Challenge testing of *Nigella sativa* fortified *Cyprinus carpio* fillets quality by various regular and atypical storage temperatures

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Abstract
Increased interest for enhancing food quality during storage, using affordable, natural solutions, lead to studies assessing Nigella sativa for its preservation properties. The present study is focused on challenge testing of Nigella sativa fortified Cyprinus carpio fillets morphological 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 carp fillet 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. The carp fillets were divided into two groups: control group (C), without NSSO and test group (T), fortified with 0.6% v/w NSSO, which was further divided into 4 groups, subjected to different storage temperatures: 3°C – group T1, 5°C – group T2, 7°C – group T3 and stress temperature trial (STT) – group T4. Group C (control) was stored at 0°C throughout the monitoring period. Histological evaluation of fish muscle revealed no significant differences between sample groups after three days of storage. This study shows promising results for the possible use of Nigella sativa seed oil, as a natural solution for promoting longer shelf life and better quality for cold-stored fresh fish.

Introduction

Fish and fishery products have always been one of the most preferred food commodities for their nutritional value (EUMOFA, 2018), being the choice of dish for the most sensible consumer categories (Romania Insider, 2019). However, fish products are extremely perishable compared with other types of meats and among various types of animal derived products (Sulieman H.M.A, 2012). As a consequence, extending fish shelf life without altering quality parameters, by using natural products, is currently a major research topic, as reflected by recent scientific literature.

The antimicrobial effects of *Nigella sativa* have extensively been studied *in vitro* (Bakal SN, 2017) and *in vivo* (Rafati S, 2014) against various 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 promising studies indicating *Nigella sativa* as an efficient antimicrobial solution for some commodities (Georgescu M. et al., 2018b; Georgescu M et al., 2019), we assessed its influence on the histological structure of *Cyprinus carpio* fillets subjected to various regular and atypical cold storage temperatures, during two weeks storage time.

Materials and methods

The present study is focused on challenge testing of *Nigella sativa* fortified *Cyprinus carpio* fillets 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 carp fillet supplemented with 0.6% v/w *Nigella sativa* seed oil (NSSO).

Sample preparation

Cyprinus carpio weighing 1-2 kg/fish was caught during May 2019, in a private pond close to Bucharest, "Bila 2 Pond" (Naipu village, Ghimpați commune, Giurgiu county). Fish were transported to laboratory in Bucharest, in ice boxes. Sample preparation included gutting, filleting and washing. The carp fillets were divided into two groups: control group (C), without NSSO and test group (T), fortified with 0.6% v/w NSSO, which was further divided into 4 groups, subjected to different storage temperatures: 3°C ± 1°C – group T1, 5°C ± 1°C – group T2, 7°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 7 ± 1°C, and keeping them at room temperature (21-24°C) for 5 minutes, daily, throughout the monitoring period. Group C (control) was stored at 0°C ± 1°C throughout the monitoring period. Each group of fish included 2-2.5 kg fillets.

Nigella sativa cold pressed seed oil (NSSO), marketed under the name "Negriol", was purchased from a Romanian company, Aghoras Invent SRL, Bucharest. NSSO was displayed to the surface of fresh carp (*Cyprinus carpio*) fillet 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 vacuum packed (using high barrier nylon polyethylene bags) and stored at designated temperatures until analysis.

Histological analysis

Fish fillets 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 carp fillet samples: control group (without NSSO), stored at 0°C ± 1°C (group C) and test groups T1-T4, test group (T), fortified with 0.6% v/w NSSO, and subjected to 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

The organization of *Cyprinus carpio* fillets muscle tissue exhibited a typical morphological pattern found in fish. Striated muscle from *Cyprinus carpio* samples exhibited the typical morphologic pattern, multinucleated fibers with peripheral nuclei (Fig. 1-5).

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

All samples reveal the integrity of the muscle fibers and of the sarcolemma. The flattened nuclei appear peripheral and the capillary vessels are seen located at endomysium level.

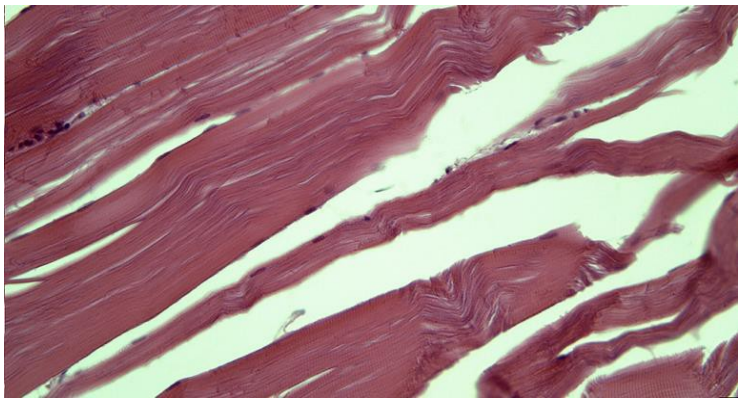

Figure 1. Muscle tissue organization in *Cyprinus carpio* fillet - control sample (Multinucleated fibers with peripheral nuclei. Longitudinal section. HE.)

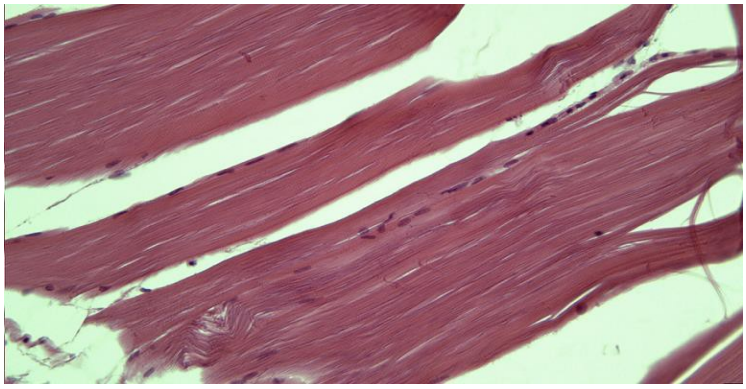

Figure 2. Muscle tissue organization in *Cyprinus carpio* fillet -T1 sample (Multinucleated fibers with peripheral nuclei. Longitudinal section. HE.)

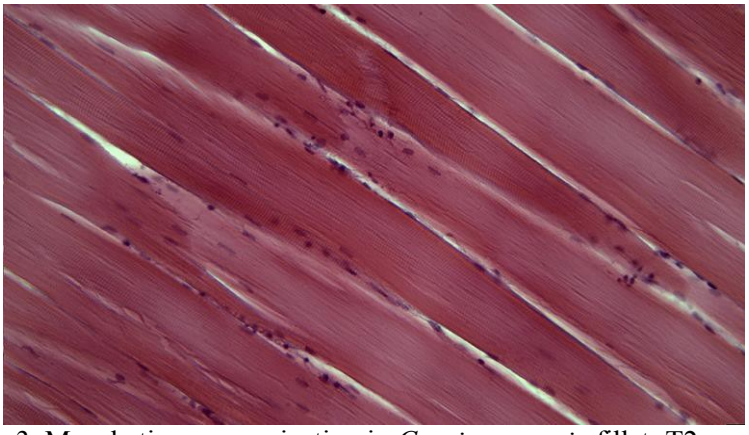

Figure 3. Muscle tissue organization in *Cyprinus carpio* fillet -T2 sample (Multinucleated fibers with peripheral nuclei. Longitudinal section. HE.)

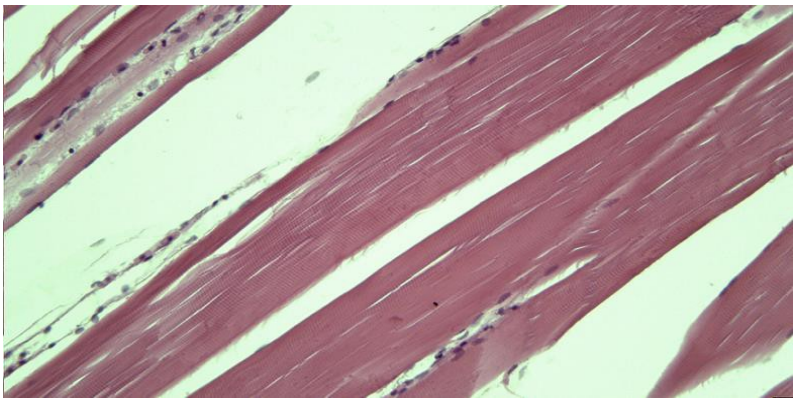

Figure 4. Muscle tissue organization in *Cyprinus carpio* fillet -T3 sample (Multinucleated fibers with peripheral nuclei. Longitudinal section. HE.)

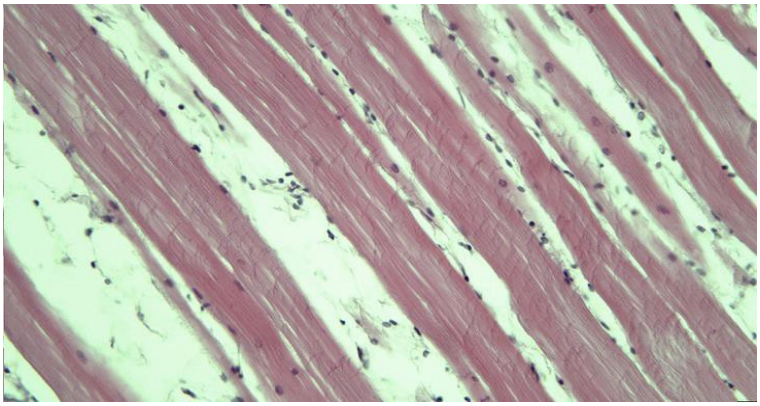

Figure 5. Muscle tissue organization in *Cyprinus carpio* fillet -T4 sample (Multinucleated fibers with peripheral nuclei. Longitudinal section. HE.)

FAO recommends a 16-21 days shelf-life for adequately refrigerated (on ice) carp, while for commercially refrigerated fish, while the use-by date is between 3-4 days, and 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, while marginally acceptable products may be obtained by day 25 of 0-4°C storage (Sulieman H.M.A et al., 2012). Very similar results are communicated by Kaifeng Li (2012), which found carp samples to be acceptable after 16 days of storage at 4°C and 24 days of storage on ice. Our results indicate that considering the histological structure of the muscle fibers, all treatment groups maintain the normal morphological structure by day 3 of storage (including the ST group). Considering the literature data, the T1-T3 samples were expected to undergo certain degrees of morphological alterations of the muscle fiber, while the ST group sample was expected to reveal significant alterations of the muscle tissue morphology. Our results suggest that *Nigella sativa* may be considered an efficient solution for prolonging the muscle quality and the shelf-life of fresh fish.

Conclusions

Our results reveal that treatment groups T1-T4 keep normal morphological structure of fish muscle throughout the monitoring period, despite the higher temperatures to which these treatments

were subjected, thus suggesting that fortification with NSSO might be associated with better fish quality. NSSO fortification of carp fillets 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 fresh fish.

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