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Shelf-Life Evaluation of Sliced Cold-Smoked Rainbow Trout (*Oncorhynchus mykiss*) Under Vacuum (Pv) and Modified Atmosphere Packaging (MAP).

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Abstract

Cold smoked trout (***Oncorhynchus mykiss***) is a traditional product from the San Daniele del Friuli area (Italy), usually packaged under vacuum, and its shelf life is 60 days. Aim of this study was to evaluate the influence of two different packaging systems (Vacuum packaging-VP and Modified atmosphere packaging-MAP) on the microbial, physico-chemical and sensorial changes of sliced cold smoked rainbow trout during storage. MAP packaged sample showed lower microbial loads than VP packaged sample throughout storage. Microbial count of VP packaged sample exceeded the limit of 6 log CFU/g after 60 days of storage. The total volatile base-nitrogen (TVB-N) values increased over time in both treatments, reaching values close to the limit of 40 mg N/100 g after 45 days. Also TBARS values did not were up to 10 nmol/g in both VP and MAP. The shelf life of 60 days seems to be too long, particularly for the VP samples. A panel composed of 12 non-professional assessors perceived significant differences in the sensorial characteristic of the samples, and concluded that the sensorial quality of MAP packaged products were better than the VP ones ($p < 0.05$).

Keywords: cold smoked Rainbow trout, MAP, vacuum packaging, shelf life

Introduction

Smoked rainbow trout (***Oncorhynchus mykiss***) is a traditional product of Friuli, a region of northeastern Italy. Rainbow trout is farmed in that region, processed by cold-smoking and packaged after slicing under vacuum. The product is stored at 4 ± 2°C and has a shelf life of approximately 60 days. Being a cold-smoked fish, the product belongs to the category of mildly processed foods (Truelstrup Hansen and Huss, 1998). Fish is one of the most perishable food products: its initial microbial population reflects the microflora of the environment at the time of capture or harvest, and it is modified by the ability of various microorganisms (mainly bacteria) to multiply in the sub-environments provided by the skin/shell surface, gill areas, and intestinal content. The muscle tissue is normally sterile: microorganisms can be found on the skin and gills and in the intestinal tract (Baross and Liston, 1970; Shewan, 1977). The microbial loads and growth is influenced by the water activity (aw), moisture, salt concentration and temperature; and these parameters favour the sequential growth of the different bacteria. The microbial ecology of cold-smoked fishery products has been intensively studied (Bernardi, Ripamonti, Campagnoli, Stella & Cattaneo, 2009; Gonzàles-Rodrìguez, Sanz, Santos, Otero & Garcia-Lopez, 2002; Joffraud, Leroi, Roy &Berdaguè, 2006; Lannelongue, hanna, Finne, Nickelson & Vanderzant,1982; Leroi, Jooffraud, Chevalier &Cardinal, 1998; Leroi, Joffraud, Chevalier & Cardinal, 2001; Leroi, 2010; Lyhs, Björkroth, Hyytiä,

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& Korkeala,1998; Truelstrup Hansen and Huss, 1998). The initial bacterial load closely depends on the hygienic conditions of processing and production (Leroi et al., 2001; Truelstrup Hansen and Huss, 1998), and it is represented by lactic acid bacteria (LAB), *Enterobacteriaceae*, *Shewanella putrefaciens*, *Aeromonas* spp., *Pseudomonas* spp., *Photobacterium phosphoreum*, and *Brochothrix thermosphacta* (Cardinal, Gunnlaugsdottir, Bjoernevik, Ouisse, Vallet, & Leroi, 2004; Jorgensen and Huss, 1989; Leroi *et al.*, 2001; 2010; Lyhs *et al*., 2007; Jaffrès, Sohier, Leroi, Pilet, Prévost, Joffraud &Dousset, 2008; Laursen, Bay, Cleenwerck, Vancanneyt, Swings, Dalgard &Leisner, 2005; Truelstrup Hansen, Gill, Drewer Røntved &Huss, 1996; Civera, Parisi, Amerio, & Giaccone, 1995).

The refrigeration of cold smoked salmon and trout does not prevent the growth of abundant microflora, particularly LAB, which have a focal role in the microbial evolution occurring in the product. The shelf life of several cold smoked fish products is limited by the presence and growth of specific microflora, and although a certain correlation between these microflora and spoilage development has not yet been established. A level of microorganisms approximately 7-8 log CFU/g is considered to determine consumer rejection. These microflora could also present at high loads without causing product spoilage; the humidity, the presence of non-proteic nitrogen and residual sugars of the product, the storage temperature are the main responsible for important microbial spoilage (Gram,1991; Gonzàles-Rodrìguez *et al* , 2002; Joffraud *et al* , 2001).

The dominant microflora in both vacuum-packed and MAP-packed smoked fish at the end of the retention period was often found to be LAB: the predominance of this group is not totally clear, but it seems that they are well suited to the particular pH, water phase salt (WPS) and salt concentration of smoked products (Bernardi *et al*., 2009; Cardinal *et al*., 2004; Laursen *et al*., 2005; Leroi *et al*., 2000; Lyhs, Lahtinen & Schelvis Smith, 2007; Samelis, Maurogenakis & Metaxopoulos, 1994). Also *Pseudomonas* spp., which initially contaminate the raw fish meat, could cause the spoilage in VP due to residual oxygen or oxygen permeability of the protective film. *Enterobacteriaceae* (Bernardi *et al*., 2009; Gimenez and Dalgaard, 2004; Leroi, 2010) could be also present, they can growth either in VP or in MAP because they are anaerobic facultative, and consequently can produce acids and gas (CO_2). Among them *Enterobacter* spp., *Serratia* spp., *Hafnia* spp. and *Proteus* spp. are the bacteria mainly present and are indicative of the level of hygiene in the processing (Bernardi *et al*., 2009). In the case of smoked salmon and related products, such as smoked trout, their role has not yet been clarified and they seem to have a minor role in the process of spoilage; many authors believe that there is no direct correlation between shelf-life and total counts of LAB (Leroi *et al*., 2001), even though the LAB cause spoilage with the production of volatile sulphur compounds and amines (Bernardi, Ripamonti, Marzano & Cattaneo, 2011). The LAB, however, should not be considered harmful exclusively for the purpose of preservation, as they represent an important ally against potentially pathogenic microorganisms. Many studies (Gimenez and Dalgard, 2004; Joffraud, Cardinal, Cornet, Chasles, Léon, Gigout & Leroi, 2006; Leroi, 2010; Leroi *et al.*, 1996) have reported that the presence of LAB, or their inoculation in smoked fishery products, results in growth inhibition of *Listeria monocytogenes*, *Clostridium botulinum* type E and *Salmonella* spp. This paper focuses on cold smoked trout fillets, a traditional product of the San Daniele del Friuli area that is usually VP packaging after slicing.

For the convenience of the consumer, because modified atmosphere packaging (MAP) allows for the easier separation of the slices, this study compared the shelf life of sliced cold-smoked rainbow trout packaged under vacuum to that of the same product with MAP packaging.

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Material and Methods

Samples

Samples consisted of portions (200 g) of sliced cold-smoked rainbow trout packaged under vacuum (- 1.0 bar) or in MAP (- 0.3 bar; 70% N_2 and 30% CO_2) , by Orved VM53 vacuum machine (Italy). The packaging consisted of Ecoterm VP 300 film (vacuum) and Multofog GA 170 (MAP; SUDPACK, Italy). The packages were stored at 4 ± 2 °C for 60 days and analysed after 0, 15, 30, 45, and 60 days. Three packages at each time were used for microbiological and physico-chemical analyses. To produce the sliced cold-smoked rainbow trout used in the tests., two lots of raw material, were used. The trouts were farmed by Italian farm of San Daniele area (Friuli, Italy), they sized of 55 ± 5 cm , weighted 4.5 ± 5 kg. The trouts were harvested, killed and beadhead, after a ice water bath. The post-mortem period was about 24 h at 4 °C. Then they were salted and cold smoked (29 °C for 24 h). The smoked trouts were sliced, VP or MAP packaged and stored at different time before analysing.

Microbiological analysis

Total aerobic mesophilic microbial counts (TMC) were evaluated on plate count agar (Oxoid, Italy) incubated at 30 °C for 48-72 h. LAB were counted onto De Man Rogosa Sharpe (MRS, Oxoid, Italy) after incubation under anaerobic conditions at 30 °C for 48 h; yeasts and moulds were counted onto malt agar (MA) (Oxoid, Italy) after incubation at 25 °C for 72-96 h. Total coliforms were counted on violet red bile lactose agar (VRBLA, Oxoid, Italy) and incubated at 37 °C for 24 h; coagulase-positive Staphylococci were enumerated on Baird-Parker agar medium (BP, Oxoid, Italy) supplemented with egg yolk tellurite emulsion (Oxoid, Italy) and incubated at 35 °C for 24-48 h; *Pseudomonas* spp. were counted on Pseudomonas agar base (Oxoid, Italy) supplemented with CFC (Oxoid, Italy) and incubated at 30 °C for 48 h; sulphite-reducing Clostridia were quantified on differential reinforced clostridial medium (DRCM, VWR, USA) after incubation at 37 °C for 24-48 h in an anaerobic jar using an anaerobic kit (gas pack anaerobic system, BBL, Becton Dickinson, USA). *Listeria monocytogenes* and *Salmonella* spp. were investigated according to ISO methods 11290/1 and 6579-1, respectively.

Physico-chemical analysis

The pH value was measured using a pH meter (Basic 20, Crison Instruments, Spain), by inserting the pH meter probe into 3 different points on each sample. The aw was measured by Aqua Lab 4 TE (Decagon Devices, USA). The moisture content was measured by the A.O.A.C. (1990) method. NaCl and TVB-N (total volatile basic nitrogen) contents were measured by the Pearson method (1973). WPS (water phase salt) was determined by the formula WPS = % salt/(% salt + % moisture) x 100, as described by Huss, Dalgaard and Gram, (1997). Analyses were performed in triplicate per each sampling point. To evaluate the oxidation stability during storage, the thiobarbituric acid–reactive substances (TBARS) were determined in triplicate (Ke, Cervantes & Robles-Martines, 1984).

Sensorial analysis

To evaluate the influence of VP and MAP packaging on the organoleptic characteristics of the sliced cold-smoked rainbow trout samples, a sensory analysis was performed using the triangle test methodology (ISO 4120:2004). Sensorial analyses were performed by 12 non-professional assessors (6 women and 6 men, representing Food Technology students with an age between 22 and 24 years). Four additional samples per each treatment were also evaluated after 60 days of

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storage at 4 ± 2 °C. The non-professional assessors were presented with three products, two of which were identical and the other one different. The assessors were asked to state which product they believed was the odd one out ($p < 0.05$). The assessors who identified the different samples were asked to indicate their preference.

Statistical analysis

The values of the various parameters were compared by one-way analysis of variance. The averages were compared with Tukey's honest significance test using the Statistical Graphics software package.

Results and Discussion

The microbial loads of cold smoked trout fillets packaged under VP and MAP are reported in Table 1. In the VP samples, the total mesophilic count (TMC) was below the detection limit (<100 CFU/g) at day 0, but a rapid increase after day 15 was found, reaching at the end of the storage period a microbial count higher than the threshold limit of 6 log CFU/g often used in food industries to indicate the end of shelf life of fish products (Olafsdottir, Chaine, Westad, Jonsdottir, Thalmann, Bazzo, Labreche, Marcq, Lundby, & Haugen, 2005). And in samples under MAP, the TMC was slightly higher at day 0, but the loads remained almost stable (approximately 2-3 log CFU/g) until day 45, at which point a rapid increase to a value of 4.52±0.43 log CFU/g at day 60 was observed. It needs to be highlighted that a TMC of 7-8 log CFU/g, often associated with sensory rejection, was never reached. From day 15 until the end of the storage period, the TMC values were significantly higher for the VP samples than for the MAP samples ($p < 0.05$). Exactly the same trend was observed for the LAB counts. In fact, a rapid increase was observed during the storage period, with an increasing of approximately 3 log from day 0 to day 15 and another jump of approximately 3 log from day 45 to day 60, when they reached a concentration of 6.42 ± 0.67 log CFU/g. Further, in this case, the LAB counts were significantly different between the treatments, thus confirming significantly higher values in the VP samples compared with the MAP samples ($p< 0.05$), which were characterised by limited LAB growth throughout the period. Our values are similar or slightly lower than those reported in the literature for smoked vacuum-packed salmon: in those studies, the TMC values were approximately 7 to 8 log CFU/g, and LAB levels were between 5 and 6 log CFU/g (Bernardi *et al.*, 2009; Jaffrès *et al.*, 2008; Leroi *et al.*, 1998, 2001; Leroi, 2010; Truelstrup Hansen *et al*., 1996; Truelstrup Hansen and Huss, 1998). Dondero, Cisterna,s, Carvajal and Simpson (2004) and Gonzàles-Rodrìguez *et al.*, (2002) obtained similar results in cold-smoked salmon and trout, even if the initial bacterial load, an index of the quality of the raw material and the hygiene of the process, was lower in our work than in these studies.

High-quality raw materials and good manufacturing practices were confirmed by the values of Coliforms and coagulase-positive Staphylococci (1 log CFU/g), which were always below the detection limit in both the VP and MAP samples during the monitored period, and by the absence of *Salmonella* spp. and *Listeria monocytogenes* in all samples.

The different types of packaging had an effect on *Pseudomona*s spp. growth (Table 1), particularly at the last two time points, where a rapid increase in the VP samples was observed. Conversely, in the MAP samples, *Pseudomonas* spp. were counted only at day 15 (2.00 ± 0.07), then they were no longer detected until the end of the storage period (< 100 CFU/g). The contamination could either derive from the processing or originate from the raw materials.

According to data obtained from previous studies, their growth depends on the oxygen permeability of the vacuum packaging (Gonzàles-Rodrìguez *et al*., 2002; Leroi *et al*., 1998, 2001). Sulphite-reducing clostridia were found only at

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day 60 in the VP samples. This result suggests that their presence cannot be connected to real growth but was likely sample-dependent.

Considering the physico-chemical analyses (Table 2), both pH and aw showed a slight decrease over time without significant differences between the VP and MAP samples. The pH values started from 6.16 in both the VP and MAP products with no significant differences ($p > 0.05$) during the storage period. Only at the end of the shelf life (day 60), the pH decreased to 6.03 and 5.97 in the VP and MAP samples, respectively, and a significant difference was noted ($p < 0.05$); this slight decrease could be due to the increase of Lactic Acid Bacteria that generally exert a buffer activity onto the food substrate. Leroi et al. (1998) found quite stable pH values (from 6.04 to 6.25), as did Bernardi *et al*., (2009), who reported constant values in the middle and at the end of the shelf life (6.12-6.11) in their study.

Similarly, the aw values detected in the MAP samples were slightly lower than those measured for the VP samples, but the noticed differences were only significant at days 15 and 60 ($p < 0.05$). The small observed Aw differences at the various sampling points probably depend on the samples' variability and are not related to real water loss. Moisture remained fairly constant over time. As far as the salt content and the WPS are concerned, the literature emphasises the importance of considering a WPS value of 3.5% as the minimum value able to control *Clostridium botulinum*, and in particular psychotropic type E, when combined with storage temperatures below 4.4 °C (Centre for Food Safety and Applied Nutrition, 2001). In this study, the values of salt and WPS varied during the storage period without displaying a specific trend, indicating that the observed differences depend only on the variability of the samples. The salt content was affected by the variability of the samples and of the salting procedure; for these reasons the decrease observed could not be considered a trend but a random heterogeneity of the samples.

Most of the studies met the guidelines suggested by the Centre for Food Safety and Applied Nutrition: Bernardi *et al*., (2009) reported WPS values in Italian smoked salmon products equal to 4.93%, whereas in French products the averages were lower but still approximately 4% (Cornu, Beaufort, Rudelle, Laloux, Bergis, Miconnet, Serot & Delignette, 2006; Hespe, Kiessking, Lunestad, Torrissen &Benccze Røra, 2004). Moreover, differently from what found in the present study, Bernardi *et al.,* (2009) highlighted that WPS values lower than 3.5% were correlated with low bacterial counts. The TBARS values did not increased up to 10 nmol/g at the end of the storage (60 days) either in PV or in MAP samples. According to different authors (Ke *et al*., 1984; Che Man & Ramadas, 1998) for quality evaluation, TBARS values of < 8 nmol/g sample are considered not rancid, 9– 20 nmol/g slightly rancid but still acceptable and >21 nmol/g rancid and unacceptable, consequently for the TBARS values, both the VP and MAP samples tested can be considered still acceptable at 60 days, despite at 60 days 10 out of 12 assessors perceived a light rancidity and fluid loss in PV products and only 1 out of 12 perceived a light rancidity in MAP products. In PV products, the TBARS means at 60 days were significantly different compared to the ones at 0,15,30,45 days. In contrast no significant difference was present among TBARS means in MAP products at each tested times.

Considering the TVB-N results (Table 2), a moderate increase over time in both the treatments was observed. As early as day 45, the values were close to 40 mg N/100 g, which corresponds to the maximum value proposed by Cantoni, Moret and Comi (1993) for this parameter, with no significant differences observed between the treatments. Additionally, Bernardi *et al*., (2009) found that at half the shelf life, the TVB-N values were close to the initial TVB-N limit (38.2 mg N/100 g), and at the end, they largely exceeded the suggested maximum value (49.8 mg N/100 g). The Chilean authorities (Sernapesca, 1996) established a limit of 30 mg N/100 g for cold smoked salmon, and this value was just present in our trial at day 0 for both the VP and MAP products. The observed TVB-N data were different from those reported by Leroi

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et al., (1998), who found initial TVB-N values of approximately 15.5 mg N/100 g and final higher values equal to 52.8 mg N/100 g.

A multiple compound quality index was proposed by Leroi *et al.,* (2001) to estimate the remaining shelf life time considering TVB-N and *Lactobacillus* spp. loads at storage temperatures < 5 °C. Applying this model and using the data of T30 for the VP and MAP products, the remaining shelf life was found to be 52 days and 55 days, respectively.

Considering the threshold limit of 6 log CFU/g (Olafsdottir *et al.,* 2005) for TMC and the TVB-N limit of 40 mg N/100 g (Cantoni *et al.,* 1993), the MAP products can be accepted until the end of their shelf life (60 days), but because the VP products exceeded the threshold limit of 6 log CFU/g, this shelf life period is not adequate. MAP allowed to maintain higher structural and physical qualities than VP, wherein higher fluid loss occurred and less juiciness was noticed. However, the MAP composition must be carefully studied, as it can create unfavourable conditions for the microflora, hindering their ability to compete with pathogens, the replication of which may be facilitated to in this substrate, e.g., *C. botulinum* under anaerobic conditions (Huss, 1980), especially without a proper salt concentration.

The sensorial acceptability of both types of smoked rainbow trout, packaged VP or in MAP, was determined by the triangular test. The panel was composed of 12 non-professional assessors.

Ten out 12 assessors established that a difference exists in the sensorial characteristic between the trout packaged in VP versus that in MAP. This result must be accepted because the minimum numbers of correct judgments to establish significance at 0.05 level was 8. The sensorial results have been just confirmed by the physico-chemical and microbiological results. In addition at 45 day off-flavor and off-odor were perceived. At 60 days, 10 out 12 assessors perceived light rancidity/fluid loss/ less juiciness in PV products, and 1 out 12 assessors perceived a light rancidity in MAP products. Consequently the panel identified that the MAP products had a higher sensorial quality than the VP also at the end of shelf-life.

Conclusion

The sliced smoked rainbow trout samples, both VP and MAP packaged, have good hygienic quality and respect the EC Regulation no. 2073/2005 for *Listeria monocytogenes, Salmonella* spp., coagulase-positive Staphylococci limits. MAP packaging preserved the product better compared with VP, showing lower microbial loads throughout the storage period and at the end of shelf life.

However, some samples showed less than 3.5% WPS, the limit for the growth of psychrotrophic (non-proteolytic) *Clostridium botulinum*. As this bacterium could grow during the product's 60-day commercial life, it is essential to optimise salting during the production process to ensure that an adequate concentration of NaCl is distributed homogenously throughout the raw material.

The deadline of 60 days appears to be too long, particularly for the VP samples, as confirmed by the sensory analysis. Consequently, a reduction of the "use-by" date could allow the manufacturer to guarantee the quality and safety of this product for its entire commercial life. For PV products a shelf-life of 45 days is more reliable.

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Table 1: Microbial loads (Log CFU/g) of the sliced cold smoked rainbow trout VP or MAP packaged.

Days	Total Mesophilic Count		*Pseudomonas* spp.		Lactic Acid Bacteria		Sulphite-reducing clostridia	
	VP	MAP	VP	MAP	VP	MAP	VP	MAP
0	< 100*	2.70±0.51	< 100*	< 100*	< 10*	1.30±0.41	< 10*	< 10*
15	4.29±0.50^{a}	2.24±0.34^{b}	< 100*	2.00±0.07	3.57±0.95^{a}	1.30±0.27^{b}	< 10*	< 10*
30	4.14±0.42^{a}	2.93±1.31^{b}	< 100*	< 100*	4.14±0.65^{a}	3.37±0.52^{a}	< 10*	< 10*
45	4.78±0.22^{a}	2.50±0.71^{b}	4.34±0.26	< 100*	3.53±1.27^{a}	1.97±1.68^{a}	< 10*	< 10*
60	6.21±0.29^{a}	4.52±0.43^{b}	6.66±0.01	< 100*	6.42±0.67^{a}	4.16±0.61^{b}	2.23±0.31	< 10*

Data represent the means ± standard deviations of the total samples; Mean with the same letters within a row (following the values), considering each single parameter, are not significantly differently (P< 0.05).*Value are expressed as CFU/g. Analyses were conducted in triplicate on three different samples per each sampling point.

Table 2: Physico-chemical analyses of the sliced, cold smoked rainbow trout

	Days				
	0	15	30	45	60
% Water	59.84±0.25^{b}	63.64±0.86^{a}	63.31±0.86^{a}	62.43±1.89^{a}	63.59±0.81^{a}
% Salt	3.11±0.03^{b}	2.18±0.59^{a}	2.69±0.14^{a}	2.78±0.16^{a}	2.59±0.23^{a}
pH	6.16±0.03^{a}	6.03±0.09^{a}	6.06±0.07^{a}	5.91±0.01^{a}	6.03±0.04^{a}
Aw	0.973±0.007^{a}	0.971±0.006^{a}	0.966±0.006^{a}	0.967±0.009^{a}	0.966±0.004^{a}
% WPS	4.94±0.15^{b}	3.32±0.91^{a}	4.07±0.16^{a}	4.27±0.21 ^{a}	3.85±0.28^{a}
TVB-N mg N/100 g	32.9±0.33^{a}	37.55±3.55^{a}	35.86±0.87^{a}	40.52±1.28^{a}	39.00±1.19^{a}
TBARS nmol/g	7.8 ± 1.2^{a}	8.1 ± 1.2^{a}	8.6 ± 0.5^{a}	9.2 ±0.6^{a}	10.6 ± 03^{b}

Legend: WPS: Water Salt Phase; TVB-N: Total volatile basic nitrogen; Data represent the means ± standard deviations of the total samples; Mean with the same letters within a column (following the values), considering each single parameter, are not significantly differently (P< 0.05). Analyses were conducted in triplicate on three different samples per each sampling point.