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FRESHNESS INDICATORS OF DEFROSTED FILLETS OF *LEPIDOCYBIUM FLAVOBRUNNEUM* IN VACUUM SKIN PACKAGING/ VSP PACKAGING DURING COLD STORAGE

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The aim of study was to monitor chemical parameters (pH, total volatile basic nitrogen, trimethylamine, free fatty acids, peroxide value, and thiobarbituric acid assay) in the samples of defrosted fillets of escolar (*Lepidocybium flavobrunneum*) fish, packed in vacuum skin packaging (VSP) during 15 days of storage at +2±2 ºC. Comparison was done with fillets packed in vacuum (control samples). The obtained results from our research showed that VSP packaging can be recommended, as it showed slightly better stability than vacuum packaging. The initial values of total volatile basic nitrogen (TVBN) content (in mg/100 g) in fish samples were low (vacuum 16.36±0.65; VSP 16.67±0.45) and these values did not change significantly until day 9. TVBN was significantly higher ($P<0.05$: vacuum 23.31±2.41, VSP 21.76±2.08) in samples analysed after 9 days of storage. Similar results were found during estimation of free fatty acids content. Trimethylamine (TMA) content was in the correlation with storage time in the same way as total volatile basic nitrogen content. During the experiment, the highest peroxide value expressed in $meqO_2\ kg^{-1}$ (vacuum 1.77±1.38; VSP2.16±0.11) and thiobarbituric acid assay content expressed in $mg\ kg^{-1}$ (vacuum 4.20±0.43; VSP 4.10±2.61) were relatively low.

Keywords: escolar fish, freshness, chemical changes, lipid oxidation

Escolar fish, *Lepidocybium flavobrunneum*, fillets without skin are a regular part of seafood menu in the Czech Republic. The transportation of this fish to the Czech Republic may last several days (ca. 2–4 days) due to the remote areas of the catch of this fish (Pacific Ocean) and follow-up manipulations within the logistic flow of materials. After thawing in the Czech Republic, fish are filleted, re-vacuum packaged (consumer packages of various weights), and sold with the warning that it is a thawed product (EC, 2011). The shelf-life of this repacked product is 9 days from the date of packaging. Escolar fish contains very high amounts of oil and the oil composition of this species differs from most seafood due to the presence of wax esters, which are responsible for diarrheal effects on consumers (Nichols et al., 2001).

Fish packed in vacuum are commonly sold in the Czech Republic in contrast to fish packaged by vacuum skin manner. Darfresh vacuum skin packaging (VSP) of fish is an innovative method, which also extends the shelf-life of fish, while offering the possibility of microwave heating of fish together with the packaging.

The aim of this research was to evaluate freshness parameters of defrosted escolar fish samples during 15 days of storage at +2±2 ºC. Samples were VSP as experimental samples and vacuum packed as control samples, because escolar (*Lepidocybium flavobrunneum*) fish can only be placed on the market in packaged form. In conformity with Regulation (EC, 2008), the fillets of escolar fish are placed on the market in vacuum packaging and labelled

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to provide information to the consumer on the risk related to the presence of substances with adverse gastrointestinal effects.

1. Materials and methods

The study was carried out by using 6 skinless fillets (A, B, C, D, E, F) of escolar fish (*Lepidocybium flavobrunneum*), which were purchased on the market as vacuum packaged thawed product (vendor: Seafood AG, Prague, Czech Republic). The country of origin of fish was Vietnam, the fishing area was Pacific Ocean FAO 71. Shelf-life period provided by the vendor was nine days from the date of packaging when stored at +2±2 ºC. Further processing (cutting), vacuum and VSP, storage at +2±2 ºC (98% vacuum), and laboratory analysis were conducted at the Department of Meat Hygiene and Technology (Faculty of Veterinary Hygiene and Ecology, Veterinary and Pharmaceutical University in Brno). Easy-open, microwavable package for a quick and easy preparation was used as vacuum skin packaging (Simple Steps® VSP Package for Produce, 2014). Vacuum skin packaging consisted of CRYOVAC® DARFRESH® base web structure (RSB 03x56; thickness 280 μm; base weight 305 g m^{-2}) and top film CRYOVAC® DARFRESH® top web (TC201; thickness 100 μm; basis weight 95.5 g m^{-2}). Cranial part (behind the head) with a thickness of 2.5 cm from each fillet (separated from the rest of fillet cross section) was analysed on the day of purchase in order to determine the input values of monitored parameters (initial data). The remaining muscle of each fillet was cut transversally in cranio-caudal direction and portioned in 12 parts (each with a thickness of about 2.5 cm). In this manner, from each fillet anatomically identical samples were formed for vacuum packaging (1st, 3rd, 5th, 7th, 9th, and 11th), and for VSP (2nd, 4th, 6th, 8th, 10th, and 12th). Anatomically identical parts obtained from two different fillets were packed together in each type of packaging, e.g. 1. mixed sample after 2 days of storage; vacuum packaging: 1. cut from fillets A+B (C+D, E+F), VSP: 2. cut from fillets A+B (C+D, E+F), etc. In that way, 36 mixed samples (18 samples for vacuum and 18 for VSP) were formed, which were analysed after 2, 4, 7, 9, 11, and 15 days of storage at +2±2 ºC (3 mixed samples for each type of packaging and for each day of storage).

The following chemical parameters of freshness were investigated: pH, TVBN (total volatile basic nitrogen in mg/100 g), TMA (trimethylamine in mg/100 g), FFA (free fatty acids in % total lipid as oleic acid), PV (peroxide value in mekvO_2 kg^{-1}), and TBA (thiobarbituric acid assay in mg kg^{-1}). The pH values were measured using the inoLab pH 730 digital pH-metre (WTW GmbH, Germany). The total volatile basic nitrogen (TVBN) was determined by direct distillation followed by titration on a Kjeltec 2300 (FOSS Analytical AB, Höganäs, Sweden) according to regulation (EC, 2005), Chapter III. Trimethylamine (TMA) was determined using the same method as for TVBN determination after formaldehyde was added to samples to unbind primary and secondary biogenic amines. Free fatty acids (FFA) and peroxide values (PV) were determined after fat extraction with diethyl ether. FFA were determined in accordance with CSN ISO (2009). Peroxide values were determined by a modified method according to CSN ISO (2010). The thiobarbituric acid assay (TBA) value was determined by the distillation method and oxidation products were quantified as malondialdehyde equivalents (MDA).

Statistical significance at $P<0.05$, resp., $P<0.01$ were evaluated using *t*-test and one-way ANOVA analysis of variance. Post hoc Tukey's test was used for finding difference among groups. Statistical analyses were done using IBM SPSS Statistics 20 software.

2. Results and discussion

Values and concentrations of tested parameters (pH, total volatile basic nitrogen (TVBN), trimethylamine (TMA), free fatty acids (FFA), peroxide value (PV), and thiobarbituric acid assay (TBA)) and statistical comparisons are shown in Fig. 1 to Fig. 6.

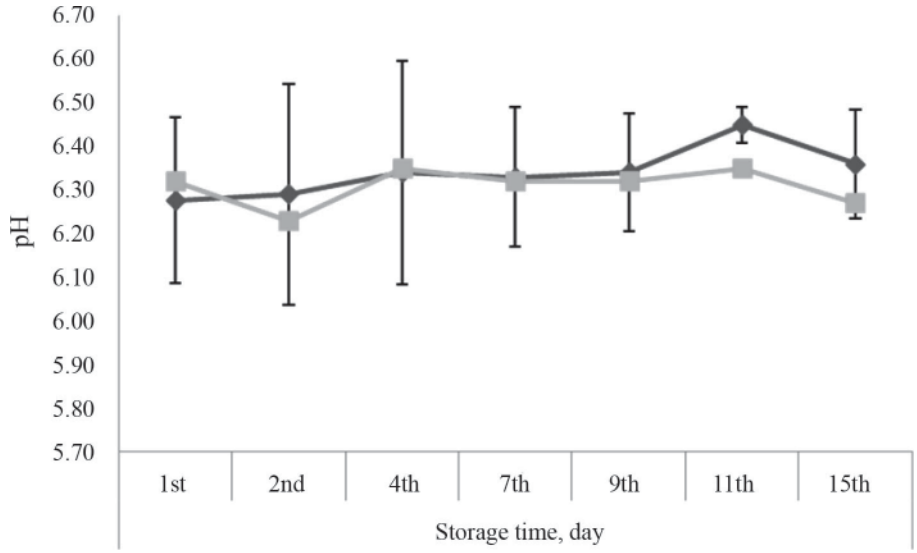

Fig. 1. pH value of escolar fish samples packed in vacuum and VSP
—◆—: vacuum; —■—: darfresh

Low pH can be an indicator of fish freshness and good sign for extended fish shelf-life (Abbas et al., 2008). The value of pH did not differ much during the 15 days of storage, either in vacuum resp. VSP, ranging from 6.28±0.19 resp. 6.32±0.11 (at the beginning of experiment) to 6.36±0.12 resp. 6.28±0.06 (after 15 days of storage).

Our results for pH values are slightly higher in comparison with previously published results (Hwang et al., 2012), where pH value was 5.6±0.3 in escolar fish steaks, though analysis were not done after the same period of time as our experiment but immediately after the purchasing. It can be assumed that such low pH values were associated with the decomposition of glycogen to lactic acid, with biochemical process named as "the maturation of meat", which occur in fish post-mortem metabolism. The pH values over 6 measured in our experiment (Fig. 1) may be associated with the later stages of meat maturation, during which lactic acid is decomposed to carbon dioxide (CO_2) and water (H_2O).

There was no difference between the TVBN content (in mg/100 g) determined on day 1 of vacuum (16.36±0.65) and VSP (16.67±0.45). Also, until the 7th day of experiments, the TVBN content in vacuum and VSP did not differ significantly ($P>0.05$). However, from the 7th until the 11th day of experiment, low molecular weight nitrogenous substances were formed more intensively in the samples packed in vacuum packaging than in VSP (Fig. 2).

Our results (Fig. 2) show much higher TVBN (in mg/100 g) concentrations (day 1/15: vacuum: 16.36±0.65 / 26.53±2.35, VSP: 16.67±0.45 / 27.72±2.46) in comparison with

previous studies, in which level of TVBN ranged from 3.2 to 8.8 (in mg/100 g) in escolar fish samples that were analysed immediately after the purchase (Hwang et al., 2012). TVBN content can be influenced by these parameters: the time that passes between slaughtering/ processing of fish and the start of laboratory analysis, storage conditions (temperature conditions and its fluctuation), the character of microbial contamination (type and number of microorganisms), and autolytic activity of native microbial proteolytic enzymes, which can vary depending on environmental conditions (Bremner, 2002).

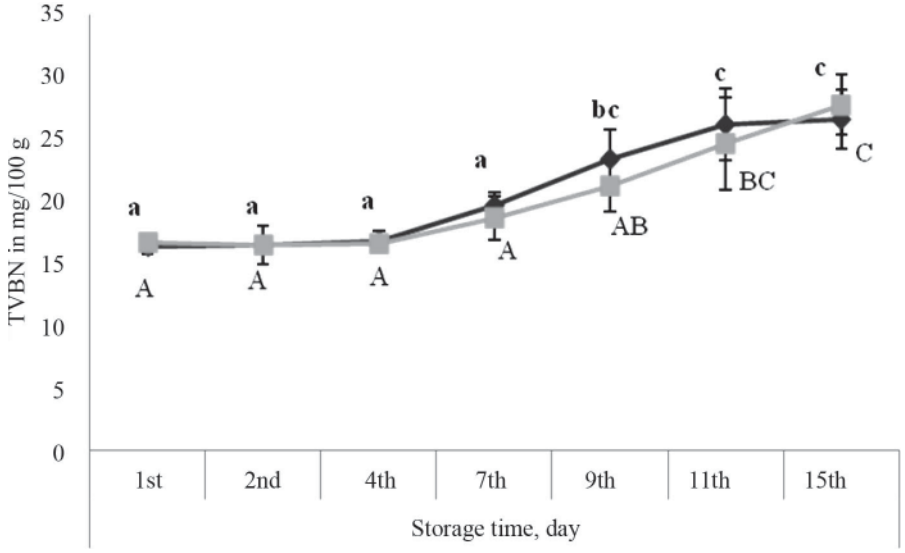

Fig. 2. Total volatile basic nitrogen (TVBN) level in the samples of escolar fish, packed in vacuum and VSP —◆—: vacuum; —■—: darfresh. Parameters values a, b, c / A, B, C are indicators for statistical significance at P<0.05 for vacuum / VSP samples in dependence on day of storage; lowercase letters indicate the lower values of the particular parameter

TMA content (in mg/100 g) showed the same fluctuation and trend as TVBN content, depending on the storage period. TMA content in the samples was in case of vacuum packed and VSP from the first day (vacuum 12.05±0.48; VSP 12.47±0.70) to 4th day (vacuum 13.06±1.54) and 11th day (VSP 21.43±3.16), respectively, almost without significant changes. Afterwards its content increased, whereas in vacuum packed samples of fish the formation of this parameter was more intensive in comparison with VSP. Levels of TMA in vacuum packaging after 9 and 11 days of storage were significantly higher (P<0.05) in comparison with the samples stored in VSP (Fig. 3).

TVBN and TMA concentrations, as freshness parameters, were increasing gradually during the storage period of the experiment, which is in accordance with other studies (Bremner, 2002). Huss (1988) reported that flat and pelagic fish have the lowest TMAO level, cod has a greater amount, and elasmobranchs and squids have the greatest (up to 250 mg/100 g). According to the same author, a good quality coldwater fish contains <1.5 mg TMA/100 g; the limit of acceptability is 10–15 mg TMA/100 g (Mokrani et al., 2012). In our experiment, this limit for vacuum and VSP samples was exceeded after 7 and 9 days of storage, respectively (Fig. 3).

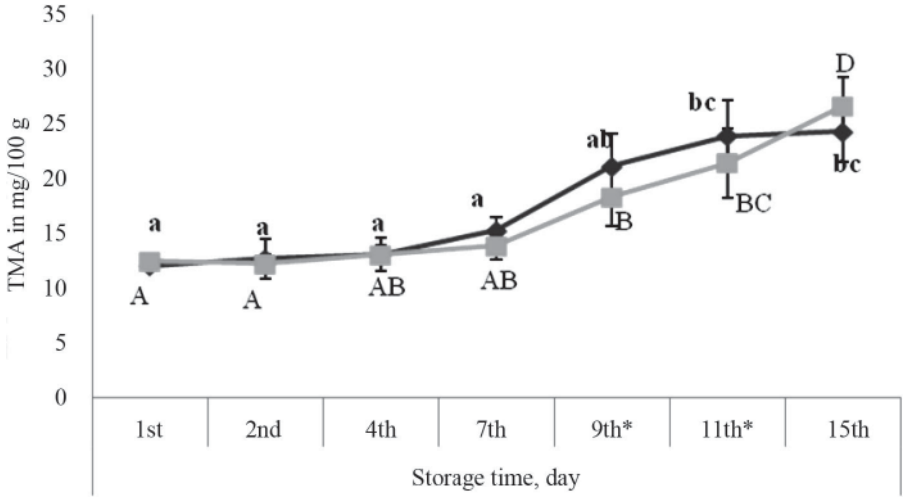

Fig. 3. Trimethylamine (TMA) level in the samples of escolar fish, packed in vacuum and VSP —◆—: vacuum; —■—: darfresh. *Significant difference (P<0.05) between vacuum and VSP packaging. Parameters values a, b, c / A, B, C, D are indicators for statistical significance at P<0.05 for vacuum / VSP samples in dependence on day of storage; lowercase letters indicate the lower values of the particular parameter.

FFA (% total lipid as oleic acid) content in fish samples from the beginning of the experiment (vacuum 1.44±0.09; VSP 1.32±0.14) up to the 9th day (vacuum 1.49±0.03; VSP 1.86±0.07) of storage did not change significantly (P>0.05). However, FFA content was higher in VSP packed samples after each measurement with the exception of measurements taken after 9 days of storage. On the 9th day of storage, FFA content was statistically significantly higher (P<0.01) in VSP packed samples than in vacuum packed samples. Between 9 and 11 days of storage FFA content increased significantly (P<0.01) to values 5.39±0.43 (vacuum) and 5.17±0.40 (VSP); after that the FFA content till the end of the experiment stayed stable (Fig. 4). Increase in FFA content can be explained with the possibility that at the beginning of the storage endogenous enzymes (lipases and phospholipase) were influencing the increase of FFA content, but after the end of lag phase FFA are produced by microbiological activity (*Photobacterium phosphoreum*, lactic acid bacteria) (Etemadi et al., 2013). This statement is supported by the higher TMA level after 9 days of storage in the samples of escolar fish.

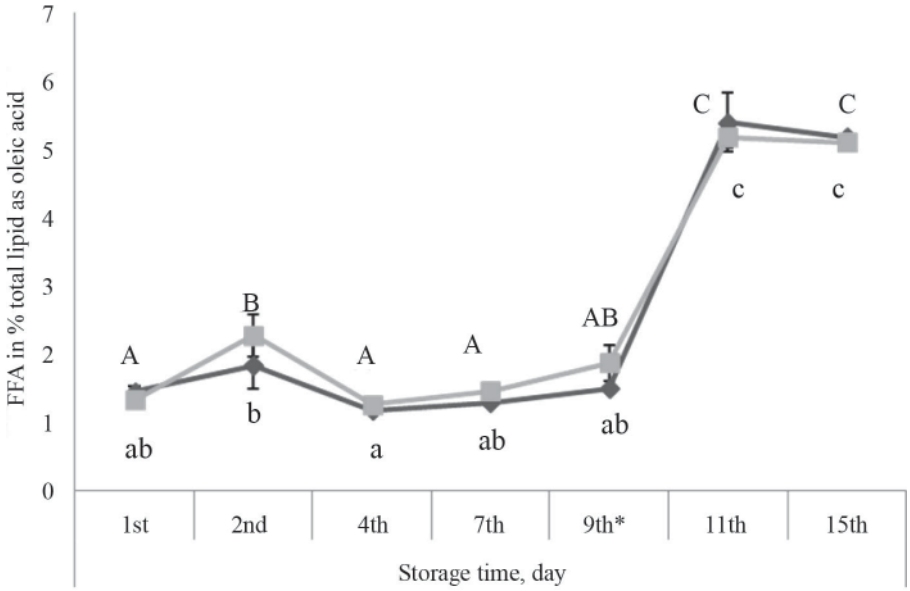

Fig. 4. Free fatty acids (FFA) content in escolar fish samples packed in vacuum and VSP —◆—: vacuum; —■—: darfresh. Parameters values a, b, c / A, B, C are indicators for statistical significance at P<0.05 for vacuum / VSP samples in dependence on day of storage; lowercase letters indicate the lower values of the particular parameter **t*-test P<0.05

In our study, the peroxide value (PV in mekvO_2 kg^{-1}) was used to determine primary products of oxidation, which dominated during the first (to the 7th day) storage period (Fig. 5); the thiobarbituric acid assay (TBA in mg kg^{-1}) value was used to determine secondary products of oxidation that dominated in the second half (from the 7th day) of our experiment (Fig. 6).

At the beginning (day 1), PV values (in mekvO_2 kg^{-1}) were very low (vacuum: 0.55±0.10; VSP: 0.60±0.10). Subsequently, values of this parameter were increasing until 7 (VSP: 2.16±0.11) and 9 (vacuum: 1.77±1.38) days of storage. Afterwards, primary products of oxidation were degraded and peroxide value started to decrease (Fig. 5).

Secondary lipid oxidation (TBA in mg kg^{-1}) occurred in the same way in both types of packaging. Secondary oxidation processes were inhibited by the lack of oxygen, and its intensity increased during the second phase of experiment storage period (day 1: vacuum 3.52±0.98; VSP 2.88±1.06, day 7: vacuum: 2.20±0.59; VSP 1.80±0.35, day 11: vacuum 4.20±0.43; VSP 3.98±0.24).

An increase in TBA values (in mg kg^{-1}) was caused by malondialdehyde production, its content was the highest after 11 days of storage in vacuum packed samples (4.20±0.43) and after 15 days of storage in VSP packaging (4.10±2.61) (Fig. 6). Our results are in agreement with the statement that storage time and freezing result in increased accumulation of MDA in fish (Paulinus et al., 2013).

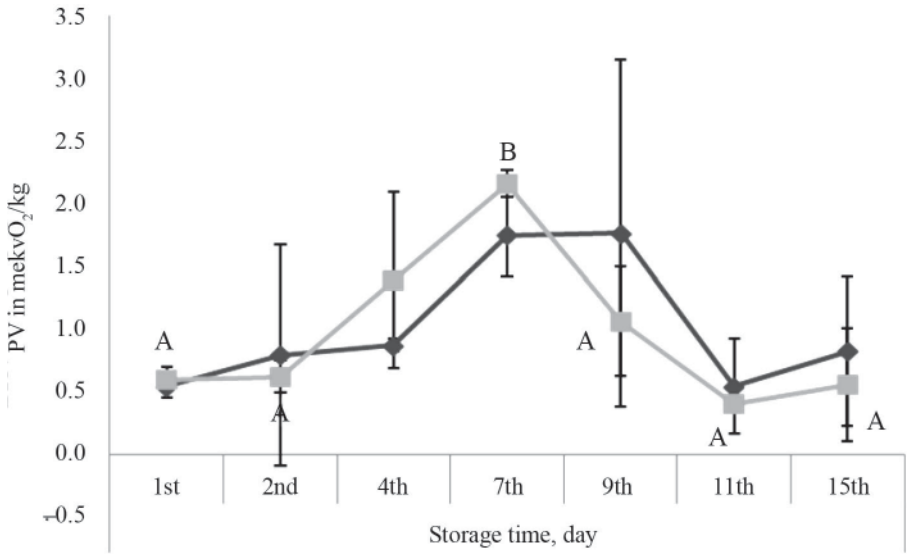

Fig. 5. Peroxide value in the samples of escolar fish, packed in vacuum and VSP
—◆—: vacuum; —■—: darfresh. Parameters values A, B are indicators for statistical significance at P<0.05 for VSP samples in dependence on day of storage; lowercase letters indicate the lower values of the particular parameter

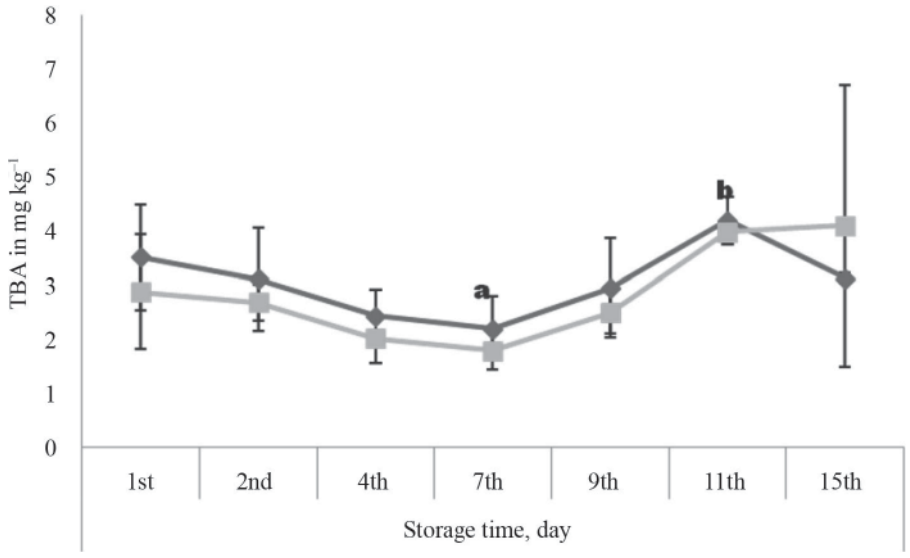

Fig. 6. Thiobarbituric acid assay (TBA) in the samples of escolar fish, packed in vacuum and VSP
—◆—: vacuum; —■—: darfresh. Parameters values a, b are indicators for statistical significance at P<0.05 for vacuum packaged samples in dependence on day of storage; lowercase letters indicate the lower values of the particular parameter

Bahmani et al. (2011) stated that there is a direct relationship between FFA formation and the loss of freshness in fish. It is known that FFA content causes texture deterioration by interacting with proteins and have a significant effect on the sensory quality of fish. In our experiment the preservative effect of temperature (+2±2 °C) and the absence of oxygen (O_2) was evident on lipid damage in both packaging. Lipolytic activity was shown to be sensitive to the time of storage in both types of packaging, and FFA content showed an accelerated development after 9 days of storage. From the experiment results, a positive finding of the shelf-life evaluation of escolar fish fillets is that this parameter was almost at the same level until the 9th day of monitoring (Fig. 4). The reason may be the immediate oxidation of products from hydrolytic cleavage of fat (lipolysis) and formation of primary oxidation products, as evidenced by the monitoring parameter PV (Fig. 5).

3. Conclusions

This study has shown that 9 days expiry period of *Lepidocybium flavobrunneum* defrosted fish fillets after packaging, specified by the manufacturer, is the maximum storage period for the given storage conditions (vacuum, +2±2 ºC) due to significant changes occurring after this storage period in FFA, PV, TVBN, TMA, and TBA levels. According to our results, VSP packaging can be recommended due to the fact that the samples packed in this type of packaging showed a slightly better stability when monitoring levels of TVBN, TMA, and TBA. A complex influence of these parameters on the human health could only be made on the basis of further analyses (e.g. biogenic amines, *Listeria monocytogenes*, *Salmonella* spp., and other microbiological tests, also sensory examination). Considering the fact that thawed fish meat is less biochemically and microbiologically stable than fresh fish meat, it should be stressed that an important prerequisite for food security is the fish handling after consumers' purchase. Proper handling should be followed during transportation home and during culinary preparation of fillets (maintenance of cold chain, elimination of secondary contamination, sufficient heat treatment).

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