

Effect of lemongrass and mint essential oils combined with food additives soaking solution on the quality of *Pangasius* fillets during cold storage

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Abstract

Pangasius fillet has high demand in the international market due to its tender flesh, delicate taste and as an affordable substitute for cod and other more expensive white fishes. The preliminary study, it was found that the optimized condition for soaking was NaCl 1.32%, STPP 1.88%, and sorbitol 4.68% for 30 mins which give a significant effect ($p < 0.05$) to the yield, water activity, pH, and improvement in the sensory of the treated fillet. However, the soaking itself was not contributed to the extension of shelf life. This study aimed to examine the effects of *Pangasius* fillet soaked in essential oil on the quality and shelf life with simulated storage similar to retail conditions. Lemongrass essential oil was showing the best result in improving the quality and shelf life of *Pangasius* fillet stored in refrigerated storage (0-4°C). The addition of 0.2% lemongrass showed 7 days extension of shelf-life or longer compared to fillet without soaking and fillet soaking without essential oil. In addition, it also reduces the Total Volatile Based-Nitrogen (TVB-N) content, improves the sensory quality (raw and cooked) of *Pangasius* fillet. Mint essential oil was showing the shelf-life extension (7 days longer) and sensory improvement at 0.3% concentration. However, the mint essential oil was not showing better TVB-N reduction in treated fillet compared to fillet soaking without essential oil. Therefore, it is recommended to incorporate 0.2% lemongrass essential oil for the *Pangasius* industry to extend the shelf life of *Pangasius* fillet followed with quality improvement.

1. Introduction

Pangasius hypophthalmus or also known as sutchi catfish, striped catfish, or tra fish is mainly produced in Vietnam (Phan *et al.*, 2009). Frozen fillets and thawed fillets are the most popular *Pangasius* product sold in supermarkets and fresh markets in Western countries (Orban *et al.*, 2008). *Pangasius* fillet has tender flesh, sweet taste, absence of fishy odour and spines, delicate flavour and firm texture when cooked. Therefore, *Pangasius* is now largely accepted as an affordable substitute for cod and other more expensive white fishes and also due to their good nutritional quality (Phan *et al.*, 2009; Karl *et al.*, 2010).

As a highly perishable food (Orban *et al.*, 2008), extra attention should be given to prolong the shelf life of *Pangasius* fillet. Food antimicrobial agent is one of the chemicals added to prolong shelf life. However, several questions about the safety of chemicals used for food preservation were raised due to the suspected negative effects on consumer's health (Cassens, 1997).

The ability of plant essential oils to protect foods against pathogenic and spoilage microorganisms has been reported by Burt (2004) and Ahmad *et al.* (2012) The essential oils (EOs) contain phenolic compounds such as thymol, carvacrol, p-cymene, and α -terpinene and have been known to inhibit both gram-negative and gram-positive microorganisms (Burt, 2004). Therefore, the usage of essential oils as natural food additives became common, since they possess antibacterial, antiviral, and antimycotic properties (Ahmad *et al.*, 2012).

In industry, soaking fillet with food additives such as sorbitol, sodium tripolyphosphate (STPP) and sodium chloride (NaCl) is the common practices to increase the yield, maintain the texture and prolong the shelf life of meat, chicken fillet and fish fillet products (Zhou *et al.*, 2006; Smith and Acton, 2010; Yusop *et al.*, 2011). As soaking with salt solution has no increasing of microbial shelf life, the addition of essential oils is recommended (Van Haute *et al.*, 2017). By adding essential oils, the usage of chemical food additives can be reduced

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(Chouliara et al., 2007).

Therefore, this study aimed to examine the effects of *Pangasius* fillet soaked in essential oil on the quality and shelf life with simulated storage similar to retail conditions.

2. Materials and methods

2.1 Raw materials

Fresh *Pangasius hypophthalmus* fillets were purchased from Pangasius company in An Giang City, Vietnam. Each well-trimmed fillet (skinless, belly off, red meat off and fat off) was about 200-220 g weight. Then, the samples were stored with ice in the polystyrene box and directly transported to the laboratory within 3 hrs. Food grade sodium tripolyphosphate (STPP) (France), sodium chloride (NaCl) (Vietnam), sorbitol (France) were used for the soaking solution. Lemongrass essential oil (LGEO) and mint essential oil (MTEO) were purchased from Vietnam Essential Oil Joint Stock Company, Vietnam.

2.2 Soaking and storage conditions

Based on the preliminary study, the optimum condition for *Pangasius* fillet was soaking for 30 mins with 1.32% NaCl, 1.88% STPP, and 4.68% sorbitol. The solutions were combined with lemongrass and mint essential oil (0.1-0.4%) with ratio 1.5:1 (w/w) and emulsified with 0.01% (w/w) Tween 80 (Germany). The fillets were maintained in $4\pm 1^\circ\text{C}$ in a refrigerator (Sanaky, Japan).

The soaking solution should only be used once to avoid contamination. The soaked fillets were then drained for 1 min and followed by packing in Polystyrene (PS) tray with cellulose moisture absorbent paper (Japan), then wrapped with Polyethylene (PE) film. The packed fillets were stored at refrigerated storage ($0-4^\circ\text{C}$). The quality changes were tested with an interval period of 0, 2, 4, 6, 9, and 11 days. The experiment was done with two independent replicates.

2.3 Microbial analysis

At each interval, a packed fillet from each treatment were aseptically opened and 25 g of the fish samples were taken using sterile scalpels and tweezers and placed in a sterile stomacher bag (filter 0.5 mm pore size) (Interscience, France). About 225 mL of sterile Maximum Recovery Diluent (MRD, Merck, Darmstadt, Germany) was added and the mixture was homogenized for 1 minute. Further decimal dilutions were prepared in MRD. The total viable counts were determined on Plate Count Agar (PCA) (Merck, Darmstadt, Germany) by

pour plating. The plates were incubated for 2 days at 37°C to determine the total mesophilic counts. Bacterial counts were expressed as \log_{10} colony-forming units (CFU) per g of sample.

2.4 Sensorial analysis

The sensory evaluation of cooked fish was conducted following the method described by Neumann et al. (1983) and using the scoring table by Khalafalla et al. (2015). Cooked fillets were evaluated for the attributes of odour, colour, texture, and taste using a 5-point unipolar hedonic scale, with a score range from 1 (very dislike) to 5 (very like). Acceptability and grade of fillets were gained from the total of attributes. The overall score is the total score of all attributes with range 4-20, with 4-7.1 considered as unacceptable and 7.2-20 as acceptable. The fillets were covered in foil and cooked individually in a steamer pot for 6 mins at 100°C . Then, samples were cut into a size of $3\times 3\times 1$ cm before serving to the 20 panelists.

2.5 Physicochemical analysis

The peroxide value (PV) was determined according to Rahman et al. (2015). Total Volatile Based-Nitrogen (TVB-N) was based on the steam distillation of an alkalinized samples and absorbed by boric acid solution (1%) and further titration using the sulfuric acid solution (0.1N) according to AOAC (1990). The pH was determined using a calibrated pH-electrode (Metler Toledo, Schwerzenbach, Switzerland).

2.6 Statistical analysis

Data were presented as means \pm standard deviation and a probability value of 0.05 ($p < 0.05$) were considered significant. One-way analysis of variance (ANOVA) was performed and the mean comparisons were done by Tukey's method. Statistical analysis was performed using Minitab 16 for windows (Minitab, LLC, Pennsylvania, USA)

3. Results and discussion

3.1 Microbial changes

Total viable counts (TVC) of treated *Pangasius* fillets during 11 days of refrigerated storage is presented in Figure 1. The initial total viable count of the fillets in this study was 5.5-6.8 log CFU/g. This indicated that the raw material was possibly contaminated during processing (Ahmad et al., 2012; Cai et al., 2015). Fillet without treatment and fillet soaked without essential oil was 7 log CFU/g at day 6 of the storage, indicating that limit of acceptability for consumption (Skovgaard, 2003). It is evident that

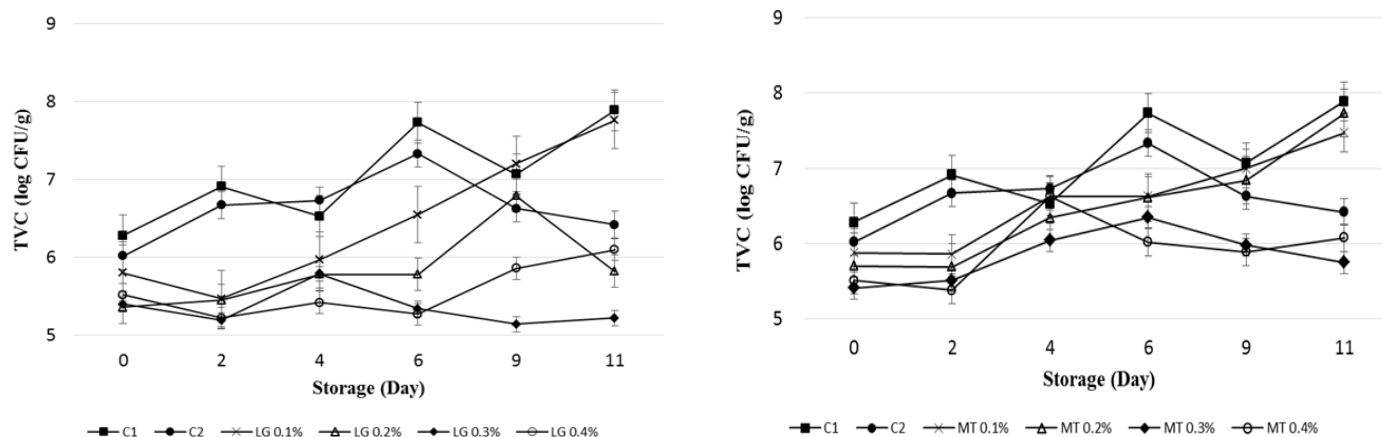


Figure 1. Microbial changes of treated *Pangasius* fillet during cold storage. C1: Control without soaking; C2: Control soaking without essential oil; MT: Soaking with mint essential oil; and LG: Soaking with lemongrass essential oil.

soaking with STPP, NaCl, and sorbitol without essential oil itself was not enough to extend the shelf life. Lee *et al.* (1994) reported that gram-positive bacteria are more susceptible to phosphate, meanwhile, Noseda *et al.* (2012) mentioned that dominant spoilage bacteria in freshwater fish are gram-negative.

Fillets treated with 0.2-0.4% of lemongrass essential oil (LGEO) showed the extension of shelf life up to 11 days (or 7 days longer) compared to both the controls without soaking (C1) and soaked without essential oil (C2). The strong antimicrobial activity of lemongrass attributed to the high citral content. The citral changes the intracellular pH, membrane potential, intracellular ATP concentration, and membrane integrity (Adukwu *et al.*, 2016; De Silva *et al.*, 2017). Mint essential oil (MTEO) showed a positive effect in extending the shelf life of *Pangasius* fillets). İşcan *et al.* (2002) found that menthol was responsible for the antimicrobial activity of MTEO. At the end of storage, the fillets that soaked with 0.3% and 0.4% mint essential oil showed an excellent effect compared to the other samples by remaining below the limit of acceptability for consumption more than 11

days. In conclusion, the 0.3% concentration of MTEO was effective to apply in soaking solutions to extend the microbial shelf life of *Pangasius* fillets.

3.2 Physicochemical properties

3.2.1 pH value

Variations in the value of pH during the refrigerated storage are presented in Table 1. No significant difference ($p>0.05$) was observed between the treated fillets and the control samples during each period of storages. However, the pH of C1 was consistently low and decrease during storage. The initial pH was 6.95 ± 0.15 then dropped to 6.67 ± 0.01 on day 11. The changes in pH during storage depend on many factors, such as storage temperature, soaking concentration, species, physiological condition, degradation of protein and enzyme activity (Biscalchin-Grýschek *et al.*, 2003). The pH of soaked fillets has a slightly higher pH than the controls, due to the pH of soaking solution coming from STPP and essential oils. The microbial loads also affected the pH of fillets. The accumulation of alkaline compounds (ammonia and TMA, etc.) generated from microbial and enzymatic actions which contribute to an

Table 1. pH changes of treated *Pangasius* fillet during cold storage

Sample	Storage (day)						
	0	2	4	6	9	11	
C1	6.95 ± 0.15^a	6.9 ± 0.02^a	6.65 ± 0.36^a	6.68 ± 0.36^a	6.68 ± 0^a	6.67 ± 0.01^a	
C2	6.94 ± 0.25^a	6.79 ± 0.11^a	6.95 ± 0.03^a	6.74 ± 0.21^a	6.99 ± 0.18^a	6.60 ± 0.05^a	
LG	0.10%	6.82 ± 0.14^a	6.98 ± 0.2^a	6.84 ± 0.13^a	6.89 ± 0.23^a	6.82 ± 0.10^a	6.7 ± 0.09^a
	0.20%	7.12 ± 0.45^a	6 ± 0.27^a	7.09 ± 0.01^a	6.98 ± 0.27^a	7.04 ± 0.02^a	6.97 ± 0.35^a
	0.30%	7.04 ± 0.3^a	7.04 ± 0^a	7.11 ± 0.18^a	7.11 ± 0.14^a	6.96 ± 0.00^a	7.10 ± 0.52^a
	0.40%	7.00 ± 0.35^a	7 ± 0.03^a	7.04 ± 0.01^a	6.96 ± 0.19^a	6.84 ± 0.05^a	6.80 ± 0.30^a
MT	0.10%	6.91 ± 0.08^a	6.91 ± 0.07^a	6.86 ± 0.15^a	6.87 ± 0.19^a	6.93 ± 0.26^a	6.78 ± 0.09^a
	0.20%	6.78 ± 0.23^a	6.78 ± 0.17^a	6.99 ± 0.36^a	6.83 ± 0.24^a	6.82 ± 0.13^a	6.66 ± 0.06^a
	0.30%	6.91 ± 0.02^a	6.91 ± 0.13^a	6.77 ± 0.14^a	6.9 ± 0.23^a	6.89 ± 0.32^a	6.65 ± 0.06^a
	0.40%	6.96 ± 0.2^a	6.96 ± 0.34^a	7.07 ± 0.03^a	6.98 ± 0.16^a	7.04 ± 0.14^a	6.85 ± 0.18^a

C1: Control without soaking; C2: Control soaking without essential oil; MT: Soaking with mint essential oil; and LG: Soaking with lemongrass essential oil. Values followed by the same letter within the same row are not significantly different from each other ($p>0.05$).

increase of pH (Ahmad *et al.*, 2012). The compounds of each essential oil also contribute to pH changes (De Silva *et al.*, 2017).

3.2.2 Peroxide value (PV)

The peroxide value of treated fillets and the controls was shown in Table 2. There was no significant difference in peroxide value among samples ($p > 0.05$). Peroxide formation is one of the signs of oxidative rancidity. During storage, all of the fillets remain below 10 mEq/kg which means it did not exceed the limit for human consumption. Low peroxide value can be a sign of early oxidation or late stage of severely oxidized fillets where more H_2O_2 have been oxidized due to lipid composition of *Pangasius* itself (Waindu and Jamala, 2013; Rathod *et al.*, 2018). Domiszewski *et al.* (2011) mentioned, despite a substantial influence of heat treatment on the amount of both primary and secondary oxidation products, the lipids maintained good quality with peroxide value of sample was below 3 mEq/kg. Variation of PV also depends on the composition of feed, particularly its lipid profile which determines the amount and composition of fish lipid fatty acids. Orban *et al.* (2008), reported lipid content of *Pangasius* fillets were dominantly saturated fatty acids (41.1-47.8%) and weakly unsaturated fatty acids (12.5-18.85%) from total 1.1-3% of lipid content.

3.2.3 Total Volatile Based-Nitrogen (TVB-N) content

TVB-N content serves as an indicator for the assessment of the freshness of fish. Enzymes from spoilage microorganism, particularly proteolytic enzyme can metabolize the amino acids of the fish muscle producing a wide variety of volatile compounds resulting in off-flavours and odours. TVB-N in freshwater fish and their product commonly comes from ammonia (Tokur *et al.*, 2006).

TVB-N content of treated *Pangasius* fillets during refrigerated storage for 11 days shown in Table 3. The significant difference ($p < 0.05$) shows that C1 had the highest TVB-N content and show a rapid increase from 7.4 to 14.7 mgN/100g on day 0 to day 11. Abbas *et al.* (2009) explained that the higher a_w tend to support more microorganisms. It can be seen that the C1 had the highest amount of TVC which resulted in the highest TVB-N compared to others (Table 3).

During storage, the TVB-N content of all samples was below the limit of acceptability for fish; 35 mg N/100g muscle (Commission of the European Communities, 1995). TVB-N content values for *Pangasius* fillets remained consistently low during storage indicating that *Pangasius* fish may not produce nitrogenous compounds to the same during storage (Tobin and Gormley, 2016).

The addition of MTEO and LGEO to the soaking solution did not give a significant effect on reducing TVB-N content. Ahmad *et al.* (2012) reported that sea bass slices wrapped with gelatine film incorporated 25% lemongrass essential oil during the 12 days of storage at 4°C have a significant effect in lowering TVB-N. Meanwhile, in this study, the concentration of essential oil was much lower to give effect in TVB-N. The intrinsic properties of the food such as fat, protein, pH, as well as the environment (storage temperature, packaging, etc.), may influence the function of essential oils (Burt and Reinders, 2003). Factors such as low pH, temperature, O_2 concentrations; and high salt content enhance the antimicrobial effect of the essential oils. Meanwhile, low water activity and high content of protein and fat were one of the barriers for bacteria from essential oils (Gómez-Estaca *et al.*, 2010).

Table 2. Peroxide value of treated *Pangasius* fillet during cold storage

Sample	Storage (day)						
	0	2	4	6	9	11	
C1	0.33±0.04 ^a	0.55±0.21 ^a	0.50±0.20 ^a	0.77±0.29 ^a	0.85±0 ^a	0.63±0.00 ^a	
C2	0.36±0.04 ^a	0.68±0.49 ^a	0.46±0.18 ^a	0.61±0.40 ^a	0.68±0.40 ^a	0.35±0.16 ^a	
LG	0.10%	0.3±0.04 ^a	0.45±0.25 ^a	0.48±0.02 ^a	0.69±0.08 ^a	0.81±0.58 ^a	0.61±0.30 ^a
	0.20%	0.33±0.14 ^a	0.65±0.16 ^a	0.51±0.02 ^a	0.73±0.37 ^a	0.53±0.33 ^a	0.48±0.25 ^a
	0.30%	0.25±0.07 ^a	0.55±0.21 ^a	0.65±0.44 ^a	0.58±0.35 ^a	0.5±0.33 ^a	0.56±0.47 ^a
	0.40%	0.26±0 ^a	0.6±0.37 ^a	0.56±0.18 ^a	0.4±0.23 ^a	0.45±0.16 ^a	0.7±0.42 ^a
MT	0.10%	0.25±0.02 ^a	0.65±0.35 ^a	0.7±0.28 ^a	0.71±0.40 ^a	0.78±0.40 ^a	0.53±0.28 ^a
	0.20%	0.18±0.11 ^a	0.75±0.44 ^a	0.86±0.51 ^a	0.61±0.35 ^a	0.81±0.44 ^a	0.46±0.04 ^a
	0.30%	0.33±0.04 ^a	0.3±0.09 ^a	0.51±0.21 ^a	0.48±0.11 ^a	0.9±0.23 ^a	0.43±0.09 ^a
	0.40%	0.31±0.21 ^a	0.5±0 ^a	0.61±0.11 ^a	0.53±0.23 ^a	0.65±0.11 ^a	0.48±0.07 ^a

C1: Control without soaking; C2: Control soaking without essential oil; MT: Soaking with mint essential oil; and LG: Soaking with lemongrass essential oil. Values followed by the same letter within the same row are not significantly different from each other ($p > 0.05$).

Table 3. TVB-N of treated *Pangasius* fillet during cold storage

Sample	Storage (day)						
	0	2	4	6	9	11	
C1	7.36±2.67 ^a	10.6±1.03 ^a	10.9±1.19 ^a	12.3±0.74 ^a	11.8±0 ^a	14.7±0 ^a	
C2	6.72±0.59 ^a	7.98±2.37 ^a	9.87±2.67 ^a	9.67±1.78 ^{ab}	9.14±0.44 ^a	9.03±0.89 ^b	
LG	0.10%	5.46±1.18 ^a	6.51±1.48 ^a	9.03±0.23 ^a	9.67±1.19 ^{ab}	10.9±0 ^a	10.7±0.89 ^{ab}
	0.20%	6.72±2.37 ^a	6.93±0.29 ^a	8.40±0 ^a	8.83±1.19 ^{ab}	7.98±0.59 ^a	8.30±1.93 ^b
	0.30%	5.88±1.19 ^a	7.35±3.26 ^a	8.93±0.45 ^a	7.78±1.49 ^b	9.24±2.37 ^a	8.19±0.89 ^b
	0.40%	6.51±2.08 ^a	7.98±1.18 ^a	8.40±0.6 ^a	9.46±0.89 ^{ab}	10.9±0.59 ^a	9.24±1.18 ^b
MT	0.10%	5.67±3.27 ^a	8.19±0.89 ^a	8.41±1.78 ^a	9.04±0.89 ^{ab}	9.77±2.52 ^a	8.82±1.18 ^b
	0.20%	5.25±2.09 ^a	7.35±0.29 ^a	8.2±0.29 ^a	9.46±0.89 ^{ab}	9.25±1.18 ^a	11.9±1.48 ^{ab}
	0.30%	5.67±0.89 ^a	8.40±2.37 ^a	8.62±1.48 ^a	8.41±0 ^{ab}	10.5±0 ^a	9.14±1.33 ^b
	0.40%	6.93±3.86 ^a	7.98±0.59 ^a	8.30±0.45 ^a	11.0±1.04 ^{ab}	9.9±0.29 ^a	9.66±0.59 ^b

C1: Control without soaking; C2: Control soaking without essential oil; MT: Soaking with mint essential oil; and LG: Soaking with lemongrass essential oil. Values followed by the same letter within the same row are not significantly different from each other ($p>0.05$).

3.3 Sensory evaluation

The overall score of cooked treated *Pangasius* fillets is shown in Table 4. There is no significant difference ($p>0.05$) between each sample during storage. However, C1 showed significant overall sensory degradation during storage compared to the others. C1 was categorized as unacceptable on day 9. Although C2 was still within the acceptable limit on day 9, sensory evaluation ended on that day since it has reached the limit of acceptability in terms of microbial load. The soaking solution with or without EO contributes to a higher preference of panellists, as a result of STPP which increased the water retention in the fillets to make the fillets juicier and a better appearance (Gonçalves *et al.*, 2018). Therefore, to keep overall sensory of fillets within an acceptable score until 11 days, the addition of LGEO from 0.2%-0.4% and MTEO into the soaking solution is favourable. The microorganisms as the spoilage bacteria of fillets contribute to the degradation of odour,

appearance, texture, and taste (Jouki *et al.*, 2014). The natural compounds of each EO reduce the action of endogenous enzymes and microbial activity, which also inhibit microbial-induced softening in the fillets (Cai *et al.*, 2015). The strong volatile compounds such as menthol and citral in both MTEO and LGEO capable to disguise the fishy odour of fillets. Another advantage of soaking is the NaCl, sorbitol, STPP, and EO contribute to the flavour which enriched the taste of treated fillets compared to C1 (Abbas *et al.*, 2009; Yusop *et al.*, 2011).

In this study, it is evident that it takes a longer period of storage for the panellist to figure out the spoilage in the fillets. Therefore, Mai and Huynh (2017) suggested that the shelf life of *Pangasius* fillets stored at low temperatures should be based on an acceptable level of TVC instead of sensory evaluation, especially sensory quality index.

4. Conclusion

Table 4. Overall sensory changes of cooked *Pangasius* fillet during cold storage

Sample	Storage (day)						
	0	2	4	6	9	11	
C1	14.3±1.29 ^a	13.9±2.47 ^a	12.5±0.99 ^a	12.0±0.47 ^a	6.08±0 ^a		
C2	15.9±0.11 ^a	15.0±2.15 ^a	14.6±1.7 ^a	13.9±0.11 ^a	14.5±0 ^a		
LG	0.10%	14.7±2.23 ^a	15.0±2.38 ^a	15.5±0.99 ^a	14.4±0.58 ^a	11±0 ^a	10±0 ^a
	0.20%	16.1±0.46 ^a	14.5±3.09 ^a	15.7±0.42 ^a	15.1±1.41 ^a	14.1±2.12 ^a	13.7±0.29 ^a
	0.30%	15.0±0.34 ^a	13.7±0.90 ^a	14.8±1.41 ^a	15.0±0.35 ^a	15.1±1.27 ^a	13.1±1.92 ^a
	0.40%	14.9±0.43 ^a	14.9±1.12 ^a	15.7±0.42 ^a	13.7±1.39 ^a	15.1±1.65 ^a	13.7±0 ^a
MT	0.10%	17.2±1.03 ^a	15.8±2.22 ^a	14.1±0.99 ^a	14.5±0.23 ^a	15.4±0 ^a	14.4±0 ^a
	0.20%	16.6±0.94 ^a	15.9±1.14 ^a	16.2±1.7 ^a	14.9±1.76 ^a	15.4±1.29 ^a	14.4±0 ^a
	0.30%	18±0.94 ^a	15.2±0.35 ^a	15.2±1.41 ^a	15±2.59 ^a	14.8±0 ^a	15.0±0 ^a
	0.40%	16.1±0.24 ^a	16.7±0.64 ^a	13.4±1.02 ^a	15.6±0.47 ^a	17±0 ^a	14.0±0.04 ^a

C1: Control without soaking; C2: Control soaking without essential oil; MT: Soaking with mint essential oil; and LG: Soaking with lemongrass essential oil. Values followed by the same letter within the same row are not significantly different from each other ($p>0.05$).

The incorporation of essential oil to the soaking solution indicated that lemongrass and mint essential oil improves the quality of *Pangasius* fillet. However, the lemongrass essential oil has a better performance in inhibiting microbial activity and also inhibiting TVB-N formation compared to the mint essential oil. Therefore, it is recommended to incorporate 0.2% lemongrass essential oil for the *Pangasius* fillets to extend the shelf life during cold storage.

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