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## Effects of Hot and Cold Smoking Processes and Thymus Oil's Addition, on Microbiological and Sensory Properties of *Sander lucioperca*'s Fillets

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**Abstract:** The objective of this study is to investigate the modifications made by smoking on hygienic and sensory quality of freshwater fish: Zander. Zander fillets were divided into two groups, the first was hot smoked while the second was cold smoked. Sensory attributes were evaluated by hedonic test and triangular test while the hygienic quality was assessed by research and monitoring of certain bacteria. Sensory analysis showed that the smoking process improve the organoleptic quality of this freshwater fish. Furthermore, distillate thyme addition's contributes to the development of superior taste, especially in the case of cold smoking. The microbiological results showed that the fresh sample bacterial populations charges' are reduced after smoking processes from 0.5-2.5 ULog. During storage, lactic acid bacteria inhibited after hot smoking, weren't detected during storage. The proliferation of *Staphylococci* slowed for hot smoked fish fillets with the addition of the distillate. But, a significant proliferation of coliforms (>3 U Log) was noted all processes combined.

**Keys words:** *Sander lucioperca*, smoking process, sensorial quality, microbiological quality

### INTRODUCTION

In Tunisia, the first *Sander*'s introduction took place at the dam of Nebhana (Kairouan) in 1968, following the immersion of 30 000 eggs, of German origin, in bins incubators (M'Hetli, 2001; Zaouali, 1981). Given the suitability of individuals to perch with their new environment, several stockings were made in various other waters (Turki *et al.*, 2009). Currently, the pike is present in reservoirs of Sidi Salem, Nebhana, BniMtir, BouHertma, SilianaLakhmess, Bzirek and Lebna (CTA, 2012). Since its introduction, the recruitment of pike in dam reservoirs is naturally replenishes the stock of himself. Production of pikeperch in dam reservoirs, had a first phase characterized by an increase exponentially to 192.4 tons in 2006, followed by a phase of weak growth in subsequent years. The majority of the production comes from Sidi Salem's dam with an average of 84.28 tons year<sup>-1</sup> followed by holding SidiBarrak with an average of 36.83 tons year<sup>-1</sup> (CTA, 2012).

Its presence in different Tunisian dams is due to its good adaptation to the environment. These nutritional qualities seem to be ignored by the Tunisian consumer. Over its absence in Tunisian culinary traditions and its organoleptic characteristics that differ from saltwater fish which is used the consumer hinder its commercialization which is limited to villages near dams. Production of pike

in Tunisian dams reached in 2010, 188.7 tons representing 16.9% of the national production of freshwater fish. It represents an exploitable resource capable of providing raw materials to processing industries and promotion of seafood. Different treatment techniques applied by this kind of industry on fish such as drying, salting, pickling and smoking would bring added value to the raw material, giving it a taste and a specific and same time prolong its shelf life. So, the objective of this work is to study the effect of smoking and the addition of the distillate thyme on the organoleptic and hygienic quality of zander's fillets.

### MATERIALS AND METHODS

**Samples collection and preparation:** The fish were brought from Sidi Salem dam, located north-west of Tunisia, belonging to the governorate of Beja (35°24'N, 9°47' E) and being on the course of Oued Madjerda.

Fishing was conducted using nets of mesh combined variable (40, 45 and 60 mm). The fishing operation takes 24 h on average and it takes place in two stages, spinning and hauling. Wiring is to extend the nets in the water. While hauling means nets withdrawal for recovering fish. Once recovered, the fish are placed on ice for transport to the laboratory to undergo transformation.

Fish undergoes a first step operations heading and gutting. These two operations must be performed on air-conditioned room at +12°C. They are a source of contamination: manipulation shed blood, mucus and bacteria on the parts of the viscera and freshly cut within the abdominal cavity. The peritoneum is the skin as a barrier to the penetration of bacteria is torn. The second step is to detach fish fillets own.

The fillets are currency in two batches. The first is immersed in brine at a concentration of 25 g L<sup>-1</sup> sodium chloride, 1 g pepper and 30 mL thyme distillate for 1 h 30, the second batch fillets are immersed in a brine having the same concentration of NaCl and pepper and without the distillate during the same period as for the first batch.

For smoking, each batch has been unscrewed to turn two. The first part was used for cold smoking at a temperature of 35°C for 5 h. The second part served for smoking hot at a temperature of 70°C for 3 h. These two temperatures were chosen after a series of preliminary test smoking later in which we tried to vary the temperature by scanning the temperature limits between the two types of smokes. This step is carried out using a smokehouse Belonia model "Raucher FV.V2 Heizung" belonging to the laboratory value of seafood from the Higher Institute of Fisheries and Aquaculture, Bizerte. The sawdust used is from the mahogany tree.

**Microbiological analysis:** The tested samples are collected aseptically a weekly basis for 28 days from the four batches prepared as described previously.

Microbiological analyzes were performed initially on the raw zander then samples having undergone smoking and flavoring stored at 4°C at equal time intervals. For each sample, 10 g of a chair are cut aseptically and ground in 90 mL of saline (9 g L<sup>-1</sup>). Appropriate dilutions were subsequently made. The numbers of the different bacterial populations is performed by taking 100 µL of the diluted sample and spread using a spreading board according to the method of plate count agar (ISO-15214, 1998).

The calculation is performed on concentrations using the AFNOR standard formula:

$$N = \frac{\sum C}{V(0.1 n1 n2) d}$$

Where:

- ΣC = Number of colonies counted on the boxes used
- n1 = Number of boxes used in the first dilution
- n2 = Number of boxes used at the second dilution
- d = Dilution of the first dilution
- V = Volume of the test socket

The interpretation of the results is carried out using a sampling plan for three classes. In this plan the studied samples are divided into three categories: good, acceptable and unsatisfactory. It is used if it is acceptable that some samples exceeded the lower limit (m) since the level of contamination risk (M) is not exceeded. Sampling units with a result of less than "m" are satisfactory or good bacteriological quality. Units showing a score between "m" and "M" are deemed to be acceptable (poor) and units containing counts above "M" are unsatisfactory.

**Sensory analysis:** The prepared samples were analyzed by two sensory tests, triangular and hedonic.

**Triangle test:** The triangle test is part of the difference tests which are designed to determine whether it is possible to distinguish two samples from each other by means of the sensory analysis. These tests may be used to determine if there was a perceptible change in the appearance, flavor or texture of a food as a result of a change in the methods of preparation (Watts *et al.*, 1989).

Three coded samples, two identical and one different, are presented to the tasters and the identification of the different sample is requested. They must choose a sample, even if they cannot distinguish the difference between the samples.

**Statistical analysis:** The significance of the results is studied using a binomial test. It totals the number of tasters who identified the different sample and check the meaning of the total by using the Table 1. In this table, X represents the number of tasters who choose different sample and n is the total number of tasters participating in the test. The table of probabilities gives 3 decimals for certain combinations of X and n. The decimal point has been omitted due to space limitations and must therefore be read 0.868 as 868. Difference is significant when Probability is less than or equal to 0.05.

**Hedonic test:** Hedonic tests are designed to measure the degree of appreciation of a product. We use scales categories ranging from "very good" to "bad" through "neutral" with a variable number of intermediate categories. Tasters choose, for each sample, the category that corresponds to their level of appreciation (Watts *et al.*, 1989).

Samples are presented in identical containers, coded with 3-digit random numbers. Each sample must have a separate number. The order has not been set but it was to choose the tasters each in his own way. Each product has been coded with a three digit number from 511-515.

Table 1: Binomial test statistic table's (Watts *et al.*, 1989)

n/X	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
5	868	509	210	045	004																									
6	912	649	120	100	018	001																								
7	941	737	429	173	045	007																								
8	961	805	532	259	088	020	003																							
9	974	805	532	259	088	020	003																							
10	983	896	701	441	213	077	020	003																						
11	988	925	766	527	289	122	039	009	001																					
12	992	946	819	607	368	178	066	019	004	001																				
13	995	961	861	678	448	241	104	035	009	002																				
14	997	973	895	739	524	310	149	058	017	004	001																			
15	998	981	921	791	596	382	203	088	031	008	002																			
16	998	986	941	934	661	453	263	126	050	016	004	001																		
17	999	990	956	870	719	522	326	172	075	027	008	002																		
18	999	993	961	989	769	598	391	223	108	043	014	004	001																	
19	995	926	921	812	648	457	279	146	065	024	007	002																		
20	992	892	940	848	703	521	339	191	002	038	013	004	001																	
21	998	982	954	829	751	581	399	240	125	056	021	007	002																	
22	998	991	965	904	794	638	460	293	163	079	033	012	003	001																
23	999	993	974	924	831	690	519	349	206	107	048	019	006	002																
24	999	985	980	941	862	737	576	406	254	140	068	028	010	003																
25	999	996	985	954	888	778	630	462	304	178	092	042	016	006	002															
26	997	989	964	910	815	679	518	357	220	121	058	025	009	002	001															
27	998	992	972	928	847	725	572	411	266	145	079	036	014	005	002															
28	999	994	979	943	874	766	623	464	314	191	104	060	022	006	003															
29	999	996	984	955	897	801	670	517	364	232	133	068	031	013	006	001														
30	999	997	988	955	916	833	714	568	415	276	166	090	043	019	002	002														
31	998	991	972	932	861	754	617	466	322	203	115	059	022	011	004	001														
32	998	993	978	946	885	789	662	516	370	243	144	078	038	016	006	002														
33	999	995	983	957	905	821	105	565	419	285	177	100	051	023	010	004														
34	999	996	987	965	922	849	744	612	468	330	213	126	067	033	014	006	002													
35	999	997	990	973	937	873	779	656	516	376	252	155	082	044	020	009	003	001												
36	998	992	978	949	885	789	662	516	370	243	144	078	038	016	006	002														
37	998	994	963	939	913	838	735	607	469	336	223	135	075	038	018	002	003	001												
38	999	996	987	967	928	863	769	650	515	381	261	164	095	051	025	013	004	002	001											
39	999	992	990	973	941	885	800	689	560	425	301	196	118	066	033	016	007	003	001											
40	999	992	992	979	962	903	829	726	603	470	342	231	144	083	044	021	010	004	001											
41	998	994	963	961	920	854	761	644	515	385	268	173	104	057	029	014	006	002	001											
42	999	995	987	968	933	876	791	683	558	428	307	205	127	073	038	019	008	003	001											
43	999	996	990	974	945	895	820	719	600	471	347	239	153	091	050	025	012	005	002	001										
44	999	997	992	980	955	912	845	752	639	514	389	275	182	111	063	033	016	007	003	001										
45	999	998	994	984	963	926	867	783	677	556	430	313	213	135	079	043	022	010	004	002	001									
46	998	995	987	970	938	887	811	713	596	472	352	246	161	098	055	029	014	006	003	001										
47	999	996	990	976	949	904	836	745	635	514	392	252	189	105	070	070	038	019	009	004	002	001								
48	999	992	980	958	919	859	776	672	554	433	318	220	142	086	048	025	012	006	002	001										
49	999	998	994	984	965	922	879	803	706	593	473	356	253	168	105	061	033	012	008	003	001									
50	999	998	995	987	972	943	896	829	739	631	513	395	287	196	126	076	042	022	011	005	002	001								

Table 2: Descriptive categories conversion into numerical ratings

Descriptive categories	Numerical ratings
Very good	5
Good	4
Neutral	3
Medium	2
Bad	1

Table 3: Descriptive categories conversion into numerical ratings for each taster

Taster	Steamed sander	Cold smoked sander+ distillate thyme	Cold smoked sander	Hot smoked sander +distillate thyme	Hot smoked sander
1	3	3	4	2	4
2	1	2	2	5	5
3	3	5	5	4	5
4	5	4	4	4	4
5	4	4	5	1	5
6	2	1	4	4	4
7	2	1	2	5	4
8	1	1	2	4	2
9	2	4	5	4	5
10	2	4	4	5	5
11	5	4	4	4	4
12	1	4	5	4	5
13	4	5	5	2	2
14	4	2	4	1	2
15	2	1	1	1	1
16	4	4	2	4	5
17	4	5	2	4	4
18	5	2	1	4	4
19	1	5	4	5	5
20	2	3	4	5	5
21	2	4	4	4	4
22	2	5	2	2	4
23	2	4	4	4	5
24	2	3	2	4	4
25	1	2	4	4	4
26	5	2	2	4	5
27	1	5	3	4	5
28	5	4	4	5	5
29	2	5	4	4	4
30	2	4	5	5	5
31	5	4	4	4	5
32	3	4	4	4	4

Table 4(a-d): Analysis of variance report obtained from Table 3 EXCEL 2010 analysis

(a)					
Groups	No. of samples	Sum	Average	Variance	
511	32	89	2,78125	2,047379032	
512	32	110	3,4375	1,802419355	
513	32	111	3,46875	1,547379032	
514	32	120	3,75	1,419354839	
515	32	134	4,1875	1,125	
(b)					
Source of variation	Sum of squares	Degrees of freedom	Mean square		
Lines	80,3	31	2,590322581		
Columns	33,7125	4	8,428125		
Error	165,8875	124	1,337802419		
Total	279,9	159			
(c)					
F	Probability	Critical value for F			
1,936251978	0,005852935	1,543950052			
6,299977394	0,000119709	2,444766161			
(d)					
Product	515	514	513	512	511
Average	4,1875	3,75	3,46875	3,4375	2,78125

**Statistical analysis:** After samples' evaluation, presented in tabular form (Table 3) and descriptive categories were converted into numerical submitted to analysis of variance in EXCEL 2010 ratings (Table 2). All tasters' results were (Table 4).

**RESULTS AND DISCUSSION**

**Microbiological analysis results**

**Mesophilic total flora:** The application of different methods of smoking has led to the reduction of total mesophilic charge initially present in the pulp of fresh fish, the Fig. 1 shows that in samples smoked at 35°C, the mesophilic total flora was 4 log UFC g<sup>-1</sup> in the beginning in fresh fish so after the smoking process the value decrease to reach 3.5 and 1.9 log UFC g<sup>-1</sup> for fish smoked at 35°C and fish smoked at 35°C with distillate thyme, respectively, then this values increase for two samples smoked with and without distillate de thyme to reach a maximum at 5.5 log UFC g<sup>-1</sup>, the product was judged satisfying after 3 weeks of storage. While for hot smoking (Fig. 2), the mesophilic total flora decreased in samples from 4 and 2 log UFC g<sup>-1</sup> for smoked fish at 70°C and to 3 log UFC g<sup>-1</sup> for smoked fish at 70°C with distillate thyme, then from the second week of storage the concentration exceeds the value of "m" while remaining below the upper value "M", so the product was judged acceptable. The decrease was greater in samples treated with distillate thyme for cold smoking and one without thyme for hot smoking. During storage, there is a gradual evolution of the microbial load in function of time, however, growth does not exceed the lower limit "m" (Fig. 2) of the sample into three classes (NHL, 2007) and different manufacturing practices are satisfactory for cold smoking. The addition of thyme distillate has no effect on the hot smoked samples, but this is not the case for those cold smoked since we see a slowdown in the development of the total mesophilic charge in samples were treated with the distillate. This result leads to the following assumptions: the distillate thyme has a bacteriostatic action and inhibiting the growth of bacteria, but this action could be inhibited by higher temperatures.

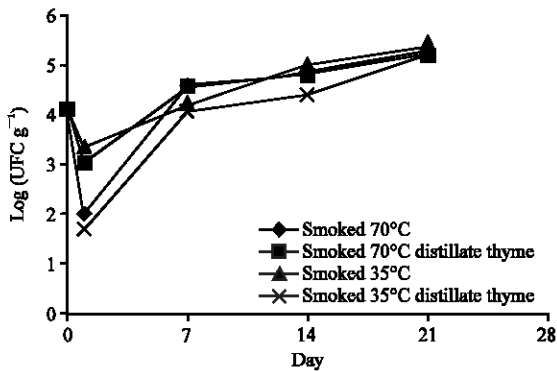


Fig. 1: Evolution of the total mesophilic charge function of time

**Lactic acid bacteria:** The results of the counts of lactic acid bacteria present in the different samples (Fig. 3) show that hot smoking causes total destruction (0 log UFC g<sup>-1</sup>) of the initial charge of lactic acid bacteria (2 and 1 log UFC g<sup>-1</sup>) which remain absent in the steps of monitoring for 3 weeks storage. However, the cold

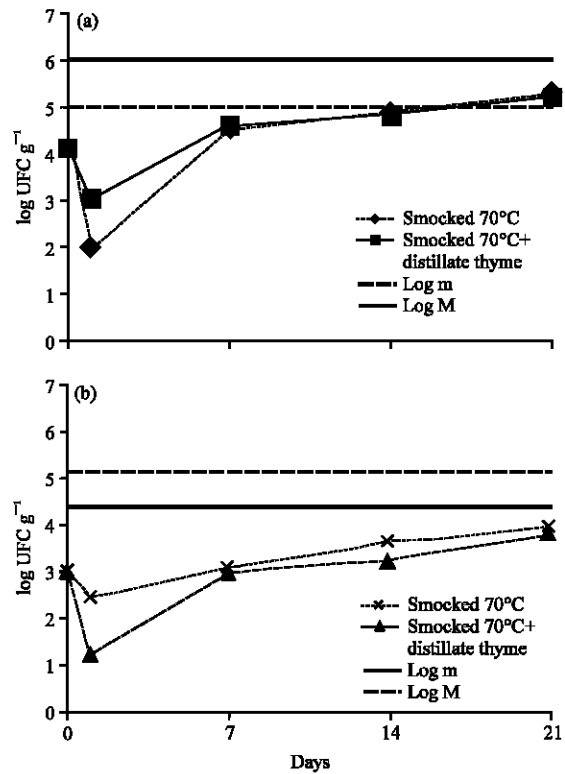


Fig. 2(a-b): (a) Evolution of the total mesophilic charge in cold smoked fish (35°C) and (b) Hot smoked fish (70°C) in relation to the limits of sampling three classes

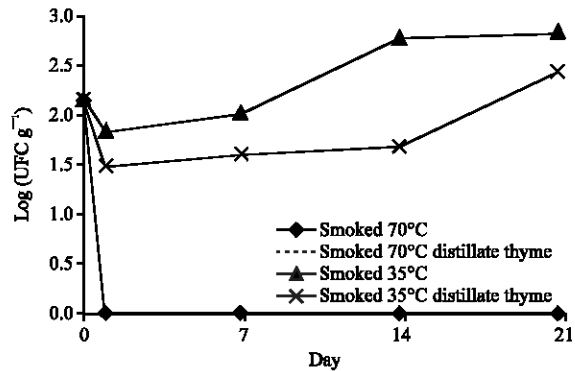


Fig. 3: Evolution of the growth of lactic acid bacteria function of time

smoking does not give the same results, but a load reduction of lactic acid bacteria with an average of 0.6 U Log from 2.1 log UFC g<sup>-1</sup> to 1.5 log UFC g<sup>-1</sup>, then we can see a development and growth from the first week of storage which translates the implementation up the weathering process. The effect of the addition of thyme distillate yields results similar to those obtained for total mesophilic flora. Microbial growth slowed in samples that have been processed by the distillate.

**Total coliforms:** The smoking process has allowed the reduction of total coliforms from 2.5-0.7 log UFC g<sup>-1</sup> for the fish smoked at 70°C and to 1 log UFC g<sup>-1</sup> for the fish smoked at 35°C (Fig. 4). The cold smoked products obtained are satisfactory, it was not until the second week that the content of total coliform exceeds the lower 'm' of sampling three classes (NHL, 2007) (Fig. 5). For hot smoked products the value of "m" specific to this type of food is not exceeded until the third week (Fig. 5). In the two types of smoking process the distillate thyme show no effect at all during the different procedures.

**Staphylococci:** The population of *Staphylococci* initially present in the pulp raw fish was 2.1 log UFC g<sup>-1</sup> then reduced with an average of 1.4 U Log for hot smoking and 0.3 U Log for cold smoking to reach 0.7 and 1.8 log UFC g<sup>-1</sup>, respectively (Fig. 6). The increase in temperature allowed during hot smoking better neutralization of *Staphylococci*. Thus, during storage the concentration of the latter, remains below the lower limit "m" (2 log UFC g<sup>-1</sup>) sampling three classes (NHL, 2007) and the product is then said satisfactory (Fig. 7). This is not the case for samples cold smoked fish; including *Staphylococci* concentration exceeds the "m" in an increasing manner throughout storage (Fig. 7). The product is then called "acceptable."

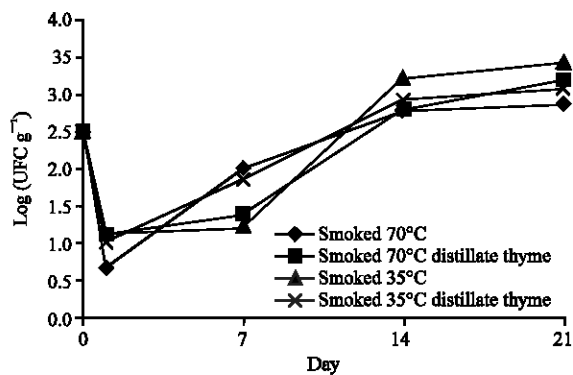


Fig. 4: Evolution of Total coliform growth function of time

Microbiological results showed that smoking technique allowed reduction of microbial charge initially contained in the pulp raw fish and allowed to obtain a satisfactory product especially for the case of hot

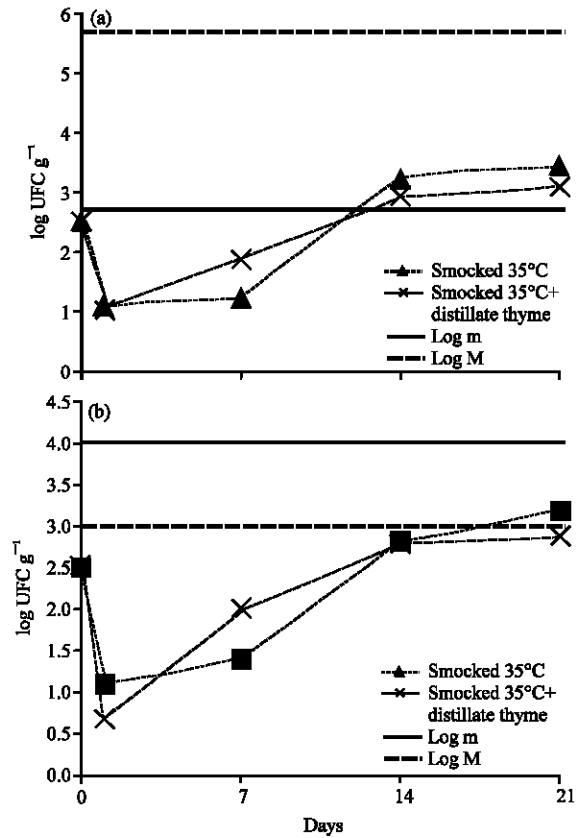


Fig. 5(a-b): (a) Evolution of the concentration of total coliforms in the cold smoked fish and (b) Hot smoked fish in relation to the limits of sampling three classes

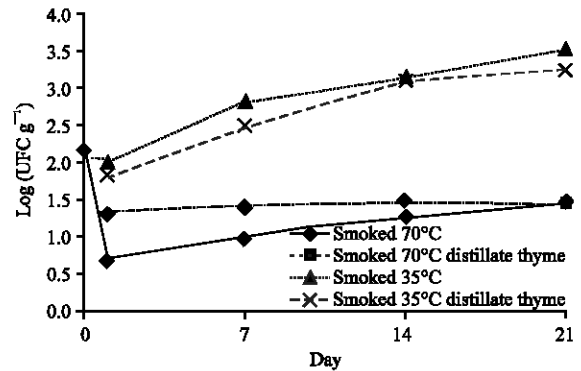


Fig. 6: Evolution of the *Staphylococcus* concentration function of time

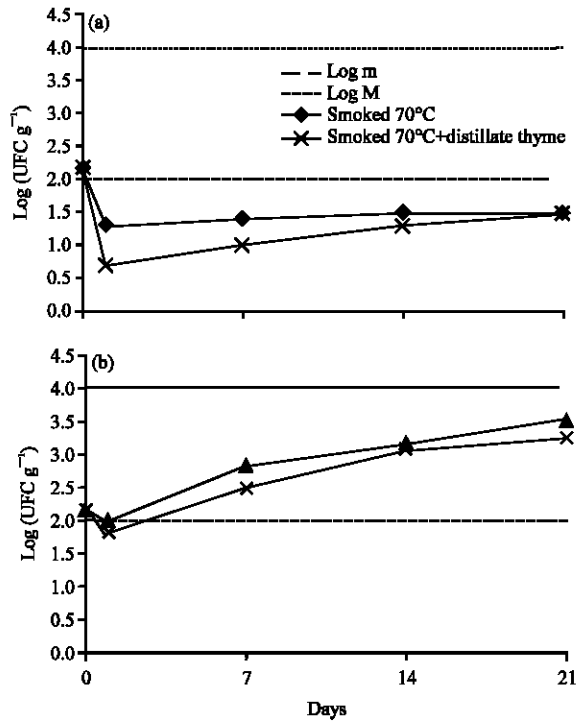


Fig. 7(a-b): (a) Evolution of the concentration of *Staphylococci* in the hot smoked fish and (b) Cold smoked fish in relation to the limits

smoking by the temperature's raising. Until the third week, the product has not yet attained its use-by date, in fact the federation of enterprises businesses and the distribution set thresholds for concentrations of total mesophilic charge to 106 CFU g<sup>-1</sup> and report total mesophilic load/lactic bacteria 102 CFU g<sup>-1</sup>. However, these results should be reinforced by other analyzes related to the presence of pathogens such as *Salmonella* and *Escherichia coli* which would ensure good hygienic quality of the resulting product.

Moreover, the quality bacteriostatic and/or bactericidal of distillate thyme has been shown on lactic acid bacteria and *Staphylococci* but not for total coliforms, this result is consistent with that reported by Burt (2004) which states that Gram-are less sensitive to the action of essential oils than gram+. The addition of the distillate thyme to the brine would lengthen the shelf life of smoked fish. In addition, this natural product is obtained by steam distillation of the plant thyme. This technique is inexpensive and easy to implement.

The use of this kind of natural food additives could replace synthetic ones that are still subject to doubts

about the health risks they may pose and especially with the daily doses restriction imposed by legislation, a natural product would be tolerated of sampling three classes.

Tests that will vary the volume of distillate thyme added to the brine would carefully study the impact of bacterial action distillate but also to explore the possibility of exploiting its aromatic qualities.

**Sensory analysis results:** The results of both tests for sensory analysis were converted to digital format to be processed.

**Triangle test:** The significance of the total number of correct answers is determined as follows, X (number of tasters who choose different sample) and n = 26 (total number of tasters participating in the test) = 33, p = 0.001 (Table 1). This test showed that the difference between the perch steamed and smoked that is significant. Thus, we can conclude that our product has distinctive qualities of fish cooked in a conventional manner.

**Hedonic test:** Processing results is done using analysis of variance (ANOVA) (Table 1, 2).

The coefficient F = 6.29 is higher than the critical value of F which is 2.44, the difference between the five modes of preparation of pikeperch is significant. However, this test cannot determine whether smoking has improved organoleptic characteristics of perch and determine the best method of preparation of fish. For this reason we taking a second test said Duncan's multiple comparison. To calculate the Duncan test, average preparation methods obtained in Table 2 were placed in descending order in Table 3 from 2.78-4.18.

To compare the 5 medium of this example, we calculated the value of goodwill for a range of 5, 4, 3 and 2 medium with the following equation:

$$\text{Deviation} = \sqrt{Q \text{ CM (E)} \div t} = Q \sqrt{1.337802419 \div 32} = 0.042 Q$$

Where:

CM = Mean square

t = Number of individual responses used to calculate each average

We obtained Q values from Table 4 for the same level of significance that was used in the analysis of variance p<0.05. The degree of freedom obtained from Table 2 is equal to 124. The Table 4 provides for a degree of freedom of 124:



- Value of Q = 3.089 for 5 medium
- Value of Q = 3.017 4 medium
- Value of Q = 2.918 3 medium
- Value of Q = 2.772 for 2 medium

We could then calculate the value of goodwill:

- Gap = Q (0.042)
- Mean difference for 5 = 3.089 (0.042) = 0.129
- Gap to 4 medium = 3.017 (0.042) = 0.126
- Gap to 3 medium = 2.918 (0.042) = 0.122
- Away for 2 medium = 2.772 (0.042) = 0.116

We applied the value of the standard five medium to medium with the largest differences between them, 4.1875 and 2.78125, since these values cover the gap 5 medium. The difference, 1.4, was above 0.129. The difference between these two means was therefore significant.

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The following comparison focused on average 4.1875 3.4375 and by using the value of the mean difference of 4 (0.126). The difference between these averages (0.75) is greater than 0.126, the difference was also significant.

Comparison of three averages was on the average 4.1875 and 3.46875:

$$4.1875-3.46875 = 0.71 > 0.122$$

Comparison of two means was on the average 4.1875 and 3.75:

$$4.1875-3.75 = 0.43 > 0.116$$

The next highest average was then compared with the lowest average and the difference was compared to the value of the gap for 4 medium:

$$3.75-2.78125 = 0.96 > 0.126$$

Was performed as described below until we made comparisons among all means:

$$3.75-3.4375 = 0.31 > 0.122$$

$$3.75-3.46875 = 0.128 > 0.116$$

$$3.46875-2.78125 = 0.687 > 0.122$$

$$3.46875-3.4375 = 0.0312 < 0.116$$

$$3.4375-2.78125 = 0.656 > 0.116$$

Calculations in the same way have been made to the texture, smell and color.

Calculations relative to various parameters of the sensory analysis showed that smoked were significantly more appreciated by the tasters steamed fish (Table 4).

For taste, Sander hot smoked without distillate was more appreciated than which cooked in the same way but without the distillate. For both cold smoked products the difference in the degree of appreciation was not significant. But the hot smoked fish was more appreciated than cold smoked. For the second parameter namely the texture, tasters liked the samples cold smoked appearance for moist and tender those hot smoked and the addition of distillate had no significant effects.

The smell of the products that we have added the thyme distillate was more popular than those who do not. But it should be noted that the cold-smoked fish with the distillate is better than hot smoked with distillate. Thyme flavoring properties play a role in modifying the odor of the final product and contribute to improving the assessment of fish. However, the color of hot smoked products which is more pronounced was most appreciated by the tasters compared to cold smoked products so the color is considered bland.

## CONCLUSION

This result shows that the smoking process can help the improve of organoleptic quality of this freshwater fish. Furthermore, the addition of flavoring additives such as distillate thyme contributes to the development of superior taste fish especially in the case of cold smoking.

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