The effect of vacuum packaging on physicochemical changes in rainbow trout (*Oncorhynchus mykiss*) during cold storage

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Abstract

The aim of the study was to monitor changes in selected physical (a_w water activity, pH values) and chemical (TVBN total volatile basic nitrogen, TMA-N trimethylamine nitrogen, FFA free fatty acids, PV peroxide values, TBA thiobarbituric acid value) properties in the shelf life of rainbow trout (*Oncorhynchus mykiss*) muscle. A total of 192 trout were examined. Control samples (96 samples) were simply packaged in contact with atmospheric oxygen, while experimental samples (96 samples) were packaged in a commercial vacuum (98%). All the samples were stored at 2 ± 2° C for 11 days. Analyses were performed on storage days 1, 2, 4, 7, 9, and 11. During the experiment, a_w values increased in both types of packaging (in air: 0.982; vacuum-packaged: 0.989). At the end of storage, TVBN and TMA concentrations were at 28.88 ± 4.42 and 19.28 ± 3.00 g mg·100^{-1}, respectively, in the muscle of vacuum-packaged trout; and at 30.52 ± 2.91 and 19.94 ± 2.05 mg·100 g^{-1}, respectively, in fish in simple packaging. The FFA content in vacuum-packaged fish initially declined before increasing to 3.67 ± 2.37% of total fat as oleic acid later in the experiment. The pattern of PV changes was inconclusive, and significant changes ($P < 0.01$) were observed in both types of packaging. On monitoring day 11, TBA values had increased to 7.34 ± 3.10 mg·kg^{-1} in vacuum-packaged fish and to 26.03 ± 8.00 mg·kg^{-1} in fish in simple packaging. Free fatty acids are not a good indicator of spoilage because they are converted to hydroperoxides. Vacuum packaging effectively slowed down oxidative changes in rainbow trout muscle. The peroxide content is not a suitable indicator of shelf life as peroxides are decomposed to secondary products. Total volatile basic nitrogen and thiobarbituric acid value can be recommended as suitable indicators of freshness and shelf life.

Fish, modified atmosphere, shelf life, fresh water

The rainbow trout (*Oncorhynchus mykiss*) was first introduced in Germany in 1882 and is now, along with the common carp (*Cyprinus carpio*), one of the most important aquaculture fish raised in cold flowing water in Europe. A total of 448 tons of live weight of rainbow trout were produced in the Czech Republic in 2012 (Kottelat and Freyhof 2007; Ženíšková and Gall 2011).

Fresh fish are traditionally stored in air at the temperature of melting ice, and their shelf life ranges from 2 to 10 days depending on the species, fishing grounds, season of the year, initial microbiological quality, and storage temperature (Masniyom 2011). The time from killing and the thermal "history" of the fish are considered key factors determining the final qualitative characteristics of fish products (Rezaei et al. 2008). Throughout their period of sale, fresh fish must satisfy the sensory requirements for the fish freshness categories of Council Regulation (EC) No. 2406/1996 and the chemical requirements set out in Commission Regulation (EC) 2074/2005 (Ježek and Buchtová 2012).

Irreversible organoleptic and physicochemical and microbiological changes that shorten the shelf life of fish occur during the process of fish spoilage (Arashisar et al. 2004;

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Chytiri et al. 2004). Vacuum packaging, in combination with low temperatures, is one of the ways in which this period can be extended (Ježek and Buchtová 2007). Removing air from the interior of the package protects the fish muscle and fat from oxidation by atmospheric oxygen and from the proliferation of aerobic bacteria with proteolytic activity (Oğuzhan and Angiş 2012; Ravi-Sankar et al. 2008). The vacuum packaging of rainbow trout reduces the number of psychrotrophic and mesophilic bacteria (Masniyom 2011; Arashisar et al. 2004).

Microorganisms present on the surface of fish produce a large variety of hydrolytic enzymes, in particular proteases. Endogenous proteases also play an important role in the *post mortem* degradation of fish muscle protein (Godiksen et al. 2009). These processes lead to a change to the textural and sensory characteristics of fish muscle.

The typical "fishy odour" is associated with the presence of trimethylamine nitrogen (TMA-N) produced mainly by the enzymatic activity of certain bacteria. These bacteria reduce trimethylamine oxide (TMAO) to TMA-N, which, together with dimethylamine and ammonia, comprises part of the total volatile basic nitrogen (TVBN) (Huss 1995).

Hydrolytic changes in lipids are the cause of the release of free fatty acids (FFA), which are much more susceptible to oxidative changes (Rezaei et al. 2008). Fish oil contains large amounts of polyunsaturated fatty acids which lead to the initiation of oxidation reactions and the formation of hydroperoxides of fatty acids and other, often toxic, secondary oxidation products. The formation of peroxides (PV) is considered an indicator of the rate of primary oxidation, while the thiobarbituric acid (TBA) value is an indicator of secondary oxidation (Huss 1995; Ježek and Buchtová 2012). Vacuum packaging prevents oxidative rancidity, protects the organoleptic quality of fish products and can effectively extend their shelf life while maintaining their characteristic aroma and flavour (Masniyom 2011).

The main objective of the study was to assess the effect of vacuum packaging on selected physicochemical properties of the shelf life of the muscle of rainbow trout (storage for 11 days at 2 ± 2 °C).

Materials and Methods

Rainbow trout samples weighing 287.20 ± 45.62 g were obtained from the fish farm Rybníkářství Pohořelice and were processed at the Mušov Freshwater Processing Plant using a standard technological procedure. The fish were electrically stunned, slaughtered and eviscerated. Each trout was individually packaged. One half of the samples were placed in microtene bags (simple packaging), the other half were vacuum-packaged. The samples prepared in this way were chilled to 2 ± 2 °C. The samples were then shipped to the Institute of Meat Hygiene and Technology at the University of Veterinary and Pharmaceutical Sciences Brno without any interruption in the cold chain, where they were stored in a cold chamber at a controlled temperature (2 ± 2 °C).

A total of 96 trout in simple packaging and 96 vacuum-packaged samples were examined. Samples were analyzed on days 1, 2, 4, 7, 9, and 11 *post mortem*. The samples were measured for water activity (a_w) with a LabMaster-aw (Novasina Ltd., Switzerland). Trout muscle pH values were measured using an inoLab pH 730 digital pH meter (WTW GmbH, Germany). The total volatile basic nitrogen (TVBN) was determined by direct distillation followed by titration on a Kjeltec 2300 analyzer unit (FOSS, Sweden). Trimethylamine nitrogen (TMA-N) was determined by the same method as TVBN after adding formaldehyde to the sample to bind the primary and secondary amines. Free fatty acids (FFA) and the peroxide value (PV) were determined after lipid extraction with diethyl ether. Determination of FFA was performed according to CSN ISO 660. Peroxide value (PV) was determined by a modified method according to CSN ISO 3960. The thiobarbituric acid (TBA) number was determined by a distillation method (Castellini et al. 2002), and oxidation products were quantified as malondialdehyde (MDA, $mg \cdot kg^{-1}$) equivalents.

The results of these analyses were statistically evaluated using one-way factor analysis in the ANOVA program (Microsoft Office EXCEL 2010). Differences were considered significant at $P < 0.05$.

Results

The water activity value (a_w) increased significantly ($P < 0.05$) during the experiment in both types of packaging (in air and in vacuum). Significantly higher ($P < 0.01$) a_w values were found until day 4 of the experiment in vacuum-packaged samples, but no significant differences ($P > 0.05$) were ascertained on subsequent days (Fig. 1).

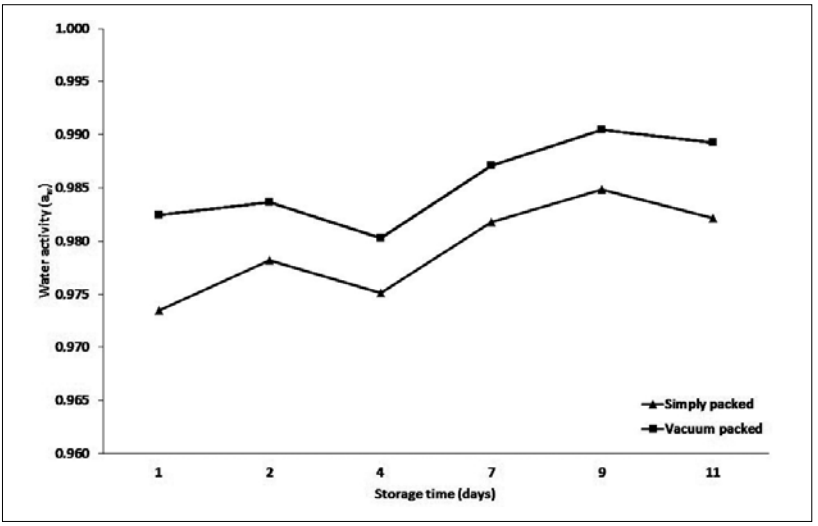

Fig. 1. Water activity in the muscle tissue of rainbow trout

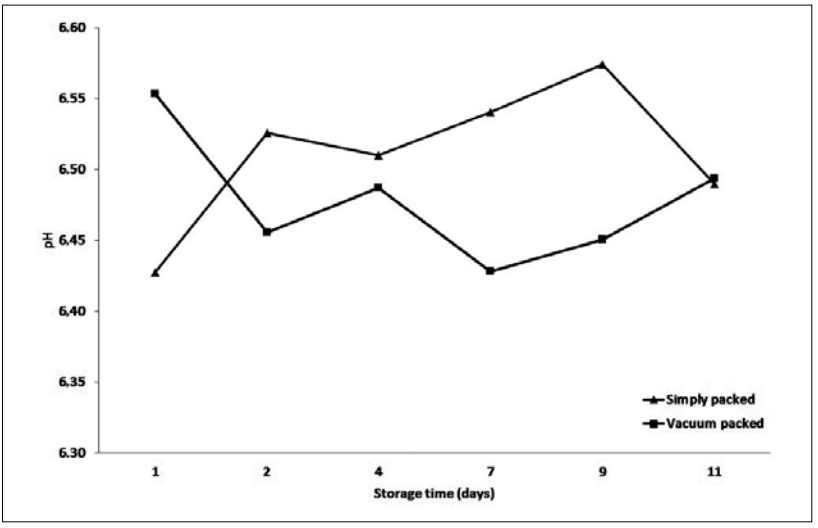

Fig. 2. Acidification of the muscle tissue of rainbow trout during cold storage

The pattern of pH decrease in the muscle of vacuum-packaged rainbow trout was different to that in trout in simple packaging. The initial pH values (day 1) in vacuum-packaged fish were significantly ($P < 0.05$) higher (6.55 ± 0.18) than those in trout in simple packaging (6.43 ± 0.11). While the pH in vacuum-packaged fish decreased until day 7 (6.43 ± 0.13), pH values in trout in simple packaging increased until day 9 (6.57 ± 0.17). At the end of the experiment (day 11), pH values in both types of packaging were practically the same (Fig. 2).

The course of proteolytic reactions in vacuum-packaged rainbow trout muscle was very similar to that in simple packaging. The total volatile basic nitrogen (TVBN) and

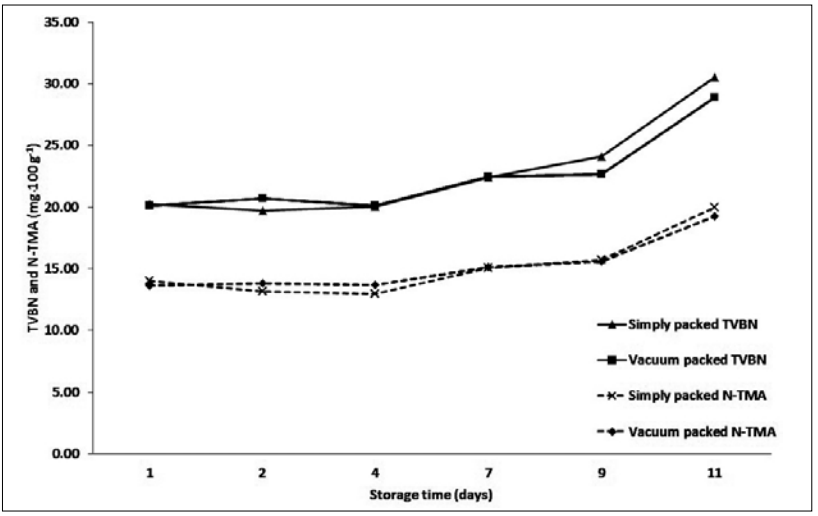

Fig. 3. Proteolytic changes in the muscle tissue of rainbow trout during cold storage

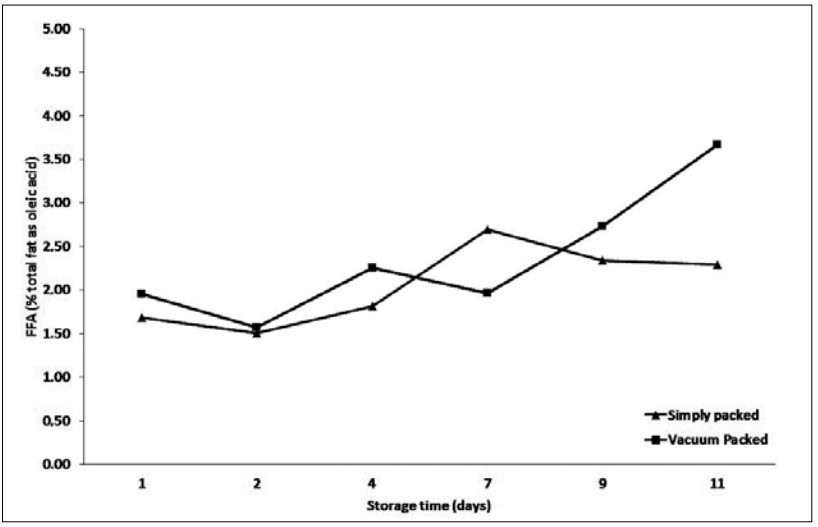

Fig. 4. Hydrolytic changes of lipids in the muscle tissue of rainbow trout during cold storage

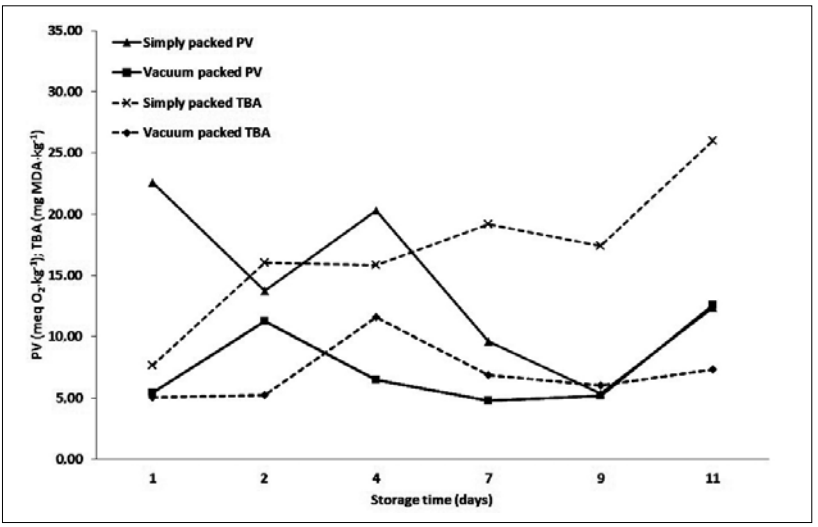

Fig. 5. The course of primary and secondary oxidation of chilled muscle tissue in rainbow trout

trimethylamine nitrogen (TMA-N) content was very similar in both types of packaging, with no significant differences. During the experiment, TVBN content increased significantly ($P < 0.01$) to a final value of 28.88 ± 4.42 mg·100 g^{-1} in vacuum-packaged samples, and to 30.52 ± 2.91 mg·100 g^{-1} in samples in simple packaging. Differences ($P < 0.05$) in TVBN content between the two types of packaging were found only on experimental days 2 and 9 (Fig. 3). During the experiment, TMA-N increases copied the growth in TVB-N content ($P < 0.01$) in both types of packaging. The only significant difference ($P < 0.05$) between TMA-N in dependence on the type of packaging was observed on day 4 of the experiment (Fig. 3).

The results of lipid hydrolysis monitoring were mixed. The differences that were found between the vacuum packaging and simple packaging were not significant in terms of statistical analysis ($P > 0.05$). In case of vacuum packaging, however, the FFA content (% total fat as oleic acid) in the trout muscle increased significantly from 1.96 ± 1.29 to 3.67 ± 2.37 between experimental days 1 and 11. In simple packaging, the difference was not significant (Fig. 4).

The content of primary lipid oxidation products calculated as the peroxide value (PV) was significantly lower ($P < 0.01$) for vacuum packaging at the beginning of the experiment than for simple packaging (5.46 ± 0.47 and 22.60 ± 2.59 meq $O_2 \cdot kg^{-1}$, respectively). Differences ($P < 0.01$) between the two types of packaging were also found on days 4 and 7. While the PV for vacuum packaging increased during the experiment (meq $O_2 \cdot kg^{-1}$) to 12.54 ± 9.16 ($P < 0.05$), the PV for simple packaging decreased significantly to 12.40 ± 3.12 ($P < 0.01$). The PV values at the end of the experiment were very similar (Fig. 5).

Discussion

Water activity (a_w) plays an important role in the spoilage of fish, and in many cases it is a critical control point in the HACCP (Hazard Analysis and Critical Control Points) system. It is also an important criterion for the evaluation of food safety and quality (Abbas et al. 2009). Water activity in fish flesh is high and there are often no significant changes in

water activity during storage (Gonzáles-Fandos et al. 2004; Hernández et al. 2009). Differences in a_w in vacuum-packaged rainbow trout during the first half of the experiment may be due to damage to cell structures and the release of interstitial fluid due to increased pressure during the packaging of samples. An increase of a_w during storage is probably related to autolytic and subsequent proteolytic microbial processes.

The pH values are close to 7.0 in the muscle tissues of live fish, but the *post mortem* pH generally ranges from 6.0 to 7.0 depending on the season of the year, fish species, and other factors. Variations in pH values in rainbow trout muscle during refrigerated storage are caused by the development of degradation changes of energy-rich compounds (formation of lactic acid and its breakdown into carbon dioxide and water) and protein (formation of volatile basic nitrogen that is alkaline in nature) and, at the end of the experiment, also by the dissolution of carbon dioxide in interstitial fluids leading to the formation of carbonic acid which causes muscle pH values to drop again. The pH values of rainbow trout muscle, as well as inconclusive differences in pH values during storage, correspond to the findings of other authors (Giménez et al. 2002; Arashisar et al. 2004; Chytiri et al. 2004; Jasour et al. 2011; Masniyom 2011) under various experimental conditions.

Changes in the content of TMA-N and TVBN in chilled rainbow trout in simple packaging and vacuum packaging during the 11 days of the experiment are shown in Fig. 3. The highest TMA-N value in both types of packaging was recorded on day 11. The values are similar to those found for other species of freshwater fish (Ježek and Buchtová 2011, 2012), but are higher than those found in uneviscerated and filleted rainbow trout by other authors (Chytiri et al. 2004). The increase in TMA-N values during storage reflects the concentration of TMAO-N (trimethylamine-N-oxide-nitrogen) present in fish muscle. Trimethylamine nitrogen is produced by the decomposition of TMAO-N in dependence on bacterial and enzymatic activity. A population of 108-109 cfu/g (colony-forming unit per gram) of *Shewanella putrefaciens* is essential for the production of TMA-N. Higher values of TMA-N may be related to processing conditions after the killing of the fish, when fish muscle may be contaminated with body fluids, and also with conditions of transport to the laboratory. The TMA-N is not a particularly useful indicator of trout muscle freshness (Chytiri et al. 2004).

The TVBN values we observed of 19.74 ± 0.58 to 30.52 ± 2.91 mg·100 g^{-1} in simple packaging and 20.07 ± 2.17 to 28.88 ± 4.42 mg·100 g^{-1} in vacuum packaging correspond to the values found in trout in experiments by a number of other authors (Giménez et al. 2002; Arashisar et al. 2004; Chytiri et al. 2004; Rezaei et al. 2008; Oğuzhan and Angiş 2012). Values of 25-40 mg TVBN·100 g^{-1} is considered the maximum limit depending on the fish species, type of treatment and processing (Fan et al. 2008). Commission Regulation (EC) 2074/2005 regards as unfit for human consumption those fish (Sebastes spp., *Salmo salar,* species belonging to the families Pleuronectidae, Merlucidae and Gadidae) in which organoleptic assessment has raised doubts as to their freshness and chemical checks have revealed that TVBN levels (25 to 35 mg·100 g^{-1}) have been exceeded (Ježek and Buchtová 2007, 2011, 2012). No limit has been stipulated for the TVBN value in trout, but most authors state 25 mg TVBN·100 g^{-1} as the highest acceptable value (Giménez et al. 2002; Arashisar et al. 2004; Chytiri et al. 2004; Oğuzhan and Angiş 2012). This value was exceeded in trout irrespective of the packaging type (simple or vacuum packaging) only on day 11. In view of major fluctuations during trout storage, the TVBN content is considered a poor indicator of freshness by some authors (Chytiri et al. 2004).

The process of lipid hydrolysis is accompanied by the release of FFA. The FFA content was not significantly affected by vacuum packaging. The values obtained were consistent with the values reported for trout by other authors. An increasing amount of FFA is often associated with a loss of freshness in fish muscle. The release of FFA may accelerate the

oxidation of lipids and cause odour variations (Rezaei et al. 2008; Jasour et al. 2011). Some authors (Özyurt et al. 2009; Bahmani et al. 2011) believe that there is a direct relationship between FFA formation and the loss of freshness in fish, and a significant effect on the sensory quality of fish. Fagan et al. (2004), on the other hand, claim that FFA concentrations as certained have no effect on sensory scores.

Lipid oxidation is often the main reason for a shorter shelf life in fish or fish products. The peroxide value (PV) was used to determine primary products of lipid oxidation, mainly hydroperoxides. Significant fluctuations in PV were recorded throughout the experiment, particularly in simple packaging. Similar fluctuations have also been found by other authors (Rezaei et al. 2008). Initial PV indicates oxidation already occurring during the course of handling and processing, and a subsequent decline in PV is then caused by competing reactions and increases in TBA values (Chaijan 2011). The PV value cannot be considered a suitable indicator of fish muscle freshness (Ježek and Buchtová 2007, 2012).

The TBA is a widely used indicator for the determination of secondary oxidation. It is apparent from Fig. 5 that vacuum packaging slowed secondary oxidation and TBA values were low. A similar result has also been recorded in other studies (Giménez et al. 2002; Arashisar et al. 2004). Giménez et al. (2002) suggest that lower concentrations of malondialdehyde (MDA) in the muscle of rainbow trout than in fish stored in air are the consequence of a low O_2 content. The sharp decline of peroxides in simple packaging resulted in an increase in TBA due to the presence of oxygen. Some bacterial enzymes may also contribute to oxidation in addition to oxygen (Huss 1995; Hernández et al. 2009). The TBA value may not show the actual degree of oxidation, because malondialdehyde can interact with other components (amines, nucleosides, nucleic acids, proteins, phospholipids, aldehydes) which are the end products of lipid oxidation. These interactions vary for different fish species (Chytiri et al. 2004).

Vacuum packing slowed the course of oxidative changes in particular in rainbow trout muscle. Values of FFA fluctuated due to their decomposition to hydroperoxides and PV can fluctuate due to peroxide decomposition to secondary oxidation products. The values of FFA and PV cannot be recommended as oxidation indicators. The TBA value can be recommended as a suitable indicator of oxidative changes. Based on our findings, vacuum packaging can be recommended as appropriate protection against trout muscle oxidation. In view of the gradual increase in its values, TVBN can be considered a suitable indicator of freshness and shelf life. Assessment of freshness must always take into account the sensory changes in fish.

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