

## Volatile Organic Compounds as Biomarkers of the Freshness of Poultry Meat Packaged in a Modified Atmosphere

JANA TOMÁNKOVÁ, GABRIELA BOŘILOVÁ, IVA STEINHAUSEROVÁ and LEO GALLAS

*Department of Meat Hygiene and Technology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences in Brno, Brno, Czech Republic*

### Abstract

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The volatile organic compounds (VOCs) in the packing of chicken meat in a modified atmosphere was qualitatively and quantitatively evaluated. The total number of 72 samples of chicken hindquarters were stored under two different modified atmospheres (70% O<sub>2</sub>, 30% CO<sub>2</sub>, and 70% argon, 30% CO<sub>2</sub>) for 20 days. Analyses were performed on Days 0, 4, 8, 12, 16, and 20. VOCs in the headspace samples were detected and quantified by gas chromatography/mass spectrometry (GC/MS) every fourth day of storage. Pentamethylheptane, dimethylsulphide, dimethyl disulphide, dimethyl trisulphide, dimethyl tetrasulphide, hydrogen sulphide and ammonia were detected. Pentamethylheptane and ammonia had similar values for both modified atmospheres (MA). The other compounds were found only in argon MA from the Day 16 of storage with a subsequent increase of values. The measured values for dimethylsulphide were 10.7 and 13.8 mg/l, for dimethyl disulphide they were 1.9 and 10.7 mg/l, dimethyl trisulphide levels were 15.7 and 19.3 mg/l and dimethyl tetrasulphide levels were 93.2 and 418.3 mg/l for Day 16 and 20. The hydrogen sulphide level was detected from 80 to 370 mg/l after the 8<sup>th</sup> day of storage. We showed that the argon MA is less suitable for packaging raw chicken parts than the oxygen MA in view of the increased amount of microflora and unpleasant odour as assessed by sensory analysis. Oxygen prolonged the shelf life by about four days in comparison with argon. Sensory evaluation was similar for both atmospheres after air exhaustion. The argon MA did not extend the shelf life as compared to the oxygen MA.

**Keywords:** VOCs; gas chromatography/mass spectrometry; argon atmosphere

Food safety is one of the major challenges in the meat industry. Public concern leads to increased requirements for high quality, safety and stability during the storage period. An extended shelf life of meat products is of great economic importance for both consumers and producers. This can be achieved by improved packaging in a gaseous environment while a gas mixture of O<sub>2</sub>, CO<sub>2</sub> and N<sub>2</sub> is commonly used. Previous studies were performed on the use of different ratios and combinations of gases to delay spoilage by aerobic microbial flora.

CO<sub>2</sub> decreases microbial growth by extending lag phase and increasing generation time (FRAQUEZA *et al.* 2008). The shelf life of packaged products is improved by modified atmosphere packaging (MAP) high in CO<sub>2</sub> for chicken meat (SMOLANDER *et al.* 2004; RAJAMÄKI *et al.* 2006). Including O<sub>2</sub> in the MAP improves colour, but it also increases lipid oxidation (SAUCIER *et al.* 2000; BALAMATSIA *et al.* 2007). Lipid oxidation is one of the main factors limiting the quality and acceptability of meat. Recently, argon has been suggested having

beneficial properties in MAP. Previous research revealed that argon is biochemically active due to its enhanced solubility in water as compared to N<sub>2</sub>. Argon appears to be a suitable compound for increasing the quality of these packaged food products. FERNÁNDEZ-LÓPEZ *et al.* (2008) and ZHOU *et al.* (2010) used argon for packaging of chicken meat. A modified atmosphere (MA) with argon has also been used for packaging of pork and beef.

Previous studies reported volatile organic compounds (VOCs) and non-volatile compounds such as biogenic amines, which are primarily a result of enzymatic decarboxylation of the amino acids by exogenous enzymes of bacterial origin. VOC relate with the microbiological and physicochemical profile of packaged meat in MA (BOOTHE & ARNOLD 2002; RAJAMÄKI *et al.* 2006; BALATSMANIA *et al.* 2007). Increased production of H<sub>2</sub>S and sulphur-based, ammonia-based, methane-based, organic acid-based, mercaptan-based, acetone-based organic compounds and others sensorial important organic compounds also belong to main limiting factors of meat quality and indicate the decay of packaged meat (LOVESTAD & BRUNO 2010). These volatile organic compounds can be assessed by gas chromatography/mass spectrometry (GC/MS). Increasing the amount of VOCs leads to an increased health hazard for consumers by negative effects of carcinogenic compounds (SESTER *et al.* 2000). Analyses of VOCs may therefore provide important information on the sensory and hygienic quality of packaged food products. Additionally, qualitative assessment of VOCs can potentially be used for the identification of microorganisms in packaged meat e.g. *E. coli*, *Enterobacteriaceae*, *Pseudomonas* sp., and lactic acid bacteria (LAB) (SESTER *et al.* 2000; MADDULA *et al.* 2009). These bacteria are prevalent in MAP. LAB cause “souring”, which is less invasive than aerobic putrefaction. LAB are the primary objective as preservative agents for modified atmosphere packaging (PATSIAS *et al.* 2008). VIHAVAINEN and BJÖRKROTH (2009) reported that high levels of psychrotrophic LAB in packaged meat produce adverse sensory changes such as an off-odour.

The aim of this study was to investigate the effect of MA packaging on the VOC profile in relation to growth measurements of microorganisms and the shelf life of packaged chicken meat. We also investigated the effect of replacing oxygen with the same amount of argon.

## MATERIAL AND METHODS

**Raw material and storage conditions.** Fresh chicken hindquarters including only bone, muscle and skin were obtained from a standard processing plant and packaged in AMILEN-OX 80 with an EVA sealant layer (VF Verpackungen GmbH, Kempten, Germany). The total number of 72 samples of chicken hindquarters were divided into portions and packaged immediately. A half of the samples were packaged using an MA containing 70% O<sub>2</sub> and 30% CO<sub>2</sub>. The other half of the samples was packaged using an MA containing 70% Ar and 30% CO<sub>2</sub>. All the samples were stored at a temperature of 4 ± 1 °C for 20 days. Six samples from each type of packaging were taken for VOCs analysis, microbiological and sensory and chemical analyses (Table 1). Each of the analyses described below was performed on fresh samples (Day 0) and the same analyses were then repeated on days 4, 8, 12, 16, and 20 of storage. The analyses were performed separately on the skin and muscle of the hindquarters.

**Analyses of volatile compounds.** Analysis of the concentration of volatile organic compounds in MAP was performed on two samples from each type of packaging at each sampling occasion. The headspace samples were pumped through a needle on a sorptive tube with active carbon with a flow of 200 ml/min for 10 minutes. Desorption of these captured compounds was carried out by 0.5 ml carbon disulphide. The analyses were performed using GC-MS according to LI and FINGAS (2003).

**Microbiological analysis.** Ten grams of skin and muscle from the hindquarters from each package were treated separately. To quantify the growth

Table 1. An overview of sampling (72 samples in total)

	Day					
	0	4	8	12	16	20
<b>Argon MA36 samples</b>						
VOC	2	2	2	2	2	2
MB	2	2	2	2	2	2
S+CH	2	2	2	2	2	2
<b>Oxygen MA 36 samples</b>						
VOC	2	2	2	2	2	2
MB	2	2	2	2	2	2
S+CH	2	2	2	2	2	2

VOC – volatile organic compounds; MB – microbiological analysis, S+CH – sensory and another chemical analysis

of microflora, total viable counts (TVC) were cultured according to norm ISO 4833:2003, psychrotrophic bacteria were cultured according to norm ISO 17410:2001, lactic acid bacteria (LAB) were cultured according to norm ISO 13721:1995, *Enterobacteriaceae* were cultured according to norm ISO 21528-2:2004, coliform bacteria were cultured according to norms ISO 4831:2006 and ISO 4832:2006, and *E. coli* were cultured according to norm ISO 16649-1, 2, 3:2003 on coliform agar ES (Merk, Darmstadt, Germany). All microbial counts were converted to logarithms of colony-forming units per gram (log CFU/g).

**Measurement of gases.** The concentrations of CO<sub>2</sub> and O<sub>2</sub> were measured by the incision of a probe inside the packaging using a Check Point II gas analyser (PBI Dansensor AS, Ringsled, Denmark) every day before the beginning of sensory evaluation and meat product analyses.

**pH.** The pH was measured by incision into the muscle with a pH meter electrode (pH 340i; WTW, Weilheim, Germany). The pH was measured in duplicate.

**Colour.** The instrumental colour measurement of the fresh poultry hindquarters was performed with a Konica Minolta CM 2600d (Konica Minolta, Tokyo, Japan) spectrophotometer. CIE-LAB L\*, a\*, and b\* values were determined as indicators of lightness, redness and yellowness, respectively. The colour was always measured at the same place on the skin and underlying muscle, respectively. The data given are means of 6 measurements for the skin and muscle surface.

**TBARS (Thiobarbituric Acid Reactive Substances).** TBARS were determined at Day 0, 4, 8, 12, 16, and 20 according to CASTELLINI *et al.* (2002). Ten grams of minced muscle and skin from the hindquarters were homogenized separately with distilled water and diluted with HCl (2:1). Distillation was performed to the final volume of 50 ml of distillate. 5 ml of the distillate and 5 ml 0.02M of thiobarbituric acid (TBA) were then heated in a boiling water bath

for 35 minutes. After cooling in the water bath, the intensity of the colour of the TBARS solution was measured in duplicate at 538 nm against a blank. The TBA content was calculated as malondialdehyde (MDA in mg/kg muscle) equivalent.

**Sensory analysis.** Sensory evaluation was performed by a sensory panel, composed of 6 assessors (only one training session before testing). Sealed packages were opened with a scalpel. Evaluation of the overall odour of the raw product was performed immediately, then a further evaluation followed: general appearance, odour of hindquarters, colour and surface slime on the skin, and colour of muscle. The intensity of each trait was evaluated on a five-stage scale from very low or bright (1) to very high or dark (5).

**Statistical analysis.** The results were evaluated using ANOVA test with packaging and storage time as fixed factors. Interaction between packaging and storage time was also included in the model. Differences among the groups were tested by Tukey's test. The analyses were performed for sample types (skin and muscle) separately. Significance was established at  $P < 0.05$ . The data was analysed using Statistica StatSoft, Inc. (2005). STATISTICA. Cz (software system for data analyses) Vers. 7.1 (StatSoft CZ, Prague, Czech Republic).

## RESULTS AND DISCUSSION

### Analyses of volatile organic compounds

Eleven VOCs were identified in the headspace of the packaged chicken hindquarters. Seven compounds were found as decaying chicken tissue in the argon MA and two compounds in the oxygen MA. The concentrations of pentamethylheptane and ammonia did not differ between the argon and oxygen MA and decreased during storage (Table 2). The concentration of pentamethylheptane correlated with the redness and yellowness values of the skin in the oxygen MA ( $P < 0.001$ ). The con-

Table 2. The content of pentamethylheptane and ammonia in package with argon and oxygen modified atmosphere during storage

Modified atmosphere	Volatile organic compounds	Time (days)					
		0	4	8	12	16	20
Argon (mg/l)	pentamethylheptane	2.758	0.198	0.169	0.143	0.14	0.079
	NH <sub>3</sub>	< 0.1	< 0.1	< 0.1	0.1	0.2	0.5
Oxygen (mg/l)]	pentamethylheptane	0.279	0.155	0.111	0.103	0.099	0.056
	NH <sub>3</sub>	< 0.1	< 0.1	< 0.1	< 0.1	0.3	0.5

centration of ammonia was slightly higher from day 16 of storage onwards for both atmospheres.

The compounds dimethylsulphide, dimethyl disulphide, dimethyl trisulphide, and dimethyl tetrasulphide were found in the argon MA only (Table 3). The concentration of these compounds was significantly higher ( $P < 0.001$ ) from Day 16 of storage, while hydrogen sulphide increased from Day 8 of storage. SENTER *et al.* (2000) and RAJAMÄKI *et al.* (2006) reported similar results for  $H_2S$  and dimethylsulphide in a vacuum MA and nitrogen MA for chicken thighs and chicken meat, respectively. LOVESTEAD and BRUNO (2010) found higher values of dimethyl disulphide and dimethyl trisulphide in 12 chicken samples during two weeks of storage. The headspaces of these samples were sampled by cryoadsorption for 10, 20, or 30 minutes. The generation of VOCs is related with the growth of bacteria, mainly with their microbial decomposition of sulphur-containing amino acids, degradation of peptides, oxidation and enzymatic activity. The components of VOCs were influenced significantly ( $P < 0.001$ ) by the time and content of carbon dioxide. These results correspond to the sensory analysis. The odour of the packages with an argon MA was very unpleasant from Day 8 of storage in comparison with Day 12 in an oxygen MA.

### Microbiological analysis

The growth of TVC, LAB and *Enterobacteriaceae* is shown in Figures 1–3. The increases of bacterial growth were similar in both MA, but the argon

MA showed lower counts of TVC during storage for skin and muscle. The initial level was 5.44 log CFU/g for skin and 3.20 log CFU/g for muscle in both MA. The TVC for muscle was lower than reported by PATSIAS *et al.* (2008). The growth of TVC on skin significantly increased ( $P < 0.05$ ) from Day 8 of storage in the argon MA and from Day 4 in the oxygen MA. The growth on muscle started from Day 12 in the argon MA and from Day 8 in the oxygen MA. BALAMATSIA *et al.* (2007) stated that the microbiological shelf life of fresh poultry is 7–8 log CFU/g. We obtained higher values from day 4 for the oxygen MA and from Day 12 on the skin for the argon MA, and from Day 12 and Day 16 on muscle for the oxygen and argon MA, respectively.

LAB and coliform bacteria grew in the oxygen MA slowly and showed lower counts than in the argon MA. The initial levels of LAB on skin and muscle were 2.8 and 1.4 log CFU/g, and the levels of coliform bacteria were 1.19 and 0 log CFU/g, respectively for both MA. These values are below the values reported by PATSIAS *et al.* (2008). The bacteria were significantly ( $P < 0.01$ ) growing on skin in the argon MA during storage. The accepted limit for coliform counts is 3 log CFU/g (SAUCIER *et al.* 2000). We obtained higher values from Day 8 for skin in the argon MA and from Day 12 of storage for skin in the oxygen MA and muscle in both MA. The growth of these bacteria was not affected by the MA composition for muscle. The amount of bacteria in both MA was influenced by the content of gas in the MA ( $P < 0.001$ ) in skin and ( $P < 0.01$ ) in muscle. Psychrotrophic LAB are an important component of the spoilage flora and cause an unpleasant odour (VIHAVAINEN & BJÖRKROTH 2009). Coliform

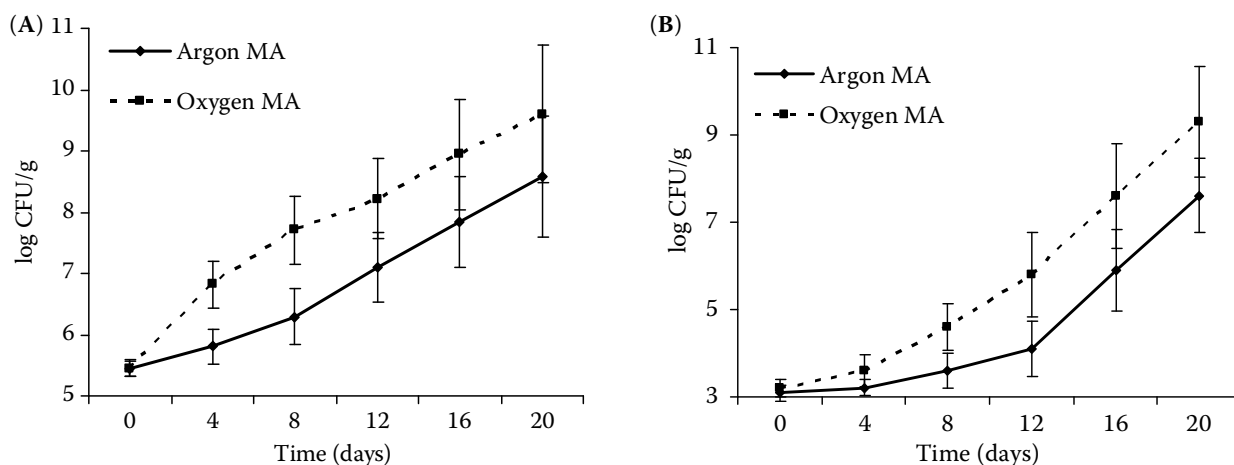


Figure 1. The profile of total viable counts growth for skin (A) and muscle (B) during storage

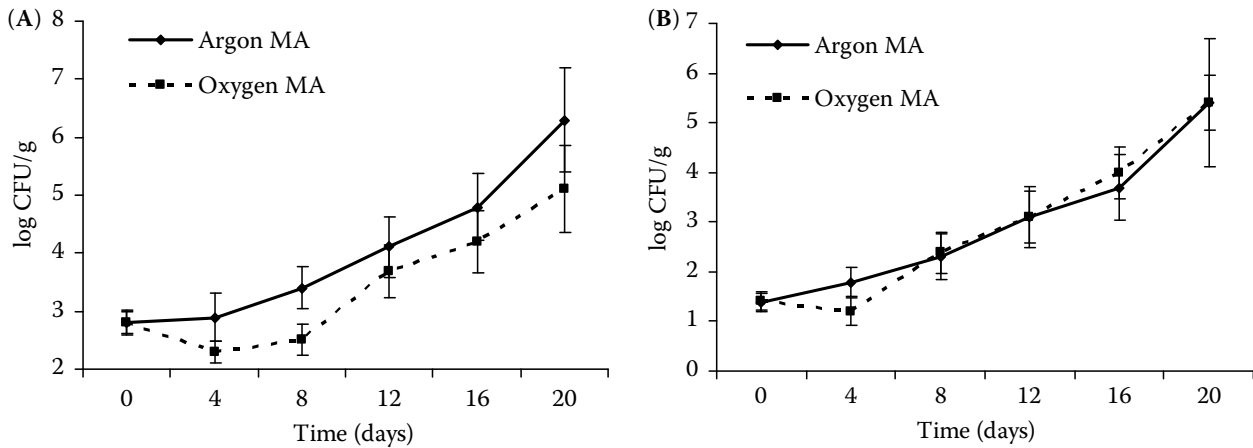


Figure 2. The profile of lactic acid bacteria growth for skin (A) and muscle (B) during storage

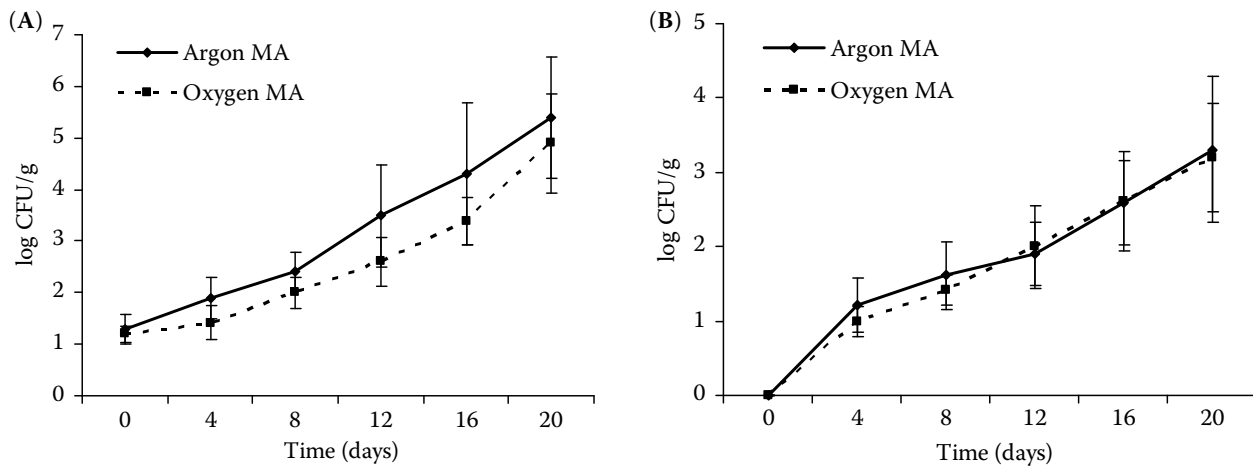


Figure 3. The profile of coliform bacteria growth for skin (A) and muscle (B) during storage

bacteria belong to *Enterobacteriaceae*, which are able to adapt themselves to environmental changes and can therefore be used as hygiene indicators. A similar growth of these bacteria was reported by SMOLANDER *et al.* (2004) in MAP. SAUCIER *et al.* (2000) reported that poultry meat had a long shelf life in a MA containing oxygen, nitrogen and CO<sub>2</sub> when compared to an MA containing only nitrogen and CO<sub>2</sub>.

#### Measurement of gases

A constant quantity of CO<sub>2</sub> is important for maintaining the poor growth of bacteria during storage due to its bacteriostatic effect. The proportion of gases and microbiological growth had an effect on the colour of the products. The amount of CO<sub>2</sub> decreased slightly in the argon MA, whereas the

amount of CO<sub>2</sub> increased from day 8 of storage in the oxygen MA ( $P < 0.001$ ) (Figure 4). This reduction of CO<sub>2</sub> in the argon MA was also observed by RUIZ-CAPILLAS and JIMÉNEZ-COMMENERO (2010) for fresh pork sausages. The exhaustion of O<sub>2</sub> and conversion to CO<sub>2</sub> is related to the rapid growth and metabolism of bacteria, enzymatic activity of muscle and also decarboxylation of biogenic amines. This increase in CO<sub>2</sub> also inhibited the growth of aerobic spoilage bacteria, e.g. of those related with a lower count of LAB and coliform bacteria in the oxygen MA (BALAMATSIYA *et al.* 2007).

#### pH

The pH of fresh chicken muscle is about 6.32 according to BALAMATSIYA *et al.* (2007). The pH values in our study ranged from 5.8 to 6.6. We

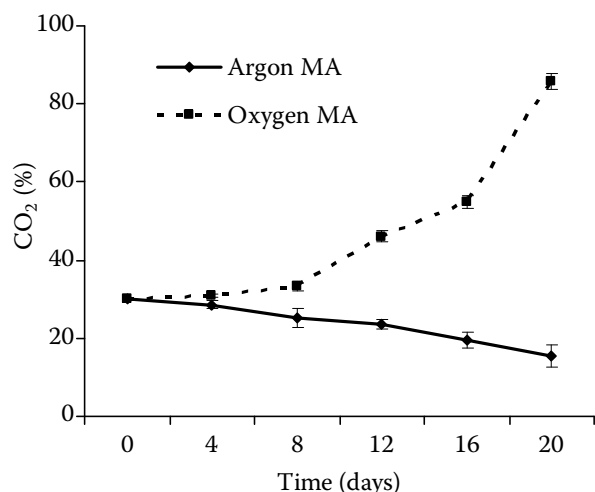


Figure 4. The amount of CO<sub>2</sub> for both MA during storage

found no significant differences between chicken meats packed in the argon and oxygen MA.

#### Instrumental colour measurement

Colour is one of the most important criteria for evaluating the quality and acceptability of packaged meat, as consumers often select meat products according to colour. The initial lightness values  $L^*$  for chicken skin were 71.80 in both atmospheres, and remained fairly stable in the argon MA during storage and rapidly increased on Day 8 of storage in the oxygen MA ( $P < 0.01$ ). The initial values  $L^*$  for muscle were 57.39 in both MA and showed similar trends as for skin (Figure 5). The initial redness ( $a^*$ ) values were 0.5 for skin and 1.6 for muscle, and those of yellowness ( $b^*$ ) 5.55 for skin

and 5.75 for muscle. Lower redness values were detected for skin and higher values for muscle in the oxygen atmosphere, because added oxygen to MA sustains the colour aspect of fresh meat (CASTELLINI *et al.* 2002). These values significantly ( $P < 0.001$ ) decreased from Day 4 of storage for muscle in the oxygen atmosphere and from Day 8 for skin in both MA (Figure 6). This decrease of redness was caused by lipid peroxidation, which produces reactive oxygen species and free radicals developmental myoglobin oxidation, producing rancid odour and surface discoloration. The results are in agreement with those of FILGUERAS *et al.* (2010) for raw rhea meat. A similar decrease of  $a^*$  values was described by SAUCIER *et al.* (2000) for an atmosphere containing oxygen and they confirmed that the modified atmospheres used did not cause any changes in yellowness ( $b^*$ ) on skin or muscle.

#### TBARS (Thiobarbituric Acid Reactive Substances)

A significant increase in TBARS was observed. This increase was more pronounced in samples stored in the oxygen MA than in the argon MA ( $P < 0.01$ ) (Figure 7). The final values increased to 100 and 180 mg MDA/kg for muscle and skin in the oxygen MA, respectively. The final values for the argon MA were 28 and 36 mg MDA/kg for muscle and skin, respectively. Similar results were reported by FERNÁNDEZ-LOPÉZ *et al.* (2008) for ostrich steaks and VEBERG *et al.* (2006) for turkey meat stored in an oxygen MA. The TBARS value correlated with the period of storage and the growth of bacteria.

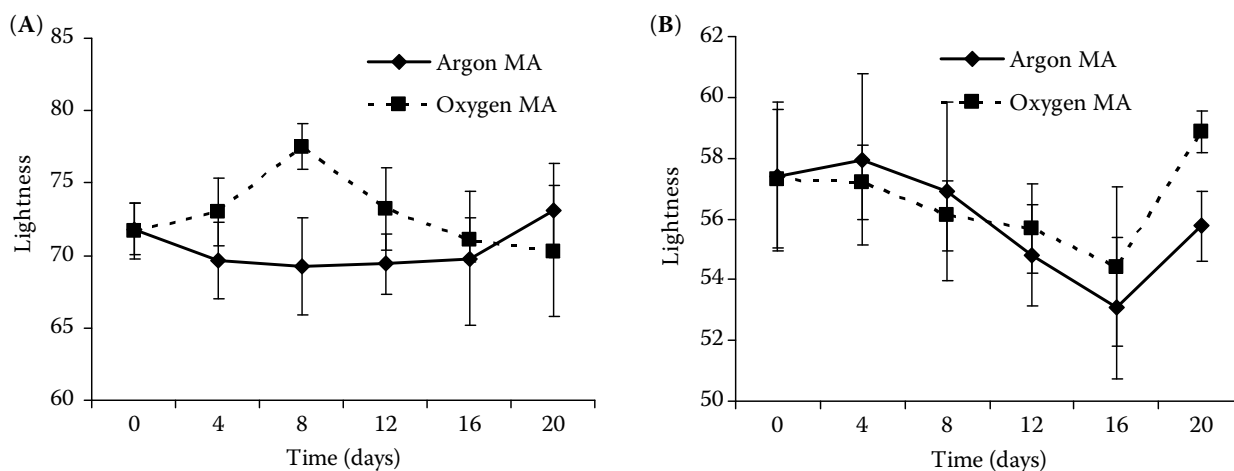


Figure 5. Lightness values for skin (A) and muscle (B) during storage

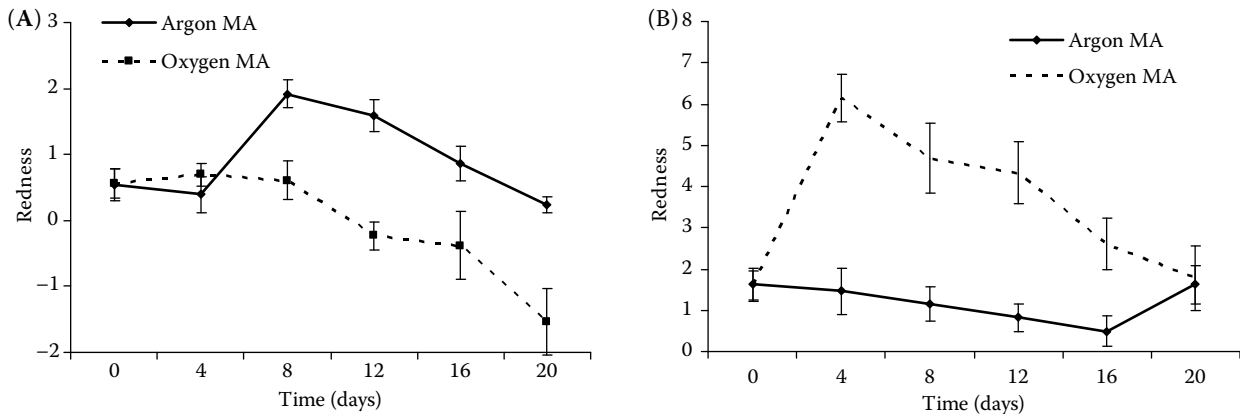


Figure 6. Redness values for skin (A) and muscle (B) during storage

Furthermore, they significantly ( $P < 0.01$ ) correlated with redness and yellowness in the oxygen MA.

### Sensory analysis

The overall odour was described as more unpleasant and unacceptable in the argon MA from day 8 of storage as compared to Day 12 in the oxygen MA. The general appearance of the chicken skin was lighter in the argon atmosphere. The increasing odour of the chicken hindquarters significantly ( $P < 0.001$ ) correlated with storage time, growth of bacteria and successive formation of VOCs in the argon MA as compared to the oxygen MA. The beginning of surface degradation alias surface slime on skin was recorded from Day 12 of storage in the oxygen MA, whereas the chicken packed in the argon MA showed it from Day 16 of storage. An argon MA causes the release of VOCs and a worse sensory profile of

the raw materials. Sensory evaluation was similar in both MA after air exhaustion packages. The results indicated that chicken meat was better preserved in an oxygen MA, maintaining the acceptability of package odour for consumers. According to SMOLANDER *et al.* (2004), sulphuric compounds with both microbial interaction and increased production of volatiles had the largest share in odour deterioration. The appearance of MA packaged chicken hindquarters is important for purchase, whereas the odour is important for consumption. The sensory shelf life was determined till Day 8 and Day 12 of storage in the argon and oxygen MA, respectively.

### CONCLUSION

VOCs were detected from day 16 of storage in the argon MA (70% argon and 30% CO<sub>2</sub>), whereas VOCs were not detected at the same storage time

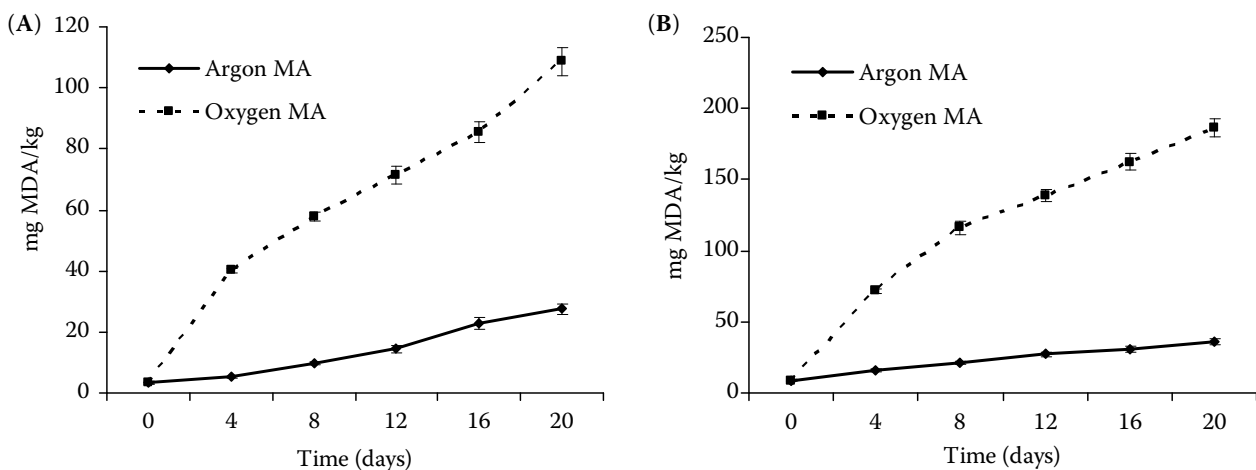


Figure 7. The amounts of malondialdehyde for skin (A) and muscle (B) during storage

Table 3. The amount of volatile organic compounds (VOC) in argon MA during storage

Modified atmosphere	VOC	Time (days)					
		0	4	8	12	16	20
Argon (mg/l)	dimethyl disulphide	< 0.030	< 0.030	< 0.030	< 0.030	1.957	10.707
	dimethyl trisulphide	< 0.045	< 0.045	< 0.045	< 0.045	15.777	19.324
	dimethylsulphide	< 0.030	< 0.030	< 0.030	< 0.030	10.766	13.835
	dimethyl tetrasulphide	< 0.045	< 0.045	< 0.045	< 0.045	93.225	418.336
	H <sub>2</sub> S	< 1	< 1	80	285	295	370

in the oxygen MA (70% O<sub>2</sub> and 30% CO<sub>2</sub>). Lipid oxidation measured as changes in TBARS values was slower in the argon MA than in the oxygen MA. However, sensory and microbiological analyses revealed that the oxygen MA is more effective in maintaining the high hygienic quality of the product at least until 12 days of storage. The analyses of VOCs are appropriate for using as biomarker.

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*Corresponding author:*

Mgr. Ing. JANA TOMÁNKOVÁ, Veterinární a farmaceutická univerzita Brno, Fakulta veterinární hygieny a ekologie, Ústav hygieny a technologie masa, Palackého 1/3, 612 42 Brno, Česká republika  
tel. + 420 541 562 752, e-mail: H09032@vfu.cz

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