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Name of the authors: Isny Maulidevi, Nurul Andriyani, Ryan Salfarino, Veriani Aprilia

Hereby I would like to submit the manuscript entitled "**The residue of formalin in catfish (*Clarias gariepinus*) after a short period processing and storage**" to Aquaculture, Aquarium, Conservation & Legislation - International Journal of the Bioflux Society.

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Date October 1st, 2020

The residue of formalin in catfish (*Clarias gariepinus*) after a short period processing and storage

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Abstract. Fresh fish is a highly perishable product due to its high protein content. A preservative may be needed to inhibit it. Nowadays, many fishes were reported to be exposed to formalin. This study aimed to determine the effect of short period processing (washing, frying, boiling in short-period) and storage (in the room and freezing temperature) on the residue of formalin in catfish, as a food model. Catfish were divided into 8 groups, e.g. before treatment, washing in airflow for 1 minute, frying in 200°C for 8 minutes, boiling in 100°C for 7 minutes, storage at room temperature (29±1°C) for 24 and 48 hours, storage at freezing temperature (-3°C) for 24 and 48 hours. Before treatment, the catfish was soaked in 1480 ppm of formalin solutions. The residue of formalin was measured at the end of each treatment by using a spectrophotometer. Data were then analyzed with analysis of variance with SPSS software. The results showed that there were significant effects of processing and storage treatment on the formalin residue of catfish. The formalin residue of all treatments was lower than that of before treatments. The frying process and storage at freezing temperatures were effective treatments to reduce the formalin residue.

Keyword: formalin, fish, processing, storage

Background. Fresh fish is a highly perishable product due to its high protein content for microbial activity (Wijayanti & Lukitasari, 2016). A preservative maybe needs to keep the freshness, inhibit the decay, and longer the shelf life (Antoni, 2010). Nowadays, there are many kinds of food-grade preservatives available in the market. Unfortunately, formalin is still frequently found in food products, including fresh fish. The main reason for the use among fishermen is cheaper, easy to find, and its effectivity to preserve the product (Male et al., 2017).

Formalin is a forty percent solution of formaldehyde in water. This compound is detected in the animal body as an intermediary metabolite. It also uses for several purposes, such as in medicine for specimen preservation and the treatment of hemorrhage (Pandey *et al.*, 2000). The preservatives activity of formalin because of its ability to react with protein generating methylene compound which is more difficult to be decayed by microbes (Ichya'uddin, 2016). In low concentration, formalin is not toxic for humans, but in higher concentrations, it had negative effects such as acute toxicity and death (Pandey *et al.*, 2000). It also decreased the levels of body antioxidants that resulting in liver damage (Yulisa et al., 2014). Other studies also reported the cases of eczema, eye irritation, respiratory tract irritation, cancer, increased the growth rate of malignant neoplastic disease, and denaturation of DNA (Suwanaruang, 2018; Wilianto & Yudianto, 2013). Therefore, its uses are forbidden.

Formalin solution was not stable during storage and exposed to high temperatures (Kaneko et al., 1977; Laksmiani et al., 2015). Those are caused by desolvation or evaporation of formalin during washing, boiling, or frying (Fellows, 2015; Harmita, 2016; Muntaha et al., 2015; Sundari et al., 2015) and enzymatic process in storage (Rachmawati et al., 2007). Formalin that was exposed to the fish decreased up to 43-53% after 5 times rinsing in 250 mL of distilled water and boiling for 30 minutes (Yusuf et al., 2015). Other studies were also reported that soaking in warm water and frying decreased the concentration of formalin up to 63,27% and 83.03% (Levita et al., 2010). In daily practices, fish is usually washed in flowing water before processing, continued to frying/boiling in a shorter time. However, there was no study explained the influences of these processing and storage on the concentration of formalin.

The purpose of this study was to know the effect of processing in a short time and storage (room temperature and freezing) on the residue of formalin. This study used catfish as a food model.

Material and Method

***Material*.** The study used fresh catfish, purchased from the traditional market in Yogyakarta. Another ingredients used were formalin solution and Schiff's reagent. Formalin solution was made from 4 mL of formalin 37% in 1,000 mL of distilled water (Rachmawati, Riyanto, and Ariyani, 2007).

Method. The study was divided into two sections, i.e. 1) study the effect of processing and 2) storage treatment. Fresh catfish were slaughtered, eviscerated, cleaned, and soaked in 1,480 ppm formalin solution (Rachmawati et al., 2007; Sugiarti et al., 2014). For the first study, catfish were divided into four groups, i.e.: controls (before treatment), washing, frying, and boiling treatments. Washing was done by watering the samples with running water for 1 minute, while frying was done by frying the samples in oil 200°C for 8 minutes. Boiling treatment was done by putting the samples into boiled water (100°C) for 7 minutes (Sugiarti, Anggo, and Riyadi, 2014). For the second study, storage treatments, the catfish was also divided into four groups, they were room temperature storage (29°C) for 24 hours and 48 hours and freezing temperatures storage (-3°C) for 24 hours and 48 hours (Handayani et al., 2010). At the end of treatments, the concentration of formalin in samples was analyzed using a spectrophotometer. As the preparation step, a 5 g sample was dissolved in 25 mL of distilled water. One mL of supernatant was mixed with 2 mL of Schiff's reagent, then heated in a water bath at 60°C for 5 minutes. It was incubated for 10 minutes before measuring the absorbance at 520 nm (Shita, 2016; Mudaffar, 2018).

Statistical Analysis. Data were presented in mean±SD and analyzed with One-Way analysis of variance (ANOVA) to know the differences among all processing treatments and the effect of storage periods on the concentration of formalin; paired sample T-test to know the differences between the same samples before and after processing treatments; independent sample t-test to know the effect of temperature at the same storage period, and two-way ANOVA to know the combination effect of storage temperature and period on the concentration of formalin. All data were analyzed with SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL, USA) at the 5% significance level.

Results. ***Effect of processing on the formalin residue of catfish.*** Table 1 shows that all treatments had significant effects on the residue of formalin ($p<0.05$). It can be seen from the p-value between the same samples before and after treatments. Among all treatments, washing shows the lowest residue of formalin ($p<0.05$). Both boiling and frying had a similar residue of formalin ($p>0.05$). The higher residue of formalin in boiling and frying may be due to heating exposure.

Table 1.
Effect of processing on the formalin residue of catfish

Processing treatments	Residue of formalin (ppm)		Δ residue of formalin before and after treatment (%)	p-value	
	Before treatment	After treatment		Among all samples after treatments	Between the same samples before and after treatments
Washing	1,420.17±66.94^{c}	310.42±77.61^{b}	78.14	0.01	0.00
Frying	1,420.17±66.94^{c}	14.18±6.69^{a}	99.01		0.02
Boiling	1,420.17±66.94^{c}	27.50±9.11^{a}	98.10		0.02

Note: Mean±SD in the same column with different superscripts are significantly different ($p<0.05$). p-value among all samples was obtained from one-way ANOVA analyses,

while p-value between the samples before and after treatments were obtained from paired T-test analyses.

Effect of storage temperatures and periods on the formalin residue of catfish. Table 2 shows the effect of storage temperatures, periods, and the combination of both on the concentration of formalin. Based on this data, the storage period influenced the residue of formalin ($p<0.05$). It could be seen from the decrease of formalin residue during 24 hours of storage. However, the decrease did not significantly different when the storage continued to 48 hours ($p>0.05$).

On the other hand, there were no differences in the residue of formalin at room and freezing temperature. It was indicated that the temperature of storage did not affect the formalin residue ($p>0.05$). Although there were differences in statistical results between the effect of temperature and periods of storage, the combined effect of both variables showed no impact on the concentration of formalin ($p>0.05$)

Tabel 2.

Effect of storage temperature and period on the formalin residue of catfish

Temperature	Periods (t) (hours)			p-value		
(T)	0	24	48	T	t	T*t
Room (29°C)	1,420.17±66.94^{a1}	52.18±28.78^{a2}	40.64±3.93^{a2}	0.20	0.00	0.76
Freezing (-3°C)	1,420.17±66.94^{a1}	39.04±7.07^{a2}	13.65±4.23^{a2}			

Note: Mean±SD in the same column with different alphabetical superscripts (a, b, or c) are significantly different ($p<0.05$) and so are in the same row with different number superscript (1,2, or 3). The p-value for T, t, and T*t was obtained from independent T-test, one-way ANOVA, and two-way ANOVA, respectively.

Discussion

***The residue of formalin before treatment*.** The residue of formalin before and after treatment (either fish processing or storage) was significantly different (Table 1 and Table 2). In this study, 1,480 ppm of formalin solution was added to the catfish and submerged for 60 minutes. The 60 minutes submersion was chosen because the aldehyde compound may be readily bonded to the amino acids of fish in the small amount (Rahmadhani et al, 2017; Baroroh & Kresnadipayana, 2016). Formalin would attack lysin for the first time, followed by histidine and tyrosine, which led to the formation of methylene compound (Ichya'uddin, 2016). The protein-containing methylene compounds could not be digested by living things and microbes, especially pathogens, so that the shelf life would be longer (Purawisastra & Sahara, 2016). The beneficial bacteria in the fish may also be killed by dehydration mechanism as another effect of the existence of formalin in the body (Mudzkirah, 2016).

The effect of processing on the formalin residue of catfish. The residue of formalin reduced to 78.14% during washing treatment. The reduction was significant ($p< 0.05$). The formalin was easily washed out because of its high solubility in the water, which is 24×10^5 mg L^{-1} at 20°C (BPOM, 2008; Sugiarti et al, 2014; Yusuf et al., 2015). Another study conducted by Yusuf et al (2015) also showed the same trend, but the reduction was lower (43%). The differences in the level of reduction can be affected by the length of formalin submersion. The longer the submersion time, the smaller the decreasing as there was more formalin bonded with protein in the fish.

In this study, frying also significantly reduced the residue of formalin ($p<0.05$). The reduction level was 99% that indicated most formalin was disappeared during the frying process. Formalin was included in a volatile compound, especially at the above of its boiling point (Joshi et al., 2015). This study was also the same with Sugiarti et al (2014) that found the reduction level of formalin up to 99.28% after 1 hour of submersion followed by the frying process (no information about the period of frying process) in squid. Frying could denature the protein and also hydrolyze its bonding with formalin (Levita et al., 2010). The high temperature in frying increased the kinetic energy and led to faster movement of the molecules that could damage the molecular bonds (Sugiarti et al*.,* 2014).

Boiling was another heat processing that was done in this study. The results showed that this process also reduced the residue of formalin up to 98.1%. The residue of formalin after treatment was different from the sample after the washing process ($p<0.05$), but the same with that after the frying process ($p>0.05$). In the boiling process, the reduction of formalin was higher than that was in only washing or soaking treatment because there was more formalin dissolved in higher temperature water (Annisak, 2019). The amount of loosing would be higher when it combined with the opened pan during the boiling (Kamal et al., 2017).

The effect of storage on the formalin residue of catfish. This research found that only a period of storage that influenced the residue of formalin ($p<0.05$), while the temperature of storage and combination between temperature and period of storage did not (Table 2). The previous different studies showed that either room temperature or freezing temperature of storage had a similar effect on the decreasing of formalin residue (Jawahar, et al, 2017; Murtini et al., 2014). It may be due to the degradation of protein during both in the room and freezing temperature, resulting in the loss of the bonds between protein and formalin and releasing free compounds. In the room temperature, the protein degradation was more caused by microbial activity (Murtini et al., 2014), while in the freezing temperature was more caused by physical destruction leading to the conformational and functional changes (Yeasmin et al., 2010). Formalin was easier to break down into another compound or reacts with other compounds in the free form (Riyanto et al., 2006; Rachmawati et al., 2007).

Conclusion. This research concluded that short time process and storage treatments decreased the concentration of formalin. Heat involvement made the decrease of concentration became more effective. In the storage study, the residue of formalin was influenced significantly by the period of storage, mainly during 24 hours of storage.

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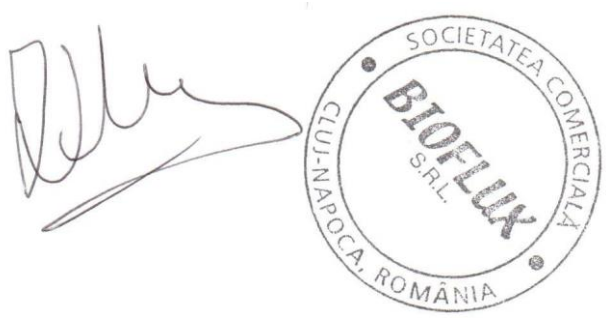

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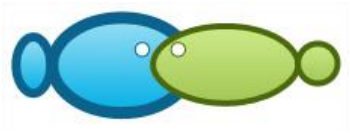

The residue of formalin in catfish (*Clarias gariepinus*) after ~~a short period~~ processing and storage for short periods

Isny Maulidevi, Nurul Andriyani, Ryan Salfarino, Veriani Aprilia

Department of Nutrition, Faculty of Health Sciences, Alma Ata University, Jakarta, Indonesia. Corresponding author: V. Aprilia, verianiaprilia@almaata.ac.id

Abstract. Fresh fish is a highly perishable product due to its high protein content. A preservative may be needed to inhibit it. Nowadays, many fishes were reported to be exposed to formalin. This study aimed to determine the effect of short period processing (washing, frying, boiling in short-period) and storage (~~in~~ at the room and at freezing temperature~~s~~) on the residue of formalin in catfish, as a food model. Catfish, *Clarias gariepinus,* were divided into 8 groups, e.g. before treatment, washing in airflow for 1 minute, frying in 200°C for 8 minutes, boiling in 100°C for 7 minutes, storage at room temperature (29±1°C) for 24 and 48 hours, storage at freezing temperature (-3°C) for 24 and 48 hours. Before treatment, the catfish was soaked in 1,480 ppm of formalin solutions. The residue of formalin was measured at the end of each treatment by using a spectrophotometer. Data were then analyzed with analysis of variance with SPSS software. The results showed that there were significant effects of processing and storage treatment on the formalin residue of catfish. The formalin residue of all treatments was lower than ~~that of~~ before treatments. The frying processing and storage at freezing temperatures were the most effective treatments ~~to~~ in reducing the formalin residue.
Key Words: formalin, fish, processing, storage.

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Introduction. Fresh fish is a highly perishable product due to its high protein content for microbial activity (Wijayanti & Lukitasari 2016). A preservative maybe needs to keep the freshness, inhibit the decay~~,~~ and prolonge~~r~~ the shelf life (Antoni 2010). Nowadays, there are many kinds of food-grade preservatives available in the market. Unfortunately, formalin is still frequently found in food products, including fresh fish~~.~~, being used among fishermen ~~The~~ mainly because~~reason for the use among fishermen is cheaper, easy to find, and~~ it is ~~effectivity~~ effective ~~to~~ in ~~preserve~~ preserving the product, cheaper and accessible (Male et al 2017).

Formalin is a forty percent solution of formaldehyde in water. This compound is detected in the animal body as an intermediary metabolite. It also uses for several purposes, such as in medicine for specimen preservation and the treatment of hemorrhage (Pandey et al 2000). The preservatives activity of formalin ~~because of~~is due to its ability to react with protein generating methylene compounds, which ~~is~~ are more difficult to be decayed by microbes (Ichya'uddin 2016). In low concentrations, formalin is not toxic for humans, but in higher concentrations, it had negative effects such as acute toxicity and death (Pandey et al 2000). It also ~~decreased~~ decreases the levels of body antioxidants ~~that~~, resulting in liver damage (Yulisa et al 2014). Other studies also reported the cases of eczema, eye irritation, respiratory tract irritation, cancer, increased ~~the~~ growth rate of malignant neoplastic diseases~~,~~ and denaturation of DNA (Suwanaruang 2018; Wilianto & Yudianto 2013). Therefore, its uses are forbidden.

Formalin solution ~~was~~ is not stable during storage and ~~exposed~~ exposure to high temperatures (Kaneko et al 1977; Laksmiani et al 2015). Those are caused by desolvation or evaporation of formalin during washing, boiling, or frying (Fellows 2015; Harmita 2016; Muntaha et al 2015; Sundari et al 2015) and enzymatic process in storage (Rachmawati et al 2007). Formalin that was ~~exposed to the~~in contact with fish decreased up to 43-53% after 5 times rinsing in 250 mL of distilled water and boiling for 30 minutes

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(Yusuf et al 2015). Other studies were also reported that soaking in warm water and frying decreased the concentration of formalin up to 63~~,~~ 27% and 83.03%, respectively (Levita et al 2010). In daily practices, fish is usually washed in flowing water before processing, ~~continued~~ then ~~to frying~~fryed/~~boiling~~ boiled ~~in a~~for a short ~~er~~ time. However, there was no study ~~explained~~ explaining the influences of these processing and storage methods on the concentration of formalin.

The purpose of this study was to ~~know~~ identify the effect of processing ~~in~~ for a short time and storage (at room and freezing temperature~~s and freezing~~) on the residue of formalin. This study used catfish as a food model.

Material and Method

Material. The study used fresh catfish, purchased from the traditional market in ~~Yogyakarta~~Jakarta. The~~An~~ other used ingredients ~~used~~ were formalin solution and Schiff's reagent. Formalin solution was made from 4 mL of formalin 37% in 1,000 mL of distilled water (Rachmawati et al 2007).

Method. The study was divided into two sections, i.e. 1) ~~study~~ analyzing the effect of processing and 2) analyzing the effect of storage treatment. Fresh catfish were slaughtered, eviscerated, cleaned, and soaked in 1,480 ppm formalin solution (Rachmawati et al 2007; Sugiarti et al 2014). For the first study, catfish were divided into four groups, i.e.: controls (before treatment), washing, frying, and boiling treatments. Washing was done by watering the samples with running water for 1 minute, while frying was done by frying the samples in oil at 200°C for 8 minutes. Boiling treatment was done by putting the samples into boiled water (100°C) for 7 minutes (Sugiarti et al 2014). For the second study, storage treatments, the catfish was also divided into four groups~~, they: were~~ room temperature storage (29°C) for 24 hours and 48 hours and freezing temperatures storage (-3°C) for 24 hours and 48 hours (Handayani et al 2010). At the end of treatments, the concentration of formalin in samples was analyzed using a spectrophotometer. As the preparation step, a 5 g sample was dissolved in 25 mL of distilled water. One mL of supernatant was mixed with 2 mL of Schiff's reagent, then heated in a water bath at 60°C for 5 minutes. It was incubated for 10 minutes before measuring the absorbance at 520 nm (Shita 2016; Mudaffar 2018).

Statistical analysis. Data were presented in mean±SD and analyzed with: (1) one-way analysis of variance (ANOVA) to ~~know~~ determine the differences among all processing treatments and the effect of storage periods on the concentration of formalin; (2) paired sample T-test to determine~~know~~ the differences between the same samples before and after processing treatments; (3) independent sample t-test to know the effect of temperature at the same storage period, and (4) two-way ANOVA to determine~~know~~ the combination effect of storage temperature and period on the concentration of formalin. All data were analyzed with SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL, USA) at the 5% significance level.

Results

Effect of processing on the formalin residue of catfish. Table 1 shows that all treatments had significant effects on the residue of formalin ($p<0.05$). It can be seen from the p-value between the same samples before and after treatments. Among all treatments, washing shows the ~~lowest~~ highest residue of formalin ($p<0.05$). Both boiling and frying had an almost similar residue of formalin ($p>0.05$). The ~~higher~~ lower residue of formalin in boiling and frying may be due to heating exposure.

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Table 1

Effect of processing on the formalin residue of catfish

Processing treatments	*Residue of formalin (ppm)*		*Δ residue of formalin before and after treatment (%)*	*p-value*	
	Before treatment	*After treatment*		*Among all samples after treatments*	*Between the same samples before and after treatments*
Washing	1,420.17±66.94^{c}	310.42±77.61^{b}	78.14		0.00
Frying	1,420.17±66.94^{c}	14.18±6.69^{a}	99.01	0.01	0.02
Boiling	1,420.17±66.94^{c}	27.50±9.11^{a}	98.10		0.02

Mean±SD in the same column with different superscripts are significantly different (p<0.05); p-value among all samples was obtained from one-way ANOVA analyses, while p-value between the samples before and after treatments were obtained from paired T-test analyses.

Effect of storage temperatures and periods on the formalin residue of catfish. Table 2 shows the effect of storage temperatures, of periods~~,~~ and of their combination ~~of both~~ on the concentration of formalin. Based on this data, the storage period influenced the residue of formalin decrease (p<0.05)~~.~~ during the first 24 hours~~It could be seen from the decrease of formalin residue during 24 hours of storage. However~~, but after a storage period of 48 hours the decrease ~~did~~ was not significantly different ~~when the, storage continued to 48 hours~~ (p>0.05).

On the other hand, there were no differences in the residue of formalin stored at room and freezing temperature~~. It was indicated~~, suggesting that the temperature of storage did not affect the formalin residue (p>0.05). Although there were differences in the statistical results between the ~~effect of~~ temperature effects and the storage periods ~~of storage~~ effects, the combined effect of both variables showed no impact on the concentration of formalin (p>0.05).

Table 2

Effect of storage temperature and period on the formalin residue of catfish

Temperature (T)	*Periods (t) (hours)*			*p-value*		
	0	*24*	*48*	*T*	*t*	*T*t*
Room (29°C)	1,420.17±66.94^{a1}	52.18±28.78^{a2}	40.64±3.93^{a2}	0.20	0.00	0.76
Freezing (-3°C)	1,420.17±66.94^{a1}	39.04±7.07^{a2}	13.65±4.23^{a2}			

Mean±SD in the same column with different alphabetical superscripts (a, b, or c) are significantly different (p<0.05) and so are in the same row with different number superscript (1, 2, or 3). The p-value for T, t, and T*t was obtained from independent T-test, one-way ANOVA, and two-way ANOVA, respectively.

Discussion

The residue of formalin before treatment. The residue of formalin before and after treatment (either fish processing or storage) was significantly different (Table 1 and Table 2). In this study, the catfish specimens were submerged for 60 minutes in ~~1,480 ppm of~~ formalin solution of 1,480 ppm.~~was added to the catfish and submerged for 60 minutes.~~ The 60 minutes submersion period was chosen because the aldehyde compound may be readily bonded to the amino acids of fish in ~~the~~ a small amount (Rahmadhani et al 2017; Baroroh & Kresnadipayana 2016). Formalin would attack lysin for the first time, followed by histidine and tyrosine, ~~which led~~leading to the formation of a methylene compound (Ichya'uddin 2016). The protein-containing methylene compounds could not be digested by living things and microbes, especially pathogens, so that the shelf life would be longer (Purawisastra & Sahara 2016). The beneficial bacteria in the fish may

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also be killed by dehydration mechanism, as another effect of the existence of formalin in the body (Mudzkirah 2016).

The effect of processing on the formalin residue of catfish. The residue of formalin was reduced ~~to~~ by 78.14% during washing treatment. The reduction was significant ($p<0.05$). The formalin was easily washed out because of its high solubility in the water, which is 24 x 105 mg L^{-1} at 20°C (BPOM 2008; Sugiarti et al 2014~~; Yusuf et al 2015~~). A~~nother~~ study conducted by Yusuf et al (2015) ~~also~~ showed the same trend, but the reduction was lower (with only 43%). The differences in the level of reduction can be affected by the ~~length~~ duration of formalin submersion. The longer the submersion time, the smaller the ~~decreasing~~ decrease, as ~~there was~~ more formalin was bonded with the fish protein ~~in the fish~~.

In this study, frying also significantly reduced the residue of formalin ($p<0.05$). The reduction level was 99% that indicated most of the formalin ~~was~~ disappeared during the frying process. Formalin was included in a volatile compound, especially ~~at the~~ above ~~of~~ its boiling point (Joshi et al 2015). This study ~~was also the same with~~confirmed the results of Sugiarti et al (2014), showing a ~~that found the~~ reduction level of formalin up to 99.28% after 1 hour of submersion followed by the frying process (no information about the period of frying process) in squid. Frying could denature the protein and also hydrolyze its bonding with formalin (Levita et al 2010). The high temperature in frying increased the kinetic energy and led to faster movement of the molecules that could damage the molecular bonds (Sugiarti et al 2014).

~~Boiling was another heat~~ Heat processing by boiling ~~that was done in this study. The results showed that this process also~~ reduced the residue of formalin with up to 98.1%, more than~~. The residue of formalin after treatment was different from the sample after~~ the washing process ($p<0.05$), but at the same degree ~~with as~~~~that after~~ the frying process ($p>0.05$). ~~In the boiling process, t~~The high reduction of formalin in the boiling process ~~was higher than that was in only washing or soaking treatment because there was more~~was due to the accelerated formalin ~~dissolved~~ dissolution rate ~~in~~ at higher temperature~~s~~ ~~water~~ (Annisak 2019). This effect could be enhanced by~~e amount of losing would be higher when it combined~~ boiling the sample ~~with the~~in an opened pan ~~during the boiling~~ (Kamal et al 2017).

The effect of storage on the formalin residue of catfish. This ~~research~~ experiment ~~found~~ demonstrated that only ~~a~~ the period of storage ~~that~~ significantly influenced the residue of formalin ($p<0.05$), while the temperature of storage and the combination between temperature and period of storage did not (Table 2). The previous different studies showed that ~~either~~ a storage at the room temperature or at the freezing temperature ~~of storage~~ had ~~a~~ similar effect~~s~~ on the ~~decreasing of~~ formalin residue decrease (Jawahar et al 2017; Murtini et al 2014), possibly ~~. It may be~~ due to the same ~~degradation~~ level of protein degradation~~during both in the room and freezing temperature, resulting in the loss of~~ by breaking the bonds between protein and formalin and releasing free compounds. ~~In~~ At the room temperature, the protein degradation was ~~more~~ essentially caused by the microbial activity, while ~~in~~ at the freezing temperature it was ~~more~~ caused mostly by physical destruction, leading to ~~the~~ conformational and functional changes (Murtini et al 2014; Yeasmin et al 2010). Formalin in the free form is more reactive and can also be more easily degraded ~~was easier to break down into another compound or reacts with other compounds in the free form~~ (Riyanto et al 2006; Rachmawati et al 2007).

Conclusions. This research concluded that short time processing and storage treatments decreased the concentration of formalin. Heat involvement ~~made~~ determined a more effective ~~the~~ decrease of concentration ~~became more effective~~. In the storage study, the residue of formalin was ~~influenced~~ significantly reduced by the period of storage, mainly during the first 24 hours, then the formalin concentration reduction was insignificant, up to 48 hours of storage.

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Acknowledgements. The authors are grateful to the Indonesia Ministry of Research, Technology, and Higher Education for providing the financial support in pursuing this research project (Program Kreativitas Mahasiswa Kemeristekdikti 2019 Program).

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Revision

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Kepada: Eni Kovacs <ek.bioflux@gmail.com>

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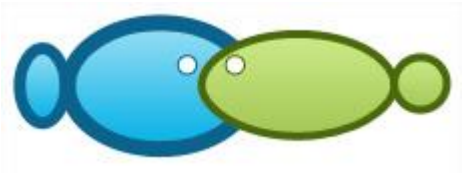

The residue of formalin in catfish (*Clarias gariepinus*) after processing and storage for short periods

Isny Maulidevi, Nurul Andriyani, Ryan Salfarino, Veriani Aprilia

Department of Nutrition, Faculty of Health Sciences, Alma Ata University, Yogyakarta, Indonesia. Corresponding author: V. Aprilia, verianiaprilia@almaata.ac.id

Abstract. Fresh fish is a highly perishable product due to its high protein content. A preservative may be needed to inhibit it. Nowadays, many fishes were reported to be exposed to formalin. This study aimed to determine the effect of short period processing (washing, frying, boiling in short-period) and storage (at the room and at freezing temperatures) on the residue of formalin in catfish, as a food model. Catfish, *Clarias gariepinus,* were divided into 8 groups, e.g. before treatment, washing in airflow for 1 minute, frying in 200°C for 8 minutes, boiling in 100°C for 7 minutes, storage at room temperature (29±1°C) for 24 and 48 hours, storage at freezing temperature (-3°C) for 24 and 48 hours. Before treatment, the catfish was soaked in 1,480 ppm of formalin solutions. The residue of formalin was measured at the end of each treatment by using a spectrophotometer. Data were then analyzed with analysis of variance with SPSS software. The results showed that there were significant effects of processing and storage treatment on the formalin residue of catfish. The formalin residue of all treatments was lower than before treatments. The frying processing and storage at freezing temperatures were the most effective treatments in reducing the formalin residue.
Key Words: formalin, fish, processing, storage, formaldehyde.

Introduction. Fresh fish is a highly perishable product due to its high protein content for microbial activity (Wijayanti & Lukitasari 2016). A preservative maybe needs to keep the freshness, inhibit the decay and prolong the shelf life (Antoni 2010). Nowadays, there are many kinds of food-grade preservatives available in the market. Unfortunately, formalin is still frequently found in food products, including fresh fish, being used among fishermen mainly because it is effective in preserving the product, cheaper and accessible (Male et al 2017).

Formalin is a forty percent solution of formaldehyde in water. This compound is detected in the animal body as an intermediary metabolite. It also uses for several purposes, such as in medicine for specimen preservation and the treatment of hemorrhage (Pandey et al., 2000). The preservatives activity of formalin is due to its ability to react with protein generating methylene compounds, which are more difficult to be decayed by microbes (Ichya'uddin 2016). In low concentrations, formalin is not toxic for humans, but in higher concentrations, it had negative effects such as acute toxicity and death (Pandey et al 2000). It also decreases the levels of body antioxidants, resulting in liver damage (Yulisa et al 2014). Other studies also reported the cases of eczema, eye irritation, respiratory tract irritation, cancer, increased growth rate of malignant neoplastic diseases and denaturation of DNA (Suwanaruang 2018; Wilianto & Yudianto 2013). Therefore, its uses are forbidden.

Formalin solution is not stable during storage and exposure to high temperatures (Kaneko et al., 1977; Laksmiani et al., 2015). Those are caused by desolvation or evaporation of formalin during washing, boiling, or frying (Fellows 2015; Harmita 2016; Muntaha et al 2015; Sundari et al 2015) and enzymatic process in storage (Rachmawati et al 2007). Formalin that was in contact with fish decreased up to 43-53% after 5 times rinsing in 250 mL of distilled water and boiling for 30 minutes (Yusuf et al 2015). Other studies were also reported that soaking in warm water and frying decreased the

concentration of formalin up to 63.27% and 83.03%, respectively (Levita et al 2010). In daily practices, fish is usually washed in flowing water before processing, then fryed/boiled for a short time. However, there was no study explaining the influences of these processing and storage methods on the concentration of formalin.

The purpose of this study was to identify the effect of processing for a short time and storage (at room and freezing temperatures) on the residue of formalin. This study used catfish as a food model.

Material and Method

Material. The study used fresh catfish, purchased from the traditional market in Yogyakarta, Indonesia. The other used ingredients were formalin solution and Schiff's reagent. Formalin solution was made from 4 mL of formalin 37% in 1,000 mL of distilled water (Rachmawati et al 2007).

Method. The study was divided into two sections, i.e. 1) analyzing the effect of processing and 2) analyzing the effect of storage treatment. Fresh catfish were slaughtered, eviscerated, cleaned, and soaked in 1,480 ppm formalin solution (Rachmawati et al 2007; Sugiarti et al 2014). For the first study, catfish were divided into four groups, i.e.: controls (before treatment), washing, frying, and boiling treatments. Washing was done by watering the samples with running water for 1 minute, while frying was done by frying the samples in oil at 200°C for 8 minutes. Boiling treatment was done by putting the samples into boiled water (100°C) for 7 minutes (Sugiarti et al 2014). For the second study, storage treatments, the catfish was also divided into four groups: room temperature storage (29°C) for 24 hours and 48 hours and freezing temperatures storage (-3°C) for 24 hours and 48 hours (Handayani et al 2010). At the end of treatments, the concentration of formalin in samples was analyzed using a spectrophotometer. As the preparation step, a 5 g sample was dissolved in 25 mL of distilled water. One mL of supernatant was mixed with 2 mL of Schiff's reagent, then heated in a water bath at 60°C for 5 minutes. It was incubated for 10 minutes before measuring the absorbance at 520 nm (Shita 2016; Mudaffar 2018).

Statistical analysis. Data were presented in mean±SD and analyzed with: (1) one-way analysis of variance (ANOVA) to determine the differences among all processing treatments and the effect of storage periods on the concentration of formalin; (2) paired sample T-test to determine the differences between the same samples before and after processing treatments; (3) independent sample t-test to know the effect of temperature at the same storage period, and (4) two-way ANOVA to determine the combination effect of storage temperature and period on the concentration of formalin. All data were analyzed with SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL, USA) at the 5% significance level.

Results

Effect of processing on the formalin residue of catfish. Table 1 shows that all treatments had significant effects on the residue of formalin ($p<0.05$). It can be seen from the p-value between the same samples before and after treatments. Among all treatments, washing shows the highest residue of formalin ($p<0.05$). Both boiling and frying had an almost similar residue of formalin ($p>0.05$). The lower residue of formalin in boiling and frying may be due to heating exposure.

Table 1

Effect of processing on the formalin residue of catfish

Processing treatments	*Residue of formalin (ppm)*		*Δ residue of formalin before and after treatment (%)*
	Before treatment	*After treatment*	
Washing	1,420.17±66.94^{c1}	310.42±77.61^{b2}	78.14
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Effect of storage temperatures and periods on the formalin residue of catfish. Table 2 shows the effect of storage temperatures, of periods and of their combination on the concentration of formalin. Based on this data, the storage period influenced the residue of formalin decrease ($p<0.05$) during the first 24 hours, but after a storage period of 48 hours the decrease was not significantly different , ($p>0.05$).

On the other hand, there were no differences in the residue of formalin stored at room and freezing temperature, suggesting that the temperature of storage did not affect the formalin residue ($p>0.05$). Although there were differences in the statistical results between the temperature effects and the storage periods effects, the combined effect of both variables showed no impact on the concentration of formalin ($p>0.05$).

Table 2

Effect of storage temperature and period on the formalin residue of catfish

Temperature (T)	*Periods (t) (hours)*			*p-value*		
	0	*24*	*48*	*T*	*t*	*T*t*
Room (29°C)	1,420.17±66.94^{a1}	52.18±28.78^{a2}	40.64±3.93^{a2}	0.20	0.00	0.76
Freezing (-3°C)	1,420.17±66.94^{a1}	39.04±7.07^{a2}	13.65±4.23^{a2}			

Mean±SD in the same column with different alphabetical superscripts (a, b, or c) are significantly different ($p<0.05$) and so are in the same row with different number superscript (1, 2, or 3). The p-value for T, t, and T*t was obtained from independent T-test, one-way ANOVA, and two-way ANOVA, respectively.

Discussion

The residue of formalin before treatment. The residue of formalin before and after treatment (either fish processing or storage) was significantly different (Table 1 and Table 2). In this study, the catfish specimens were submerged for 60 minutes in formalin solution of 1,480 ppm. The 60 minutes submersion period was chosen because the aldehyde compound may be readily bonded to the amino acids of fish in a small amount (Rahmadhani et al 2017; Baroroh & Kresnadipayana 2016). Formalin would attack lysine for the first time, followed by histidine and tyrosine, leading to the formation of a methylene compound (Ichya'uddin 2016). The protein-containing methylene compounds could not be digested by living things and microbes, especially pathogens, so that the shelf life would be longer (Purawisastra & Sahara 2011). The beneficial bacteria in the fish may also be killed by dehydration mechanism, as another effect of the existence of formalin in the body (Mudzkirah 2016).

The effect of processing on the formalin residue of catfish. The residue of formalin was reduced by 78.14% during washing treatment. The reduction was significant ($p<0.05$). The formalin was easily washed out because of its high solubility in the water, which is 24 x 105 mg L^{-1} at 20°C (BPOM 2008; Sugiarti et al 2014). A study conducted by Yusuf et al (2015) showed the same trend, but the reduction was lower (with only 43%). The differences in the level of reduction can be affected by the duration of formalin submersion. The longer the submersion time, the smaller the decrease, as more formalin was bonded with the fish protein.

In this study, frying also significantly reduced the residue of formalin ($p<0.05$). The reduction level was 99% that indicated most of the formalin disappeared during the frying process. Formalin was included in a volatile compound, especially above its boiling point (Joshi et al 2015). This study confirmed the results of Sugiarti et al (2014), showing a reduction level of formalin up to 99.28% after 1 hour of submersion followed by the frying process (no information about the period of frying process) in squid. Frying could denature the protein and also hydrolyze its bonding with formalin (Levita et al 2010). The high temperature in frying increased the kinetic energy and led to faster movement of the molecules that could damage the molecular bonds (Sugiarti et al 2014).

Heat processing by boiling reduced the residue of formalin with up to 98.1%, more than the washing process ($p<0.05$), but at the same degree asthe frying process ($p>0.05$).The high reduction of formalin in the boiling process was due to the accelerated formalin dissolution rate at higher temperatures (Annisak 2019). This effect could be enhanced by boiling the sample in an open pan (Kamal et al 2017).

The effect of storage on the formalin residue of catfish. This experiment demonstrated that only the period of storage significantly influenced the residue of formalin ($p<0.05$), while the temperature of storage and the combination between temperature and period of storage did not (Table 2). The previous different studies showed that a storage at the room temperature or at the freezing temperature had similar effects on the formalin residue decrease (Jawahar et al 2017; Murtini et al 2014), possibly due to the same level of protein degradation by breaking the bonds between protein and formalin and releasing free compounds. At the room temperature, the protein degradation was essentially caused by the microbial activity, while at the freezing temperature it was caused mostly by physical destruction, leading to conformational and functional changes (Murtini et al 2014; Yeasmin et al 2010). Formalin in the free form is more reactive and can also be more easily degraded (Riyanto et al 2006; Rachmawati et al 2007).

Conclusions. This research concluded that short time processing and storage treatments decreased the concentration of formalin. Heat involvement, especially during boiling and frying process determined a more effective decrease of concentration (the decrease level was >90%). In the storage study, the residue of formalin was significantly reduced by the period of storage, mainly during the first 24 hours, then the formalin concentration reduction was insignificant, up to 48 hours of storage.

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Revision

Eni Kovacs <ek.bioflux@gmail.com> 7 Desember 2020 pukul 04.02
Kepada: verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id>

Dear Veriani Aprilia,

Please find the manuscript attached and make the necessary adjustments.

Thank you.

Kind regards,
Editor AACL Bioflux
Eniko Kovacs

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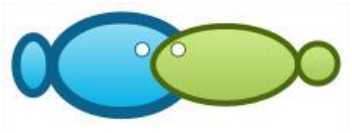

The residue of formalin in catfish (*Clarias gariepinus*) after processing and storage for short periods

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Abstract. Fresh fish is a highly perishable product due to its high protein content. A preservative may be needed to inhibit it. Nowadays, many fishes were reported to be exposed to formalin. This study aimed to determine the effect of short period processing (washing, frying, boiling in short-period) and storage (at the room and at freezing temperatures) on the residue of formalin in catfish, as a food model. Catfish, *Clarias gariepinus,* were divided into 8 groups, e.g. before treatment, washing in airflow for 1 minute, frying in 200°C for 8 minutes, boiling in 100°C for 7 minutes, storage at room temperature (29±1°C) for 24 and 48 hours, storage at freezing temperature (-3°C) for 24 and 48 hours. Before treatment, the catfish was soaked in 1,480 ppm of formalin solutions. The residue of formalin was measured at the end of each treatment by using a spectrophotometer. Data were then analyzed with analysis of variance with SPSS software. The results showed that there were significant effects of processing and storage treatment on the formalin residue of catfish. The formalin residue of all treatments was lower than before treatments. The frying processing and storage at freezing temperatures were the most effective treatments in reducing the formalin residue.
Key Words: fresh fish, preservative, food model, treatments, formalin residue.

Introduction. Fresh fish is a highly perishable product due to its high protein content for microbial activity (Wijayanti & Lukitasari 2016). A preservative maybe needs to keep the freshness, inhibit the decay and prolong the shelf life (Antoni 2010). Nowadays, there are many kinds of food-grade preservatives available in the market. Unfortunately, formalin is still frequently found in food products, including fresh fish, being used among fishermen mainly because it is effective in preserving the product, cheaper and accessible (Male et al 2017).

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Discussion

The residue of formalin before treatment. The residue of formalin before and after treatment (either fish processing or storage) was significantly different (Table 1 and Table 2). In this study, the catfish specimens were submerged for 60 minutes in formalin solution of 1,480 ppm. The 60 minutes submersion period was chosen because the aldehyde compound may be readily bonded to the amino acids of fish in a small amount (Rahmadhani et al 2017; Baroroh & Kresnadipayana 2016). Formalin would attack lysine for the first time, followed by histidine and tyrosine, leading to the formation of a methylene compound (Ichya'uddin 2016). The protein-containing methylene compounds could not be digested by living things and microbes, especially pathogens, so that the shelf life would be longer (Purawisastra & Sahara 2011). The beneficial bacteria in the fish may also be killed by dehydration mechanism, as another effect of the existence of formalin in the body (Mudzkirah 2016).

The effect of processing on the formalin residue of catfish. The residue of formalin was reduced by 78.14% during washing treatment. The reduction was significant ($p<0.05$). The formalin was easily washed out because of its high solubility in the water, which is 24 x 105 mg L^{-1} at 20°C (BPOM 2008; Sugiarti et al 2014). A study conducted by Yusuf et al (2015) showed the same trend, but the reduction was lower (with only 43%). The differences in the level of reduction can be affected by the duration of

formalin submersion. The longer the submersion time, the smaller the decrease, as more formalin was bonded with the fish protein.

In this study, frying also significantly reduced the residue of formalin ($p<0.05$). The reduction level was 99% that indicated most of the formalin disappeared during the frying process. Formalin was included in a volatile compound, especially above its boiling point (Joshi et al 2015). This study confirmed the results of Sugiarti et al (2014), showing a reduction level of formalin up to 99.28% after 1 hour of submersion followed by the frying process (no information about the period of frying process) in squid. Frying could denature the protein and also hydrolyze its bonding with formalin (Levita et al 2010). The high temperature in frying increased the kinetic energy and led to faster movement of the molecules that could damage the molecular bonds (Sugiarti et al 2014).

Heat processing by boiling reduced the residue of formalin with up to 98.1%, more than the washing process ($p<0.05$), but at the same degree as the frying process ($p>0.05$).The high reduction of formalin in the boiling process was due to the accelerated formalin dissolution rate at higher temperatures (Annisak 2019). This effect could be enhanced by boiling the sample in an open pan (Kamal et al 2017).

The effect of storage on the formalin residue of catfish. This experiment demonstrated that only the period of storage significantly influenced the residue of formalin ($p<0.05$), while the temperature of storage and the combination between temperature and period of storage did not (Table 2). The previous different studies showed that a storage at the room temperature or at the freezing temperature had similar effects on the formalin residue decrease (Jawahar et al 2017; Murtini et al 2014), possibly due to the same level of protein degradation by breaking the bonds between protein and formalin and releasing free compounds. At the room temperature, the protein degradation was essentially caused by the microbial activity, while at the freezing temperature it was caused mostly by physical destruction, leading to conformational and functional changes (Murtini et al 2014; Yeasmin et al 2010). Formalin in the free form is more reactive and can also be more easily degraded (Riyanto et al 2006; Rachmawati et al 2007).

Conclusions. This research concluded that short time processing and storage treatments decreased the concentration of formalin. Heat involvement determined a more effective decrease of concentration (the decrease level was >90%). In the storage study, the residue of formalin was significantly reduced by the period of storage, mainly during the first 24 hours, then the formalin concentration reduction was insignificant, up to 48 hours of storage.

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Dear Kovacs,
Here my revised article. thank you very much

Regards
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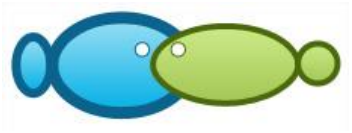

The residue of formalin in catfish (*Clarias gariepinus*) after processing and storage for short periods

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Abstract. Fresh fish is a highly perishable product due to its high protein content. A preservative may be needed to inhibit it. Nowadays, many fishes were reported to be exposed to formalin. This study aimed to determine the effect of short period processing (washing, frying, boiling in short-period) and storage (at the room and at freezing temperatures) on the residue of formalin in catfish, as a food model. Catfish, *Clarias gariepinus,* were divided into 8 groups, e.g. before treatment, washing in airflow for 1 minute, frying in 200°C for 8 minutes, boiling in 100°C for 7 minutes, storage at room temperature (29±1°C) for 24 and 48 hours, storage at freezing temperature (-3°C) for 24 and 48 hours. Before treatment, the catfish was soaked in 1,480 ppm of formalin solutions. The residue of formalin was measured at the end of each treatment by using a spectrophotometer. Data were then analyzed with analysis of variance with SPSS software. The results showed that there were significant effects of processing and storage treatment on the formalin residue of catfish. The formalin residue of all treatments was lower than before treatments. The frying processing and storage at freezing temperatures were the most effective treatments in reducing the formalin residue.
Key Words: fresh fish, preservative, food model, treatments, formalin residue.

Introduction. Fresh fish is a highly perishable product due to its high protein content for microbial activity (Wijayanti & Lukitasari 2016). A preservative maybe needs to keep the freshness, inhibit the decay and prolong the shelf life (Antoni 2010). Nowadays, there are many kinds of food-grade preservatives available in the market. Unfortunately, formalin is still frequently found in food products, including fresh fish, being used among fishermen mainly because it is effective in preserving the product, cheaper and accessible (Male et al 2017).

Formalin is a forty percent solution of formaldehyde in water. This compound is detected in the animal body as an intermediary metabolite. It also uses for several purposes, such as in medicine for specimen preservation and the treatment of hemorrhage (Pandey et al 2000). The preservatives activity of formalin is due to its ability to react with protein generating methylene compounds, which are more difficult to be decayed by microbes (Ichya'uddin 2016). In low concentrations, formalin is not toxic for humans, but in higher concentrations, it had negative effects such as acute toxicity and death (Pandey et al 2000). It also decreases the levels of body antioxidants, resulting in liver damage (Yulisa et al 2014). Other studies also reported the cases of eczema, eye irritation, respiratory tract irritation, cancer, increased growth rate of malignant neoplastic diseases and denaturation of DNA (Suwanaruang 2018; Wilianto & Yudianto 2013). Therefore, its uses are forbidden.

Formalin solution is not stable during storage and exposure to high temperatures (Kaneko et al 1977; Laksmiani et al 2015). Those are caused by desolvation or evaporation of formalin during washing, boiling, or frying (Muntaha et al 2015; Sundari et al 2015) and enzymatic process in storage (Rachmawati et al 2007). Formalin that was in contact with fish decreased up to 43-53% after 5 times rinsing in 250 mL of distilled water and boiling for 30 minutes (Yusuf et al 2015). Other studies were also reported that soaking in warm water and frying decreased the concentration of formalin up to

63.27% and 83.03%, respectively (Levita et al 2010). In daily practices, fish is usually washed in flowing water before processing, then fryed/boiled for a short time. However, there was no study explaining the influences of these processing and storage methods on the concentration of formalin.

The purpose of this study was to identify the effect of processing for a short time and storage (at room and freezing temperatures) on the residue of formalin. This study used catfish as a food model.

Material and Method

Material. The study used fresh catfish, purchased from the traditional market in Yogyakarta, Indonesia. The other used ingredients were formalin solution and Schiff's reagent. Formalin solution was made from 4 mL of formalin 37% in 1,000 mL of distilled water (Rachmawati et al 2007).

Method. The study was divided into two sections, i.e. 1) analyzing the effect of processing and 2) analyzing the effect of storage treatment. Fresh catfish were slaughtered, eviscerated, cleaned, and soaked in 1,480 ppm formalin solution (Rachmawati et al 2007; Sugiarti et al 2014). For the first study, catfish were divided into four groups, i.e.: controls (before treatment), washing, frying, and boiling treatments. Washing was done by watering the samples with running water for 1 minute, while frying was done by frying the samples in oil at 200°C for 8 minutes. Boiling treatment was done by putting the samples into boiled water (100°C) for 7 minutes (Sugiarti et al 2014). For the second study, storage treatments, the catfish was also divided into four groups: room temperature storage (29°C) for 24 hours and 48 hours and freezing temperatures storage (-3°C) for 24 hours and 48 hours (Handayani et al 2010). At the end of treatments, the concentration of formalin in samples was analyzed using a spectrophotometer. As the preparation step, a 5 g sample was dissolved in 25 mL of distilled water. One mL of supernatant was mixed with 2 mL of Schiff's reagent, then heated in a water bath at 60°C for 5 minutes. It was incubated for 10 minutes before measuring the absorbance at 520 nm (Shita 2016; Mudaffar 2018).

Statistical analysis. Data were presented in mean±SD and analyzed with: (1) one-way analysis of variance (ANOVA) to determine the differences among all processing treatments and the effect of storage periods on the concentration of formalin; (2) paired sample T-test to determine the differences between the same samples before and after processing treatments; (3) independent sample t-test to know the effect of temperature at the same storage period, and (4) two-way ANOVA to determine the combination effect of storage temperature and period on the concentration of formalin. All data were analyzed with SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL, USA) at the 5% significance level.

Results

Effect of processing on the formalin residue of catfish. Table 1 shows that all treatments had significant effects on the residue of formalin ($p<0.05$). It can be seen from the p-value between the same samples before and after treatments. Among all treatments, washing shows the highest residue of formalin ($p<0.05$). Both boiling and frying had an almost similar residue of formalin ($p>0.05$). The lower residue of formalin in boiling and frying may be due to heating exposure.

Table 1

Effect of processing on the formalin residue of catfish

Processing treatments	*Residue of formalin (ppm)*		*Δ residue of formalin before and after treatment (%)*
	Before treatment	*After treatment*	
Washing	1,420.17±66.94^{c1}	310.42±77.61^{b2}	78.14
Frying	1,420.17±66.94^{c1}	14.18±6.69^{a2}	99.01
Boiling	1,420.17±66.94^{c1}	27.50±9.11^{a2}	98.10

Mean±SD in the same column with different alphabetical superscripts (a, b, or c) are significantly different ($p<0.05$) by using one-way ANOVA statistical analysis. Mean±SD in the same row with different number superscripts (1 or 2) are significantly different ($p<0.05$) by using paired sample T-test statistical analysis.

Effect of storage temperatures and periods on the formalin residue of catfish. Table 2 shows the effect of storage temperatures, of periods and of their combination on the concentration of formalin. Based on this data, the storage period influenced the residue of formalin decrease ($p<0.05$) during the first 24 hours, but after a storage period of 48 hours the decrease was not significantly different ($p>0.05$).

On the other hand, there were no differences in the residue of formalin stored at room and freezing temperature, suggesting that the temperature of storage did not affect the formalin residue ($p>0.05$). Although there were differences in the statistical results between the temperature effects and the storage periods effects, the combined effect of both variables showed no impact on the concentration of formalin ($p>0.05$).

Table 2

Effect of storage temperature and period on the formalin residue of catfish

Temperature (T)	*Periods (t) (hours)*			*p-value*		
	0	*24*	*48*	*T*	*t*	*T*t*
Room (29°C)	1,420.17±66.94^{a1}	52.18±28.78^{a2}	40.64±3.93^{a2}	0.20	0.00	0.76
Freezing (-3°C)	1,420.17±66.94^{a1}	39.04±7.07^{a2}	13.65±4.23^{a2}			

Mean±SD in the same column with different alphabetical superscripts (a, b, or c) are significantly different ($p<0.05$) and so are in the same row with different number superscript (1, 2, or 3). The p-value for T, t, and T*t was obtained from independent T-test, one-way ANOVA, and two-way ANOVA, respectively.

Discussion

The residue of formalin before treatment. The residue of formalin before and after treatment (either fish processing or storage) was significantly different (Table 1 and Table 2). In this study, the catfish specimens were submerged for 60 minutes in formalin solution of 1,480 ppm. The 60 minutes submersion period was chosen because the aldehyde compound may be readily bonded to the amino acids of fish in a small amount (Rahmadhani et al 2017; Baroroh & Kresnadipayana 2016). Formalin would attack lysine for the first time, followed by histidine and tyrosine, leading to the formation of a methylene compound (Ichya'uddin 2016). The protein-containing methylene compounds could not be digested by living things and microbes, especially pathogens, so that the shelf life would be longer (Purawisastra & Sahara 2011). The beneficial bacteria in the fish may also be killed by dehydration mechanism, as another effect of the existence of formalin in the body (Mudzkirah 2016).

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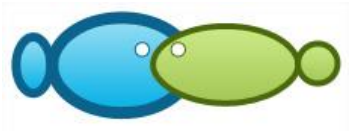

The residue of formalin in catfish (*Clarias gariepinus*) after processing and storage for short periods

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Abstract. Fresh fish is a highly perishable product due to its high protein content. A preservative may be needed to inhibit it. Nowadays, many fishes were reported to be exposed to formalin. This study aimed to determine the effect of short period processing (washing, frying, boiling in short-period) and storage (at the room and at freezing temperatures) on the residue of formalin in catfish, as a food model. Catfish, *Clarias gariepinus,* were divided into 8 groups, e.g. before treatment, washing in airflow for 1 minute, frying in 200°C for 8 minutes, boiling in 100°C for 7 minutes, storage at room temperature (29±1°C) for 24 and 48 hours, storage at freezing temperature (-3°C) for 24 and 48 hours. Before treatment, the catfish was soaked in 1,480 ppm of formalin solutions. The residue of formalin was measured at the end of each treatment by using a spectrophotometer. Data were then analyzed with analysis of variance with SPSS software. The results showed that there were significant effects of processing and storage treatment on the formalin residue of catfish. The formalin residue of all treatments was lower than before treatments. The frying processing and storage at freezing temperatures were the most effective treatments in reducing the formalin residue.
Key Words: fresh fish, preservative, food model, treatments, formalin residue.

Introduction. Fresh fish is a highly perishable product due to its high protein content for microbial activity (Wijayanti & Lukitasari 2016). A preservative maybe needs to keep the freshness, inhibit the decay and prolong the shelf life (Antoni 2010). Nowadays, there are many kinds of food-grade preservatives available in the market. Unfortunately, formalin is still frequently found in food products, including fresh fish, being used among fishermen mainly because it is effective in preserving the product, cheaper and accessible (Male et al 2017).

Formalin is a forty percent solution of formaldehyde in water. This compound is detected in the animal body as an intermediary metabolite. It also uses for several purposes, such as in medicine for specimen preservation and the treatment of hemorrhage (Pandey et al 2000). The preservatives activity of formalin is due to its ability to react with protein generating methylene compounds, which are more difficult to be decayed by microbes (Ichya'uddin 2016). In low concentrations, formalin is not toxic for humans, but in higher concentrations, it had negative effects such as acute toxicity and death (Pandey et al 2000). It also decreases the levels of body antioxidants, resulting in liver damage (Yulisa et al 2014). Other studies also reported the cases of eczema, eye irritation, respiratory tract irritation, cancer, increased growth rate of malignant neoplastic diseases and denaturation of DNA (Suwanaruang 2018; Wilianto & Yudianto 2013). Therefore, its uses are forbidden.

Formalin solution is not stable during storage and exposure to high temperatures (Kaneko et al 1977; Laksmiani et al 2015). Those are caused by desolvation or evaporation of formalin during washing, boiling, or frying (Fellows 2015; Harmita 2016; Muntaha et al 2015; Sundari et al 2015) and enzymatic process in storage (Rachmawati et al 2007). Formalin that was in contact with fish decreased up to 43-53% after 5 times rinsing in 250 mL of distilled water and boiling for 30 minutes (Yusuf et al 2015). Other studies were also reported that soaking in warm water and frying decreased the

concentration of formalin up to 63.27% and 83.03%, respectively (Levita et al 2010). In daily practices, fish is usually washed in flowing water before processing, then fryed/boiled for a short time. However, there was no study explaining the influences of these processing and storage methods on the concentration of formalin.

The purpose of this study was to identify the effect of processing for a short time and storage (at room and freezing temperatures) on the residue of formalin. This study used catfish, *Clarias gariepinus*, as a food model.

Material and Method

Material. The study used fresh catfish, purchased from the traditional market in Yogyakarta, Indonesia. The other used ingredients were formalin solution and Schiff's reagent. Formalin solution was made from 4 mL of formalin 37% in 1,000 mL of distilled water (Rachmawati et al 2007).

Method. The study was divided into two sections, i.e. 1) analyzing the effect of processing and 2) analyzing the effect of storage treatment. Fresh catfish were slaughtered, eviscerated, cleaned, and soaked in 1,480 ppm formalin solution (Rachmawati et al 2007; Sugiarti et al 2014). For the first study, catfish were divided into four groups, i.e.: controls (before treatment), washing, frying, and boiling treatments. Washing was done by watering the samples with running water for 1 minute, while frying was done by frying the samples in oil at 200°C for 8 minutes. Boiling treatment was done by putting the samples into boiled water (100°C) for 7 minutes (Sugiarti et al 2014). For the second study, storage treatments, the catfish was also divided into four groups: room temperature storage (29°C) for 24 hours and 48 hours and freezing temperatures storage (-3°C) for 24 hours and 48 hours (Handayani et al 2010). At the end of treatments, the concentration of formalin in samples was analyzed using a spectrophotometer. As the preparation step, a 5 g sample was dissolved in 25 mL of distilled water. One mL of supernatant was mixed with 2 mL of Schiff's reagent, then heated in a water bath at 60°C for 5 minutes. It was incubated for 10 minutes before measuring the absorbance at 520 nm (Shita 2016; Mudaffar 2018).

Statistical analysis. Data were presented in mean±SD and analyzed with: (1) one-way analysis of variance (ANOVA) to determine the differences among all processing treatments and the effect of storage periods on the concentration of formalin; (2) paired sample T-test to determine the differences between the same samples before and after processing treatments; (3) independent sample t-test to know the effect of temperature at the same storage period, and (4) two-way ANOVA to determine the combination effect of storage temperature and period on the concentration of formalin. All data were analyzed with SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL, USA) at the 5% significance level.

Results

Effect of processing on the formalin residue of C. gariepinus. Table 1 shows that all treatments had significant effects on the residue of formalin ($p<0.05$). It can be seen from the p-value between the same samples before and after treatments. Among all treatments, washing shows the highest residue of formalin ($p<0.05$). Both boiling and frying had an almost similar residue of formalin ($p>0.05$). The lower residue of formalin in boiling and frying may be due to heating exposure.

Table 1

Effect of processing on the formalin residue of *Clarias gariepinus*

Processing treatments	*Residue of formalin (ppm)*		*Δ residue of formalin before and after treatment (%)*
	Before treatment	*After treatment*	
Washing	1,420.17±66.94^{c1}	310.42±77.61^{b2}	78.14
Frying	1,420.17±66.94^{c1}	14.18±6.69^{a2}	99.01
Boiling	1,420.17±66.94^{c1}	27.50±9.11^{a2}	98.10

Mean±SD in the same column with different alphabetical superscripts (a, b, or c) are significantly different ($p<0.05$) by using one-way ANOVA statistical analysis. Mean±SD in the same row with different number superscripts (1 or 2) are significantly different ($p<0.05$) by using paired sample T-test statistical analysis.

Effect of storage temperatures and periods on the formalin residue of C. gariepinus. Table 2 shows the effect of storage temperatures, of periods and of their combination on the concentration of formalin. Based on this data, the storage period influenced the residue of formalin decrease ($p<0.05$) during the first 24 hours, but after a storage period of 48 hours the decrease was not significantly different ($p>0.05$).

On the other hand, there were no differences in the residue of formalin stored at room and freezing temperature, suggesting that the temperature of storage did not affect the formalin residue ($p>0.05$). Although there were differences in the statistical results between the temperature effects and the storage periods effects, the combined effect of both variables showed no impact on the concentration of formalin ($p>0.05$).

Table 2

Effect of storage temperature and period on the formalin residue of *Clarias gariepinus*

Temperature (T)	*Periods (t) (hours)*			*p-value*		
	0	*24*	*48*	*T*	*t*	*T*t*
Room (29°C)	1,420.17±66.94^{a1}	52.18±28.78^{a2}	40.64±3.93^{a2}	0.20	0.00	0.76
Freezing (-3°C)	1,420.17±66.94^{a1}	39.04±7.07^{a2}	13.65±4.23^{a2}			

Mean±SD in the same column with different alphabetical superscripts (a, b, or c) are significantly different ($p<0.05$) and so are in the same row with different number superscript (1, 2, or 3). The p-value for T, t, and T*t was obtained from independent T-test, one-way ANOVA, and two-way ANOVA, respectively.

Discussion

The residue of formalin before treatment. The residue of formalin before and after treatment (either fish processing or storage) was significantly different (Table 1 and Table 2). In this study, the *C. gariepinus* specimens were submerged for 60 minutes in formalin solution of 1,480 ppm. The 60 minutes submersion period was chosen because the aldehyde compound may be readily bonded to the amino acids of fish in a small amount (Rahmadhani et al 2017; Baroroh & Kresnadipayana 2016). Formalin would attack lysine for the first time, followed by histidine and tyrosine, leading to the formation of a methylene compound (Ichya'uddin 2016). The protein-containing methylene compounds could not be digested by living things and microbes, especially pathogens, so that the shelf life would be longer (Purawisastra & Sahara 2011). The beneficial bacteria in the fish may also be killed by dehydration mechanism, as another effect of the existence of formalin in the body (Mudzkirah 2016).

The effect of processing on the formalin residue of C. gariepinus. The residue of formalin was reduced by 78.14% during washing treatment. The reduction was significant ($p<0.05$). The formalin was easily washed out because of its high solubility in the water, which is 4×10^5 mg L^{-1} at 20°C (BPOM 2008; Sugiarti et al 2014). A study conducted by Yusuf et al (2015) showed the same trend, but the reduction was lower (with only 43%). The differences in the level of reduction can be affected by the duration

of formalin submersion. The longer the submersion time, the smaller the decrease, as more formalin was bonded with the fish protein.

In this study, frying also significantly reduced the residue of formalin ($p<0.05$). The reduction level was 99% that indicated most of the formalin disappeared during the frying process. Formalin was included in a volatile compound, especially above its boiling point (Joshi et al 2015). This study confirmed the results of Sugiarti et al (2014), showing a reduction level of formalin up to 99.28% after 1 hour of submersion followed by the frying process (no information about the period of frying process) in squid. Frying could denature the protein and also hydrolyze its bonding with formalin (Levita et al 2010). The high temperature in frying increased the kinetic energy and led to faster movement of the molecules that could damage the molecular bonds (Sugiarti et al 2014).

Heat processing by boiling reduced the residue of formalin with up to 98.1%, more than the washing process ($p<0.05$), but at the same degree as the frying process ($p>0.05$). The high reduction of formalin in the boiling process was due to the accelerated formalin dissolution rate at higher temperatures (Annisak 2019). This effect could be enhanced by boiling the sample in an open pan (Kamal et al 2017).

The effect of storage on the formalin residue of C. gariepinus. This experiment demonstrated that only the period of storage significantly influenced the residue of formalin ($p<0.05$), while the temperature of storage and the combination between temperature and period of storage did not (Table 2). The previous different studies showed that a storage at the room temperature or at the freezing temperature had similar effects on the formalin residue decrease (Jawahar et al 2017; Murtini et al 2014), possibly due to the same level of protein degradation by breaking the bonds between protein and formalin and releasing free compounds. At the room temperature, the protein degradation was essentially caused by the microbial activity, while at the freezing temperature it was caused mostly by physical destruction, leading to conformational and functional changes (Murtini et al 2014; Yeasmin et al 2010). Formalin in the free form is more reactive and can also be more easily degraded (Riyanto et al 2006; Rachmawati et al 2007).

Conclusions. This research concluded that short time processing and storage treatments decreased the concentration of formalin. Heat involvement determined a more effective decrease of concentration (the decrease level was >90%). In the storage study, the residue of formalin was significantly reduced by the period of storage, mainly during the first 24 hours, then the formalin concentration reduction was insignificant, up to 48 hours of storage.

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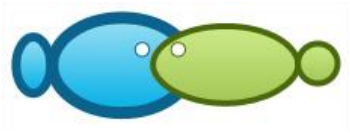

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Material and Method

Material. The study used fresh catfish, purchased from the traditional market in Yogyakarta, Indonesia. The other used ingredients were formalin solution and Schiff's reagent. Formalin solution was made from 4 mL of formalin 37% in 1,000 mL of distilled water (Rachmawati et al 2007).

Method. The study was divided into two sections, i.e. 1) analyzing the effect of processing and 2) analyzing the effect of storage treatment. Fresh catfish were slaughtered, eviscerated, cleaned, and soaked in 1,480 ppm formalin solution (Rachmawati et al 2007; Sugiarti et al 2014). For the first study, catfish were divided into four groups, i.e.: controls (before treatment), washing, frying, and boiling treatments. Washing was done by watering the samples with running water for 1 minute, while frying was done by frying the samples in oil at 200°C for 8 minutes. Boiling treatment was done by putting the samples into boiled water (100°C) for 7 minutes (Sugiarti et al 2014). For the second study, storage treatments, the catfish was also divided into four groups: room temperature storage (29°C) for 24 hours and 48 hours and freezing temperatures storage (-3°C) for 24 hours and 48 hours (Handayani et al 2010). At the end of treatments, the concentration of formalin in samples was analyzed using a spectrophotometer. As the preparation step, a 5 g sample was dissolved in 25 mL of distilled water. One mL of supernatant was mixed with 2 mL of Schiff's reagent, then heated in a water bath at 60°C for 5 minutes. It was incubated for 10 minutes before measuring the absorbance at 520 nm (Shita 2016; Mudaffar 2018).

Statistical analysis. Data were presented in mean±SD and analyzed with: (1) one-way analysis of variance (ANOVA) to determine the differences among all processing treatments and the effect of storage periods on the concentration of formalin; (2) paired sample T-test to determine the differences between the same samples before and after processing treatments; (3) independent sample t-test to know the effect of temperature at the same storage period, and (4) two-way ANOVA to determine the combination effect of storage temperature and period on the concentration of formalin. All data were analyzed with SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL, USA) at the 5% significance level.

Results

Effect of processing on the formalin residue of C. gariepinus. Table 1 shows that all treatments had significant effects on the residue of formalin ($p<0.05$). It can be seen from the p-value between the same samples before and after treatments. Among all treatments, washing shows the highest residue of formalin ($p<0.05$). Both boiling and frying had an almost similar residue of formalin ($p>0.05$). The lower residue of formalin in boiling and frying may be due to heating exposure.

Table 1

Effect of processing on the formalin residue of *Clarias gariepinus*

Processing treatments	*Residue of formalin (ppm)*		*Δ residue of formalin before and after treatment (%)*
	Before treatment	*After treatment*	
Washing	1,420.17±66.94^{c1}	310.42±77.61^{b2}	78.14
Frying	1,420.17±66.94^{c1}	14.18±6.69^{a2}	99.01
Boiling	1,420.17±66.94^{c1}	27.50±9.11^{a2}	98.10

Mean±SD in the same column with different alphabetical superscripts (a, b, or c) are significantly different ($p<0.05$) by using one-way ANOVA statistical analysis. Mean±SD in the same row with different number superscripts (1 or 2) are significantly different ($p<0.05$) by using paired sample T-test statistical analysis.

Effect of storage temperatures and periods on the formalin residue of C. gariepinus. Table 2 shows the effect of storage temperatures, of periods and of their combination on the concentration of formalin. Based on this data, the storage period influenced the residue of formalin decrease ($p<0.05$) during the first 24 hours, but after a storage period of 48 hours the decrease was not significantly different ($p>0.05$).

On the other hand, there were no differences in the residue of formalin stored at room and freezing temperature, suggesting that the temperature of storage did not affect the formalin residue ($p>0.05$). Although there were differences in the statistical results between the temperature effects and the storage periods effects, the combined effect of both variables showed no impact on the concentration of formalin ($p>0.05$).

Table 2

Effect of storage temperature and period on the formalin residue of *Clarias gariepinus*

Temperature (T)	*Periods (t) (hours)*			*p-value*		
	0	*24*	*48*	*T*	*t*	*T*t*
Room (29°C)	1,420.17±66.94^{a1}	52.18±28.78^{a2}	40.64±3.93^{a2}	0.20	0.00	0.76
Freezing (-3°C)	1,420.17±66.94^{a1}	39.04±7.07^{a2}	13.65±4.23^{a2}			

Mean±SD in the same column with different alphabetical superscripts (a, b, or c) are significantly different ($p<0.05$) and so are in the same row with different number superscript (1, 2, or 3). The p-value for T, t, and T*t was obtained from independent T-test, one-way ANOVA, and two-way ANOVA, respectively.

Discussion

The residue of formalin before treatment. The residue of formalin before and after treatment (either fish processing or storage) was significantly different (Table 1 and Table 2). In this study, the *C. gariepinus* specimens were submerged for 60 minutes in formalin solution of 1,480 ppm. The 60 minutes submersion period was chosen because the aldehyde compound may be readily bonded to the amino acids of fish in a small amount (Rahmadhani et al 2017; Baroroh & Kresnadipayana 2016). Formalin would attack lysine for the first time, followed by histidine and tyrosine, leading to the formation of a methylene compound (Ichya'uddin 2016). The protein-containing methylene compounds could not be digested by living things and microbes, especially pathogens, so that the shelf life would be longer (Purawisastra & Sahara 2011). The beneficial bacteria in the fish may also be killed by dehydration mechanism, as another effect of the existence of formalin in the body (Mudzkirah 2016).

The effect of processing on the formalin residue of C. gariepinus. The residue of formalin was reduced by 78.14% during washing treatment. The reduction was significant ($p<0.05$). The formalin was easily washed out because of its high solubility in the water, which is 4 x 10^5 mg L^{-1} at 20°C (BPOM 2008; Sugiarti et al 2014). A study conducted by Yusuf et al (2015) showed the same trend, but the reduction was lower (with only 43%). The differences in the level of reduction can be affected by the duration

of formalin submersion. The longer the submersion time, the smaller the decrease, as more formalin was bonded with the fish protein.

In this study, frying also significantly reduced the residue of formalin ($p<0.05$). The reduction level was 99% that indicated most of the formalin disappeared during the frying process. Formalin was included in a volatile compound, especially above its boiling point (Joshi et al 2015). This study confirmed the results of Sugiarti et al (2014), showing a reduction level of formalin up to 99.28% after 1 hour of submersion followed by the frying process (no information about the period of frying process) in squid. Frying could denature the protein and also hydrolyze its bonding with formalin (Levita et al 2010). The high temperature in frying increased the kinetic energy and led to faster movement of the molecules that could damage the molecular bonds (Sugiarti et al 2014).

Heat processing by boiling reduced the residue of formalin with up to 98.1%, more than the washing process ($p<0.05$), but at the same degree as the frying process ($p>0.05$). The high reduction of formalin in the boiling process was due to the accelerated formalin dissolution rate at higher temperatures (Annisak 2019). This effect could be enhanced by boiling the sample in an open pan (Kamal et al 2017).

The effect of storage on the formalin residue of C. gariepinus. This experiment demonstrated that only the period of storage significantly influenced the residue of formalin ($p<0.05$), while the temperature of storage and the combination between temperature and period of storage did not (Table 2). The previous different studies showed that a storage at the room temperature or at the freezing temperature had similar effects on the formalin residue decrease (Jawahar et al 2017; Murtini et al 2014), possibly due to the same level of protein degradation by breaking the bonds between protein and formalin and releasing free compounds. At the room temperature, the protein degradation was essentially caused by the microbial activity, while at the freezing temperature it was caused mostly by physical destruction, leading to conformational and functional changes (Murtini et al 2014; Yeasmin et al 2010). Formalin in the free form is more reactive and can also be more easily degraded (Riyanto et al 2006; Rachmawati et al 2007).

Conclusions. This research concluded that short time processing and storage treatments decreased the concentration of formalin. Heat involvement determined a more effective decrease of concentration (the decrease level was >90%). In the storage study, the residue of formalin was significantly reduced by the period of storage, mainly during the first 24 hours, then the formalin concentration reduction was insignificant, up to 48 hours of storage.

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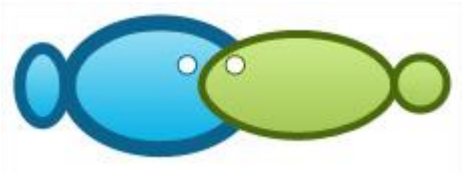

The residue of formalin in catfish (*Clarias gariepinus*) after processing and storage for short periods

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Abstract. Fresh fish is a highly perishable product due to its high protein content. A preservative may be needed to inhibit it. Nowadays, many fishes were reported to be exposed to formalin. This study aimed to determine the effect of short period processing (washing, frying, boiling in short-period) and storage (at the room and at freezing temperatures) on the residue of formalin in catfish, as a food model. Catfish, *Clarias gariepinus,* were divided into 8 groups, e.g. before treatment, washing in airflow for 1 minute, frying in 200°C for 8 minutes, boiling in 100°C for 7 minutes, storage at room temperature (29±1°C) for 24 and 48 hours, storage at freezing temperature (-3°C) for 24 and 48 hours. Before treatment, the catfish was soaked in 1,480 ppm of formalin solutions. The residue of formalin was measured at the end of each treatment by using a spectrophotometer. Data were then analyzed with analysis of variance with SPSS software. The results showed that there were significant effects of processing and storage treatment on the formalin residue of catfish. The formalin residue of all treatments was lower than before treatments. The frying processing and storage at freezing temperatures were the most effective treatments in reducing the formalin residue.
Key Words: fresh fish, preservative, food model, treatments, formalin residue.

Introduction. Fresh fish is a highly perishable product due to its high protein content for microbial activity (Wijayanti & Lukitasari 2016). A preservative maybe needs to keep the freshness, inhibit the decay and prolong the shelf life (Antoni 2010). Nowadays, there are many kinds of food-grade preservatives available in the market. Unfortunately, formalin is still frequently found in food products, including fresh fish, being used among fishermen mainly because it is effective in preserving the product, cheaper and accessible (Male et al 2017).

Formalin is a forty percent solution of formaldehyde in water. This compound is detected in the animal body as an intermediary metabolite. It also uses for several purposes, such as in medicine for specimen preservation and the treatment of hemorrhage (Pandey et al 2000). The preservatives activity of formalin is due to its ability to react with protein generating methylene compounds, which are more difficult to be decayed by microbes (Ichya'uddin 2016). In low concentrations, formalin is not toxic for humans, but in higher concentrations, it had negative effects such as acute toxicity and death (Pandey et al 2000). It also decreases the levels of body antioxidants, resulting in liver damage (Yulisa et al 2014). Other studies also reported the cases of eczema, eye irritation, respiratory tract irritation, cancer, increased growth rate of malignant neoplastic diseases and denaturation of DNA (Suwanaruang 2018; Wilianto & Yudianto 2013). Therefore, its uses are forbidden.

Formalin solution is not stable during storage and exposure to high temperatures (Kaneko et al 1977; Laksmiani et al 2015). Those are caused by desolvation or evaporation of formalin during washing, boiling, or frying (Fellows 2000; Hayun et al 2017; Muntaha et al 2015; Sundari et al 2015) and enzymatic process in storage (Rachmawati et al 2007). Formalin that was in contact with fish decreased up to 43-53% after 5 times rinsing in 250 mL of distilled water and boiling for 30 minutes (Yusuf et al 2015). Other studies were also reported that soaking in warm water and frying decreased

the concentration of formalin up to 63.27% and 83.03%, respectively (Levita et al 2010). In daily practices, fish is usually washed in flowing water before processing, then fryed/boiled for a short time. However, there was no study explaining the influences of these processing and storage methods on the concentration of formalin.

The purpose of this study was to identify the effect of processing for a short time and storage (at room and freezing temperatures) on the residue of formalin. This study used catfish, *Clarias gariepinus*, as a food model.

Material and Method

Material. The study used fresh catfish, purchased from the traditional market in Yogyakarta, Indonesia. The other used ingredients were formalin solution and Schiff's reagent. Formalin solution was made from 4 mL of formalin 37% in 1,000 mL of distilled water (Rachmawati et al 2007).

Method. The study was divided into two sections, i.e. 1) analyzing the effect of processing and 2) analyzing the effect of storage treatment. Fresh catfish were slaughtered, eviscerated, cleaned, and soaked in 1,480 ppm formalin solution (Rachmawati et al 2007; Sugiarti et al 2014). For the first study, catfish were divided into four groups, i.e.: controls (before treatment), washing, frying, and boiling treatments. Washing was done by watering the samples with running water for 1 minute, while frying was done by frying the samples in oil at 200°C for 8 minutes. Boiling treatment was done by putting the samples into boiled water (100°C) for 7 minutes (Sugiarti et al 2014). For the second study, storage treatments, the catfish was also divided into four groups: room temperature storage (29°C) for 24 hours and 48 hours and freezing temperatures storage (-3°C) for 24 hours and 48 hours (Handayani et al 2010). At the end of treatments, the concentration of formalin in samples was analyzed using a spectrophotometer. As the preparation step, a 5 g sample was dissolved in 25 mL of distilled water. One mL of supernatant was mixed with 2 mL of Schiff's reagent, then heated in a water bath at 60°C for 5 minutes. It was incubated for 10 minutes before measuring the absorbance at 520 nm (Shita 2016; Mudaffar 2018).

Statistical analysis. Data were presented in mean±SD and analyzed with: (1) one-way analysis of variance (ANOVA) to determine the differences among all processing treatments and the effect of storage periods on the concentration of formalin; (2) paired sample T-test to determine the differences between the same samples before and after processing treatments; (3) independent sample t-test to know the effect of temperature at the same storage period, and (4) two-way ANOVA to determine the combination effect of storage temperature and period on the concentration of formalin. All data were analyzed with SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL, USA) at the 5% significance level.

Results

Effect of processing on the formalin residue of C. gariepinus. Table 1 shows that all treatments had significant effects on the residue of formalin ($p<0.05$). It can be seen from the p-value between the same samples before and after treatments. Among all treatments, washing shows the highest residue of formalin ($p<0.05$). Both boiling and frying had an almost similar residue of formalin ($p>0.05$). The lower residue of formalin in boiling and frying may be due to heating exposure.

Table 1~~1~~

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Processing treatments	*Residue of formalin (ppm)*		*Δ residue of formalin before and after treatment (%)*
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Washing	1,420.17±66.94^{c1}	310.42±77.61^{b2}	78.14
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Effect of storage temperatures and periods on the formalin residue of C. gariepinus. Table 2 shows the effect of storage temperatures, of periods and of their combination on the concentration of formalin. Based on this data, the storage period influenced the residue of formalin decrease ($p<0.05$) during the first 24 hours, but after a storage period of 48 hours the decrease was not significantly different ($p>0.05$).

On the other hand, there were no differences in the residue of formalin stored at room and freezing temperature, suggesting that the temperature of storage did not affect the formalin residue ($p>0.05$). Although there were differences in the statistical results between the temperature effects and the storage periods effects, the combined effect of both variables showed no impact on the concentration of formalin ($p>0.05$).

Table 2~~2~~

Effect of storage temperature and period on the formalin residue of *Clarias gariepinus*

Temperature (T)	*Periods (t) (hours)*			*p-value*		
	0	*24*	*48*	*T*	*t*	*T*t*
Room (29°C)	1,420.17±66.94^{a1}	52.18±28.78^{a2}	40.64±3.93^{a2}	0.20	0.00	0.76
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Discussion

The residue of formalin before treatment. The residue of formalin before and after treatment (either fish processing or storage) was significantly different (Table 1 and Table 2). In this study, the *C. gariepinus* specimens were submerged for 60 minutes in formalin solution of 1,480 ppm. The 60 minutes submersion period was chosen because the aldehyde compound may be readily bonded to the amino acids of fish in a small amount (Rahmadhani et al 2017; Baroroh & Kresnadipayana 2016). Formalin would attack lysine for the first time, followed by histidine and tyrosine, leading to the formation of a methylene compound (Ichya'uddin 2016). The protein-containing methylene compounds could not be digested by living things and microbes, especially pathogens, so that the shelf life would be longer (Purawisastra & Sahara 2011). The beneficial bacteria in the fish may also be killed by dehydration mechanism, as another effect of the existence of formalin in the body (Mudzkirah 2016).

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Dear Kovacs,

Thank you for your politeness.
We decided to erase the reference from the list for a reason that we have written in the document.

Regards,
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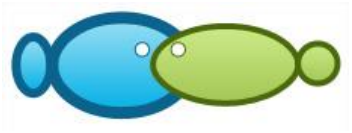

The residue of formalin in catfish (*Clarias gariepinus*) after processing and storage for short periods

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Abstract. Fresh fish is a highly perishable product due to its high protein content. A preservative may be needed to inhibit it. Nowadays, many fishes were reported to be exposed to formalin. This study aimed to determine the effect of short period processing (washing, frying, boiling in short-period) and storage (at the room and at freezing temperatures) on the residue of formalin in catfish, as a food model. Catfish, *Clarias gariepinus,* were divided into 8 groups, e.g. before treatment, washing in airflow for 1 minute, frying in 200°C for 8 minutes, boiling in 100°C for 7 minutes, storage at room temperature (29±1°C) for 24 and 48 hours, storage at freezing temperature (-3°C) for 24 and 48 hours. Before treatment, the catfish was soaked in 1,480 ppm of formalin solutions. The residue of formalin was measured at the end of each treatment by using a spectrophotometer. Data were then analyzed with analysis of variance with SPSS software. The results showed that there were significant effects of processing and storage treatment on the formalin residue of catfish. The formalin residue of all treatments was lower than before treatments. The frying processing and storage at freezing temperatures were the most effective treatments in reducing the formalin residue.
Key Words: fresh fish, preservative, food model, treatments, formalin residue.

Introduction. Fresh fish is a highly perishable product due to its high protein content for microbial activity (Wijayanti & Lukitasari 2016). A preservative maybe needs to keep the freshness, inhibit the decay and prolong the shelf life (Antoni 2010). Nowadays, there are many kinds of food-grade preservatives available in the market. Unfortunately, formalin is still frequently found in food products, including fresh fish, being used among fishermen mainly because it is effective in preserving the product, cheaper and accessible (Male et al 2017).

Formalin is a forty percent solution of formaldehyde in water. This compound is detected in the animal body as an intermediary metabolite. It also uses for several purposes, such as in medicine for specimen preservation and the treatment of hemorrhage (Pandey et al 2000). The preservatives activity of formalin is due to its ability to react with protein generating methylene compounds, which are more difficult to be decayed by microbes (Ichya'uddin 2016). In low concentrations, formalin is not toxic for humans, but in higher concentrations, it had negative effects such as acute toxicity and death (Pandey et al 2000). It also decreases the levels of body antioxidants, resulting in liver damage (Yulisa et al 2014). Other studies also reported the cases of eczema, eye irritation, respiratory tract irritation, cancer, increased growth rate of malignant neoplastic diseases and denaturation of DNA (Suwanaruang 2018; Wilianto & Yudianto 2013). Therefore, its uses are forbidden.

Formalin solution is not stable during storage and exposure to high temperatures (Kaneko et al 1977; Laksmiani et al 2015). Those are caused by desolvation or evaporation of formalin during washing, boiling, or frying (Fellows 2000; Hayun et al 2017; Muntaha et al 2015; Sundari et al 2015) and enzymatic process in storage (Rachmawati et al 2007). Formalin that was in contact with fish decreased up to 43-53% after 5 times rinsing in 250 mL of distilled water and boiling for 30 minutes (Yusuf et al 2015). Other studies were also reported that soaking in warm water and frying decreased

the concentration of formalin up to 63.27% and 83.03%, respectively (Levita et al 2010). In daily practices, fish is usually washed in flowing water before processing, then fryed/boiled for a short time. However, there was no study explaining the influences of these processing and storage methods on the concentration of formalin.

The purpose of this study was to identify the effect of processing for a short time and storage (at room and freezing temperatures) on the residue of formalin. This study used catfish, *Clarias gariepinus*, as a food model.

Material and Method

Material. The study used fresh catfish, purchased from the traditional market in Yogyakarta, Indonesia. The other used ingredients were formalin solution and Schiff's reagent. Formalin solution was made from 4 mL of formalin 37% in 1,000 mL of distilled water (Rachmawati et al 2007).

Method. The study was divided into two sections, i.e. 1) analyzing the effect of processing and 2) analyzing the effect of storage treatment. Fresh catfish were slaughtered, eviscerated, cleaned, and soaked in 1,480 ppm formalin solution (Rachmawati et al 2007; Sugiarti et al 2014). For the first study, catfish were divided into four groups, i.e.: controls (before treatment), washing, frying, and boiling treatments. Washing was done by watering the samples with running water for 1 minute, while frying was done by frying the samples in oil at 200°C for 8 minutes. Boiling treatment was done by putting the samples into boiled water (100°C) for 7 minutes (Sugiarti et al 2014). For the second study, storage treatments, the catfish was also divided into four groups: room temperature storage (29°C) for 24 hours and 48 hours and freezing temperatures storage (-3°C) for 24 hours and 48 hours (Handayani et al 2010). At the end of treatments, the concentration of formalin in samples was analyzed using a spectrophotometer. As the preparation step, a 5 g sample was dissolved in 25 mL of distilled water. One mL of supernatant was mixed with 2 mL of Schiff's reagent, then heated in a water bath at 60°C for 5 minutes. It was incubated for 10 minutes before measuring the absorbance at 520 nm (Shita 2016; Mudaffar 2018).

Statistical analysis. Data were presented in mean±SD and analyzed with: (1) one-way analysis of variance (ANOVA) to determine the differences among all processing treatments and the effect of storage periods on the concentration of formalin; (2) paired sample T-test to determine the differences between the same samples before and after processing treatments; (3) independent sample t-test to know the effect of temperature at the same storage period, and (4) two-way ANOVA to determine the combination effect of storage temperature and period on the concentration of formalin. All data were analyzed with SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL, USA) at the 5% significance level.

Results

Effect of processing on the formalin residue of C. gariepinus. Table 1 shows that all treatments had significant effects on the residue of formalin ($p<0.05$). It can be seen from the p-value between the same samples before and after treatments. Among all treatments, washing shows the highest residue of formalin ($p<0.05$). Both boiling and frying had an almost similar residue of formalin ($p>0.05$). The lower residue of formalin in boiling and frying may be due to heating exposure.

Table 1

Effect of processing on the formalin residue of *Clarias gariepinus*

Processing treatments	*Residue of formalin (ppm)*		*Δ residue of formalin before and after treatment (%)*
	Before treatment	*After treatment*	
Washing	1,420.17±66.94^{c1}	310.42±77.61^{b2}	78.14
Frying	1,420.17±66.94^{c1}	14.18±6.69^{a2}	99.01
Boiling	1,420.17±66.94^{c1}	27.50±9.11^{a2}	98.10

Mean±SD in the same column with different alphabetical superscripts (a, b, or c) are significantly different ($p<0.05$) by using one-way ANOVA statistical analysis. Mean±SD in the same row with different number superscripts (1 or 2) are significantly different ($p<0.05$) by using paired sample T-test statistical analysis.

Effect of storage temperatures and periods on the formalin residue of C. gariepinus. Table 2 shows the effect of storage temperatures, of periods and of their combination on the concentration of formalin. Based on this data, the storage period influenced the residue of formalin decrease ($p<0.05$) during the first 24 hours, but after a storage period of 48 hours the decrease was not significantly different ($p>0.05$).

On the other hand, there were no differences in the residue of formalin stored at room and freezing temperature, suggesting that the temperature of storage did not affect the formalin residue ($p>0.05$). Although there were differences in the statistical results between the temperature effects and the storage periods effects, the combined effect of both variables showed no impact on the concentration of formalin ($p>0.05$).

Table 2

Effect of storage temperature and period on the formalin residue of *Clarias gariepinus*

Temperature (T)	*Periods (t) (hours)*			*p-value*		
	0	*24*	*48*	*T*	*t*	*T*t*
Room (29°C)	1,420.17±66.94^{a1}	52.18±28.78^{a2}	40.64±3.93^{a2}	0.20	0.00	0.76
Freezing (-3°C)	1,420.17±66.94^{a1}	39.04±7.07^{a2}	13.65±4.23^{a2}			

Mean±SD in the same column with different alphabetical superscripts (a, b, or c) are significantly different ($p<0.05$) and so are in the same row with different number superscript (1, 2, or 3). The p-value for T, t, and T*t was obtained from independent T-test, one-way ANOVA, and two-way ANOVA, respectively.

Discussion

The residue of formalin before treatment. The residue of formalin before and after treatment (either fish processing or storage) was significantly different (Table 1 and Table 2). In this study, the *C. gariepinus* specimens were submerged for 60 minutes in formalin solution of 1,480 ppm. The 60 minutes submersion period was chosen because the aldehyde compound may be readily bonded to the amino acids of fish in a small amount (Rahmadhani et al 2017; Baroroh & Kresnadipayana 2016). Formalin would attack lysine for the first time, followed by histidine and tyrosine, leading to the formation of a methylene compound (Ichya'uddin 2016). The protein-containing methylene compounds could not be digested by living things and microbes, especially pathogens, so that the shelf life would be longer (Purawisastra & Sahara 2011). The beneficial bacteria in the fish may also be killed by dehydration mechanism, as another effect of the existence of formalin in the body (Mudzkirah 2016).

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The effect of processing on the formalin residue of C. gariepinus. The residue of formalin was reduced by 78.14% during washing treatment. The reduction was significant ($p<0.05$). The formalin was easily washed out because of its high solubility in the water, which is 4 x 10^5 mg L^{-1} at 20°C (BPOM 2008; Sugiarti et al 2014). A study conducted by Yusuf et al (2015) showed the same trend, but the reduction was lower (with only 43%). The differences in the level of reduction can be affected by the duration

of formalin submersion. The longer the submersion time, the smaller the decrease, as more formalin was bonded with the fish protein.

In this study, frying also significantly reduced the residue of formalin ($p<0.05$). The reduction level was 99% that indicated most of the formalin disappeared during the frying process. Formalin was included in a volatile compound, especially above its boiling point (Joshi et al 2015). This study confirmed the results of Sugiarti et al (2014), showing a reduction level of formalin up to 99.28% after 1 hour of submersion followed by the frying process (no information about the period of frying process) in squid. Frying could denature the protein and also hydrolyze its bonding with formalin (Levita et al 2010). The high temperature in frying increased the kinetic energy and led to faster movement of the molecules that could damage the molecular bonds (Sugiarti et al 2014).

Heat processing by boiling reduced the residue of formalin with up to 98.1%, more than the washing process ($p<0.05$), but at the same degree as the frying process ($p>0.05$). The high reduction of formalin in the boiling process was due to the accelerated formalin dissolution rate at higher temperatures (Annisak 2019). This effect could be enhanced by boiling the sample in an open pan (Kamal et al 2017).

The effect of storage on the formalin residue of C. gariepinus. This experiment demonstrated that only the period of storage significantly influenced the residue of formalin ($p<0.05$), while the temperature of storage and the combination between temperature and period of storage did not (Table 2). The previous different studies showed that a storage at the room temperature or at the freezing temperature had similar effects on the formalin residue decrease (Jawahar et al 2017; Murtini et al 2014), possibly due to the same level of protein degradation by breaking the bonds between protein and formalin and releasing free compounds. At the room temperature, the protein degradation was essentially caused by the microbial activity, while at the freezing temperature it was caused mostly by physical destruction, leading to conformational and functional changes (Murtini et al 2014; Yeasmin et al 2010). Formalin in the free form is more reactive and can also be more easily degraded (Riyanto et al 2006; Rachmawati et al 2007).

Conclusions. This research concluded that short time processing and storage treatments decreased the concentration of formalin. Heat involvement determined a more effective decrease of concentration (the decrease level was >90%). In the storage study, the residue of formalin was significantly reduced by the period of storage, mainly during the first 24 hours, then the formalin concentration reduction was insignificant, up to 48 hours of storage.

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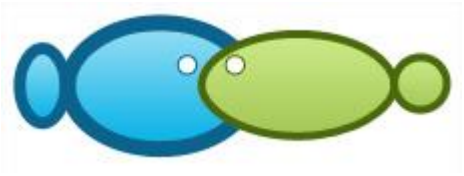

The residue of formalin in catfish (*Clarias gariepinus*) after processing and storage for short periods

Isny Maulidevi, Nurul Andriyani, Ryan Salfarino, Veriani Aprilia

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Abstract. Fresh fish is a highly perishable product due to its high protein content. A preservative may be needed to inhibit it. Nowadays, many fishes were reported to be exposed to formalin. This study aimed to determine the effect of short period processing (washing, frying, boiling in short-period) and storage (at the room and at freezing temperatures) on the residue of formalin in catfish, as a food model. Catfish, *Clarias gariepinus,* were divided into 8 groups, e.g. before treatment, washing in airflow for 1 minute, frying in 200°C for 8 minutes, boiling in 100°C for 7 minutes, storage at room temperature (29±1°C) for 24 and 48 hours, storage at freezing temperature (-3°C) for 24 and 48 hours. Before treatment, the catfish was soaked in 1,480 ppm of formalin solutions. The residue of formalin was measured at the end of each treatment by using a spectrophotometer. Data were then analyzed with analysis of variance with SPSS software. The results showed that there were significant effects of processing and storage treatment on the formalin residue of catfish. The formalin residue of all treatments was lower than before treatments. The frying processing and storage at freezing temperatures were the most effective treatments in reducing the formalin residue.
Key Words: fresh fish, preservative, food model, treatments, formalin residue.

Introduction. Fresh fish is a highly perishable product due to its high protein content for microbial activity (Wijayanti & Lukitasari 2016). A preservative maybe needs to keep the freshness, inhibit the decay and prolong the shelf life (Antoni 2010). Nowadays, there are many kinds of food-grade preservatives available in the market. Unfortunately, formalin is still frequently found in food products, including fresh fish, being used among fishermen mainly because it is effective in preserving the product, cheaper and accessible (Male et al 2017).

Formalin is a forty percent solution of formaldehyde in water. This compound is detected in the animal body as an intermediary metabolite. It also uses for several purposes, such as in medicine for specimen preservation and the treatment of hemorrhage (Pandey et al 2000). The preservatives activity of formalin is due to its ability to react with protein generating methylene compounds, which are more difficult to be decayed by microbes (Ichya'uddin 2016). In low concentrations, formalin is not toxic for humans, but in higher concentrations, it had negative effects such as acute toxicity and death (Pandey et al 2000). It also decreases the levels of body antioxidants, resulting in liver damage (Yulisa et al 2014). Other studies also reported the cases of eczema, eye irritation, respiratory tract irritation, cancer, increased growth rate of malignant neoplastic diseases and denaturation of DNA (Suwanaruang 2018; Wilianto & Yudianto 2013). Therefore, its uses are forbidden.

Formalin solution is not stable during storage and exposure to high temperatures (Kaneko et al 1977; Laksmiani et al 2015). Those are caused by desolvation or evaporation of formalin during washing, boiling, or frying (Fellows 2000; Hayun et al 2017; Muntaha et al 2015; Sundari et al 2015) and enzymatic process in storage (Rachmawati et al 2007). Formalin that was in contact with fish decreased up to 43-53% after 5 times rinsing in 250 mL of distilled water and boiling for 30 minutes (Yusuf et al 2015). Other studies were also reported that soaking in warm water and frying decreased

the concentration of formalin up to 63.27% and 83.03%, respectively (Levita et al 2010). In daily practices, fish is usually washed in flowing water before processing, then fryed/boiled for a short time. However, there was no study explaining the influences of these processing and storage methods on the concentration of formalin.

The purpose of this study was to identify the effect of processing for a short time and storage (at room and freezing temperatures) on the residue of formalin. This study used catfish, *Clarias gariepinus*, as a food model.

Material and Method

Material. The study used fresh catfish, purchased from the traditional market in Yogyakarta, Indonesia. The other used ingredients were formalin solution and Schiff's reagent. Formalin solution was made from 4 mL of formalin 37% in 1,000 mL of distilled water (Rachmawati et al 2007).

Method. The study was divided into two sections, i.e. 1) analyzing the effect of processing and 2) analyzing the effect of storage treatment. Fresh catfish were slaughtered, eviscerated, cleaned, and soaked in 1,480 ppm formalin solution (Rachmawati et al 2007; Sugiarti et al 2014). For the first study, catfish were divided into four groups, i.e.: controls (before treatment), washing, frying, and boiling treatments. Washing was done by watering the samples with running water for 1 minute, while frying was done by frying the samples in oil at 200°C for 8 minutes. Boiling treatment was done by putting the samples into boiled water (100°C) for 7 minutes (Sugiarti et al 2014). For the second study, storage treatments, the catfish was also divided into four groups: room temperature storage (29°C) for 24 hours and 48 hours and freezing temperatures storage (-3°C) for 24 hours and 48 hours (Handayani et al 2010). At the end of treatments, the concentration of formalin in samples was analyzed using a spectrophotometer. As the preparation step, a 5 g sample was dissolved in 25 mL of distilled water. One mL of supernatant was mixed with 2 mL of Schiff's reagent, then heated in a water bath at 60°C for 5 minutes. It was incubated for 10 minutes before measuring the absorbance at 520 nm (Shita 2016; Mudaffar 2018).

Statistical analysis. Data were presented in mean±SD and analyzed with: (1) one-way analysis of variance (ANOVA) to determine the differences among all processing treatments and the effect of storage periods on the concentration of formalin; (2) paired sample T-test to determine the differences between the same samples before and after processing treatments; (3) independent sample t-test to know the effect of temperature at the same storage period, and (4) two-way ANOVA to determine the combination effect of storage temperature and period on the concentration of formalin. All data were analyzed with SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL, USA) at the 5% significance level.

Results

Effect of processing on the formalin residue of C. gariepinus. Table 1 shows that all treatments had significant effects on the residue of formalin ($p<0.05$). It can be seen from the p-value between the same samples before and after treatments. Among all treatments, washing shows the highest residue of formalin ($p<0.05$). Both boiling and frying had an almost similar residue of formalin ($p>0.05$). The lower residue of formalin in boiling and frying may be due to heating exposure.

Table 1

Effect of processing on the formalin residue of *Clarias gariepinus*

Processing treatments	*Residue of formalin (ppm)*		*Δ residue of formalin before and after treatment (%)*
	Before treatment	*After treatment*	
Washing	1,420.17±66.94^{c1}	310.42±77.61^{b2}	78.14
Frying	1,420.17±66.94^{c1}	14.18±6.69^{a2}	99.01
Boiling	1,420.17±66.94^{c1}	27.50±9.11^{a2}	98.10

Mean±SD in the same column with different alphabetical superscripts (a, b, or c) are significantly different ($p<0.05$) by using one-way ANOVA statistical analysis. Mean±SD in the same row with different number superscripts (1 or 2) are significantly different ($p<0.05$) by using paired sample T-test statistical analysis.

Effect of storage temperatures and periods on the formalin residue of C. gariepinus. Table 2 shows the effect of storage temperatures, of periods and of their combination on the concentration of formalin. Based on this data, the storage period influenced the residue of formalin decrease ($p<0.05$) during the first 24 hours, but after a storage period of 48 hours the decrease was not significantly different ($p>0.05$).

On the other hand, there were no differences in the residue of formalin stored at room and freezing temperature, suggesting that the temperature of storage did not affect the formalin residue ($p>0.05$). Although there were differences in the statistical results between the temperature effects and the storage periods effects, the combined effect of both variables showed no impact on the concentration of formalin ($p>0.05$).

Table 2

Effect of storage temperature and period on the formalin residue of *Clarias gariepinus*

Temperature (T)	*Periods (t) (hours)*			*p-value*		
	0	*24*	*48*	*T*	*t*	*T*t*
Room (29°C)	1,420.17±66.94^{a1}	52.18±28.78^{a2}	40.64±3.93^{a2}	0.20	0.00	0.76
Freezing (-3°C)	1,420.17±66.94^{a1}	39.04±7.07^{a2}	13.65±4.23^{a2}			

Mean±SD in the same column with different alphabetical superscripts (a, b, or c) are significantly different ($p<0.05$) and so are in the same row with different number superscript (1, 2, or 3). The p-value for T, t, and T*t was obtained from independent T-test, one-way ANOVA, and two-way ANOVA, respectively.

Discussion

The residue of formalin before treatment. The residue of formalin before and after treatment (either fish processing or storage) was significantly different (Table 1 and Table 2). In this study, the *C. gariepinus* specimens were submerged for 60 minutes in formalin solution of 1,480 ppm. The 60 minutes submersion period was chosen because the aldehyde compound may be readily bonded to the amino acids of fish in a small amount (Rahmadhani et al 2017). Formalin would attack lysine for the first time, followed by histidine and tyrosine, leading to the formation of a methylene compound (Ichya'uddin 2016). The protein-containing methylene compounds could not be digested by living things and microbes, especially pathogens, so that the shelf life would be longer (Purawisastra & Sahara 2011). The beneficial bacteria in the fish may also be killed by dehydration mechanism, as another effect of the existence of formalin in the body (Mudzkirah 2016).

The effect of processing on the formalin residue of C. gariepinus. The residue of formalin was reduced by 78.14% during washing treatment. The reduction was significant ($p<0.05$). The formalin was easily washed out because of its high solubility in the water, which is 4×10^5 mg L^{-1} at 20°C (BPOM 2008; Sugiarti et al 2014). A study conducted by Yusuf et al (2015) showed the same trend, but the reduction was lower (with only 43%). The differences in the level of reduction can be affected by the duration

of formalin submersion. The longer the submersion time, the smaller the decrease, as more formalin was bonded with the fish protein.

In this study, frying also significantly reduced the residue of formalin ($p<0.05$). The reduction level was 99% that indicated most of the formalin disappeared during the frying process. Formalin was included in a volatile compound, especially above its boiling point (Joshi et al 2015). This study confirmed the results of Sugiarti et al (2014), showing a reduction level of formalin up to 99.28% after 1 hour of submersion followed by the frying process (no information about the period of frying process) in squid. Frying could denature the protein and also hydrolyze its bonding with formalin (Levita et al 2010). The high temperature in frying increased the kinetic energy and led to faster movement of the molecules that could damage the molecular bonds (Sugiarti et al 2014).

Heat processing by boiling reduced the residue of formalin with up to 98.1%, more than the washing process ($p<0.05$), but at the same degree as the frying process ($p>0.05$). The high reduction of formalin in the boiling process was due to the accelerated formalin dissolution rate at higher temperatures (Annisak 2019). This effect could be enhanced by boiling the sample in an open pan (Kamal et al 2017).

The effect of storage on the formalin residue of C. gariepinus. This experiment demonstrated that only the period of storage significantly influenced the residue of formalin ($p<0.05$), while the temperature of storage and the combination between temperature and period of storage did not (Table 2). The previous different studies showed that a storage at the room temperature or at the freezing temperature had similar effects on the formalin residue decrease (Jawahar et al 2017; Murtini et al 2014), possibly due to the same level of protein degradation by breaking the bonds between protein and formalin and releasing free compounds. At the room temperature, the protein degradation was essentially caused by the microbial activity, while at the freezing temperature it was caused mostly by physical destruction, leading to conformational and functional changes (Murtini et al 2014; Yeasmin et al 2010). Formalin in the free form is more reactive and can also be more easily degraded (Riyanto et al 2006; Rachmawati et al 2007).

Conclusions. This research concluded that short time processing and storage treatments decreased the concentration of formalin. Heat involvement determined a more effective decrease of concentration (the decrease level was >90%). In the storage study, the residue of formalin was significantly reduced by the period of storage, mainly during the first 24 hours, then the formalin concentration reduction was insignificant, up to 48 hours of storage.

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Final draft

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Kepada: Eni Kovacs <ek.bioflux@gmail.com>

Dear Kovacs

This is our revision. Several grammatical errors were found after using the Grammarly software.
thank you

Regards
Veriani Aprilia
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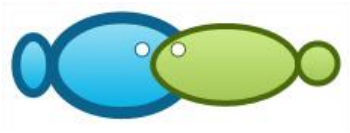

The residue of formalin in catfish (*Clarias gariepinus*) after processing and storage for short periods

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Boiling	1,420.17±66.94^{c1}	27.50±9.11^{a2}	98.10

Mean±SD in the same column with different alphabetical superscripts (a, b, or c) are significantly different ($p<0.05$) by using one-way ANOVA statistical analysis. Mean±SD in the same row with different number superscripts (1 or 2) are significantly different ($p<0.05$) by using paired sample T-test statistical analysis.

Effect of storage temperatures and periods on the formalin residue of C. gariepinus. Table 2 shows the effect of storage temperatures, of periods and of their combination on the concentration of formalin. Based on this data, the storage period influenced the residue of formalin decrease ($p<0.05$) during the first 24 hours, but after a storage period of 48 hours the decrease was not significantly different ($p>0.05$).

On the other hand, there were no differences in the residue of formalin stored at room and freezing temperature, suggesting that the temperature of storage did not affect the formalin residue ($p>0.05$). Although there were differences in the statistical results between the temperature effects and the storage periods effects, the combined effect of both variables showed no impact on the concentration of formalin ($p>0.05$).

Table 2

Effect of storage temperature and period on the formalin residue of *Clarias gariepinus*

Temperature (T)	*Periods (t) (hours)*			*p-value*		
	0	*24*	*48*	*T*	*t*	*T*t*
Room (29°C)	1,420.17±66.94^{a1}	52.18±28.78^{a2}	40.64±3.93^{a2}	0.20	0.00	0.76
Freezing (-3°C)	1,420.17±66.94^{a1}	39.04±7.07^{a2}	13.65±4.23^{a2}			

Mean±SD in the same column with different alphabetical superscripts (a, b, or c) are significantly different ($p<0.05$) and so are in the same row with different number superscript (1, 2, or 3). The p-value for T, t, and T*t was obtained from independent T-test, one-way ANOVA, and two-way ANOVA, respectively.

Discussion

The residue of formalin before treatment. The residue of formalin before and after treatment (either fish processing or storage) was significantly different (Table 1 and Table 2). In this study, the *C. gariepinus* specimens were submerged for 60 minutes in formalin solution of 1,480 ppm. The 60 minutes submersion period was chosen because the aldehyde compound may be readily bonded to the amino acids of fish in a small amount (Rahmadhani et al 2017). Formalin would attack lysine for the first time, followed by histidine and tyrosine, leading to the formation of a methylene compound (Ichya'uddin 2016). The protein-containing methylene compounds could not be digested by living things and microbes, especially pathogens, so that the shelf life would be longer (Purawisastra & Sahara 2011). The beneficial bacteria in the fish may also be killed by dehydration mechanism, as another effect of the existence of formalin in the body (Mudzkirah 2016).

The effect of processing on the formalin residue of C. gariepinus. The residue of formalin was reduced by 78.14% during washing treatment. The reduction was significant ($p<0.05$). The formalin was easily washed out because of its high solubility in the water, which is 4×10^5 mg L^{-1} at 20°C (BPOM 2008; Sugiarti et al 2014). A study conducted by Yusuf et al (2015) showed the same trend, but the reduction was lower (with only 43%). The differences in the level of reduction can be affected by the duration

of formalin submersion. The longer the submersion time, the smaller the decrease, as more formalin was bonded with the fish protein.

In this study, frying also significantly reduced the residue of formalin ($p<0.05$). The reduction level was 99% that indicated most of the formalin disappeared during the frying process. Formalin was included in a volatile compound, especially above its boiling point (Joshi et al 2015). This study confirmed the results of Sugiarti et al (2014), showing a reduction level of formalin up to 99.28% after 1 hour of submersion followed by the frying process (no information about the period of frying process) in squid. Frying could denature the protein and also hydrolyze its bonding with formalin (Levita et al 2010). The high temperature in frying increased the kinetic energy and led to faster movement of the molecules that could damage the molecular bonds (Sugiarti et al 2014).

Heat processing by boiling reduced the residue of formalin by up to 98.1%, more than the washing process ($p<0.05$), but at the same degree as the frying process ($p>0.05$). The high reduction of formalin in the boiling process was due to the accelerated formalin dissolution rate at higher temperatures (Annisak 2019). This effect could be enhanced by boiling the sample in an open pan (Kamal et al 2017).

The effect of storage on the formalin residue of C. gariepinus. This experiment demonstrated that only the period of storage significantly influenced the residue of formalin ($p<0.05$), while the temperature of storage and the combination between temperature and period of storage did not (Table 2). The previous different studies showed that a storage at the room temperature or at the freezing temperature had similar effects on the formalin residue decrease (Jawahar et al 2017; Murtini et al 2014), possibly due to the same level of protein degradation by breaking the bonds between protein and formalin and releasing free compounds. At the room temperature, the protein degradation was essentially caused by the microbial activity, while at the freezing temperature it was caused mostly by physical destruction, leading to conformational and functional changes (Murtini et al 2014; Yeasmin et al 2010). Formalin in the free form is more reactive and can also be more easily degraded (Riyanto et al 2006; Rachmawati et al 2007).

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Conclusions. This research concluded that short-time processing and storage treatments decreased the concentration of formalin. Heat involvement determined a more effective decrease in concentration (the decrease level was >90%). In the storage study, the residue of formalin was significantly reduced by the period of storage, mainly during the first 24 hours, then the formalin concentration reduction was insignificant, up to 48 hours of storage.

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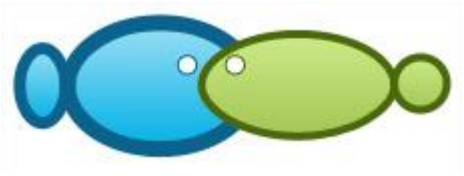

The residue of formalin in catfish (*Clarias gariepinus*) after processing and storage for short periods

Isny Maulidevi, Nurul Andriyani, Ryan Salfarino, Veriani Aprilia

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Abstract. Fresh fish is a highly perishable product due to its high protein content. A preservative may be needed to inhibit it. Nowadays, many fishes were reported to be exposed to formalin. This study aimed to determine the effect of short period processing (washing, frying, boiling in short-period) and storage (at the room and at freezing temperatures) on the residue of formalin in catfish, as a food model. Catfish, *Clarias gariepinus,* were divided into 8 groups, e.g. before treatment, washing in airflow for 1 minute, frying in 200°C for 8 minutes, boiling in 100°C for 7 minutes, storage at room temperature (29±1°C) for 24 and 48 hours, storage at freezing temperature (-3°C) for 24 and 48 hours. Before treatment, *C. gariepinus* was soaked in 1,480 ppm of formalin solutions. The residue of formalin was measured at the end of each treatment by using a spectrophotometer. Data were then analyzed with analysis of variance with SPSS software. The results showed that there were significant effects of processing and storage treatment on the formalin residue of *C. gariepinus*. The formalin residue of all treatments was lower than before treatments. The frying processing and storage at freezing temperatures were the most effective treatments in reducing the formalin residue.
Key Words: fresh fish, preservative, food model, treatments, formalin residue.

Introduction. Fresh fish is a highly perishable product due to its high protein content for microbial activity (Wijayanti & Lukitasari 2016). A preservative maybe needs to keep the freshness, inhibit the decay and prolong the shelf life (Antoni 2010). Nowadays, there are many kinds of food-grade preservatives available in the market. Unfortunately, formalin is still frequently found in food products, including fresh fish, being used among fishermen mainly because it is effective in preserving the product, cheaper and accessible (Male et al 2017).

Formalin is a forty percent solution of formaldehyde in water. This compound is detected in the animal body as an intermediary metabolite. It also uses for several purposes, such as in medicine for specimen preservation and the treatment of hemorrhage (Pandey et al 2000). The preservative activity of formalin is due to its ability to react with protein generating methylene compounds, which are more difficult to be decayed by microbes (Ichya'uddin 2016). In low concentrations, formalin is not toxic for humans, but in higher concentrations, it had negative effects such as acute toxicity and death (Pandey et al 2000). It also decreases the levels of body antioxidants, resulting in liver damage (Yulisa et al 2014). Other studies also reported the cases of eczema, eye irritation, respiratory tract irritation, cancer, increased growth rate of malignant neoplastic diseases and denaturation of DNA (Suwanaruang 2018; Wilianto & Yudianto 2013). Therefore, its uses are forbidden.

Formalin solution is not stable during storage and exposure to high temperatures (Kaneko et al 1977; Laksmiani et al 2015). Those are caused by desolvation or evaporation of formalin during washing, boiling, or frying (Fellows 2000; Hayun et al 2017; Muntaha et al 2015; Sundari et al 2015) and enzymatic process in storage (Rachmawati et al 2007). Formalin that was in contact with fish decreased up to 43-53% after 5 times rinsing in 250 mL of distilled water and boiling for 30 minutes (Yusuf et al 2015). Other studies were also reported that soaking in warm water and frying decreased

the concentration of formalin up to 63.27% and 83.03%, respectively (Levita et al 2010). In daily practices, fish is usually washed in flowing water before processing, then fried/boiled for a short time. However, there was no study explaining the influences of these processing and storage methods on the concentration of formalin.

The purpose of this study was to identify the effect of processing for a short time and storage (at room and freezing temperatures) on the residue of formalin. This study used catfish, *Clarias gariepinus*, as a food model.

Material and Method

Material. The study used fresh *C. gariepinus*, purchased from the traditional market in Yogyakarta, Indonesia. The other used ingredients were formalin solution and Schiff's reagent. Formalin solution was made from 4 mL of formalin 37% in 1,000 mL of distilled water (Rachmawati et al 2007).

Method. The study was divided into two sections: 1) analyzing the effect of processing and 2) analyzing the effect of storage treatment. Fresh *C. gariepinus* were slaughtered, eviscerated, cleaned, and soaked in 1,480 ppm formalin solution (Rachmawati et al 2007; Sugiarti et al 2014).

For the first study, *C. gariepinus* were divided into four groups: controls (before treatment), washing, frying, and boiling treatments. Washing was done by watering the samples with running water for 1 minute, while frying was done by frying the samples in oil at 200°C for 8 minutes. Boiling treatment was done by putting the samples into boiled water (100°C) for 7 minutes (Sugiarti et al 2014).

For the second study, storage treatments, *C. gariepinus* was also divided into four groups: room temperature storage (29°C) for 24 hours and 48 hours and freezing temperatures storage (-3°C) for 24 hours and 48 hours (Handayani et al 2010). At the end of treatments, the concentration of formalin in samples was analyzed using a spectrophotometer. As the preparation step, a 5 g sample was dissolved in 25 mL of distilled water. 1 mL of supernatant was mixed with 2 mL of Schiff's reagent, and then heated in a water bath at 60°C for 5 minutes. It was incubated for 10 minutes before measuring the absorbance at 520 nm (Shita 2016; Mudaffar 2018).

Statistical analysis. Data were presented in mean±SD and analyzed with: (1) one-way analysis of variance (ANOVA) to determine the differences among all processing treatments and the effect of storage periods on the concentration of formalin; (2) paired sample T-test to determine the differences between the same samples before and after processing treatments; (3) independent sample t-test to know the effect of temperature at the same storage period, and (4) two-way ANOVA to determine the combined effect of storage temperature and period on the concentration of formalin. All data were analyzed with SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL, USA) at 5% significance level.

Results

Effect of processing on the formalin residue of C. gariepinus. Table 1 shows that all treatments had significant effects on the residue of formalin ($p<0.05$). It can be seen from the p-value between the same samples before and after treatments. Among all treatments, washing shows the highest residue of formalin ($p<0.05$). Both boiling and frying had an almost similar residue of formalin ($p>0.05$). The lower residue of formalin in boiling and frying may be due to heating exposure.

Table 1

Effect of processing on the formalin residue of *Clarias gariepinus*

Processing treatments	*Residue of formalin (ppm)*		*Δ residue of formalin before and after treatment (%)*
	Before treatment	*After treatment*	
Washing	1,420.17±66.94^{c1}	310.42±77.61^{b2}	78.14
Frying	1,420.17±66.94^{c1}	14.18±6.69^{a2}	99.01
Boiling	1,420.17±66.94^{c1}	27.50±9.11^{a2}	98.10

Mean±SD in the same column with different alphabetical superscripts (a, b, or c) are significantly different ($p<0.05$) by using one-way ANOVA statistical analysis. Mean±SD in the same row with different number superscripts (1 or 2) are significantly different ($p<0.05$) by using paired sample T-test statistical analysis.

Effect of storage temperatures and periods on the formalin residue of C. gariepinus. Table 2 shows the effect of storage temperatures, of periods and of their combination on the concentration of formalin. Based on this data, the storage period influenced the residue of formalin decrease ($p<0.05$) during the first 24 hours, but after a storage period of 48 hours the decrease was not significantly different ($p>0.05$).

On the other hand, there were no differences in the residue of formalin stored at room and freezing temperature, suggesting that the temperature of storage did not affect the formalin residue ($p>0.05$). Although there were differences in the statistical results between the temperature effects and the storage periods effects, the combined effect of both variables showed no impact on the concentration of formalin ($p>0.05$).

Table 2

Effect of storage temperature and period on the formalin residue of *Clarias gariepinus*

Temperature (T)	*Periods (t) (hours)*			*p-value*		
	0	*24*	*48*	*T*	*t*	*T*t*
Room (29°C)	1,420.17±66.94^{a1}	52.18±28.78^{a2}	40.64±3.93^{a2}	0.20	0.00	0.76
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Discussion

The residue of formalin before treatment. The residue of formalin before and after treatment (either fish processing or storage) was significantly different (Table 1 and Table 2). In this study, the *C. gariepinus* specimens were submerged for 60 minutes in formalin solution of 1,480 ppm. The 60 minutes submersion period was chosen because the aldehyde compound may be readily bonded to the amino acids of fish in a small amount (Rahmadhani et al 2017). Formalin would attack lysine for the first time, followed by histidine and tyrosine, leading to the formation of a methylene compound (Ichya'uddin 2016). The protein-containing methylene compounds could not be digested by living things and microbes, especially pathogens, so that the shelf life would be longer (Purawisastra & Sahara 2011). The beneficial bacteria in the fish may also be killed by dehydration mechanism, as another effect of the existence of formalin in the body (Mudzkirah 2016).

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In the present study, frying also significantly reduced the residue of formalin ($p<0.05$). The reduction level was 99% indicating that most of the formalin disappeared during the frying process. Formalin was included in a volatile compound, especially above its boiling point (Joshi et al 2015). This study confirmed the results of Sugiarti et al (2014), showing a reduction level of formalin up to 99.28% after 1 hour of submersion followed by the frying process (no information about the period of frying process) in squid. Frying could denature the protein and also hydrolyze its bonding with formalin (Levita et al 2010). The high temperature in frying increased the kinetic energy and led to faster movement of the molecules that could damage the molecular bonds (Sugiarti et al 2014).

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Conclusions. The present research concluded that short-time processing and storage treatments decreased the concentration of formalin. Heat involvement determined a more effective decrease in concentration (the decrease level was >90%). In the storage study, the residue of formalin was significantly reduced by the period of storage, mainly during the first 24 hours, and then the formalin concentration reduction was insignificant, up to 48 hours of storage.

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