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Antioxidant Capacity and Lipid Peroxidation Products of Carp (*Cyprinus carpio* L.) Meat Stored in Refrigeration Conditions with Addition of Herbs or Vegetables

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Abstract

Ochrem A., Zapletal P., Żychlińska-Buczek J., Maj D., Czerniejewska-Surma B., Pokorska J., Kułaj D. (2017): Antioxidant capacity and lipid peroxidation products of carp (*Cyprinus carpio* L.) meat stored in refrigeration conditions with addition of herbs or vegetables. Czech J. Food Sci., 35: 251–258.

The aim of the study was to evaluate the antioxidant capacity and the extent of lipid peroxidation of carp meat with plant additives. The research was conducted in two stages: January and April. Fish meat was mixed with additives, put into bags and held in refrigeration conditions for two weeks. Trolox Equivalent Antioxidant Capacity (TEAC) values on the first day of research in January ranged from 2.46 µM TE/g (w/w) to 4.60 µM TE/g (w/w). In April it was from 1.75 µM TE/g (w/w) to 3.21 µM TE/g (w/w), depending on the additives and method of storage (air-stored or vacuum-packed). TEAC values were higher in air-stored groups. The highest peroxide value was indicated in the control group at the end of research in January (6.06 mEq O_2/kg lipids). The TOTOX index of meats on the last day of research was higher in April (18.24) than in January (max. 17.22). The antioxidants present in herbs prevent from the formation of secondary lipid oxidation products.

Keywords: fish; functional food; food additives

Fish are among the most important sources of nutrients in the human diet. High contents of proteins and unsaturated fatty acids make them particularly suitable to be consumed at least once a week (Raatz *et al.* 2013). The consumption of fish meat in Poland (12.2 kg/per capita) is still lower than that of pork (35.5 kg/per capita) and poultry (26.5 kg/per capita). Poles consume particularly little meat of freshwater fish (approx. 2.5 kg/person). Only during the Christmas time due to the sale of carp, demand for freshwater fish revives for a while. Due to the intensive fishing activity, fish stocks are being depleted. It leads to increasing importance of aquaculture, which is dominated by the cyprinids family (freshwater fish) all over the world (Lirski 2011).

The high level of polyunsaturated fatty acids (PUFAs), which is observed especially in the fish meat, also implies negative consequences of changes in their meat and in their products. The compounds are particularly susceptible to oxidation, which in the case of lipids is called peroxidation. Bartosz (2013) explains that it is a free radical oxidation process of unsaturated fatty acids or other lipids which leads to the formation of peroxide compounds. According to Laguerre *et al.* (2007), oxidation processes can run in three different ways: spontaneous, non-enzymatic oxidation via free radicals; non-enzymatic and without the participation of radical photooxidation or enzymatic oxidation. Research on the use of natural antioxidants such as herbs or their extracts is still expanding (Kumar *et al.* 2015).

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Antioxidants are substances which are capable of delaying, slowing down, or preventing oxidation processes. Antioxidants are compounds that are capable of donating hydrogen radicals for pairing with other available free radicals to prevent the propagation reaction during the oxidation process (Balev *et al*. 2011; Dragoev *et al*. 2014; Kumar *et al*. 2015).

The aim of the present study was to evaluate the antioxidant capacity and lipid peroxidation products of carp meat with plant additives (herbs or vegetables).

MATERIAL AND METHODS

Carps (*Cyprinus carpio* L.) were obtained from the ponds of the Experimental Station of the Department of Ichthyobiology and Fisheries of University of Agriculture in Kraków (Mydlniki, 50°05'N, 19°50'E). The fish were harvested in January and April (n = 30/month).

Because some fish stay in the ponds after the Christmas season, the authors decided to check their changes. The experiment was carried out in two months: in January (1st stage), the carp weighed 1699.93 g ± 173.82 g (min. 1314.00 g; max. 2152.00 g); and in April (2nd stage), the carp weighed 2398.00 g ± 333.49 g (min. 1906.00 g; max. 3096.00 g).

Immediately after catching and killing, the carp were transported to the laboratory, weighed (± 2 g) (Axis, Poland), and then filleted. Fillets were weighed, rinsed under running water, and left to drain for 15 minutes. Afterwards all the fillets were minced using a Zelmer 586.5 meat mincer with a 3 mm mesh diameter (Zelmer, Poland). The meat thus obtained was made uniform by mixing and divided into four parts, or groups. Then the following ingredients were added to the meat (m/m): group C (control; without additives), group H1 (0.1% black pepper, 0.2% white mustard, and 0.2% juniper), group H2 (0.1% juniper, 0.1% rosemary, 0.1% thyme), group V (8.0% onion, 0.1% garlic, 0.07% parsley, 10.0% carrots, and 8.0% leek).

After adding the vegetables and herbs in each group, the meat was packed into food bags of 20 × 29 cm in size (Food Saver, USA) and vacuum-sealed or left open to the air.

Laboratory analyses of meat were performed every two days during two weeks of storage. All samples were stored in a refrigerator with controlled temperature (4°C).

Determination of antioxidant capacity using the TEAC method. (a) Extraction of antioxidants from the meat. The extraction was carried out with distilled water at a ratio of 1 : 10 (meat to water) at 8600 rpm for 2.5 min using an X120 homogeniser (Danlab, Poland). The homogenate was then subjected to MPW 223E centrifugation (MPW Med. Instruments, Poland) at 4000 rpm for 20 minutes. (b) Determination of antioxidant capacity using the TEAC method with ABTS radical (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (Re *et al*. 1999).

The basic reagent was made by dissolving ABTS (Sigma-Aldrich, China) in distilled water to achieve a concentration of 7 mM. Then, potassium persulfate (Chemland, Poland) was added for a final concentration of 2.45 mM. The obtained solution was left in a dark place for 16 h to generate free radicals. Prior to the test, the basic reagent was diluted with methanol (Chemland, Poland) to an absorbance of 0.700 ± 0.02, measured at a wavelength of 734 nm against distilled water, to prepare the working reagent. Determination of the antioxidant capacity involved mixing the aqueous antioxidant extract with the working solution. The absorbance value was measured after 30 min at a wavelength of 734 nm using a Genesys 20 UV-VIS spectrophotometer (Spectro-Lab, Poland) (Plust 2007). The results were expressed in μM TE/g (w/w) based on a standard curve, with Trolox as the standard substance (Sigma-Aldrich, Denmark).

Determination of peroxide value (PV). Fish fat extraction was carried out using the Bligh-Dyer method (Bligh & Dyer 1959). The resulting extracts were intended to determine the peroxide and anisidine value.

This was performed in accordance with BN-74/8020-07. The method involves oxidation by peroxides present in rancidifying fat of ferrous to ferric ions and colorimetric determination of the concentration of coloured complex ions forming from ferrous ions in the presence of ammonium thiocyanate.

Determination of anisidine value (AV). Because peroxides undergo further transformations, the determination of peroxide value should be complemented by additional tests of secondary oxidation products (Saeed & Howell 2002).

The test was carried out in accordance with PN-93/A-86926. The principle of the method consists in reacting the aldehydes present in the test sample with a solution of *p*-anisidine in ice-cold acetic acid and spectrophotometric measurement of the absorbance of the resulting yellow-coloured complex.

Determination of total oxidation value (TOTOX). The TOTOX value was calculated on the basis of PN-93/A-86926: $\text{TOTOX} = 2.6 \cdot \text{PV} + \text{AV}$

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Sensory evaluation. The sensory evaluation was performed according to PN-ISO 6564:1999. The evaluation was carried out by 5–7 persons with taste sensitivity as per PN-ISO 3972:1998 and olfactory sensitivity as per PN-ISO 5496:1997. Intensity of the characteristic notes was classified using the following scale: 0 = none, 1 = slightly perceptible, 2 = weak, 3 = moderate, 4 = strong, 5 = very strong. As per the Appendix to the Polish Norm 6564:1999, selected results are presented graphically in the form of radar charts created in Excel 2010.

Statistical analysis. The results were statistically analysed using the SAS statistical package (SAS System for Windows, Release 8.2., 2001). Analysis of variance with repeated measures was also conducted. The model included fixed effects such as: month, group, and packaging, and interactions: month × group, month × packaging, group × packaging, month × group × packaging.

Table 1. Trolox Equivalent Antioxidant Capacity (μM TE/g (w/w)) of the vacuum-packed or air-stored forcemeat during cold storage (4°C)

Month	Group	Packing	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
January	C	air	3.27^{Aai}	2.43^{Ba}	2.15^{Bag}	2.45^{Ba}	7.69^{Ca}	6.24^{Da}	10.76^{Ea}
		vacuum	2.46^{Abg}	3.35^{Bbd}	2.26^{Aa}	2.22^{Ab}	1.80^{Cb}	2.40^{Ab}	2.33^{Ab}
	H1	air	3.74^{Af}	3.99^{Ac}	3.69^{Ab}	3.65^{Acg}	5.37^{Bc}	10.89^{cc}	7.61^{Dc}
		vacuum	3.02^{Ac}	3.54^{Bd}	2.22^{Cag}	2.29^{Cab}	2.48^{Cd}	3.04^{Ad}	2.81^{Adh}
	H2	air	4.60^{Ae}	4.51^{Af}	3.27^{Bd}	4.31^{Ae}	6.61^{Cf}	9.08^{Df}	7.95^{Ec}
		vacuum	3.91^{ABf}	4.38^{Af}	3.60^{BDb}	2.93^{Cf}	3.34^{Deh}	3.90^{ABg}	3.47^{BDf}
	V	air	2.52^{Ab}	2.87^{Ae}	2.04^{BCag}	1.79^{Cd}	3.58^{De}	3.69^{De}	2.65^{Ad}
		vacuum	2.73^{Ad}	3.27^{Bb}	1.39^{Cc}	2.48^{Aa}	1.59^{Cb}	2.52^{Ab}	1.99^{De}
April	C	air	2.25^{Ag}	2.61^{Aa}	2.54^{Ae}	2.53^{Aa}	1.28^{Bg}	2.33^{Abj}	3.42^{Cf}
		vacuum	1.89^{Ah}	2.37^{Ba}	2.31^{Ba}	1.89^{Ad}	1.70^{Ab}	1.74^{Ah}	1.71^{Aeg}
	H1	air	2.34^{Ag}	3.06^{BCeg}	3.53^{Bb}	3.31^{Bch}	2.64^{Ad}	2.84^{CDi}	2.99^{Dh}
		vacuum	1.75^{Ah}	3.25^{Bbdg}	2.94^{Bf}	3.10^{Bf}	1.58^{Ab}	2.31^{ACbj}	1.90^{Ae}
	H2	air	3.21^{Aci}	4.34^{Bf}	4.21^{Bh}	3.72^{Cg}	3.17^{Ah}	2.83^{Ai}	3.05^{Ak}
		vacuum	2.86^{Ad}	4.26^{Bf}	3.65^{Cb}	3.17^{Afh}	2.14^{Di}	2.76^{Ai}	3.03^{Ak}
	V	air	1.76^{Ah}	2.83^{Be}	2.05^{Ag}	1.89^{Ad}	1.67^{Ab}	2.84^{Bi}	4.82^{Ci}
		vacuum	2.20^{Ag}	3.08^{Beg}	2.68^{Ce}	2.22^{ACb}	1.37^{Dg}	2.08^{Aj}	1.75^{Dgj}
SEM			0.14	0.11	0.10	0.10	0.12	0.15	0.20
P-value		M	< 0.0001	< 0.0001	< 0.0001	0.4309	< 0.0001	< 0.0001	< 0.0001
		G	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		P	< 0.0001	0.0620	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		M × G	0.0013	0.0079	0.0126	0.0159	< 0.0001	< 0.0001	< 0.0001
		M × P	0.0381	0.1821	0.0207	0.0023	< 0.0001	< 0.0001	< 0.0001
		G × P	< 0.0001	0.0025	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		M × G × P	0.8353	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Contrast		C vs. H1	0.3481	0.0007	0.0009	0.0526	< 0.0001	0.0017	< 0.0001
		C vs. V	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		C vs. H2	0.0004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		H1 vs. V	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.5947
		H1 vs. H2	0.0613	< 0.0001	< 0.0001	< 0.0001	0.6090	< 0.0001	< 0.0001
		V vs. H2	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.6071	0.0011

$^{A–C}$values in the same row with different letters differ significantly ($P \leq 0.05$); $^{a–c}$values in the same column with different letters differ significantly ($P \leq 0.05$); M – month; G – group; P – packaging

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The least squares means (LSM) for the tested groups were compared using contrast analysis.

RESULTS AND DISCUSSION

***Trolox equivalent antioxidant capacity* (*TEAC*).** The results of the TEAC are shown in Table 1.

The lowest value of the antioxidant capacity in January was determined in the vacuum-packed control group. The highest value was determined in the air-stored H_2 group. In all groups continuous changes in the values were reported. The lowest antioxidant capacity value of the last day of research was obtained in the V Group (vacuum-packed and air-stored).

In April, the highest value on the first day was recorded in the air-stored H2 group, and the lowest in the vacuum-packed H1 group. On the last day of research, the highest antioxidant capacity value in April was significantly lower than the highest value in January. Similar antioxidant capacity to the present study was reported by Plust (2007) and

Table 2. Peroxide value (PV) (mEq O_2/kg lipids) of the vacuum-packed or air-stored forcemeat during cold storage (4°C)

Month	Group	Packing	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
January	C	air	1.26^{Aa}	2.05^{Ba}	1.83^{Ba}	1.84^{Bag}	1.60^{ABa}	1.15^{Aa}	6.06^{Ca}
		vacuum	1.32^{Aa}	2.13^{Ba}	1.72^{ACa}	1.92^{Cag}	1.43^{Aa}	2.31^{Bb}	4.17^{Db}
	H1	air	1.61^{ACa}	1.41^{Aa}	1.59^{ACa}	1.89^{CDag}	2.05^{Dad}	1.95^{Dbe}	2.52^{Ecf}
		vacuum	2.17^{Aac}	3.50^{Bbd}	4.13^{BDb}	3.43^{Bbd}	2.98^{Bcg}	5.03^{Cc}	3.93^{Db}
	H2	air	6.94^{Ad}	3.92^{Bbde}	3.75^{Bbe}	2.75^{Cc}	2.86^{Ccd}	2.62^{Cbd}	3.91^{Bb}
		vacuum	4.31^{Ae}	4.00^{Abde}	4.25^{Abf}	3.98^{Ad}	3.99^{Ae}	5.15^{Bc}	3.83^{Ab}
	V	air	3.87^{Abe}	1.91^{ba}	4.75^{Cbf}	3.27^{Abc}	3.38^{Ace}	4.53^{Ccg}	5.32^{Dd}
		vacuum	2.89^{Abc}	2.86^{Aab}	2.31^{ABa}	2.15^{Bac}	2.76^{Acd}	3.08^{Adh}	1.42^{Ce}
April	C	air	4.19^{Ae}	7.45^{Bc}	7.15^{Bc}	7.33^{Be}	9.40^{Cf}	2.89^{Dbdh}	1.83^{Ece}
		vacuum	3.75^{Abe}	4.00^{Abde}	1.80^{Bad}	1.73^{Ba}	2.43^{Cdg}	0.84^{Da}	2.78^{Cf}
	H1	air	3.73^{Abe}	4.36^{Bde}	3.56^{ACbe}	2.75^{Cc}	3.11^{Ccg}	1.38^{Dae}	2.61^{Cf}
		vacuum	3.66^{Abe}	3.69^{Abd}	2.76^{Bde}	1.66^{Cah}	1.48^{Ca}	0.85^{Da}	1.82^{Cce}
	H2	air	3.50^{Abe}	6.85^{Bc}	4.61^{Cbf}	4.97^{Cf}	6.66^{Bh}	4.80^{Ccg}	1.50^{De}
		vacuum	4.04^{Ae}	9.08^{Bf}	4.02^{Ab}	1.06^{Ch}	5.94^{Dh}	3.54^{Agh}	1.64^{Ee}
	V	air	4.17^{Ae}	5.07^{Be}	5.18^{Bf}	5.03^{Bf}	6.58^{Ch}	6.94^{Cf}	4.25^{Ab}
		vacuum	3.33^{Abe}	4.43^{Bde}	2.00^{Cad}	2.39^{Ccg}	2.00^{Cad}	4.24^{Bg}	7.02^{Dg}
SEM			0.23	0.25	0.21	0.13	0.17	0.16	0.17
P-value		M	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.5710	< 0.0001
		G	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		P	< 0.0001	0.5033	< 0.0001	< 0.0001	< 0.0001	0.0536	0.0403
		M × G	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		M × P	0.0182	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		G × P	0.0002	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0008
		M × G × P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Contrast		C vs. H1	0.7402	0.0013	0.8537	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		C vs. V	< 0.0001	0.2122	0.0194	0.9999	0.9908	< 0.0001	< 0.0001
		C vs. H2	< 0.0001	< 0.0001	< 0.0001	0.9989	< 0.0001	< 0.0001	< 0.0001
		H1 vs. V	< 0.0001	0.2397	0.0017	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		H1 vs. H2	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	1.0000
		V vs. H2	< 0.0001	< 0.0001	0.0194	0.9967	< 0.0001	< 0.0001	< 0.0001

$^{A–C}$values in the same row with different letters differ significantly ($P \le 0.05$); $^{a–c}$values in the same column with different letters differ significantly ($P \le 0.05$); M – month; G – group; P – packaging

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Martínez *et al.* (2014). Plust (2007) also proved that the TEAC values decreased until the seventh day of the study, and then there was a subsequent increase which continued until the end of the study. Similar tendencies were observed in our study, but with the difference that the antioxidant capacity remained constant until the seventh day in the air-stored control group. It could be associated with a reaction between reducing sugars and free amino acids or free amino and protein groups.

Probably the increase of the TEAC value may be an indicator of spoilage. The antioxidative potential of meat may be increased by some additives (for example herbs or vegetables), but during the research it should be stable. Huang *et al.* (2011) stated that the oxidation process leads to the formation of compounds capable of scavenging free radicals.

Peroxide and anisidine value and the TOTOX index. The peroxide and anisidine value and the TOTOX index are shown in Tables 2–4.

Table 3. Anisidine value (AV) of the vacuum-packed or air-stored forcemeat during cold storage (4°C)

Month	Group	Packing	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
January	C	air	1.72^{Aa}	2.62^{Bad}	1.94^{Aa}	1.10^{Ca}	1.64^{Aa}	4.63^{Da}	1.48^{Aad}
		vacuum	1.30^{Aaf}	1.23^{Ab}	1.11^{Ab}	1.72^{Bb}	1.52^{Ba}	1.27^{Ab}	1.67^{Ba}
	H1	air	0.00^{Ab}	0.00^{Ac}	0.00^{Ac}	0.00^{Ac}	0.00^{Ab}	0.00^{Ac}	0.00^{Ab}
		vacuum	0.00^{Ab}	0.00^{Ac}	0.00^{Ac}	0.00^{Ac}	0.00^{Ab}	0.00^{Ac}	0.00^{Ab}
	H2	air	0.00^{Ab}	2.11^{Bd}	0.00^{Ac}	0.00^{Ac}	0.00^{Ab}	0.00^{Ac}	0.00^{Ab}
		vacuum	0.00^{Ab}	0.00^{Ac}	0.00^{Ac}	0.00^{Ac}	0.00^{Ab}	0.00^{Ac}	0.00^{Ab}
	V	air	0.00^{Ab}	3.06^{Ba}	0.00^{Ac}	0.00^{Ac}	0.00^{Ab}	1.44^{Cb}	0.00^{Ab}
		vacuum	0.00^{Ab}	2.26^{Bad}	0.00^{Ac}	0.00^{Ac}	0.00^{Ab}	0.00^{Ac}	0.00^{Ab}
April	C	air	2.67^{Ace}	4.14^{Be}	4.05^{Bd}	6.00^{Cd}	0.65^{Dc}	1.68^{Ed}	2.22^{Ac}
		vacuum	2.08^{Aac}	2.18^{Aad}	1.03^{Bb}	0.75^{BDa}	0.22^{Cd}	0.68^{De}	1.06^{Bd}
	H1	air	0.00^{Ab}	0.00^{Ac}	0.00^{Ac}	0.00^{Ac}	0.00^{Ab}	0.00^{Ac}	0.00^{Ab}
		vacuum	0.00^{Ab}	0.00^{Ac}	0.00^{Ac}	0.00^{Ac}	0.14^{Bd}	0.00^{Ac}	0.00^{Ab}
	H2	air	0.00^{Ab}	0.63^{Bb}	0.00^{Ac}	0.78^{Ba}	0.00^{Ab}	0.00^{Ac}	0.00^{Ab}
		vacuum	0.88^{Af}	0.00^{Bc}	0.00^{Bc}	0.00^{Bc}	0.00^{Bb}	0.00^{Bc}	0.00^{Bb}
	V	air	3.69^{Ad}	2.26^{Bad}	0.65^{Ce}	1.72^{Db}	0.00^{Eb}	0.00^{Ec}	0.00^{Eb}
		vacuum	3.17^{Ade}	1.06^{Bb}	0.00^{Cc}	0.00^{Cc}	0.00^{Cb}	0.00^{Cc}	0.00^{Cb}
SEM			0.16	0.17	0.07	0.10	0.07	0.08	0.09
P-value		M	< 0.0001	0.1481	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.7258
		G	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		P	0.3089	< 0.0001	< 0.0001	< 0.0001	0.1473	< 0.0001	0.0128
		M × G	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.9457
		M × P	0.7621	0.4686	< 0.0001	< 0.0001	0.5465	< 0.0001	0.0007
		G × P	0.0006	< 0.0001	< 0.0001	< 0.0001	0.0049	< 0.0001	0.0005
		M × G × P	0.0202	0.0002	< 0.0001	< 0.0001	0.1635	< 0.0001	< 0.0001
Contrast		C vs. H1	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		C vs. V	0.1812	0.0127	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		C vs. H2	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		H1 vs. V	< 0.0001	< 0.0001	0.0055	< 0.0001	0.9056	< 0.0001	1.0000
		H1 vs. H2	0.2172	< 0.0001	1.0000	0.0286	0.9056	1.0000	1.0000
		V vs. H2	< 0.0001	< 0.0001	0.0055	0.0053	1.0000	< 0.0001	1.0000

$^{A–C}$values in the same row with different letters differ significantly ($P \leq 0.05$); $^{a–c}$values in the same column with different letters differ significantly ($P \leq 0.05$); M – month; G – group; P – packaging

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Table 4. TOTOX value of the vacuum-packed or air-stored forcemeat during cold storage (4°C)

Month	Group	Packing	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
January	C	air	4.99^{Aa}	7.96^{Ba}	6.69^{Cae}	5.88^{Aab}	5.80^{Aa}	7.62^{Bad}	17.22^{Da}
		vacuum	4.73^{Aa}	6.78^{Ba}	5.59^{Aae}	6.70^{Ba}	5.23^{Aa}	7.28^{Ba}	12.52^{Cb}
	H1	air	4.19^{ABa}	3.67^{Ab}	4.14^{ABa}	4.90^{BCb}	5.34^{Ca}	5.07^{Cb}	6.55^{Dcf}
		vacuum	5.63^{Aa}	9.09^{Bce}	10.73^{Cb}	8.91^{BDc}	7.74^{Dbe}	13.07^{Ec}	10.21^{Cd}
	H2	air	18.05^{Ad}	12.31^{Bd}	9.75^{Cbd}	7.15^{Da}	7.44^{Dbe}	6.81^{Da}	10.15^{Cd}
		vacuum	11.20^{Ab}	10.40^{Ade}	11.04^{Ab}	10.35^{Ad}	10.37^{Ac}	13.40^{Bc}	9.96^{Ad}
	V	air	10.05^{Abf}	8.03^{Bac}	12.35^{Cbf}	8.51^{Bc}	8.79^{Bb}	13.21^{Cc}	13.84^{Cb}
		vacuum	7.52^{Ac}	9.69^{Bce}	6.01^{ACa}	5.59^{Cab}	7.17^{ADbe}	8.02^{Dad}	3.69^{Ee}
April	C	air	13.58^{Ae}	23.51^{Bf}	22.65^{Bc}	25.06^{Ce}	25.09^{Cd}	9.19^{Ddg}	6.98^{Ec}
		vacuum	11.84^{Ab}	12.58^{Ad}	5.72^{Bae}	5.25^{Bb}	6.54^{Bae}	2.86^{Ce}	8.29^{Dc}
	H1	air	9.70^{Af}	11.34^{Bd}	9.26^{Abd}	7.15^{Ca}	8.07^{ACb}	3.58^{De}	6.78^{Ccf}
		vacuum	9.51^{Af}	9.59^{Ace}	7.18^{Bde}	4.31^{Cb}	3.98^{Ca}	2.20^{De}	4.72^{Cef}
	H2	air	9.10^{Af}	18.44^{Bh}	11.99^{Cbf}	13.70^{Cf}	17.31^{Bf}	12.47^{Ccg}	3.90^{De}
		vacuum	11.38^{Ab}	23.60^{Bf}	10.44^{AEb}	2.76^{Cg}	15.45^{Df}	9.20^{Ed}	4.25^{Ce}
	V	air	14.53^{Ae}	15.44^{Ag}	14.12^{Af}	14.79^{Af}	17.11^{Bf}	18.05^{Bf}	11.04^{Cd}
		vacuum	11.84^{Ab}	12.58^{Ad}	5.19^{Ba}	6.22^{Ba}	5.19^{Ba}	11.01^{Ag}	18.24^{Ca}
SEM			0.61	0.66	0.54	0.34	0.44	0.41	0.45
P-value		M	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0005	< 0.0001
		G	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		P	< 0.0001	0.0185	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0129
		M × G	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		M × P	0.0193	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		G × P	0.0013	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0002
		M × G × P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Contrast		C vs. H1	0.0040	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0514	< 0.0001
		C vs. V	< 0.0001	0.0405	0.2196	< 0.0001	0.0035	< 0.0001	0.4939
		C vs. H2	< 0.0001	< 0.0001	0.3391	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		H1 vs. V	< 0.0001	< 0.0001	0.0005	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		H1 vs. H2	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	1.0000
		V vs. H2	0.0069	< 0.0001	0.0028	0.6439	< 0.0001	< 0.0001	< 0.0001

$^{A–C}$values in the same row with different letters differ significantly ($P \leq 0.05$); $^{a–c}$values in the same column with different letters differ significantly ($P \leq 0.05$); M – month; G – group; P – packaging

Statistically significant differences were recorded in the peroxide values on the first day between groups in January. The lowest PV in January on the first day was recorded in the C group. On the last day of the study, the values showed statistically significant differences as compared with the first day. The vacuum-packed H2 group was an exception. No statistically significant differences between the groups were noted on the first day of the study in April. On the last day, the lowest value was noted in the air-stored H2 Group and the highest in the V group also stored in air. In the case of both months, the values first increased and then decreased in all groups. The anisidine value was determined mainly in the C and V groups, both in January and April. In April, fish meat from the air-stored C group exhibited higher AVs than the vacuum-packed meat throughout the entire study. The values in the control group between the vacuum-packed and air-stored fish were statistically significantly different from the third day. The TOTOX index was higher in April in the control group, and in the H1 and V groups. The H2 group exhibited higher values of the TOTOX index in January.

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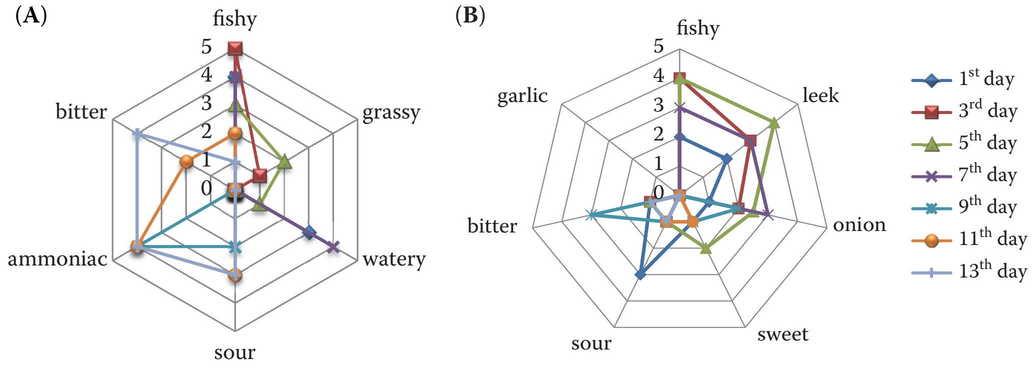

Figure 1. Sensory profiling of flavour (**A**) in the control group stored in air in January and (**B**) in the V group vacuum-packed in April

An initial increase and subsequent decrease of the peroxide value (PV) was also noted by Shi *et al.* (2014) in silver carp (*Hypophthalmichthys molitrix*) meat. The study of Çoban (2013) confirmed the effectiveness of natural antioxidants to extend the freshness of fish meat.

In the present study, in the H2 group in January PVs were higher on the first day. These results suggest that excessive concentrations of antioxidants can have a pro-oxidative effect. Alghazeer *et al.* (2008) also reported that a higher portion of natural antioxidants to fillets showed a higher PV. According to Gokoglu *et al.* (2012) the colorimetric determination of AV depends on the concentration of secondary lipid oxidation products and their structural features, and it should not exceed 2.

In our research AVs were almost undetected in the H1 and H2 groups. It was so because meats were vacuum-packed and held in a refrigerator where they were protected from light, oxygen, and high temperatures. But the main reason was the addition of herbs, which revealed their antioxidant potential and prevented the production of secondary lipid oxidation products (Aranda *et al.* 2006). Pereira de Abreu *et al.* (2011) claimed that antioxidants and phenolic compounds act as free radical acceptors, which ends the process of oxidation at the initial stage.

Sensory evaluation. The results of sensory evaluation are shown in Figure 1. In January, fishy odour was the most dominant in the control group, and it was more perceptible in the vacuum-sealed group. Sour and ammonia notes were recorded from the seventh day in the air-stored meat. Ammonia smell was noted only in the air-stored group. In the H2 group, the herbal additives were perceptible until the ninth day, while the predominant note was rosemary.

In April, less sensory notes were recorded in the control group as compared with the research in January. Fishy smell was scored higher in the initial period of the study, while watery smell scored lower. Compared with January, bitter taste was also more perceptible. An ammonia note was perceptible in the air-stored group stored from the seventh day of the study.

In our study, the appearance of sweet taste was noted in the V and H2 groups. It is associated with the formation of ketones, alcohols, and aldehydes. Characteristic notes of freshwater fish odours came from unsaturated carbonyl compounds and 6-, 8- and 9-carbon alcohols (Ólafsdóttir & Jónsdóttir 2010). Moreover, the oxidation processes occurring during fish storage cause the accumulation of aldehydes, such as hexanol, cis-4-heptenal, 2,4-heptadienal, and 2,4,7-decadienal, which contribute to the formation of rancid, cold aftertaste associated with storage.

In our study, the application of plant additives contributed to a reduction of the intensity of the ammonia note. Rosemary, juniper, and thyme contributed to inhibiting the formation of this odour even in the air-stored groups. Özogul *et al.* (2010) also showed the application of rosemary extract to keep good sensory evaluation.

CONCLUSION

The addition of black pepper (0.1%), mustard (0.2%), and juniper (0.2%) inhibited the formation of secondary lipid oxidation products and vacuum packing restrained changes in the antioxidant capacity of meat. For short-term meat storage it is justified to add vegetables to mask the taste and aroma of fish.

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