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Lipid and protein oxidation in ostrich meat under various packaging types during refrigerated storage and *in vitro* gastrointestinal digestion*

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The aim of the study was to evaluate lipid and protein oxidation during refrigerated storage (0, 4, 8, 12 and 16 days) and *in vitro* gastrointestinal digestion of ostrich meat (*M. iliofibularis*), which was packed either under vacuum (VP) or modified atmosphere (MAP) in two combination of gases O_2:CO_2:N_2, being 40:40:20 (MAP 1) and 60:30:10 (MAP 2). Lipid oxidation was assessed by malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), and hexanal (HEX), and protein oxidation was evaluated by the formation of protein **carbonyl content compounds (**PCC**). In raw ostrich meat packed under both MAP conditions, an increase of MDA and 4-HNE levels was observed during storage, while the MDA value under vacuum was generally stable during 16-day storage. The PCC concentration under MAP1 and MAP2 after *in vitro* digestion was at the comparable level during the storage period. In summary, packaging and storage methods influenced the development of oxidation processes affecting the final quality of ostrich meat during storage.**

KEY WORDS: *in vitro* digestion / lipid / meat / ostrich / packaging system / protein oxidation / storage

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Ostrich meat is characterized by low fat level, with a high proportion of polyunsaturated fatty acids (PUFA) [Horbańczuk *et al*. 1998, 2018, 2019]. It becomes popular among consumers worldwide [Cooper *et al*. 2004, 2007, 2008, Horbańczuk *et al*. 2007, 2008, 2021ab]. This dark red meat is also characterized by higher pH (about 6) as compared to beef or pork [Sales and Horbańczuk 1998, Sales *et al*. 1999, Horbańczuk and Wierzbicka 2016, 2017]. Above mentioned features negatively affected the quality of ostrich meat during storage [Fernandez-Lopez *et al.* 2006]. Ostrich meat is also rich in heme iron, the pro-oxidative factor which may promote the degradation of lipids and proteins, leading to undesirable aromas and off-flavors during storage [Hoffman *et al*. 2014]. Lipid and protein oxidation in meat damages the cellular structures of the muscle, which results in loss of the meat quality and its decreased shelf life [Vuorela *et al*. 2005, Seydim *et al*. 2006].

The most common markers of oxidative processes are malondialdehyde (MDA, 4-hydroxy-2-nonenal (4-HNE), hexanal (HXE) and protein carbonyl content (*PCC*). Malondialdehyde is formed during the decomposition of lipid hydroxides produced during peroxidation of polyunsaturated fatty acids (especially of long-chain polyunsaturated fatty acids), whereas, 4-HNE is the product of the oxidative breakdown of hydroperoxides derived from n-6 acids.

Oxidation continues during digestion in the gastrointestinal tract. Method of meat *in vitro* digestion simulates the physiological conditions of digestion *in vivo* and is very useful for studying and understanding changes, interactions, as well as the bioaccessibility of nutrients and non-nutritive compounds [Bornhorst *et al*. 2014, Van Hecke *et al.* 2014, 2015, 2017b, 2019, Lucas-González *et al.* 2018, Li *et al*. 2020]. The oxidation products emerging during the meat digestion processes may negatively affect health status of the consumers. The aim of the study was to assess the changes of lipid- (MDA, 4-HNE, HEX) and protein oxidation (*PCC*) during refrigerated storage of ostrich meat in various packaging types, i.e. vacuum (VP) and modified atmosphere (MAP) in two combination of gases O_2:CO_2:N_2 i.e. 40:40:20 (MAP 1) and 60:30:10 (MAP 2). In order to investigate the lipid and protein oxidation in ostrich meat also during gastrointestinal digestion, *in vitro* digestion model was applied.

Material and methods

Samples and packaging

Meat samples were obtained from the *M. iliofibularis* from ostrich (8 in each group) slaughtered at the age of 10-12 months, weighing between 90 and 95 kg. Muscles were collected from carcasses 24 h after slaughter. External fat and visible connective tissue were removed and muscles were cut starting from the proximal side into 2.5 cm thick steaks (sample weight: 150±15 g). Then, each group of steaks (from 8 ostriches) was either packed under vacuum or under two conditions of modified atmosphere packaging (MAP). Vacuum packaging was performed within 1 min after

cut by using a Vac-20SL2A packaging machine (Edesa Hostelera S.A., Barcelona, Spain), during which each steak was packed individually in polyamide/polyethylene bags (thickness 90μm [20/70], oxygen permeability 50 cm^3/m2/24 h, CO_2 permeability 140 cm^3/m2/24 h, water vapor permeability 6-8 g/m2/24 h). The in-package vacuum level was 2.5 kPa. Modified atmosphere packaging (MAP) was performed in two combinations of O_2:CO_2:N_2 gases in ratios of 40:40:20 (MAP1) and 60:30:10 (MAP2). The steaks were placed on PET/PE trays (parameters: 187×137×50 mm), and the film used was a 44 μm thick PET/CPP + AF laminate with maximum oxygen permeability not exceeding 10 cm^3/m^2/24 h/bar (EC04, Corenso, Helsinki, Finland). Samples were packed with an M3 packaging machine (Sealpack, Oldenburg, Germany). The muscle samples were stored in a refrigerator at 2°C and samples were collected on the days 0 (24 h after slaughter), 4, 8, 12 and 16 of refrigerated storage.

In vitro gastrointestinal digestion

The *in vitro* digestions were performed according to a previously described protocol, specific for studying oxidation processes during passage in the gastrointestinal system [Van Hecke *et al*. 2018]. The digestions were performed in quadruplicate. In brief, 4.5 g of ostrich meat was sequentially incubated at 37°C for 5 min with 6 mL saliva, 2 h with 12 mL gastric juice (initial stomach pH of 2.5 to final stomach pH of 3.5), and 2 h with 2 mL bicarbonate buffer (1 m, pH 8.0), 12 mL duodenal juice, and 6 mL bile juice (small intestinal pH of 6.5). After completion, samples were homogenized with an ultra-turrax (9500 rpm) and aliquots were stored at -80°C for further analysis.

Oxidation products in meat and digests

Malondialdehyde (free + bound) was measured spectrophotometrically in meat and digests as TBARS (thiobarbituric acid reactive substances) at 532 nm following the reaction with 2-thiobarbituric acid in an acid environment after hydrolysis with NaOH, extraction in 1-butanol, and quantification using a standard curve with 1,1,3,3-tetramethoxypropane and concentrations are expressed as nmol MDA/ml. [Van Hecke 2017a]. Unbound 4-HNE and HEX in raw ostrich meat and digests were measured by HPLC following their derivatization with cyclohexanedione, as previously described [Van Hecke 2017a] with slight modifications, and each compound was quantified with corresponding analytical standards. Concentrations of PCC in raw ostrich meat and digests were determined spectrophotometrically following reaction with 2,4-dinitrophenylhydrazine according to Ganhão *et al.* [2010]

Statistical analysis

A generalized mixed model analysis (repeated measures ANOVA) was performed on all measured parameters in order to determine the fixed effect of packaging treatment and storage time as a repeated measure, as well as their interaction, separately for the raw stored ostrich meat and *in vitro* digested ostrich meat (SAS v 9.4). Ostriches' identity (bird number) was included in the model as a random factor. There were

no outliers present in the dataset. Normality and homogeneity of residual variance assumptions were checked using the Shapiro test and examination of the normal plot, and these were met by all variables under investigation. The Tukey's adjustment option was used to conduct the analysis. For all analyses, results are reported as means ± standard error of the mean (SEM).

Results and discussion

The changes in MDA, 4-HNE, HEX and PCC values in raw and after *in vitro* digestion ostrich meat samples, as influenced by type of packaging and refrigerated storage are shown in Figure 1-4.

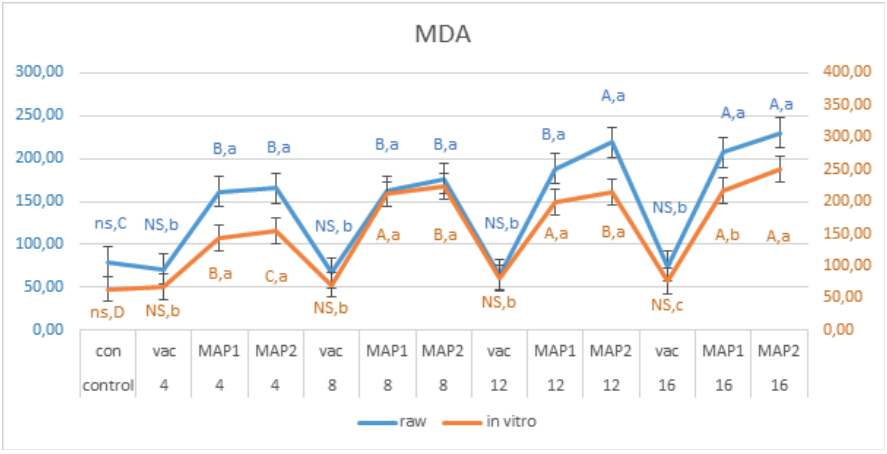

Fig. 1. Levels of MDA (nmol /g) in raw and *in vitro* digestion ostrich samples, as related to the type of packaging and refrigerated storage. Mean values bearing different letters for each day within the same packaging conditions (A, B, C, D), or between packaging systems within the same day (a, b, c,). differ significantly at $p<0.05$.

A significant increase of MDA and 4-HNE levels were observed along the storage of meat packed in MAP1 and MAP2. The MDA value in ostrich raw meat under vacuum was generally stable during 16-day storage, and no significant differences were observed across days of storage. Similar results for ostrich meat packed in vacuum and modified atmosphere without oxygen, stored at 2°C until day 8th was reached by Fernandez-Lopez *et al*. [2008]. Moreover, in the study conducted by Seydim *et al.* [2006] ostrich meat, which was vacuum packed and stored at 4°C up to 9 days, was characterized by lower changes regarding lipid oxidation processes as compared to air packaging system. Overall, the value of 4-HNE increased under MAP1 and MAP2, whereas in VAC system it was stable during storage. This is in line with the results obtained for goose meat stored under vacuum and modified atmosphere conditions which had a higher oxidation rate for MAPs than vacuum [Orkusz *et al*. 2017]. The

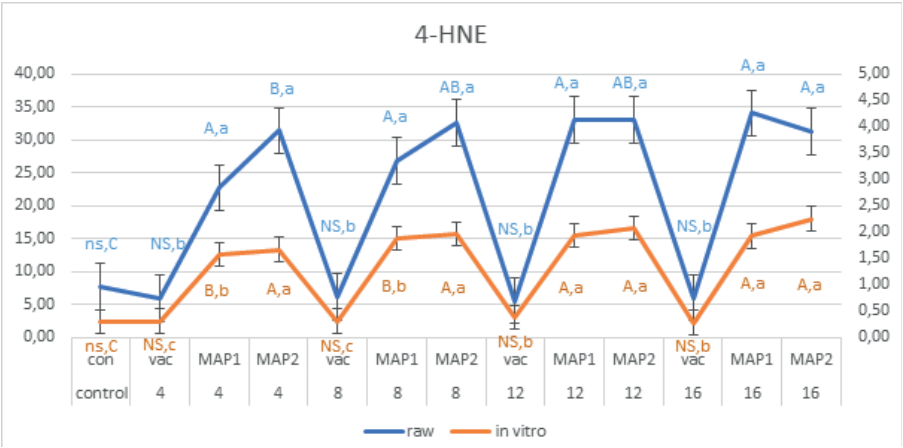

Fig. 2. Levels of 4-HNE (µmol/kg meat) value in raw and in vitro digestion ostrich samples, as related to the type of packaging and refrigerated storage. Mean values bearing different letters for each day (A, B, C, NS-not significant) or between packaging systems (a, b, c, ns-not significant) differ significantly at $p<0.05$.

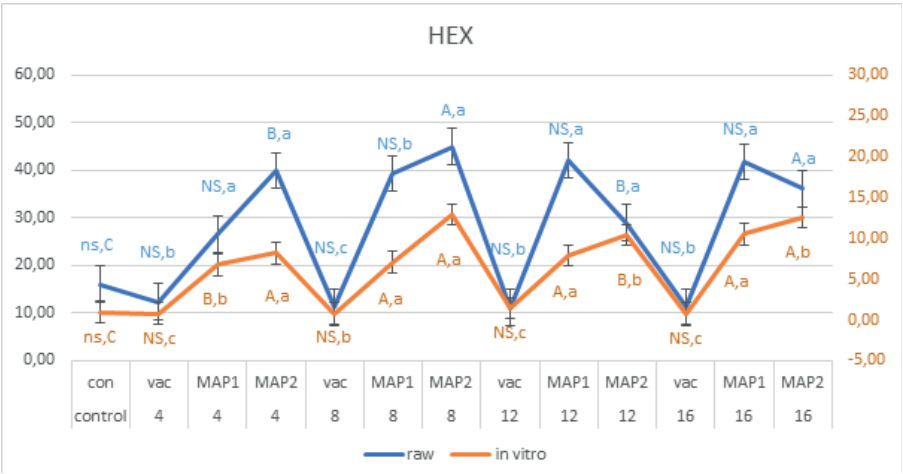

Fig. 3. Levels of HEX (µmol/kg meat) value in raw and in vitro digestion ostrich samples, as related to the type of packaging and refrigerated storage. Mean values bearing different letters for each day (A, B, C, NS-not significant) or between packaging systems (a, b, c, ns-not significant) differ significantly at $p<0.05$.

HEX under MAP1 raised during storage days, while under MAP2 on the 8th day, a statistically significant raise of this parameter level was observed, as compared to previous storage days. PCC level (Fig. 4) in raw meat samples under MAP2 grew unsteadily over the days until the level of 4.76 nmol DNPH/mg protein on the last storage day (16).

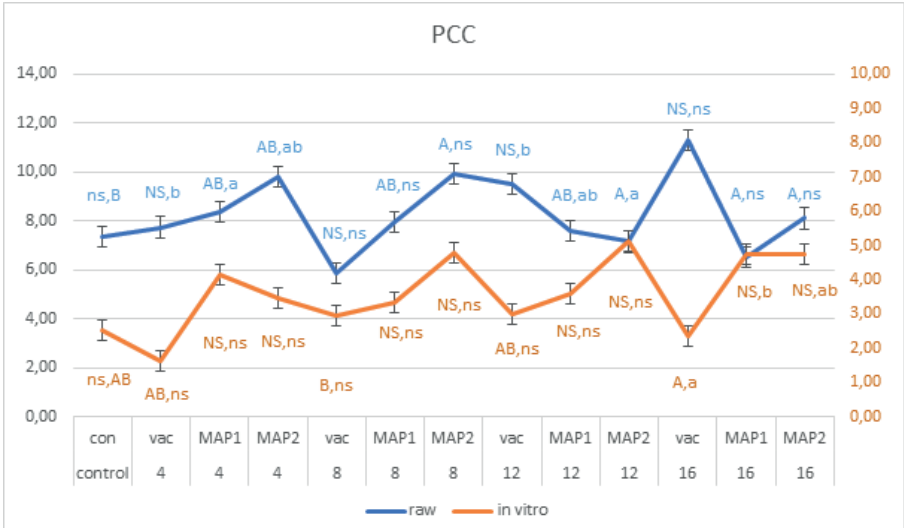

Fig. 4. Levels of PCC (nmol DNPH/mg protein) levels in raw and in vitro digestion ostrich samples, as related to the type of packaging and refrigerated storage. Mean values bearing different letters for each day (A, B, C, NS-not significant) or between packaging systems (a, b, c, ns-not significant) differ significantly at $p<0.05$.

Values of all measured parameters were significantly higher on all experimental days under both MAP types of packaging, as compared to vacuum one, except for days 8 and 12 for PCC, when no significant differences between methods were observed. These results are in line with shelf-life duration of vacuum-packed ostrich meat, as described by Capita *et al.* [2018].

The significant effect of the interaction between packaging method and day in *in vitro* digested ostrich meat samples was observed for the MDA and 4-HNE (Fig. 1 and 2). An increase of the MDA levels after *in vitro* digestion of the meat samples between all control points was observed under MAP 2 packaging method. Similarly, in MAP1 the MDA levels increased regularly over the experimental days, and no significant difference in the MDA levels were observed between day 8 and 12 (Fig. 1). 4-HNE levels were significantly lower under vacuum at the onset of the experiment (day 0), as compared to all other experimental days, both in MAP1 and MAP2 (Fig. 2). However, no significant differences in 4-HNE levels were observed along storage time in vacuum packed samples. The MDA values in MAP and MAP2 after digestion *in vitro* on the final experimental day (16) increased significantly in comparison with the day 0. In case of 4-HNE and HEX, their values raised significantly during storage time, either in samples after digestion process or in the raw ones. The results obtained in our study are generally in agreement with the results of digested chicken meat samples achieved by Sobral *et al.* [2020]. The value of HEX in the digested samples under MAP1 and MAP2 was over three times higher at the end of storage (day 16th),

as compared to the raw meat samples (Fig. 3). The PCC value for MAP1 and MAP2 after *in vitro* digestion was generally at the same level during the storage period (Fig. 4). Furthermore, according to results of Filgueras *et al.* [2011] the storage time had less impact than cooking on protein changes in rhea meat.

Conclusion

Based on obtained results in our study, it can be concluded that the effect of the interaction between packaging method and day in raw and after *in vitro* digestion of ostrich meat was observed for the MDA, 4-HNE and HEX parameters. In MAP 1 and MAP2 the increase of MDA and 4HNE levels was observed during storage, whereas the MDA value in ostrich raw meat in vacuum it was generally on the same level along 16-day storage. The value of 4-HNE overall increased in MAP1 and MAP2, whereas in VAC system it was stable during storage time. The PCC values for MAP1 and MAP2 after in vitro digestion were generally at the same level during the storage period.

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