Bleeding of Common Carp (Cyprinus carpio) Improves Sensory Quality of Fillets and Slows Oxidative and Microbiological Changes During Refrigerated Aerobic Storage

Running title: Common carp bleeding improves fillet quality

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SUMMARY
Common carp (Cyprinus carpio) aquaculture is one of the most important and rapidly growing productions around the world. However, for consumers, carp is often not acceptable due to its distinctive colour and odour. In our study, bleeding of common carp with the effect on fillet quality was studied. Obtained results show, that carp bleeding by cutting the gill arches is an effective way of reducing the total haem content, which here decreased from (9.63±1.55) μmol haemoglobin/kg in unbled carp to (2.35±0.79) μmol haemoglobin/kg in bled carp. Furthermore, fillets from bled carp showed reduced formation of primary and secondary lipid oxidation products and growth of microorganisms during 12 days of refrigerated aerobic storage. On the last day of storage, lipid hydroperoxides decreased from (88.88±4.19) μmol cumene hydroperoxide/kg in unbled to (62.13±2.86) μmol cumene hydroperoxide/kg in bled carps; TBARS decreased from (4.18±0.54) μmol malondialdehyde/kg in unbled to

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(2.63±0.35) μmol malondialdehyde/kg in bled carps; mesophilic and psychrotrophic bacteria count decreased from (6.37±0.10) log CFU/g and (6.22±0.26) log CFU/g in unbled to (4.00±0.21) log CFU/g and (4.23±0.16) log CFU/g in bled carps. These raw bled fillets showed increased lightness $L^*$, and reduced redness $a^*$ and yellowness $b^*$, compared to unbled fillets. Sensory analysis showed improved colour, odour and overall acceptability of raw bled fillets. Overall, bleeding improves the quality of carp fillets. Thus, inclusion of bleeding into processing of carp fillets has the potential to improve their acceptance by consumers and prolong their shelf-life.

**Key words:** common carp, bleeding, sensory quality, colour, lipid oxidation, microbiological quality

**INTRODUCTION**

Cyprinids are the most cultivated fish group worldwide and their production is constantly increasing. The production of common carp (*Cyprinus carpio*) through aquaculture reached 4 556 622 tonnes in 2016, which made carp the third most produced aquacultured fish worldwide, after grass carp (*Ctenopharyngodon idellus*) and silver carp (*Hypophthalmichthys molitrix*) (1). To provide fresh and safe common carp product of good quality, development and application of effective species-specific processes before, during and after slaughter are needed (2). For common carp, many studies of pre-slaughter and slaughter processes have been investigated, which have emphasised the importance for meat quality of reducing the stress (3-5). One of the processes that can be included in the slaughter is bleeding, and for common carp this is most commonly achieved by cutting the gill arches or by direct gutting (2). However, to date, there have been no studies on the effects of bleeding on the quality of the fillet meat of common carp.

The presence of haem proteins in fish meat promotes lipid oxidation, discolouration, and odour development (6-8). Haem is part of both myoglobin and haemoglobin, with haemoglobin recognized as the better promoter of lipid oxidation (9). Iron is included in haem, and it is also an important source of nutrients for microorganisms (10). With removal of the blood from the fish body, both erythrocytes (which contain the haemoglobin) and plasma are removed, which can be sources of lipid oxidation in fish (6). Thus it can be expected that removal of blood from the fish meat will slow lipid oxidation and colour changes, improve the sensory characteristics, and inhibit microbial growth. Although different haem pro-oxidation mechanisms and factors that influence these have been investigated (for
review see 11), reports of various effects of haem on lipid oxidation across different fish species indicates the need for further species-specific studies.

The colour and odour of the raw common carp meat are important factors for consumers. Despite its affordability and high nutritional value, it is often ignored because of its unattractive look (2). A more 'appealing' look and improved sensory characteristics, with emphasis on odour development, might improve the acceptability of common carp fillets. The effects of bleeding during the slaughter of common carp on fillet quality, in terms of instrumental colour, sensory characteristics, lipid oxidation and microbiological parameters during refrigerated aerobic storage were investigated. With selected methods, the influence of bleeding of carp on the quality of fillets was evaluated through comparison of bled and unbled carp. Only by confirming that bleeding of common carp is the key processing step to improve the quality, further optimization of the process and research for mechanisms behind it can be done.

MATERIALS AND METHODS

Fish and experimental plan

Four-year-old common carp (N = 80; body mass, (2395.60±287.02) g) that were bred under pond aquaculture and purged for 2 months were bought from a local producer in November 2016. These were transported to the Institute of Aquaculture and Protection of Waters, University of South Bohemia (České Budějovice, Czech Republic; authorization for use of experimental animals: 35085/2016-MZE-17214). These carp were rested for 24 h in a water tank (6 °C, 96 % O₂) before the start of the experiments.

All of the carp were killed by a blow to the head. Forty randomly selected carp were bled by cutting the gill arches and hanging them vertically with the head down for 30 min, to allow gravity to help the bleeding. After that, all of the carp were stored in ice, positioned straight, with their bodies upright (i.e. natural swimming position), and in a refrigerated chamber. The further processing was performed after 22 h, to let the blood coagulate and to prevent bleeding during the gutting in the unbled group. All of these carp were then gutted, descaled and filleted by the same person, to ensure consistency. The mean filleted yield was (42.24±2.14) %. The fillets were packed individually in non-sealed polyethylene vacuum bags (size 250 mm x 400 mm) to prevent drying of the fillet surface and stored in a straight horizontal position (i.e. 'flat') in a refrigerated chamber (Nordline UR600, Nosreti, Ostrava, Czech Republic) at (2.0±0.5) °C.
The analyses of these carp fillets were carried out on days 1, 3, 6, 9 and 12 of storage. Randomly selected right fillets from the bled and unbled groups (N = 6, from each group) were used for colour analyses. Similarly randomly selected left fillets were used for sensory evaluation (N = 6, from each group), and right fillets for chemical and microbiological analyses (N = 6, from each group). For microbiological analyses, approximately the first 5 cm of the fillets were cut and used fresh. The haem content analysis was carried out only on light dorsal muscle, and the rest of the fillets were used for lipid oxidation analysis, after being homogenised and stored at -80 °C (Igloo U570, Telstar, Sant Cugat, Spain) until analysis.

**Chemicals**

Chloroform, methanol, ammonium thiocyanate, iron (II) sulphate, cumene hydroperoxide, bovine haemoglobin, 2,6-di-tert-butyl-4-methylphenol (BHT), 1,1,3,3-tetraethoxypropane (TEP), phosphoric acid, plate count agar, peptone water (Sigma Aldrich, St. Louis, MO, USA), sodium chloride, acetone (Lach-Ner, Neratovice, Czech Republic), hydrochloric acid, barium chloride dihydrate (Penta, Prague, Czech Republic), trichloracetic acid (TCA; Fisher Chemical, Leicestershire, UK), 2-thiobarbituric acid (TBA; Merck, Darmstadt, Germany) were used.

**Colour analysis**

The colour of the carp fillet meat was measured at three locations along the dorsal part of each fillet, above the lateral line (*i.e.* frontal, middle, caudal), using a colour spectrophotometer (CM-600d; Konica Minolta, Tokyo, Japan). Each spot was measured in duplicate. Colour space CIELAB was used, with measurement of the colour parameters $L^*$ (lightness, from black [0] to white [100]), $a^*$ (redness, on the red to green axis, from green [-a] to red [+a]), and $b^*$ (yellowness, on the yellow to blue axis from blue [-b] to yellow [+b]).

**Sensory evaluation**

The sensory qualities of the raw meat and cooked meat of the carp fillets were evaluated by a panel of 10 members of the staff of the Institute of Aquaculture and Protection of Waters. These were carried out in individual cubicles, to separate the panellists from each other, and under controlled conditions of temperature, light and humidity (12). Each sample was labelled randomly with a 3-digit or 4-digit code, and the evaluations were performed in triplicate. During the evaluations, there was a 40-min break, and with each new assessment, the same sample was evaluated under a different code.
The whole raw carp fillets were examined for texture (from 5, as firm; to 1, as soft), colour (from 5, as no discolouration; to 1, as extreme discolouration), odour (from 5, as extremely desirable; to 1, as extremely undesirable), and overall acceptability (from 5, as extremely acceptable; to 1, as extremely unacceptable) (13).

To evaluate the cooked meat of the carp fillets, they were cut into small pieces (approximately 2 cm × 2 cm). Each sample comprised three pieces of meat, with each from a different part of the fillets. These were placed in 0.2-L glass jars, and cooked in an electric oven for 15 min at 150 °C, without any added salt, oil or spices (14). The panelists evaluated four parameters of the cooked carp meat from the fillets, as flavour (from 5, as extremely desirable; to 1, as extremely undesirable), odour (from 5, as extremely desirable; to 1, as extremely undesirable), aftertaste (from 5, as extremely desirable; to 1, as extremely undesirable) and consistency (from 5, as firm; to 1, as soft).

**Haem content**

Haem protein can originate from myoglobin or haemoglobin, although myoglobin is not present in light muscle (6,15,16). Therefore, only raw light muscle was used to determine the fillet meat haem content, using acidified acetone according to a previously described method (16,17). Briefly, 5 g of sample was homogenised (Ultra Turax basic T18, IKA, Staufen im Breisgau, Germany) with 20 mL of acid acetone for 15 s and incubated for 1 h in the refrigerator. Afterwards, homogenate was filtered through Whatman no.1 filter paper and centrifuged (Hereus Megafuge 16R, Thermo Scientific, Waltham, Massachusetts, USA) at 10 000 xg for 15 min. Absorbance was measured at 640 nm (DR2800, Hach Lange, Düsseldorf, Germany). A standard curve was prepared with bovine haemoglobin, and the data were expressed as μmol haemoglobin/kg. Each sample was analysed in duplicate.

**Lipid oxidation parameters**

Lipid hydroperoxides and thiobarbituric acid reactive substances (TBARS) were determined using previously described spectrophotometrical methods (18,19). For lipid hydroperoxides, 11 mL of 65 % chloroform in methanol was added to 1 g of sample, homogenised for 3 min and filtered. To 7 mL of supernatant 2 mL of 0.5 % NaCl was added, vortexed (Vortex GENIUS 3, IKA, Staufen im Breisgau, Germany) and centrifuged at 3 000 xg for 5 min. To 5 mL of lower phase, 0.25 μL solution of ammonium thiocyanate and iron (II) chloride (for detailed instructions of preparation see 18) were added and incubated for 20 min. Absorbance was measured at 500 nm. The standard curve was prepared using cumene.
hydroperoxide (CHP), and the data were expressed as μmol CHP/kg (18). For TBARS, 0.2 mL of 0.02 M BHT and 9.1 mL of 10 % TCA in 0.2 M H₃PO₄ were added to 1 g of sample, homogenised for 30 s and filtered. To 1.5 mL of supernatant same volume of TBA was added and incubated overnight in the dark at room temperature. Absorbance was measured at 530 nm. The standard curve was prepared with TEP, and the data were expressed as μmol malondialdehyde (MDA)/kg (19). Each sample was analysed in duplicate.

**Microbiological analysis**

To determine the total numbers of aerobic mesophilic and psychrotrophic bacteria, analyses were done according to standardised procedures (20,21). Shortly, approximately 10 g of raw fillet meat was taken aseptically from the dorsal muscle and homogenised for 3 min (Stomacher Classic Panoramic IU 500, IUL Instruments, Barcelona, Spain) with 90 mL of 0.1 % peptone water. Serial dilutions were done and plated on agar plates. For mesophilic bacteria, the plates were incubated (NB 203 XL, N-Biotek, Pyeongcheon-ro, South Korea) for 3 days at 30 °C, and for psychrotrophic bacteria, they were incubated for 10 days at 6.5 °C (Nordline UR600, Nosreti, Ostrava, Czech Republic). These data were expressed as logarithmic colony forming units (log CFU)/g raw carp fillet meat. Each sample was analysed in duplicate.

**Statistical analysis**

The colour, haem content, lipid oxidation and microbiological parameters are presented as means ± standard deviation. Data assumptions of normality (Shapiro Wilk test) and homogeneity of variance (Levene’s test) were met. These data were analysed using general linear models (GLM) to test the influences of Group (bled, unbled) and Time (storage days 1, 3, 6, 9, 12), and their interactions. For colour, this also included Location of measurement (frontal, middle, caudal). Pearson’s correlation coefficient was calculated to correlate haem content with lipid oxidation and microbiological parameters. The sensory analyses data were obtained on the ordinal scale, and are presented as median and interquartile range. These data were further analysed using nonparametric Mann-Whitney U test to compare each evaluated parameter between bled and unbled group on each assessment day, and with Kruskal-Wallis H test to compare sensory attribute between sampling days. Calculated probability p ≤0.05 was considered as statistically significant. All statistical analyses were carried out following the instructions (22,23). Statistical analyses were performed using the SPSS V23 (24).
RESULTS AND DISCUSSION

Colour of raw carp fillet meat

The bleeding of the carp during slaughter significantly affected the colour of the carp fillet meat \((p <0.001; \text{Fig. 1; Tables 1, 2})\). The raw bled fillets showed higher \(L^*\) (lightness) and lower \(a^*\) (redness) and \(b^*\) (yellowness), compared to the raw unbled fillets. Both \(a^*\) and \(b^*\) increased significantly over time \((p <0.001)\); however, \(L^*\) did not change significantly in any of the groups. Higher whiteness was reported for bled Atlantic cod \((Gadus morhua)\), compared to the unbled cod with calculated whiteness index \((71.29\ \text{bled}, 64.68\ \text{unbled})\) from measurements of hyperspectral image \((25)\). Similarly, higher \(L^*\) was shown for immediately bled \((L^* 47)\) Atlantic salmon \((Salmo salar)\), compared to those bled after 12 h, which can be considered as an unbled \((L^* 44)\) group \((26)\). Similarly again, more pronounced redness was reported for unbled fillets in yellowtail \((Seriola quinqueradiata)\) and Asian seabass \((Lates calcarifer)\) \((7,27)\). Also in Digre et al. \((28)\) significantly higher \(L^*\) and lower \(a^*\) values were observed in gill cutting compared to direct gutting as bleeding method.

Fig. 1: Representative photographs of bled (left) and unbled (right) pieces of common carp dorsal muscle.

Table 1: CIEXYZ colour space parameters measured at the three locations for the meat of the raw bled and unbled carp fillets after up to 12 days of refrigerated aerobic storage.

Table 2: Effect of bleeding, time and their interaction on quality attributes and effect of location and interactions for colour parameters of bled and unbled carp fillets during the storage using GLM.

There were also differences between the three sample locations of the raw carp fillet meat used for the colour measurements. The \(L^*\) at all three locations was comparable within each group \((p >0.05)\). On the contrary, location significantly affected both \(a^*\) and \(b^*\), which were significantly higher for the caudal location than for the other two locations \((p <0.001)\). A similar pattern was described for Atlantic cod \((25,28)\), where whiteness index in the belly part was higher compared to loins. These changes seen for the whole carp fillet surface colour might be partially due to fat deposition, which is lower towards the tail section \((26)\).
Furthermore, the thickness of the fillet is reduced for the tail section, which will result in an additional contribution to the fillet colour from the dark muscle, compared with the frontal and middle sections.

**Sensory quality**

The sensory evaluations of the raw bled and unbled carp fillet meat showed significant differences (Fig. 2; Table 3; p <0.05), with the raw bled fillets evaluated by better (i.e. higher) scores overall, compared to the raw unbled fillets. The greatest improvements between the groups were seen for colour evaluation of the bled fillets and overall acceptability, where 2 points of difference were observed. Colour improvement and less discolouration were also described as main sensory characteristics improved by bleeding of yellowtail (7), Atlantic cod (25), and skipjack (*Katsuwonus pelamis*) (29). Therefore, bleeding is efficient processing step for improvement of fillet colour, which is one of the important factors for consumers acceptability of carp meat. Another very important factor for sensory acceptability of carp is odour, which was also improved for one score on day 6 and 9. Also for Asian seabass, more pronounced odour of unbled fillets was reported, compared to bled fillets with odour score on day 9 and 15 of storage reaching average of 5 and 10 in unbled, and 2 and 7 in bled cod (27). Also in sensory evaluation of rancid odour in rainbow trout (*Onchorhynchus mykiss*) and Atlantic mackerel (*Scomber scombrus*), rancid odour was significantly greater in unbled samples. After 15 days of storage rancid odour was detected in 60 % of unbled and 30 % of bled trout. For Atlantic mackerel this was 100 % for unbled and 40 % for bled on day 8 of refrigerated storage (6). There were no changes in texture of raw carp fillets. Similarly was also shown for red sea bream (*Pagrus major*), flatfish (*Paralichthys olivaceus*) and rudder-fish (*Girella punctata*) (30), all of which are demersal fish as well as carp. Bleeding of common carp had a significant effect on improving the sensory acceptability of raw carp fillets.

Fig. 2: Sensory quality of the raw (a) and cooked (b) bled (shades of blue) and unbled (shades of red) common carp fillets on storage days 1, 3, 6 and 9 under refrigerated aerobic storage.

Table 3: Sensory attributes comparison of raw and cooked carp fillets between bled and unbled group during the storage using Mann-Whitney U test.
For the cooked carp fillet meat, the bled and unbled meat showed comparable sensory qualities on days 1 and 3 of storage (Fig. 2; Table 3; p >0.05). However, there were improvements (i.e. higher scores) for bleeding in all of the characteristics by day 6 of storage, for consistency and flavour this improvement was significant (p <0.05). On day 12, these cooked meat samples were considered inedible, and so the sensory analysis was not performed. Therefore, the last sensory evaluation of the cooked meat was on day 9, with no significantly different attributes between the groups (p >0.05). The sensory evaluation of cooked fish meat has not been studied in the previous studies of bleeding influence on fish meat quality. The most pronounce difference was observed on day 6, where there was an improvement in the sensory evaluation of the bled meat. So, bleeding also had a positive effect on the cooked carp meat, which further increases the possibility of better acceptability of carp meat from the consumers.

**Haem content**

Total haem content was determined for all of the raw fillet meat samples, to show the effectiveness of the bleeding. As the haem content in the raw bled and unbled fillet meat did not change over time, the data from all of the sampling days were combined. For the raw bled meat, the haem content (2.35±0.79) μmol haemoglobin/kg was significantly lower (p <0.001) compared to the raw unbled meat (9.63±1.55) μmol haemoglobin/kg. This confirmed effectiveness of bleeding and successful removal of residual blood from carp meat. Obtained data correspond to data from other studies of bleeding efficiency (6,16,27,31). Haem content as lowered by bleeding from (11.10±4.59) μmol haemoglobin/kg in unbled to (7.39±2.93) μmol haemoglobin/kg in bled rainbow trout, from (6.07±1.02) μmol haemoglobin/kg in unbled to (3.40±0.48) μmol haemoglobin/kg in bled Atlantic mackerel (6), and from (1.01±0.19) mg haemoglobin/kg in unbled to (0.07±0.05) mg haemoglobin/kg in bled skipjack (31). In bled Asian seabass total haem content was observed as over 0.100 decrease in measured absorbance at 525 nm compared to unbled seabass (27). In Atlantic salmon and Atlantic cod counting bloodspots has been shown as reliable, non-invasive method for evaluation of residual blood (16,25,26), and for both fish significantly higher number of bloodspots was found in unbled fish compared to bled. However, contrary to the present data for raw carp meat, the haem content in Asian seabass was reported to decrease with longer storage time. This will be due to the degradation of the fish meat, which lowers the solubility of the haem proteins (11,32).
Haemoglobin is a protein made from four haem subunits, each carrying iron which enables its main function – transfer of oxygen (11). At the same time, haemoglobin also gives red colour to the blood, and consequently to the tissue in which it is present. Therefore, effective bleeding reduces the amount of residual blood in fish meat, and the colour of the meat is improved as there is less or no discoloration as a result of the breakdown of haemoglobin. Additionally, the removal of blood and thus haemoglobin is positively related to other sensory attributes, especially odour and overall acceptability of carp fillets. Thus, removal of haemoglobin from carp meat positively affects its sensory quality.

**Lipid oxidation**

The data for the two lipid oxidation parameters of lipid hydroperoxides and TBARS are given in Tables 2 and 4. For the lipid hydroperoxides, these significantly increased in both the raw bled and unbled carp fillet meat with increased storage time (p <0.001). As well as these increases in both groups over time, the lipid hydroperoxides in the bled fillets were significantly lower for all of the sampling days, compared to the unbled fillets (p <0.001). Increases in lipid hydroperoxides were also reported for Asian seabass over 15 days of storage on ice with similar initial and final values in bled and unbled seabass at day 0 and 15 (4.5 and 14 mg of hydroperoxide/kg), but with significantly lower values in the bled seabass meat during storage (27). However, contrary to the present study in carp, for the Asian seabass, the lipid hydroperoxides reached a plateau on day 9 for the unbled fillets, and on day 12 for the bled fillets. Conversely, for yellowtail muscle, the effects of bleeding on the lipid hydroperoxides depended on the muscle type: bled light (0.08 μmol of hydroperoxide/g) and dark (3 μmol of hydroperoxide/g) muscle were more pro-oxidative than unbled light (0.04 μmol of hydroperoxide/g) and dark muscle (4 μmol of hydroperoxide/g) after 72 hours (7). In washed mince of bighead carp (*Hypophthalmichthys nobilis*) added haemoglobin was shown as more prooxidative compared to added myoglobin, reaching over 80 μmol CHP/kg after day 1 of storage, which was not reached in myoglobin treated samples during all 9 days of observation. On day 3 of storage in mince treated with haemoglobin values raised to over 200 μmol CHP/kg and increased until day 5, followed by slight decrease (33). Thus, the importance of muscle type and species specificity for process of lipid oxidation are expressed. For common carp, the removal of blood and thus highly prooxidative haemoglobin proved to be positively correlated (r = 0.649, p = 0.003) with the formation of primary oxidation products.
Table 4: Lipid oxidation and microbiological parameters of the raw bled and unbled carp fillets after up to 12 days of refrigerated aerobic storage.

TBARS also increased in both the raw bled and unbled carp fillet meat with increased storage time, with significantly lower values again for the bled fillets (p <0.001). The difference here was more pronounced from the third storage day, onwards. Similar changes to TBARS were reported in previous research on Asian seabass bleeding, where after 15 days of storage TBARS in bled sample reached 10 mg MDA/mg in bled and 32 mg MDA/kg in unbled seabass (27). In rainbow trout TBARS were greater in the unbled group from day 7 to 15, and in mackerel already from day 2, but with no significance due to high variations (6). In skipjack after 2 days of storage at 2 °C no changes were seen in MDA levels, with (1.53±0.29) μmol MDA/kg in unbled and (1.35±0.17) μmol MDA/kg in bled fish (31). For common carp no high variations were observed in TBARS, additionally positive correlation (r = 0.763, p <0.001) between haem content and TBARS confirms efficiency of bleeding for delaying secondary lipid oxidation in carp.

Microbiological growth

The data for the colony counts for mesophilic and psychrotrophic bacteria in the raw bled and unbled carp fillet meat at 6.5 °C and 30 °C, respectively, are given in Tables 2 and 4. The bleeding and storage time significantly affected the growth of these bacteria in the raw carp fillets (p <0.001). On the first sampling day, the mesophilic and psychrotrophic bacteria were higher in the bled group. This was probably due to the cut for the bleeding and the overnight storage of the carp, which will have given the bacteria on the surface of the carp an easy way to invade the raw carp meat. On all of the other sampling days, the mesophilic and psychrotrophic bacteria were higher in the unbled group. Such slowing of growth of mesophilic and psychrotrophic bacteria in raw fillets of bled fish was also reported for Asian seabass, where initial counts were around 3 log CFU/g (27), showing higher initial bacterial contamination compared to common carp initial load below 2 log CFU/g. After 15 days of storage mesophilic count reached 7 log CFU/g in unbled and 6 log CFU/g in bled seabass, and psychrotrophic count 8 log CFU/g in unbled and 7 log CFU/g in bled seabass (27), showing 1 log CFU/g difference between bled and unbled fish. In common carp this difference was with 2 log CFU/g more pronounce, and until day 12 of storage, these values did not exceed 7 log CFU/g as upper acceptable limit for mesophilic count in fish meat (34). Despite of this limit, 6 log CFU/g has been shown as critical point in fish meat storage (35),
which was not exceeded in bled carp until day 12 of storage. This reduction was also likely responsible for the improvement of sensory acceptability of raw fillets from bled carp, as microbial activity is responsible for development of fishy odour, discolouration and slime development.

The data in Table 4 show that the mesophilic and psychrotrophic bacteria both increased in the same way, and reached comparable logarithmic values. This showed that the bacteria in both the bled and unbled carp fillets can grow across a wide temperature range, and that it was the same population of bacteria that grew at both temperatures. From this, it would appear that these were bacteria of the genus *Pseudomonas*, which are the main bacterial spoilers of fish meat stored under refrigerated aerobic conditions (36,37). This was later on confirmed by isolation of strains, determination of their phenotypic characteristics and sequencing for bacterial identification (Sterniša M and Smole Možina S, unpublished). In addition to their growth across a wide temperature range, the importance of the presence of iron has also been highlighted for these bacteria (38,39). Therefore, in the present study, the reduction in haem, and thus also in iron, positively contributed (mesophilic count r = 0.686, p = 0.001; psychrotrophic count r = 0.808, p <0.001) to the reduced growth rate of these bacteria in the raw fillets of the bled carp.

CONCLUSION

To conclude, this bleeding of common carp affected all of the parameters investigated in the present study. The reduction in haem in the raw carp fillets as a result of the bleeding improved the colour characteristics and sensory qualities, and it also slowed down lipid oxidation and growth of bacteria, which indicates that the residual blood is a promoter of lipid oxidation and bacterial growth in these raw carp fillets. As well as higher \( L^* \) (lightness) and lower \( a^* \) (redness) and \( b^* \) (yellowness) for the raw bled carp fillet meat, there was less discolouration. Together with the improved sensory qualities, this positively contributed to the general acceptability of common carp in the present study. The inclusion of bleeding in the processing of common carp fillets might thus provide meat of better quality that is more acceptable to consumers. However, there remains the need to determine and optimise the best bleeding technique during the slaughter of common carp. In addition, the bleeding has the potential to prolong the shelf-life of common carp fillets, which should have been further investigated.
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**Table 1.** CIELAB colour space parameters measured at the three locations for the meat of the raw bled and unbled carp fillets after up to 12 days of refrigerated aerobic storage.

<table>
<thead>
<tr>
<th>Colour parameter</th>
<th>t(storage)/day</th>
<th>Raw carp fillet meat</th>
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<td>Frontal</td>
<td>Middle</td>
<td>Caudal</td>
<td>Frontal</td>
<td>Middle</td>
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<tr>
<td>L*</td>
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<td>41.70±3.74</td>
<td>42.16±2.32</td>
<td>42.07±1.76</td>
<td>37.78±1.87</td>
<td>37.76±1.57</td>
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<td>43.98±5.27</td>
<td>41.54±1.91</td>
<td>42.70±1.62</td>
<td>36.83±2.33</td>
<td>37.46±1.76</td>
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<tr>
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<td>6</td>
<td>43.80±4.67</td>
<td>42.72±1.87</td>
<td>43.28±1.50</td>
<td>36.95±1.65</td>
<td>37.58±1.41</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>44.45±3.78</td>
<td>42.37±1.19</td>
<td>44.77±1.08</td>
<td>38.26±1.60</td>
<td>39.55±2.95</td>
</tr>
<tr>
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<td>12</td>
<td>44.75±3.90</td>
<td>42.54±1.90</td>
<td>43.62±1.20</td>
<td>37.75±2.24</td>
<td>37.56±0.98</td>
</tr>
<tr>
<td>a*</td>
<td>1</td>
<td>-1.35±0.38</td>
<td>-1.72±0.37</td>
<td>-1.26±0.65</td>
<td>2.03±1.18</td>
<td>1.64±1.37</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-1.73±0.84</td>
<td>-2.00±0.57</td>
<td>-1.11±0.46</td>
<td>3.21±1.95</td>
<td>2.47±1.98</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>-1.47±0.74</td>
<td>-1.86±0.62</td>
<td>-0.94±0.87</td>
<td>3.34±1.51</td>
<td>3.02±2.38</td>
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<tr>
<td></td>
<td>9</td>
<td>-0.96±0.92</td>
<td>-1.16±1.15</td>
<td>0.02±1.08</td>
<td>4.58±1.76</td>
<td>3.62±1.65</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>-1.60±0.92</td>
<td>-1.26±0.98</td>
<td>0.23±1.14</td>
<td>2.89±1.23</td>
<td>3.33±1.13</td>
</tr>
<tr>
<td>b*</td>
<td>1</td>
<td>2.68±1.08</td>
<td>2.15±1.51</td>
<td>3.45±0.83</td>
<td>5.58±0.78</td>
<td>5.52±1.22</td>
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<tr>
<td></td>
<td>3</td>
<td>3.43±1.93</td>
<td>2.77±1.02</td>
<td>4.86±0.41</td>
<td>6.42±1.84</td>
<td>6.36±1.63</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4.01±1.47</td>
<td>2.33±0.68</td>
<td>4.67±0.83</td>
<td>6.47±1.29</td>
<td>6.36±1.70</td>
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<tr>
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<td>9</td>
<td>4.21±1.09</td>
<td>3.55±1.13</td>
<td>5.79±1.31</td>
<td>6.79±1.32</td>
<td>6.85±0.97</td>
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<tr>
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<td>12</td>
<td>4.38±1.34</td>
<td>4.41±0.75</td>
<td>6.31±0.87</td>
<td>5.83±1.08</td>
<td>7.01±0.52</td>
</tr>
</tbody>
</table>

Data are means±standard deviation

*L*: Significantly affected by Group (p<0.001)

*a*: Significantly affected by Group, Location and Time (p<0.001); significant interactions between Group and Location (p<0.01) and Group and Time (p<0.05)

*b*: Significantly affected by Group, Location and Time (p<0.001); significant interactions between Group and Location (p<0.05)
Table 2. Effect of bleeding, time and their interaction on quality attributes and effect of location and interactions for colour parameters of bled and unbled carp fillets during the storage using GLM.

<table>
<thead>
<tr>
<th>Quality attribute</th>
<th>Bleeding x Time</th>
<th>Bleeding x Location</th>
<th>Bleeding x Time x Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>a*</td>
<td>***</td>
<td>***</td>
<td>*</td>
</tr>
<tr>
<td>b*</td>
<td>***</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td>Lipid hydroperoxides</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>TBARS</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Mesophilic bacteria</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Psychrotrophic bacteria</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

TBARS, thiobarbituric acid reactive substances

Within each row, for each factor and their interactions: *, p <0.05; **, p <0.01; ***, p <0.001; NS, not significant (p >0.05)
Table 3. Sensory attributes comparison of raw and cooked carp fillets between bled and unbled group during the storage using Mann-Whitney U test.

<table>
<thead>
<tr>
<th>Fillet</th>
<th>Sensory attribute</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Me</td>
<td>IQR</td>
<td>U</td>
<td>Me</td>
<td>IQR</td>
<td>U</td>
</tr>
<tr>
<td>Raw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Texture</td>
<td>B</td>
<td>5</td>
<td>1</td>
<td>NS</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>UB</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Colour</td>
<td>B</td>
<td>5</td>
<td>1</td>
<td>***</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>UB</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Odour</td>
<td>B</td>
<td>5</td>
<td>1</td>
<td>NS</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>UB</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Overall</td>
<td>B</td>
<td>5</td>
<td>0</td>
<td>***</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>acceptability</td>
<td>UB</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>3.75</td>
</tr>
<tr>
<td>Cooked</td>
<td>Consistency</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>5</td>
<td>0.5</td>
<td>NS</td>
<td>4.5</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td>UB</td>
<td>5</td>
<td>0.5</td>
<td>4.5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Odour</td>
<td>B</td>
<td>4.75</td>
<td>1</td>
<td>NS</td>
<td>4.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>UB</td>
<td>4.5</td>
<td>0.5</td>
<td>4.25</td>
<td>1.5</td>
<td>4</td>
</tr>
<tr>
<td>Flavour</td>
<td>B</td>
<td>4.5</td>
<td>1.38</td>
<td>NS</td>
<td>4</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>UB</td>
<td>4.5</td>
<td>1</td>
<td>4.25</td>
<td>1.88</td>
<td>3.75</td>
</tr>
<tr>
<td>Aftertaste</td>
<td>B</td>
<td>4.5</td>
<td>1</td>
<td>NS</td>
<td>4</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>UB</td>
<td>4.5</td>
<td>1</td>
<td>4.5</td>
<td>1.38</td>
<td>3.75</td>
</tr>
</tbody>
</table>

B, bled; UB, unbled; T, comparison of sensory attribute between sampling days; Me, median; IQR, interquartile range, U, results of Mann-Whitney U test; H, results of Kruskal-Wallis H test.

For U and H: *, p <0.05; **, p <0.01; ***, p <0.001; NS, not significant (p >0.05).
Table 4. Lipid oxidation and microbiological parameters of the raw bled and unbled carp fillets after up to 12 days of refrigerated aerobic storage.

<table>
<thead>
<tr>
<th>(storage)/day</th>
<th>Lipid oxidation parameters</th>
<th>Microbiological parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n(CHP)/m(fillet))/ (μmol/kg)</td>
<td>(n(MDA)/m(fillet))/ (μmol/kg)</td>
</tr>
<tr>
<td></td>
<td>Bled</td>
<td>Unbled</td>
</tr>
<tr>
<td>1</td>
<td>12.38±1.73</td>
<td>16.37±1.80</td>
</tr>
<tr>
<td>3</td>
<td>21.55±2.19</td>
<td>33.48±3.50</td>
</tr>
<tr>
<td>6</td>
<td>26.38±1.63</td>
<td>37.00±2.46</td>
</tr>
<tr>
<td>9</td>
<td>36.08±1.84</td>
<td>55.92±2.76</td>
</tr>
<tr>
<td>12</td>
<td>62.13±2.86</td>
<td>88.88±4.19</td>
</tr>
</tbody>
</table>

Data are means±standard deviation

CHP, cumene hydroperoxide; MDA, malondialdehyde; CFU, colony-forming units

Parameters significantly affected by Group, Time and their interactions (p <0.001)
Fig. 2. Sensory quality of the raw (a) and cooked (b) bled (shades of blue) and unbled (shades of red) common carp fillets on storage days 1, 3, 6 and 9 under refrigerated aerobic storage.