1. Submission received in MDPI Journal(9-2-2019)
2. Manuscript rejected in MDPI Journal (11-2-2019)
3. Decline submission in Jurnal Teknologi (7-7-2019)
    - Put article into layout using the latest template
    - Required a minimum 50 references and tagged in the article
    - Follow this path : 1.0 Introduction, 2.0 Metodhology, 3.0 Results and Discussion, 4.0 Conclusion
4. Peer reviewed in AACL Bioflux (15-10-2019)
    - Preliminary acceptance letter AACL
5. Review process (4-12-2019)
6. Revised process (24-12-2019)
7. Received the manuscript (29-12-2019)
8. Published (30-12-2019)

Fwd: [Nutrients] Manuscript ID: nutrients-452632 - Submission Received

Dari: Retno Setyowati (retnosetyo@fk.undip.ac.id)

Kepada: fronthea_thp@yahoo.co.id

Tanggal: Selasa, 12 Februari 2019 07.44 WIB

---------- Forwarded message ---------
From: **fronthea swastawati** <fronthea.sw.undip@gmail.com>
Date: Sen, 11 Feb 2019 pukul 20.45
Subject: Fwd: [Nutrients] Manuscript ID: nutrients-452632 - Submission Received
To: <retnosetyo@fk.undip.ac.id>

---------- Forwarded message ---------
From: **Editorial Office** <nutrients@mdpi.com>
Date: Sab, 9 Feb 2019 14:48
Subject: [Nutrients] Manuscript ID: nutrients-452632 - Submission Received
To: Fronthea Swastawati <fronthea.sw.undip@gmail.com>
Cc: Fronthea Swastawati <fronthea.sw.undip@gmail.com>, Lukita Purnawati <lukitapnw.undip@gmail.com>, Ratna Dama Purnamayati <ratnadama.fkip.undip@gmail.com>

Dear Dr. Swastawati,

Thank you very much for uploading the following manuscript to the MDPI submission system. One of our editors will be in touch with you soon.

Journal name: Nutrients
Manuscript ID: nutrients-452632
Type of manuscript: Article
Title: THE TOXICITY ANALYSIS OF LIQUID SMOKE NANOCAPSULES AND ITS APPLICATION TO THE CHEMICAL CHARACTERISTICS OF CATFISH (PANGASIUS SP.) FILLET
Authors: Fronthea Swastawati *, Lukita Purnawati, Ratna Dama Purnamayati
Received: 9 February 2019
E-mails: fronthea.sw.undip@gmail.com, lukitapnw.undip@gmail.com, ratnadama.fkip.undip@gmail.com
Nanomedicine-Based Strategies for Improving Nutrient Bioavailability and Health Effects
http://www.mdpi.com/journal/nutrients/special_issues/Nanomedicine_Nutrient_Health

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Tel. +41 61 683 77 34
Fax: +41 61 302 89 18

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Dari: Retno Setyowati (retnosetyo@fk.undip.ac.id)

Kepada: fronthea_thp@yahoo.co.id

Tanggal: Selasa, 12 Februari 2019 07.44 WIB

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From: **fronthea swastawati** <fronthea.sw.undip@gmail.com>
Date: Sen, 11 Feb 2019 pukul 20.45
Subject: Fwd: [Nutrients] Manuscript ID: nutrients-452632 - Declined for Publication
To: <retnosetyo@fk.undip.ac.id>

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From: **Sally Cheng** <sally.cheng@mdpi.com>
Date: Sen, 11 Feb 2019 15:44
Subject: [Nutrients] Manuscript ID: nutrients-452632 - Declined for Publication
To: Fronthea Swastawati <fronthea.sw.undip@gmail.com>
Cc: Lukita Purnawati <lukitapnw.undip@gmail.com>, Ratna Dama Purnamayati <ratnadama.fkip.undip@gmail.com>, Nutrients Editorial Office <nutrients@mdpi.com>, Crystal Feng <crystal.feng@mdpi.com>

Dear Dr. Swastawati,

Thank you for submitting the following manuscript to Nutrients:

Manuscript ID: nutrients-452632
Type of manuscript: Article
Title: THE TOXICITY ANALYSIS OF LIQUID SMOKE NANOCAPSULES AND ITS APPLICATION TO THE CHEMICAL CHARACTERISTICS OF CATFISH (PANGASIUS SP.) FILLET
Authors: Fronthea Swastawati *, Lukita Purnawati, Ratna Dama Purnamayati
Received: 9 February 2019
E-mails: fronthea.sw.undip@gmail.com, lukitapnw.undip@gmail.com, ratnadama.fkip.undip@gmail.com
Nanomedicine-Based Strategies for Improving Nutrient Bioavailability and Health Effects
http://www.mdpi.com/journal/nutrients/special_issues/Nanomedicine_Nutrient_Health
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We are writing to inform you that we will not be able to process your paper further. Papers sent for peer-review are selected on the basis of discipline, novelty and general significance, in addition to the usual criteria for publication in scholarly journals. Therefore, our decision is not necessarily a reflection of the quality of your research.

We wish you every success if you choose to submit the paper elsewhere.

Kind regards,
Ms. Sally Cheng
Managing Editor

Nutrients 2019 – Nutritional Advances in the Prevention and Management of Chronic Disease 25–27 Sept 2019, Barcelona, Spain
https://nutrients2019.sciforum.net/

Ms. Sally Cheng
MDPI Branch Office, Beijing
Room 201, Building No. 4,
Zijin Digital Park, No. 18,
Nansi Avenue, Zhongguancun,

Haidian District, 100190 Beijing, China
Email: sally.cheng@mdpi.com

MDPI
St. Alban-Anlage 66
4052 Basel
Postfach, CH-4020 Basel
Switzerland

Trs: [JT] Editor Decision (D) #13860

Dari: Retno Setyowati (retnosetyo@fk.undip.ac.id)
Kepada: fronthea_thp@yahoo.co.id
Tanggal: Senin, 29 Juli 2019 14.37 WIB

Dikirim dari Ponsel Huawei saya

-------- Pesan Asli --------
Subjek: Fwd: [JT] Editor Decision (D) #13860
Dari: fronthea swastawati
Kepada: retnosetyo@fk.undip.ac.id
CC:

> ---------- Forwarded message ---------
> Dari: **Professor Dr. Rosli Md Illias** <r-rosli@utm.my>
> Date: Min, 7 Jul 2019 pukul 09.50
> Subject: [JT] Editor Decision (D) #13860
> To: Fronthea Swastawati <fronthea.sw.undip@gmail.com>
>
> Fronthea Swastawati:
>
> We have reached a decision regarding your submission to Jurnal Teknologi,
> "THE TOXICITY ANALYSIS OF LIQUID SMOKE AND ITS APPLICATION TO THE CHEMICAL CHARACTERISTICS OF CATFISH (PANGASIUS SP.) FILLET".
>
> Our decision is to: DECLINE SUBMISSION
>
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>
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>
> - If this is not a review article, please follow this path :
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> This will help us and our audience have a better understanding what your

article is all about.

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Universiti Teknologi Malaysia
r-rosli@utm.my

Jurnal Teknologi, Penerbit UTM Press
https://jurnalteknologi.utm.my/

Fwd: AACL Bioflux - manuscript reception confirmation and formatting request

Dari: Retno Setyowati (retnosetyo@fk.undip.ac.id)

Kepada: fronthea_thp@yahoo.co.id

Tanggal: Kamis, 31 Oktober 2019 13.14 WIB

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Dari: **fronthea swastawati** <fronthea.sw.undip@gmail.com>
Date: Kam, 31 Okt 2019 12:52
Subject: Fwd: AACL Bioflux - manuscript reception confirmation and formatting request
To: <retnosetyo@fk.undip.ac.id>

---------- Forwarded message ---------
Dari: **Tudor Papuc** <ptudor2008@yahoo.com>
Date: Sel, 15 Okt 2019 02:23
Subject: Re: AACL Bioflux - manuscript reception confirmation and formatting request
To: fronthea swastawati <fronthea.sw.undip@gmail.com>

Hello,

At this moment, the manuscript has passed the preliminary evaluation and it is preliminary accepted for publication.
Please find attached the preliminary acceptance letter.

After this acceptance, you should make the payment. The payment details are below. Only after the payment is made the manuscript will be sent to reviewers.

However, the English is still very bad, and I am afraid that the reviewers might reject the manuscript. So I propose this: please recommend the name of one or 2 reviewers that you know and you are confident that they will really help in improving the manuscript, and I will consider sending them the manuscript for evaluation.

The final acceptance or rejection will be confirmed after receiving the double-blind peer reviews.

Let me know if you need more details.

Thank you for publishing with us!

Best regards,

Tudor Papuc

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preliminary acceptance letter AACL.pdf
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Bioflux (publishing house)
54 Ceahlau Street,
Cluj-Napoca 400488,
Romania, European Union

Certificate

This certificate shows that the manuscript entitled "**The toxicity analysis of liquid smoke and its application to the chemical characteristics of catfish (pangasius sp.) Fillet**", authored by Fronthea Swastawati, Lukita Purnawati, Ratna Dama Purnamayati, submitted to our journal Aquaculture, Aquarium, Conservation & Legislation - International Journal of the Bioflux Society (AACL Bioflux), has been preliminary accepted for publication.

Thank you for your participation!

Cordially yours,

Editor,
Tudor Păpuc

Senior Researcher
Ioan Valentin Petrescu-Mag, PhD
editor-in-chief

SOCIETATEA COMERCIALĂ BIOFLUX S.R.L. CLUJ-NAPOCA ROMÂNIA

Fwd: AACL Bioflux - manuscript reception confirmation and formatting request

Dari: Retno Setyowati (retnosetyo@fk.undip.ac.id)

Kepada: fronthea_thp@yahoo.co.id

Tanggal: Sabtu, 9 Mei 2020 13.10 WIB

---------- Forwarded message ---------
Dari: **fronthea swastawati** <fronthea.sw.undip@gmail.com>
Date: Sab, 9 Mei 2020 11:17
Subject: Fwd: AACL Bioflux - manuscript reception confirmation and formatting request
To: <retnosetyo@fk.undip.ac.id>

---------- Forwarded message ---------
Dari: **Tudor Papuc** <ptudor2008@yahoo.com>
Date: Sel, 24 Des 2019 06:09
Subject: Re: AACL Bioflux - manuscript reception confirmation and formatting request
To: fronthea swastawati <fronthea.sw.undip@gmail.com>

Ok, I will try to publish, but first you need to make some corrections. As was working on your manuscript as you wrote me the message.

Please find attached the manuscript with comments from the reviewers and me.
Make the corrections then send me back the manuscript, with your agreement to publish it in the form you send it to me. What will be further changed will regard the formatting and the new correction of English for the new paragraphs and corrections that you will make.

Please note that this is the last time you can make any changes before publication. After publication, we cannot make anymore changes.

Thank you,

Best Regards,
Tudor Păpuc
Editor, Bioflux SRL

On Monday, December 23, 2019, 05:38:23 AM GMT+2, fronthea swastawati <fronthea.sw.undip@gmail.com> wrote:

Dear Editor

We would like to remind you to publish our article since December issue of AACL Bioflux was released.
We are looking forward to hearing from you soon.

Best regards

Fronthea

Pada tanggal Rab, 4 Des 2019 14:01, fronthea swastawati <fronthea.sw.undip@gmail.com> menulis:

> Dear Editor,
>
> Thank you for the update.
>
> Best regards,
>
> Fronthea
>
> Pada tanggal Rab, 4 Des 2019 13:55, Tudor Papuc <ptudor2008@yahoo.com> menulis:

Hello,

The manuscript has not been published yet.
It is still at the reviewers.

I wrote to the reviewers to hurry up with the evaluations. I have one positive review and the second reviewer told me the evaluation will be positive, but he still has some work to do on it.

After I receive the evaluations, I will send you the reviewer comments, and I will also correct the language and formatting. You will make the corrections, send the manuscript to me, and I will check it and if everything is ok, I will publish it.

My goal is to publish your manuscript in this issue (Nov-Dec).

For now, we need to wait for the second evaluation.

Best Regards,
Tudor Păpuc
Editor, Bioflux SRL

On Sunday, December 1, 2019, 12:03:11 AM GMT+2, fronthea swastawati <fronthea.sw.undip@gmail.com> wrote:

Dear Editor,

We would like to confirm have our article published? Please give us the link of paper publication so we can check it out.

We are looking forward to hearing from you as soon as possible.

Best regards,

Fronthea

Pada tanggal Kam, 31 Okt 2019 12:51, fronthea swastawati <fronthea.sw.undip@gmail.com> menulis:

---------- Forwarded message ---------
Dari: **Tudor Papuc** <ptudor2008@yahoo.com>
Date: Sel, 15 Okt 2019 02:23
Subject: Re: AACL Bioflux - manuscript reception confirmation and formatting request
To: fronthea swastawati <fronthea.sw.undip@gmail.com>

Hello,

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Let me know if you need more details.

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Best regards,

Tudor Papuc

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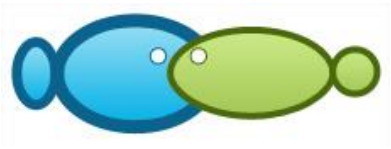

The toxicity analysis of liquid smoke and its application to the chemical characteristics of catfish (*Pangasius* sp.) fillet

[1]Fronthea Swastawati, [1]Lukita Purnawati, [2]Ratna D. Purnamayati

[1] Faculty of Fisheries and Marine Science, Diponegoro University, Tembalang, Semarang, Central Java, Indonesia; [2] Faculty of Medicine, Diponegoro University, Tembalang, Semarang, Central Java, Indonesia. Corresponding author: F. Swastawati, fronthea.sw.undip@gmail.com

Abstract. Liquid smoke is known to have several compounds, such as phenols, acids and carbonyls, with antibacterial and antioxidant properties. This study aims to discover the liquid smoke toxicity and determine the effect of liquid smoke nanocapsule addition on the chemical characteristics of catfish (*Pangasius* sp.) fillet. The liquid smoke combination (corncob and coconut shell) was processed into nanocapsules and was applied on catfish fillet in various concentrations 0%, 0.5%, 1% and 1.5%. The fillet then was stored at room temperature for 24 hours. The results showed that after 14 days of observation, there was no animal experiment who die because of liquid smoke treatment. The application of liquid smoke (LS) nanocapsules on catfish fillet maintained the moisture, lipid and protein content of catfish fillet, with the following values: 62.49%, 6.71% and 30.55%, respectively. The Aw, pH, TBA and TVBN analysis values of catfish fillet with 1.5% of liquid smoke nanocapsules were 0.707, 6.2, 3.593 mg. mal/kg and 82.237 mgN/100 g, respectively. This shows that 1.5% liquid smoke nanocapsules are able to maintain the chemical composition, as well as inhibit lipid oxidation in catfish fillet. However, it could not inhibit microbial growth, shown by the TVBN value, which exceeds the standard. According to the Scanning Electron Microscopy analysis, the morphology of catfish fillet with the addition of nanocapsules was more compact and solid compared to the control sample.
Key Words: toxicity, catfish fillet, chemical composition, nanocapsule.

Introduction. Catfish is a great source of many nutrients. Tenyang et al (2013) state that catfish contains high levels of fat, represented mostly by unsaturated fatty acids, especially EPA and DHA. Catfish also contains a high level of protein. The texture of catfish fillet is easily damaged due to the high content of lipids and proteins. Therefore, preservation methods are necessary in maintaining its chemical characteristics.

One of the preservation methods is smoking. Smoking is a preservation technique using smoke compounds as a medium. Smoke can be condensed in liquid smoke, which is easy to apply on food. Liquid smoke contains active compounds which serve as food preservatives, such as phenols, acids and carbonyls that have antibacterial and antioxidant properties. The compounds are volatile and not durable during storage, therefore, nanoencapsulation should be conducted in order to protect the compounds. However, sometimes liquid smoke is suspected to contain toxic compounds which appear during the pyrolysis process. Toxicity analysis usually needs to be conducted in the case of liquid smoke, together with the analysis of the chemical composition of the food to which liquid smoke is applied.

Nanoencapsulation is a technology that traps the bioactive compound, so it could be transferred appropriately on food until reaching the cells. One of the nanoencapsulation methods is spray drying, which produces nanoparticles. The nanoparticle is in the form of powder so it can be easily applied to the catfish fillet. This study aims to discover the liquid smoke toxicity and determine the concentration of liquid smoke nanocapsulses, which preserve the chemical characteristics of catfish fillet.

Comment [u1]: see next comment

Comment [u2]: You should remove everything related to the mice experiment, because it is not clear what effects there are (just that the mice didn't die); the rest of the manuscript is ok, it is about what changes in the fish fillet when you use the liquid smoke; but all related to toxicity in mice should be removed. Both reviewers disagree with the method used to prove the safety of the liquid smoke, saying it is not complete, and one reviewer gives a positive evaluation only if this part is removed.

moreover, many approvals from bioethical committees are needed for experimentation on mammals (mice)

I suggest to also change the title to "Application of liquid smoke from corn cobb and coconut shells to the fillet of catfish (Pangasius sp.)", because all related to toxicity will be removed

so please delete this phrase

Comment [u3]: in all concentrations?

Comment [u4]: mention the full names and write these abbreviations in brackets; except for pH, that can remain as it is

Comment [u5]: what is this measurement unit? i cannot find it

Comment [u6]: you have only one reference in the introduction; please use at least 7-8 references here, and make the introduction longer (by at least 1 paragraph – you can write about the production of Pangasius, or its chemical composition, or how liquid smoke is usually seen in negative terms and insist on its positive aspects)

Comment [u7]: remove

Material and Method

Research materials. The materials used in this study are comprised of: catfish, coating materials, liquid smoke produced from corn cobs and coconut shells and adult mice to analyze the toxicity. Catfish or "patin fish" (pangasiaus hipophtthalmus) were purchased from a local market in Semarang, Central Java, Indonesia. The research samples were five fresh live catfish, weighing approximately 2 kg each. Liquid smoke was obtained by the pyrolysis of corncobs and coconut shells. The process was conducted in a laboratory of Diponegoro University. The coating materials for the nanoencapsulation process were Na-alginate provided by PT Selalu Lancar Maju Karya, Jakarta, Indonesia. Maltodextrin DE 10 and Arabic gum were purchased from CV Multi Kimia Raya, Semarang, Indonesia. Other chemicals used were Kjeldahl tablets, acetic acid, sodium hydroxide, methyl red indicator, benzene, total volatile base nitrogen (TVB-N), chloric acid (TVB), phenolphthalein, silicon anti-foaming indicator and sodium hydroxide. The experiment was conducted in 2017.

Liquid smoke production process. The liquid smoke was produced by pyrolysis process with a set of pyrolysis tools: a drum unit made of stainless steel with a 100 kg capacity, a unit condenser, a pipe to release smoke, a set of distillation and ignition tools. Corncobs and coconut shells were introduced in the pyrolysis set. The pyrolysis process was conducted at 400°C for 5 hours. Liquid smoke produced from the process was distillated to be applied on the catfish fillets.

Toxicity Analysis of Liquid Smoke. The toxicity assay was performed by using male and female mice Balb/C weigh up to 20-30 gram, from Unit Pemeliharaan Hewan Percobaan (UPHP) of Gadjah Mada University Yogyakarta, Indonesia. First, the laboratory mice were acclimatized for 7 days to adapt to laboratory atmosphere and to eliminate stress due to transportation. The mice were fasted for 4 hours before giving the material test, while water feeding was still allowed. The mice were randomly grouped into 5 groups of dosage and one group of control. Each group consisted of 12 mice (6 male & 6 female).
The liquid smoke was diluted and given by per-oral for 1 ml with 10% and 20% concentration, while the control group was only given the solvent. The distribution of prepared material was performed by oral feeding feeding tube) and only given once, while food and drink were given by ad libitum during the experiment. The observation of behavior was intensively conducted for the first 6 hours, the observation of toxic effect and mortality was done for 24 hours and daily observation was carried out for 14 days on liver and kidney macroscopically.

Liquid smoke nanoencapsulation. The liquid smoke (combination of corncobs and coconut shells) nanoencapsulation process was performed according to Saloko et al (2013), using maltodextrin, Arabic gum and Na-alginate as a coating material with a ratio of 0.167:0.667:0.167. The solution was homogenized and centrifuged at 3000 rpm for 30 minutes at room temperature. The supernatant was separated and filtered to obtain a pure nanoparticle solution. The nanoparticle solution was heated at 50°C in a water bath for 15 minutes and homogenized using a homogenizer at 4000 rpm for 2.5 minutes. The sample was then dried using a spray dryer at 130°C for the inlet temperature, the outlet temperature was 70°C. The nanocapsules obtained were then collected into a sealed glass bottle and stored at room temperature.

The application of liquid smoke nanocapsules on catfish fillet. Catfish fillets with an approximate size of 25x15x1 cm and a weight of around 100 g were smeared with liquid smoke in a concentration of 0%, 0.5%, 1% and 1.5% of the fillet weight. The fillet was heated in an oven at 90°C for 4 hours. It was stored at room temperature for 24 hours.

Comment [u8]: remove

Comment [u9]: check the name again and write the genus with a capital letter, and italic, in a scientific manner

Comment [u10]: also provide a month

Comment [u11]: this should be removed… it was not a detailed study, other effects were not monitored, the long term effect is not known and even if these were, more test should be conducted before using the product for human consumption

Chemical composition. Moisture content analysis was carried out using the thermogravimetric method, protein content was determined using the Kjeldahl method, and fat content was determined using the Soxhlet extraction with petroleum ether.

pH and Aw analysis. A digital pH meter (pH meter TPX-90i Chemical Laboratories Co., Ltd.), was used to analyze the pH, while a digital Aw meter (Rotronic HYGROPALM) was used to analyze the Aw.

TVBN analysis. The TVBN analysis was conducted according to the Indonesian National Standard (2009) and Achmadi et al (2013). About 10 g of sample were mixed with mortar then 90 mL of perchloric acid 6% was added. The solution was homogenized for 2 minutes, then filtered and settled at room temperature for 30 minutes. Afterwards, the solution was filtered with filter paper number 2-3. About 50 mL of filtrate was poured into a distillation tube and several drops of phenolphthalein and silicone anti-foaming indicator were added. Distillation was carried out by adding 10 mL of NaOH 20%. An Erlenmeyer containing 100 mL of H_3BO_4 3% and 3-5 drops of Tashiro indicator (purple solution) were prepared. Distillation lasted 10 minutes, until 100 mL of distillate and 200 mL of a final volume (green solution) were obtained. The distilled sample was then titrated using HCl 0.02 N. Titration was terminated when a purple solution was formed.

TVB–N(mg/100g)=[(Vc-Vb)xNx14.007x100]/W

Where: Vc - HCl volume for sample titration; Vb - HCl volume for blank titration; N - HCl normality; W - sample weight (g); 14.007 - the nitrogen weight.

TBA analysis. The TBA analysis was measured according to Swastawati et al (2012). About 5 g of sample were added to 50 mL of distilled water and the mix was homogenized. The solution was poured into a distillation tube and 2.5 mL of HCl 4 N was added until a pH of 1.5 was reached. It was heated for 10 minutes until 50 mL of distillate was obtained. The distillated mixture was then filtered and 5 mL of distillate was transferred into a 50 mL Erlenmeyer in sterile conditions. 5 mL of TBA reagent was added and heated for 35 minutes. Afterwards, the solution was cooled and measured spectrophotometrically at 528 nm. Lastly, the TBA value was calculated (7.8 D).

Morphology. The catfish fillet morphology was observed using Scanning Electron Microscopy (SEM) with 10 kV of voltage.

Statistical analysis. This study used a completely randomized design with one factor of liquid smoke nanocapsule concentration. The experiment was conducted in triplicate. The SPSS analysis was conducted and the advanced test was performed using the Tukey analysis.

Results and Discussion

Toxicity of liquid smoke. Liquid smoke is the result of pyrolysis using high temperature. According to Dhabbah (2015), fire effluents are sometimes able to produce acute toxicity in the human body. Toxicity is affected by the components contained in liquid smoke. From a previous study, the dominant components of liquid smoke produced by pyrolysis at a temperature of 100-400°C are acetic acid and phenols (Budaraga et al 2016; Yuniningsih & Anggaini 2013). According to toxicity test result in mice with 14 days of observation, there are no dead mice, either with treatment 10% or 20%. Some negative results are observed by Budaraga et al (2016) who analyzed the toxicity of liquid smoke from cinnamon, and reported mortality in mice at concentrations higher than 1500 ppm. The toxicity assay on liquid smoke should be conducted before applying on food, and will be the object of a future study, in the case of liquid smoke from corncob and coconut shells.

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Moisture, crude protein and crude fat of smoked catfish fillet. The catfish fillet smeared with liquid smoke nanocapsules in various concentration was analyzed for its chemical composition. The analysis results are presented in Table 3.

Table 1

Result of Degenerative and Necrosis Hepar

No. Slide	Degenerative						Necrosis					
	Male			Female			Male			Female		
	K	10%	20%	K	10%	20%	K	10%	20%	K	10%	20%
I	4	3	4	4	3	4	0	0	0	0	0	0
I I	4	3	4	3	3	4	0	0	0	0	0	0
I I I	4	3	4	4	4	4	0	0	0	0	0	0

Table 2

Result of Degenerative and Necrosis Kidney

No. Slide	Degenerative						Necrosis					
	Male			Female			Male			Female		
	K	10%	20%	K	10%	20%	K	10%	20%	K	10%	20%
I	4	3	3	4	3	3	0	0	0	0	0	0
I I	4	4	3	4	3	4	0	0	0	0	0	0
I I I	3	3	4	4	4	4	0	0	0	0	0	0

Table 3

Moisture, crude protein and crude fat content of smoked catfish fillet

Concentration	*Moisture (%)*	*Crude protein (%)*	*Crude fat (%)*
0%	65.303±0.405^{d}	28.107±1.17^{a}	7.573±0.297^{d}
0.5%	61.147±0.301^{a}	28.530±0.306^{a}	4.677±0.282^{a}
1.0	63.597±0.325^{c}	29.853±0.525^{ab}	5.737±0.208^{b}
1.5%	62.490±0.248^{b}	30.547±0.759^{b}	6.713±0.337^{c}

Note:

The moisture content of catfish fillet with different liquid smoke nanocapsule concentrations decreased due to the heat, which reduced the moisture level in fish fillet. The moisture content of catfish fillet is around 61.147-65.303%, in accordance with Isamu et al (2012), who processed smoked tuna and the moisture level obtained after smoking was around 63-68%. The moisture level in stingray is around 61-73% (Swastawati et al 2012). The moisture level of stingray fillet decreases inversely proportional to the protein and lipid content. Protein and lipid contents of catfish increase along with the decline of the moisture level. According to Swastawati et al (2012), the decline in moisture level and the increase of protein and lipid contents of smoked fish are because of heating in the smoking process.

Aw. Water activity (Aw) holds an important role in fish deterioration due to microbial growth (Abbas 2009). The Aw value in this research is presented in Table 4.

Table 4

Data Value of Smoked Catfish Fillet

Concentration	*Aw*	*pH*	*TVBN mgN/100 g*	*TBA mg.mal/kg*
0%	0.914±0.004^{d}	6.733±0.057^{c}	95.167±0.511^{d}	10.563±0.025^{c}
0.5%	0.890±0.003^{c}	6.533±0.057^{b}	88.363±0.565^{c}	4.960±0.223^{b}
1.0	0.777±0.009^{b}	6.433±0.057^{b}	81.417±0.281^{a}	4.007±0.111^{a}

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all tables should be renumbered, and mentioned properly in the text

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1.5%	0.707±0.007[a]	6.200±0.100[a]	82.237±1.521[b]	3.593±0.225[a]

Note:

Based on Table , the higher liquid smoke nanocapsule concentration applied on catfish fillet is, the lower the Aw value is. On 1.5% nanocapsule concentration, the Aw value was 0.707, compared with that of 0% concentration, 0.914. This result is similar to others, which show that the Aw value of hot smoked rainbow trout is around 0.7-0.9 (Esmailnia 2015). This indicates that liquid smoke nanocapsules are capable to inhibit microbial growth. Aw is closely related with the moisture content. According to Lisa et al (2015), the relation between the moisture content and water activity is indicated by a directly proportional tendency: the Aw increases with the moisture value. The Aw value expressed in the decimal on scale range 0-1.0.

pH. The pH value of the catfish fillet decreases along with the increasing of nanocapsule concentration. The pH value of smoked catfish with 1.5% nanocapsule concentration was 6.2. This result was significantly different from the pH value of the fillet with 0% nanocapsule concentration, of 6.7. Liquid smoke contains acid compounds (Swastawati et al 2007). This indicates that nanocapsules are able to trap acid compounds in liquid smoke and can be absorbed into the tissues.

TVBN value. TVBN measures the bacterial activity able to degrade proteins (Ariestya 2016). Based on the table, the TVBN of catfish fillet decreased along with the increase of liquid smoke nanocapsule concentration after 24 hours of storage. The TVBN value of catfish fillet with 1.5% liquid smoke nanocapsules was 82.237 mgN/100g. This result is higher compared with those of other studies, where 8% liquid smoke concentration on pomfret produces a TVBN value of approximately 23 mgN/100g (Achmadi et al 2013).

The increasing value of TVBN caused by bacterial activity on muscle tissues produce ammonia, trimethylamine and dimethylamine. The nanocapsule concentration and the TVBN values are inversely proportional. This because liquid smoke contains phenols able to inhibit microbial activities. Saloko et al (2014) state that phenols from liquid smoke are antimicrobial agents able to inhibit microbial activities. The maximum limit for TVBN in fish is 35 mgN/100g (Gunsen & Aydin 2011). In this study, the TVBN exceeded the maximum limit for standard consumption. This is allegedly due to phenols, which partially evaporated during the nanoencapsulation process with spray dryer in high temperature, thus not inhibiting microbial activities.

TBA value. TBA indicates the lipid oxidation degree (Bilgin 2008). Based on the table above, the liquid smoke nanocapsule concentration and TBA values are inversely proportional. This indicates that liquid smoke nanocapsules are able to inhibit lipid oxidation in catfish fillet. The TBA of smoked catfish with 1.5% nanocapsule concentration was 3.593 mg paraformaldehyde/kg. This result is significantly different from the one in the control sample. The maximum limit of TBA is 5 mg paraformaldehyde/kg (Gunsen & Aydin 2011). The catfish fillet with nanocapsule addition produces TBA values below the maximum limit. It shows that the application of liquid smoke nanocapsules in catfish fillet works well in inhibiting lipid oxidation.

Morphology. The morphology of catfish fillet are visible in Figure 1. According to the figure, the muscle tissues of catfish without liquid smoke nanocapsule addition were fragile and easily damaged, whereas the muscle tissues with liquid smoke addition were compact and solid. This indicates that the addition of liquid smoke nanocapsules positively affects the catfish muscle tissue texture. This is related to the decline of the moisture content in catfish fillet with the addition of nanocapsules. The decreasing level of moisture affects the texture, making it more compact and solid. Martinez et al (2011) state that liquid smoke affects the texture of muscle tissue. Furthermore, liquid smoke compounds, especially carbonyl, are able to affect the texture of the product.

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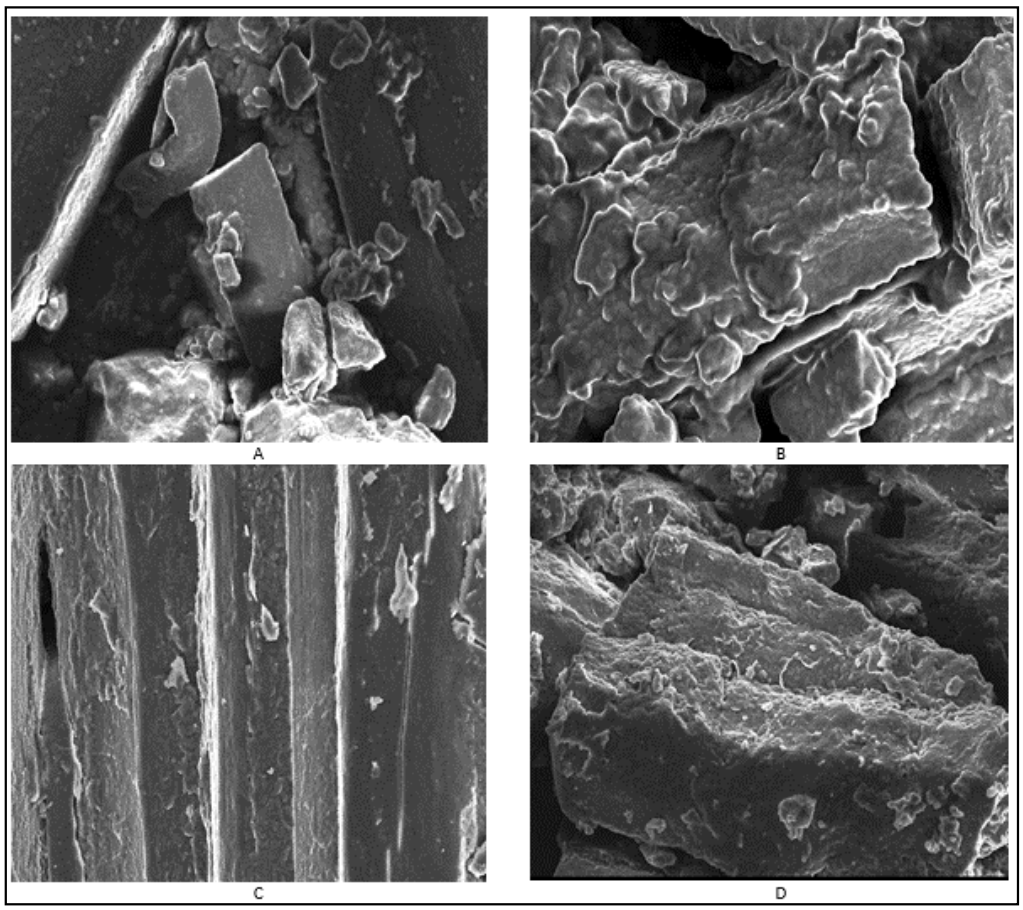

Figure 1. The Morphology of catfish muscle tissue; A - 0% liquid smoke nanocapsule concentration; B – 0.5% liquid smoke nanocapsule concentration; C – 1.0% liquid smoke nanocapsule concentration; D – 1.5% liquid smoke nanocapsule concentration.

Conclusions. In terms of toxicity, liquid smoke still needs to be tested. The 1.5% concentration of liquid smoke nanocapsule applied to catfish fillet is able to maintain the crude protein and crude fat content, as well as to inhibit lipid oxidation. However, microbial growth is still active, as shown by the TVBN value, which exceeds the Indonesian standard. Scanning Electron Microscopy analysis shows that the morphology of catfish fillet with the addition of nanocapsules was more compact and solid compared to the control sample.

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On Sunday, December 29, 2019, 04:36:23 PM GMT+2, fronthea swastawati <fronthea.sw.undip@gmail.com> wrote:

Dear Editor,
Thank you for your information.

Best regards,

Fronthea

Pada tanggal Min, 29 Des 2019 19:23, Tudor Papuc <ptudor2008@yahoo.com> menulis:

I have received the manuscript and I am working on it.
I am trying to publish it today or tomorrow.
I will let you know.

Best Regards,
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Editor, Bioflux SRL

On Saturday, December 28, 2019, 08:03:35 AM GMT+2, fronthea swastawati <fronthea.sw.undip@gmail.com> wrote:

Dear Editor,

We would like to send the revised manuscript.
We have addressed all comments from reviewer, making the paper much more improved.

We hope this revision will be satisfactory and the paper will be published in this month issue.

We are looking forward to hearing from you as soon as possible.

Best regards,

Fronthea Swastawati

Pada tanggal Sel, 24 Des 2019 18:54, Tudor Papuc <ptudor2008@yahoo.com> menulis:

Thank you! Merry Christmas!
If you finish the corrections soon, before New Year, you can send the manuscript (we are working on the holidays too).

Best Regards,
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Editor, Bioflux SRL

On Tuesday, December 24, 2019, 01:52:28 PM GMT+2, fronthea swastawati <fronthea.sw.undip@gmail.com> wrote:

Dear Editor,
Thank you for the update.
We will revise the paper soon.

Merry Christmas and Happy New Year.

Best regards,

Fronthea

Pada tanggal Sel, 24 Des 2019 06:09, Tudor Papuc <ptudor2008@yahoo.com> menulis:

Ok, I will try to publish, but first you need to make some corrections. As was working on your manuscript as you wrote me the message.

Please find attached the manuscript with comments from the reviewers and me.
Make the corrections then send me back the manuscript, with your agreement to publish it in the form you send it to me. What will be further changed will regard the formatting and the new correction of English for the new paragraphs and corrections that you will make.

Please note that this is the last time you can make any changes before publication. After publication, we cannot make anymore changes.

Thank you,

Best Regards,
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On Monday, December 23, 2019, 05:38:23 AM GMT+2, fronthea swastawati <fronthea.sw.undip@gmail.com> wrote:

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We would like to remind you to publish our article since December issue of AACL Bioflux was released.
We are looking forward to hearing from you soon.

Best regards

Fronthea

Pada tanggal Rab, 4 Des 2019 14:01, fronthea swastawati <fronthea.sw.undip@gmail.com> menulis:

Dear Editor,

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Best regards,

Fronthea

Pada tanggal Rab, 4 Des 2019 13:55, Tudor Papuc <ptudor2008@yahoo.com> menulis:

Hello,

The manuscript has not been published yet.
It is still at the reviewers.

I wrote to the reviewers to hurry up with the evaluations. I have one positive review and the second reviewer told me the evaluation will be positive, but he still has some work to do on it.

After I receive the evaluations, I will send you the reviewer comments, and I will also correct the language and formatting. You will make the corrections, send the manuscript to me, and I will check it and if everything is ok, I will publish it.

My goal is to publish your manuscript in this issue (Nov-Dec).

For now, we need to wait for the second evaluation.

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On Sunday, December 1, 2019, 12:03:11 AM GMT+2, fronthea swastawati <fronthea.sw.undip@gmail.com> wrote:

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Best regards,

Fronthea

Pada tanggal Kam, 31 Okt 2019 12:51, fronthea swastawati <fronthea.sw.undip@gmail.com> menulis:

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Dari: **Tudor Papuc** <ptudor2008@yahoo.com>
Date: Sel, 15 Okt 2019 02:23
Subject: Re: AACL Bioflux - manuscript reception confirmation and formatting request
To: fronthea swastawati <fronthea.sw.undip@gmail.com>

Hello,

At this moment, the manuscript has passed the preliminary evaluation and it is preliminary accepted for publication.
Please find attached the preliminary acceptance letter.

After this acceptance, you should make the payment. The payment details are below. Only after the payment is made the manuscript will be sent to reviewers.

However, the English is still very bad, and I am afraid that the reviewers might reject the manuscript. So I propose this: please recommend the name of one or 2 reviewers that you know and you are confident that they will really help in improving the manuscript, and I will consider sending them the manuscript for evaluation.

The final acceptance or rejection will be confirmed after receiving the double-blind peer reviews.

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Thank you for publishing with us!

Best regards,

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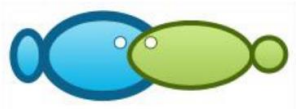

Application of liquid smoke from corncob and coconut shell to the fillet of catfish (*Pangasius* sp.)

[1]Fronthea Swastawati, [1]Lukita Purnamayati, [2]Ratna D. Purnawati

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Abstract. Liquid smoke is known to have several compounds, such as phenols, acids and carbonyls, with antibacterial and antioxidant properties. This study aims to discover the liquid smoke toxicity and determine the effect of liquid smoke nanocapsule addition on the chemical characteristics of catfish (*Pangasius* sp.) fillet. The liquid smoke combination (corncob and coconut shell) was processed into nanocapsules and was applied on catfish fillet in various concentrations 0%, 0.5%, 1% and 1.5%. The fillet then was stored at room temperature for 24 hours. The application of liquid smoke (LS) nanocapsules in a concentration of 1.5% on catfish fillet maintained the moisture, lipid and protein content of catfish fillet, with the following values: 62.49%, 6.71% and 30.55%, respectively. The water activity, pH, thiobarbituric acid (TBA) and total volatile base nitrogen (TVBN) analyses values of catfish fillet with 1.5% liquid smoke nanocapsules were 0.707, 6.2, 3.593 mg malonaldehyde/kg and 82.237 mgN/100 g, respectively. This shows that 1.5% liquid smoke nanocapsules are able to maintain the chemical composition, as well as inhibit lipid oxidation in catfish fillet. However, it could not inhibit microbial growth, shown by the TVBN value, which exceeds the standard. According to the Scanning Electron Microscopy analysis, the morphology of catfish fillet with the addition of nanocapsules was more compact and solid compared to the control sample.
Key Words: toxicity, catfish fillet, chemical composition, nanocapsule.

Introduction. Catfish (*Pangasius hypophthalmus*) is a farmed freshwater fish in Indonesia with a high demand for its consumption (Tran et al 2017). According to the 2012 Asia Fish Model data, total catfish production (*Pangasius* sp.) in Indonesia reached 384000 tons, while the level of public consumption of catfish reached 243000 tons. It shows that the consumption of catfish reaches 63% of the total production. The rate of production and consumption of catfish is expected to increase until 2028. The level of consumption of catfish in Indonesia is mainly in the form of fillets. Nurilmala et al (2015) stated that Indonesians consume around 700 tons of catfish fillet per month. The high demand for catfish is due to the good taste and soft texture of the white meat of catfish (Rathod et al 2018).

Catfish is a great source of many nutrients. Catfish has a 75.3% moisture content, 7.8% crude fat, 17.2% crude protein, and 0.8% ash (Manthey-Karl et al 2016). Tenyang et al (2013) stated that catfish contains high levels of fat, mostly unsaturated fatty acids in the form of EPA and DHA. Hashim et al (2015) stated that besides EPA and DHA, the unsaturated fatty acids in catfish are omega-7 fatty acids, like palmitoleic acid and omega-9 fatty acids, such as oleic acid. Catfish also contains a high level of protein. Catfish contain high amounts of essential amino acids (AA), such as lysine, isoleucine, leucine, valine, glutamic acid, aspartic acid, alanine and arginine (Pratama et al 2018). The high nutrient content in catfish can lead to rapid degradation or damaged during storage. Ikasari & Suryaningrum (2015) stated that catfish fillets experience a decrease in panelist ratings of appearance, odor and texture during storage. This is also supported

by the increasing value of total volatile base nitrogen (TVBN) in the filet. Therefore, preservation methods are necessary for maintaining their chemical characteristics.

One of the preservation methods is smoking. Smoking is a preservation technique using smoke compounds as a medium (Rizo et al 2015). Smoke can be condensed in liquid smoke, which is easy to apply to food. Liquid smoke contains active compounds that serve as food preservatives, such as phenols and acids (Lombok et al 2014) and also carbonyls (Budaraga et al 2017) that has antibacterial (Zuraida et al 2011) and antioxidant properties (Saloko et al 2014). Sometimes, liquid smoke is suspected to contain toxic compounds that appear during the pyrolysis process. However, sometimes, the components contained in liquid smoke have significant benefits. Besides being able to extend shelf life, the liquid smoke flavor is also very preferred. Liquid smoke has been widely applied to food products. Desniorita & Maryam (2015) applied 2% of liquid smoke to extend the shelf life of flour sauce for seven days. Maryam (2015) added 2% liquid powder smoke to sponge cake to produce a sponge cake with a flavor and taste liked by panelists. The sponge cake had a shelf life of 8 days. The liquid smoke compounds are volatile and not durable during storage. Therefore, nanoencapsulation should be carried out in order to protect the compounds.

Nanoencapsulation is a technology that traps the bioactive compound, so it can be transferred appropriately on food until reaching the cells. One of the nanoencapsulation methods is by spray drying, which produces nanoparticles (Ezhilarasi et al 2013). The nanoparticles are in the form of powder so that they can be easily applied to food. Saloko et al (2014) applied liquid smoke nanocapsules from coconut shells to fresh tuna and produced tuna that can survive for 48 hours at room temperature. There is no research yet on the application of nanocapsules from liquid coconut shell smoke combined with corncobs. Therefore, this study aimed to determine the concentration of liquid smoke nanocapsules from coconut shells and corncob, which can preserve the chemical characteristics of catfish fillets.

Material and Method

Research materials. The materials used in this study are comprised of: catfish, coating materials, liquid smoke produced from corncobs and coconut shells. Catfish or "patin fish" (*Pangasius hypophthalmus*) were purchased from a local market in Semarang, Central Java, Indonesia. The research samples were five fresh live catfish, weighing approximately 2 kg each. Liquid smoke was obtained by the pyrolysis of corncobs and coconut shells. The process was conducted in a laboratory of Diponegoro University. The coating materials for the nanoencapsulation process were Na-alginate provided by PT Selalu Lancar Maju Karya, Jakarta, Indonesia. Maltodextrin DE 10 and Arabic gum were purchased from CV Multi Kimia Raya, Semarang, Indonesia. Other chemicals used were Kjeldahl tablets, acetic acid, sodium hydroxide, methyl red indicator, benzene, total volatile base nitrogen (TVB-N), chloric acid (TVB), phenolphthalein, silicon anti-foaming indicator and sodium hydroxide. The experiment was conducted from April to November 2017.

Liquid smoke production process. The liquid smoke was produced by pyrolysis process with a set of pyrolysis tools: a drum unit made of stainless steel with a 100 kg capacity, a unit condenser, a pipe to release smoke, a set of distillation and ignition tools. Corncobs and coconut shells were introduced in the pyrolysis set. The pyrolysis process was conducted at 400°C for 5 hours. Liquid smoke produced from the process was distillated to be applied on the catfish fillets.

Liquid smoke nanoencapsulation. The liquid smoke (combination of corncobs and coconut shells) nanoencapsulation process was performed according to Saloko et al (2013), using maltodextrin, Arabic gum and Na-alginate as a coating material with a ratio of 0.167:0.667:0.167. The solution was homogenized and centrifuged at 3000 rpm for 30 minutes at room temperature. The supernatant was separated and filtered to obtain a pure nanoparticle solution. The nanoparticle solution was heated at 50°C in a water bath

for 15 minutes and homogenized using a homogenizer at 4000 rpm for 2.5 minutes. The sample was then dried using a spray dryer at 130°C for the inlet temperature, the outlet temperature was 70°C. The nanocapsules obtained were then collected into a sealed glass bottle and stored at room temperature.

The application of liquid smoke nanocapsules on catfish fillet. Catfish fillets with an approximate size of 25x15x1 cm and a weight of around 100 g were smeared with liquid smoke in a concentration of 0%, 0.5%, 1% and 1.5% of the fillet weight. The fillet was heated in an oven at 90°C for 4 hours. It was stored at room temperature for 24 hours.

Chemical composition. Moisture content analysis was carried out using the thermogravimetric method, protein content was determined using the Kjeldahl method, and fat content was determined using the Soxhlet extraction with petroleum ether.

pH and Aw analysis. A digital pH meter (pH meter TPX-90i Chemical Laboratories Co., Ltd.), was used to analyze the pH, while a digital Aw meter (Rotronic HYGROPALM) was used to analyze the Aw.

TVBN analysis. The TVBN analysis was conducted according to established methods (Badan Standarisasi Nasional 2009; Achmadi et al 2013). About 10 g of sample were mixed with ceramics mortar then 90 mL of perchloric acid 6% was added. The solution was homogenized for 2 minutes, then filtered and settled at room temperature for 30 minutes. Afterwards, the solution was filtered with filter paper number 2-3. About 50 mL of filtrate was poured into a distillation tube and several drops of phenolphthalein and silicone anti-foaming indicator were added. Distillation was carried out by adding 10 mL of NaOH 20%. An Erlenmeyer containing 100 mL of H_3BO_4 3% and 3-5 drops of Tashiro indicator (purple solution) were prepared. Distillation lasted 10 minutes, until 100 mL of distillate and 200 mL of a final volume (green solution) were obtained. The distilled sample was then titrated using HCl 0.02 N. Titration was terminated when a purple solution was formed.

TVBN (mg/100 g)=[(Vc-Vb)xNx14.007x100]/W

Where: TVBN - total volatile base nitrogen; Vc - HCl volume for sample titration; Vb - HCl volume for blank titration; N - HCl normality; W - sample weight (g); 14.007 - the nitrogen weight.

TBA analysis. The thiobarbituric acid (TBA) analysis was measured according to Swastawati et al (2012). About 5 g of sample were added to 50 mL of distilled water and the mix was homogenized. The solution was poured into a distillation tube and 2.5 mL of HCl 4 N was added until a pH of 1.5 was reached. It was heated for 10 minutes until 50 mL of distillate was obtained. The distillated mixture was then filtered and 5 mL of distillate was transferred into a 50 mL Erlenmeyer in sterile conditions. 5 mL of TBA reagent was added and heated for 35 minutes. Afterwards, the solution was cooled and measured spectrophotometrically at 528 nm. Lastly, the TBA value was calculated.

Morphology. The catfish fillet morphology was observed using Scanning Electron Microscopy (SEM) with 10 kV of voltage.

Statistical analysis. This study used a completely randomized design with one factor of liquid smoke nanocapsule concentration. The experiment was conducted in triplicate. The SPSS 17 software was used to analyze the parametric data. If there were significant differences, the analysis was continues with the Tukey test.

Results and Discussion

Toxicity of liquid smoke. Liquid smoke is the result of pyrolysis using high temperature. According to Dhabbah (2015), fire effluents are sometimes able to produce acute toxicity in the human body. Toxicity is affected by the components contained in liquid smoke. The dominant components of liquid smoke produced by pyrolysis at a temperature of 100-400°C are acetic acid and phenols (Budaraga et al 2016a; Yuniningsih & Anggaini 2013). Some negative results are observed by Budaraga et al (2016b) who analyzed the toxicity of liquid smoke from cinnamon, and reported mortality in mice at concentrations higher than 1500 ppm. The toxicity assay on liquid smoke should be conducted before applying on food, and will be the object of a future study, in the case of liquid smoke from corncob and coconut shells.

Moisture, crude protein and crude fat of smoked catfish fillet. The catfish fillet smeared with liquid smoke nanocapsules in various concentration was analyzed for its chemical composition. The analysis results are presented in Table 1.

Table 1

Moisture, crude protein and crude fat content of smoked catfish fillet

Concentration	*Moisture (%)*	*Crude protein (%)*	*Crude fat (%)*
0%	65.303±0.405^{d}	28.107±1.17^{a}	7.573±0.297^{d}
0.5%	61.147±0.301^{a}	28.530±0.306^{a}	4.677±0.282^{a}
1.0	63.597±0.325^{c}	29.853±0.525^{ab}	5.737±0.208^{b}
1.5%	62.490±0.248^{b}	30.547±0.759^{b}	6.713±0.337^{c}

Note: different superscript letters in the same column show significant differences (P<0.05).

The moisture content of catfish fillet with different liquid smoke nanocapsule concentrations decreased due to the heat, which reduced the moisture level in fish fillet. The moisture content of catfish fillet is around 61.147-65.303%, in accordance with Isamu et al (2012), who processed smoked tuna (*Katsuwonus pelamis*) and the moisture level obtained after smoking was around 63-68%. The moisture level in stingray (*Dasyatis blekeery*) is around 61-73% (Swastawati et al 2012). The moisture level of stingray fillet decreases inversely proportional to the protein and lipid content. Protein and lipid contents of catfish increase along with the decline of the moisture level. According to Swastawati et al (2012), the decline in moisture level and the increase of protein and lipid contents of smoked fish are because of heating in the smoking process.

Aw. Water activity (Aw) holds an important role in fish deterioration due to microbial growth (Abbas 2009). The Aw value in this research is presented in Table 2.

Table 2

Aw, pH, TVBN and TBA of smoked catfish fillet

Concentration	*Aw*	*pH*	*TVBN mgN/100 g*	*TBA mgmalonaldehyde/kg*
0%	0.914±0.004^{d}	6.733±0.057^{c}	95.167±0.511^{d}	10.563±0.025^{c}
0.5%	0.890±0.003^{c}	6.533±0.057^{b}	88.363±0.565^{c}	4.960±0.223^{b}
1.0	0.777±0.009^{b}	6.433±0.057^{b}	81.417±0.281^{a}	4.007±0.111^{a}
1.5%	0.707±0.007^{a}	6.200±0.100^{a}	82.237±1.521^{b}	3.593±0.225^{a}

Note: Aw – water activity; TVBN - total volatile base nitrogen; TBA - thiobarbituric acid. Different superscript letters in the same column show significant differences (P<0.05).

Based on Table 2, the higher liquid smoke nanocapsule concentration applied on catfish fillet is, the lower the Aw value is. On 1.5% nanocapsule concentration, the Aw value was 0.707, compared with that of 0% concentration, 0.914. This result is similar to others, which show that the Aw value of hot smoked rainbow trout (*Oncorhynchus mykiss*) is

around 0.7-0.9 (Esmailnia 2015). This indicates that liquid smoke nanocapsules are capable to inhibit microbial growth. Aw is closely related with the moisture content. According to Lisa et al (2015), the relation between the moisture content and water activity is indicated by a directly proportional tendency: the Aw increases with the moisture value.

pH. The pH value of the catfish fillet decreases along with the increasing of nanocapsule concentration. The pH value of smoked catfish with 1.5% nanocapsule concentration was 6.2. This result was significantly different from the pH value of the fillet with 0% nanocapsule concentration, of 6.7. Liquid smoke contains acid compounds (Swastawati et al 2007). This indicates that nanocapsules are able to trap acid compounds in liquid smoke and can be absorbed into the tissues.

TVBN value. TVBN measures the bacterial activity able to degrade proteins (Ariestya 2016). Based on Table 2, the TVBN of catfish fillet decreased along with the increase of liquid smoke nanocapsule concentration after 24 hours of storage. The TVBN value of catfish fillet with 1.5% liquid smoke nanocapsules was 82.237 mgN/100g. This result is higher compared with those of other studies, where 8% liquid smoke concentration on pomfret (*Colossoma macropomum*) produces a TVBN value of approximately 23 mgN/100g (Achmadi et al 2013). The smoke was obtained from palm oil and shells.

The increasing value of TVBN caused by bacterial activity on muscle tissues produce ammonia, trimethylamine and dimethylamine. The nanocapsule concentration and the TVBN values are inversely proportional. This because liquid smoke contains phenols able to inhibit microbial activities. Saloko et al (2014) state that phenols from liquid smoke are antimicrobial agents able to inhibit microbial activities. The maximum limit for TVBN in fish is 35 mgN/100g (Gunsen et al 2011). In this study, the TVBN exceeded the maximum limit for standard consumption. This is allegedly due to phenols, which partially evaporated during the nanoencapsulation process with spray dryer in high temperature, thus not inhibiting microbial activities.

TBA value. TBA indicates the lipid oxidation degree (Bilgin et al 2008). Based on Table 2, the liquid smoke nanocapsule concentration and TBA values are inversely proportional. This indicates that liquid smoke nanocapsules are able to inhibit lipid oxidation in catfish fillet. The TBA of smoked catfish with 1.5% nanocapsule concentration was 3.593 mg paraformaldehyde/kg. This result is significantly different from the one in the control sample. The maximum limit of TBA is 5 mg paraformaldehyde/kg (Gunsen et al 2011). The catfish fillet with nanocapsule addition produces TBA values below the maximum limit. It shows that the application of liquid smoke nanocapsules in catfish fillet works well in inhibiting lipid oxidation.

Morphology. The morphology of catfish fillet are visible in Figure 1. According to the figure, the muscle tissues of catfish without liquid smoke nanocapsule addition were fragile and easily damaged, whereas the muscle tissues with liquid smoke addition were compact and solid. This indicates that the addition of liquid smoke nanocapsules positively affects the catfish muscle tissue texture. This is related to the decline of the moisture content in catfish fillet with the addition of nanocapsules. The decreasing level of moisture affects the texture, making it more compact and solid. Martinez et al (2011) state that liquid smoke affects the texture of muscle tissue. Furthermore, liquid smoke compounds, especially carbonyl, are able to affect the texture of the product.

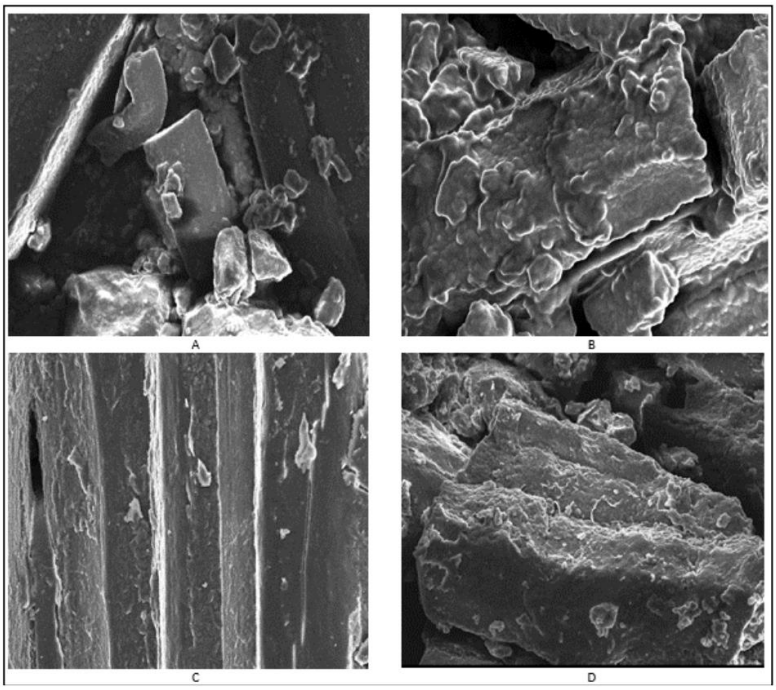

Figure 1. The Morphology of catfish muscle tissue; A - 0% liquid smoke nanocapsule concentration; B – 0.5% liquid smoke nanocapsule concentration; C – 1.0% liquid smoke nanocapsule concentration; D – 1.5% liquid smoke nanocapsule concentration.

Conclusions. In terms of toxicity, liquid smoke still needs to be tested. The 1.5% concentration of liquid smoke nanocapsule applied to catfish fillet is able to maintain the crude protein and crude fat content, as well as to inhibit lipid oxidation. However, microbial growth is still active, as shown by the TVBN value, which exceeds the Indonesian standard. Scanning Electron Microscopy analysis shows that the morphology of catfish fillet with the addition of nanocapsules was more compact and solid compared to the control sample.

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