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Research Article

EFFECTS OF SMOKING PROCESS ON PRODUCING POLYCYCLIC AROMATIC HYDROCARBONS (PAH), NUTRITIONAL VALUE VARIATIONS, FATTY ACIDS COMPOUNDS, AND MICROBIAL LOAD OF SILVER CARP (*HYPOPHTHALMICHTHYS MOLITRIX)*'S TEXTURE

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ABSTRACT
In this study, effects of smoking process on production of polycyclic aromatic hydrocarbons (PAH) and nutritional value changes, microbial load, and fatty acids profile of silver carp's texture was investigated. Fresh silver carp was obtained from a local store and was smoked using *cold-smoking* method. Then it was conserved for 30 days. Nutritional value evaluation of the fish was done by measuring protein, moisture, fat, and ash during smoking and conservation. Carcinogenic PAH production was also done via liquid chromatography with high efficiency. Also variations in fatty acids profile were done using gas chromatography with the purpose of determining the effect of smoking process and fish conservation. Microbial counting was done before, after smoking and after 30 days of conservation. Results revealed that during smoking, moisture reduced significantly and hence protein and fat increased, but ash amount did not change significantly. PAHs with high molecular weight for fresh fish, smoked fish, and fish conserved over 30 days were 0.25, 2.14, and 1.67 micrograms per kg, which were much less than allowed limitations set by the European Union. In terms of total Omega-3fat acids, fresh fish, smoked fish, and the fish conserved for 30 days had significant differences with values of 6.48, 4.66, and 4.77 grams per 100 grams, respectively ($P<0.05$). Cold smoking process significantly affected composition amount of most fat acid groups for silver carp. It also resulted in reduced microbial load and considerably extended duration of fish preservation.

Keywords: *Fatty Acid, Cold Smoking, Silver Carp, Polycyclic Aromatic Hydrocarbons*

INTRODUCTION
Silver carp (Hypophthalmichthysmolitrix), with a production of over 4 million tons in the world and 50,000 tons in Iran (Binsi *et al.*, 2007), is a fish species that is extensively used in many countries of the world due to its use of the first level of nutrition pyramid (phytoplanktons), quick growth, easy development, high nutritional conversion coefficient and appropriate nutritional value (Boran and Regenstein, 2009). This fish is bred massively in farms and it is usually sold as fresh or frozen, whole or fillet and therefore it is more susceptible to decay compared with other meat products. For this reason, it is especially important to use different fish conservation methods. In this regard, various techniques like salting, freezing, packing, smoking, and other methods are used. Making use of the smoke produced by wood has a long history and it is used with the purpose of conservation and persistency expansion, and creating special and desirable tastes. Smoke is a carbonic material that is produced by burning organic elements present in wood e.g. phenols, aldehydes, acids, volatile hydrocarbons, etc. (Cheow *et al.*, 2007). From among these compounds, polycyclic aromatic hydrocarbons have been identified for their toxicity and carcinogenic traits as a qualitative criterion of smoked foods. Utilizing PAH-contaminated smoked food and even inhaling air contaminated with PAH's produced from wood smoke and by burning agricultural products or taking roasted meats, and foods processed with a smoke imbrued with these compounds causes them to infiltrate human body. Grilling, smoking, and roasting food on flames increases the number of PAHs in food (Cho *et al.*, 2004; Cho *et al.*, 2005). Studies conducted on animals suggest that PAHs do not tend to remain in different textures for a long time, and therefore most of these

compounds are excreted from the body after several days. However, type and extent to which PAHs affect human health depends on various factors such as amount imported, duration of contact with these materials, body's response to them which differs with age, gender, nutrition, individual health, and finally source type or way of contact with such materials (Cho *et al.*, 2004). Effect intensity of smoke compounds is dependent on smoke density and temperature. Type of fish chosen for smoking and type of wood shavings typically affects texture, odor (aroma), and other organoleptic properties such as taste (Choi and Regenstein, 2000). Lower temperature, little oxygen, certain temperature, appropriate moisture, and accurate smoke density control give better and more pleasant taste to the product and increase its conservation property (Gimenez *et al.*, 2005). Today 360 chemical compounds have been identified in smoke most important of which are phenolic, acidic, and carbonyl compounds (Gimenez *et al.*, 2005). Phenols have antioxidant properties, delaying putrefaction in fish. Permitted limit set by the European Commission for all PAHs in smoked fish textures is 30 µg per kg, and acceptable limit of Benzo(a)pyrene, which is the carcinogenic criterion of smoke, has been stated as 5 µg per kg in smoked fish (Gilsenan and Ross-Murphy, 2000). Fish's specific propertythat makes it outstanding among other foods is the type of fat in it (Gomez-Guillen *et al.*, 2002). Fatty Omega-3 acids contained in fish oil have very important effects on human health and play an important and constructive role in preventing diseases and helping ameliorate different disorders and complications (Gomez- Guillen and Montero, 2001).

MATERIALS AND METHODS

Fish Preparation

12 fresh silver carps were purchased from pisciculture center of Babolsar region in Mazandaran Province in August. Purchased fish samples were measured biometrically. Medium fish length was determined to be 36.5 cm with medium weight of 1.2 kg. Once prepared, the fish were placed in polystyrene boxes covered with ice, and were transferred to laboratory for smoking.

Fish Cold Smoking

Cold smoking method was used in this project, where an indirect heat source was used for heating and smoking. Temperature was usually about 25-27 °C. To keep temperature fixed and ensure consistent drying of the product and desired color, smoking was stopped in less than 24 hours. To smoke, fish were suspended using threads in the smoking room (cabin) (Gornall *et al.*, 1949).

Determining Nutritional Value

To measure protein contained in the sample the Macro-Kjeldahl method was used. In this method, in presence of sulfuric acid and a catalyst, nitrogen atoms are converted to aluminum sulfate in organic nitrogen-rich compounds, and then ammonia is distilled from an alkaline medium and is absorbed in choleric acid or boric acid, and through titration with an acid its amount is determined. Determining protein value is done in three stages: digestion, distillation, and titration. In the digestion stage, one gram of the sample along with Kjeldahl catalyst was poured into special flask and was heated. After this stage, distillation was done in which about 250 ml of water and 75 ml of sodium was added to the digested sample and heated again until sample protein was gradually distilled, and it entered a flask containing boric acid and methyl, with its color changing from red to yellow. Distillation ended after about 20 minutes and titration of the sample protein was done with 0.1-normal acid sulfuric. Protein amount was measured using the following formula (Haiying *et al.*, 2008). 5 grams of minced fish was placed in oven at 105°C and removed after 4 hours and transferred to desiccators. The sample is weighed again after cooling, and drying goes on until no considerable change is seen in the sample (Haiying *et al.*, 2008). To determine the amount of ash 5 grams of the uniformed sample was poured inside a crucible with constant weight. It was then kilned for one hour until the sample was formed into ash. Next, the container of the incinerated sample was removed from oven and weighed after cooling in desiccators (Haiying *et al.*, 2008):

40 grams of minced fish meat was transferred to a 500-milliliter decanter, and 160 milliliters of methanol and the same amount of chloroform were added to the decanter. By adding distilled water to the compound, phases were segregated. Ratio of methanol chloroform and water was 2:6:1.2. The fat-

containing chloroform layer was segregated. By extrusion of solvent and re-weighing of the flask, amount of oil and sample fish fat percent were calculated as follows (Jamilah and Harvinder, 2002):

Measurement and Identification of Poly-Aromatic Hydrocarbons

Measuring and identification of PAH values were done by HPLC (Cecil Instruments, made in England, model Ce-4100), and detection was done by two fluorescence and UV detectors (Ladislaus *et al.,* 2007). The samples were completely dried in vacuum under cold conditions. In a crucible, required amount of powder was prepared. Then a soap was made using N-hexane. Finally, they were identified in terms of quantity and quality by injection to the HPLC machine and using column chromatography (Laemli, 1970).

Preparing Fatty Acids Profile

Identification and blending of fatty acids using gas chromatography machine (USP26-NF21 Supplement-Capillary Gas) via the FID detector was performed using capillary pipe and a 50m×0.25 mm column. After extracting fat and fatty acid methyl ester by esterization, analysis of samples' fatty acids was done by GC. Helium was used as a carrier gas. During the heating process, temperature of injection, detector, and columnwere 250 °C, 260 °C, and 155 °C, respectively, with an injection volume of 1 µl. Column temperature was first raised to 150 °C for two minutes. This temperature was constant for 3 minutes and it was raised to 210 °C after 3 minutes, and was kept at this temperature for 29 minutes. Rate of the carrier gas was 0.5, with an injection amount of 1 µl and fission rate was 1:10 (Liu *et al.,* 2007).

Microbial Load Determination

Total microbial count was done via the typical *total count* method and the culture environment of *nutrient agar* according to instructions given as follows: First 5 grams of initial sample was blended well with 45 ml of physiological serum in a flask until the solution got a good consistency (initial suspension with tenuity of 0.1). After several minutes the suspension was kept fixed so particles deposited. Next with a sterilized pipette, one milliliter of the initial suspension was transferred to pipes containing 9 milliliter of sterilized thinner (physiologic serum). So, sequential tenuities of 0.01, 0.001, and 0.0001 were obtained. Now for bacterial growth with *Pure plate* method (1 milliliter from experiment pipe), the sample was placed in the culture environment of *nutrient agar* so that it was pulled at 45 °C into pipette and agar in the shape of lambda (Λ) several times (it must be fluent so no anaerobic microbes enter it). Inside oven, it was then placed for 24-48 hours at 37 °C (as it is human food). Next counting generated colonies was done. For example, if 7 bacterial colonies were observed, this number (7) was multiplied by inverse of tenuity. Then it is multiplied by 1 ml, thus yielding number of colonies in each gram of the food material.

Statistical Analysis

All results were obtained in three iterations. Statistical reviews were done using SPSS 19 and ANOVA was computed with confidence level of 95%. Significant statistical differences were evaluated using Duncan's new multiple range test in confidence level of 95%. Charts were drawn using Excel™ 2013.

RESULTS AND DISCUSSION

Effects of Smoking Process on Nutritional Value of Silver Carp

In table 1, effects of smoking process on nutritional value and biochemical compounds of silver carp's texture have been shown. Treatments were reviewed and compared as fresh fish, smoked fish, and 30-day conserved smoked fish. Results show that the highest amount of protein belonged to fresh smoked silver carp, and 30-day smoked fish with protein amounts of 43.89, and 43.14 respectively. In statistical terms no significant difference was observed between fresh smoked fish and conserved ones ($P<0.05$). However, significant difference was observed between these two samples and protein amount of fresh fish (20.02 percent). In terms of fat, results were similar to protein's trend, in a way that 4.18, and 4.14 percent fat were determined in fresh smoked fish and conserved one respectively, which was in significant contrast to fresh fish (2.63) ($P<0.05$). This amount of protein and fat difference between fresh fish and smoked one is due t removal of fish's water during smoking. This moisture changes in fresh fish was equal to 74.2 percent. Smoked fish samples and conserved ones had 48.80 and 49.49 percent of moisture respectively. In terms of ash amount no significant difference was observed among samples.

This shows ineffectiveness of smoking process and its preservation on ash amount of fish texture ($P<0.05$).

Results revealed that fresh fish's moisture was equal to 74.2 percent. During smoking and conserving it for 30 days, the moisture reached 48.80 and 49.49. Over the period of fish smoking and conservation, fish texture loses water and dries to some extent, thus reducing moisture. This process reduced moisture in a desirable way. In another similar study, during salting and drying of cold-smoked fish, moisture increased to 64 percent, and for hot-smoked fish, this rose to 60 percent (Muyonga *et al.*, 2008). One important factor of nutritional value is amount of its protein. Highest values of protein relates to fresh smoked silver carp and 30-day smoked silver carp with protein values of 43.89 and 43.14%, respectively. Fresh fish sample's protein was determined to be 21.12 percent, which shows considerable increase of protein during smoking, so that protein value had increased to twice as much as that of fresh silver carp. This increase is in connection with water shrinking and loss over smoking process and fish conservation (ShahiriTabarestani *et al.*, 2010). Ash percent in different treatments varied over 2.15-2.31 and no significant difference was observed. Generally, ash amount does not change considerably during hot and cold smoking processes, with numbers close to results of this study (ShahiriTabarestani *et al.*, 2010). Fat amount increased considerably during smoking. In raw fish this value was 2.63 which rose to 4.18 during smoking process. Fat percent for both smoking methods showed increasing trend affected by processing. On the other hand, records have shown that fish drying has positive effects on its quality, thus causing increased nutritional parameters (Shahidi and Botta, 1994; Schrieber and Gareis, 2007).

Table 1: Nutritional values variation for silver carp during smoking and conservation

Silver carp sample	protein	fat	Moisture	Ash
fresh sample	$21.12^{b}\pm0.14$	$2.63^{b}\pm0.08$	$74.2^{a}\pm0.45$	$2.15^{a}\pm0.05$
fresh cold smoked sample	$43.89^{a}\pm0.21$	$4.18^{a}\pm0.05$	$48.80^{b}\pm0.11$	$2.31^{a}\pm0.06$
30-day cold smoked sample	$43.14^{a}\pm0.18$	$4.14^{a}\pm0.05$	$49.49^{b}\pm0.38$	$2.26^{a}\pm0.08$

Difference in letters denotes significant difference in means of each column ($p<0.05$)

Effects of Cold-Smoking on the Amount of Poly-Aromatic Hydrocarbons

As seen from table 2, the amount of poly-aromatic hydrocarbons for fresh, smoked and 30-day-conserved silver carps have been shown separately. No Benzo[a]pyrene PAHs were seen in fresh fish. In smoked fish, however, this value reached 0.61 µg per kg and in 30-day conserved fish this reached 0.52 µg per kg, showing production of Benzo[a]pyrene during cold smoking of the fish. As regards *Cyclpentapyrene* PAH's results were completely similar to those of *Benzo[a]pyrene* i.e. amount of Cyclpentapyrene in fresh fish, smoked fish, and fish conserved over 30 days were 0, 0.47, and 0.34 respectively, where smoked and conserved fish had significant differences with the control sample ($p<0.05$). In fresh fish, the amount of *chrysene* PAHs has been determined to be 0.1 µg per kg. This value has reached 0.44 and 0.39 µg per kg over 30 days of conservation. Other polycyclic aromatic hydrocarbon is *Benzo[b]fluoranthene* which was not detected in fresh fish, but was calculated as 0.5 µg per kg in smoked fish and 0.35 µg per kg in conserved smoked fish. Smoking process has affected the amount of this hydrocarbon specifically, thus increasing it. *Benzo[a]anthracene* is another important hydrocarbon which increases considerably during the smoking process. In this study it was determined that these hydrocarbons increased with smoking process, and decreased over 30-day conservation period. That is in fresh fish, smoked fish, and the fish conserved for 30 days, 0.15, 0.59, and 0.51 µg per kg of *Benzo[a]anthracene* were determined, respectively. Cold smoking had significant effect on the amount of these hydrocarbons ($P<0.05$). In this study, value of PAH4 (a fourfold group of PAHs with high molecular weight consisting of *Benzo(a)pyrene, Benzo(a)anthranene, Benzo9b)fluoranthene*and *Chrysene* for fresh fish, smoked fish, and fish conserved for 30 days was 0.25, 2.14, and 1.67 µg per kg respectively, which is far less than the limit permitted by the European Union.

The hypothesis for creation of poly-aromatic hydrocarbons is that melted fat resulted from heated meat drops on hot surface of smokehouse and is analyzed thermally, thus leading to production of PAHs,

which ultimately settle on fish skin during smoking. Food compounds e.g. fat have the effect of PAHs being generated through thermal analysis or polymerization, and these different stages affect PAH production. So, the more fat value is, the more PAHs will exist (Sims *et al.*, 1997). It is stated in literature that as fat volume of biologic textural membrane increases, contaminators are absorbed more quickly. Highest number of PAHs is smoked products is obtained immediately after smoking. Although PAHs concentration gradually decreases due to decomposition and interaction with other elements present in environment, part of PAHs enter fish textures and get guarded against light and oxygen. So PAHs get fixed after a certain time in fish body. Making use of wood for smoke production leads to a considerable amount of PAHs.

In this study, amount of PAH4 (a fourfold group of PAHs with high molecular weight consisting of *Benzo(a)pyrene*, *Benzo(a)anthranene*, *Benzo(b)fluoronthene*, and *Chrysene*) for fresh fish, smoked fish, and fish conserved for 30 days was 0.25, 2.14, and 1.67 µg per kg respectively, which was far lower than the limit set by the European Union for all of these compounds in smoked fish's texture (30 µg per kg) (Songchotikunpan *et al.*, 2007). Most carcinogenic PAHs have high molecular weight. In current study, highest HMW concentration was yielded in silver carp. Total concentration of HMW is typically higher than other molecular weights. Type of smoking method also affects PAHs concentration. For example samples smoked with charcoal of 250 °C have lowest PAHs value compared with those smoked via wood and straw.

According to EU's 2011 announcement, permitted and acceptable amount of *Benzo[a]pyrene*, which is the carcinogenic criterion, was set to 50 µg per kg. Comparisons for the current study showed less of the above compounds in smoked and conserved fish than permitted limit. *Benzo[a]pyrene* has also been seen less in other researches regarding smoked fish. For example, Asuha and Charles Ikenna only extracted 0.022 and 0.032 µg per kg of this compound for two samples from four samples. However, Mihalca et al estimated BaP value in 6 samples from 15 samples under investigation, all of which had been smoked via smoke of direct heat, more than permitted amount (Stainsby, 1987). On the other hand, amount of fish's fatty acid and smoking temperature also affect PAHs amount (12). One of the noticeable limitations in study of smoked fish's quality in Iranian markets is lack of knowledge concerning raw fish and materials before purchase. Furthermore, production date and its time of delivery to consumers are not clear for shoppers and consumers.

Table 1: Measuring the amount of poly-aromatic hydrocarbons in fresh fish, smoked fish, and 30-day fish

Polycyclic aromatic hydrocarbons	Abbreviations	Fresh sample	fresh smoked	30-day smoked sample
Benzo[a]pyrene	BAP	n.d	0.61[a]	0.52[b]
Cyclpentapyrene	CPP	n.d	0.47[a]	0.34[b]
Chrysene	CHR	0.1[b]	0.44[a]	0.39[a]
5-methylchrysene	5MC	n.d	n.d	n.d
Benzo[b]fluoranthene	BBF	n.d	0.50[a]	0.35[b]
Benzo[k]fluroanthene	BKF	n.d	n.d	n.d
Benzo[g]fluroanthene	BGF	n.d	n.d	n.d
Ideno[cd]pyrene	ICP	n.d	n.d	n.d
Dibenzo[ah]anthracene	DHA	n.d	n.d	n.d
Dibenzo[ghi]pyrene	bgp	n.d	n.d	n.d
Dibenzo[ae]pyrene	dep	n.d	n.d	n.d
Dibenzo[ai]pyrene	Dip	n.d	n.d	n.d
Dibenzo[ah]pyrene	dhp	n.d	n.d	n.d
Benzo[a]anthracene	baa	0.15[c]	0.59[a]	0.51[b]

Letters difference denotes significant difference in means for each column ($p<0.05$)

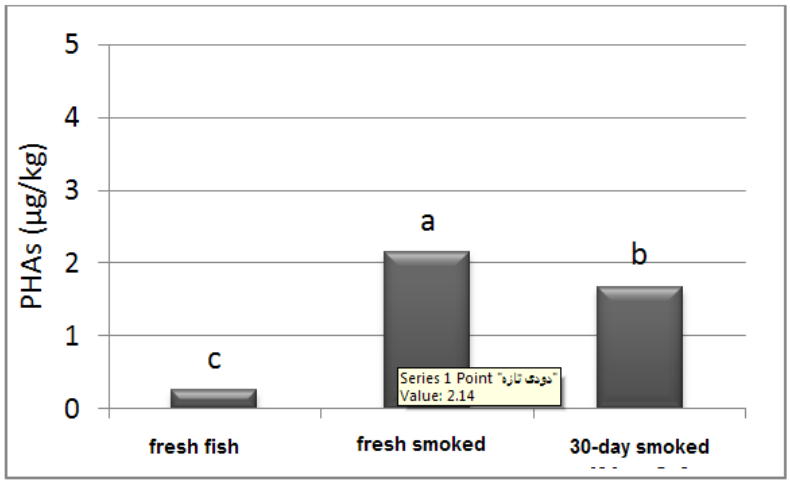

Figure 1: PAHs variation with high molecular weight of carp during smoking and conservation. Letters difference denotes significant difference in means of each column ($p<0.05$)

Effects of Smoking on Composition of Fatty Acids for Silver Carp

Table 3: Profile analysis of fresh, smoked, and 30-day fish

Fatty acid	Fatty acid type	fresh sample	fresh smoked sample	30-day smoked sample
Myristic	C14:0	2.55	2.59	2.57
Palmitic	C16:0	17.13	17.19	17.15
Stearic	C18:0	6.04	6.09	6.07
Oleic	C18:1	38.3	38.6	38.5
Linoleic	C18:2n6	4.32	2.40	2.54
Alpha-linolenic	C18:3 n3	1.03	0.50	0.55
Arachidonic	C20:0	0.11	0.17	0.14
Gadoleic	C20:1	1.65	1.64	1.62
Eicosadienoic	C20:2	0.31	0.35	0.33
Eicosatrienoic	C20:3	0.81	0.87	0.85
EPA	C20:5 n3	2.40	1.88	1.96
Sitoletic	C22:1	1.49	1.54	1.52
Dicosapentaenoic acid	C22:5	0.57	0.59	0.54
DHA	C22:6 n3	3.05	2.28	2.16

Profile analysis for fresh fish, smoked fish and 30-day fish has been shown in table 3. As seen from the table, in fresh fish, smoked fish, and fish conserved over 30 days, oleic acid, has been the dominant fat with values of 38.3, 38.6, and 38.5 grams per 100 grams respectively, have had the highest amounts among fatty acids. No significant difference was evident among samples in terms of amount of fatty acid ($p<0.05$). After oleic acid, palmitic acid was ranked second in terms of amount. In terms of total Omega-3 fatty acids, fresh fish, smoked fish, and the fish conserved for 30 days had significant differences with values of 6.48, 4.66, and 4.77 grams per 100 grams ($p<0.05$). Results of experiments for fatty acids showed that the process of smoking and conserving fish over 30 days affected the amount of fish's fatty acid, thus reducing Omega-3 fatty acids and increasing Omega 6 fatty acids. As an example, amounts of two useful unsaturatedmultiplex fatty acids (EPA and DHA) have had certain changes during smoking and conservation, with value of the Eicosapentaenoicacid (EPA) in fresh fish being 2.40 grams per 100 grams and 1.88 and 1.96 percent for smoked and conserved fish respectively. Also, the amount of fatty acid of *Docosahexaenoicacid*(DHA) are, as shown in the table 3.05, 2.28, and 2.16 grams per 100 grams for fresh fish, smoked fish, and fish conserved over 30 days respectively. This suggests structural change and complex fixation of silver carp's fatty acid during cold-smoking process. Total saturated fatty acids of

fresh fish, smoked fish and fish conserved for 30 days was determined to be 25.83, 26.04, and 25.93 percent, with no changes occurred fir carp's textures during performed processes, in this case neither. Steiner et al found by conducting studies on 3 smoked fishes sardine (sardinellamelanura), bream (Abramisbramaorientalis), and tilapia (oreochromisniloticus) that smoking reduces amounts of unsaturatedfatty acids. This reduction depends varies depending on fish type in terms of fat amount (Wasswa *et al.,* 2007). The Study mentioned showed that fish type is of high importance, as it is seen in this study that smoking did not decrease amounts of unsaturated fatty acids. Amount of unsaturated fatty acids in smoked fish is one of the factors leading to increased PAH in smoked fish's textures (12). Since PAHs are fat-friendly compounds, increase in amount of fatty acids in fish's textures leads to more absorption of these compounds during smoking (Yata *et al.,* 2007).

Table 4: Composition percentage of fatty acid groups for fresh, smoked, and 30-day-conserved fish

fatty acid groups	Treatment		
	fresh fish	fresh smoked fish	30-day conserved smoked fish
Saturated(SFA)	25.83^{a}	27.51^{a}	25.93^{a}
Monounsaturated (MUFA)	41.44^{a}	41.78^{a}	41.64^{a}
Unsaturated (UFA)	52.83^{a}	51.52^{a}	51.37^{a}
Polyunsaturated (PUFA)	11.39^{a}	9.74^{b}	9.73^{b}
Ratio of Polyunsaturated to saturated (PUFA/SFA)	0.44^{a}	0.35^{b}	0.37^{b}
Ratio of unsaturated to saturated (UFA/SFA)	2.04^{a}	1.87^{a}	1.98^{a}
Omega-3 (ω-3)	6.48^{a}	4.66^{B}	4.77^{B}
Omega-6 (ω-6)	4.32^{a}	2.40^{b}	2.74^{b}
EPA+DHA	5.90^{a}	4.16^{b}	3.68^{b}
Ratio of omega-3 to omega-6 (ω-3/ω-6)	1.5^{b}	1.94^{a}	1.74^{ab}
Poly-n criterion: DHA+EPA/C16	0.34^{a}	0.24^{b}	0.21^{b}

**Letters difference denotes significant difference of means in each column (p<0.05)*

Effects of Smoking Process on Microbial Load of Silver Carp

As seen in table 5, total count of micro-organisms in fresh fish and fresh smoked fish and preserved fish has been 4.6×10^{3}, 2.8×10^{3}, and 6.8×10^{5} Log cfu/g (logarithm of colonies count in each gram of sample), respectively. These values had significant differences in probability level of 0.5 % ($p<0.05$). Results showed that by smoking fish, fish's microorganisms count decreased and by preserving it for 30 days, this count increased colossally. During the smoking process, fish's microorganisms count decreased. Over 30 days of conservation also, microorganisms count increased over two logarithmic growths, thus reaching 6.8×10^{5} Log cfu/g. Results of this study revealed that by smoking fish, fish's microorganisms count decreased and by preserving it for 30 days, it increased colossally. Smoking process retards growth of deterioration-causing bacteria and extends fish's permanency duration. Permitted limit for bacteria count in smoked fish is 10^{6} colony units formed per gram (Cho *et al.,* 2004). In this study, bacterial total count varied over the range of 2.8×10^{3}-6.8×10^{5} Log cfu/g, with the lowest count relating to fresh smoked fish and highest count for smoked fish preserved over 30 days. Of course microorganisms' amount was within allowed and consumable range. Reports suggest that compounds such as hydrogen, acids, carbonyl, aldehydes, and their derivatives are generated in the process of smoke production. For this reason, fungicide and antimicrobial property exists specially in smoke compounds. Smoke conveys also phenolic elements which hinder microbial growth, and amount of phenolic elements are considered a quality factor for smoked fish (20, 23).

Table 5: Variations of microbial load (Log cfu/g) for silver carp during smoking and conservation

Sample	fresh fish	fresh smoked	30-day smoked
microbial count	$4.6^{b}\times10^{3}$	$2.8^{c}\times10^{3}$	$6.8^{a}\times10^{5}$

Letters difference denotes significant difference in means of each column (p<0.05)

Conclusion

According to the results of this study, it was clarified that during smoking moisture decreased and hence protein and fat increased, but ash amount did not change considerably. PH difference between fresh, smoked, and preserved fish was not significant. PAHs with high molecular weight were 0.25, 2.14, and 1.67 for fresh, smoked, and preserved fish respectively, which was far less than the limit set by the EU. A certain amount of Benzo[a]pyrene has not been seen in fresh fish, but in smoked fish this was 0.61 µg per kg and in smoked fish conserved for 30 days this reached 0.52 µg per kg, showing Benzo[a]pyrene production during fish smoking. In terms of total omega-3 fatty acids count, fresh fish, smoked fish, and 30-day fish were significantly different with values of 6.48, 4.66, and 4.77 grams per 100 grams, respectively ($p<0.05$). Results of experiments done for fatty acid compounds showed that smoking process and preserving smoked fish for 30 days did not affect the amount of fatty acids, and led to preservation of useful fatty acids including omega-3 and omega-6. In short, cold smoking of silver carp did not have destructive effects on fish health, and no threat is observed concerning consumption of this fishery product.

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