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MONITORING *CARASSIUS GIBELIO* MUSCLE TISSUE QUALITY UNDER BLACK CUMIN OIL FORTIFICATION AND TYPICAL COLD STORAGE

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Abstract

On the background of the growing need for natural alternatives to fresh fish preservatives, the present study is focused on the assessment of Nigella sativa seed oil effect over the freshness and morphological structure of Carassius gibelio (Prussian carp) muscle tissue, during typical cold storage. The monitoring involved storage of fish samples at regular temperature conditions, recommended for the food industry (0 ± 1°C, on ice). The Carassius gibelio fish samples were divided into three groups: control group (C), without NSSO; test group 1, fortified with 0.1% v/w NSSO (T1) and test group 2, fortified with 0.6% v/w NSSO (T2). Histological and freshness monitoring reveal that treatment groups T1-T2 keep normal morphological structure of fish muscle throughout the monitoring period, while showing slightly improved freshness indicators (pH and TVB-N), compared to control samples. This study strengthens the proposal for NSSO as a natural enhancer of fresh fish quality and shelf life.

Key words*: Carassius gibelio, NSSO, freshness indicators.*

Introduction

Carassius gibelio is known as the Gibel carp, or the Silver Prussian carp and belongs to the *Cyprinidae* family, being pretty common for freshwater ecosystems, mostly in Europe, Russia, Korea and north-east China.

In Romania, Prussian carp fishing is the most practiced amateur fishing, while for consumption, it is known as the most preferred local fresh water fish (Tăpăloagă D, 2017; 2018).

As fish and fishery products are on the top listed food preferences, even for the sensitive categories of consumers (EUMOFA, 2018; Romania Insider, 2019), these are highly perishable food commodities (Sulieman H.M.A., 2012), which require shelf life extension solutions.

Current research literature is rich in proposals of natural preservation methods, among which is *Nigella sativa* seed oil. *Nigella sativa* has recognized *in vitro* (Bakal S.N., 2017) and *in vivo* (Rafati S., 2014) antimicrobial effect against various microorganisms and was proposed as antibacterial solution for various types of commodities, such as cheeses (Georgescu M. et al., 2018a) and fresh fish (Ozpolat and Duman, 2017).

Considering our promising previous results indicating *Nigella sativa* as an efficient antimicrobial solution for other food commodities (Georgescu M. et al., 2018b; Georgescu M et al., 2019), this paper is focused on assessment of *Nigella sativa* seed oil effect over the freshness and morphological structure of *Carassius gibelio* (Prussian carp) muscle tissue, during typical cold storage.

MATERIALS AND METHODS

The conducted research presented in this article is focused on assessment of *Black cumin seed oil (Nigella sativa seed oil - NSSO)* fortified *Carassius gibelio* (Prussian carp) muscle tissue quality, during the storage period of six days. The monitoring involved storage at regular temperature conditions, ideally applied in the food industry (0 ± 1°C, on ice), for fresh Prussian carp whole fish samples, supplemented with various amounts of black cumin seed oil (0.1 and 0.6% v/w).

Sample preparation

Carassius gibelio whole fish weighing 50-80 g/fish (Figure 1) was caught in July 2019, from marsh located close to Bucharest, "Chiroiu 4 Marsh" (Chiroiu-Pământeni commune,

Ialomița county). Fish were transported to laboratory in Bucharest, in ice boxes. Sample preparation included gutting, removal of head and gills, and washing. The *Carassius gibelio* individual fish were divided into two groups: control group (C), without NSSO and test group (T), fortified with *Nigella sativa* seed oil (NSSO). The test group (T) was further divided into 2 groups, depending on the type of NSSO fortification: group T1 - fortified with 0.1% v/w NSSO and group T2, fortified with 0.6% v/w NSSO. All groups of samples were stored on ice in trays which were introduced in a domestic, commercial refrigerator, set at 3°C (at ideal storage conditions) throughout the monitoring period. Each group included three gutted, head-less and gill-less whole fish, one for each set of analysis.

Figure 1. *Carassius gibelio* whole fish, cought from "Chiroiu 4 Marsh" (Chiroiu-Pământeni commune, Ialomița county)

Nigella sativa cold pressed seed oil (NSSO), marketed under the name "Black caraway oil (Ulei de Negrilică)", was purchased from a Romanian company, Carmita Classic, Alba Iulia (Alba County, Romania) (Figure 2).

Figure 2. Black cumin oil -"Black caraway oil (Ulei de Negrilică)", Carmita Classic, Alba Iulia (Alba County, Romania)

NSSO was displayed to the surface of *Carassius gibelio* whole fish samples in appropriate volume/weight using a micropipette, followed by mildly massaging the oil onto each sample using a gloved hand, according to the method described by Ozpolat E. and Duman M. (2017).

Treatment groups were packed in plastic bags without using vacuum (using high barrier nylon polyethylene bags) (T2 - T4), or were covered in ice (control group, C and T1 group) and stored at designated temperatures until analysis (Figure 3).

Freshness indicators analysis

The pH of samples was measured according to an AOAC method (Association of Official Analytical Chemists, 1990). A total of 10g of sample (from each treatment group) was homogenized with 90 ml deionized water and the pH was measured with a digital pH meter (Fisher Scientific Accumet Basic).

Total volatile basic nitrogen (TVB-N, mg/100g fish flesh) was determined by direct distillation of fish after addition of magnesium oxide (MgO), according to the Romanian Standard for determination of total volatile basic nitrogen in meat (SR 9065-7:2007).

Figure 3. *Carassius gibelio* whole fish sample preparation: sample weighing (left); Treatment groups packed in high barrier nylon polyethylene bags) (right)

Histological analysis

Fish muscle samples were prepared into 1-2 cm diameter sections, immediately fixed in buffered formalin and posteriorly embedded in paraffin. Once fixed, a dehydration was performed by increase of alcohol degree (70, 80, 96, 98°), followed by immersion in xylene (twice) and two baths in paraffin, each sample remained 1 hour in each solution. Automatic processing took 5 hours. Histological sections of 5 μm in thickness, transverse and vertical, were obtained and subsequently stained with haematoxylin-eosin (HE) to evaluate the morphology patterns of the muscle fibers. To stain, a deparaffinization was carried out using a xylene immersion for three times (20, 15 and 10 minutes, respectively) and the tissue was rehydrated by decreasing of the alcohol degree, 100° (3 min), 96° (1 min), 80° (1 min) and 70° (1 min), followed by immersion in distilled water (3 min).

Data analysis

The study design included three batches of *Carassius gibelio* whole fish samples: control group (without NSSO), on ice, and placed at refrigerator (group C) and test groups T1-T2. Both test groups were fortified with NSSO: T1 - fortified with 0.1% v/w NSSO and group T2, fortified with 0.6% v/w NSSO. Test groups were subjected to the same storage conditions as the control group. The three batches of samples were considered the treatments, which were analyzed at days 1, 3 and 6 of storage. For the freshness indicators, variance analysis of data was performed by One way analysis of variance (ANOVA) using SAS (ANOVA version 9.1). The threshold of significance level between treatments was $p < 0.05$.

RESULTS AND DISCUSSIONS

TVB-N content

The values of TVB-N at study start-up were higher than those described by recent publications, for *Cyprinidae* fish, as Pramod K (2019) found an initial value of 4.57 mg/100g, compared to our study, which indicated values ranging from 8.22 to 8.87 mg/100 g. As expected, the TVB-N values followed a rising shift throughout the monitoring period (Figure 4). However, the total volatile basic nitrogen (TVB-N) limit values considered by scientific literature and current available legislation, ranging from 25 mg/100g to 30 mg/100 g (Oehlenschlager J, 1992; Regulation no. 2074/2005), were not reached by any of the treatment or control groups of samples (fig 4).

In terms of differences noted between the rising trends of TVB-N content of control samples,

and treatment samples, there is an obvious difference between T2 and control (C), as revealed in figure 4. Control group samples reached a value of 21.73 mg/100g at day 6 of refrigerated storage on ice, while T2 samples revealed a lower maximum value, of 18.36 mg/100g. However, no statistical significance is associated with this difference ($p > 0.05$).

For treatment 1, *Carassius gibelio* whole fish samples enriched with 0.1 % v/w NSSO, the TVB-N rising shift pattern is inconstant, as on day 3, the indicated value is slightly higher (13.32 mg/100g) than for control group samples (12.8 mg/100g), against expectations, while on day 6, the TVB-N content of T1 group samples is only slightly lower (20.24 mg/100g) than for control group samples (21.73 for control group samples).

Our study reveals that NSSO in the designated concentrations did not significantly influence the TVB-N pattern of fluctuation during cold storage, for *Carassius gibelio* whole fish samples.

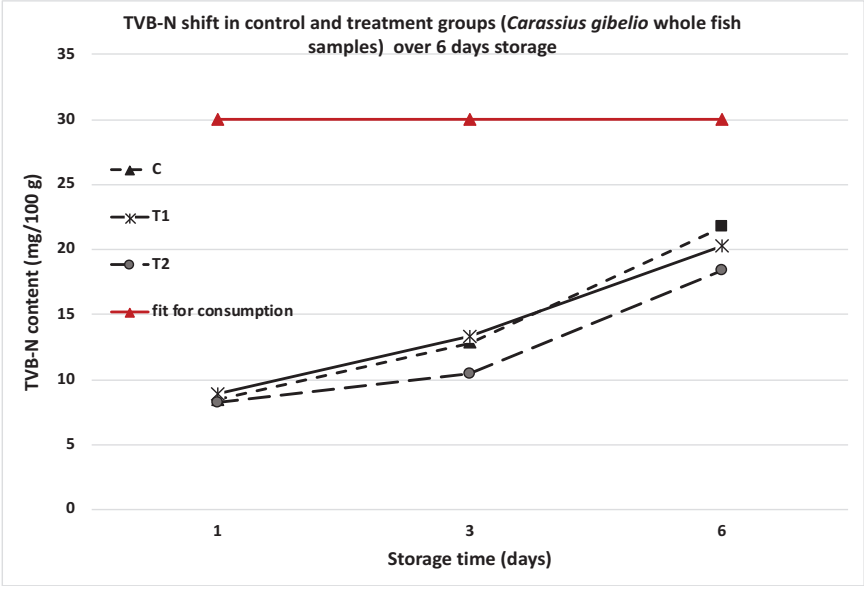

Figure 4. Results of TVB-N content in control and treatment *Carassius gibelio* whole fish samples

pH trend

The initial values of the pH for all considered samples ranged between 6.18 and 6.22 and were higher than pH values communicated by other scientific studies for *Carassius gibelio* fresh muscle samples, who report values ranging from 5.55, to 6.07, with an average of 5.77± 0.13 (Zhang Z et al., 2016). However, the values revealed in our study were much lower than those indicated for similar species, such as Crucian carp, during similar storage on ice, for which the reported initial pH value averaged 7.06 ± 0.04 (Kaifeng L et al., 2015). As there currently are no regulated maximum admitted limits of fresh fish pH, we are considering the lowest maximum admitted limit (7.0) available in international guides and regulations, considered as the threshold limit for condemnation (Goncalves A.A., 2017). None of the samples in our study exceeds this limit and therefore can be considered fit for consumption from this point of view.

Post-mortem raise in fish muscle pH follows quickly, soon after the initial drop induced by la lactic acid accumulation, and is due to accumulation of ammonia and trimethylamine, which are associated with spoilage. Our results reveal a difference in pH trend between the treatment groups, as lower pH values are seen for T1 and T2 groups of samples. There is also a positive connection between the NSSO concentration of enrichment and pH rising trend, as lower values were seen for T2 group of samples (6.22-6.49, with an average of

6.33), compared to T1 group of samples (6.18-6.54, with an average of 6.34) (Figure 5). However, no statistical significance ($p > 0.05$) was indicated for the positive correlation between the NSSO concentration and the beneficial effect on the pH of the fish muscle.

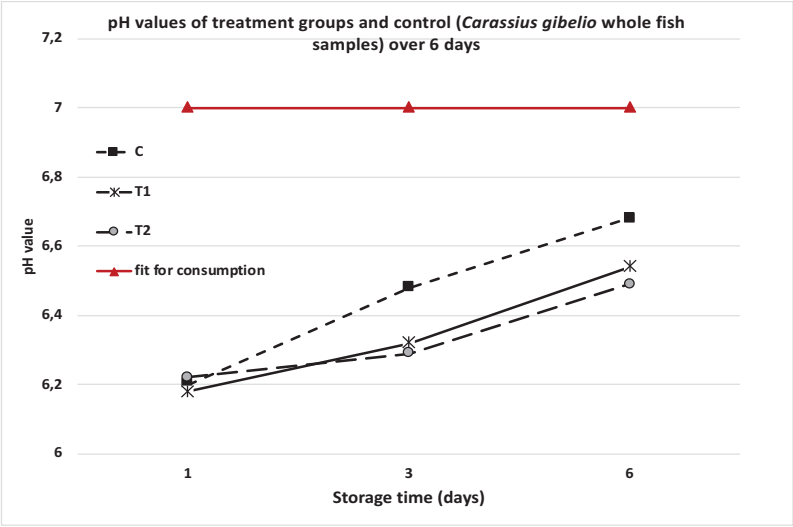

Figure 5. Results of pH trend in control and treatment *Carassius gibelio* whole fish samples

Histological analysis results

Fish

The organization of *Carassius gibelio* muscle tissue exhibited a typical morphological pattern found in fish. The muscular tissue from *Carassius gibelio* samples is composed of skeletal striated muscle fibers of fusiform shape with numerous nuclei arranged peripherally, the intermuscular connective tissue is poorly represented. (Figures 6-14).

Following histological evaluations no significant differences were observed between the samples collected on the first, the third and the sixth day of storage.

In all examined samples (9) the integrity of the muscle cells and the presence of numerous blood capillaries were observed.

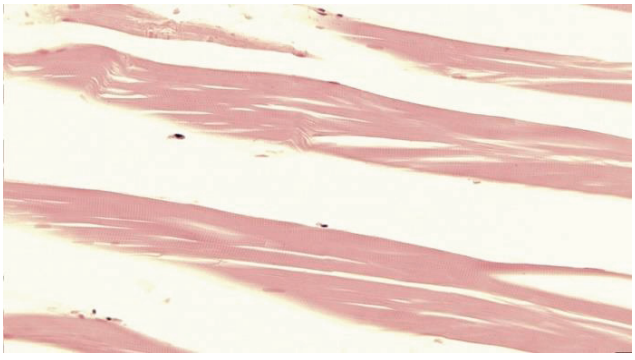

Figure 6. Histological assessment of *Carassius gibelio* whole fish samples at day 1 – Control group (Longitudinal section. Ob. 40X, Col. HE)

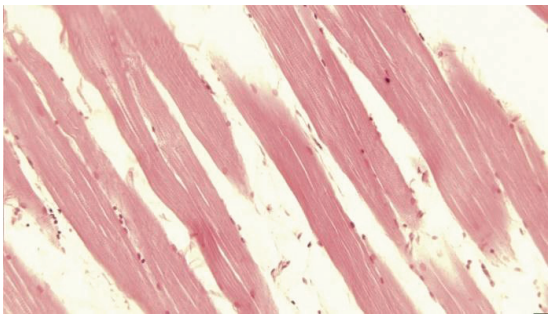

Figure 7. Histological assessment of *Carassius gibelio* whole fish samples
at day 1 – T1 group, 0.1% v/w NSSO (Longitudinal section. Ob. 40X, Col. HE)

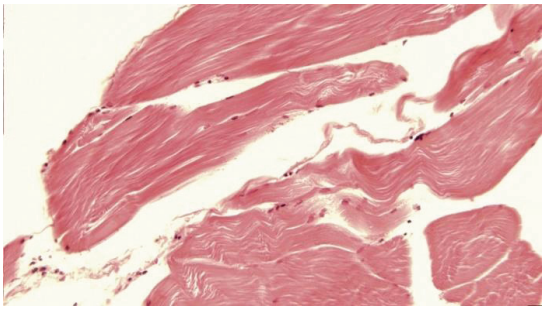

Figure 8. Histological assessment of *Carassius gibelio* whole fish samples
at day 1 – T2 group 0.6% v/w NSSO (Longitudinal section and transverse. Ob. 40X, Col. HE)

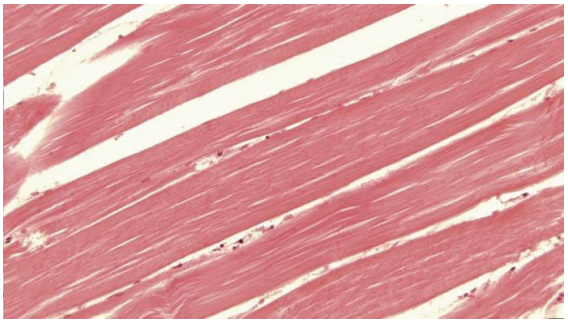

Figure 9. Histological assessment of *Carassius gibelio* whole fish samples at day 3 – Control
(Longitudinal section. Ob. 40X, Col. HE)

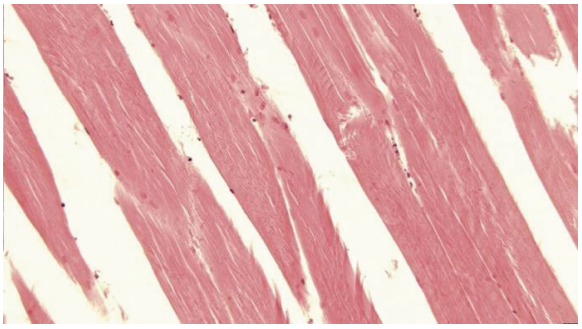

Figure 10. Histological assessment of *Carassius gibelio* whole fish samples at day 3 – T1,
0.1% v/w NSSO (Longitudinal section. Ob. 40X, Col. HE)

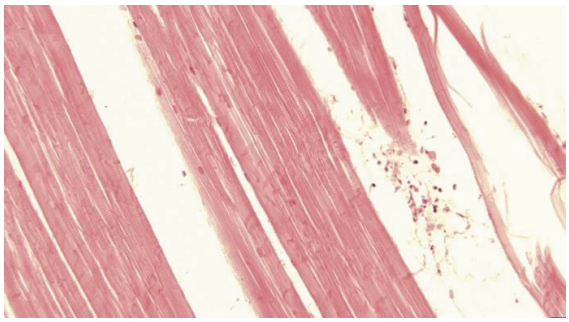

Figure 11. Histological assessment of *Carassius gibelio* whole fish samples at day 3 – T2, 0.6% v/w NSSO (Longitudinal section. Ob. 40X, Col. HE)

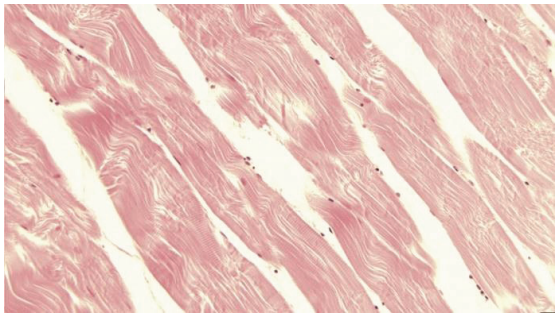

Figure 12. Histological assessment of *Carassius gibelio* whole fish samples at day 6 – Control group (Longitudinal section. Ob. 40X, Col. HE)

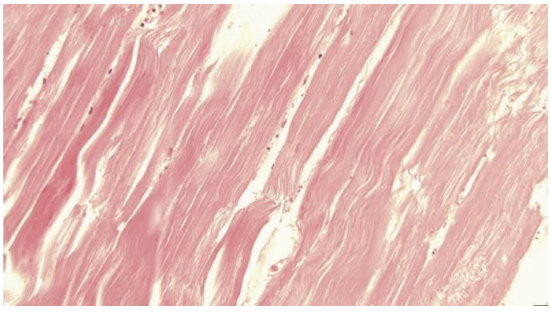

Figure 13. Histological assessment of *Carassius gibelio* whole fish samples at day 6 – T1, 0.1% v/w NSSO (Longitudinal section. Ob. 40X, Col. HE)

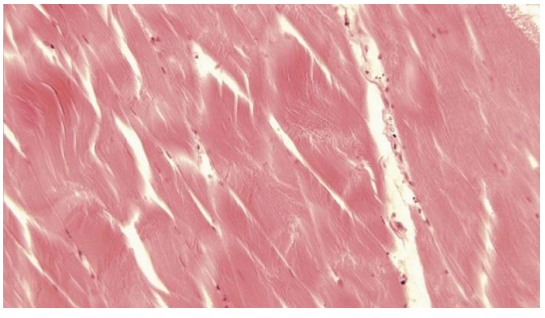

Figure 14. Histological assessment of *Carassius gibelio* whole fish samples at day 6 – T2, 0.6% v/w NSSO (Longitudinal section. Ob. 40X, Col. HE)

CONCLUSIONS

Our results reveal that treatment groups T1-T2 keep normal morphological structure of fish muscle throughout the monitoring period, while showing slightly improved freshness indicators (pH and TVB-N), compared to control samples. This study strengthens the proposal for NSSO as a natural enhancer of fresh fish quality and shelf life.

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