ผลของการลดกลิ่นไม่พึงประสงค์ในเนื้อปลานิลบด (*Oreochromis niloticus*) ด้วยโอโซน

Effect of Ozone Treatments on Nile Tilapia Mince (*Oreochromis niloticus*) Off-Odor

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บทคัดย่อ

ปลานิล(*Oreochromis niloticus*) เป็นปลาน้ำจืดที่มีกลิ่นไม่พึงประสงค์ ซึ่งเกิดได้ตามธรรมชาติ ได้แก่ กลิ่นโคลน และกลิ่นคาว เป็นต้น จึงเป็นสาเหตุที่ทำให้ไม่ได้รับการยอมรับจากผู้บริโภค งานวิจัยที่ผ่านมามีการศึกษาการลดกลิ่นในปลาด้วยการใช้สารเคมีหลายชนิด แต่อาจเกิดการตกค้างของสารเคมี และเป็นอันตรายต่อผู้บริโภค ดังนั้นจึงมีการนำโอโซนมาใช้ในการลดกลิ่น เนื่องจากโอโซนมีคุณสมบัติเป็นสารออกซิไดซ์ที่รุนแรง และไม่มีสารตกค้างในอาหาร จึงปลอดภัยต่อผู้บริโภค วัตถุประสงค์ของงานวิจัยนี้คือ การลดกลิ่นไม่พึงประสงค์ในเนื้อปลานิลบดด้วยโอโซน โดยการนำเนื้อปลาบดมาล้างด้วยน้ำ (ชุดควบคุม) และโอโซน 7 ชุดการทดลอง จากนั้นวิเคราะห์สารประกอบที่ระเหยได้ในเนื้อปลาด้วยเทคนิคแก๊สโครมาโทกราฟี-แมสสเปกโทรมิเตอร์ พร้อมทั้งวิเคราะห์ปริมาณโปรตีนและไขมันในเนื้อปลา พบว่าเนื้อปลาที่ผ่านการล้างทุกชุดการทดลองมีสารประกอบทั้งหมด 18 ชนิด ซึ่งสารประกอบที่มีปริมาณมากและชักนำการปล่อยกลิ่นไม่พึงประสงค์ในทุกชุดการทดลอง ได้แก่ methoxy phenyl oxime (fishy), hexanal (fatty)และ 1-Octen-3-ol (mushrooms)เมื่อเปรียบเทียบกับชุดควบคุมปริมาณของสารประกอบเหล่านี้ลดลงอย่างมีนัยสำคัญทางสถิติ หลังจากผ่านการล้างด้วยโอโซน อีกทั้งการล้างด้วยโอโซนมีผลต่อปริมาณโปรตีนและไขมันเพียงเล็กน้อย ซึ่งการล้างแบบ ozone flotation เป็นเวลา 30 นาที สามารถกำจัดกลิ่นไม่พึงประสงค์ได้มากที่สุด จึงเลือกวิธีนี้เพื่อใช้ในการลดกลิ่นไม่พึงประสงค์ในเนื้อปลานิลบดที่จะใช้ในการพัฒนาผลิตภัณฑ์จากเนื้อปลานิลบดต่อไป

คำสำคัญ: กลิ่นโคลน, กลิ่นคาว, ปลาบด, การใช้ประโยชน์จากเศษเหลือ, ไอโซเทอร์มอล

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ABSTRACT

Nile tilapia (*Oreochromis niloticus*)is freshwater fish that often has off-odorslikeearthy and fishy odor smellwhich causes many consumers to reject products made from this species. Therefore, different types of chemicals are used to reduce the odor. However, some treatments may leave chemical residues that are not safe for consumers. Ozone has been used to reduce the odor because it has strong oxidizing properties and leaves no residue, and therefore is safe for consumers. This study aimed to reduce off-odors in Nile tilapiamincewith different ozone treatments. Fish mince were treated with water (control) and seven ozone treatments. Then, volatile compounds were measured by GC / MS analysis, which identified a total of 18 volatile compounds in the fish mince. Volatile compounds found in high proportions in all experiments were methoxy phenyl oxime (giving fishy off-odor), hexanal (fatty odor) and 1-Octen-3-ol (mushroom odor). There was a statistically significant decrease of these compounds within different ozone treatments compared to water treatment (control). Ozone treatments had a slight effect on fat and protein content in tilapia mince. 30-minuteozone flotation treatment provided the best reduction of off-odor from fish. Therefore, ozone treatment is suitable for preparing tilapia mincein order to further develop tilapiaproducts.

Key words: earthy smell, fishy odor, fish mince,utilization of waste, isothermal

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) has rapid growth, easy cultivation, high nutritional value, relatively low price, and can be processed into several food products. Nile tilapia aquaculture in Thailand produced an annual harvest of 222.04 tons in 2013-2017, which was valued at approximately 11,284.52 million baht (Fisheries Development Policy and Strategy Division, 2017). However, freshwater fish products are often characterized by unpleasant odors that are unacceptable to many consumers, thus becoming a major restriction to the processing and consumption of Nile tilapia (Yufei *et al*., 2016). The compounds associated with off-odors are generated by enzymatic reactions, lipid autoxidation, microbial action, environmentally, and thermally derived reactions (Selli *et al*., 2009). Moreover, lipid-derived volatile compounds such as aldehydes and ketones are frequently generated by oxidative enzymatic reactions and autoxidation of lipids (Pratama *et al*., 2018).

Many researchers have reported that reducing off-flavor in aquatic animals is possible with various chemicals, i.e. salmon skin had the lowest scores for fishy odor when washed with 1% (w/v) sodium chloride solution for 5 min (Tiwtha and Usawakesmanee, 2012), as did minced Nile tilapia pretreated by alkaline solubilization (Yarnpakdee *et al*., 2012). In the same way, the pretreatment of tilapia skin with 1.5% NaCl, 0.2% NaOH, 0.2% sulfuric acid and 1% citric acid gave an odorless gelatin (Tohmadlae *et al*., 2019).

In addition, ozone was more effective than oxygen in reducing geosmin of catfish mince (Dew, 2005). Geosmin is a compound that gives off-flavor in freshwater fish especially tilapia. Similarly, Thai panga (*Pangasius conchophilus*) fish mince exposed to 400 mg ozonated water and 3% banana leaf ash for 5 min received the lowest sensory evaluation scores for earthy flavor (geosmin) (Thipbharos, 2014). Washing with ozonated water or ozone-flotation was recognized to be effective in the elimination of geosmin from the fish muscle of bighead carp. When the time of washing increased, the content of geosmin decreased (Zhang *et al*., 2016). Ozone has a very high oxidizing efficiency as it is able to interact with organic and inorganic compounds by oxidation, both directly and indirectly (Gottschalk *et al*., 2000). Many factors can impact the efficiency of ozone addition in the removal of off-odors, such as quality of the water, temperature, organic matter content of the water, and pH (Gonçalves and Gagnon, 2011). Some chemical treatments of fish products leave residual compounds, which may have a negative impact on human health (Brodowska *et al*., 2017). The advantage of ozone treatment is that it is free of chemical residues, and thus ozone is used in many food processing industries. The purpose of this study was to examine the effect of isothermal treatments using ozone on Nile tilapia off-odor. An effective treatment process would be valuable for producing tilapia products that are both safe and accepted by consumers.

MATERIALS AND METHODS

Materials

Nile tilapiamince (*Oreochromis niloticus*) wasseparated fromtilapiabone, were received from tilapia fillet processing wasteof Mankit Mankhong Co., Ltd., Samut Prakan, Thailand. The fish were packed in plastic bags and kept in an insulated box to maintain temperature below 10 °C, transported to the Department of Fishery Products, Faculty of Fisheries, Kasetsart University within 2 h, and stored at -20 °C until use.

Preparation of fish mince treated with ozone

Tilapiamincewas treated with solutions as described below in the ratio of 1:6 (w/v) at room temperature. In all experiments, water was changed every 30 min. Ozone water is water that was prepared by releasing ozone gas into the water. Ozone flotation is a process of continuously releasing ozone bubbles into the water during treatment of the mince. In preliminary experiments, tilapia mince was treated at least 30 min for reducing off-odors.Then 30 min was applied for the experiments.

Fish mince was treated with ozone in the following seven treatments along with one control: Control - water (30 min), W30 - ozone water (30 min), F30 - ozone flotation (30 min), F60 - ozone flotation (60 min), F90 - ozone flotation (90 min), WF30- ozone water (30 min) and ozone flotation (30 min), WF60 - ozone water (30 min) and ozone flotation (60 min), and WF90 - ozone water (30 min) and ozone flotation (90 min)

Determination of ozone content

Ozone content was analyzed according to Zhang *et al.* (2016). A 100 ml aliquot of ozone water was added to 20 ml of 2% potassium iodide containing 3 mL of 0.5 M sulfuric acid to keep the solution acidic. After reaction for 5 min, the solution was titrated with 0.1 N sodium thiosulfate standard solution (1% starch solution as indicator) until the blue color disappeared

Determination of volatile compounds from fish mince

Fish mince and distilled water were added in the ratio of 1:2 in 5 ml cap vials and then heated at 90 °C for 5 min. Volatile compounds were analyzed by GC-MS analysis according to Tohmadlae *et al*. (2019). Volatile compounds were extracted by solid phase microextraction with DVB/CAR/PDMS (50/30µm) at 60 °C for 10 min. After that, GC-MS analysis was performed using an Agilent Technologies 7890B coupled with Agilent Technologies 5977A unit (Agilent Technologies Inc., Santa Clara, USA). Volatile Compounds were separated on a HP-Innowax capillary column (Agilent Technologies Inc., Santa Clara, USA) (30 m × 0.25mm ID, with film thickness of 0.25 mm), using experimental parameters as follows: Oven temperature program was 40 ºC to 230 ºC by an increase of 10 ºC/min and holding for 3 min. Helium was employed as a carrier gas, with a constant flow of 1 mL/min. The injector was operated in the splitless mode and its temperature was set at 250 ºC. Transfer line temperature was maintained at 270 ºC. The quadrupole mass spectrometer was operated in the electron ionization (EI) mode and source temperature was set at 230 ºC. The area of a peak was calculated to quantify the amount of the volatile compound.

Determination of protein and fat content

Determination of protein content in fresh Nile tilapia mince and treated mincewere analyzed according to Nielsen (2010), which uses the Kjeldahl method to determine nitrogen content, using 6.25 as the conversion factor to get crude protein from total nitrogen. Fat content was analyzed according to AOAC (2012).

Statistical analysis

All experimental data were calculated in triplicate with completely randomized design (CRD). Data were subjected to one-way analysis of variance (ANOVA). Differences between means were tested by Duncan's multiple range test. The data were presented as mean ± standard deviation. A probability value of $p < 0.05$ was considered statistically significant. Statistical tests were conducted using statistic operating system.

RESULTS AND DISCUSSION

Effect of ozone on volatile compounds of fish mince

Fish mince were treated with different ozone treatments and then analyzed for volatile compounds using GC / MS analysis, with results shown in Figure 1 and Table 1. All experiments detected and identified forvolatile compounds including four aldehydes, one furan, two acids, five alcohols, three hydrocarbons, and three miscellaneous compounds. The proportions of compounds were high and included those known induce to off-odors in fish: methoxy phenyl oxime, hexanal, nonanal, and 1-Octen-3-ol. Of these, methoxy phenyl oxime produces a characteristic fishy odor. Similarly, these compounds have been identified in tilapia skin (Tohmadlae *et al*., 2019). Methoxy phenyl oxime in fish mince decreased when treated with different ozone treatments compared to soaking in plain water (control, 26.09%), with results shown in Table 2. Ozone water treatment (W30) had a slightly lower level of this compound (21.95%) compared to other ozone treatments. This treatment also had the lowest measured level of ozone (9.2 mg/L). Treatments F30 (501.8 mg/L) and WF30 (545.0 mg/L) had much higher dissolved ozone. Due to ozone is unstable molecule and disappear very fast, ozone content of ozone water treatment is less than ozone flotation treatment. Therefore, ozone flotation treatment effected to fishy odor more than ozone flotation. In addition, ozone application wasconcern in ozone concentration and contacted time. High concentration of ozone may generate undesirable odor. However, ozone flotation treatment for 30 min (4.41%) had the lowest levels of methoxy phenyl oxime compared with the control.

Similarly, levels of 1-Octen-3-ol decreased when treated with ozone treatments in all experiments.This compound is an alcoholproduced by unsaturated fatty acid oxidation (Li *et al*., 2019). Which produces an earthy or mushroom odor. Normally, alcohols have a high detection threshold, and thus affect odors in food less than aldehydes (Pratama *et al*., 2018). However, 1-Octen-3-ol is an unsaturated alcohol with lower threshold values, and so is expected to have higher impact on the overall aroma (Peinado *et al*., 2016).

In addition, aldehydes compounds were identified in the samples including hexanal and nonanal that are often found in aquatic animals such as Nile tilapia, catfish (Pratama *et al*., 2018), and Codfish (*Gadus morhua*) (Yufei *et al*., 2016).However, the source of aldehyde groups is from secondary lipid oxidation (Pratama *et al*., 2018) or reduction of carbonyl compounds (Lu *et al*., 2019). Aldehydes have a low detection threshold, and thus have many effects on odors in food (Yufei *et al*., 2016). The flavor of aldehydes depends on the number of carbon atoms. Generally, aldehydes containing 3-4 carbon atoms produce strong pungent flavors, while 5-9 carbon atoms in aldehydes emit fragrances of lipid and oil. In addition, aldehydes with high molecular weight have the unique fragrance of orange peel, such as the citrus smell of octanal (Li *et al*., 2019). Hexanal presents odor characteristics of either a grassy or fatty odor which levels of hexanal decreased when treated with ozone treatments. This compound is an oxidation product of linoleic acid which considered an important indicator of lipid oxidation (Frankel, 1993; Frankel and Tappel, 1991). The results suggested that the use of ozone flotation for 30 min produced the greatest reduction of odor-causing these compounds compared to other ozone treatments. As with the greater elimination of geosmin in the fish muscle of bighead carp with ozone-flotation than ozone water, this might be due to a higher degree of oxidation (Zhang *et al*., 2016).

Table 1 Volatile compounds in Nile tilapia mince.

Compound	Odor Description
Aldehydes	
Hexanal	Grass, Fatty
Nonanal	Fatty, Plastic
Heptanal	Fruity
Octanal	Citrus
Furans	
2,3 dihydro furan,	-
Acids	
2-Pyridinepropanoic acid, .alpha.-methyl-.beta.-oxo-, ethyl ester	-
Hexanedioic acid, bis(2-ethylhexyl) ester	-
Alcohols	
1-Octen-3-ol	Mushrooms, Earthly
2,6-bis (1,1-dimethylethyl)-4-methyl-phenol	-
1-Hexanol	Astringent, Grass
1-Heptanol	Fatty
1-Nonanol	Citrus, Citronella oil
Hydrocarbon	
Ethyl benzene	-
1,2 dimethyl benzene	-
Pentadecane	-
Miscellaneous compounds	
Trichloromethane	-
Methoxy phenyl oxime	Fishy
5-(Phenoxy)methyl-2-amino-1,3,4-thiadiazoles	-

Remark: - indicates unable to specify the odor

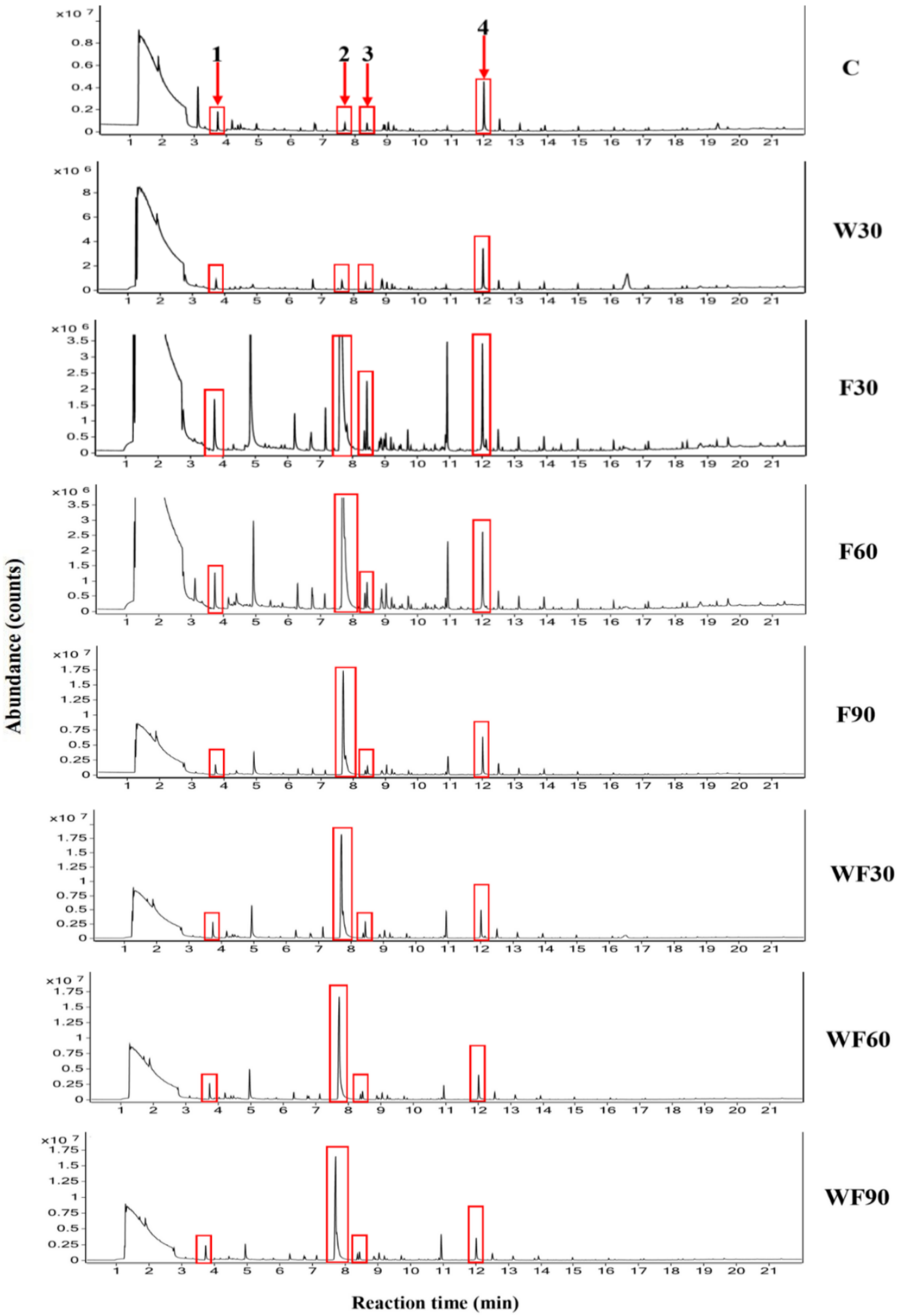

Figure 1 Chromatogram of volatile compound in Nile tilapia mince

C-Control,W30 - ozone water (30 min), F30 - ozone flotation (30 min), F60 - ozone flotation (60 min), F90 - ozone flotation (90 min), WF30- ozone water (30 min) and ozone flotation (30 min), WF60 - ozone water (30 min) and ozone flotation (60 min), and WF90 - ozone water (30 min) and ozone flotation (90 min), 1-RTat 3.729 min is Hexanal, 2-RT at 7.695 min is Nonanal, 3-RT at 8.381 min is 1-Octen-3-ol and 4-RT at 12.023 min is Methoxy phenyl oxime

On the other hand, nonanal increased when compared with the control in all experimental treatments. This compound contributes to various fatty or plastic odors. Nonanal might possibly derive from the high amount of oleic acid in tilapia (Pratama *et al*., 2018). Ozone process may increase levels of odor-causing some compounds as the generation of intermediate products resulting from the interaction between ozone, hydrogen sulfide, and methylamine (Alkoaik, 2009). Ozone can be used as a deodorizing agent through oxidation reactions and structural change of organic compounds. Oxidation processes may occur through both direct and indirect routes (Gottschalk *et al*., 2000). Ozone reacts mainly with double bonds, activated aromatic systems and non-protonated amines (Gunten, 2007). In general, ozonation mechanisms of organic compounds only comprise 1, 3-dipolar cycloaddition, electrophilic addition, and nucleophilic addition (non-aqueous solution), responsible for degrading olefins, aromatic compounds, and carbon-nitrogen bond containing compounds, respectively (Riebel *et al*., 1960; Sonntag and Gunten, 2012). Moreover, organic compounds can react indirectly with free radicals such as OH° and HO_2° that are generated upon homogenous and heterogeneous catalytic decomposition of ozone (Gunten, 2003). However, the reaction of ozone is complex, and ozone treatment effectiveness is strongly determined by many factors that may cause some limitations in the selection of a sufficiently effective ozone dose (Miller *et al*., 2013). All treated mince gave slightly plastic odor. This odor was disappeared after heating. Fish mince from ozone flotation for 30 min was reduced.In preliminary experiments, texture and pleasant odor of fish flesh were similarly to control. Therefore, ozone treatment would not affect to fish product development.

Table 2 Main volatile compounds in Nile tilapia mince after treatments with ozone.

Treatments	**Methoxy phenyl oxime (%)**	**Hexanal (%)**	**Nonanal (%)**	**1-Octen-3-ol (%)**
Control	26.09^{a}	9.13^{a}	3.58^{h}	2.99^{a}
W30	21.95^{b}	6.23^{c}	6.04^{g}	2.53^{b}
F30	4.41^{h}	2.78^{h}	59.14^{a}	0.74^{f}
F60	6.60^{g}	2.97^{g}	58.14^{c}	1.09^{e}
F90	12.29^{c}	4.22^{f}	50.86^{d}	1.15^{d}
WF30	8.24^{e}	5.31^{d}	45.37^{f}	1.08^{e}
WF60	6.97^{f}	4.49^{e}	58.92^{b}	1.08^{e}
WF90	9.08^{d}	6.44^{b}	47.51^{e}	1.93^{c}

Remark:$^{a\text{-}h}$ Different superscripts in the same column indicate significant differences (p<0.05).

W30 - ozone water (30 min), F30 - ozone flotation (30 min), F60 - ozone flotation (60 min), F90 - ozone flotation (90 min), WF30- ozone water (30 min) and ozone flotation (30 min), WF60 - ozone water (30 min) and ozone flotation (60 min), and WF90 - ozone water (30 min) and ozone flotation (90 min).

Effect of ozone on protein and fat content of fish mince

The values for protein and fat content of fish mince after the treatments with ozone are presented in Table 3. Protein content in different treatments significantly decreased from raw material. Treatment with both water and ozone water removes the blood and dissolved proteins, so the protein content of the fish decreases. Although protein content in treatments W30, F30 and F60 was not different from the control, increasing the time of treatment resulted in reduced protein content. In the same way, fat content in different treatments decreased from raw material when the time of treatment was increased. However, ozone water treatment (W30) was not different from raw material in terms of fat content. Similarly, the use of ozone for preparation of aflatoxin-contaminated peanuts had a slight effect on fat and protein content when the degree of ozone increased (Sahab *et al*., 2013). Previous studies indicated that the amino acids are oxidized, although the polyamide bond of the protein main chain is not degraded by the action of ozone (Cataldo, 2003).

Table 3 Protein and fat content in untreatedNile tilapia mince and after different treatments with ozone.

Treatments	**Protein (%)**[*]	**Fat(%)**[*]
Raw material	18.00±0.32[a]	3.18±0.13[a]
Control	14.42±0.27[b]	2.71±0.30[b]
W30	14.51±0.14[b]	2.75±0.28[a]
F30	14.61±0.14[b]	2.65±0.51[b]
F60	14.38±0.27[b]	2.47±0.21[b]
F90	14.06±0.09[c]	2.36±0.12[b]
WF30	13.85±0.26[c]	2.08±0.17[c]
WF60	13.74±0.24[c]	1.78±0.20[c]
WF90	13.79±0.26[c]	1.55±0.14[d]

Remark: [*] Values are given as mean ± SD from triplicate determinations.
[a-d] Different superscripts in the same column indicate significant differences (p<0.05).
W30 - ozone water (30 min), F30 - ozone flotation (30 min), F60 - ozone flotation (60 min), F90 - ozone flotation (90 min), WF30- ozone water (30 min) and ozone flotation (30 min), WF60 - ozone water (30 min) and ozone flotation (60 min), and WF90 - ozone water (30 min) and ozone flotation (90 min).

CONCLUSION

In the study we attempted to reduce off-odors in Nile tilapia minces with different ozone treatments, and then analyzed the volatile compound by using GC / MS analysis. In all experiments, we detected 18 volatile compounds, of which methoxy phenyl oxime (fishy odor), hexanal (fatty odor) and 1-Octen-3-ol (mushroom odor) cause offensive odors in fish mince. The level of most of these compounds decreased from the control when treated with different ozone treatments, while there was an increase in nonanal. Ozone water treatment for 30 min eliminated nonanal more effectively than other ozone treatments. In addition, increasing the time of treatment in different ozone treatments might have increased off-odors in the fish mince. However, ozone flotation treatment for 30 min had the greatest reduction of off-odors, while the protein and fat content decreased slightly compared to fresh mince. Meanwhile, there might be some off-odor emission from the reaction of ozone. Therefore, the use of ozone treatment is suitable for the preparation of tilapia mince in products that should have favorable consumer acceptance

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