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Antioxidative and antimicrobial activities of liquid smoke nanocapsules using chitosan and maltodextrin and its application on tuna fish preservation

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ABSTRACT

The aim of the study was to investigate the antioxidative and antimicrobial activities of liquid smoke (LS) nanocapsules from coconut shell using chitosan and maltodextrin as encapsulants and its application on tuna fish preservation. Nanocapsules were prepared by three variations of chitosan (CS)–maltodextrin (MD) i.e. CS (0.5% w/v) and MD (9.5% w/v) in acetic acid (1.0% v/v), only MD (10% w/v) in LS, and a mixture of CS (1.5% w/v) and MD (8.5% w/v) in LS. The experimental factor of varying nanocapsules concentration 0%; 2.5%; 5.0%; 7.5% and 10% w/w was used to evaluate the antioxidative and antimicrobial activities in fresh tuna fish stored at ambient temperature for 0, 12, 24, 36 and 48 h. Fresh tuna minced fish was observed for TVB-N, TPC and sensory evaluation. Results indicated that nanocapsules mixture of CS and MD in LS showed higher value in parameters evaluated i.e. total phenolic, total acid and radical scavenging activity. The addition of nanocapsules prepared of a mixture of CS (1.5% w/v) and MD (8.5% w/v) in LS higher than 5.0% could maintain the fish freshness until 48 h at room temperature and had the smallest particle size. Based on sensory evaluation, the score was neither like nor dislike for all nanocapsules concentrations. The results suggested that LS nanocapsule was an effective preservative agent for fresh tuna fish; therefore these nanocapsules are promising for food applications.

1. Introduction

Liquid smoke (LS) is usually obtained from the condensation of wood smoke produced by smoldering wood chips or sawdust under limited oxygen. Commercial LS is commonly fractionated, purified and concentrated to yield aqueous, oil or dry powder products. Through the refining process, undesirable polycyclic aromatic hydrocarbons (PAH) are removed, and the intensity of flavor and color in the refined LS is easily adjusted (Varlet, Serot, & Prost, 2010). Refined LS generally offers more flexible applications to particular food systems when compared with LS products (Martinez, Salmerón, Guillén, & Casas, 2007).

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The main purpose of LS application in proteinaceous food products is not only to act as a coloring and flavoring agent; but also to possess antibacterial and antioxidative properties (Darmadji & Izumimoto, 1994; Varlet et al., 2010). Various phenolic compounds present in LS lead to a lower pH and destroy the bacterial cell walls (Pszczola, 1995). LS from coconut shells has been reported to contain bioactive compounds such as phenols, carbonyls and organic acids. Therefore, the coconut shell LS has the potential in increasing the shelf life of proteinaceous food products (Zuraida, Sukarno, & Budijanto, 2011).

The bioactive compounds of LS need to be protected against deterioration during the process through the nanoencapsulation technique. Nanoencapsulation of bioactive compounds represents a viable and efficient approach to increase the physical stability of active substances, protecting them from the interactions with the food ingredients, and because of the subcellular size, increasing their bioactivities (Chuah, Kuroiwa, Ichikawa, Kobayashi, & Nakajima, 2009; Quintanilla-Carvajal et al., 2010; Sekhon, 2010).

Various biocompatible and degradable natural polymers can be employed in the nanoencapsulation technique. Chitosan (CS) has been used as a wall material for encapsulation of sensitive core ingredients such as lipophilic drugs (Ribeiro et al., 1999), vitamin D_2 (Shi & Tan, 2002), astaxanthin (Higuera-Ciapara, Felix-Valenzuela, Goycoolea, & Arguelles-Monal, 2004), and ampicillin (Anal, Stevens, & Remuan-López, 2006). Another wall material is maltodextrin (MD) that most commonly uses material for encapsulation of bioactive material because of low cost and effectiveness. MD is a water-soluble material and able to protect encapsulated ingredient from oxidation. Some studies have explored the use of MD to protect sensitive compounds like vitamin C in fruit juice and to increase product stability in acerola powder (Righetto & Netto, 2005). A recent study found that MD can enhance the phenolic and anthocyanin content during processing of purple sweet potato flour compared to without using MD (Ahmed, Akter, & Eun, 2010).

A previous study demonstrated the ability of CS assembled into nanoparticles of 400–600 nm (Grenha et al., 2010). Preparing nanoparticles in this size range is facilitated by the use of adequate cross-linking agents. TPP is a non-toxic polyanion known for its capacity to cross-link CS, a reaction mediated by electrostatic forces, resulting in the formation of ionic cross-linked networks (Rodrigues, Rosa da Costa, & Grenha, 2012).

In the present study, CS and MD have been chosen as the polycationic and anionic matrices. However, there is no study reporting the nanoencapsulation of LS components and a little is known on CS–MD as encapsulant at different concentrations into LS for antioxidative and antimicrobial activities. The aims of this study were to investigate antioxidative and antimicrobial activities of LS nanocapsules from coconut shell using CS and MD as encapsulant and its application on tuna fish preservation.

2. Materials and methods

2.1. Materials

Raw coconut shell liquid smoke used was obtained from Tropica Nucifera Industry, Yogyakarta, Indonesia. Liquid smoke was purified using the redistilation method in the laboratory. Chitosan (CS) was purchased from Biotech Surindo, Indonesia (deacetylation degree 91.5%, moisture 10.43%, ash content 0.71%). Maltodextrin (MD) with dextrose equivalent (DE) 10.8% was from the Grain Processing Corp. (Iowa, USA), Sodium tripolyphosphate (TPP) and glacial acetic acid (HOAc) were supplied by Sigma Chemicals Ltd. (Munich, Germany). The other chemicals used for analysis were of analytical grade. *Bacillus subtilis* FNCC 0059, *Escherichia coli* FNCC 0091, *Pseudomonas fluoroscens* FNCC 0070 and *Staphylococcus aureus* FNCC 0047 were obtained from the Food and Nutrition Culture Collection (FNCC), Center for Food and Nutrition Study, Gadjah Mada University, Yogyakarta, Indonesia. Tuna fish (*Euthynnus affinis*) was obtained from a commercial fish processing plant in Sadeng, Gunung Kidul Regency Yogyakarta, Indonesia.

2.2. Preparation of nanocapsules

CS–MD mixed nanoparticles were prepared with a slight modification of previously described method of Grenha et al. (2010), based on the polyelectrolyte complexation of CS with MD and additional ionic gelation of chitosan with sodium tripolyphosphate (TPP) anions. CS (0.5% w/v) and MD (9.5% w/v) dispersed in an aqueous solution of glacial acetic acid (1.0% v/v) were prepared and referred to as F1 sample code. In addition, CS and MD dissolved in coconut shell liquid smoke were also prepared at a ratio of CS: MD (0%: 10%) and referred to as F2 sample code, and (1.5%: 8.5%) referred to as F3 sample code.TPP (1.0 mg/mL) was added in these mixtures and agitated using a magnetic stirrer at 200 rpm for 30 min at room temperature. Nanoparticles were isolated by centrifugation (Centrifuge Damon/IEC Division, Connecticut, USA) at 3000 rpm in a 50 mL conical tube for 30 min at room temperature. Supernatant was discarded and nanoparticles were vacuum filtered (Gast, USA) using Whatman #2. The dispersed nanoparticles were heated at 50 °C in a waterbath for 15 min and were homogenized using homogenizer (Ultraturrax T50 Basic IKA Werke, Germany) at 4000 rpm for 2.5 min. Subsequently, the dispersed sample was fed into a Büchi B-290 mini spray dryer (Flawil, Switzerland) for drying. The operating conditions were aspirator rate 50%, drying inlet air temperature a 150 °C (±2 °C), while the outlet air temperature varied between 70 and 82 °C, feed flow rate was set at 5.1 mL/min. Then, the spray-dried powders were collected, kept in amber bottles and stored under desiccated conditions at room temperature prior to application.

2.3. Chemical analyses of nanocapsules

Phenol content of nanocapsules was determined following the procedure established by Senter, Robertson, and Meredith (1989) and carbonyl was quantified according to Lappin and Clark (1951). Total acidity was determined by using titration, by weighing 1 mg of sample in a 250 ml beaker diluted with 100 ml of distilled water. Then, it was titrated with 0.1 N sodium hydroxide solution to an equivalence point of pH 8.15, as determined by using a pH meter. Acidity was calculated as percent by weight of acetic acid using the factor: 1 ml of 0.1 N sodium hydroxide is equivalent to 60.05 mg acetic acid. A pH-meter (Schott, Deutschland,

Germany) was used to determine the pH of the nanocapsules at 26 °C.

Scavenging effect of phenolic compounds present in the nanocapsules on DPPH radical was monitored according to the method described by Yen and Chen (1995). A 0.1 ml of methanolic solution containing 0.4–2.0 mg of nanocapsules was mixed with 2 ml of methanol. A methanolic solution of α,α-diphenyl-β-picrylhydrazyl (DPPH) (1 mM, 0.250 ml) was then added. The mixture was vortexed for 15 s, and left to stand at room temperature for 30 min, and the absorbance of this solution was read at 517 nm.

2.4. Particle size measurement of nanocapsules

Particle size measurement of nanocapsules was done by suspending them in distilled water. The nanocapsules were measured using a laser particle size distribution analyzer (Malvern Zetasizer Nanoseries Nano ZS Ver 6.20, Malvern Instruments Ltd., Malvern, UK). The size distribution was determined by the span value. Triplicate measurements were conducted.

2.5. Antimicrobial assessment of nanocapsules

The antimicrobial activity of liquid smoke nanocapsules was determined by the agar disk diffusion method against *B. subtilis* FNCC 0059, *E. coli* FNCC 0091, *P. fluoroscens* FNCC 0070 and *S. aureus* FNCC 0047 by measuring the inhibition area. The culture medium used was Trypticase Soy Broth (TSB) (Cat. 129, Merck, Germany) and Mueller-Hinton Agar (MHA) (Cat. 405, Merck, Germany) autoclaved at 121° C × 15 min (MAC-5100, Eyela, Japan). Microorganisms were reactivated in TSB. Swab was distributed evenly over the agar surface; an inoculum in a tube with turbidity no. 0.5 (1×10^8 CFU/ml) of the scale was subsequently deposited with Mac Farland disks on the plate. Then, the agar plates were incubated at 37 °C for 24 h (MIR-262, Sanyo, Japan). After the incubation period, the antimicrobial activity was evaluated by observing the formation of inhibition zones and the diameter was measured in mm.

2.6. Application of nanocapsules on tuna fish preservation

After slaughter (minced cut and bleeding), the fish were eviscerated, cleaned and immediately transported to the laboratory in a cool box for preparation of the experimental samples. The fish were ground using blender and each weighing 100±0.3 g was wrapped individually in cling film. Each bag was then added with nanocapsules of different concentrations i.e. 0%, 2.5%, 5.0%, 7.5% and 10% (w/w). Then, it was stored at a room temperature for 0, 12, 24, 36 and 48 h until analysis. The fish were evaluated for total volatile based nitrogen, total plate count and sensory evaluation.

2.7. Total volatile basic nitrogen (TVB-N) of tuna fish

The TVB-N content of tuna fish minced was determined according to the method of Cobb, Alaniz, and Thompson (1973) with a slight modification. TVB-N sample extract in TCA 7% was absorbed in boric acid solution during incubation at 35 °C for 2 h. Then, it was titrated with 0.02 N HCl and the value was expressed as mg N per 100 g fish muscle.

2.8. Total plate count of tuna fish

Five grams of each homogenated sample was diluted with 45 ml of NaCl 0.85%, followed by serial dilutions (1:10) until 10^{-7} g/ml of sample was obtained. One milliliter of diluted aliquots was placed on Petridishes and approximately 15 ml of plate count agar (Cat. 0325, Oxoid, UK) was added. After the agar was solidified, all Petridishes were inverted and placed in an oven at 37 °C for 48 h (MIR-262, Sanyo, Japan). Colony forming units were counted based on ISO 11290-1:1997 for microbiological methods (Harrigan, 1998).

2.9. Sensory evaluation of tuna fish

Minced fresh tuna fish (*E. affinis*) that had been added with nanocapsules was determined for a preference using 5-point hedonic scale (Meilgaard, Civille, & Carr, 1999). Fish fresh minced without nanocapsules liquid smoke was used as the control. Twenty panelists were asked to evaluate the odor of the samples. A score of 5 was defined as very pleasant, a score 4 was defined as pleasant, a score of 3 was defined as neither like nor dislike, a score of 2 was defined as unpleasant and a score of 1 was defined as very unpleasant for human consumption.

2.10. Statistical analysis

The differences between the mean values of multiple groups were analyzed by one-way analysis of variance (ANOVA) with Tukey methods range tests. ANOVA data with a $P<0.05$ was classified as statistically significant. MINITABS 16.0 software, Origin 75 and Microsoft Excel 2007 programs were used.

3. Results and discussion

3.1. Characteristics of liquid smoke nanocapsules

The characteristics of liquid smoke nanocapsules are summarized in Table 1. The incorporation of coconut shell liquid smoke in CS and MD based nanoparticles increases the total phenol, total acid, pH and radical scavenging activity. On the other hand, the carbonyl content had a lower value. As observed in Table 1, the total phenol content of sample F1 was 0.17% and increased significantly compared to samples F2 and F3 of 5.27% and 5.46%, respectively. Coconut shell liquid smoke nanocapsules contain phenolic compounds such as phenol, 2-methoxyphenol (guaiacol), 4-methylphenol (p-cresol) and dimethylphenols (data not shown) that provide aromatic and antioxidant effects. This result was similar to the earlier reports (Kristinsson, Danyali, & Ua-Angkoon, 2007; Soldera, Sebastianutto, & Bortolomeazzi, 2008) in which phenolic compounds are prominent in liquid smoke and play a major contribution to antibacterial activity. In addition, Varlet et al. (2010) found the phenolic compounds contribute to smoke flavor and color of

Table 1 – Characteristics of produced liquid smoke nanocapsules.

Formulation	Total phenol (%)	Carbonyl (%)	Total acid (% acetic acid)	pH	Radical scavenging activity (%)	Particle size (nm)
F1	0.17 ± 0.00^{a}	2.84 ± 0.02^{a}	1.01 ± 0.00^{a}	2.49 ± 0.01^{a}	7.51 ± 0.16^{a}	16.08 ± 0.20^{a}
F2	5.27 ± 0.01^{b}	2.20 ± 0.01^{b}	4.23 ± 0.03^{b}	2.20 ± 0.01^{b}	48.12 ± 0.08^{b}	14.87 ± 0.16^{b}
F3	5.46 ± 0.00^{c}	1.01 ± 0.01^{c}	6.72 ± 0.01^{c}	2.49 ± 0.01^{a}	58.97 ± 0.16^{c}	13.43 ± 0.68^{c}

Note:
F1=CS (0.5% w/v)+MD (9.5% w/v) in an aqueous solution of glacial acetic acid (1.0% v/v).
F2=Only MD (10% w/v) in coconut shell liquid smoke.
F3=CS (1.5% w/v)+MD (8.5% w/v) in coconut shell liquid smoke.
Reported means (±standard deviations) derived from 3 replications with 3 samples per replication.
Means within column followed by different superscripts are significantly different at $P<0.05$ by Tukey Methods range test.

liquid smokes, and also posses antibacterial and antioxidant properties.

Liquid smoke consists of carbonyls that can interact with muscle foods, affect their textural properties and ultimately provide consumer acceptance (Martinez et al., 2007). Carbonyl compounds in the smoke have a role in coloring and flavoring the products. In this study, the carbonyl value of the sample F1 was approximately 2.84% and tends to decrease in F2 and F3, i.e. 2.20% and 1.01%, respectively. Visually, the coconut shell spray-dried nanoparticles were a white-yellowish powder. It was due to an ionic interaction between $-NH_3^+$ group of chitosan and carbonyls groups of liquid smoke. Carbonyls generally contribute to the color of the product once it reacts with amino groups. This result was similar to those reported by Guillen and Ibargoitia (1998) in several commercial aqueous smokes; those are reported to consist of carbonyl derivatives including aldehydes and ketones.

Organic acids are a result of the partial pyrolysis of wood cellulose and hemicellulose. Acidic compounds of liquid smoke showed antibacterial activity (Budijanto, Hasbullah, Prabawati, Setyadjit, & Zuraida, 2008). The acids content of sample F1 was 1.01% and increased significantly compared to samples F2 and F3 of 4.23% and 6.72%, respectively. Similarly, acetic acid was found in liquid smoke obtained from beechwood (Guillen & Ibargoitia, 1998) and it was the most abundant organic acid in liquid smoke prepared from walnut tree branches (Wei, Ma, & Dong, 2010). Acidity of liquid smokes depends on the wood source, processing steps and refining parameters (Sung, Huang, & Sun, 2007). Achmadi, Mubarik, Nursyamsi, and Septiaji (2013) mentioned that the content of organic acids in liquid smoke reached 9.2%. Acetic acid has been identified as the most prevalent organic acid present in smoke, followed by formic, propionic, butyric and other acids (Arnim & Marlida, 2012). Acids influence flavor (tartness), color, texture and microbial stability of food (Montazeri, Oliveira, Himelbloom, Leigh, & Crapo, 2013).

Liquid smoke is usually acidic with a pH of 1.5–5.5 (Toth & Potthast, 1984). The pH of the original coconut shell liquid smoke used in this study was 2.54. After processing smoke powder of CS–MD nanoparticles is obtained by using spray-drying; the pH of liquid smoke nanocapsules was in the range of 2.20–2.49. Our results are in agreement with Zuraida et al. (2011) and Achmadi et al. (2013) who obtained similar values of pH in the coconut shell and oil-palm shell liquid smoke, which were 2.0 and 3.2 respectively. Furthermore, the pH of some commercial liquid smoke products ranges from 2.3 to 5.7 (Montazeri et al., 2013).

As shown in Table 1, all samples of nanocapsules had strong DPPH free radical scavenging activity (7.51–58.97% inhibition). The highest radical scavenging activity was observed in coconut shell liquid smoke nanocapsules obtained by using chitosan 1.5% and maltodextrin 8.5% (sample F3) as wall material. In sample F3, the role of CS contributed for the increase in antioxidative activity due to the hydroxyl groups of phenolic compound liquid smoke. The antioxidative activity of nanocapsules was well correlated with the total phenolic content. A positive correlation was observed, whereby the antioxidant activity increased with the increase of the total phenolic content (Deladino, Anbinder, Navarro, & Martino, 2008).

3.2. *Particle size*

The mean particle size of nanocapsules is shown in Table 1. The average sizes of particles formed from F1, F2 and F3 were 16.08 nm, 14.87 nm, and 13.43 nm, respectively. Zhang, Oh, Allen, and Kumacheva (2004) reported that nanoparticle at a concentration level of 0.1% (w/w) in a mixture of low molecular weight of CS and TPP (weight ratio of 5:1) resulted in a bimodal particle size distribution ranging between 153 and 500 nm. In addition, Hu et al. (2008) found that the interactions between phenolic groups of LS and amino groups of CS (over phosphate group of TPP) may lead to decrease in the cross-linking density.

3.3. *Antibacterial activity*

Four strains of microorganisms were used for antibacterial activity test, namely *E. coli* and *P. fluorescens* (representing Gram negative) and *B. Subtilis* and *S. aureus* (representing Gram positive). Table 2 shows the antimicrobial test results performed against these bacteria by the agar disk diffusion method. The sample F1 showed no antimicrobial activity against the studied microorganisms. In addition, the effect of nanocapsule incorporated is expressed in Fig. 4. Nanocapsules of liquid smoke containing CS 1.5% (sample F3) were the most effective against microorganisms tested. The larger zones of inhibition were observed for the Gram negative bacteria (*P. fluorescens* and *E. coli*) while the lower for Gram positive (*B. subtilis* and *S. aureus*). The sample F1 containing

Table 2 – Antimicrobial activity, expressed as inhibition zone (mm), of liquid smoke nanocapsules against test microorganisms.

Formulation	*B. subtilis*	*E. coli*	*P. fluorescens*	*S. aureus*
F1	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
F2	12.00 ± 0.01^{b}	13.00 ± 0.01^{b}	17.00 ± 0.03^{b}	0.00 ± 0.00^{a}
F3	12.00 ± 0.00^{b}	13.00 ± 0.01^{b}	17.00 ± 0.01^{b}	0.00 ± 0.00^{a}

Note:
F1=CS (0.5% w/v)+MD (9.5% w/v) in an aqueous solution of glacial acetic acid (1.0% v/v).
F2=Only MD (10% w/v) in coconut shell liquid smoke.
F3=CS (1.5% w/v)+MD (8.5% w/v) in coconut shell liquid smoke.
Reported means (±standard deviations) derived from 3 replications with 3 samples per replication.
Means within column followed by different superscripts are significantly different at $P<0.05$ by Tukey Methods range test.

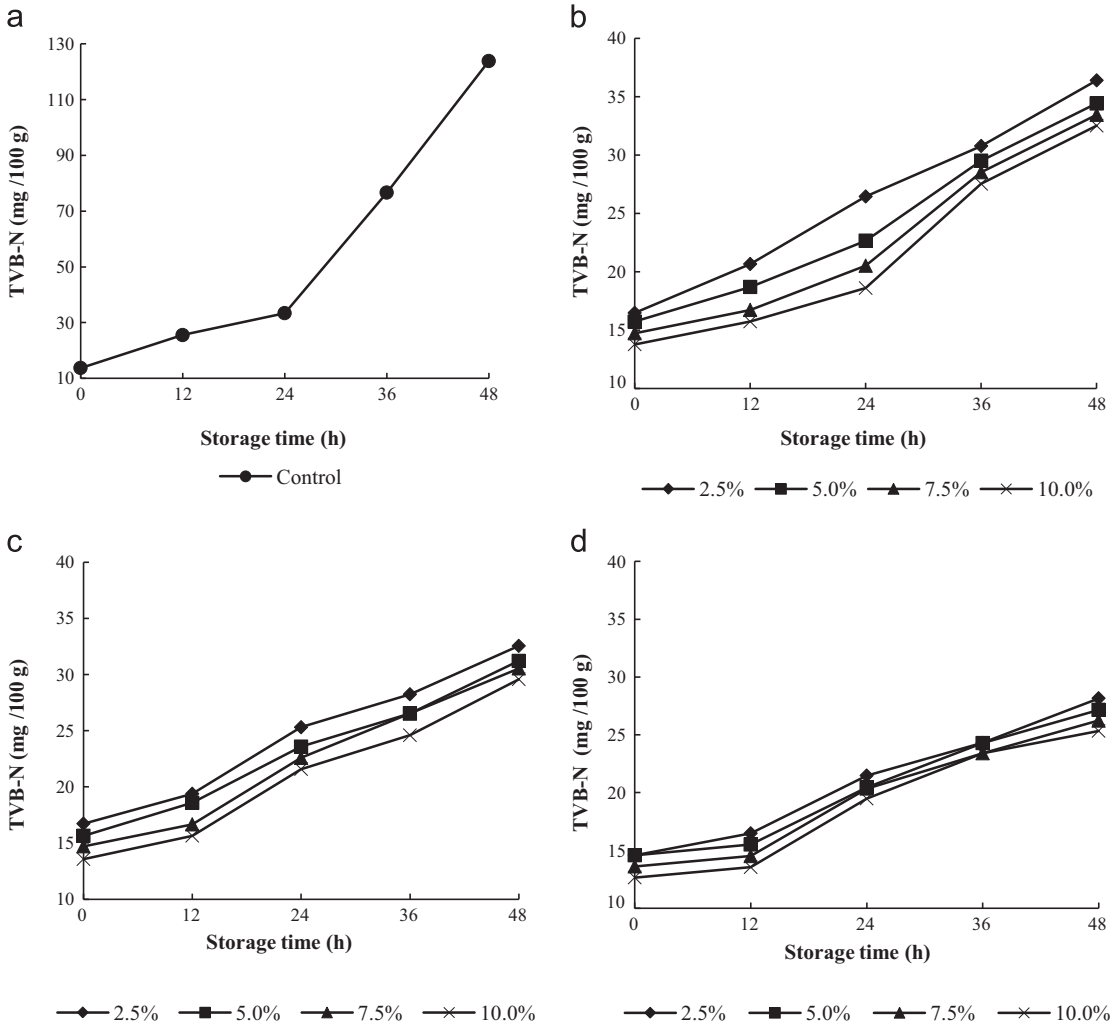

Fig. 1 – Total Volatile Base Nitrogen of liquid smoke nanocapsules-tuna fish during storage. (a) Sample control, (b) Sample F1=CS (0.5% w/v)+MD (9.5% w/v) in an aqueous solution of glacial acetic acid (1.0% v/v), (c) Sample F2=Only MD (10% w/v) in coconut shell liquid smoke, and (d) Sample F3=CS (1.5% w/v)+MD (8.5% w/v) in coconut shell liquid smoke.

CS 0.5% in acetic acid 1.0% was not effective against *S. aureus*. Numerous studies investigating the action of liquid smoke against pathogenic microorganisms agree that phenolic compounds are the most effective against Gram-negative bacteria rather than against Gram-positive bacteria (Pelissari, Grossman, Yamashita, & Pineda, 2009). Therefore, the clear zone formed on Gram negative is more distinct as compared with that on Gram positive. It is related to the effect of the liquid smoke on the bacterial growth. Based on the diameter of formed clear zone, the nanocapsule of coconut shell liquid smoke has antibacterial activity in a strong category with inhibition area of 10–20 mm (Davis & Stout, 1971).

3.4. *Total volatile basic nitrogen (TVB-N)*

Freshness is the most important attribute in assessing the quality of fish. One of the biochemical methods used to evaluate fish freshness is the total volatile basic nitrogen (TVB-N). TVB-N values of tuna fish preserved using nanocapsules liquid smoke are shown in Fig. 1. In all samples, the TVB-N value increased slowly during the first 12 h for tuna fish. Increasing TVB-N values was caused by the action of bacteria in the fish muscular tissue that produces ammonia, trimethylamine and dimethtylamine. With prolonging the storage time, the TVB-N values were also increasing. After 12 h, in untreated (sample control), the TVB-N value was 33.41 mg/100 g. However at concentration higher than 5.0% in sample F1 and sample F2 gave less than 30 mg/100 g. A less than 30 mg/100 g value has been specified for sample F3 which was no higher than 28.16 mg/100 g for all treated tuna fish with nanocapsules during 48 h of storage at room temperature (28 °C). Our results are in agreement with Ozogul and Ozogul (2000) who obtained similar TVB-N values in the rainbow trout during 6 days of storage in wrapped cling film. In addition, Achmadi et al. (2013) reported that pomfret fish preserved with various concentrations of liquid smoke 8%, 9%, 10% were still below the maximum permissible value.

According to Oehlenschlager (1992) when the concentration of TVB-N exceeds 30 mg/100 g flesh, the fish should be considered unfit for consumption. Hence, TVB-N can be used only as an indicator of fitness for consumption rather than as an index of freshness throughout the storage life of fish.

3.5. *Total plate count*

Fig. 2 highlights TPC values of nanocapsules-tuna fish during storage at room temperature (28 °C) at different concentrations. The total microorganism growth of fresh tuna fish before storage was 4.72 log CFU/g. After 12 h of storage, significant increase in microbial growth was observed in the untreated (sample control), giving 6.81 log CFU/g. This would

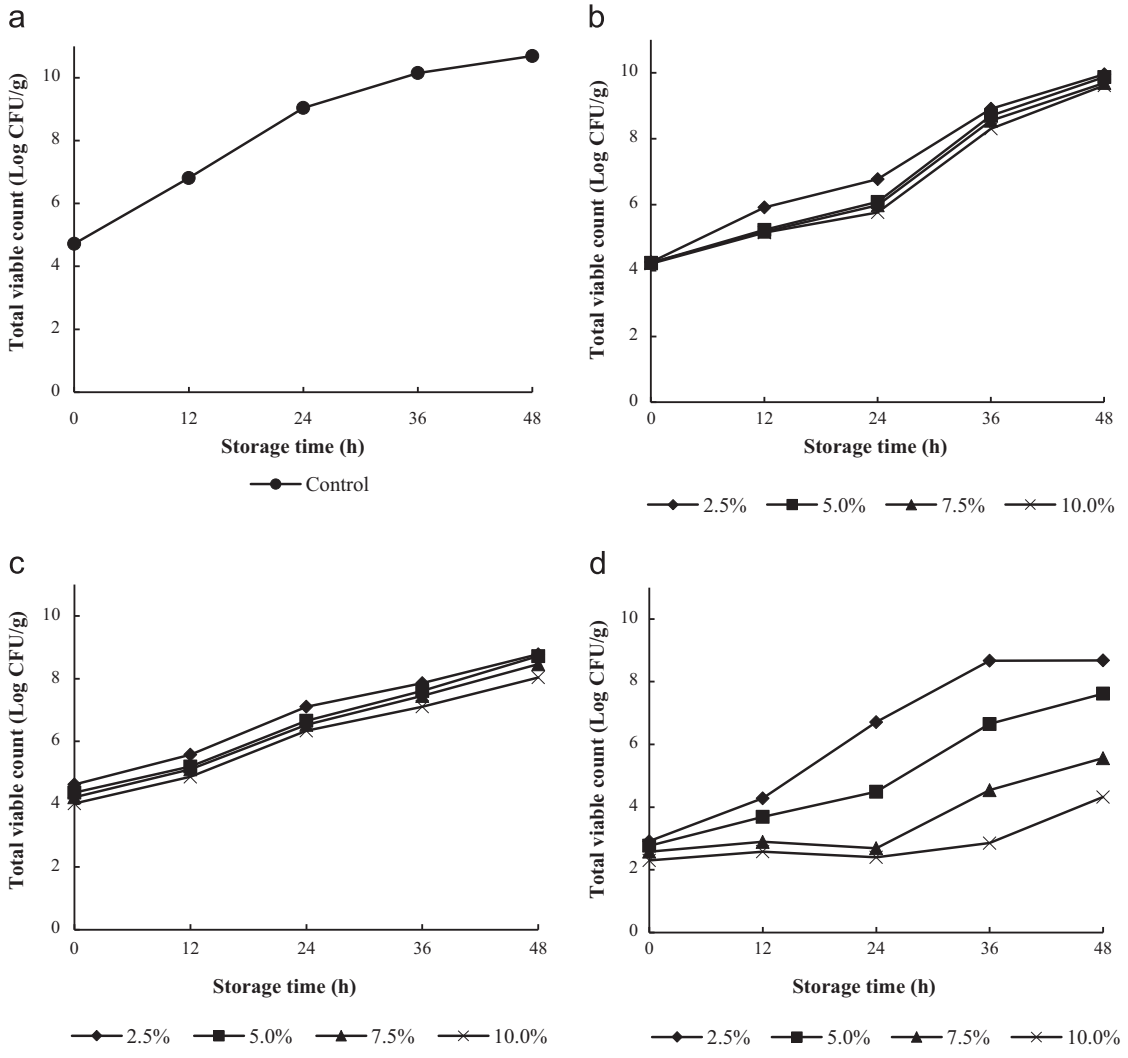

Fig. 2 – Microbial growth of liquid smoke nanocapsules-tuna fish during storage. (a) Sample control, (b) Sample F1=CS (0.5% w/v)+MD (9.5% w/v) in an aqueous solution of glacial acetic acid (1.0% v/v), (c) Sample F2=Only MD (10% w/v) in coconut shell liquid smoke, and (d) Sample F3=CS (1.5% w/v)+MD (8.5% w/v) in coconut shell liquid smoke.

indicate spoilage and possible rejection of the sample. It has been advised that microbiological threshold of ready to eat fish products is 6.00 log CFU/g (ICMSF, 1986). After 48 h storage at room temperature, TPC of untreated sample was very high (10.69 log CFU/g). Inhibitory effect on microbial growth was still shown after 24 h at concentration of 5.0% in sample F1 giving 6.08 log CFU/g and sample F2 giving 6.66 log CFU/g. Meanwhile, at the same concentration in F3 treated tuna fish showed 6.65 log CFU/g after 36 h of storage. Higher concentration of nanocapsules applied led to reduced microbial growth. In sample F3 at concentration 7.5% resulted in 5.56 log CFU/g after 48 h. It led to three log reductions on nanocapsules treated sample compared to sample control. Therefore, sample F3 was able to extend the shelf life of tuna fish 48 h longer than untreated (sample control) stored at room temperature (28 °C).

3.6. *Sensory assessment*

Sensory characteristics of whole fish are clearly visible to consumers and sensory methods are still the most satisfactory means for assessing the freshness quality since it gives the best idea of consumer acceptance. Coconut shell liquid smoke nanocapsules contain phenols, carboxylic acids, hydrocarbons among the mixture of various chemical components which can impart a smoky flavor to the product (Guillen, Manzanos, & Zabala, 1995).

Intensity of odor attributes to samples stored in room temperature was relatively stable during the test period as shown in Fig. 3. Odor profiles of samples F1, F2 and F3 did not change significantly. The lowest change of odor profile during 48 h storage was shown in sample F1 at a concentration of 2.5% stored at room temperature with average hedonic score of 2.56. Meanwhile, the highest hedonic score of sample was in sample F2 at a concentration 7.5% with average hedonic score of 3.14. The role of liquid smoke in fish enriched the aromatic compounds contained in the smoke. Toth and Potthast (1984) stated that the treatment of fish with liquid smoke leads to changes not only in its physicochemical but also in sensorial attributes.

4. Conclusion

Coconut shell liquid smoke nanocapsules had chemical specifications of phenol 5.46%, carbonyl 1.01%, total acids 6.72%, pH 2.49, radical scavenging activity 58.97%, particle size 13.43 nm, and the coconut shell liquid smoke nanocapsules are a potential safe preservative for fish. Application of 5% liquid smoke nanocapsules, extends the fish freshness up to 24 h at room temperature than that of without treatments. The coconut shell liquid smoke nanocapsules are bacteriostatics in nature and shows strong inhibition toward bacterial growth. The highest antimicrobial activity was performed against *E. coli* and *P. fluorescens* which represent Gram negative. Therefore, the nanocapsules liquid smoke of coconut shell can be applied as a natural fish preservative.

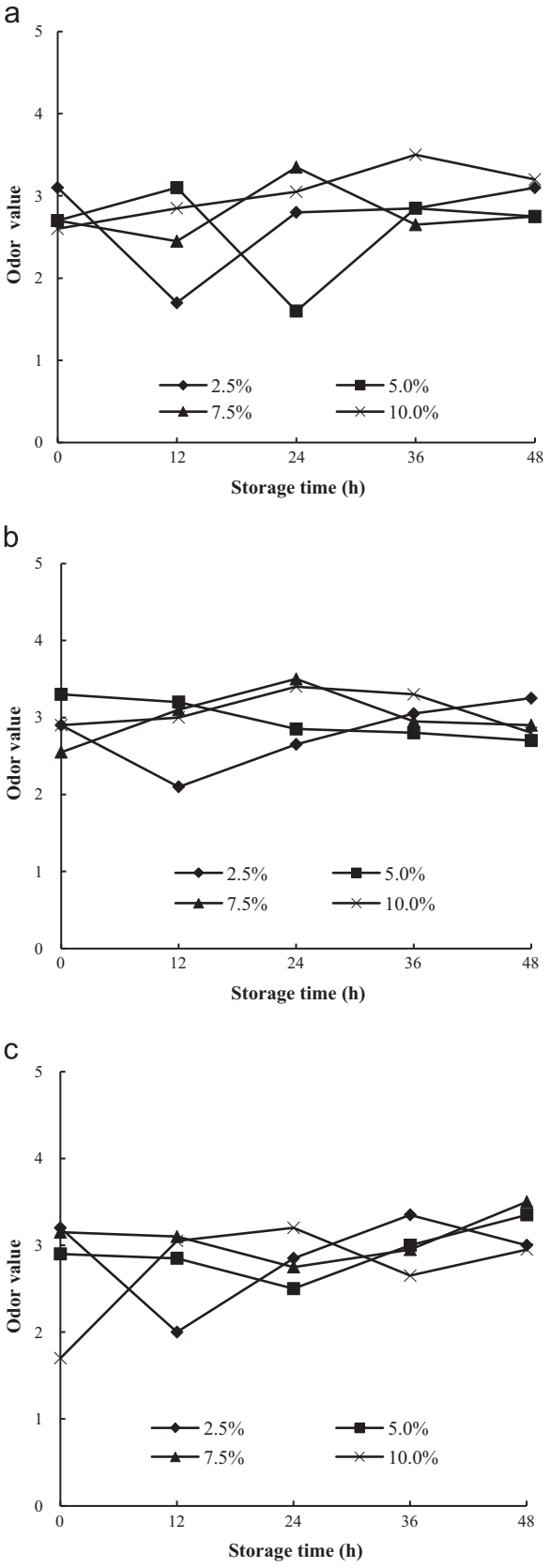

Fig. 3 – The odor of liquid smoke nanocapsules-tuna fish during storage. (a) Sample F1=CS (0.5% w/v)+MD (9.5% w/v) in an aqueous solution of glacial acetic acid (1.0% v/v), (b) Sample F2= Only MD (10% w/v) in coconut shell liquid smoke, and (c) Sample F3=CS (1.5% w/v)+MD (8.5% w/v) in coconut shell liquid smoke.

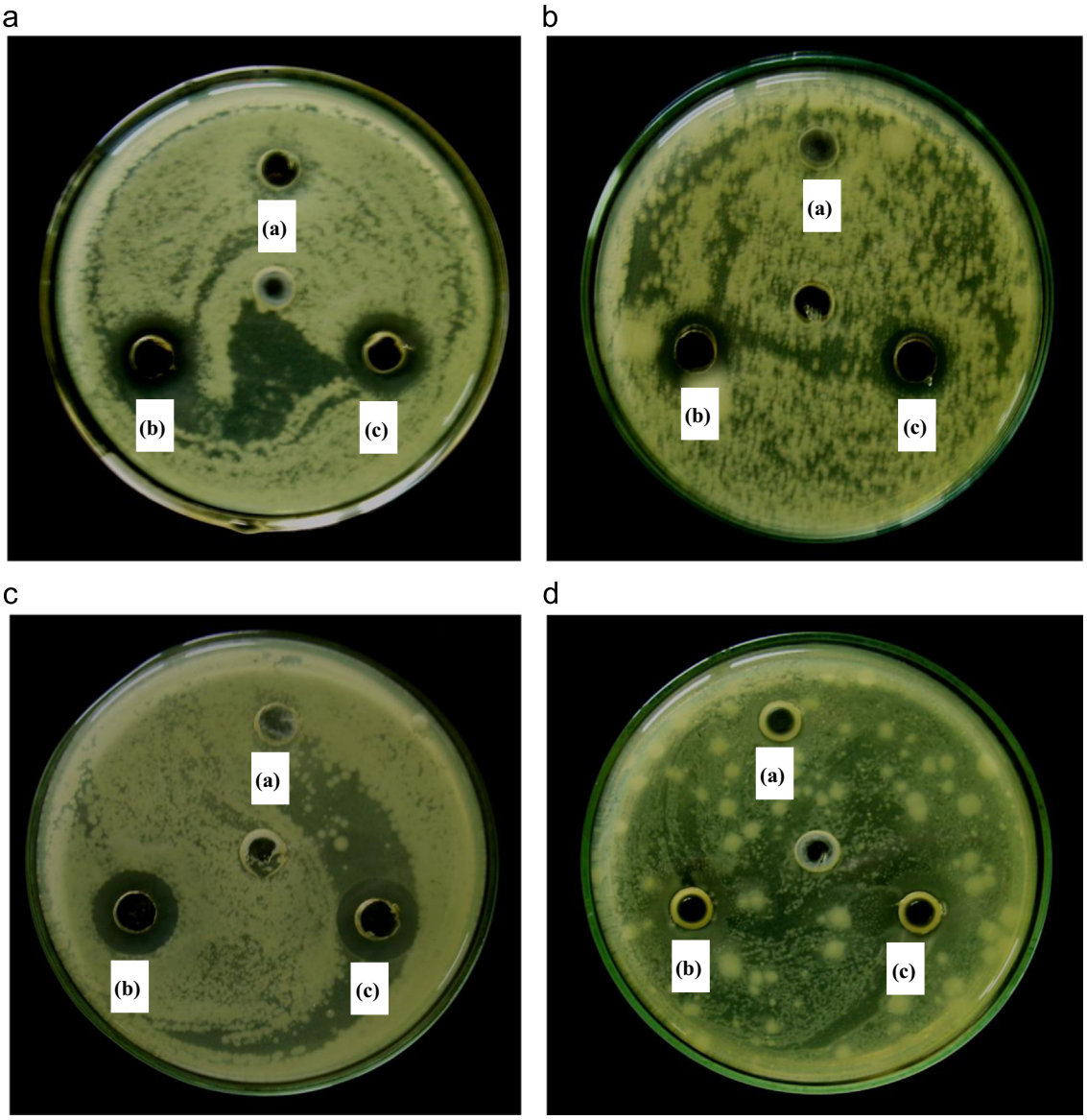

Fig. 4 – Inhibition zones against the microorganisms (A) *Bacillus subtilis*, **(B)** *Escherichia coli*, **(C)** *Pseudomonas fluorescens* **(D)** *Staphylococcus aureus* **for nanocapsules. (a) Sample F1=CS (0.5% w/v)+MD (9.5% w/v) in an aqueous solution of glacial acetic acid (1.0% v/v), (b) Sample F2=Only MD (10% w/v) in coconut shell liquid smoke, and (c) Sample F3=CS (1.5% w/v)+MD (8.5% w/v) in coconut shell liquid smoke.**

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