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HISTOLOGICAL ASSESSMENT OF *CARASSIUS GIBELIO* MUSCLE TISSUE STRUCTURE UNDER BLACK CUMIN OIL FORTIFICATION AND STORAGE UNDER REGULAR AND ATYPICAL TEMPERATURES

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Abstract

As Prussian carp (Carassius gibelio) is one of the most consumed local fish in Romania, the present study is focused on challenge testing of Nigella sativa fortified Carassius gibelio whole fish muscle sample morphological quality. The challenge testing involved exposure to atypical temperature conditions, other than those anticipated in the food industry, including stress temperature trial (exposure to storage temperature variations) for fresh Carassius gibelio whole fish, supplemented with 0.6% v/w Nigella sativa seed oil (NSSO). Histological evaluation of muscle structure was performed at day 3 of storage, for all samples. Histological assessment of dorsal skeletal fish muscle revealed no significant differences between the NSSO enriched challenged sample groups and the control group, after three days of storage. This study shows promising results for the possible use of Nigella sativa seed oil, as a natural solution for extending shelf life and enhancing the quality of cold-stored fresh Prussian carp.

Key words*: Carassius gibelio,NSSO, histological evaluation.*

INTRODUCTION

Prussian carp, *Carassius gibelio* is currently considered one of the most extensively spread fresh water species in Europe, Russia, Turkey and Asia. In Romania it is one of the most consumed species, being known as "the national fish", or "every Sunday Fisherman's fish" (Tăpăloagă D., 2017; 2018). Being one of the most recommended, nutritious and affordable food commodity, fish and fishery products must overcome their main disadvantage: the extremely high perishability (EUMOFA, 2018; Romania Insider, 2019; Sulieman H.M.A., 2012). As a consequence, extending fish shelf life without altering quality parameters, by using natural, inexpensive products, which are easily accepted by the consumers, remains a major research topic, as reflected by recent scientific literature (Jun M., 2019). Among various types of natural solutions, such as microbial-derived compounds (bacteriocins, reuterin, organic acids), algae and mushrooms, animal derived compounds, the plant-derived compounds are some of the most preferred: essential oils, plant extracts and natural wood smoke. Essential oils have antimicrobial and antioxidant properties which make them excellent candidates for shelf life extending in various perishable foods, such as fish and fishery products (Jun M., 2019).

There are numerous studies concerning the antimicrobial effects of *Nigella sativa in vitro* (Bakal S.N., 2017) and *in vivo* (Rafati S., 2014) against various spoilage and pathogenic microorganisms. *Nigella sativa* has been 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 the antimicrobial and antioxidant potential of *Nigella sativa* seed oil (Georgescu M. et al., 2018b; Georgescu M et al., 2019), this study aimed to assess its influence on the histological structure of *Carassius gibelio* (Prussian carp) muscle tissue, subjected to various atypical cold storage temperatures and stress temperature treatment, during three days storage time.

MATERIALS AND METHODS

The present study is focused on challenge testing of *Nigella sativa* fortified *Carassius gibelio* (Prussian carp) muscle tissue quality. The challenge testing involved accelerated tests

at temperature conditions other than those anticipated in the food industry (5-10°C), including stress temperature trial (exposure to storage temperature variations) for fresh Prussian carp whole fish samples, supplemented with 0.6% v/w *Nigella sativa* (Black cumin) seed oil (NSSO).

Sample preparation

Carassius gibelio whole fish weighing 50-100 g/fish was caught early June 2019, from a designated Complex located 15 km away from Bucharest, called "Pescarium Corbeanca" (Corbeanca commune). 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 0.6% v/w NSSO. The test group (T) was further divided into 4 groups, subjected to different storage temperatures: on ice, and placed at refrigerator – group T1 (fresh ice was changed daily throughout the storage period), 0-4°C ± 1°C – group T2, 5-6°C ± 1°C – group T3 and stress temperature trial (STT) – group T4. The STT involved removing the treatment group samples from the refrigerator set to 6 ± 1°C, and keeping them at room temperature (21-24°C) for 5-10 minutes, daily, throughout the storage period. Group C (control) was stored on ice, and placed at refrigerator (in the same conditions as T1 group) throughout the storage period. Each group included two gutted, head-less and gill-less whole fish.

Nigella sativa cold pressed seed oil (NSSO), marketed under the name "Egyptian Black Cumin Oil (Ulei de Chimen Negru Egiptean)", was purchased from a Romanian company, Herbal Sana SRL, Bucharest (Figure 1). 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) (Figure 2).

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 1. Black cumin oil - "Egyptian Black Cumin Oil (Ulei de Chimen Negru Egiptean)", Herbal Sana SRL, Bucharest

Figure 2. *Carassius gibelio* whole fish sample preparation: sample weighing (left); NSSO displaying onto the surface of *Carassius gibelio* whole fish samples in appropriate volume/weight using a micropipette (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 five batches of *Carassius gibelio* whole fish samples: control group (without NSSO), on ice, and placed at refrigerator (group C) and test groups T1-T4. All test groups (T1-T4), fortified with 0.6% v/w NSSO. Test groups were subjected to various temperatures: 3°C ± 1°C (T1), 5°C ± 1°C (T2), 7°C ± 1°C (T3) and stress temperature trial (STT) – group T4. The five batches of samples were considered the treatments, which were analyzed at day 3 of storage.

RESULTS AND DISCUSSION

For the control sample, the organization of *Carassius gibelio* dorsal skeletal muscle tissue exhibited a typical morphological pattern found in fish. Striated muscle from *Carassius gibelio* samples exhibited the typical morphologic pattern, multinucleated fibers with peripheral nuclei (Figure 3).

Histological evaluation of fish muscle revealed no significant differences between sample groups after three days of storage.

In all the examined samples, it is observed that the skeletal striated muscle fibers have an integral sarcolemma and the nuclei are disposed at the periphery having a flattened oval shape. The presence of numerous capillaries in the endomisium level is observed (Figures 4-7).

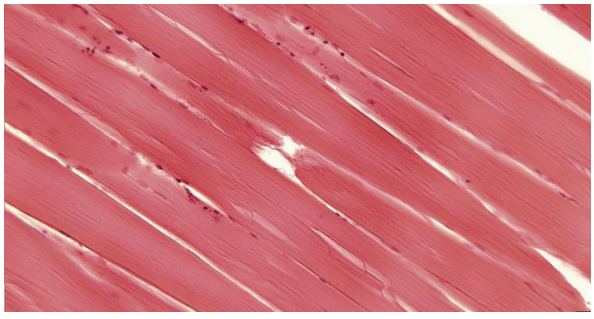

Figure 3. Muscle tissue organization in *Carassius gibelio* whole fish sample - **control sample**
(Multinucleated fibers with peripheral nuclei. Longitudinal section. Ob. 40 X, Col. HE)

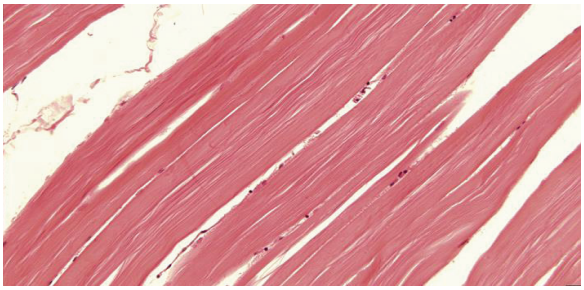

Figure 4. Muscle tissue organization in *Carassius gibelio* fillet -**T1 sample**
(Multinucleated fibers with peripheral nuclei and capillaries. Longitudinal section. Ob. 40 X, Col. HE)

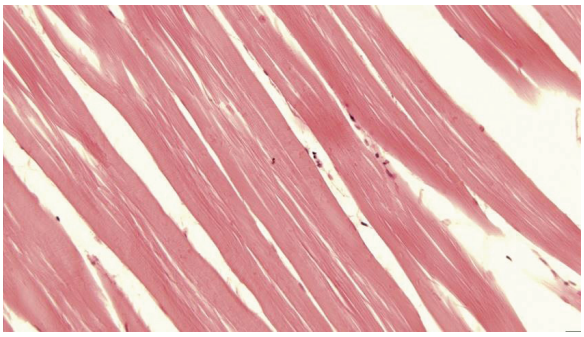

Figure 5. Muscle tissue organization in *Carassius gibelio* **whole** fish sample -**T2 sample**
(Multinucleated fibers with peripheral nuclei. Longitudinal section. Ob. 40 X, Col. HE)

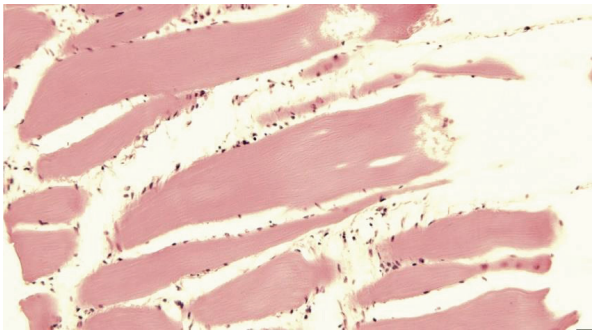

Figure 6. Muscle tissue organization in *Carassius gibelio* whole **fish** sample -**T3 sample**
(Multinucleated fibers with peripheral nuclei. Longitudinal and transverse section. Ob. 40 X, Col. HE)

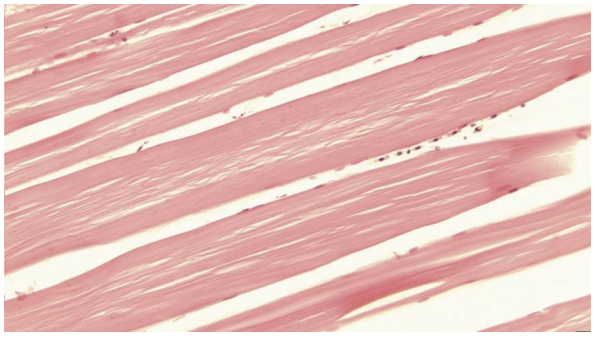

Figure 7. Muscle tissue organization in *Carassius gibelio* whole **fish** sample -**T4 sample**
(Multinucleated fibers with peripheral nuclei. Longitudinal section. Ob. 40 X, Col. HE)

Even if the recommended shelf-life for properly stored fresh fish is, according to FAO, as long as 16-21 days (adequately refrigerated on ice), the use-by date of most producers is between 3-4 days, while the sell-by date is as short as 1-2 days. Most studies indicate 15 days of adequately refrigerated storage as maximum time frame for good quality fresh fish, (Sulieman H.M.A et al., 2012). Considering the minimum sell-by period, of 1-2 days, our results of day 3 of storage were expected to reveal changes in the morphological structure of control samples and presumably differences for samples which were NSSO enriched. However, results indicated that all treatment groups maintained the normal morphological structure by day 3 of storage (including the ST group). Considering the literature data, the T2-T3 samples were expected to undergo certain degrees of morphological alterations of the muscle fiber, due to atypical temperature exposure, while the ST group sample (T4) was

expected to reveal significant alterations of the muscle tissue morphology. However, our results, which demonstrate maintaining of normal morphological structure in *Carassius gibelio* whole fish T1-T4 sample groups, suggest that *Nigella sativa* may be considered an efficient solution for prolonging the muscle quality and the shelf-life of fresh Prussian carp. Further research, involving larger study samples, could provide the statistical significance needed to support these findings. Also, sensory assessment of fresh and cooked NSSO enriched Prussian carp would be useful for selection of the most appropriate and efficient NSSO dosage.

CONCLUSIONS

The present study indicates that *Carassius gibelio* whole fish enriched with Nigella sativa seed oil and subjected to atypical and stress temperatures keep normal morphological structure of fish muscle throughout the storage period, despite the higher temperatures to which these treatments were subjected. These results suggest that fortification with NSSO might be associated with increased fish quality. NSSO fortification of *Carassius gibelio* whole fish exposed to stress temperature trial (STT), helps maintain the normal muscle tissue pattern morphology, with similar appearance to adequately stored NSSO-free fish. This study shows promising results for the possible use of NSSO as a natural solution for promoting longer shelf life and better quality for cold-stored *Carassius gibelio* whole fish.

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