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A combination of lemongrass and lemon basil essential oils inhibited bacterial growth and improved the shelf life of chicken fillets

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Abstract

Lemongrass [*Cymbopogon citratus* (DC.) Stapf] and lemon basil (*Ocimum×africanum* Lour.) are spices commonly used in Indonesian culinary and both have been known for demonstrating antimicrobial properties. Hence, the combination of both plants has the potential to be developed further as a natural food preservative. In this study, the constituents of individual lemongrass and lemon basil essential oils were analyzed and their potential use as a natural preservative of chicken fillets was evaluated. The essential oils were obtained from a steam and water distillation process. The chemical constituents of both essential oils were analyzed by the Gas Chromatography-Mass Spectroscopy (GC-MS) technique. Their application as a preservative of chicken fillets was evaluated based on their capacity to inhibit the growth of bacteria on the meat as well as the physical observation of the preserved meat during 9 d of refrigerated storage. The combination of both essential oils at an optimal ratio of 1:1 %v/v was capable of significantly inhibiting the growth of bacteria on the meat during 9-d refrigerated storage as well as extending the shelf life of the meat for up to 6 d. The preservation effects shown by the combination of both essential oils might be due to the presence of the high proportion of oxygenated compounds that have been proven to be the antibacterial active compounds of essential oils, including geranyl acetate, geraniol, citronellal, estragole, and linalool.

Keywords: Essential oil, *Cymbopogon citratus*, *Ocimum×africanum*, Preservation, Chicken fillet

1. Introduction

Chicken meat is the most popular poultry product and widely consumed worldwide [1]. However, the nature of chicken meat with its high amounts of water (75.0%), proteins (22.8%), and fats (0.9%) make it is easily spoiled by microorganisms and furthermore has a relatively short shelf life [2]. A preservation process is needed to prolong its shelf life and minimize the risk of food poisoning. Physical preservation methods, including modification of temperature and atmospheric packaging, have been well studied and are possible to be applied [3-4].

The uses of antimicrobial agents for the preservation of chicken meat, including chitosan, nisin, and essential oils of plants, are also widely described [5-7]. The uses of essential oils, particularly, is attributed to their antimicrobial properties against a wide array of microbes, including the spoilage bacteria [8]. Essential oils of spices especially gain significant interest because of our familiarity with their taste and aroma in our daily meals which might improve their acceptability as natural food preservatives. Lemongrass [*Cymbopogon citratus* (DC.)

Stapf, Poaceae] and lemon basil (*Ocimum×africanum* Lour., Lamiaceae) are two spices commonly used in Indonesian culinary, typically for meat-based meals.

Individually, the essential oil of both plants has demonstrated antimicrobial properties [9-11]. Furthermore, lemongrass essential oil has been applied for prolonging the shelf life of grapes [12]. The use of a combination of essential oils in a preservation effort is thought to produce synergistic or mutually reinforcing effects, which eventually will enhance the shelf life of the preserved food [13]. The use of the combination of lemongrass and clove essential oils was proven to be capable of prolonging the shelf life of bovine ground meat [14]. In this study, we analyzed the chemical constituents of individual lemongrass and lemon basil essential oils as well as evaluating the application of their combination to be used as a natural chicken fillet preservative. The evaluation of the preservation effect of the combination of both essential oils was based on their inhibition of bacterial growth and also their capability in extending the shelf life of the meat.

2. Materials and methods

2.1 Plant materials

Aerial parts of lemongrass and lemon basil were collected from Banyumas, Central Java, Indonesia. The identity of plant materials was determined at the Laboratory of Plant Taxonomy, Faculty of Biology, University of Jenderal Soedirman, Banyumas, Indonesia. The plant materials were dried under direct sun light until their water content was less than 10%.

2.2 Distillation of the essential oils

Lemongrass and lemon basil essential oils were obtained from a steam and water distillation process as previously described [15]. The distillation process for each plant material was run for an average of six h.

2.3 Identification of chemical constituents of the essential oils

Lemongrass and lemon basil essential oils were separately analyzed using a Gas Chromatograph-Mass Spectrometer (GC-MS) Shimadzu QP2010 SE hyphenated to SH-Rxi-5Sil MS. The condition of the chromatographic separation, ionization, as well as the identification of chemical constituents followed a previously reported method [16].

2.4 Evaluation of the preservation effects on chicken fillet by the essential oils

Lemongrass and lemon basil essential oils were evaluated for their chicken fillet preservation effects following a previous report [16]. In brief, the essential oils were dissolved in an equal volume of dimethyl sulfoxide (DMSO) and further homogenized with sterile distilled water to obtain five different concentration ratios of a combination of lemongrass and lemon basil essential oils (1:0, 0:1, 2:0.2, 1:1, and 0.2:2 %v/v). Chicken fillets were prepared from fresh chicken breast bought from a local market in Banyumas, Central Java, Indonesia. The fillets were cut into cubes in 1x1x1 cm and subjected to the surface sterilization by immersing them in boiling water for a minute. The cubes of meats were placed in 100 ml of liquid containing different concentration ratios of essential oils, as well as DMSO and sterile distilled water as negative controls, under sterile conditions. The preserved meats were kept in a refrigerator with a temperature of 5±2 ˚C for 9 d. The observation was carried out at 3 d intervals in d 0, 3, 6, and 9. The inhibition of bacterial growth on chicken meat preserved with the combination of essential oils cultured on nutrient broth (NB) was calculated in each observation d. In addition, the shelf life of the chicken fillet was subjectively observed based on the physical characteristics of the meat using color, odor, texture, and slime formation as the parameters by the researchers.

2.5 Statistical data analysis

The data was obtained from three experiments and the means were calculated from triplicate results. Means separation of the optical densities of cultured bacterial suspensions in NB and area under curve (AUC) of the aforementioned curve versus the preservation time data was accomplished by Duncan's tests. Significance was evaluated at p-value < 0.05. Statistical analysis was conducted by the general procedures of SPSS Statistics v.20 (SPSS Inc.).

3. Results and discussion

There are 24 compounds identified in lemongrass essential oil, with geranyl acetate, geraniol, citronellal, patchouli alcohol, and citronellyl acetate as the major constituents (Table 1). Typically, lemongrass essential oil is mainly consist of neral, geranial, geraniol, β-caryophyllene, and nerol. Altogether, those compounds are utilized as the quality parameter of lemongrass essential oil [17-18]. For example, geranial, neral, β-myrcene, 6-methyl-5-hepten-2-one, α-gurjunene, and germacrene-D-4-ol were detected as the main constituents of lemongrass essential oils collected in Purbalingga, Indonesia; while geranial, neral, β-myrcene, 6- methyl-5-hepten-2-one, heptanal, and α-thujene were identified from those obtained from Abomey Calavi, Benin [9, 19]. Hence, the chemical constituents of the lemongrass essential oil used in this study are different from those aforementioned reports. However, variation in the composition of essential oils are commonly reported, as differences in cultivation conditions and geographical origin will affect the chemical constituents of most plant species [20].

On the other hand, lemon basil essential oil consists of 10 compounds, with dominant constituents including estragole, linalool, α-bisabolene, β-caryophyllene, and geranial (Table 1). Our result is similar to a previously reported study, that estragole is the main constituent of lemon basil. Estragole, geranial, and neral are the major constituents of lemon basil collected in Chiang Mai, Thailand [21]. In addition, linalool, α-bisabolene, β-caryophyllene, and geranial were also found as the characteristic constituents of lemon basil essential oil originated from northern India [22].

Table 1 Chemical constituents of lemongrass and lemon basil essential oils.

No	Compound name	RT (min)	Area (%)	
			lemongrass	lemon basil
1	2-Methylpentane	7.87	0.82	-
2	Limonene	13.239	3.29	-
3	Ocimene	13.762	1.03	-
4	Linalool	17.745	1.08	22.64
5	Citronellal	21.249	17.83	-
6	Estragole	24.391	-	69.93
7	Citronellol	26.435	4.33	-
8	Neral	26.999	-	0.6
9	Geraniol	28.052	19.06	-
10	Geranial	28.881	0.97	1.03
11	p-Menthane-3,8-diol	32.237	0.96	-
12	2-Methoxy(2-propenyl)-phenol	32.776	-	0.35
13	Citronellyl acetate	32.848	6.5	-
14	Geranyl acetate	33.982	19.72	-
15	β-Elemene	34.291	0.53	-
16	β-Caryophyllene	35.322	3.14	1.22
17	Farnesol	36.507	-	0.44
18	Germacrene D	37.359	0.72	-
19	α-Bergamotene	37.7	0.48	0.78
20	Methyl Isoeugenol	37.782	0.87	-
21	α-Cadinene	38.519	1.49	-
22	α-Bisabolene	39.142	-	2.48
23	Elemol	39.355	0.53	-
24	Geranyl butyrate	39.56	1.84	-
25	3-Methoxycinnamaldehyde	39.808	-	0.5
26	Patchouli alcohol	41.709	7.46	-
27	α-Cadinol	42.226	1.04	-
28	γ-Decalactone	43.254	3.36	-
29	1-(3-Cyanophenyl)-2-phenylethane	57.983	1.73	-
30	Cinnoline	67.22	0.52	-
	Total area		99.3	99.97
	Total terpenes and phenyl propanes		92.87	99.97
	Total oxygenated compounds		82.19	95.49
	Total hydrocarbon compounds		10.68	4.48

Linalool, geranial, β-caryophyllene, and α-bergamotene are identified in both essential oils, with a significantly higher proportion was found in lemon basil essential oil. The total terpene and phenylpropane compounds were higher in lemon basil essential oil as well. The proportion of oxygenated compounds in both essential oils was considerably higher than that of the hydrocarbons (Table 1). Terpenoids and phenylpropenes are considered as the antimicrobial compounds in essential oils, with oxygenated compounds showing a higher activity than those of hydrocarbons [8]. Hence, geranyl acetate, geraniol, and citronellal from lemongrass essential oil, as well as estragole and linalool from lemon basil essential oil, might be the compounds responsible for antimicrobial activity and further potential meat preservation effect of both oils.

The capability of the combinations of lemongrass and lemon basil essential oils in inhibiting the growth of bacteria in chicken fillet is indicated by the optical density of the culture of the meats in NB. The higher optical density indicates the presence of more bacteria on the chicken fillets. There are two negative controls used in this study, the sterile water (as the medium) and 1% DMSO (as the co-solvent). Both negative controls demonstrated the highest optical density compared to other groups in most of the observation d, except in d 3. The meats treated with essential oils at any given ratios demonstrated an increasing optical density at d 3, which later gradually decreased until d 9. The wide variation in the optical density between the groups in d 0 was noticeable. It represented the insufficient surface sterilization we did, as the bacteria were still on the meats afterward. In d 3, the bacterial growth inhibition activity of the combination of the lemongrass and lemon basil essential oils were varied; those in higher ratios of lemongrass oil demonstrate a good inhibitory activity, while that at ratio of 0:1 and 1: 1 %v/v exhibited no inhibitory activity, and their optical density were even higher than those with negative control. This phenomenon might indicate that the essential oil with more dominant antibacterial activity is that from lemongrass. The presence of a lower fraction of lemongrass essential oil was not capable of inhibiting the growth of the bacteria on the meats, and hence more bacterial growth was observed. In the higher concentrated suspension, such in both samples and also negative control, the deviation of the Beer-Lambert Law had taken place and further affected the observed optical density [23]. This phenomenon highlighted the unsuitability of the optical density-based enumeration for bacterial growth on chicken fillets in this study. For a better and more accurate result, a more suitable bacterial enumeration method should have been used. The direct bacterial growth enumeration, especially using the determination of colony forming units, is more appropriate for this purpose. For example, the direct method has been successfully used in a study of the preservation of black sea bream fillets using kakadu plum water extract [24]. In d 6 and 9, the combination of both essential oils in all ratios showed lower optical densities compared to negative controls (Figure 1)

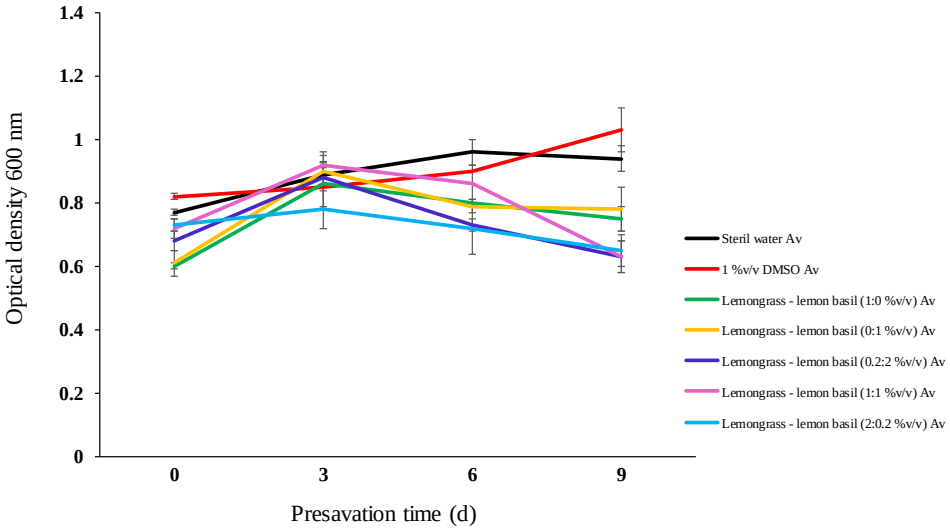

Figure 1 Profile of the optical density of culture of chicken meat in NB during 9-d of refrigerated storage.

In order to evaluate the bacterial growth inhibitory activity during 9 d of preservation, we calculated the total AUC of the optical density of cultures of chicken fillet preserved with the combination of lemongrass and lemon basil essential oils, versus the preservation time. The total AUC represents the overall bacterial growth in d 0-9. Hence, the total AUC of a given group that is significantly different from the negative control indicates that the group is capable of inhibiting the bacterial growth during a 9-d preservation period. All treatments with lemongrass and lemon basil essential oils are capable of inhibiting the bacteria on the meats. The use of essential oils in combination, in any ratios, demonstrated a better inhibitory activity than those of individual oil use.

However, the activity of those 3 ratios of lemongrass and lemon basil essential oils are statistically equal (Figure 2).

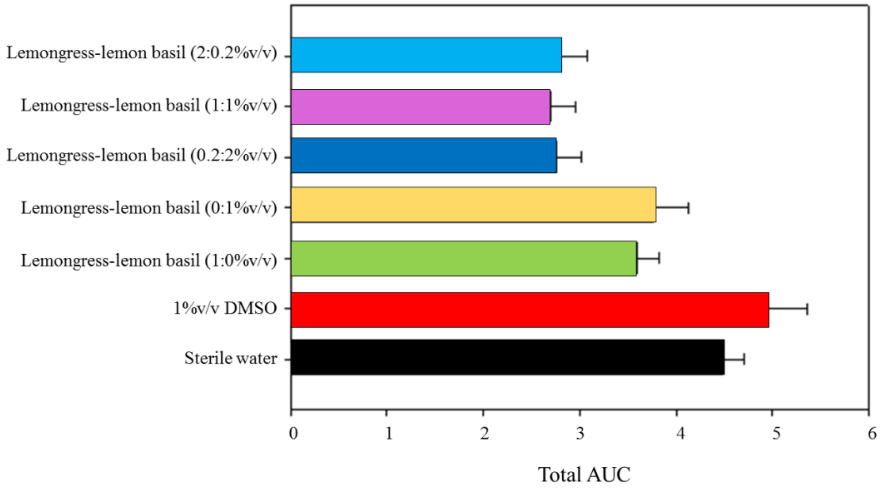

Figure 2 Total AUC of the profile optical density of culture of chicken meat in NB during 9-d of refrigerated storage.

Our results demonstrated that the use of lemongrass and lemon basil essential oils in combination have a better preservation effect than those in individual oil uses. The increasing antimicrobial activity and further food preservation effect of a combination of essential oils resulted from the additive or synergistic effects of the respective essential oil [25-26]. This strategy has been evaluated and indicates that the combination of lemongrass and clove essential oils successfully minimized the number of *Listeria monocytogenes* inoculated in bovine ground meat [14].

We calculated the relative volume of oxygenated compounds in each ratio of lemongrass and lemon basil essential oils by multiplying the % area of total oxygenated compounds to the volume of the respective essential oil used in each ratio and then calculated the results. They were calculated in order to find the relationship between the inhibitory activities with the compounds in each ratio. The relative volume of oxygenated compounds was found to support our bacterial growth inhibitory activity result; that the combination of essential oils at any given ratios demonstrated a higher relative volume of oxygenated compounds than those in individual oil use (Figure 3). The oxygenated compounds, especially the aldehydes and phenols, have been recognized as volatile compounds with the strongest antibacterial activity [25]. Hence, the presence of more oxygenated compounds in a combination of essential oils will enhance its antimicrobial activity. In our case, geranyl acetate, geraniol, citronellal, estragole, and linalool might be responsible for the antibacterial activity of the combination of lemongrass and lemon basil essential oils.

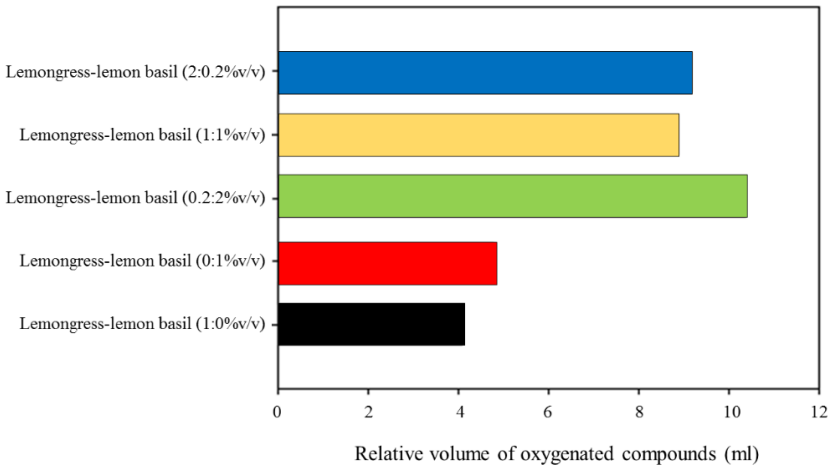

Figure 3 Relative volume of antimicrobial-active oxygenated compounds in a combination of lemongrass and lemon basil essential oil.

We evaluated the physical characteristics of preserved chicken fillets, including color, odor, texture, and slime formation, in order to determine improvement of shelf life of the meat with the combination of lemongrass and lemon basil essential oil. The changes in any of those four parameters were caused by bacterial activity. Treatment with the essential oils changed the color of the meat from pinkish-white to yellowish white. However, the color of meat in negative control was pinkish-white and started to change in d 6. The meat treated with single lemongrass essential oil started to change color into brownish-white in d 9, while that in the lemon basil group remained unchanged until d 9. The use of essential oils masked the fresh chicken meat odor after being applied. While meat in negative control started to smell slightly rotten in d 3, those preserved with lemongrass and lemon basil essential oils exhibited delayed odor changes. The delaying of odor changes in meat preserved with combinations of essential oils is longer than those preserved with individual oils. The combination of lemongrass and lemon basil at ratio of 2:0.2 %v/v was capable of retaining the original color of the yellowish-white meat which remained unchanged until d 9. Without essential oil, the texture of chicken meat in negative control quickly changed in d 3, while those preserved with essential oils demonstrated a longer delay in texture changes with the trend as in the odor parameter. The presence of slime in the meats treated with negative control group, individual essential oil, and a combination of essential oils could be detected in d 3, 6, and 9, respectively (Table 2).

Table 2 The physical characteristics of chicken fillets during 9-d of refrigerated storage.

Chicken fillet preserved with	Observed characters	Preservation time (d)			
		0	3	6	9
Sterile water	color	pinkish-white	pinkish-white	yellowish-white	yellowish-white
	odor	fresh	putrid +	putrid ++	putrid +++
	texture	firm	softer	softer	softer
	slime formation	no	yes	yes	yes
Lemongrass - lemon basil (1%:0, v/v)	color	yellowish-white	yellowish-white	yellowish-white	brownish-white
	odor	aromatic lemongrass	aromatic lemongrass	weaker aromatic lemongrass, putrid +	weaker aromatic lemongrass, putrid +
	texture	firm	firm	softer	softer
	slime formation	no	no	yes	yes
Lemongrass - lemon basil (0:1%, v/v)	color	pinkish-white	pinkish-white	pinkish-white	pinkish-white
	odor	aromatic lemon basil	aromatic lemon basil	aromatic lemon basil	weaker aromatic lemon basil, putrid +
	texture	firm	firm	softer	softer
	slime formation	no	no	yes	yes
Lemongrass - lemon basil (0.2:2%, v/v)	color	yellowish-white	yellowish-white	yellowish-white	yellowish-white
	odor	aromatic lemon basil	aromatic lemon basil	aromatic lemon basil	weaker aromatic lemon basil, putrid +
	texture	firm	firm	firm	softer
	slime formation	no	no	no	yes
Lemongrass - lemon basil (1:1%, v/v)	color	yellowish-white	yellowish-white	yellowish-white	yellowish-white
	odor	aromatic lemongrass and lemon basil	aromatic lemongrass and lemon basil	aromatic lemongrass and lemon basil	weaker aromatic lemongrass and lemon basil, putrid +
	texture	firm	firm	firm	softer
	slime formation	no	no	no	yes

Chicken fillet preserved with	Observed characters	Preservation time (d)			
		0	3	6	9
Lemongrass - lemon basil (2:0.2%, v/v)	color	yellowish-white	yellowish-white	yellowish-white	yellowish-white
	odor	aromatic lemongrass	aromatic lemongrass	aromatic lemongrass	aromatic lemongrass
	texture	firm	firm	firm	softer
	slime formation	no	no	no	yes

The improvement of the shelf life of the chicken fillets preserved with a combination of lemongrass and lemon basil essential oils was calculated from the comparison on the first d when any of the physical characteristics of the meat started to change in a given group, to that of negative control (Figure 4). The combination of lemongrass and lemon basil essential oils in all ratios are capable of prolonging the shelf life of the meat up to 6 d and are better than the single essential oil uses. The improvement of shelf life capacity of combined essential oils has been described. For example, a combination of thyme and laurel essential oils extended the shelf life of bluefish in ice by 3-4 d [27].

Combining the results of the inhibition of microbial growth and the physical properties of the meat, the combination of lemongrass and lemon basil essential oils in three different ratios demonstrated the same efficacy. The combination of both essential oils was capable of significantly inhibiting the growth of bacteria on the meat during 9 d of preservation as well as extending the shelf life of the meat for up to 6 d. Based on this result, we recommended the ratio of 1:1 %v/v as the optimal ratio for the combination of lemongrass and lemon basil essential oil.

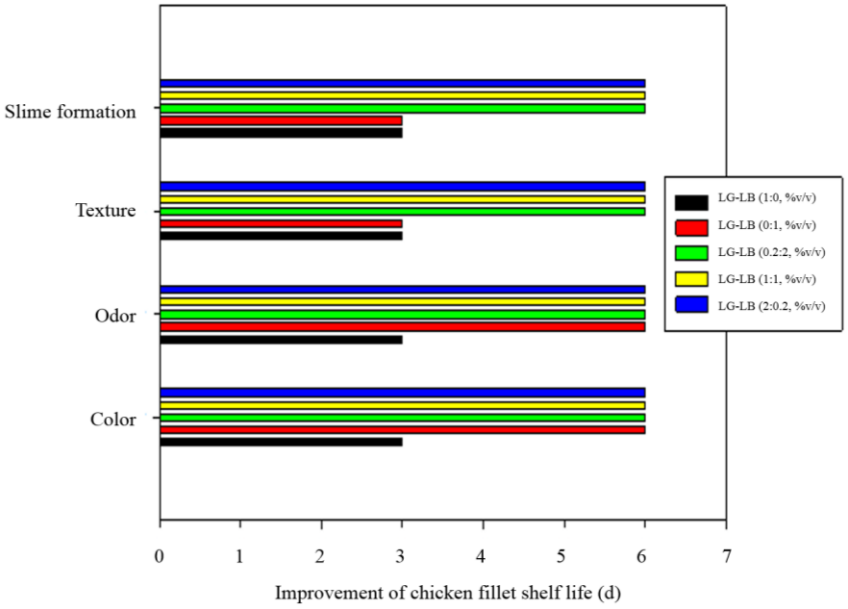

Figure 4 The effect of a combination of lemongrass and lemon basil essential oils to the shelf life of chicken fillets. LG = lemongrass and LB = lemon basil.

4. Conclusion

A combination of lemongrass and lemon basil essential oils at the optimal ratio of 1:1% v/v was capable of reducing the bacterial growth on the meat during 9 d of preservation and prolonging the shelf life of the meat for up to 6 d compared to the negative control at a temperature of 5 ˚C. These preservation effects might be related to the high proportion of oxygenated compounds, especially geranyl acetate, geraniol, citronellal, estragole, and linalool in both essential oils.

5. References

[1] OECD. Meat consumption (indicator) [Internet]. 2019 [cited 2019 Feb 14]. Available from https://data.oecd.org/agroutput/meat-consumption.htm.

[2] FAO. Composition of Meat [Internet]. 2019 [cited 2019 Feb 14]. Available from http://www.fao.org/ag /againfo/themes/en/meat/backgr_composition.html.
[3] Zouaghi F, Cantalejo MJ. Study of modified atmosphere packaging on the quality of ozonated freeze-dried chicken meat. Meat Sci. 2016;119:123-131.
[4] Smadi H, Sargeant JM, Shannon HS, Raina P. Growth and inactivation of *Salmonella* at low refrigerated storage temperatures and thermal inactivation on raw chicken meat and laboratory media: Mixed effect meta-analysis. J Epidemiol Glob Health. 2012;2(4):165-79.
[5] Alnoman M, Udompijitkul P, Sarker MR. Chitosan inhibits enterotoxigenic *Clostridium perfringens* type A in growth medium and chicken meat. Food Microbiol. 2017;64:15-22.
[6] Raeisi M, Tabaraei A, Hashemi M, Behnampour N. Effect of sodium alginate coating incorporated with nisin, *Cinnamomum zeylanicum*, and rosemary essential oils on microbial quality of chicken meat and fate of Listeria monocytogenes during refrigeration. Int J Food Microbiol. 2016;238:139-145.
[7] Al-Hijazeen M, Lee EJ, Mendonca A, Ahn DU. Effect of oregano essential oil (*Origanum vulgare* subsp. hirtum) on the storage stability and quality parameters of ground chicken breast meat. Antioxidants. 2016;5(2):article E18.
[8] Chouhan S, Sharma K, Guleria S. Antimicrobial activity of some essential oils-present status and future perspectives. Medicines 2017;4(3):article 58.
[9] Hamad A, Nuritasari A, Hartanti D. Chemical composition and antimicrobial study of essential oil of lemongrass (*Cymbopogon citratus*). der Pharm Lett. 2017;9(5):109-116.
[10] Tofiño-Rivera A, Ortega-Cuadros M, Galvis-Pareja D, Jiménez-Rios H, Merini LJ, Martínez-Pabón MC. Effect of *Lippia alba* and *Cymbopogon citratus* essential oils on biofilms of *Streptococcus mutans* and cytotoxicity in CHO cells. J Ethnopharmacol. 2016;194:749-754.
[11] Carovic´-Stanko K, Orlic´ S, Politeo O, Strikić F, Kolak I, Milos M, Satovic Z. Composition and antibacterial activities of essential oils of seven Ocimum taxa. Food Chem. 2010;119:196-201.
[12] Sonker N, Pandey AK, Singh P, Tripathi NN. Assessment of *Cymbopogon citratus* (DC.) Stapf essential oil as herbal preservatives based on antifungal, antiaflatoxin, and antiochratoxin activities and *in vivo* efficacy during storage. J Food Sci. 2014;79(4):M628-M634.
[13] Hyldgaard M, Mygind T, Meyer RL. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front Microbiol. 2012;3:1-24.
[14] de Oliveira TL, das Graças-Cardoso M, de Araújo-Soares R, Ramos EM, Piccoli RH, Tebaldi VM. Inhibitory activity of *Syzygium aromaticum* and *Cymbopogon citratus* (DC.) Stapf. essential oils against *Listeria monocytogenes* inoculated in bovine ground meat. Brazilian J Microbiol. 2013;44(2):356-365.
[15] Hamad A, Mahardika MGP, Yuliani I, Hartanti D. Chemical constituents and antimicrobial activities of essential oils of *Syzygium polyanthum* and *Syzygium aromaticum*. Rasayan J Chem. 2017;10(2):564-569.
[16] Hartanti D, Haqqi MZU, Hamad A. Potency of combination of essential oils of Ginger and Lemongrass as fresh chicken meat natural preservative. Adv Sci Lett. 2018;24(1):91-94.
[17] de Groot A, Schmidt E. Essential oils, part V: Peppermint oil, lavender oil, and lemongrass oil. Dermatitis. 2016;27(6):325-332.
[18] Abdulazeez MA, Abdullahi AS, James BD. Chapter 58 – Lemongrass (*Cymbopogon* spp.) Oils. In: Preedy VR, editor. Essential oils in food preservation, flavor and safety. Amsterdam: Academic Press; 2016. p. 509-516.
[19] Gbenou JD, Ahounou JF, Akakpo HB, Laleye A, Yayi E, Gbaguidi F, Baba-Moussa L, Darboux R, Dansou P, Moudachirou M, Kotchoni SO. Phytochemical composition of *Cymbopogon citratus* and *Eucalyptus citriodora* essential oils and their antiinflammatory and analgesic properties on Wistar rats. Mol Biol Rep. 2012;40(2):1127-1134.
[20] Bajalan I, Rouzbahani R, Pirbalouti AG, Maggi F. Variation in chemical composition and antibacterial activity of the essential oil of wild populations of *Phlomis olivieri*. Chem Biodivers. 2017;14(5):article e1600444.
[21] Tangpao T, Chung H-H, Sommano SR. Aromatic profiles of essential oils from five commonly used Thai basils. Foods. 2018;7(11):article E175.
[22] Padalia RC, Singh VR, Bhatt G, Chauhan A, Upadhyay RK, Verma RS, Chanotiya CS. Optimization of harvesting and post-harvest drying methods of Ocimum africanum Lour. for production of quality essential oil. J Essent Oil Res. 2018;30:437-443.
[23] Mäntele W, Deniz E. UV-VIS absorption spectroscopy: Lambert-Beer reloaded. Spectrochim Acta Part A Mol Biomol Spectrosc. 2017;173:965-968.
[24] Wright MH, Shalom J, Matthews B, Greene AC, Cock IE. Terminalia ferdinandiana Exell: Extracts inhibit *Shewanella* spp. growth and prevent fish spoilage. Food Microbiol. 2019;78:144-122.
[25] Bassolé IHN, Juliani HR. Essential oils in combination and their antimicrobial properties. Molecules. 2012;17(4):3989-4006.

[26] Calo JR, Crandall PG, O'Bryan CA, Ricke SC. Essential oils as antimicrobials in food systems: A review. Food Control. 2015;54:111-119.
[27] Erkan N, Tosun ŞY, Ulusoy Ş, Üretener G. The use of thyme and laurel essential oil treatments to extend the shelf life of bluefish (*Pomatomus saltatrix*) during storage in ice. J für Verbraucherschutz und Leb. 2011;6:39-48.