Research Article

THE EFFECT OF SUPPLEMENTATION GARLIC OIL AS AN ANTIBACTERIAL ACTIVITY AND SALTING TIME ON THE CHARACTERISTICS OF SALTED EGG

P. W. Harlina, Ma Mei Hu, A. M. Legowo, Y. B. Pramono

ABSTRACT: This study has been done to examine the influences of garlic oil, salting time in the characteristics of salted egg. Typical bacterial used in this study were *Escherichia coli, Salmonella enteritidis,* and *Staphylococcus aureus*. They were obtained from the culture collection of National R&D Center for Egg Processing Laboratory, Food Science and Technology College, Huazhong Agricultural University, Wuhan, China. The experimental design was completely randomized design with factorial factor A x B (3 x 5) for three replications. Factor A were supplementation of garlic oil with different concentration (0%; 0.1%; 0.5%) and factor B were different time for salting process (0; 7; 14; 21; 28 days). The research was done through several activities, the determination of garlic oil as an antibacterial activity and making salted eggs in the presence of garlic oil are performed. The results showed that garlic oil had antibacterial activity on the three bacteria used in this study. The minimum inhibitory concentration (MIC) of garlic oil was used as a determination of the concentration in salted egg presence of garlic oil. Egg white and yolk of duck eggs with different salting time and concentration of garlic oil showed slight differences in chemical composition and textural properties as salting proceeded. Both of treatment could induce solidification of yolk accompanied by oil exudation and the development of gritty texture. Thus both of treatment somehow affected the characteristics of the resulting egg white and egg yolk.

Keywords : garlic oil, salted egg, salting time

INTRODUCTION

The salted duck egg is one of the most popular preserved egg products in China and Indonesia. The principle of making salted eggs is salting. It can be made by brining eggs in saturated saline or by coating the egg with a soil paste mixed with salt for 1–4 weeks (Peh *et al.,* 1982; Lin *et al.,* 1984; Chang and Lin 1986). In addition to being eaten as the whole egg, salted egg yolk is widely used as fillings in foods such as moon cakes and glutinous rice dumplings (Chiang and Chung 1986; Chi and Tseng 1998).

Conventionally, salted eggs are made from duck eggs because they attain more desirable characteristics than do hen eggs (Li and Hsieh, 2004). Duck egg has a higher fat content than chicken eggs. According to Nutrition Directorate of the Ministry of Health Republic of Indonesia (1979) duck egg yolk has fat content is 35%, while the fat content of chicken egg yolk there was 31.9%.

During salting, the yolk gradually becomes solidified and hardened. On the other hand, egg white loses viscosity and becomes watery (Chi and Tseng 1998). Chi and Tseng (1998) also reported that salting caused moisture removal from egg yolk and the diffusion of salt into egg white and egg yolk. All changes occurring during the salting most likely determine the preferential characteristics of salted egg, both raw and cooked forms. The customer anticipates greater value in egg yolk than in egg white. The desirable characteristics of salted egg yolk include orange color, oil exudation and gritty texture. Generally, the dehydration and salt content are the major factors affecting the hardness of salted yolk (Kaewmanee *et al.,* 2009). Granulation in salted egg yolk is related to salt and the interaction with low-density lipovitellenin (Wang 1991). Wang (1991, 1992) also indicated that the formation of salted yolk might be related to the diffusion speed and final concentration of NaCl. From the stand point of customers, cooked salted egg yolks with a granular texture are generally considered to be desirable (Chiang and Chung 1986; Peh *et al.,* 1982; Wang 1992).

Rate of salt penetration into egg white and yolk governed by salting methods may have the influence on the changes in composition as well as characteristic of cooked salted egg white and yolk. Additionally, salting time more likely governs the changes in composition as well as the properties of eggs, especially after cooking.

Spices such as garlic, onion, cinnamon, cloves, thyme, and sage have been investigated for their antimicrobial activity. Garlic as an antibacterial property is widely used for a number of infectious diseases. Garlic oil, extracted from garlic bulbs using steam distillation, is composed primarily of diallyl disulfide (60%), diallyl trisulfide (20%), allyl propyl disulfide (16%), a small quantity of disulfide and probably diallyl polysulfide (Warade and Shinde, 1998) named allicin. Pure allicin is a volatile molecule that is poorly miscible in aqueous solutions and which has the typical odor of freshly crushed garlic (Block, 1985). Han, Lawson, Han, and Han (1995) reported that the

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antibiotic activity of 1 mg allicin has been equated to that of 15 IU of penicillin.

Garlic volatile oil content, *allicin*, is able to work as an antioxidant, and antibacterial (Velisek *et al.,* 1997; Sun and Ku, 2006; Aruoma *et al.,* 1997; Adler and Beuchat, 2002). Essential oils and their components are known to be active against a wide variety of microorganisms, including Gram-negative (Helander *et al.,* 1998; Sivropoulou *et al.,* 1996) and Gram-positive bacteria (Kim *et al.,* 1995).

Usage of garlic that can serve an antibacterial agent against some types of bacteria isolated from salted egg should be investigated. This needs to be done to determine the effectiveness of garlic as antibacterial agent, so hopefully the garlic oil may be used as a natural preservative in salted egg storage before consumption not only as a preservative but also to enhance the flavour of salted egg. Therefore, the aims of this study were to examine the influences of garlic oil, salting and storage time in the microbial, chemical, and physical characteristics of salted egg in the presence of garlic oil. The benefit of this research was to provide information about the effect of garlic oil on antibacterial activity and the characteristics of salted egg.

MATERIALS AND METHODS

This research has been implemented on June 2011 at the Laboratory of National R&D Center for Egg Processing, Food Science and Technology College, Huazhong Agricultural University, Wuhan, China.

Materials

The materials used in making salted eggs were: fresh duck eggs, garlic oil (Anhui Capa, Co., Ltd), and salt. And the chemicals were: nutrient agar and nutrient broth were obtained from Qingdao Hope Bio-Technology Co.Ltd, China. N-hexane, Iso-propanol, Sodium chloride, ethanol 99%, Alcohol, K_2CrO_4, $AgNO_3$, D-(+)-Glucose, and Phenol red were obtained from Sinopharm chemical reagent Co. Ltd (Shanghai, China).

Methods

The cultures of bacteria

Typical bacterial used in this study were *Escherichia coli, Salmonella enteritidis*, and *Staphylococcus aureus*. They were obtained from the culture collection of National R&D Center for Egg Processing Laboratory, Food Science and Technology College, Huazhong Agricultural University, Wuhan, China. The bacterial cultures were grown on nutrient agar slants and kept at 4°C. Sub culturing was carried out every month to maintain bacterial viability.

Determination of antibacterial activity

The bacterial cultures were prepared approximately 10^5 CFU/mL of tested bacteria. The tubes were prepared medium 2 mL which is contain 2.7 g nutrient broth, 3.1 mg phenol red and 1.5 g D–(+)-Glucose in 150 mL of distillated water. Garlic oil was initially diluted into 10000 ppm concentration using ethanol and then took 6 mL dissolved into 9 mL 0.85% of sodium chloride solution and then become final concentrations of 4000 ppm; 2000 ppm; 1000 ppm; 500 ppm; 250 ppm; 125 ppm; and 62.5 ppm. In each tube, 2 mL of diluted garlic oil, 2 mL of nutrient broth and 0.1 mL of a 10-12 hours bacterial culture 105 CFU/mL were added. The tubes were then incubated at 37°C for 48 hours and viable bacteria were observed.

Inhibitory activity of garlic oil in nutrient broth

The experiments were carried out with 50 mL of nutrient broth in a 250 mL flask. Garlic oil was initially diluted with ethanol into 20% v/v concentration. In each flask, 0.5 mL of dilute garlic oil, 49 mL of nutrient broth and 0.5 mL of a 10-12 hours grown bacterial culture were added. Growth media in flasks were incubated in an incubator shaker at 125 rpm 37°C for 10-12 hours and 0.5 mL of culture was withdrawn as periodical samplings. The samples were serially diluted in sterile distillated water and 0.1 mL of each dilution was spread on the Nutrient Agar. The plates were then incubated at 37°C for 24 hours and every 4 hours viable bacteria were counted.

Making salted egg in the presence of garlic oil with a soaking process

Fresh eggs of duck (*Anas platyrhucus*), less than 3 days after laying, having average weights of 65–75 g, were obtained from a local producer in Wuchang, Wuhan, Hubei Province, China. Duck eggs retrieved and cleaned with sand paper until the stool was missing. Duck eggs were salted by soaking with the salt solution 13% (salt solution: egg = 1:1 w/w). Garlic oil which has been prepared is inserted into the saturated salt solution at adjusted per each concentration. The prepared eggs were stored at room temperature for 28 days, followed by its cleaning and boiling.

Experimental Design

Experiments were run in triplicate with three different treatments of garlic oil on salt egg. Completely randomized design with factorial factor in three repeat was used throughout the study. Data were presented as mean values with standard deviations. One-way analysis of variance was carried out and means comparison were done by Duncan's multiple range tests (Steel and Torrie 1980). Statistical analyses were performed with the statistical program (SPSS for windows, SPSS Inc, Chicago, IL, USA).

Proximate Analysis and Determination of Salt Content

Egg yolk and egg white were analysed for water content and total solid (AOAC, 2000). Salt content in egg samples was measured by the method of (AOAC, 2000). Samples were treated with 50 ml hot water 70°C. The mixture was stirred for 15 minutes. Distillated water to the 250 mL of volumentary flasks was added. Take 25 mL into the flask using by a pipette and 25 mL distillated water was added. One mL of K_2CrO_4 was added. The mixture was titrated with the standardized $AgNO_3$ until the solution became permanently light red, as following formula:

$$X_G = \left(\frac{T \times C_{AgNO_3} \times M_{NaCl}}{W_{sampel}} \right) \times 100 \quad \%$$

Where T is volume titer of $AgNO_3$; M_{NaCl} is molecular weight of NaCl = 58,4 (g/gmol, kg/kgmol); W sample is weight of sample; C_{AgNO3} is volume $AgNO_3$ for titration (ml); N_{AgNO3} is normality of $AgNO_3$ for titration (gmol/ml).

Determination Oil Exudation of Egg Yolk

Oil exudation of egg yolk was measured according to the method of Lai *et al.*, (1999) in Kaewmanee *et al.,* (2009) with a slight modification. Yolk (5 g) is homogenized with 35 mL of n-hexane/2-propanol (3:2 v/v) at 5000 rpm for 10 minute, using a homogenizer. The filtrate obtained through Whatman No. 1 filter paper is evaporated at 55°C in a water bath and then dried at 105°C to constant weight. The residue is weighed and taken as total lipid content. To determine the oil exudation, yolk (5 g) was mixed with 25 mL of distillated water and homogenate at 5000 rpm for 30 seconds. The homogenate is centrifuged at 9500 g for 30 min at 25°C and 25 mL of n-hexane/2-propanol (3:2 v/v) are added to the supernatant to dissolve the float. The solvent lipid layer obtained is separated using a separating funnel. The solvent in the solvent-lipid layer is evaporated in a water bath and heated at 105°C until a constant weight was obtained. The residue is weighed and taken as free lipid. Oil exudation was defined as the proportion of free to total lipid content.

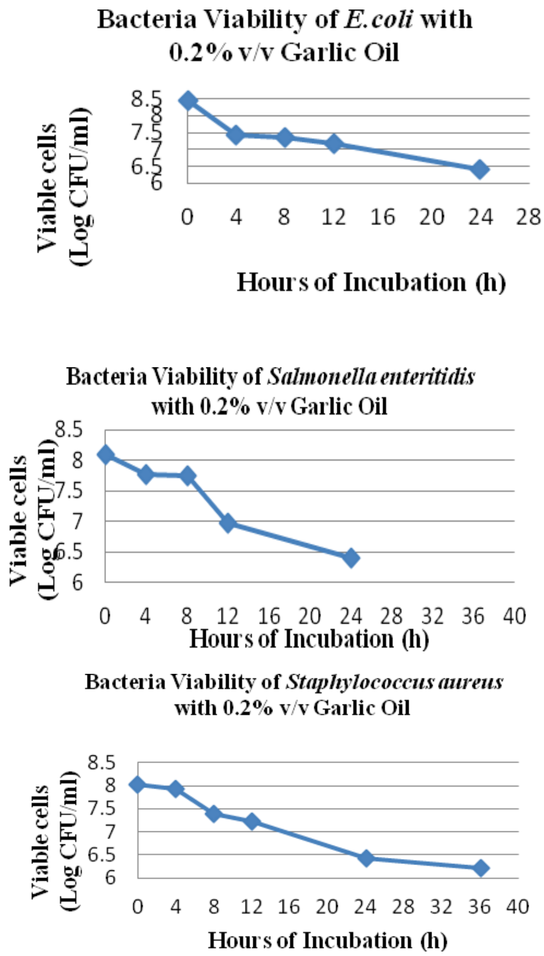
Texture Profile of Egg Yolk

Texture profile was carried out with Stable Micro System TA.XT texture analyzers plus from England. Egg yolks were cut into 2 parts. The samples were compressed twice to 50% of their original height with a compression cylindrical aluminium probe (50 mm diameter). Textural analyses were performed at room temperature. Force distance deformation curves was recorded at cross head speed of 5 mm/s and the recording speed was 5 mm/s. Textural variables obtained from force and area measurements were: hardness (g), springiness (elasticity) (mm), cohesiveness, gumminess (N), and chewiness (g.mm) (Kaewmanee *et al.,* 2009).

RESULTS AND DISSCUSSION

Inhibitory activity of garlic oil in the nutrient broth

The inhibitory effect of garlic oil against the three selected bacteria is shown in Fig. 1. Garlic oil at approximately 0.2% v/v was able to reduce the growth of all bacteria tested. The longtern inhibitory effect was observed on Staphylococcus aureus followed by Escherichia coli and then Salmonella enteritidis. The reduction of Staphylococcus aureus growth was 8.02 log cycles decreases up to 6.20 log cycles after 36 h incubation, which is Gram-Positive bacteria. The reduction of E. coli and Salmonella enteritidis growth was 8.44 and 8.10 log cycles decreases up to 6.40 and 6.39 log cycles after 24 h incubation, which are Gram Negative bacteria. It confirmed that the presence of 0.2% v/v garlic oil can inhibited growth on all bacteria tested and was dependent on the Gram character of the microorganisms and the time of incubation.



(a)

(b)

(c)

Fig. 1. Bacterial activity of (a) *Escherichia coli*, (b) *Salmonella enteritidis*, and (c) *Staphylococcus aureus* with 0.2% v/v garlic oil.

Water content and total solids of salted egg in the presence of garlic oil

Generally, Gram-positive are more sensitive than Gram-negative bacteria to the antimicrobial compounds in spices (Pranoto et al., 2005). Gram-positive bacteria tend to be more sensitive to the antibacterial component. Because the structure of Gram positive bacterial cell more simple and easier for antibacterial agents to enter the cell and found the target for work, and while the structure of Gram negative bacterial cell is more complex and layered three, namely the outer layer of lipoproteins, the middle layer of peptidoglycan and lipopolysaccharide (Pelczar and Chan, 1986 in Kusmiyati and Agustini, 2007). The fact that cysteine abolishes aliicin's antibacterial potential through a binding reaction between the SH group of cysteine with the S-allyl moiety (S-CH2-CH= CH2) of allicin suggest how it is that allicin attacks. Staphylococcus aureus more effectively than *E. coli*. The former, Gram positive, bacterium lacks protein in its cell wall, whereas the latter Gram negative one has about 9% protein in its wall (Greenstein, 1939 in Fujisawa et al., 2009). In this case, the protein prevents to some extent entry into the cell of the S-allyl moiety cleaved from allicin, and hence the protein-rich *E.coli* is expected to be less sensitive to allicin than the peptodeglycan-rich *Staphylococcus aureus* (Fujisawa et al., 2009). The main antimicrobial effect of allicin is due to the interaction with important thiol-containing enzymes. Allicin reacts very rapidly with free thiol groups, via thiol-disulphide exchange and, therefore, it is thought that its main mechanism of antimicrobial action is through interaction with thiol containing enzymes, including cysteine protease and alcohol dehydrogenase (Ankri et al., 1997; Rabinkov et al., 1998 in Bakri and Douglas, 2004). Because these enzymes tend to be essential for bacterial nutrition and metabolism it has been suggested that development of resistance to allicin arises 1000-fold less easily than it does to certain antibiotics (Gupta et al., 1995 in Bakri and Douglas, 2004). Allicin also specifically inhibits other bacterial enzymes such as the acetyl-CoA-forming system, consisting of acetate kinase and phosphotransacetyl-CoA synthetase (Focke et al., 1990 in Ankri and Mirelman, 1999).

As seen in Fig 2, at 0 days up to 28 days of salting, no difference in water content was found in egg white for control samples ($p>0.05$). Incorporation of garlic oil affected the water content of egg white on salt egg. The water content value tended to decrease as higher amounts of garlic oil were incorporated. After 7 days of salting, the decreased water content of egg white was found for all samples. For egg yolk at 0 days up to 28 days of salting, no differences in water content were found for all samples ($p>0.05$).

At 0 days up to 28 days of salting, there was difference in total solids was found in egg white for control and supplementation 0.1% v/v of garlic oil samples ($p<0.05$) (Fig. 3). During salting, the increased total solid of egg white and egg yolk were found among all samples.

During brining, the yolk gradually becomes solidified, whereas the albumen loses viscosity and becomes watery (Peh et al., 1982; Chiang and Chung, 1986; Lai et al., 1999). Solidified yolk obtained during salting might impede the migration of NaCl (Kaewmanee et al., 2011). During salting, water could migrate from egg yolk to egg white, then to the environment through the egg shell, as governed by pore sizes and structure of the shell (Chi and Tseng, 1998; Kaewmanee et al., 2009). Garlic oil has hydrophobic property. In this system, garlic oil might contribute to extend intermolecular interactions of the structural matrix on salt egg. Its probably has change of water content and total solid on salt egg.

Salt concentration of salted egg in the presence of garlic oil

As seen in Fig 4, at 0 days up to 28 days of salting, there were differences in salt concentration were found in egg white among all samples ($p<0.05$). For egg yolk at 0 days up to 28 days of salting, there was difference in salt concentration was found for control samples ($p<0.05$). The supplementation of garlic oil had no significant effect ($p>0.05$) on salt concentration was found in egg yolk. During salting, the increased salt concentration of egg white and egg yolk was found among all samples.

During the brining, NaCl migrated from saline through egg shell into albumen and then yolk. At the same time, dehydration also occurred in albumen and yolk. Both effects of NaCl diffusion and dehydration would cause hardening yolk (Chi and Tseng, 1998; Kaewmanee et al., 2009; Lai et al., 1999). High salt content in egg white might induce water migration from egg yolk. The difference in salt content was caused by the differences in osmotic pressure between both salting methods. After yolks become solidified, the migration of NaCl could be lowered. Furthermore, high lipid content in yolk might impede the migration of NaCl into the yolk.

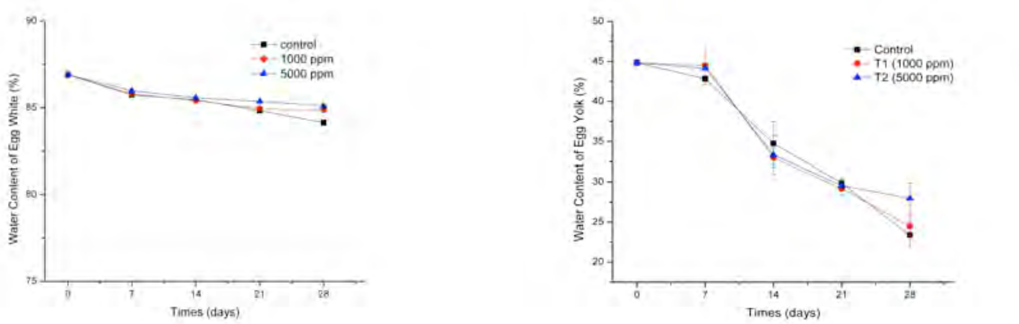
Garlic oil has hydrophobic property. In this system, garlic oil might contribute to extend intermolecular interactions of the structural matrix on salt egg. Its probably has change of salt concentration on egg white. Salt content in salted egg can be varied with salting processes. Salt content affects the acceptance of consumers. Generally, eggs salted for 7 days are recommended for pan frying owing to the less salty taste. Eggs salted for 14 days or more are ready for boiling and commonly consumed, especially with rice gruel, for breakfast (Kaewmanee et al., 2009).

Oil exudation by yolk of salted egg in the presence of garlic oil

Oil exudation increased with increasing salting time ($p<0.05$), but for control samples no differences in oil exudation by yolk were found ($p>0.05$) (Fig. 5). The supplementation 0.1% v/v and 0.5% v/v of garlic oil there had differences in oil exudation on salt egg were found ($p<0.05$). A significant difference ($p<0.05$) supplementation 0.5% v/v garlic oil with salting time. This may be due to the use of different commercial garlic products and the preparation methods of garlic oil (Yalcin et al., 2006). Garlic oil is an essential oil product extracted from garlic bulbs by using steam distillation. The compounds of garlic oil mainly are diallyl disulfide (60%), diallyl trisulfide (20%), allyl propyl disulfide (16%), a small quantity of disulfide and probably diallyl polysulfide (Warade and Shinde, 1998 in Pranoto et al., 2005).

Texture profile analysis of yolk of salted egg in the presence of garlic oil

Texture profile analyses of yolk of salt egg are shown in Table 1. The hardness of salted egg yolk decreased with increasing salting time. This result suggested that the structure of cooked yolk of salted egg became softer. Because when cooked of salt egg the oil would come out and due to structure of yolk become softer with increasing salting time. During salting, the solidification of egg yolk was initiated near the vitelline membrane and preceded toward the centre, the exterior formation. The interior yolk was still in liquid form, but became more viscous with further dehydration of exterior salted yolk. When yolk protein became more concentrated, interaction between protein molecules, including lipoproteins, could occur. This resulted in the formation of a gel like network.



a b

Fig 2. Water Content of (a) Egg White and (b) Egg Yolk during Salting Time.

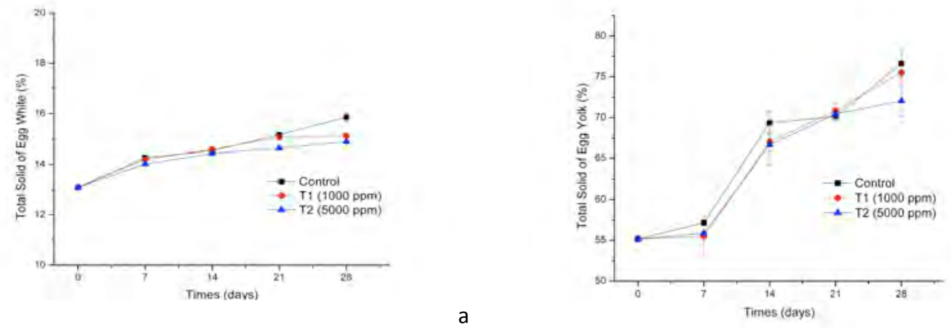


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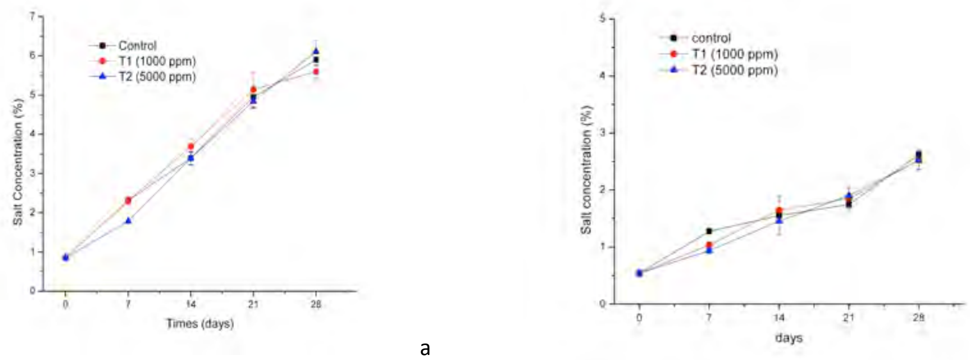
Fig 3. Total Solid of (a) Egg White and (b) Egg Yolk during Salting Time.



a b

Fig 4. Salt Concentration of (a) Egg White and (b) Egg Yolk during Salting Time.

The presence of native yolk lipids or emulsified oil droplet appears to influence the yolk gel rheological properties. Yolk protein concentrates, having relatively high lipid content, produce gel networks at low protein concentrations, suggesting that the yolk lipid molecules are somehow involved in gel structure formation (Kiosseoglou, 2003; Kaewmanee *et al.,* 2009). Salted egg yolk was more dehydrated and could form a gritty texture. Grittiness is the major factor affecting consumer acceptance of salted egg product (Chi and Tseng, 1998). Gumminess, springiness, cohesiveness, chewiness and resilience slightly decreased when the salting time increased (Kaewmanee *et al.,* 2009).

For springiness, presenting how well a product physically springs back after it has been deformed during the first compression, the decrease in cooked salted egg yolk was obtained after 21 days of salting (p<0.05). The lowest springiness was found in cooked salted egg yolk at 28 days of salting (p<0.05). The values indicate that the yolk is losing its elasticity during salting (Manju *et al.,* 2007) because springiness is the elastic or recovering property of samples during compression.

Resilience is defined as the energy accumulated that allows the sample to recover its original shape after deformation. Slight decreases in resilience were obtained in cooked salted egg yolk for all samples ($p<0.05$). For hardness of cooked salted egg yolk decreased with increasing salting time and reached the maximum at 28 days ($p<0.05$), respectively. However, had no differences in hardness was observed in supplementation 0.5% v/v of garlic oil samples ($p>0.05$).

Table 1. Texture Profile Analysis of Salted Yolk Egg in the Presence of Garlic Oil

Parameter/Group		Days				
		0 days	7 days	14 days	21 days	28 days
Hardness (g)	1	483.42±0.41^{c}	482.29±1.28^{c}	456.15±19.16^{c}	406.94±0.68^{a}	397.31±2.45^{b}
	2	483.42±0.41^{c}	478.04±10.32^{c}	456.92±2.35^{c}	376.21±21.40^{b}	246.49±78.11^{c}
	3	483.42±0.41^{c}	482.96±0.0025^{c}	451.83±7.78^{c}	325.37±5.35^{c}	266.50±10.76^{c}
Springiness (mm)	1	0.815±0.003^{b}	0.803±0.001^{b}	0.73±0.053^{a}	0.60±0.00057^{b}	0.53±0.006^{b}
	2	0.815±0.003^{b}	0.808±0.0005^{a}	0.72±0.0187^{b}	0.71±0.0675^{a}	0.53±0.0644^{b}
	3	0.815±0.003^{b}	0.806±0.0026^{a}	0.65±0.0341^{b}	0.56±0.0156^{b}	0.53±0.0395^{b}
Chewiness	1	330.16±2.03^{c}	312.145±0.83^{c}	248.67±22.95^{b}	155.38±4.12^{b}	117.55±7.71^{b}
	2	330.16±2.03^{c}	322.35±7.93^{b}	237.27±6.50^{b}	171.55±6.20^{a}	70.61±29.44^{c}
	3	330.16±2.03^{c}	312.19±1.34^{c}	191.97±10.19^{c}	109.39±7.11^{c}	78.51±9.89^{c}
Cohesiveness	1	0.838±0.0035^{c}	0.806±0.001^{b}	0.75±0.0260^{b}	0.63±0.0163^{b}	0.56±0.0286^{c}
	2	0.838±0.0035^{c}	0.834±0.002^{a}	0.72±0.0158^{b}	0.64±0.0091^{b}	0.52±0.0092^{c}
	3	0.838±0.0035^{c}	0.802±0.001^{c}	0.65±0.036^{c}	0.60±0.0205^{c}	0.55±0.023^{c}
Gumminess(N)	1	405.10±1.71^{b}	388.73±1.52^{b}	340.08±10.93^{a}	258.81±6.90^{a}	222.14±12.55^{a}
	2	405.10±1.71^{b}	398.70±9.57^{b}	331.12±7.39^{a}	241.61±16.26^{a}	130.17±42.79^{b}
	3	405.10±1.71^{b}	387.34±0.48^{b}	296.27±17.34^{b}	195.51±9.64^{b}	146.35±9.47^{b}
Resilience	1	0.465±0.002^{c}	0.446±0.007^{b}	0.38±0.030^{a}	0.28±0.019^{b}	0.22±0.017^{b}
	2	0.465±0.002^{c}	0.4015±0.0005^{c}	0.32±0.031^{b}	0.31±0.028^{b}	0.16±0.016^{c}
	3	0.465±0.002^{c}	0.394±0.001^{c}	0.26±0.011^{c}	0.25±0.08^{c}	0.23±0.030^{b}

Note: The Analyzed data showed significant difference at ($P<0.05$). $^{a,\ b}$ fronts mean ranking in all treatment groups by Duncan's Multiple Range Tests. All values are mean ± SD of data from 3 independent experiments. Group 1 : control samples; group 2 : supplementation 0.1% v/v of garlic oil; group 3 : supplementation 0.5% v/v of garlic oil.

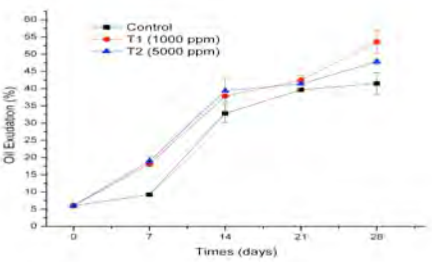


Fig 5. Oil Exudation of Egg Yolk during Salting Time

Cohesiveness slightly decreased as salting time increased. Decreases in cohesiveness were obtained after 28 days of salting ($p< 0.05$). Cohesiveness is the ratio of work done during the second compression divided by the work done during the first compression (Manju *et al.,* 2007). In general, cohesiveness indicates how well the product with stands a second deformation and is relative to how it behaves under the first deformation. The supplementation 0.5% v/v of garlic oil had no differences in cohesiveness were found in egg yolk ($p>0.05$). Decrease in gumminess were found in cooked salted egg yolk up to 14 days to 28 days of salting for control samples and supplementation 0.1% v/v of garlic oil samples ($p<0.05$) and there has no differences for supplementation 0.5% v/v of garlic oil samples ($p>0.05$). Chewiness refers to the work done (Manju *et al.,* 2007). For the chewiness, the continuous decreases were found during the salting of 28 days for all the samples ($p<0.05$), However, no differences in chewiness was observed in supplementation 0.5% v/v of garlic oil samples ($p>0.05$). Decrease in chewiness indicates that the yolk becomes soft during salting.

CONCLUSION

The results showed that garlic oil had antibacterial activity on the three bacteria used in this study. The minimum inhibitory concentration of garlic oil was used as a determination of the concentration in salted egg presence of garlic oil. Egg white and yolk of duck eggs with different salting time and concentration of garlic oil showed slight differences in chemical composition and textural properties as salting proceeded. Both of treatment could induce solidification of yolk accompanied by oil exudation and the development of gritty texture. Thus both of treatment somehow affected the characteristics of the resulting egg white and egg yolk.

This study clearly suggested that Garlic oil has a high antioxidant and anticancer also (Velisek *et al.,* 1997; Sun and Ku, 2006; Aruoma *et al.,* 1997; Adler and Beuchat, 2002). Therefore further work should be performed on the antioxidant activity and anticancer component of garlic oil in egg products.

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REFERENCES

AOAC. 2002. Official Methods of Analysis. Standard Methods for the Examination (17th ed.). Washington, DC: Association of Official Analytical Chemists. APHA.

Adler, B. Band., L. R. Beuchat. 2002. Death of *Salmonella*, *Escherichia coli* O157: H 7, and *Lesteria monocytogenes* in Garlic Butter as Affected by Storage Temperature. J. of Food Protect. 65 (12):1976-1980.

Aruoma, O. I., J. P. ESpencer., D. Warren., P. Jenner., J. Butlerand., and B. Halliwell. 1997. Characteristization of Food Antioxidants, Illustrated using Commercial Garlic and Ginger Preparation. Food Chem. 60, 149-156.

Ankri S, Mirelman D. 1999. Antimicrobial Properties of *Allicin* from Garlic. Microbes Infect, 2, 125-129.

Bakri, I. M. and Douglas, C. W. I. 2005. Inhibitory Effect of Garlic Extract on Oral Bacteria. Archives of Oral Biology, 50, 645-651.

Block E. 1985. The Chemistry of Garlic and Onions. Sci Am., 252, 114-9.

Chang, H. S.; Lin, S. M. 1986. Studies on the Manufacturing of Flavored Chicken Eggs. J. Chinese Soc. Anim. Sci., 15, 71-82.

Chiang, B. H., Chung, M. Y. 1986. Salted Egg Yolk Processing Safeasibility Study. Food Sci., 13, 1-9 (in Chinese).

Chi, S. P., and Tseng, K. H. 1998. Physicochemical Properties of Salted Pickled Yolk from Duck and Chicken Eggs. Journal of Food Science, 33, 507–513.

Direktorat Gizi, Departemen Kesehatan Republik Indonesia. 1979. Daftar Komposisi Bahan Makanan. Bharata Karya Aksara, Jakarta.

Feeney, R. E., Weaver, J. M., Jones, J. R., and Rhodes, M. B. 1956. Studies of the Kinetics of Yolk Deterioration in Shell Eggs. Poult. Sci. 35: 1061-1066.

Fujisawa, Hiroyuki, Kazuhiro, Watanabe. Kaoru, Suma. Kana, Origuchi. Hiroshi, Matsufuji., Taiichiro, Seki., and Toyohiko, Ariga. 2009. Antibacterial Potential of Garlic- Derived Allicin and Its Cancellation by Sulfhydryl Compounds. Biosci. Biotechnol. Biochem. 73, 1948-1955.

Han, J., Lawson, L., Han, G., and Han, P. 1995. A spectrophotometric Method for Quantitative Determination of Allicin and Total Garlic Thiosulfinates. Annals of Biochemistry, 225, 157–160.

Helander, I. M., Alokomi, H. L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E. J., et al. 1998. Characterization of the Action of Selected Essential Oil Components on Gram-negative Bacteria. Journal of Agriculture and Food Chemistry, 46, 3590–3595.

Kaewmanee, Thammarat. Soottawat Benjakul. And Wonnop Visessanguan. 2009a. Effect of Salting Processes on Chemical Composition, Textural Properties and Microstructure of Duck Egg. J Sci Food Agric 89, 625–633.

Kaewmanee, Thammarat. Soottawat Benjakul. And Wonnop Visessanguan. 2009b. Changes in Chemical Composition, Physical Properties and Microstructure of Duck Egg as Influenced by Salting. Food Chemistry, 112, 560–569.

Kaewmanee, Thammarat., Soottawat Benjakul., and Wonnop Visessanguan. 2011. Effect of Acetic Acid and Commercial Protease Pretreatment on Salting and Characteristics of Salted Duck Egg. Food Bioprocess Technol. DOI 10.1007/s11947-011-0510-1.

Kaewmanee, Thammarat., Soottawat Benjakul., and Wonnop Visessanguan. 2011. Effect of Salting Processes on Chemical Composition, Textural Properties and Microstructure of Cooked Duck Egg. J. Food Science, 76, 139-147.

Kim, S.-J., and Ustunol, Z. 2001. Sensory Attributes of Whey Protein Isolate and Candelilla Wax Emulsion Edible Films. Journal of Food Science, 66, 909–911.

Kiosseoglou, V. 2003. Egg Yolk Protein Gels and Emulsions. Current Opinion in Colloid and Interface Science, 8, 356–370.

Kusmiyati dan Agustini, Ni Wayan Sri. 2007. Uji Aktivitas Senyawa Antibakteri dari Mikroalga *Porphyridium cruentum*. Biodiversitas, 8, 48-53.

Lai, K. M., Chi, S. P., and Ko, W. C. (1999). Changes in Yolk States of Duck Egg during Long-Term Brining. Journal of Agricultural and Food Chemistry, 4, 733–736.

Li. J., and Hsieh Y. P. 2004. Traditional Chinese Food Technology and Cuisine. Asia Pacific J Clin Nutr., 13, 147–155.

Manju, S., Leema Jose., T. K. Srinivasa Gopal., C. N. Ravishankar., and K. V. Lalitha. 2007. Effects of Sodium Acetate Dip Treatment and Vacuum Packaging on Chemical, Microbiological, Textural and Sensory Changes of Pearlspot (*Etroplus suratensis*) during Chill Storage. Food Chemistry, 102, 27-35.

Mineki, M., and Kobayashi, M. 1997. Microstructure of Yolk from Fresh Eggs by Improved Method. Journal of Food Science, 62, 757–761.

Nychas, G. J. E. 1995. Natural Antimicrobial from Plants. In G. W. Gould (Ed.), New Methods of Food Preservation (pp. 59–89). Glasgow: Blackie Academic Professional.

Peh, H. C., Chang, H. S., and Li, S. L. 1982. Studies on the Manufacturing of Salted Chicken Egg. J. Chinese Soc. Anim. Sci., 11, 45-58.

Pranoto, Y., Vilas M Salokhe, and Sudip K. Rakshit. 2005. Physical and Antibacterial Properties of Alginate-based Edible Film Incorporated with Garlic Oil. Food Science International 38, 267-271.

Sivropoulou, A., Papanikolaou, E., Nikolanou, C., Kokkini, S., Lanaras, T., & Arsenakis, M. 1996. Antimicrobial and Cytotoxic Activities of Origanum Essential Oils.

Journal of Agricultural and Food Chemistry, 44, 1202–1205

Steel, R. D. D., and Torrie, J. H. 1980. Principle and Procedures of Statistic: A biometrical approach. New York: McGraw-Hill.

Sun, X and D. D. Ku. 2006. *Allicin* In Garlic Protects against Coronary Endothelialdys Function and Right Hearthy Pertrophy in Pulmonary Hypertensiverats. Am J Physiol Heart Circ Physiol 29, 2431-2438.

Velisek, J., R. K. J. Davidek. 1997. Chemical Composition and Classification of Culinary and Pharmaceutical Garlic-Based Product. Z Lebensm Unters Forsch A. 204, 161-164.

Wang, C. T. 1991. The Physical–Chemical Properties of Salted Egg Yolk during Granulation or Gelation Process. Journal of the Chinese Society of Animal Science, 29, 406–414.

Wang, C. T. 1992. Manufacture of Salted Hen Yolk from Fresh Hen Yolk. Journal of the Chinese Society of Animal Science, 21, 429–440.

Warade, S. D., and Shinde, K. G. 1998. Garlic. In D. K. Salunke and S. S. Kadam (Eds.), Handbook of Vegetable Science and Technology (pp. 397–413). USA: Marcel Dekker, Inc.

Yalcın, Sakine, EEbruOnba¸ Sılar, Zehra Reisli, and Suzan Yalcın. 2006. Effect of Garlic Powder on the Performance, Egg Traits and Blood Parameters of Laying Hens. J Sci Food Agric., 86, 1336–1339.