects on the lipid oxidation and microstructural characteristics of salted duck eggs, compared to the control. From those results, we posit that biological properties of clove extract might also influence the properties of egg product. Thus, objective of this study was to primarily investigate the impact of clove extract on lipid oxidation, antioxidant activity, fatty acid composition and volatile compounds of salted duck eggs.

Materials and methods

Materials

Chloroform, methanol, 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), sodium hydroxide (NaOH), sodium chloride (NaCl), ethylenediamine tetraacetic acid disodium salt, ethanol, isooctane, potassium ferricyanide, FeCl_3 , $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, NaH_2PO_4 , were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). P-anisidine was purchased from Aladdin Industrial Corporation (Shanghai, China). DPPH was purchased from Shanghai Yuanye Biological Technology, China. n-Hexane for chromatography was obtained from Tianjin Guangfu Fine Chemical Research Institute, China. Borontrifluoride-methanol ($\text{BF}_3\text{-MeOH}$) was purchased from ANPEL Scientific Instruments (Shanghai), China.

Fresh eggs of duck (*Anas platyrhynchos*) with a weight of 65–75 g were obtained within 3 days after laying, from a local producer (Wuhan, Hubei Province, China).

The clove extract was from dried buds of *Flos Caryophyllata* (*Syzygium aromaticum*) (Xian Runxu Bio-Technology, China), commonly known as public cloves. On the other hand, the extract contains volatile oil $19.0 \pm 1.0\%$, in which the main components are eugenol, acetyl eugenol, heptanone, methyl salicylate, α -valene, pepper phenol, benzyl alcohol, and benzaldehyde. Moreover, the studied clove extract composition is in agreement with Ogata et al. (2000), Lee and Shibamoto (2001) and Cortés-Rojas et al. (2014) who proved that eugenol is the main compound in the clove extract.

Sample preparation

Fresh duck eggs were manually cleaned with water, then the eggs were salted by soaking in a 13% salt solution (salt solution: egg = 1: 1, w/w). Subsequently, the egg samples were treated with 0.1 and 0.5% (w/v) of clove extract. For control samples, salted duck eggs were used without clove

extract. All samples were then stored at room temperature (25 °C) for 28 days. The salting time for TBARS experiment was extended to 49 days. The experiment was conducted in three independent replications, with the analyses performed each week. The methodology used here was the same as used by Harlina et al. (2015) with slight modification.

Antioxidant activity

Egg white and egg yolk were blended with 95% ethanol at a ratio of 1:10 (w/v) and extracted at 60 °C in a water bath with continuous shaking at a speed of 170 r/min for 2 h. The obtained slurry was centrifuged at 10,000×*g* for 10 min (Model 3740, Kubota, Osaka, Japan). The supernatant was used for antioxidant activity analysis. Antioxidant activities including DPPH radical scavenging activity and reducing power assay of salted duck eggs supplemented with clove extract was investigated *in vitro*.

The antioxidant activity was evaluated by free radical scavenging activity (DPPH method). The hydrogen atom or electron donating ability of antioxidant in egg samples was determined from bleaching of purple colored ethanol solution of DPPH. This spectrophotometric assay uses the stable radical DPPH as a reagent. DPPH was prepared at a concentration of 0.002%. Different concentrations of supernatant were taken in each test tube and volumes were made up to 2 mL. Then 2 mL of DPPH solution was added to each test tube and these solutions were kept in dark for 30 min. All the samples were tested in triplicate. Later optical density was recorded at 517 nm using UV–Visible spectrophotometer (NanoDrop 2000C spectrophotometer, Thermo Fisher Scientific, USA). Ethanol mixed with DPPH was used as a control. The method was same as used by Xiao et al. (2014), Cvetković et al. (2015), Savic et al. (2016), and Ilić-Stojanović et al. (2017) with slight modification. The formula used for calculation is:

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

where A = Optical density of control; B = Optical density of sample.

The reducing power assay was evaluated described by Xiao et al. (2014). Briefly, 2.0 mL sample was mixed with 0.2 mol/L sodium phosphate buffer (pH 6.6) 2.0 mL and 1% (w/v) potassium ferricyanide 2.0 mL. The above mixture was incubated at 50 °C for 20 min. After cooling, 10% trichloroacetic acid 2.0 mL was added. After centrifugation at 3000×*g* for 10 min, the supernatant collected 2.0 mL and mixed with distilled water 2.0 mL and 0.1% (w/v) FeCl₃ 0.4 mL. Mixtures were standing at room temperature

for 10 min; the absorbance was recorded at 700 nm. The same volume of distilled water was used as a blank.

Extraction and derivatization of lipid from egg yolk

The lipids of the yolk were extracted according to the method of Bligh and Dyer (1959) with minor modifications. Yolk samples (30 g) were homogenized in a mixture of chloroform: methanol: distilled water (120:120:60, v/v/v) at 11,000×*g* using a homogenizer for 2 min. The homogenizer was treated with ultrasound (20 °C, 80% power, 30 min) (KQ 3200E, China). The mixture was filtered through a Büchner filter funnel. The chloroform phase (bottom phase) was drained off into an Erlenmeyer flask. Anhydrous sodium sulphate (1–2 g) was added, and the mixture was shaken thoroughly to remove residual water. The lipid in chloroform was decanted into a round-bottom flask through a filter paper (Whatman No.1). Before it was evaporated at 55 °C using a rotary evaporator and the residual solvents were removed by flushing with nitrogen. The lipid was stored in an amber vial under nitrogen at – 20 °C until it was analyzed.

Fifty milligrams (50 mg) of lipid residue was weighed on a 10 mL centrifuge tubes, and a portion of 3 mL of NaOH in MeOH (0.1 mol/L) was added, which was vortex-mixed and heated for 30 min at 60 °C. After cooling in water, the mixture was supplemented with 2 mL of BF₃-MeOH, vortex-mixed and heated for 15 min at 60 °C. After rapid cooling with running water, the mixture was supplemented with 2 mL of hexane and 2 mL of NaCl 0.88%, and vortex-mixed for 30 s. Finally, the top hexane phase was collected and directly analyzed by GC. The result of each fatty acid was expressed as a percentage of the total Fatty Acid Methyl Ester (FAME). Three replicates were measured for each sample.

Fatty acid profile

The GC–MS analysis of FAME of egg yolk lipid was described by Wang et al. (2014). The experiment was performed on an Agilent 7890B/5977A/7693 Autosampler Series Gas Chromatograph (Agilent Technologies, Palo Alto, CA) using a DB-WAX capillary column (30 m × 0.25 mm × 0.25 mm film thickness, Hewlett Packard, USA). The experiment was analyzed by split injection at a ratio of 10:1, using ultra-high purity helium as the carrier gas at 1.0 mL/min of flow rate. The injector and detector temperatures were 250 and 260 °C, respectively. The initial temperature was 150 °C for 0 min, raised to 230 °C of 10 °C/min, and was maintained constant at 230 °C for 15 min. The duration of the analysis was 23 min, and the injection volume was 0.2 mL. The mass spectrometer was operated in electron impact mode at

70 eV of an ionization voltage with continuous scanning from m/z 50 to 500. The detection of FAME of egg yolk lipid was analyzed by comparing the mass spectral data to the NIST 11 library database and referencing to the literature. Only components with a similarity of more than 60% (the maximum similarity is 100%) were reported here.

Lipid oxidation of salted egg yolks

Oxidative rancidity of fat was measured by a TBARS assay of MDA as described in our previous study (Harlina et al. 2015).

The conjugated dienoic acid (CDA) of the duck eggs was analyzed as described by Kim et al. (2013). Oil samples (100 mg) were dissolved in isooctane 25 mL for 10 min. The mixtures were diluted with 10-fold isooctane (v/v), and the absorbance at 233 nm was evaluated with a UV–Vis spectrophotometer. Three replicates were measured for each sample. The CDA was calculated using following formula:

$$\text{CDA}(\%) = (0.84 \times A_{233} \text{ nm}) \div (bc/K0) \quad (2)$$

Here A_{233} = the absorbance at 233 nm, b = the path length of the cell (cm), c = the concentration (g-1L), $K0$ = the absorptive of the acid groups (0.03).

p -anisidine value (p -AV) of the duck egg oil was analyzed as described by Kim et al. (2013). The oil sample (100 mg) was dissolved in isooctane solution 25 mL, and the absorbance of the mixture was evaluated using a UV–Vis spectrophotometer at 350 nm. The 2.5 mL of the supernatant mixture was mixed with 0.5% w/v p -anisidine in acetic acid 0.5 mL, after 10 min the absorbance was analyzed at 350 nm absorbance. Three replicates were measured for each sample. The p -AV was calculated using following formula:

$$p - AV = 25 \times (1.2 \times A_2 - A_1) \div W \quad (3)$$

Here, A_1 = the absorbance at 350 nm before the addition of p -anisidine reagent, A_2 = the absorbance at 350 nm after the addition of p -anisidine reagent, W = the sample weight (g).

GC–MS analysis of salted egg yolks volatile flavor compounds

The volatile flavor compounds analysis of salted duck egg yolk supplemented with clove extract and control was analyzed by HS SPME GC–MS, on an Agilent 7890B/5977A/7693 autosampler Series Gas Chromatograph (Agilent Technologies, Palo Alto, CA) using a DB-WAX capillary column (30 m $\times$ 0.25 mm i.d $\times$ 0.25 mm film thickness, Agilent Technologies, Palo Alto, CA). The volatiles fiber was using a SPME Fiber Assembly (75 μ m

CAR/PDMS Fused Silica 24 Ga, Manual Holder, 3pk; Supelco, Bellefonte, PA). Before analysis, the fiber was inserted into the GC injector, the temperature condition was 250 °C. HS-SPME was analysis by incubating 5 g of fresh egg yolks in a 20 mL glass vial at 50 °C for 120 min. Then the volatiles was directly desorbed in the GC injection port and desorbed at 250 °C for 5 min using the splitless injection at 250 °C of an inlet temperature, and 260 °C of MS detector. The pressure of carrier gas was ultra-high purity helium at 7.07 kPa. The program temperature used started at 40 °C for 2 min, followed by 230 °C at 5 °C/min, and a 10 min hold at 230 °C. Mass spectra were recorded at an ionization voltage of 70 eV in the electron-impact mode and temperature 250 °C, with 30–500 m/z of scan range and 0.69 s of scan time. The qualification of the volatile flavor compounds was analyzed by comparing the mass spectra with NIST 11 mass-spectral library.

Electronic nose analysis of yolk flavors

Three grams of each yolk sample was accurately weighed into a 10 mL headspace vials, sealed and extracted for 30 min at room temperature. Then, the vials were loaded into the autosampler tray and extracted for 10 min at 50 °C using purified air as the carrier gas at a flow rate of 200 mL/min. The injection volume, time, and stirring rate were 2500 mL, 3 s, and 250 $\times$ g, respectively. The duration of acquisition and the delay between injections were 50 and 180 s, respectively. The maximum response points of the E-nose, automatically recorded for each of 18 sensors, were used for the analysis (Wang et al. 2014). Three replicates were measured for each sample.

The flavor of yolks was analyzed using a FOX-3000 E-nose (Alpha MOS, Toulouse, France), which consisted of a sampling apparatus, an array of sensors, a HS-100 autosampler, air generator equipment and software (Alpha Soft V11) for data recording and analyzing. The sensory array was composed of 18 metal oxide sensors divided into three chambers: LY, T, and P (Wang et al. 2014).

Statistical analysis

Experiments were run in triplicate with two different concentrations of clove extracts (0.1% and 0.5%, w/v) on salted duck eggs. Data were presented as mean values with standard deviations (S.D). One-way analysis of variance (ANOVA) was performed by using SPSS 16.0 software (SPSS, Chicago, IL). Means were compared using Duncan's multiple-range test to determine the least significant difference among means at the significance level of 0.05.

For e-nose results, PCA has been used for comparing between groups.

Results and discussion

The impact of clove extracts supplementation on the egg's radical scavenging ability (RSA)

RSA values of salted duck eggs supplemented with clove extracts were examined and compared against the control samples (Fig. 1a). The results indicated that radical scavenging activity increased with increasing salting time ($p < 0.05$) for all samples. The scavenging value of salted duck eggs supplemented with 0.5% (w/v) clove extract was significantly higher compared to the control samples ($p < 0.05$) at 28 days of salting for egg white and egg yolk samples. The antioxidant function of clove extract is in

agreement with the report by Ogata et al. (2000), Lee and Shibamoto (2001) and Cortes-Rojas et al. (2014) which proved that potential antioxidant ability of clove extracts due to the existence of eugenol. In fact, our present study (Illustrated in Table 3) also identified the presence of eugenol compound in eggs supplemented with cloves. As a chemical component belonging to the phenolic class, eugenol has been found to show antioxidant abilities like other components in the class. The eugenol component has an attractive conjugation of the chain carbon with aromatic particle ring that could engage in the phenoxyl radical element stabilization by resonance process. It degrades two and more DPPH radical molecules, in spite of the presence of only one hydrogen from a hydroxyl group. Therefore, it is suggested that the improvement of the scavenging ability might be a result of the eugenol included in the clove (Fig. 1).

Fig. 1 **a** Radical scavenging ability of egg white and egg yolk of salted duck eggs control and salted duck eggs supplemented with clove extract (0.1 and 0.5%, w/v) over 28 days of salting; **b** Reducing power assay of egg white and egg yolk of salted duck eggs control

and salted duck eggs supplemented with clove extract (0.1 and 0.5%, w/v) over 28 days of salting. Bars represent standard deviation from triplicate determinations. Different letters on the bar indicate significant differences ($p < 0.05$)

The impact of clove extracts supplementation on the egg's reducing power

Reducing power was determined by evaluating the absorbance at 700 nm after mixing the ferric solutions and samples. Figure 1b shows the reducing power of egg white and yolk of salted egg control and those treated with clove extract 0.1 and 0.5% (w/v) during 28 days of curing time, expressed as absorbance at 700 nm. Reducing power was significantly higher in salted eggs supplemented with 0.5% (w/v) clove extract than control samples ($p < 0.05$) on the 28th day of salting for both egg white and egg yolk samples. The reducing power of the salted duck eggs supplemented with clove extracts increased over salting time, however, in a concentration-dependent manner. Our results were similar to a study reported by Sun et al. (2013) on egg white protein hydrolysate.

The impact of clove extracts supplementation on the egg's TBARS values

TBARS, an indicator to assess the extent of lipid oxidation over the processing time, is mainly used to evaluate the creation of the secondary products of oxidation namely malonaldehyde, aldehydes components, and ketones. TBARS levels of three experimental groups are depicted in Fig. 2a. During 49 days of salting, the TBARS level of salted eggs supplemented with 0.5% (w/v) clove extract was significantly lower compared to the control group ($p < 0.05$). The TBARS value of all batches is increased over salting time ($p < 0.05$). This result further verified that added antioxidant spices could reduce the oxidative stability of salted egg. Natural antioxidant spices, such as polyphenol extract of cloves could be applied to delay oxidation during storage (Gouda et al. 2017) because cloves contain eugenol with the potent antioxidant capability (Ogata et al. 2000; Lee and Shibamoto 2001). Eugenol is considered safe as declared by Food and Drug Administration (FDA) (Gulcin 2011), it permits the hydrogen atom donation and stabilizes phenoxyl radicals by producing stable compounds that do not initiate or extend oxidation. Our finding of the positive impact of cloves on the lipid oxidation process is in agreement with other studies. For example, El-Maati et al. (2016); revealed that clove essential oil, at 15 mg/mL concentration, impeded 97% lipid oxidation of linoleic acid. Another study conducted by Yi et al. (2015), reported that clove extract was able to essentially prevent and decrease TBARS value as well as hydroperoxides caused by myoglobin of beef juice and beef. Lipid oxidation formerly develops via the chain of free radical attack. As the radical level increases, it can expedite the level of oxidation if there is no availability of antioxidant compounds (Feng and

Fig. 2 **a** TBARS value of salted duck egg yolks control and salted duck egg yolks supplemented with clove extract (0.1 and 0.5%, w/v) over 49 days of salting; **b** Conjugated dienes value of salted duck egg yolks control and salted duck egg yolks supplemented with clove extract (0.1 and 0.5%, w/v) over 42 days of salting; **c** p-Anisidine value of salted duck egg yolks control and salted duck egg yolks supplemented with clove extract (0.1 and 0.5%, w/v) over 42 days of salting. Bars represent standard deviation from triplicate determinations. Different letters on the bar indicate significant differences ($p < 0.05$)

Ahn 2016). Moreover, it has been found that, there is a highly significant positive correlation between the TBARS value and the radical scavenging assay of egg yolk with

different significations for salted duck egg enriched with clove extract 0.5% (w/v) ($r^2 = 0.91$), salted duck egg enriched with clove extract 0.1% (w/v) ($r^2 = 0.87$). And for salted duck egg control, it did not attend the significant level ($r^2 = 0.50$).

The impact of clove extracts supplementation on the egg's conjugated diene

Figure 2b showed that the rate of conjugated diene (CD) declined as salting time progressed, probably due to a trend of increasing diene over salting time. Clove extract was able to diminish the formation of CD in duck eggs during salting, as both treatments had lower rates of the CD ($p < 0.05$) compared to the control group (Fig. 2b). Similar observations have been reported by Juntachote et al. (2007) on cooked ground pork added with galangal extract and Pena-Ramos and Xiong (2003) on cooked pork patties; they found that the concentration of CD decreased as storage time progressed. The formation of CD has been related to the oxidation process of PUFA (Kim et al. 2013). In general, peroxide was formed at the early stage of oxidation and it had shown absorption at 233 nm which also determines the stages of primary oxidation (Yoon et al. 1991). Also, in this study it has found a highly significant negative correlation between CD and the radical scavenging assay of egg yolk for salted duck egg enriched with clove extract 0.5% (w/v) ($r^2 = -1.00$), and salted duck egg enriched with clove extract 0.1% (w/v) ($r^2 = -0.98$). However, it didn't attend the significant level for salted duck egg control ($r^2 = -0.58$) and fresh duck egg ($r^2 = -0.50$).

The impact of clove extracts supplementation on the egg's *p*-anisidine values

The increment *p*-anisidine value (*p*-AV) in all samples with increasing salting time could be clarified by the less constant products of primary oxidation during salting that further degrade to secondary oxidation products such as aldehydes components and ketones. A lower *p*-AV indicates that less rancid lipid is produced. Adding clove extract to salted duck eggs induced a significant subtraction in the anisidine values, compared to a control sample ($p < 0.05$) (Fig. 2c). The strongest inhibitory impact on the generation of secondary oxidation products was monitored in the natural antioxidant clove extracts. The potency of antioxidants from the plant in lowering *p*-AV have been investigated in numerous reports (CheMan and Jaswir 2000; Nor et al. 2009). In all the studies the *p*-AV increment level coincides in time with the level of the TBARS increase and CD decrease, probably because along with the oxidative process, a significant increment in the generation

products of primary and secondary oxidation is created and this is obtained by TBARS, CD and *p*-AV analysis.

Importantly, in this study it has found a positive significant correlation coefficient between *p*-AV of yolk lipid and the radical scavenging assay of egg yolk, salted duck egg enriched with clove extract 0.5% (w/v) ($r^2 = 1.00$), and salted duck egg enriched with clove extract 0.1% (w/v) ($r^2 = 0.94$). And for salted duck egg control, it did not attend the significant level ($r^2 = 0.64$).

The impact of clove extracts supplementation on the egg's fatty acid composition evaluated by GC-MS

Fatty acids play important features in the metabolic mechanism of living organisms namely sources of energy, structural elements in membrane polar of lipid and pioneers of bioactive particles (Tocher 2003). The fatty acid (FA) composition of salted eggs from control and supplemented with clove extract samples are presented in Table 1. Each FA has expressed as mean percentage value $\pm$ standard deviation, relative to the total fat content. 18 FAs (C12:0, C14:0, C15:0, C16:0, C16:1n-9, C17:0, C17:1, C18:0, C18:1n-9, C18:3n-6, C18:2n-6, C18:1, C20:4n-6, C20:3n-6, C20:2n-6, C20:1, C20:0 and C22:6n-3) were identified and quantified in all samples over the salting period. The predominant FAs for all samples on the 14th day of the salting process were oleic acid (18:1n-9) followed by palmitic acid (16:0) and arachidonic acid (ARA) (C20:4n-6). This result is in agreement with those reported by Liu et al. (2005) and Kaewmanee et al. (2009). Oleic, palmitic, and linoleic acids are the most dominant fatty acids present in both fresh duck egg and salted duck egg yolk. The total monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) contents were significantly higher ($p < 0.05$) in the egg samples supplemented with 0.5% (w/v) clove extract than the control sample on the 28th day of salting.

Palmitic acid was the dominant saturated fatty acid (SFA) in all samples, and the concentration of palmitic acid was significantly higher than the control sample ($p < 0.05$) on the 28th day of salting. MUFAs and PUFAs, oleic acid (18:1n-9) and ARA (C20:4n-6) were the most dominant respectively. And both their concentrations in eggs supplemented with clove extract 0.5% (w/v) were markedly higher than that in other samples ($p < 0.05$) on the 28th day of salting. PUFA to SFA ratio was also sharply increased from 1.12 in the control to 1.24 in 0.1% (w/v) clove extract samples and 1.23 in 0.5% (w/v) clove extract samples ($p < 0.05$). Similar to our findings, Harlina et al. (2015) revealed that ratio of PUFA and SFA in eggs supplemented with garlic oil increased following concentration at 0.1% (v/v) and 0.5% (v/v). A lower proportion of

Table 1 Fatty acid profile (% total fatty acid) of salted egg yolk supplemented with clove extract analyzed by GC–MS

Fatty acids		Salting time (day)	Treatment groups		
RT	Fatty acids compounds (%)		Control	0.10% w/v	0.5% w/v
21.38	C12:0	0	5.37 ± 0.31 ^a	5.37 ± 0.31 ^a	5.37 ± 0.31 ^a
		14	3.19 ± 0.02 ^c	4.32 ± 0.12 ^b	4.37 ± 0.03 ^b
		28	nd	nd	1.69 ± 0.05 ^d
29.77	C14:0	0	52.81 ± 2.64 ^b	52.81 ± 2.64 ^b	52.81 ± 2.64 ^b
		14	44.81 ± 3.65 ^c	58.88 ± 0.49 ^b	65.58 ± 0.6 ^a
		28	6.74 ± 0.37 ^e	4.04 ± 0.17 ^e	16.31 ± 0.72 ^d
33.92	C15:0	0	8.07 ± 0.17 ^c	8.07 ± 0.17 ^c	8.07 ± 0.17 ^c
		14	8.93 ± 0.73 ^{bc}	10.81 ± 0.18 ^a	9.51 ± 0.09 ^b
		28	nd	1.08 ± 0.08 ^e	3.15 ± 0.08 ^d
37.94	C16:1	0	804.89 ± 22.31 ^a	804.89 ± 22.31 ^a	804.89 ± 22.31 ^a
		14	564.76 ± 3.71 ^b	721.22 ± 6.27 ^a	812.15 ± 29.92 ^a
		28	83.93 ± 0.05 ^d	69.28 ± 7.12 ^d	199.13 ± 8.61 ^c
39.02	C16:0	0	3423.6 ± 23.2 ^b	3423.6 ± 23.2 ^b	3423.6 ± 23.2 ^b
		14	4680.72 ± 214.41 ^a	4247.64 ± 73.35 ^a	4593.7 ± 119.75 ^a
		28	825.01 ± 12.71 ^d	546.19 ± 39.39 ^d	1485.85 ± 68.37 ^c
42.48	C17:1	0	27.93 ± 0.1 ^b	27.93 ± 0.1 ^b	27.93 ± 0.1 ^b
		14	24.11 ± 2.26 ^c	35.44 ± 0.79 ^a	29.94 ± 1.4 ^b
		28	nd	nd	10.89 ± 0.86 ^d
43.4	C17:0	0	27.42 ± 1.06 ^c	27.42 ± 1.06 ^c	27.42 ± 1.06 ^c
		14	38.35 ± 3.35 ^a	35.69 ± 0.25 ^{ab}	33.71 ± 0.81 ^b
		28	nd	3.1 ± 0.01 ^e	11.19 ± 0.43 ^d
45.3	C18:3n-6	0	61.59 ± 1.06 ^b	61.59 ± 1.06 ^b	61.59 ± 1.06 ^b
		14	65.52 ± 1.46 ^b	87.56 ± 0.18 ^a	95.35 ± 0.87 ^a
		28	6.98 ± 0.43 ^d	11.24 ± 0.81 ^{cd}	18.92 ± 0.37 ^c
45.93	C18:2n-6	0	2947.96 ± 26.31 ^a	2947.96 ± 26.31 ^a	2947.96 ± 26.31 ^a
		14	28.18 ± 0.63 ^e	34.69 ± 0.18 ^c	43.48 ± 0.39 ^e
		28	411.63 ± 2.64 ^c	286.1 ± 23.95 ^d	973.43 ± 45.13 ^b
46.18	C18:1	0	nd	nd	nd
		14	1979 ± 44.34 ^{ab}	1907.98 ± 14.11 ^b	1910.77 ± 51.42 ^b
		28	949.47 ± 799.25 ^c	666.73 ± 78.91 ^c	2422.1 ± 61.16 ^a
46.453	C18:1,trans-9	0	9770.99 ± 39.72 ^{ab}	9770.99 ± 39.72 ^{ab}	9770.99 ± 39.72 ^{ab}
		14	10146.95 ± 343.51 ^{ab}	9153.16 ± 88.78 ^b	10911.85 ± 621.86 ^a
		28	90.86 ± 8.83 ^c	64.09 ± 5.91 ^c	176.67 ± 8.95 ^c
47.186	C18:0	0	949.58 ± 4.48 ^d	949.58 ± 4.48 ^d	949.58 ± 4.48 ^d
		14	1364.01 ± 68.9 ^a	1103.49 ± 20.02 ^c	1252.6 ± 40.79 ^b
		28	183.95 ± 24.69 ^f	109.5 ± 6.08 ^g	307.48 ± 14.2 ^e
51.451	C20:4n-6	0	1186.27 ± 8.23 ^c	1186.27 ± 8.23 ^c	1186.27 ± 8.23 ^c
		14	3183.98 ± 138.7 ^b	3046.62 ± 56.92 ^b	3929.88 ± 34.61 ^a
		28	700.43 ± 1.84 ^d	435.43 ± 30.45 ^e	1057.99 ± 41.03 ^c
52.058	C20:3n-6	0	150.59 ± 2.43 ^c	150.59 ± 2.43 ^c	150.59 ± 2.43 ^c
		14	245.72 ± 5.5 ^a	206.35 ± 0.56 ^b	251.6 ± 2.17 ^a
		28	32.69 ± 0.68 ^e	18.4 ± 0.93 ^e	61.48 ± 1.37 ^d
52.675	C20:2n-6	0	67.01 ± 2.8 ^c	67.01 ± 2.8 ^c	67.01 ± 2.8 ^c
		14	134.67 ± 3.01 ^a	110.22 ± 0.4 ^b	143.96 ± 1.24 ^a
		28	nd	4.3 ± 0.22 ^e	24.02 ± 1.78 ^d
52.853	C20:1	0	97.95 ± 2.6 ^b	97.95 ± 2.6 ^b	97.95 ± 2.6 ^b
		14	126.37 ± 5.1 ^a	90.27 ± 0.1 ^c	13.11 ± 0.38 ^e

Table 1 continued

Fatty acids		Salting time (day)	Treatment groups		
RT	Fatty acids compounds (%)		Control	0.10% w/v	0.5% w/v
53.672	C20:0	28	11.02 ± 0 ^f	7.1 ± 0.24 ^{ef}	29.42 ± 0.8 ^d
		0	14.92 ± 0.19 ^a	14.92 ± 0.19 ^a	14.92 ± 0.19 ^a
		14	4.91 ± 0.21 ^c	12.85 ± 0.35 ^b	14.07 ± 0.14 ^a
57.01	C22:6n-3	28	nd	nd	4.63 ± 0.21 ^c
		0	122.5 ± 2.33 ^c	122.5 ± 2.33 ^c	122.5 ± 2.33 ^c
		14	366.78 ± 2.44 ^b	491.28 ± 1.32 ^a	529.76 ± 4.61 ^a
	SFA	28	55.5 ± 0.64 ^e	67.94 ± 0.03 ^{de}	110.83 ± 1.21 ^{cd}
		0	4481.78 ± 54.69 ^c	4481.78 ± 54.69 ^c	4481.78 ± 54.69 ^c
		14	6144.91 ± 12.97 ^a	5473.68 ± 51.99 ^b	5973.52 ± 23.24 ^a
	MUFA	28	1015.7 ± 86.26 ^e	663.92 ± 5.7 ^c	1830.29 ± 26.4 ^d
		0	10701.75 ± 154.71 ^c	10701.75 ± 154.71 ^c	10701.75 ± 154.71 ^c
		14	12841.2 ± 151.76 ^{ab}	11908.06 ± 18.14 ^{bc}	13677.81 ± 375.7 ^a
	PUFA	28	1127.93 ± 158.67 ^e	807.2 ± 28.34 ^e	2779.33 ± 1.82 ^d
		0	4535.93 ± 16.01 ^b	4535.93 ± 16.01 ^b	4535.93 ± 16.01 ^b
		14	4024.85 ± 49.97 ^c	3976.72 ± 32.44 ^c	4994.02 ± 43.89 ^a
	MUFA/SFA	28	1133.86 ± 6.22 ^e	823.41 ± 0.55 ^e	2246.68 ± 6.42 ^d
		0	2.4 ± 0.18 ^a	2.4 ± 0.18 ^a	2.4 ± 0.18 ^a
		14	2.09 ± 0.03 ^a	2.17 ± 0.05 ^a	2.29 ± 0.2 ^a
	PUFA/SFA	28	1.12 ± 0.62 ^b	1.21 ± 0.14 ^b	1.52 ± 0.07 ^b
		0	1.02 ± 0.09 ^b	1.02 ± 0.09 ^b	1.02 ± 0.09 ^b
		14	0.66 ± 0.01 ^d	0.73 ± 0.02 ^{cd}	0.84 ± 0.06 ^c
	UFA/SFA	28	1.12 ± 0.03 ^{ab}	1.24 ± 0.05 ^a	1.23 ± 0.02 ^a
		0	3.42 ± 0.26 ^a	3.42 ± 0.26 ^a	3.42 ± 0.26 ^a
		14	2.75 ± 0.03 ^{bcd}	2.9 ± 0.03 ^{abc}	3.12 ± 0.23 ^{ab}
	Σn:3	28	2.24 ± 0.6 ^d	2.45 ± 0.09 ^{cd}	2.75 ± 0.09 ^{bcd}
		0	122.5 ± 2.33 ^c	122.5 ± 2.33 ^c	122.5 ± 2.33 ^c
		14	366.78 ± 2.44 ^b	491.28 ± 1.32 ^a	529.76 ± 4.61 ^a
	Σn:6	28	55.5 ± 0.64 ^e	67.94 ± 0.03 ^{de}	110.83 ± 1.21 ^{cd}
		0	4413.43 ± 9.44 ^a	4413.43 ± 9.44 ^a	4413.43 ± 9.44 ^a
		14	3658.07 ± 11.57 ^b	3485.44 ± 33.74 ^b	4464.26 ± 39.28 ^a
	n:6/n:3	28	1140.84 ± 6.24 ^d	755.47 ± 0.53 ^e	2135.84 ± 8.96 ^c
		0	36.66 ± 1.56 ^a	36.66 ± 1.56 ^a	36.66 ± 1.56 ^a
		14	10.07 ± 0.34 ^c	7.09 ± 0 ^c	8.43 ± 0 ^c
		28	20.53 ± 0.69 ^b	11.14 ± 0.02 ^c	19.28 ± 0.54 ^b

a,b,c,d,e,f fronts mean ranking in all treatment groups by Duncan's Multiple Range Tests. Values are mean ± SD, n = 3

MUFA monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *SFA* saturated fatty acid, *UFA* unsaturated fatty acid, *nd* not detected
The fatty acid data exhibited significant difference ($p < 0.05$)

SFA is important for healthful food (Ladeira and Santarosa 2014). It could be viewed from Table 1 that the total SFA was considerably lower in the egg yolk supplemented with 0.1% clove extract than control ($p < 0.05$) after 28 days of salting. The ratio of UFA/SFA gradually decreased over salting time and significantly differed from the control group ($p < 0.05$). This was possibly the result of the oxidative cleavage of fatty acids on storage (Harlina et al. 2015). FA composition significantly changed during

salting in the presence of clove extract. This phenomena might be due to presence of large amounts of active phenolic components in clove such as eugenol, eugenol acetate and β caryophyllene (Lee and Shibamoto 2001). These compounds probably contribute to extend intermolecular interactions of the structural matrix on salted eggs.

The ratio value of n-6/n-3 was significantly different in both treatment groups compared with the control group ($p < 0.05$). As shown in Table 1, n-3 and n-6 PUFAs were

decreased for all samples during salting. This result is similar to our previous study (Harlina et al. 2015) which reported that C18:2 n-6 started to decrease slightly during the salting process. The decrease in n-6 PUFA might be mainly ascribed to the decrease in C20:4 n-6 ($p < 0.05$) within the initial 28 days of salting. The human body cannot synthesize DHA. One of the excellent sources for DHA is duck egg yolk. Omega-3 PUFA, particularly DHA had a significantly high level in the eggs supplemented with 0.5% (w/v) clove extract than control samples ($p < 0.05$) after 28 days of salting. Long-chain PUFAs like DHA and eicosapentaenoic acid (EPA) may decrease the potency of heart disease, cancer, inflammation and arthritis (Harper and Jacobson 2005). Thus, the consumption of eggs contains n3, might help to achieve the recommended diet from long chain PUFAs for health gains as confirmed through the Australia and New Zealand Nutrient Reference Values (Khan et al. 2017).

The impact of clove extract supplementation on the egg's volatile flavor compound detected by GC–MS

Lipid oxidation is one of the main principal reactions of processed food products that generate volatile compounds and improve flavor development. Table 2 listed data of GC peaks monitored for fresh duck egg and salted egg control samples with the qualifications of each compound in the sample. 25 volatile compounds were qualified and were categorized into 9 groups, they were 3 esters, 3 alkenes, 8 nitrogenous compounds, 1 alcohol, 2 ethers, 5 organic acids, 1 aldehyde and 2 others for fresh duck egg and 1 esters, 5 acids, 4 alcohols, 4 alkanes, 2 ketones, 3 aldehydes, 2 nitrogenous compounds, 3 ethers and other for salted egg control samples, respectively. Among them, seven volatile compounds (n-hexadecanoic acid, 2-methylaminomethyl-1,3-dioxolane, 3-azabicyclo [3.2.2] nonane, 1,4,7,10,13,16 hexaoxacyclooctadecane, nonanal, octaethylene glycol monododecyl eter, 15-crown-5) were identified both in fresh egg and salted egg control.

The volatile constituents of salted duck eggs flavored with clove extracts at 0.1 and 0.5% (w/v) at 28 days of salting were identified by SPME GC–MS analysis (Table 3). 46 and 37 volatiles were identified respectively and they were categorized into 9 groups namely 8 esters, 12 organic acids, 5 alcohols, 2 alkanes, 3 ketones, 7 aldehydes, 4 nitrogenous compounds, 2 ethers and 2 others and 2 esters, 8 organic acids, 5 alcohols, 4 alkenes, 6 ketones, 4 aldehydes, 4 nitrogenous compounds, and 3 ethers. Fourteen volatile compounds (acetic acid, propanedioic acid, propyl, eugenol, n-hexadecanoic acid, ethanol, cyclohexanol, 2,6-dimethyl, 2-methylaminomethyl-1,3-dioxolane, 1,4, 7,10,13,16,19-hepta-2-cycloheptacosanone, hexanal,

nonanal, 2-octenal, (E), benzaldehyde, 15-crown-5) were identified in both treatment groups. The GC–MS total ion chromatogram of volatile compounds in salted duck egg supplemented with clove extract are given in Supplementary Fig. 1.

Eugenol, an organic acid, was found in duck eggs supplemented with clove extracts. According to Ogata et al. (2000), it was the main bioactive compound of clove and it exhibited potent antioxidant abilities. Huang and Frankel (1997) also reported that eugenol played a prominent role in antioxidative ability. Powerful antioxidant abilities of eugenol might be due to its belonging to the phenolic groups (Lee and Shibamoto 2001). The particle of eugenol acquires an attractive combination of the chain carbon aromatic ring by resonance which may contribute to the phenoxyl radical stabilization (Cortes-Rojas et al. 2014).

Lipid oxidation process is also established to be connected with alcohol formation (Qin et al. 2012). Alcohols and acids arise from the reduction and oxidation of their corresponding aldehydes. Five alcohols were identified in salted duck eggs supplemented with clove extracts. Moreover, acetic acid, assumed to be generated as by-product from degradation process of long chain FA, was found in duck eggs containing clove extract. In addition, hexanoic acid, enzymatically produced using PUFA through lipoxygenase pathway (Wei et al. 2013), was found in salted duck egg supplemented with clove extract. Numerous aldehydes were also found in egg samples. Hexanal, heptanal, and nonanal are the products of linoleic acid, n-6, and n-9 PUFA oxidation, respectively (Xu et al. 2014). Alkanes were qualified in this report, which provides slight contributions to food flavor systems. In addition, in duck eggs supplemented with clove extracts several alkenes and ketones compounds were found as well as four nitrogenous compounds and some ethers (Table 3).

The impact of clove extract supplementation on the egg's flavor properties detected by electronic nose

Principal component analysis of volatile compounds in salted duck eggs supplemented with clove extracts on the 28th day of salting was presented in Fig. 3a. The contribution rates of the first and second PCs were 93.55% and 3.73%, respectively. The total contribution rate was 97.28% (over 85%), suggesting that the two PCs contain important data information of flavor components. The higher rate of the contribution, the better the PCA data which show the various indigenous index of data collection. According to Liu et al. (2012), the total contribution rate of more than 85% suggested the methods probability.

The PCA of volatile flavor compounds of salted duck eggs supplemented with 0.5% (w/v) clove extract located

Table 2 Compounds identified in the fresh duck egg and salted duck egg yolk control at 28 days of salting

Compounds classes	Groups	Peak No	RT (min)	Code	Compounds names
Ester	Fresh egg	1	17.87	A1	Succinic acid, decyl tetrahydrofur furyl ester
		2	22.71	A2	L-Alanine, N-valeryl-, tetradecyl ester
Acid	Fresh egg	1	7.95	B1	Styrene
		2	22.25	B2	2-Thiophenecarboxylic acid, 5-(1,1-dimethylethoxy)-
		3	25.12	B3	Propanedioic acid, propyl-
		4	36.94	B4	Octadec-9-enoic acid
		5	44.51	B5	n-Hexadecanoic acid
	Control	1	15.97	B1	Acetic acid
		2	21.48	B2	4-(Methylamino) butyric acid
		3	33.83	B3	Pterin-6-carboxylic acid
		4	36.93	B4	6-Octadecenoic acid
		5	44.50	B5	n-Hexadecanoic acid
Alcohol	Control	1	3.99	C1	Ethanol
		2	7.14	C2	1-Propanol, 2-methyl-
		3	10.10	C3	1-Butanol, 3-methyl-
		4	19.75	C4	3,4-Dimethylcyclohexanol
Alkane	Fresh egg	1	25.63	D1	3-Azabicyclo[3.2.2]nonane
		2	29.48	D2	2-Methylaminomethyl-1,3-dioxolane
		3	40.92	D3	1,4,7,10,13,16-Hexaoxacyclooctadecane
	Control	1	30.79	D1	2-Methylaminomethyl-1,3-dioxolane
		2	31.00	D2	3-Azabicyclo[3.2.2]nonane
		3	35.19	D3	1,3-Dioxolane, 4-methyl-
		4	36.48	D4	1,4,7,10,13,16-Hexaoxacyclooctadecane
Ketone	Fresh egg	1	nd	E1	nd
	Control	1	11.71	E1	Acetoin
		2	14.51	E2	2-Nonanone
Aldehyde	fresh egg	1	13.65	F1	Nonanal
	Control	1	9.10	F1	Butanal, 3-methyl-
		2	14.63	F2	Nonanal
		3	17.77	F3	Benzaldehyde
Nitrogen	Fresh egg	1	16.85	G1	Cyanoacetylurea
		2	20.39	G2	Methylpent-4-enylamine
		3	23.12	G3	Butanamine, 2,2-dinitro-N-methyl
		4	24.38	G4	Propanamide
		5	27.36	G5	Ethylamine, 2-(adamantan-1-yl)-1-m-ethyl
		6	28.98	G6	N-Formyl-dl-alanine
		7	31.00	G7	p-Hydroxyamphetamine
		8	31.80	G8	Ethyl oxamate
	Control	1	20.22	G1	N-Acetyl norephedrine
		2	35.33	G2	Dimethylamine
Ether	Fresh egg	1	41.17	H1	Octaethylene glycol monododecyl eter
		2	43.82	H2	15-Crown-5
	Control	1	38.33	H1	Octaethylene glycol monododecyl eter
		2	41.58	H2	15-Crown-5
		3	44.01	H3	3,6,9,12-Tetraoxatetradecan-1-ol
Others	fresh egg	1	8.70	I1	Methallyl cyanide
		2	15.49	I2	1-Butene, 4-isothiocyanato-
	Control	1	22.85	I1	Naphthalene

nd not detected

Table 3 Compounds identified in the salted duck egg yolk supplemented with clove extracts 0.1 and 0.5% (w/v) at 28 days of salting

Compound class	Groups	Peak no	RT (min)	Code	Compounds names
Ester	Clove extract 0.1% (w/v)	1	6.76	A1	Ethyl oxamate
		2	27.95	A2	Fumaric acid, dodecyl 3-methylbut-3-enyl ester
		3	28.37	A3	Methyl tetradecanoate
		4	30.54	A4	Pentadecanoic acid, methyl ester
		5	32.51	A5	Pentadecanoic acid, 14-methyl-, methyl ester
		6	32.97	A6	9-Hexadecenoic acid, methyl ester,(Z)-
		7	36.47	A7	9-Octadecenoic acid (Z)-, methyl ester
		8	37.24	A8	9,12-Octadecadienoic acid (Z,Z)-,methyl ester
	Clove extract 0.5% (w/v)	1	15.91	A1	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate
		2	23.62	A2	Methyl salicylate
Acid	Clove extract 0.1% (w/v)	1	6.95	B1	DL-Cystine
		2	15.99	B2	Acetic acid
		3	20.96	B3	Propanedioic acid, propyl-
		4	24.37	B4	Hexanoic acid
		5	24.72	B5	D-Mannoheptulose
		6	27.00	B6	Heptanoic acid
		7	29.32	B7	Octanoic acid
		8	31.49	B8	Eugenol
		9	34.10	B9	Phenol, 2,6-bis(1,1-dimethylethyl)
		10	36.94	B10	Oleic Acid
		11	39.45	B11	Imidodicarbonic acid, diethyl este
		12	44.50	B12	n-Hexadecanoic acid
	Clove extract 0.5% (w/v)	1	16.00	B1	Acetic acid
		2	25.15	B2	Propanedioic acid, propyl-
		3	28.40	B3	Phenol
		4	31.49	B4	Eugenol
		5	34.67	B5	Phenol, 2-methoxy-4-(1-propenyl)-,(Z)-
		6	36.92	B6	Octadec-9-enoic acid
Acid	Clove extract 0.5% (w/v)	7	37.40	B7	6-Octadecenoic acid, (Z)-
		8	44.51	B8	n-Hexadecanoic acid
Alcohol	Clove extract 0.1% (w/v)	1	3.99	C1	Ethanol
		2	11.84	C2	Ethanol, 2-(vinylloxy)-
		3	19.74	C3	Cyclohexanol, 2,6-dimethyl-
		4	27.59	C4	Cyclohexanol, 1-ethynyl-
		5	37.74	C5	Ethanol, 2-bromo-
	Clove extract 0.5% (w/v)	1	4.09	C1	Ethanol
		2	10.08	C2	1-Butanol, 3-methyl-, formate
		3	18.50	C3	1,6-Octadien-3-ol, 3,7-dimethyl-
		4	19.74	C4	Cyclohexanol, 2,6-dimethyl-
		5	41.73	C5	Ethanol, 2-[2-(2-ethoxyethoxy)ethoxyl
Alkane	Clove extract 0.1% (w/v)	1	7.66	D1	2-Methylaminomethyl-1,3-dioxolane
		2	42.80	D2	1,4,7,10,13,16-Hexaoxacyclooctadecane
	Clove extract 0.5% (w/v)	1	18.91	D1	2-Methylaminomethyl-1,3-dioxolane
		2	20.00	D2	Hexadecane
		3	42.23	D3	1,4,7,10,13,16-Hexaoxacyclooctadecane
		4	16.13	D4	Octadecane, 1-iodo-

Table 3 continued

Compound class	Groups	Peak no	RT (min)	Code	Compounds names
Ketone	Clove extract 0.1% (w/v)	1	25.36	E1	2(3H)-Furanone, 5-butylidihydro-
		2	40.92	E2	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
		3	41.81	E3	1,4,7,10,13,16,19-Hepta-2-cyclo heneicosanone
	Clove extract 0.5% (w/v)	1	8.87	E1	2-Heptanone
		2	20.25	E2	Acetaldehyde, dimethylhydrazone
		3	20.81	E3	Acetophenone
Aldehyde	Clove extract 0.1% (w/v)	4	22.31	E4	Hentriacontane
		5	22.81	E5	3-Pentanone, dimethylhydrazone
		6	41.57	E6	1,4,7,10,13,16,19-Hepta-2-cyclo heneicosanone
		1	6.48	F1	Hexanal
		2	8.91	F2	Butanal, 3-methyl-
		3	14.53	F3	Nonanal
Aldehyde	Clove extract 0.1% (w/v)	4	17.75	F4	Benzaldehyde
		5	19.49	F5	2-Octenal, (E)-
		6	20.52	F6	2,4-Decadienal, (E,E)-
	Clove extract 0.5% (w/v)	7	37.09	F7	3,5-di-tert-Butyl-4-hydroxybenzaldehyde
		1	6.44	F1	Hexanal
		2	14.45	F2	Nonanal
Nitrogen	Clove extract 0.1% (w/v)	3	15.41	F3	2-Octenal, (E)-
		4	17.74	F4	Benzaldehyde
	Clove extract 0.5% (w/v)	1	3.38	G1	N-Dimethylaminomethyl-N-methylformamide
		2	4.67	G2	3-Amino-6-nitro-4-phenyl-1H-quinolin-2-one
		3	7.54	G3	Benzeneethanamine, 2,5-difluoro-.beta.,3,4-trihydroxy-N-methyl-
	Clove extract 0.5% (w/v)	4	7.74	G4	4-Fluorohistamine
Ether	Clove extract 0.1% (w/v)	1	6.79	G1	Butanoic acid, 3-amino-
		2	6.97	G2	Metaraminol
		3	17.23	G3	3,3-Dimethyl-4-methylamino-butan-2-one-
	Clove extract 0.5% (w/v)	1	38.91	H1	15-Crown-5
		2	45.87	H2	Octaethylene glycol monododecyl eter
		1	41.33	H1	3,6,9,12-Tetraoxatetradecan-1-ol
Others	CLOVE extract 0.1% (W/V)	2	43.10	H2	15-Crown-5
		3	43.30	H3	12-Crown-4
	Clove extract 0.5% (w/v)	1	26.87	I1	2-(1-Hydroxycycloheptyl)-furan
		2	34.00	I2	(2E,4E)-3,7-Dimethyl-2,4-octadiene
		1	nd	I1	nd

nd not detected

in the left area of our figure, while the control group in the other areas, which indicated the differences in the volatile flavor of the control sample and those supplemented with clove extracts. The above results revealed that PCA from the e-nose data information was able to differentiate between the salted egg control and salted eggs enhanced with clove extract at 28 days of salting.

Typical curves generated by E-nose for volatile compounds are shown in Fig. 3b. Constant transforming signals over salting period were observed; representing eighteen different types of sensors. Salted duck eggs fortified with

cloves were found to be significantly different than fresh and control duck eggs, which indicated the obvious transformation of volatile flavor compounds in duck egg during salting time. The discrepancies found were due to the establishment of the unique volatile flavor components of distinct concentrations in each of the tested samples (Cui et al. 2015).

As can be seen from Table 4, the high positive significant correlation coefficients were obtained between E-nose and fatty acid (MUFA) ($r = 0.80$, $p < 0.01$). The significant correlation coefficients were also observed between

Fig. 3 **a** PCA of E-nose data of salted duck eggs supplemented with clove extract on the 28th day of salting. 0.1-Fresh egg, 4.1-Salted egg control, 4.2-salted egg supplemented with 0.1% clove extract, w/v, 4.3- salted egg supplemented with 0.5% clove extract, w/v. **b** E-nose

sensor intensity for volatile compounds of: **(a)** Fresh duck egg, **(b)** control group, **(c)** supplementation of clove extract 0.1%, w/v, **(d)** supplementation of clove extract 0.5%, w/v

PUFA and lipid oxidation (CD) ($r = 0.75$, $p < 0.05$) (Table 4). According to Kim et al. (2013), which reported that the formation of CD has been related to the oxidation process of PUFA. Inverse significant correlation coefficients were also found between PUFA and antioxidant activity (DPPH) ($r = -0.77$, $p < 0.01$). Lipids are responsive to oxidation process as it contain high amounts of PUFAs. Oxidation of lipid is the main deteriorating problem in food and poses a huge challenge to the food industry and food scientists. Lowered nutritional value, changed texture, and color are the results from oxidation of

lipid. Supplementation of antioxidants in food lipids is one way to inhibit rancidity, delay the development of toxic oxides, protecting nutritional level, and improve the shelf life of food (Shahid et al. 2008). And inverse significant correlation coefficients were also observed between E-Nose and TBARS ($r = -0.80$, $p < 0.01$). In flavor analysis, aldehyde compound such as hexanal can be pointed as an evaluator for oxidation and is associated with TBARS level (Muguerza et al. 2003). Aldehydes were qualified as the main compounds in cooked egg in numerous earlier research (Wang et al. 2014). In the

Table 4 Correlation coefficients between the parameters: E-Nose, fatty acids, antioxidant activity, and lipid oxidation

Parameters	E-nose	SFA	MUFA	PUFA	Omega 3	Omega 6	DPPH	TBARS	CD	P-AV
E-Nose	1.00	0.75*	0.80**	0.74*	0.62*	0.73*	− 0.55	− 0.80**	0.63*	− 0.88**
SFA	0.75*	1.00	0.98***	0.99***	0.83**	0.99***	− 0.82**	− 0.91***	0.80**	− 0.95***
MUFA	0.80**	0.98***	1.00	0.98***	0.76**	0.98***	− 0.84**	− 0.94***	0.84**	− 0.97***
PUFA	0.74*	0.99***	0.98***	1.00	0.84**	0.99***	− 0.77**	− 0.95***	0.75*	− 0.95***
Omega 3	0.62*	0.83**	0.76**	0.84**	1.00	0.82**	− 0.36	− 0.78**	0.33	− 0.80**
Omega 6	0.73*	0.99***	0.98***	0.99***	0.82**	1.00	− 0.79**	− 0.95***	0.77**	− 0.95***
DPPH	− 0.55	− 0.82**	− 0.84**	− 0.77**	− 0.36	− 0.79**	1.00	0.68*	− 0.99***	0.74*
TBARS	− 0.80**	− 0.91***	− 0.94***	− 0.95***	− 0.78**	− 0.95***	0.68*	1.00	− 0.69*	0.96***
CD	0.63*	0.80**	0.84**	0.75*	0.33	0.77**	− 0.99***	− 0.69*	1.00	− 0.75*
P-AV	− 0.88**	− 0.95***	− 0.97***	− 0.95***	− 0.80**	− 0.95***	0.74*	0.96***	− 0.75*	1.00

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

current study, a number of aldehydes were detected. Noticeably, the aldehydes were established from the Strecker degradation process of amino acids or decomposition of oxidative materials of UFAs during processing (Wang et al. 2014).

Conclusion

In this study, clove extract supplementation has demonstrated various benefits such as preventing the lipid oxidation in egg and improving the unique flavor of the egg product. Enhancement of salted duck egg products with extra health promoting agents is one of the aims of egg industry, which could be successfully achieved by clove extract supplementation without quality deterioration. Supplementation of clove extract can be used as a potential antioxidant agent and a healthy natural food additive, as exemplified by this study. Moreover, this study also demonstrate that salted duck egg preservation in terms of shelf life with extra health benefits incorporated by supplementation of clove extract ultimately improves the food quality. In terms of providing flavor alternatives to consumers as well as avoiding potential health risks thereby adding natural antioxidant agents during salting process, this study provides a good example.

However, clove extracts at only two concentrations namely 0.1 and 0.5% (w/v) were included in the present study, and it remains unknown whether the application of the clove extract at higher or lower concentration might take similar effect because of the possible alteration in the quality of egg products. Moreover, the isolation, characterization, and purification of major phenolic components from clove extract are recommended.

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Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest.

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