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ORIGINAL RESEARCH PAPER

NUTRITIONAL ASSESSMENT OF SMOKED MEAT PRODUCTION OBTAINED FROM FARMED AND WILD EUROPEAN CATFISH (*SILURUS GLANIS*)

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Abstract: *Silurus glanis* is a freshwater fish native to Europe and Western Asia. Its meat is highly appreciated by consumers. By smoking it, food products with particularly good sensory qualities are obtained. To carry out this study, comparisons were made between fish originated from aquaculture (A group - Aquaculture group) and from the natural environment (Prut river, Romania) (C group - Capture group). Fillets of the studied fish were smoked and stored at a temperature of +2 °C ÷ +5 °C. The samples were analyzed for sensory and physicochemical traits at 5, 15, 25, 30, 35 and 40 days of storage. The smoked catfish presented particularly good sensory qualities, and the origin of the meat did not significantly influence it. The products were considered optimal for consumption up to 30 days of storage. The chemical analysis revealed moisture loss at the end of the analyzed period by 4.78 % (A) and 4.70 % (C), whilst the dry matter compounds concentrated at a reversed pace, accordingly. The high values for PUFA (34.97 % in A and 30.68 % in C), make smoked European catfish meat an important source of "good fats", a fact also highlighted by the values of Polyunsaturated Index (33.86 in A and 45.23 in C), Atherogenic Index (0.41 in A and 0.35 in C) and Thrombogenic Index (0.22 in A and 0.27 in C).

Keywords: *European catfish; fatty acids, freshness status, sanogenic indices, smoked, wild and cultivated*

INTRODUCTION

The European catfish (*Silurus glanis*) is a member of the *Siluridae* family and lives in lakes and rivers in Europe as well as in Western Asia. The European catfish is a freshwater fish but is also occasionally found in the salt waters of the Black Sea and Baltic Sea. It is a very aggressive carnivore and therefore can take advantage of some food sources that other species cannot use [1 – 3].

This species is not highly demanding on the physical-chemical qualities of the water, compared to other fish species, it easily tolerates cloudy waters with a low content of dissolved oxygen [2].

Although it is an autochthonous species for Romania, its economic importance is limited to polyculture rearing with cyprinid species, where it ensures a good state of health of the biocenosis of which it is a part. As a predatory, ichthyophage species par excellence, the catfish brooding density in a fish farm cannot be very high, in order not to harm the main aquaculture species. This aspect greatly limits the availability of the species on the market, a fact that indirectly influences consumer preferences in choosing European catfish for consumption [2, 4].

Catfish is a boneless, tasty, high-protein fish species that is traded in European and West Asian countries. Catfish is generally bought fresh but is a highly perishable food product and therefore requires immediate cooking or preservation [5]. Thus, catfish meat is marketed in many countries after being processed, which increases its shelf life and develops different tastes and flavors [3, 4].

Smoking is one of the oldest methods of preserving fish meat. The products are particularly appreciated by consumers, especially due to the specific color and taste they acquire and develop throughout storage [6 – 9]. The exposure of the fish to the action of smoke leads to the extension of its acceptability term through the lens of the combined effect of two categories of factors: dehydration of the product, which occurs due to the temperature in the smoking chamber, as well as the antimicrobial effect exerted by the smoke. Thus, the bacterial microflora is greatly diminished, and the remaining one does not find a suitable environment for development, considering the relatively low humidity of the finished product [10 – 14]. However, it must be admitted that, currently, smoking represents rather a way of superior capitalization of products, by providing specific taste and flavors, than a mean of preservation per se [15, 16], smoking products being catalogued, not infrequently, as delicacies.

Smoking represents the technological operation that takes place in the exposure of a food to the action of aerosol smoke, with the aim of ensuring preservation, aromatization and the formation of a specific color. The main element of this preservation method is smoke. From a physical point of view, smoke is a polydisperse aerosol consisting of a dispersion phase and a dispersible phase [15, 16]. From a chemical point of view, about 1000 substances have been identified in the composition of the smoke. They are of great importance because they are directly involved in the coloring and flavoring of smoked products, while providing a bacteriostatic role. Wood smoke contains a combination of antioxidant and antimicrobial chemicals but also some harmful compounds such as polycyclic aromatic hydrocarbons (PAHs). Regulation (EC) no. 1881/2006, supplemented by Commission Regulation 835/2011, specifies the maximum levels (ML) of PAHs in various food products. In 2008, the European Food Safety Authority (EFSA) concluded that Benzo(a)Pyrene (BaP) alone is not an adequate indicator for the

occurrence and toxicity of PAHs in food. Therefore, additional MLs for the sum of 4 PAHs (BaP, benz(a)anthracene (BaA), chrysene (CHR) and benzo(b)fluoranthene (BbFA)) were set in Commission Regulation (EU) no. 835/2011 [17 – 20].
Most of the time, hard wood (beech, oak, ash, maple) is used in smoking fish, providing more aromatic compounds, and some researchers have highlighted the sensory improvement of European catfish meat subjected to smoking with sawdust from hardwood [21 – 23].
The aim of this study was to analyze the sensory and physical-chemical changes that occur in wild and aquacultured European catfish meat, preserved by traditional smoking with hardwood and stored at 2 - 5 °C, 80 - 85 % relative humidity, throughout 40 days, as well as to assess the fatty acid profile and to calculate sanogenic indices.

MATERIALS AND METHODS

Biological material origin, sampling and preparation

In this study, 300 specimens of the species *Silurus glanis*, of various sizes and weights, were analyzed, coming from two different rearing environments in Romania, represented by a fish farm (47°19'23.4"N 27°31'08.8"E) located in Iași County (aquaculture biological material - group A), as well as by the Prut River on the Bivolari-Gorban sector (47°32'01"N 27°26'29"E - 46°53'43"N 28°05'07"E) (for catch wild biological material - C group). In order to assess the dynamics of European catfish meat quality, preserved by smoking, 36 samples were selected from both originating systems. Later, the biological material was divided into numerically equal groups, for each storage interval within the preservation method, the storage period was divided into six time intervals for the evaluation of the meat quality, each interval comprising six specimens. The body mass of these individuals was between 1300 g and 2200 g. A decisive role in the selection of specimens with this body mass was played by other research indicating that fish with this body mass are optimal from the point of view of the relationship between the rate of growth and the economic aspects related to the production process [24, 25], as well as the average sales weight per copy concretely found in specialty stores in Romania [25]. Fillets (muscle portions taken parallel to the backbone) were sampled and smoked. The smoking involved the use of a traditional method, which initially involved salting (6 - 8 %), washing, draining, hanging and slinging at a temperature lower than 30 °C. Smoking was done at a temperature of 25 - 40 °C for 4 days, followed by wiping and packing in plastic pans with lids, dimensions 116/116/60 cm. This was followed by storage at a temperature of +2 °C ÷ +5 °C and a relative air humidity of 80 - 85 %, throughout 40 days. The samples were analyzed at: 5, 15, 25, 30, 35 and 40 days after storage.
Beech wood (*Fagus silvatica*) was used for smoking, the traditional method pre-submitted the setting up of a smokehouse made of a wooden barrel, the smoke being directed through a trench dug approximately 1 m long at the end of which a fire was ignited, about 2 hours a day for 4 days.

Sensory analysis of cold smoked fillets

The hedonic scale with a simple score, between 9 and 1, where 9 indicates the maximum desired quality of the analyzed sensory attribute, according to the product standard, was used in sensory analysis. Obtaining the minimum score, i.e. 1, indicates absolutely unacceptable parameters, for each type of sensory attribute [10, 17, 21, 26 – 28]. The sensory evaluation was carried out by a team made up of thirty-six members, familiar with fish products. The analysis was done in a specific room, respecting the technical conditions necessary to perform this operation. Each of the members of the analysis committee received a coded sample, related to each storage interval. The samples had a rectangular shape and an average weight of 50 g. Certain sensory traits were assessed: general appearance, color, smell, consistency and taste. The score calculation is based on the relation (1):

$$ACS = AGS \times RC \times TF \quad (1)$$

whereas:
ACS = average corrected score;
AGS = average given score – the arithmetic mean of the results issued from tasters' individual scoring for a certain sensory trait;
RC = relevance coefficient; it indicates to what extent each sensory trait participates in defining the sensorial quality of the product (general aspect and color, Rc = 0.1; consistency, Rc = 0.2; smell and taste, Rc = 0.3).
TF = transformation factor, to translate the 9 points scale into a 20 points scale, to assess the overall product quality (Tf = 2.22, issued from the 20/9 division).
Based on the results obtained from the sensory evaluation, the Total Average Score (TAS) was calculated, by summing the values of the ACS from all the sensory traits [29]. The resulting TAS values are interpreted based on the scoring scale for qualitative assessment (Table 1).

Table 1. *Score scale for qualitative evaluation on a scale of 0 - 20 points* [29]

Total average score	Provided grade
18.1 – 20	Very well
15.1 – 18	Well
11.1 – 15	Satisfactory
7.1 – 11	Not satisfactory
0 – 7	Inappropriate (rotten)

Physical and chemical analysis of cold smoked fillets

To assess the *p*H value of the meat, an InoLab 740 *p*H-oximeter (Xylem Analytics Germany Sales GmbH & Co. KG, WTW, Weilheim, Germany) was used, equipped with the function of automatic adjustment of the pH value in correlation with the temperature of the solution to be measured, kit two-point calibration and distilled water according to SR ISO 2917:2007 [30]. Six analytical repetitions were carried per group/moment of analysis.
Free ammonia was assessed via the Eber and Nessler method. In Eber protocol, ammonia from the sample to be analyzed, in contact with hydrochloric acid vapors, forms ammonium chloride which has the appearance of a smoky-grey cloud, similar to cigarette

smoke [31 – 33]. In the Nessler method, free ammonia from the aqueous extract of the sample to be analyzed forms a yellow-orange complex with dipotassium tetraiodo-mercury (Nessler's reagent) (ammonium mercury oxyiodide) [31 – 33]. For the determination of the free state hydrogen sulphide (H_2S) in the free state. Hydrogen sulphide from the sample interacts with lead acetate, and forms lead sulphide (composed of blackish-brown color) [31 – 33]. Six analytical repetitions were carried per group/moment of analysis.

The easily hydrolysable nitrogen (NH_3) (ammonia nitrogen) was determined by setting it free with a weak base, entrainment with pore vapor, and trapping in an acid solution [31 – 33]. Six analytical repetitions were carried per group/moment of analysis.

Meat proximate composition was gravimetrically assessed. First, the water/dry matter content was measured (oven drying at +105 °C) [SR ISO 1442/1997]. Then, total minerals content was assessed through ashing at +550 °C (AOAC 981:10/1990) [23 – 26]. Total nitrogen content was transformed in crude protein content, consecutive to Kjeldahl analytical method (AOAC 981:10/1990) [23 – 26]. Total lipids content was measured through the Soxhlet method, using petroleum ether as extraction solvent (AOAC 981:10/1990) [34 – 39]. Each chemical assessment was carried out in 6 repetitions.

Fatty acids profile analysis and lipids nutritional quality

Fatty Acid Methyl Esters (FAME) from fillet samples were extracted and measured by gas chromatography and mass spectrometry detection. As devices, a Perkin Elmer Chromatographic system coupled with mass spectrometer detector (GC-MS) (Clarus 680 gas chromatograph and Clarus SQ8T quadrupole mass spectrometer (manufacturer, Perkin Elmer Inc., USA, for both devices) were used. The chromatographic column was an Elite-Wax with stationary polar phase Polyethylene glycol (PEG) (30 m length, with film thickness of 1.0 µm and 0.25 mm inner diameter). The injecting temperature was set at 220 °C, for a volume of sample of 1.0 µL. Helium flow rate was set at 1.5 $mL \cdot minute^{-1}$, for a splitting ratio of 40:1. Temperature gradient was set at 100 °C, throughout 2 minutes, standstill, and stationary 1 minute at 250 °C. Mass Spectrometer was characterized by the following operational values: transfer line and source operated at 150 °C temperature; 0-1.5 minutes' delay of the solvent < multiplier 1500 [39 – 43].

Sample fatty acids quantification issued by comparison of FAME retention time against a FAME Supelco 37 Mix) (homologated mix). Each fatty acid was expressed as g FAME/100 g of total identified FAME [39-42]. To outline the lipid profile, the fatty acids were grouped accordingly: Saturated Fatty Acids – SFA, as sum (Σ SFA = C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C18:0 + C20:0 + C22:0); Mono Unsaturated Fatty Acids – MUFA, as sum (Σ MUFA = C16:1 + C18:1 cis-9 + C20:1 n-9 + C22:1 n-9); Poly Unsaturated Fatty Acids, as sum (Σ PUFA = C18:2 n-6 + C20:2 n-6 + C18:3 n-3 + C20:3 n-3 + C20:5 n-3 + C18:3 n-6), total Unsaturated Fatty Acids, as sum of MUFA and PUFA [40 – 43].

Omega - 3 and Omega - 6 PUFA series was expressed as ratio (n-3 / n-6).

Polyunsaturated Index (PI) was calculated by using the Timmons relation (2) [29, 32, 33]:

$$PI = C18:2\ n\text{-}6 + (C18:3\ n\text{-}3 \times 2) \quad (2)$$

The Atherogenic Index (AI) and Thrombogenic Index (TI) of fats were calculated, using the data issued from FAME GC analysis of the European catfish meat, applying the relations proposed by Ulbricht and Southgate [39, 44 – 46]:

$$AI = (C12:0+C16:0+4\times C14:0)/[\Sigma MUFA+\Sigma(n-6)+\Sigma(n-3)] \quad (3)$$

$$TI = (C14:0+C16:0+C18:0)/[0.5\times\Sigma MUFA+0.5\times\Sigma(n-6)+3\times\Sigma(n-3)+\Sigma(n-3)/\Sigma(n-6)] \quad (4)$$

According to Fernandez *et al*. [35], the relation (5) was applied to compute the ratio between hypocholesterolemic (h) FA and hypercholesterolemic (H) FA.

$$h / H = (C18:1 + PUFA)/(C12:0 + C14:0 + C16:0) \quad (5)$$

Statistical analysis

The experimental data were treated using the MS Excel Data analysis package, to obtain the statistical descriptors (mean, variance, standard error of mean, coefficient of variation. The differences between groups were tested for significance using the ANOVA single factor method incorporated in MS Excel Data analysis package, that runs in fact a Fisher regular test for one to one comparisons.

RESULTS

Sensory evaluation and changes throughout the storage period

The results of the sensory analysis for the samples from aquaculture (A) and the wild environment (C) are found in Table 2. No significant differences ($P > 0.05$) occurred between groups, in all assessment moments.

Table 2. *Sensorial evaluation of smoked European catfish fillets (results issued from 36 tasters/trait)*

Group	Scores	General appearance	Color	Smell	Consistency	Taste
1st storage period (P > 0.05)						
A	AGS (Mean±SEM)	8.67±0.52	8.83±0.41	9.00±0.00	8.67±0.52	9.00±0.00
	ACS	1.92	1.96	5.99	3.85	5.99
	TAS	19.7				
C	AGS (Mean±SEM)	8.83±0.41	8.67±0.52	9.00±0.00	8.50±0.55	8.50±0.55
	ACS	1.96	1.92	5.99	3.77	5.66
	TAS	19.3				
2nd storage period (P > 0.05)						
A	AGS (Mean±SEM)	7.50±0.55	7.83±0.41	7.67±0.52	8.33±0.52	7.83±0.41
	ACS	1.67	1.74	5.11	3.70	5.22
	TAS	17.4				
C	AGS (Mean±SEM)	7.33±0.52	7.67±0.52	7.67±0.52	8.17±0.41	7.67±0.52
	ACS	1.63	1.70	5.11	3.63	5.11
	TAS	17.1				

3rd storage period (P > 0.05)						
A	AGS (Mean±SEM)	6.67±0.52	6.33±0.52	6.83±0.41	7.33±0.52	6.33±0.52
	ACS	1.48	1.41	4.55	3.26	4.22
	TAS	14.9				
C	AGS (Mean±SEM)	6.83±0.41	6.50±0.55	6.83±0.41	7.17±0.41	6.83±0.41
	ACS	1.52	1.44	4.55	3.18	4.55
	TAS	15.2				
4th storage period (P > 0.05)						
A	AGS (Mean±SEM)	5.83±0.41	5.67±0.52	5.83±0.41	6.83±0.41	5.50±0.55
	ACS	1.30	1.26	3.89	3.03	3.66
	TAS	13.1				
C	AGS (Mean±SEM)	5.50±0.55	5.50±0.55	6.00±0.00	6.67±0.52	5.83±0.41
	ACS	1.22	1.22	4.00	2.96	3.89
	TAS	13.2				
5th storage period (P > 0.05)						
A	AGS (Mean±SEM)	4.83±0.41	4.67±0.52	4.83±0.41	6.33±0.52	4.83±0.41
	ACS	1.07	1.04	3.22	2.81	3.22
	TAS	11.3				
C	AGS (Mean±SEM)	4.83±0.41	4.50±0.55	5.33±0.52	5.83±0.41	4.83±0.41
	ACS	1.07	1.00	3.55	2.59	3.22
	TAS	11.4				
6th storage period (P > 0.05)						
A	AGS (Mean±SEM)	3.33±0.52	3.17±0.41	2.83±0.41	2.33±0.52	2.67±0.52
	ACS	0.74	0.70	1.89	1.04	1.78
	TAS	6.1				
C	AGS (Mean±SEM)	3.67±0.52	3.83±0.41	2.50±0.55	2.50±0.55	2.83±0.41
	ACS	0.81	0.85	1.67	1.11	1.89
	TAS	6.3				

According to the data presented in Table 2, all 5 sensory traits of aquacultured European catfish fillets were better appreciated by tasters during the first third of the storage interval, while the fillets issued from the wild environment became better appreciated since the 25th day of storage.

Freshness status traits

The *p*H values analyzed for smoked European catfish fillets are presented in Table 3. Following the six sessions of *p*H value evaluation, it was found that smoked European catfish fillets were very stable throughout the 40 days of storage. The samples from aquaculture obtained average *p*H values between 6.31 ± 0.12 *p*HU (*p*H units) and 6.38 ± 0.07 *p*HU, while those from the wild environment varied between 6.32 ± 0.09 *p*HU and 6.39 ± 0.07 *p*HU.

Table 3. *pH dynamics in smoked European catfish meat*

Storage days	Group	Statistical descriptors				*P value*
		Mean±SEM (*p*H units)	CV [%]	Min	Max	
5	A	6.35±0.09	1.36	6.28	6.47	0.1294
	C	6.32±0.09	1.35	6.22	6.45	
15	A	6.37±0.07	1.09	6.25	6.45	0.0892
	C	6.32±0.11	1.80	6.15	6.45	
25	A	6.31±0.12	1.97	6.15	6.45	0.5830
	C	6.29±0.06	0.93	6.22	6.39	
30	A	6.35±0.17	2.69	6.05	6.49	0.6991
	C	6.36±0.09	1.41	6.22	6.44	
35	A	6.35±0.07	1.18	6.22	6.44	0.6286
	C	6.36±0.08	1.25	6.25	6.47	
40	A	6.38±0.07	1.17	6.29	6.45	0.7835
	C	6.39±0.07	1.09	6.30	6.48	

The dynamics of readily hydrolysable nitrogen in European catfish meat stored as a smoked product can be found in Table 4.

Table 4. *Mean values of NH_3 in smoked European catfish*

Storage days	Group	Statistical descriptors				*P* value
		Mean±SEM	CV [%]	Min	Max	
5	A	35.34±1.60	4.53	32.15	36.55	0.0415
	C	32.42±2.45	7.57	29.87	36.54	
15	A	34.30±1.54	4.50	32.30	36.74	0.8762
	C	34.43±2.37	6.89	30.15	36.70	
25	A	35.62±1.42	3.98	33.90	37.16	0.7461
	C	34.95±1.43	4.11	33.16	36.72	
30	A	37.41±1.54	4.11	34.68	39.16	0.2359
	C	36.19±2.21	6.10	32.15	38.69	
35	A	39.36±1.78	4.52	36.29	41.23	0.1097
	C	37.19±2.51	6.76	33.55	40.12	
40	A	40.80±3.03	6.74	40.80	48.15	0.0083
	C	40.47±1.50	3.70	38.26	42.64	

Except for the second storage interval (A), the ammonia values showed an upward trend, as samples were stored longer (Table 4). The average values varied between 34.30 ± 1.54 mg/100g and 40.80 ± 3.03 mg/100g, in aquacultured fish, respectively between 32.42 ± 2.45 mg/100g and 40.47 ± 1.50 mg/100g, in the wild caught fish. The minimum level of NH_3 was in C group (29.87 mg/100g), and the maximum one in A group (48.15 mg/100g). Significant differences were identified between the two batches in the first storage interval ($P < 0.05$), and in the last storage interval ($P < 0.01$) (Table 4).

The qualitative test results of Eber, Nessler and H_2S identification methods are displayed in Table 5 and could be observed that from 35th days of storage, the smoked filled becomes unfit for human consumption.

Table 5. *Adjuvant chemical reactions for freshness state assessment in smoked European catfish*

Storage days	Group	Eber reaction	Nessler reaction	H_2S
5	A	-	-	-
	C	-	-	-
15	A	-	-	-
	C	-	-	-
25	A	-	-	-
	C	-	-	-
30	A	-	-	-
	C	-	-	-
35	A	-	±	±
	C	±	±	±
40	A	±	+	+
	C	+	±	+

"-" fresh meat. "±" quite fresh meat. "+" low alteration degree. "++" moderate alteration degree. "+++" high alteration degree

Proximate composition

The results of the comparative evaluation and the dynamics of water and dry matter in smoked European catfish meat during different storage periods can be found in Table 6.

Table 6. *Comparative evaluation of water and dry matter from European catfish smoked meat in different storage periods*

Storage days	Group	Sample mass vs. storage onset [%]		Water [%]		Dry matter [%]	
		Mean±SEM	CV [%]	Mean±SEM	CV [%]	Mean±SEM	CV [%]
5	A	100±0.00	0.00	60.78±1.94	3.28	39.22±1.54	3.92
	C	100±0.00	0.00	59.71±1.60	2.67	40.29±1.47	3.65
15	A	98.85±1.74	1.76	60.08±2.36	3.93	39.92±2.30	5.96
	C	99.08±3.07	3.09	59.16±2.40	4.06	40.84±1.26	3.13
25	A	97.99±1.99	2.03	59.56±1.39	2.33	40.44±0.92	2.39
	C	97.91±3.45	3.50	58.46±2.39	4.09	41.54±1.53	3.82
30	A	96.81±1.87	1.92	58.84±1.59	2.71	41.16±1.01	2.63
	C	97.54±1.38	1.41	58.24±1.49	2.55	41.76±1.61	4.03
35	A	96.35±2.92	3.03	58.56±2.32	3.96	41.44±1.18	3.13
	C	96.75±3.31	3.40	57.77±1.98	3.43	42.23±1.95	4.96
40	A	95.21±2.46	2.59	57.87±1.86	3.22	42.13±0.90	2.42
	C	95.31±2.74	2.88	56.91±2.02	3.55	43.09±1.02	2.68

$P > 0.05$ in all comparisons between groups.

During 40-day storage at 2 - 5 °C, product mass losses of 4.94 percentage points (A) and 4.89 percentage points (C) occurred (Table 6).

In batch A, the dry matter concentrate 39.22 % to 42.13 %, due to moisture loss from 60.78 % to 57.87 % throughout 40 days of storage. In batch C, during storage, the

moisture content of the samples decreased from 59.71 % to 56.91 %, which means a reduction of 4.69 % and the dry matter concentrated, subsequently, from 40.29 % till 43.09 %. Throughout storage, the initial mass of the samples diminished till 95.21 % - 95.31 % due to dehydration (Table 6).
The proportions of the main micro-constituents that make up the dry matter of the cold-smoked catfish meat and their dynamics are shown in Table 7.

Table 7. *Comparative evaluation of dry matter components from European catfish smoked meat in different storage periods*

Storage period (days)	Group	Crude ash [%]			Proteins [%]			Lipids [%]		
		Mean ± SEM		V [%]	Mean ± SEM		V [%]	Mean ± SEM		V [%]
5	A	2.17	0.03	2.93	27.23	0.58	5.21	9.82	0.08	1.92
	C	2.23	0.03	3.06	28.30	0.56	4.81	9.76	0.17	4.35
15	A	2.21	0.03	3.48	27.72	0.96	8.5	10.00	0.27	6.72
	C	2.26	0.03	3.09	28.69	0.63	5.42	9.89	0.15	3.63
25	A	2.24	0.03	3.09	28.08	0.45	3.91	10.13	0.17	4.22
	C	2.3	0.04	3.82	29.18	0.65	5.44	10.06	0.18	4.33
30	A	2.28	0.03	3.5	28.58	0.52	4.49	10.31	0.23	5.48
	C	2.31	0.06	6.4	29.33	0.64	5.31	10.12	0.22	5.42
35	A	2.29	0.04	4.38	28.77	0.36	3.04	10.38	0.17	4.1
	C	2.34	0.05	5.09	29.66	0.75	6.23	10.23	0.12	2.91
40	A	2.33	0.09	9.33	29.25	0.27	2.24	10.55	0.17	4.05
	C	2.38	0.06	6.36	30.27	0.48	3.86	10.44	0.22	5.05

$P > 0.05$ in all comparisons.

Throughout storage, the finished product became more concentrated in minerals, due to moisture loss, by 0.16 percentage points in the case of batch A and by only 0.15 percentage points in batch C.
Proteins increased from 27.23% after 5 days of storage till 29.25% in 40 days of storage, in group A and from 28.30% till 30.27% in group C.
The same concentrating trend was noticed in lipids proportion in dry matter and they varied accordingly, from 9.82% till 10.55% in group A, and from 9.76 till 10.44% in group C, respectively.

Fatty acids profile and sanogenic indices

Table 8 shows the profile in fatty acids and sanogenic indices of smoked European catfish meat after 5 days of storage. The meat comes from two different, namely A - fish farm and C - wild environment (Prut river).
According to Table 8, from the total of fatty acids highlighted in the samples from the two groups (A and C), the highest share was represented by MUFA (40.15 % for A and 43.2 % for C), followed by PUFA (34 .97 % in A and 30.68 % in C) and SFA (24.88 % in A and 26.12 % in C), which indicates the presence of good quality fat in the aquaculture fish.
According to Table 8, from the total of fatty acids highlighted in the samples from the two groups (A and C), the highest share was represented by MUFA (40.15 % for A and 43.2 % for C), followed by PUFA (34.97 % in A and 30.68 % in C) and SFA (24.88 %

in A and 26.12 % in C), which indicates the presence of good quality fat in the aquaculture fish.

Table 8. *Fatty acids profile (g/100 g total FAME) and sanogenic indices of smoked European catfish*

Fatty acid	A	C	*P value*
C 14:0	8.94 ± 2.37	5.61 ± 2.15	5.34×10^{-9}
C 14:1	ND	1.15 ± 0.81	-
C 16:0	33.53 ± 5.02	55.07 ± 8.63	3.06×10^{-9}
C 16:1	10.63 ± 1.89	30.48 ± 4.24	7.46×10^{-12}
C 18:0	8.49 ± 2.18	13.40 ± 2.49	6.43×10^{-9}
C 18:1 n-9	58.04 ± 8.72	90.23 ± 12.27	8.71×10^{-9}
C 18:2 n-6	23.97 ± 4.83	17.41 ± 4.08	1.61×10^{-7}
C 18:3 n-3	4.95 ± 1.98	13.91 ± 2.79	8.54×10^{-12}
C 20:0	0.52 ± 0.06	0.67 ± 0.08	1.31×10^{-6}
C 20:1 n-9	16.69 ± 4.52	6.60 ± 1.74	1.82×10^{-11}
C 20:2 n-6	1.28 ± 0.24	1.76 ± 0.27	1.67×10^{-7}
C 20:4 n-6	0.74 ± 0.09	7.69 ± 2.05	1.69×10^{-13}
C 20:3 n-3	16.00 ± 2.63	0.99 ± 0.07	1.14×10^{-13}
C 20:5 n-3	10.37 ± 4.01	11.22 ± 1.10	0.0092
C 22:0	1.43 ± 0.31	2.92 ± 0.25	1.47×10^{-10}
C 22:5 n-6	0.50 ± 0.04	ND	-
C 22:5 n-3	6.06 ± 2.04	6.96 ± 1.93	0.0002
C 22:6 n-3	20.83 ± 5.39	31.28 ± 4.98	1.83×10^{-8}
Σ SFA	52.90	77.67	
Σ MUFA	85.36	128.47	
Σ PUFA	74.33	91.23	
n-3	58.21	64.36	
n-6	26.49	26.86	
n3/n6	2.20	2.40	
n6/n3	0.46	0.42	
PUFA/SFA	1.40	1.17	
USFA/SFA	3.02	2.83	
PI	33.86	45.23	
AI	0.41	0.35	
TI	0.22	0.27	
hFA	132.37	181.46	
HFA	42.47	60.68	
h/H	3.12	2.99	

PI: polyunsaturated index. TI: thrombogenic index. AI: Atherogenic Index. hFA: hypocholesterolemic Fatty Acids (C18:1 + polyunsaturated FA). HFA: Hypercholesterolemic Fatty Acids (C12:0 + C14:0 + C16:0). h/H: hypocholesterolemic/Hypercholesterolemic FA.

DISCUSSION

Sensory evaluation and changes throughout the storage period

Cold-smoked European catfish fillets were evaluated by a panel of thirty-six members using the simple scoring hedonic scale ranging from 9 to 1 qualitative points. The first

sensory evaluation session took place five days after the biological material was sacrificed. The technological process of smoking took place over a period of four days, so that the time elapsed from the moment of finishing smoking to the moment of sensory evaluation was one day. The scores given by the team of evaluators during the six sessions of sensory evaluation indicated that the five sensory characteristics recorded quite similar degradation dynamics, related to the source of the biological material.

If, throughout the six sessions of sensory evaluation, no statistically significant differences were recorded between the two batches in terms of the overall quality of the samples, between the storage intervals there is a much more pronounced dynamic of product quality degradation, which it is also reflected by the statistical indicators.

According to the interpretation provided by the scoring scale for the overall assessment of the quality of smoked preserved fish, it can be considered as "very good" at the beginning of the storage period, "good" on the 15th day and "satisfactory" for the following 3 storage intervals. The total weighted average score for day 35, which amounts to 11.3 points (A) and 11.4 points (C), respectively, is at the lower end of the scoring scale for the qualitative assessment, for the satisfactory qualification, which indicates that the product can still be consumed, but it can no longer be delivered. The 6.1 points (A) and 6.3 points (C) obtained by the batches related to the last storage interval indicate that the analyzed samples are qualitatively inadequate, being qualified as "altered".

The interpretation of the statistical differences between the samples with the same source of provenance preserved in the form of a smoked product indicates that there are statistically significant differences between the batches of the six storage intervals ($P < 0.01$). These results highlight the fact that the qualitative changes occurring in fish meat are very intense, referring to the length of time existing between storage intervals.

The specialized literature provides information related to the valorization of European catfish in the form of a smoked product [21, 22], but most of the time the emphasis falls on the sensory, physical-chemical and microbiological characteristics of the finished product and less on the recorded degradation dynamics by it during storage. However, a study similar to the present one was carried out by Daramola *et al*. (2007) [47] in which the quality of the smoked meat of African catfish (*Clarias gariepinus*) was evaluated, with the help of sensory analysis over a period of six weeks. Interestingly, although the samples were stored at an ambient temperature that varied between 25 and 32 °C, they kept their minimum quality properties for a period of about four weeks.

Freshness status traits

It was observed that the storage period did not have a major influence on *p*H dynamics, as no statistically significant differences were obtained between the samples analyzed within the six evaluation sessions. However, Kök *et al*. (2009) [3] as well as Swastawati *et al*. (2019) [48] attribute the development of bacteria from the *Lactobacillaceae* family as the cause of *p*H fluctuations in processed fish meat. A comparative analysis of the *p*H dynamics of smoked catfish meat from this study with the *p*H dynamics of fresh catfish meat from aquaculture [49] kept under refrigeration conditions (at a temperature of 2 °C ÷ +4 °C) during 15 days of storage, indicates that the *p*H value considered unsuitable for consuming fresh fish meat (6.8 *p*HU) was recorded after 9 days of storage in refrigerated conditions (6.88 *p*HU) and at the end of the analyzed period (after 15 days) the *p*H values reached values indicating an altered meat (7.14 *p*HU).

Results similar to those obtained in the current research are also highlighted in literature. Thus Yasemen Y. (2007) [50] analyzing the *p*H value of the meat of African catfish (*Clarias gariepinus*), previously processed by smoking, indicated a constant maintenance of the value of this indicator throughout the interval of storage (24 days). Similarly, Kołodziejska *et al*. (2002) [51] identified a *p*H level in mackerel meat processed by smoking, which started from the value of 6.13 *p*HU and reached 6.22 *p*HU after 21 days of storage, at a temperature of 2 °C.

Easily hydrolysable nitrogen is frequently used as an indicator of the level of quality degradation for fish meat preserved and stored in various forms.

The statistical analysis performed between the batches made up of biological material from aquaculture, demonstrated that the average values of NH_3 are maintained at a close level until the 25th day of storage, after which the statistical differences are at an insignificant threshold only between adjacent intervals. However, in the last evaluation session, a remarkable increase in the amount of ammonia was found, resulting in statistically significant differences between this batch and all other batches.

In the case of batches originating from the wild environment, statistically significant differences were also recorded between the NH_3 values of the batch evaluated in the last storage interval and all other batches. And this time, no statistically significant differences were obtained between the other pairs of lots.

Taking into account the fact that the legislation specific to the field [47] admits a maximum value of ammonia of 65 mg NH_3/100 g at the level of fish products processed in smoked form, it was found that the samples evaluated in the present study did not accumulate amounts close to this maximum limit until the end of the storage interval.

A comparative analysis of the dynamics of easily hydrolysable nitrogen (NH_3) in the smoked catfish meat from this study, which at the end of the analyzed period (40 days at a temperature of 2 ÷ 5 °C) was below the maximum limit allowed by the legislation in the field, with the dynamics of easily hydrolysable nitrogen for fresh catfish meat from aquaculture [49], kept under refrigeration conditions throughout 15 days of storage shows that, for fresh meat the value considered unsuitable for consumption (25 mg NH_3/100g) was recorded much earlier, after only 6 days of storage under refrigeration conditions.

Results located below this limit are highlighted by the specialized literature. Thus, Yasemen Y. (2007) [50] obtained values of nitrogen compounds in the case of *Clarias gariepinus* preserved by hot smoking, between 17.67 ± 0.81 and 29.16 ± 1.68 mg/100g, in a period of 24 days of storage, when the product was no longer compliant from the point of view of sensory analysis. Similar results were identified in numerous other works [52 – 56], this aspect suggesting that it is recommended that ammonia values be correlated with sensory and microbiological analyses in establishing the optimal acceptability time of products preserved by smoking.

A comparative analysis of the dynamics for the Eber, Nessler and H_2S reactions in the case of smoked catfish in this study that came out slightly positive ("±" relatively fresh meat) and positive ("+" low alteration degree) only from day 35 of storage, with the dynamics for Eber, Nessler and H_2S reactions in the case of fresh catfish meat from aquaculture [49], kept under refrigeration conditions (at a temperature of 2 °C ÷ +4 °C), indicated negative reactions for fresh meat ("-" fresh meat) or slightly positive "±" relatively fresh meat) only up to 6 days of storage under refrigeration conditions and after 9 days of storage under refrigeration conditions and until the end of the analyzed period

(15 days) the Eber reactions, Nessler and H_2S were positive ("+" low alteration degree, "++" moderate alteration degree, "+++" high alteration degree).
Thus, it was found that in the case of fresh meat kept in refrigeration conditions, the freshness indicators indicate a storage of a maximum of 6 days, while the smoked catfish meat can be kept in similar conditions for a much longer period of up to 30 - 35 days.

Proximate composition

The mass losses had similar dynamics between the two batches with different sources of origin in similar storage intervals, because the statistical interpretation of the average values of these constituents did not indicate the existence of significant differences at the minimum threshold of significance (Table 6). After smoking, the amount of water in the product represented 60.78 %, for fish from aquaculture, respectively 59.71 %, for those caught from the Prut River, while the amount of dry matter varied between 39.22 % (A), and 40.29 % (C).
The low rate of water losses related to the finished product of 4.79 % (A) and 4.69 % (C), is primarily due to the high water losses that occur during the application of the conservation method, when the specific processes of the method (blasting, salting), as well as the actual smoking to a great extent influences the water content, but not the other tissue constituents, which are prone to degradation with the installation of alteration processes.
A comparative analysis of the dynamics of water loss for smoked catfish in this study (kept at temperatures of 2 ÷ 5°C, for 40 days) with the dynamics of water loss for fresh catfish [49], kept under conditions of refrigeration (at a temperature of 2 °C ÷ +4 °C) over 15 days of storage, indicates that water losses in the case of fresh meat storage recorded much higher losses, up to 85.56 % in in the case of fish from aquaculture and up to 83.15 % in the case of fish from the natural environment.
The dynamics of loos of water that makes up the muscle tissue of the catfish meat is highlighted by the statistical interpretation of the values obtained, which indicated in most cases statistically significant differences only between the samples analyzed initially and those analyzed at the end of the storage period. In terms of dry matter compounds, no statistical significance of the dynamics throughout storage was recorded for the crude ash (total minerals) ($P > 0.05$). However, in the case of organic matter representatives, i.e. lipids and proteins, there were found statistically significant differences between storage onset moment and last assessment, even if they concentrated as well, due to water loss. This completes the main image of organic matter degradation throughout storage, especially of the protein decay, in concordance with NH_3 and H_2S formation. It would be interesting to assess the oxidation dynamics of lipids and this could be a research follow-up. Given that a similar qualitative deterioration was also found following the sensory analysis, it can be said that the lipid oxidation process had an important role in the qualitative denaturation of samples from aquaculture, a phenomenon also found in other specialized articles [50, 57, 58]. However, such degradative phenomenon could be avoided if special materials or altered atmosphere is used when products are packaged for increase shelf life and to warrant sanogenity and safety [59, 60].
Literature highlighted a similar evolution of the main chemical constituents, following the application of smoking on fish meat. Thus, Besharati *et al*. (2004) [59] applying hot and cold smoking to the meat of rainbow trout (*Onchorhyncus mykiss*) highlighted a

decrease of water content by 3.61 %, respectively 3.93 % after two weeks of storage. Although they do not refer to the influence of storage on the dynamics of degradation of the main constituents that make up the chemical composition of smoked fish meat, numerous other works indicate that smoking influences to the greatest extent the amount of water present in the samples [50, 61 – 67].

Fatty acids profile and sanogenic indices

The high values of MUFA and PUFA, groups of fatty acids with beneficial effect on human health, in particular a protective role against cardiovascular system diseases [68 – 70], make European catfish an important source of "good fats". This fact was also highlighted by calculating the polyunsaturated index (PI) which had values of 33.86 for A and 45.23 for C, which are in close correlation with the high level of PUFA, an important aspect for human health due to implications in the regulation of blood cholesterol levels [71 – 74].
AI and TI indicate the nutritional value of a lipid source in relation to human health; high values indicate a higher risk of developing cardiovascular diseases [42, 75]. These indices are described in detail by Ulbricht and Southgate [45], who reported values of 0.6 for AI and 1.37 for TI for pork, AI 0.72 and TI 1.27 for beef, and AI 0.50 and TI 0.95 for chicken. In the present work, the results of these indices were low for the two categories of European catfish smoked meat. In aquacultured fish, AI was 0.41 and TI 0.22, respectively 20 % and 331 % lower compared to chicken meat. In the case of wild catfish meat, the values were 0.35 for AI and 0.27 for TI, respectively 43 % and 252 % lower than in chicken meat. These data indicate fish meat as a more suitable product for healthy human nutrition than pork, beef and chicken [76].

CONCLUSIONS

Preserved by smoking, European catfish can be a product with special sensory attributes that are appropriate for consumption up to approximately 15 days of storage under refrigeration conditions, aspect also confirmed by the results on the state of freshness. From a chemical point of view, the preservation technique provided a reduced rate of degradation due to the specific processes of smoking; the main factor influencing shelf life was the water content.
The evaluation of the fatty acid profile as well as of the sanogenic indices highlighted the special properties of the lipids in the smoked European catfish meat, AI and TI had significantly higher values than meats from other species (pork, beef, poultry).

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