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Lipid- and protein oxidation during storage and *in vitro* gastrointestinal digestion of ostrich, beef and chicken jerky snacks*

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The oxidative stability of jerky made from ostrich, beef and chicken meat with (sea salt or tomato-pepper mixture) or without additives during storage (room temperature, 9 months) and after *in vitro* gastrointestinal digestion was investigated. Lipid oxidation (thiobarbituric acid reactive substances and hexanal) and protein oxidation (protein carbonyl compounds) were evaluated in both jerky and gastrointestinal digests. Lower lipid oxidation was observed in jerky with flavor additives. The susceptibility to oxidation was the highest in ostrich, lower in beef and the lowest in chicken jerky. Nine months storage time increased both lipid and protein oxidation products levels,

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further increased by the *in vitro* digestion of jerky. We showed, that shelf-life of jerky depends on the species while some additives (tomato-pepper mixture) that can inhibit the oxidation process in jerky may not warrant their antioxidant effect after digestion.

KEY WORDS: dry meat / flavor additives / hexanal / protein carbonyl compounds / TBARS

Drying as a form of food preservation is one of the oldest methods available to humanity [Akhtar and Pandey 2015]. According to Sørensen [2009], Neanderthals already had the ability to dry the meat which they hunted during summer, in order to transfer it to the winter settlements without the risk of it getting spoiled. According to Rodriguez-Hidalgo *et al*. [2015], drying meat in Europe can be dated to the Middle Pleistocene, probably prior to the time when *Homo sapiens* was present on the old continent. Removing the water from the meat made the transport easier by reducing the meat size and weight (about three times) and limited bacterial growth [Akhtar and Pandey 2015]. Soffer [1989] indicates that it was the ability to handle meat and its drying that enabled high mobility of people in the late Paleolithic era. Drying meat gave many possibilities (easier transport and increased mobility, lower risk of spoilage, concentration of nutrients) thanks to which this method of preservation gained great recognition and was used in various forms on all continents, while the modern jerky originates from this old method.

Currently, the production of dried meat is not forced by the need to secure the product. There are many possibilities for food preservation these days, and meat's long-term viability is not a challenge. Despite this, dried meat is still produced because it is part of the snack food which is characterized by a high concentration of nutrients, especially protein [Zdanowska-Sąsiadek *et al*. 2018].

Meat of different animal species is characterized by different susceptibility to oxidation which may be similar in the case of jerky. Lipid stability of meat mainly depends on the balance of antioxidants, oxidation substrate, cholesterol content, as well as heme pigment [Sohaib *et al*. 2017]. Ostrich (high heme-Fe and high PUFAs content), beef (high heme-Fe but low PUFAs) and chicken (low heme-Fe but high PUFAs) meat show different sensitivity to oxidation [Min *et al*. 2010, Nair *et al*. 2014, Cooper and Horbańczuk 2004, Horbańczuk *et al.* 2007, 2008, Kawka *et al.* 2007, Cooper *at al.* 2008]. It may be suggested that similar results will be observed in jerky. In order to reduce oxidative processes in meat, many authors point to the use of antioxidant additives. The antioxidant properties of vegetables and herbs like tomatoes (lycopene), peppers, oregano have been extensively described [Van Hecke *et al*. 2017, Tewari *et al.* 2017ab, Al-Hijazeen *et al*. 2018, Aminzare *et al*. 2018, Mozos *et al.* 2018, 2021, Wang *et al.* 2018, 2020, Yeung *et al*. 2018, 2019, 2020abc, 2021ab, Pieczyńska *et al*. 2020, Li *et al*. 2021, Martini *et al*. 2021, Chopra et al. 2022]. The use of these additives in jerky probably can positively affect reduction of lipid and protein oxidation and at the same time enrich jerky's taste. During drying, water is removed from meat, thanks to which its activity significantly decreases, which contributes to a significant extension of the shelf life of meat. Jerky can be stored for several months, but in order to determine the maximum storage time, a number of factors should be taken into account, including type

of meat or type of muscle (oxidative or glycolytic muscles), the degree of drying (water content), as well as presence of antioxidant additives, both natural and also inorganic chemicals (such as sodium nitrate). Due to the fact that the most important issue of lipid oxidation is generation of harmful compounds that implicate several human diet-related diseases, including atherosclerosis, cancer, inflammation and aging processes, among others [Alfaia *et al*. 2010, Van Hecke *et al*. 2014], it also seems very important to determine the level of oxidation after jerky digestion.

Rate and degree of jerky oxidation depends on the species, moreover, the use of flavor additives such as salt or herbs-vegetables mixtures may change the dynamics of oxidation.

The aim of the current study was to assess oxidative stability of jerky made from ostrich, beef and chicken with or without flavor additives during nine-month storage and *in vitro* digestion.

Material and methods

Preparation of the jerky samples

Jerkies were produced according to Horbańczuk *et al*. [2015] from one type of muscle, obtained from nine animals per species: ostrich (*m. ambiens*), beef (*m. semimembranosus*), and broiler chicken (*m. pectoralis major*). The exact procedure as well as the chemical composition, heme-Fe content and fatty acid profile of jerky were determined and published [Zdanowska-Sąsiadek *et al*. 2018]. After drying jerkies were divided according to the scheme: 1 – groups with no flavor additives: natural chicken, natural beef and natural ostrich; 2 – groups sprinkled with flakes of sea salt: salted chicken, salted beef and salted ostrich; 3 – groups sprinkled with mix of dried tomatoes with pepper: spiced chicken, spiced beef and spiced ostrich. All jerky were packed separately into 50g bags, and stored in vacuum. Analyses were performed on freshly produced jerky samples and stored samples. Due to the lack of preservatives in the tested jerky, a storage period has been set for 9 months at 21°C. In industrial conditions, when preservatives (e.g. potassium sorbate or sodium nitrite) are used, the jerky has a shelf life of 2 years.

In vitro digestion

In vitro digestion of the jerky after preparation and after 9 months storage was performed according to a previously described protocol, specifically designed for studying oxidation processes during passage in the gastrointestinal system [Van Hecke *et al.* 2014]. Digestions consisted of an enzymatic digestion simulating the mouth, stomach and duodenum gastro-intestinal tract compartments. Briefly, jerky samples (4.5 g) were sequentially incubated at 37°C for 5 min with 6 mL saliva, 2 h with 12 mL gastric juice, and 2 h with 2 mL bicarbonate buffer (1 M, pH 8.0), 12 mL duodenal juice and 6 mL bile juice. The incubations were performed in triplicate. After completion, samples were homogenised with an Ultra Turrax (9500 rpm) and aliquots were stored in Eppendorf tubes at -80°C pending analysis.

Oxidation products

TBARS. Total (free + bound) malondialdehyde (MDA) concentrations in jerky samples and digests were measured colorimetrically as thiobarbituric acid reactive substances (TBARS), by a modified method in accordance with Grotto *et al.* [2007]. Results were expressed as μg of MDA per g of jerky.

Hexanal. Level of hexanal (HEX) were analyzed in jerky samples and digests following the formation of their fluorescent derivatives with 1,3-cyclohexanedione through HPLC, based on the method by Holley *et al*. [1993] and adaptations by Van Hecke *et al.* [2017].

Total carbonyls. Protein carbonyl compounds (PCC) were determined by subjecting meat and digest samples to 2,4- dinitrophenylhydrazine (DNPH) derivatization as described by Ganhãoa *et al.* [2010]. Protein concentration of the samples (mg mL^{-1}) was determined spectrophotometrically at 280 nm. DNP hydrazine absorbance was measured at 370 nm, and protein carbonyl concentration (nmol mL^{-1}) was calculated as [A_{370}/ $\varepsilon_{hydrazone}$*10^6], where $\varepsilon_{hydrazone}$ is 22000 M^{-1} cm^{-1}. Protein carbonyl concentration expressed as nmol of carbonyl per mg of protein was calculated from the above-mentioned concentrations, and DNPH-treated samples were corrected by subtracting the protein carbonyl concentration of their blank equivalents.

Statistical analysis

A generalized linear mixed model analysis, using repeated measures ANOVA, was performed on all measured parameters, including flavor group and species of jerky as fixed factors and storage time as a repeated measure as well as their interactions. The validity of the models was tested by using Akaike's information criterion. Animal identity was included into 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-Wilk test and examination of the normal plot and it was met by all variables under investigation. PROC GLIMMIX of SAS v 9.3 (SAS Institute Inc., Cary, NC, USA) including the Tukey adjustment option was used to conduct the analysis. The least square means for all significant effects in the models ($p \leq 0.05$) were computed using the LSMEANS option. The trend of a significant effect was considered for $p < 0.10$.

Results and discussion

Lipid and protein oxidation in fresh and stored jerky

Jerky used in the study was characterized by different oxidative stability both within lipids (TBARS, HEX) and proteins (PCC) (Fig. 1). In general ostrich jerky contained higher lipid- and protein oxidation products, compared to beef jerky, demonstrated by higher TBARS (3 to 6-fold) and HEX (4- to 8-fold) and compared to chicken jerky, demonstrated by higher TBARS (7- to 50-fold), HEX (2- to almost 5-fold) and PCC (2- to 7-fold). Storage for 9 months generally increased oxidation by increasing TBARS average 5-fold and PCC average 3-fold. Overall, flavor additives

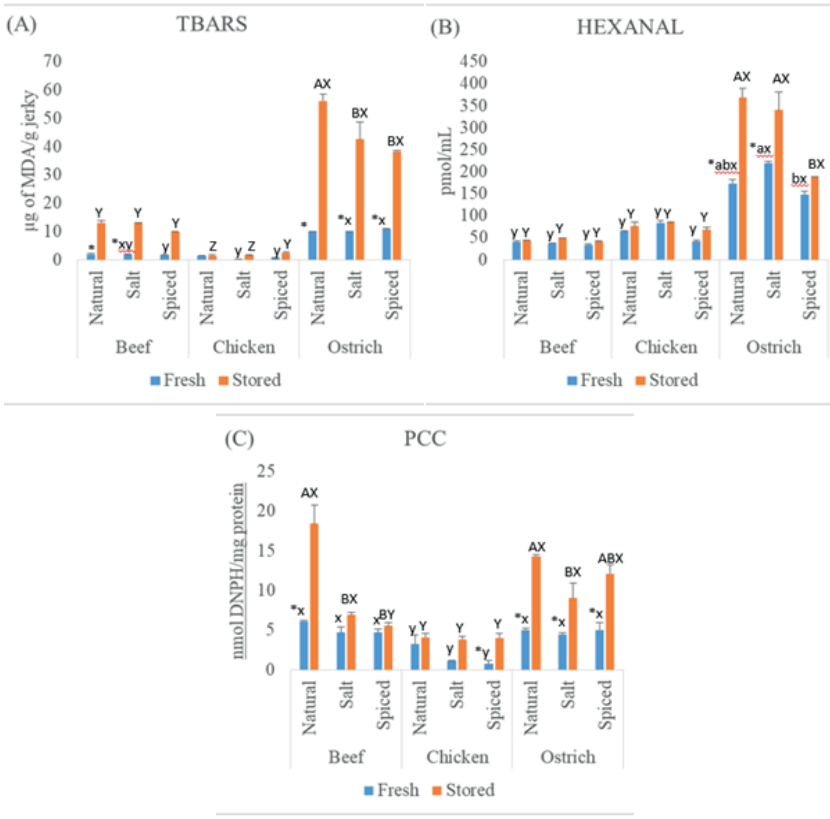

Fig. 1. Lipid and protein oxidation in fresh and stored beef, chicken and ostrich jerky (mean±SEM). PCC – protein carbonyl compounds; a, b – different letters in the bars within the same species for different flavor additives in fresh jerky indicate significant differences at P<0.05; A, B – different letters in the bars within the same species for different flavor additives in storage jerky indicate significant differences at P<0.05; x, y – different letters in the bars within the same flavor additives for different species in fresh jerky indicate significant differences at P<0.05; X, Y, Z – different letters in the bars within the same flavor additives for different species in storage jerky indicate significant differences at P<0.05; * – differences between fresh and storage jerky within the same species and flavor additives at P<0.05.

showed antioxidant properties. Use of salt resulted in average reduction of TBARS by 6% and PCC by 35% while spice reduction of TBARS by 7%, HEX by 22% and PCC by 30%. It should be noted that species plays an important role in the course of oxidative processes in jerky. Use of flavor additives such as salt, herbs or vegetables in the production of jerky has a positive effect on the inhibition of oxidation processes, and the amount of oxidation products increases with time.

Lipid and protein oxidation in fresh and stored jerky after in vitro digestion

The degree of lipid and protein oxidation after *in vitro* digestion of fresh and stored jerky is shown in Figure 2. In general *in vitro* digestion increase lipid and

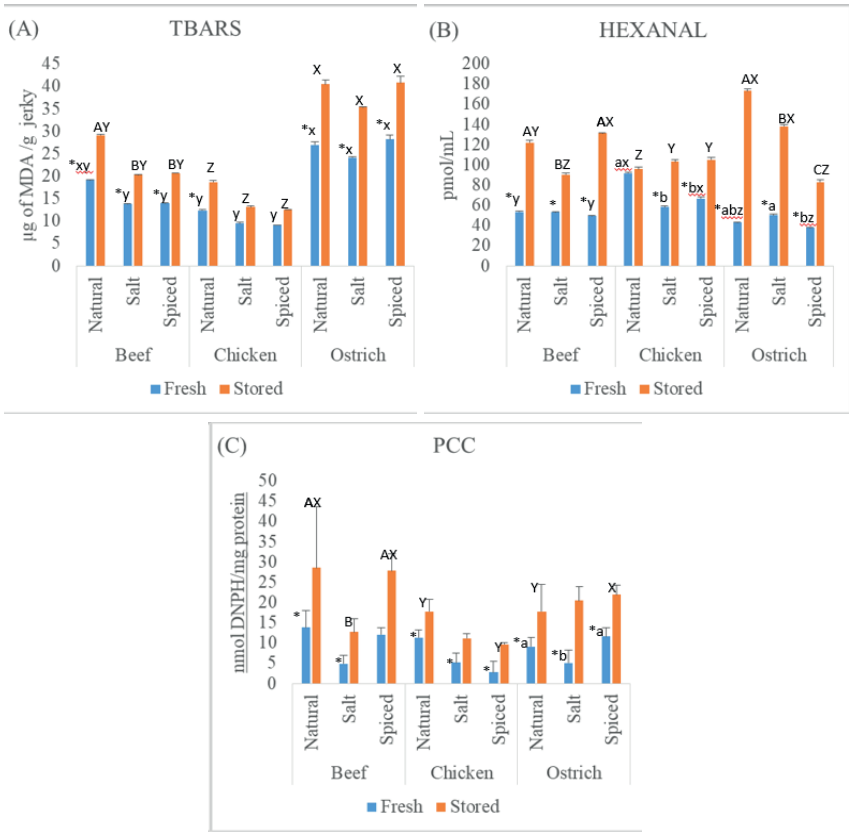

Fig. 2. Lipid and protein oxidation in fresh and stored beef, chicken and ostrich jerky after in vitro digestion (mean±SEM). PCC – protein carbonyl compounds; a,b – different letters in the bars within the same species for different flavor additives in fresh jerky after digestion indicate significant differences at P<0.05; A, B, C – different letters in the bars within the same species for different flavor additives in storage jerky after digestion indicate significant differences at P<0.05; x, y, z – different letters in the bars within the same flavor additives for different species in fresh jerky after digestion indicate significant differences at P<0.05; X, Y, Z – different letters in the bars within the same flavor additives for different species in storage jerky after digestion indicate significant differences at P<0.05; * – differences between fresh and storage jerky after digestion within the same species and flavor additives at P<0.05.

protein oxidation however, some relationships were observed which were not found in stored jerky. The antioxidant effect of salt and tomato-pepper additive on TBARS in digested stored beef was observed which was not noted in stored jerky. Inhibition effect of all measured oxidation parameters was observed in stored beef after addition of salt flakes. In this group an increase in TBARS, HEX and PCC was also observed, but it was limited compared to the analogous natural and spice group. It should be mentioned that the inhibitory effect of salt in fresh beef was only observed for PCC. The antioxidant effect of salt was also observed for PCC in fresh ostrich and for HEX in fresh chicken. It may be interesting that the antioxidant character of the tomato-pepper additive was

eliminated after *in vitro* digestion of beef on PCC of the stored samples (Fig. 1C vs 2C). The result, that deviates from the previously shown trends is the level of HEX for *in vitro* digestion ostrich. In all ostrich groups there was a decrease in HEX after *in vitro* digestion, in the fresh groups it was 4-fold decrease and in the stored 2-fold . In the stored jerky the highest level of lipid and protein oxidation products was found in ostrich groups (Fig. 1). After *in vitro* digestion, this effect was only observed in relation to TBARS. The storage time influenced the growth of lipid and protein oxidation after *in vitro* digestion. In many studied groups, similarly to jerky, the protective role of salt and tomato-pepper mixture was observed after *in vitro* digestion, although, as noted earlier, the antioxidant effect of flavor additives was not always confirmed.

The study was conducted to investigate the oxidative stability of jerky prepared from three species: chicken, ostrich, beef, before and after 9 months storage. The most important finding was that chicken breast meat has the highest oxidative stability, regardless of the use of preservatives, in this case, salt or dried tomatoes in combination with colored pepper, as compared to meat from other species. The reason may be the difference in the content of heme pigment, since its higher content in meat causes an increased production of hydrogen peroxide (H_2O_2) during oxymyoglobin autooxidation. Raw beef contained up to 220 mg kg^{-1} heme iron [Valenzuela *et al*. 2009], raw ostrich meat around 400 mg kg^{-1} [Sales and Horbańczuk, 1998], while raw poultry meat even ten times less [Min *et al*. 2008]. Earlier Zdanowska-Sąsiadek *et al.* [2018] confirmed similar differences in heme iron content in jerky from poultry and beef. These authors also showed that the heme iron content in dried ostrich meat was even twice as high (858 mg kg-1) as compared to beef. Apart from the differences in the level of heme iron, a different profile of fatty acids was observed in the examined jerky samples. Beef jerky was low in polyunsaturated fatty acids while chicken and ostrich jerkies were rich sources of PUFA [Zdanowska-Sąsiadek *et al.,* 2018]. This may explain the lowest oxidative stability of ostrich meat obtained in this study. Analysis of jerky made from ostrich meat showed the highest level of the lipid oxidation. This is due to the fact that H_2O_2 can react with metmyoglobin to generate ferryl myoglobin, which in turn initiates lipid peroxidation. The susceptibility of muscle lipids to peroxidation depends on the PUFA content and the simultaneous intensity of the formation of ferryl myoglobin through the interaction of H_2O_2 with metmyoglobin. It is because ferryl myoglobin can extract a hydrogen atom to initiate lipid peroxidation [Baron and Andersen 2002, Møller and Skibsted 2006]. This may explain the lowest TBARS in jerky and in *in vitro* digested jerky produced from chicken meat. Although chicken meat overall contains more fat compared to the ostrich meat, it contains less PUFA, especially PUFA n-3 [Horbańczuk *et al.*, 1998] and significantly lower level of heme pigments that limits the formation of ferryl myoglobin and initiation of oxidation processes.

The level of toxic aldehydes such as hexanal, which are formed as a result of damage to n-6 PUFA, as well as PCC which are products of protein oxidation, can be used as indicators of which meat species and which jerky production technology is the

most beneficial and safest for humans. Our research clearly showed that chicken jerky contained the least PCC of all meat species. We also obtained such results after *in vitro* digestion. This is of great importance because the gastric fluid is a strong oxidation catalyst due to its low pH and oxygen, H_2O_2, ascorbate and iron content. Mentioned unfavorable factors occurring during the digestion influenced the oxidation process with different results. An interesting observation was conducted in the storage beef jerky. Studies have shown that while in jerky, after adding the tomato-pepper mixture, a reduced level of PCC was observed compared to the control group, in digest the level of PCC was similar in both mention groups. It seems that the antioxidant properties of the tomato-pepper mixture were inhibited during digestion. Kopec *et al.* [2017] in studies showed that antioxidant compounds such as lycopene or β-carotene during *in vitro* digestion in the presence of metmyoglobin are effectively reduced by 30% and 80%, respectively. Therefore, the use of a tomato-pepper mixture may have an effective effect on jerky, but its effectiveness may be significantly inhibited after digestion, especially in the presence of a large amount of metmyoglobin, as is the case of beef. Level of lipid oxidation products in chicken jerky were relatively low. High PCC and high levels of lipid oxidation products, especially hexanal in stored jerky from ostrich meat, indicate that this meat should be stored for a shorter period or better protected by antioxidants.

Looking for the possibility of producing jerky from red meat, like beef and ostrich meat, is also directed to obtain a product with an increased iron content. Unfortunately, this is associated with a significant reduction in time during which this product can be stored without reducing its quality due to oxidation. Based on markers of oxidative stability, the use of salt flakes for ostrich reduced TBARS and PCC. Many studies showed an increase in the lipid and protein oxidation after the use of salt [Jiang *et al.* 2021; Poljanec *et al*. 2021, Zhao *et al*. 2020], while others do not show such effect [Ripollés *et al*. 2011]. The authors of the studies confirming the pro-oxidative effect of NaCl indicates that responsible mechanism of action is thought to be related to: cell membrane disruption, decreased activity of antioxidant enzymes, and the increased release of Fe^{3+} ions and the formation of ferrylmyoglobin and metmyoglobin. Amaral, de Sillva and Lannes [2018] observed a relationship between the concentration of NaCl and the intensity of oxidation. The use of less NaCl (less than 2%) resulted in an increase in oxidation, while a higher amount of salt reduced this effect.

Our research showed, that shelf-life of jerky depends on the species of meat. The most stable material for jerky production is chicken meat because it contains low level of heme iron. The higher content of heme iron in beef and ostrich jerky (which was confirmed in earlier study [Zdanowska-Sąsiadek *et al*. 2018]) resulted in the faster lipid oxidation process. It is most visible for the ostrich jerky, which high content of heme iron and PUFA is a factor of high oxidative instability. We hypothesized that red meat can be stored in jerky form without preservatives but no for so long (9 months).

Current research showed how important it is to determine the products of lipid and protein oxidation not only in jerky but also after digestion. The mechanisms that take place during the digestion process significantly changed the oxidative status

compared to the initial one, which was observed in the case of the tomato-pepper mixture. The antioxidant effect of the mixture present in the jerky was eliminated during the digestion process. It should be emphasized that different substances react differently in the digestive environment (acidic pH, action of enzymes, etc.).

Additional research on jerky from various types of meat extended by the analysis of microbiological status and activity of antioxidant enzymes seem necessary and helpful in determining the optimal time of jerky storage.

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