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Original Scientific Article

THE EFFECTS OF TWO WAYS OF STORAGE ON PHYSICOCHEMICAL CHANGES IN PHEASANT MEAT

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Summary: In this study the effects of two ways of storage by chilling and vacuum packaging on the physicochemical changes of hunted pheasant meat were determined during the refrigerate storage. Lactic acid, phosphate and pH value in breast and thigh muscle of chilled (n=10) and vacuum packed pheasants (n=10) were evaluated at 1st, 7th and 14th day after weaning. On day 7 lactic acid concentrations significantly increased in thigh (1.63 ± 0.41 g/100g; $p \leq 0.05$). On day 14, lactic acid in the non-vacuum packed thigh significantly decreased (0.73 ± 0.56g/100g; $p \leq 0.05$) compared to day 7. Significantly higher ($p \leq 0.01$) concentrations of lactic acid were recorded in vacuum packed than non-vacuum packed thigh muscles on day 14. In breast, significant difference ($p \leq 0.01$) in lactic acid between vacuum and non-vacuum packed meat was observed on day 14 (1.88 ± 0.18, respectively 1.15 ± 0.67g/100g). Increased pH value in non-vacuum packed thigh was observed on day 7 (6.49 ± 0.15g/100g), and in vacuum packed on day 14 (6.42 ± 0.13g/100g). Significantly higher concentrations of phosphates in thigh were recorded on day 7 in both groups, vacuum (0.90 ± 0.17; $p \leq 0.05$) and non-vacuum packed meat (0.91 ± 0.10; $p \leq 0.05$), in comparison with day 1. The significant decrease (0.68 ± 0.18; $p \leq 0.01$) of phosphates in thigh were observed on day 14 in non-vacuum packed thigh. In vacuum-packed thigh the phosphates showed significantly increased level (0.89 ± 0.15; $p \leq 0.05$) on day 14 compared with day 1. Our results indicate that the most suitable method of storage of pheasant meat is a combination of chilling and vacuum packing.

Key words: pheasant; meat; lactic acid; pH; vacuum

Introduction

Game birds are hunted mainly for recreational reasons (1) but have also been used as a food source. One of the worlds's most hunted and the most important game bird species in many countries in Europe, Australia, Asia, North and South America and New Zealand is the pheasant *(Phasianus colchicus).* This bird is selected for breeding stock in many countries to produce high nutritive meat (2).

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Pheasant meat ranks among highly appreciated food and mainly breast and thighs of pheasant are highly valued meat portions. Meat is characterized by a high concentration of protein and low content of intramuscular fat and low cholesterol content. Pheasant meat is considered also a good nutritional source of iron (3). These data are valuable for characterizing the nutritional quality of pheasant meat.

However, the other most important traits of quality of pheasant meat are also the pH value, lactic acid and phosphates concentrations. Lactic acid level reflects quantitative transformation

of glycogen and indicates typical or atypical processes of meat ripening (4). The pH value of a muscle is an accepted parameter to identify normal and deviating meat qualities (5). The conversion of muscle to meat is also an energy-demanding process and in the muscle after death, as well as in life, the energy is provided by splitting of ATP to ADP and inorganic phosphorus. In the muscle after death, the ATP is replenished by the conversion of ADP to ATP by the transfer of the higher energy phosphate for creatine phosphate and by degradation of glycogen (6).The maturing processes are running in muscles up to the point of time after death of game, until the supplies of glycogen and energetically valuable phosphates are available.

Physicochemical properties of meat change most intensely during the first hours after the killing of pheasants. This is an interval of most intensive glycolysis and lactic acid increase in the muscle tissues. The speed of this biochemical processes and degree of decreasing of pH values are important for optimal properties of pheasant meat.

One way to maintain the acceptable quality of pheasant meat is chilling. Chilled meat of pheasant should be stored at no more than 4°C in a hygienic manner and this temperature should be maintained throughout the supply chain. Storage of meat at low temperature is a prerequisite for the development of the major eating qualities including tenderness and flavour (7).

The length of refrigeration storage has also a significant impact on the activity of lactate dehydrogenase (LDH) isoenzymes in the blood serum of game after 7 days and the concentrations of LDH isoenzymes - LDH 4 and LDH 5 are reduced (8).

The present study is also focused on the role of vacuum packing of cold-stored pheasant meat. There are many advantages to vacuum packing. The main advantages of vacuum package are that it assists in the preservation of meat, improves tenderness during ageing process as well as shelf life of the meat. Using these modern techniques of packaging we can maintain the microbial and also the sensory quality of the products during the storage (9).

The aim of this study was to compare the effects of vacuum packaging and cold storage of pheasant meat on the physical and biochemical changes (pH value, lactic acid, phosphates concentrations) of meat.

Materials and methods

Pheasants were obtained from regular drive hunts during the hunting season (autumn – winter) in the eastern Slovakia. They had been killed by lead shots. Hunted pheasants were 8 – 9 month-old. Carcass were delivered at 4 °C to the Department of Food Hygiene and Technology in the University of Veterinary Medicine and Pharmacy in Košice (Slovakia). Pheasants were eviscerated 24 hours post mortem and processed. Evisceration (the complete removal all internal organs) is widely recommended method for treatment of feathered game carcasses, to ensure high hygienic quality of the pheasant meat during storage and it is in compliance with the Regulation (EC) of No. 853/2004 (10). The determination of concentrations of lactic acid, phosphates and pH values was carried out 24 hour after death on day 1 of the experiment in all pheasants. Afterwards, samples of muscles from pheasants (20) were divided into 2 groups.

First group consisted of non-vacuum packed pheasant thigh and breast muscle (n=10) . The thigh and breast muscles of the second group of pheasants (n=10), were removed and vacuum packed. The temperature for storing the meat in refrigerator was in both cases 4°C for 14 days.

The concentrations of lactic acid, phosphates and pH values were determined 24 hour after death on day 1 of experiment, as well as on day 7 and on day 14 of the experiment.

Measuring the pH value of meat

Samples of 50g from each group of meat (thigh muscle and breast muscle) were homogenized for 10 minutes. Afterwards, 10g was used for extraction by 100 ml distilled water, and then filtrated**.** The pH values were analysed in a watery meat extract by using a pH meter (InoLab pH720, WTW, Weilheim, Germany) with glass electrode (11).

Capillary electrophoretic analysis

Electrophoretic analyser, Type EA 102 (Villa Labeco, Spišská Nová Ves, Slovak Republic) with a conductive detector was used for measurement of lactic acid and phosphates in meat. The capillary electrophoretic analysis is an appropriate method of determining of lactic acid and phosphates in

meat as an important indicator of quality (12). This method is suitable for analysis of lactic acid changes in the game meat during ageing process (13). The watery extract from the pH measurements was diluted 1:10, and injected into an electrophoretic analyser. The separation analytical system used in analyser consisted of leading electrolyte 10mM HCL, β-alanine and 0.1% methylhydroxyethylcellulose, pH 3.2. Terminator electrolyte consisted of 5mM caproic acid and 5mM hydroxymethyl-aminomethane. The direct currents used in pre-separation and analytical columns were 250 μA and 50 μA. Time of analysis was 10 – 15 min. The results of analysis were evaluated by ITP - Pro 32 software (KasComp. Bratislava, Slovak Republic). The concentrations of lactic acid and phosphates were expressed in $g.100g^{-1}$ of meat.

Statistical evaluation

The results were statistically analysed using GraphPad Prism Software, Version 4.00, San Diego, CA, USA, (2003). One-way analysis of variance (ANOVA) with the post hoc Tukey's multiple comparison test was used to evaluate statistical significance of differences between non-vacuum packed pheasant meat and vacuum packed meat (statistically significant differences are illustrated in the tables by numbers), and between days of the experiment statistically significant differences are illustrated in the tables by letters). The data were presented as mean and standard deviation.

Results and discussion

The course of changes in the concentration of lactic acid and pH during refrigeration storage of pheasant breast muscle is shown in Table 1. The initial mean value of lactic acid concentration measured in the breast muscle within 24 hours after hunting was 1.55 ± 0.69g/100g. On the day 7 of storage, lactic acid concentration increased in both groups. Higher values were measured in the vacuum packed breast muscle (1.92 ± 0.53g/100g), however, there were found no significant differences compared with non-vacuum packed breast muscle (1.84 ± 0.30g/100g). Significantly higher concentrations of lactic acid ($p \leq 0.01$) were observed in vacuum packed than non-vacuum packed breast muscles on day 14 of storage (1.88 ± 0.18, respectively 1.15 ± 0.67g/100g).

On this day of the experiment lactic acid concentration, as a product of lactate dehydrogenase (LDH) activity decreased in both groups, but more significantly in non-vacuum packed breast meat ($p \leq 0.05$).

The initial mean value of phosphates recorded in the breast muscle within 24 hours after hunting (on day 1 of storage) was 0.81 ± 0.22 g/100g (Tab. 1).

Assessment of dynamic phosphates concentrations in vacuum and non-vacuum packed breast meat showed a significant increase (1.01 ± 0.10; $p \leq 0.05$) in non-vacuum packed meat on day 7 of the experiment compared with day 1. An insignificant increase of levels of phosphates was observed also in vacuum packed breast meat on day 7 of experiment.

We observed a significant increase (1.11 ± 0.09; $p \leq 0.01$) of concentrations of phosphates in breast muscle in vacuum packed meat on day 14 of the experiment, as compared with day 7 and day 1.

On the other hand, the significant decrease (0.75 ± 0.14; $p \leq 0.001$) in concentrations of phosphates in breast were observed on day 14 of the experiment in non-vacuum packed meat.

The initial mean value of breast muscle pH was 5.93 ± 0.32. This parameter in both monitored groups did not change significantly during storage at 4 °C. Also, there were observed no significant differences of pH value between breast muscles which were vacuum-packed and those non-vacuum packed (Table 1).

The mean concentration of lactic acid measured in thigh muscles of pheasants within 24 hours after the hunt was 1.14 ± 0.48 g/100g. During refrigeration storage, amount of the lactic acid varied depending on packing method (Table 2). On day 7 of storage, concentration of lactic acid measured in vacuum packed thigh muscles significantly increased (1.63 ± 0.41 g/100g; $p \leq 0.05$). Increase of the quantity of lactic acid was recorded also in non-vacuum packed thigh muscle (1.34 ± 0.28g/100g), but was not statistically significant. The mean value of lactic acid concentration measured in vacuum packed thigh muscle on day 14 of storage was about the same as on day 7 (1.62 ± 0.27g/100g). On the other hand, on day 14 of storage a significant decrease in lactic acid was observed in non-vacuum packed thighs (0.73 ± 0.56g/100g; $p \leq 0.05$) compared to day 7. The most significant difference ($p \leq 0.01$) in

Table 1: Lactic acid and phosphates concentration (g/100g) and pH value monitored in breast muscle of pheasants during storage, V – Vacuum packed, NV – non-vacuum packed

Monitored parameter	Storage method	Day of storage		
		1.	7.	14.
Lactic acid	V	1.55 ± 0.69	1.92 ± 0.53	1.88 ± 0.18 [1]
	NV	1.55 ± 0.69 [ab]	1.84 ± 0.30 [a]	1.15 ± 0.67 [b;2]
Phosphates	V	0.81 ± 0.22 [b]	0.96 ± 0.06 [b]	1.11 ± 0.09 [a,1]
	NV	0.81 ± 0.22 [b]	1.01 ± 0.10 [a]	0.75 ± 0.14 [b;2]
pH	V	5.93 ± 0.32	6.05 ± 0.29	5.91 ± 0.20
	NV	5.93 ± 0.32	5.87 ± 0.26	6.10 ± 0.27

Statistical significant differences between two packing method are illustrated in the lines of tables by numbers and between days of the experiment by letters. Means in the lines with the same superscript (a. b) and means in the columns with the same superscript (1.2) do not differ significantly. Means with different superscripts differ significantly.

Table 2: Lactic acid and phosphates concentration (g/100g) and pH value monitored in thigh muscle of pheasants during storage V – Vacuum, NV – non-vacuum packed

Monitored parameter	Storage method	Day of storage		
		1.	7.	14.
Lactic acid	V	1.14 ± 0.48[b]	1.63 ± 0.41[a]	1.62 ± 0.27[a;1]
	NV	1.14 ± 0.48[ab]	1.34 ± 0.28[a]	0.73 ± 0.56[b;2]
Phosphates	V	0.71 ± 0.15 [b]	0.90 ± 0.17 [a]	0.89 ± 0.15 [a,1]
	NV	0.71 ± 0.15 [b]	0.91 ± 0.10 [a]	0.68 ± 0.18 [b;2]
pH	V	6.38 ± 0.20	6.35 ± 0.18	6.42 ± 0.13
	NV	6.38 ± 0.20[b]	6.49 ± 0.15[ab]	6.62 ± 0.26[a]

Statistical significant differences between two packing method are illustrated in the lines of tables by numbers and between days of the experiment by letters. Means in the lines with the same superscript (a. b) and means in the columns with the same superscript (1.2) do not differ significantly. Means with different superscripts differ significantly.

the concentration of lactic acid measured in the vacuum packed thigh and non-vacuum packed thigh muscle was observed on day 14 of storage, as was ascertained in the breast muscle.

The initial mean concentration of phosphates recorded in the thigh on day 1 was 0.71 ± 0.15 g/100g (Tab. 2). Significantly higher concentrations of phosphates in thigh muscle were recorded on day 7 of the experiment in both groups, vacuum (0.90 ± 0.17; $p \leq 0.05$) and non-vacuum packed meat (0.91 ± 0.10; $p \leq 0.05$), in comparison with day 1.

Similar course of changes in levels of phosphates such as those recorded in breast muscle were recorded in thigh muscle in non-vacuum packed meat. The significant decrease (0.68 ± 0.18; $p \leq 0.01$) in concentrations of phosphates in thigh were observed on day 14 of the experiment.

The concentrations of phosphates in thigh muscle in vacuum packed meat showed significantly increased levels (0.89 ± 0.15; $p \leq 0.05$) on day 14 of the experiment, as compared with day 1.

The pH value measured in the thigh muscle within 24 hours after hunt was 6.38 ± 0.20. During cold storage this value was increasing in both thigh muscle groups. While the increase of pH value in non-vacuum packed thigh was observed already on day 7 (6.49 ± 0.15g/100g), the increase in pH value in vacuum packed thigh was observed on day 14 (6.42 ± 0.13g/100g). As Table 2 shows, more pronounced increase in pH was recorded in thigh muscle which was stored non-vacuum packed, but

statistically significant differences in pH value of vacuum packed thigh and non-vacuum packed thigh muscle were not observed.

From the data presented in tables 1 and 2, pH values measured in the breast muscles were higher throughout the entire storage period, compared to those that were found out in thigh meat. These results are in accordance with studies of other authors (14, 15).

The pH values obtained in our study are closely related with the amount of the lactic acid present in the individual muscles. The concentration of lactic acid was higher in the breast muscle. These differences might result from the different patterns of myofibres. Breast muscles of pheasants are predominantly composed of fast-twitch, glycolytic fibres, whereas thigh muscles have higher percentage of oxidative fibre types (16).

Lactic acid concentration and pH value is influenced by ante-mortem carbohydrate depletion caused by physical activity as a result of chase during hunting, as noted by Paulsen et al. 2008 (17). They recorded, that pH values of hunted non-vacuum packed pheasant muscles were increased during storage, and these values were similar to our results. On the other hand, pH values of vacuum packed meat were almost the same throughout the entire storage period, which is in agreement with the result reported by Pfeifer et al. 2014 (18).

On day 7 of storage the concentration of lactic acid in the vacuum and also in the non-vacuum packed thigh and breast muscles increased. However, pH values measured in those muscles did not decrease; on the contrary, they remained the same as on the first day, or increased. The amount of lactic acid should influence pH value of the muscles, but there are also other factors which have impact on this quality indicator. The decomposition of nitrogenous compounds and formation of alkaline compounds, such as NH_3 causes pH increasing (19). Based on our measured values of pH we can suggest, that the decomposition changes in vacuum packed pheasant meat proceed more slowly compared to non-vacuum packaged meat.

The monitoring of one of indicators of ripening process - the concentrations of lactic acid showed statistically highest differences in vacuum and not-vacuum packed pheasant meat on the day 14 of storage. Lactic acid concentrations measured on day 14 in the vacuum packed breast and thigh meat were almost the same as on the day 7 of storage and were significantly higher ($p \leq 0.01$) compared to values measured in non-vacuum packed meat. The presence of atmospheric oxygen probably affects the degradation of lactic acid, which has impact on sensory properties of meat (20).

These results of dynamic phosphates in non-vacuum packed breast and thigh muscle are in accordance with studies by Januška et al. 2014 (21) who compared physico-chemical changes during maturation of shot and slaughtered pheasants.

In our study we recorded important differences in concentrations of phosphates between vacuum packed and non-vacuum packed meat after 7 day of storage of pheasants meat. The vacuum packed breast meat showed a significantly higher ($p \leq 0.001$) concentrations of phosphates compared with the meat packed in normal atmosphere on day 14 of storage.

Similar, significantly higher ($p \leq 0.01$) concentrations of phosphates were recorded in vacuum packed thigh meat compared with non-vacuum packed meat on day 14 of storage.

Glycolytic pathway plays a key role in skeletal muscle energy metabolism by converting glucose to pyruvate to generate ATP (22). ATP is the essential metabolite for *rigor mortis.* The *rigor* starts when the ATP level decreased less than 85% of the *in vivo* level. The temporal pattern of the post mortem ATP concentration depends on the glycogen and phosphocreatine reserve at the onset of death, as well as the period of anoxia (23).

Inadequate glycogen content in muscle at the moment of death results in little production of lactic acid; therefore meat has a high pH value. The pH value of muscle at 24 h after death is used to determine meat quality and take further processing decisions. Scheffler et al., 2011 (24) reported another explanation for the increased pH decline, instead of lactic acid production, namely the free protons and heat, originating from the ATP hydrolysis.

The rate of post-slaughter degradation of glycogen depends on the *post-mortem* muscle metabolism, is affected by *post-mortem* technologies, such as chilling conditions, which can affect directly the activity of the enzymes that regulate glycogenolysis (25, 26).

During storage, ripening of the pheasant meat occurs, progressively increasing tenderness and developing taste through the proteolytic activity of meat enzymes. Ripening process depends on temperature and can be accelerated by increasing it. However, for hygienic reasons it is recommended, that temperature of 4°C should be

used with a relative humidity of 85–95 %. Like the ageing of meat during cold storage at 4°C, other complementary treatments, maintaining quality of game meat and reducing the risk of microbial spoilage are used.

The reason for the preference of vacuum – packing method is the fact that it can remarkably extend the storage time. In this way stored meat and meat products can be safe and keep quality. For this reason special airtight synthetic films have been developed which can be heat-sealed after removing the air around the packed meat, thus keeping it practically out of contact with the surrounding atmosphere.

The shelf-life of vacuum packed pheasant meat and at the same time stored under 4°C can be remarkably influenced.

Conclusion

In our study we compared the effects of two different methods of treating and storing of feathered game after hunting. These methods impact on the course of physico – chemical changes during ripening process of pheasant meat. It can be concluded that storing pheasant meat by chilling and vacuum packing is the most advantageous combination of the methods that preserves the high hygiene quality of the game meat for a longer period of time. This storage method of pheasant meat enables the final product to become both successful in the wild game meat market and attractive to the consumers.

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UČINKI DVEH NAČINOV SKLADIŠČENJA NA FIZIKALNO-KEMIJSKE SPREMEMBE V MESU FAZANOV

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Povzetek: V raziskavi so bili proučeni učinki dveh načinov shranjevanja fazanjega mesa, in sicer hlajenega v hladilniku ter vakuumsko pakiranega, na fizikalno-kemične spremembe. Koncentracija mlečne kisline in fosfatov ter pH vrednosti prsnih in stegenskih mišic so bile izmerjene pri ohlajenih (n = 10) in vakuumsko pakiranih fazanih (n = 10) 1., 7. in 14. dan po začetku shranjevanja. Sedmi dan so se koncentracije mlečne kisline v stegnu znatno povišale (1,63 ± 0,41 g / 100 g; p ≤ 0,05). Štirinajsti dan so se koncentracije mlečne kisline v nevakuumsko pakiranih stegnih občutno znižale (0,73 ± 0,56 g/100 g; p ≤ 0,05) v primerjavi s 7. dnevom. Značilno višja (p ≤ 0,01) koncentracija mlečne kisline je bila ugotovljena 14. dan v nevakuumsko pakiranih mišicah stegna. Pri prsih je bila značilna razlika (p ≤ 0,01) v koncentraciji mlečne kisline med vakuumsko in nevakuumsko pakiranim mesom opažena 14. dan (1,88 ± 0,18 ter 1,15 ± 0.67 g/100 g). pH vrednost v nevakuumsko pakiranih stegnih je bila 7. dan 6,49 ± 0,15, pri vakuumsko pakiranih pa 14. dan 6,42 ± 0,13 g/100 g). Občutno višje koncentracije fosfatov so bile v stegnih izmerjene 7. dan pri vakuumsko (0,90 ± 0,17; p ≤ 0,05) in nevakuumsko pakiranem mesu (0,91 ± 0,10; p ≤ 0,05) v primerjavi s 1. dnem. Značilno znižanje (0,68 ± 0,18; p ≤ 0,01) fosfatov je bilo opaženo pri nevakuumsko pakiranih stegnih 14. dan. Pri vakuumsko pakiranih stegnih je bila bistveno povišana raven fosfatov (0,89 ± 0,15; p ≤ 0,05) 14. dan v primerjavi s prvim dnem. Rezultati kažejo, da je najprimernejši način shranjevanja fazanjega mesa kombinacija hlajenja in vakuumskega pakiranja.

Ključne besede: fazan; meso; mlečna kislina; pH; vakuum