AJSR
AFRICAN JOURNAL OF SCIENCE AND RESEARCH

African Journal of Science and Research ,2014,(3)2:16-22
ISSN: 2306-5877
Available Online: http://ajsr.rstpublishers.com/

EFFECTIVENESS OF VACUUM DEVICES FOR HOME STORAGE OF RAINBOW TROUTS FROM GAME FISHING LAKES.

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Received:24 ,Feb,2014 ***Accepted: 21,Mar,2014.***

Abstract
The aim of this work was to study the effectiveness of vacuum devices for home storage of rainbow trouts from sport fishing lakes located near Avellino, in the South of Italy. The trout were divided in two groups: one was vacuum-packaged by using a "Food Vacuum System", while the other was stored in plastic trays covered with plastic wrap. Both the fish samples were stored at 4°C for 6 days. Rainbow trout purchased in a local supermarket were used as control. The trout quality was evaluated by microbiological, chemical and sensory analyses. Fatty acids and volatile compounds were characterized by GC and SPME-GC/MS. Results proved the efficiency of the vacuum system in preserving the quality characteristics of the trout, limiting the formation of off-odors and off-flavors related to spoilage and oxidative processes.

Keywords: *Oncorhynchus mykiss, vacuum device, spoilage, Volatile Organic Compounds, quality.*

INTRODUCTION

The rainbow trout (*Oncorhynchus mykiss*) is a freshwater salmonid native to inland waters including sport fishing lakes or introduced for food and restocking in almost every continent. Its good adaptability and high growth rate, make the rainbow trout the most cultured fish species around the world.

Easy management of the species during growth/culture is however accompanied by difficulties during post-catch shelf storage. Similarly to seafood products, trout are highly perishable due to their chemical and structural characteristics. The neutral pH, high value of Aw (water activity), presence of autolytic enzymes and low percentage carbohydrate (<0.5%) in their meat, make them particularly susceptible to rapid spoilage processes (Gram and Huss 1996). The immediate post-mortem flesh changes are related to both endogenous chemical and enzymatic autolysis, and then to bacterial processes, which together alter the fish meat characteristics and cause formation of off-odors and off-flavors during storage (Lougovois and Kyrana 2005).

Seafood products in particular contain high concentrations of unsaturated fatty acids which, being very reactive and unstable, easily undergo hydrolysis and oxidation. Phospholipids are the first to be hydrolyzed, followed by triacylglycerols, cholesterol esters and wax esters. Free fatty acids (FFAs), being virtually absent in the fat of the living fish, constitute the main degradation products and their presence can be used to evaluate fish freshness. Fatty acids are typically analyzed after conversion into their methyl esters prior to analysis by Gas Chromatography with Flame Ionization Detection (GC/FID) or Mass Spectroscopy (GC/MS)(Turchini *et al.* 2004; Saglik *et al.* 2007).

The process of degradation is accelerated by specific Gram-positive and Gram-negative psychrotrophic bacteria (Gram and Huss 1996). Their growth is facilitated by the fact that the fish substrate is rich in free amino acids, non-protein nitrogenous compounds and lipids. The volatile compounds (VOCs) released can be considered relevant quality indicators in the assessment of fish freshness and recently many studies have been undertaken to identify and quantify these compounds by using different analytical methods such as Static Head Space Analysis (SHA), Dynamic Head Space Analysis (DHA) and electronic nose (Duflos *et al.* 2010) Solid Phase MicroExtraction (SPME), a relatively new tool, can also be used to perform rapid, simple and solvent-free extraction of VOCs from food. SPME combined with GC/MS was first used to assess fish quality by Bene *et al.* (2001), who characterized volatile products in salmon and whiting. Iglesias and Medina (2008), have applied this methodology successfully to study the volatile fractions in rainbow trout.

Inhibiting these degenerative processes and increasing shelf life is a matter of great importance for consumer health and economic return. In view of this, the present study evaluates the effectiveness of a domestic vacuum device in preserving fish meat quality. Samples were assessed by sensory criteria and further by microbiological analysis and measurement of volatiles and free fatty acids at 0, 3 and 6 days preservation at 4 °C. Volatile compounds were determined by SPME/GC/MS and free fatty acids by GC/FID.

Trout were caught from sport fishing lakes located in hill and mountain areas near Avellino, in southern Italy. Samples were divided in two groups and stored in two different ways. One group was vacuum-packaged by using the "Food Vacuum System", while the other group was stored in plastic trays covered with plastic wrap.

Chemicals

Culture media and Ringer solution were from Oxoid (Hampshire, UK). Chemical reagents, standards and solvents were all of analytical grade and from Sigma-Aldrich (St.Louis, MO, USA). SPME fibers, Carboxen (CAR)/polydimethylsiloxane (PDMS) StableFlex fibre (85 m), were obtained from Supelco (Bellofonte, PA, USA) and the columns for GC and GC/MS were purchased from Agilent J&W (Agilent Technologies Inc.).

Animals

Rainbow trout (average size 300 g) were sampled from three sport fishing lakes in Prata di Principato Ultra, Cesinali and Serino (290 m,

360 m and 752 m respectively above sea level) near Avellino. They were sacrificed by anoxia and transported to the laboratory at low temperature in sterile box cooler . Rainbow trout (average size 300 g) purchased in local supermarkets were used as controls.

Sensorial analysis

Sensory evaluation was made according to the European Union grading system (EC No 2406/96). This system distinguishes between three freshness categories, E, A and B, corresponding to increasing levels of spoilage, with fish grading below B being considered non-edible. In order to rate the rainbow trout product discard level, the sea weedy odor reported as a reference parameter for whitefish (table A, Annex 1) was changed to "soil and mud" smell (EC No 2406/96). At Day 0 (D0) the skin, skin mucus, eye, gills, peritoneum and abdominal cavity smells were evaluated. On Day 3 (D3) and Day 6 (D6) the skin, flesh, peritoneum and abdominal cavity smells were assessed. These assessments were performed by an untrained panel.

Preparation and storage of samples

After sensory evaluation, the fish were manually eviscerated, beheaded, filleted, mechanically peeled and washed with tap water. Samples were divided into three identical portions and randomly allotted to one of the three storage period groups.

The first samples (time zero, D0) were subjected immediately to pH measurement, and then to microbiological and volatile compounds/free fatty acid analyses. The second samples were placed in a tempered glass container and Vacuum-Packaged (VP) by the "Food Vacuum System" (IMCO Free Net SpA; EN), while the third samples (F, Fridge) were stored in a plastic tray covered with plastic wrap. The samples VP and F were stored at 4 °C for 3 or 6 days (max 6 days as the limit for home storage of highly perishable food) prior to the analyses described above. Each sample was analyzed in triplicate.

pH value

The pH values of homogenates obtained from skin, muscle and gills samples, collected at different storage times, were recorded by using a pH-meter (Hanna Instruments).

Microbial analyses

At time zero (D0) 10 grams each of skin, gills and muscles were removed aseptically, weighed into sterile stomacher bags and homogenized in 90 mL of Ringer solution in a Stomacher Model 400 (Lab Stomacher Blender 400-BA7021, Seward-Medical U.K). The homogenates were serially diluted 10-fold in Ringer solution and spread onto the surface of agar plates. Specific culture media and conditions were used: PCA (for Total Mesophilic Aerobic Bacteria), MSA (for *Micrococcaceae*), YPD (for yeast and mould) and the plates were incubated for 3-5 days at 28 °C. For Total Coliform Bacteria, VRBGA and MacConkey Sorbitol Agar was used for incubation of 48 h at 37 °C; VRBA was used for Fecal Coliform Bacteria, incubation for 48 h at 44 °C and PBA for *Pseudomonas* spp., 5 days incubation at 20 °C. MRS (for Lactic Acid Bacteria) and SPS (for Sulfate-Reducing Bacteria) were incubated anaerobically at 28 °C and 20 °C, respectively, for 5 days. Microbial populations were enumerated and were recorded as $\log_{10}$ colony forming units/g (cfu/g) .

At day three and six (D3 and D6) microbiological analyses were performed on skin and muscles maintained under the two different storage conditions (F and VP at 4 °C). The analyses were performed in triplicate.

Analysis of volatile compound by Solid Phase Microextraction (SPME)–GasChromatography/MassSpectrometry

Trout fillets were cut into 1 cm cubes and then 15 g of flesh were homogenized into a stomacher bag with 8 mL of ultrapure water saturated with NaCl, for 2 min. The mixture was centrifuged (10 min, 3500 rpm) and 5 g of the supernatant was transferred into a 20 mL screw-on cap vial (Supelco, Bellefonte, PA, USA), sealed with a hole cap PTFE/silicone septum for the production of headspace and the successive analyses.

The vial was placed in the dry block-heater and held at 60 °C for 30 min to equilibrate. The extraction and injection processes were automatically performed using an autosampler MPS 2 (Gerstel, Mülheim, Germany). The CAR/PDMS fiber was, then, automatically inserted into the septum of the vial for 10 min, to allow the volatile compounds absorption onto the SPME fibre surface. Each SPME fiber was conditioned before first use, as recommended by the manufacturer. In order to desorb the volatile metabolites, the SPME fibre was introduced into the injector port of the gas chromatograph device, model GC 7890A, Agilent (Agilent Technologies, Santa Clara, USA) coupled with a mass spectrometer 5975 C (Agilent) and analysed. The desorbed metabolites were analysed on a capillary column HP-Innowax (30m×0.25 mm×0.5µm Agilent J&W). The oven temperature program was initially set at 40 °C for 3 min, ramped to 180 °C at 5 °C/min, increased to 260 °C at 10 °C/min and held for 0 min. The temperature of the ion source and the quadrupole were kept at 230 °C and 150 °C respectively; helium was used as carrier gas with a flow of 1.3 mL/min; injector temperature was held at 260 °C and the splitless mode was used for the analysis. The fiber was maintained in the injector for 10 min (Duflos *et al.* 2010). Mass Spectra were acquired at an ionization energy of 70 eV and volatile components were detected by mass selective detector. The detector operated in a mass range between 33 and 300 amu with a scan rate of 2 scans/s.

Compounds were identificated by comparing mass spectra with library databases (WILEY7/NIST05). The data were obtained from triplicate analyses. The peak area of each compound was represented in $\log_{10}$ transformed values, according to Weber-Fechner law (Miyasaki *et al.* 2011).

Fatty acid analysis

Analyses were carried out on the fillets of three different trout samples at D0 and D6. The lipids were extracted according to Bligh and Dyer method (Bligh and Dyer 1959) and methylated using sulforic acid/methanol solution at 2% held to 80 °C for 2 (Christie 1989).

Sample spectra were obtained from a Fisons GasChromatograph (Model 8000) fitted with a FID detector (Fisons Instruments, USA) using the following conditions: HP-Innowax column (30m×0.25mm×0.25µm Agilent J&W), split (1:20), temperature programmed from 150 °C to 180 °C at 3 °C/min, from 180 °C to 250 °C at 2.5 °C/min and held for 10 min. Fatty acids were identified using known external standards (FAME 37 MIX). Data were obtained in triplicate and reported as percentage area for each compound detected. The Index of Atherogenicity and the Index of Thrombogenicity were calculated with the following equations (Ulbricht and Southgate 1991), taking into account the different effect of different fatty acids on human health:

IA (Index of Atherogenicity) = (C12:0 +4xC14:0+C16:0)/(∑ω-6+∑ω-3+C18:1+∑MUFA)

IT(Index of Thrombogenicity) =(C14:0+C16:0+ C18:0)/(0.5 x ∑ω-6 + 0.5 x C18:1 + 0.5x∑MUFA +3 ∑ω-3 + ω-3/ω-6)

Statistical analysis

All experimental data were reported as mean ± SD (Standard Deviation). Significant differences among the data were estimated by applying analysis of variance (ANOVA). Tukey's Test was used to evaluate the differences (at $P<0.05$). Statistical analyses were carried out using the Microsoft Excel software package (Microsoft Corp, Redmont, WA) with Daniel's XL toolbox.

Principal Component Analysis of the GasChromatographic and GasChromatographic/ MassSpectrometry data was carried out using the software Tanagra 1.4

RESULTS AND DISCUSSION

Sensory analysis

Results from sensory evaluation (Table1) showed that at D0 all samples from the three sport fishing lakes could be classified as "Extra" (Annex 1, EC No 2406/96). This is in agreement with the literature reports on the effect of time between the capture and the sale, on fish freshness (Gram and Huss 1996; Òlafsdottir *et al.* 2005).

For freshly caught trout, the sensory analysis performed on F and VP samples stored at 4°C until D3 showed them characteristic of an "E (Extra)" product but by D6 they had deteriorated to the "A" category. Trout from the Market (M) at D0 belonged to the freshness category "A" and remained so to D3. By D6 however they were classified "B (not edible)" in both F and in VP storage modes due to an acceleration of the spoilage process of the peritoneum as indicated by the pH values (data not shown) and microbial counts (Fig1.). The findings for these market trout probably derive from the longer time elapsed between the capture and sale, as well as from the transportation and handling modes of the fish samples as discussed by Tiecco (2000).

Although VP storage best preserved skin color and texture of the muscles of trout sampled in sport fishing lakes, compared to those purchased at the Market, but in the sensory evaluation data do not show significant differences between the two preservation methods. These results generally agree with literature reports showing non edibility after 6 days storage at 4°C (Poli *et al.* 2005).

Table 1. Sensory analysis: sport fishing lakes S (Serino), P (Prata di Principato Ultra), C (Cesinali) and control M(Market)

	D0		D3								D6							
	M	S-C-P	M		S		C		P		M		S		C		P	
			F	VP	F	VP	F	VP	F	VP	F	VP	F	VP	F	VP	F	VP
Skin	E	E	E-A	E-A	E-A	E	E	E	E	E-A	A	E-A	A	A	A	E-A	A	A
Skin mucus	E-A	E	A	A	E-A	E	E	E	A	A	A	A	A	A	E-A	E-A	E-A	A
Eye	A	E	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Gills	A	E	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Peritoneum (in gutted fish)	nd	nd	E	A	E	E	E	E	E	E	B-NA	B-NA	E	E	E	E	A	E-A
Smell of gills(D0) and abdominal cavity	E-A	E	A	A	E-A	A	E	E	E	E-A	B	A-B	A-B	A-B	A	A	A	A
Flesh	E-A	E	A	E-A	A	E	E	E	E	A	B	B	A-B	A	A	E	A	E-A

[a]Freshness category : E (Extra Fresh); A (Fresh); B (Admitted); NA (Not Admitted); nd (not determined)

pH value

No significant differences were observed among pH values of the different fish samples, notwithstanding the storage conditions (data not shown). As reported in literature, pH values do not influence fish storage (Lugovois and Kyrana 2005). Variations of pH values can depend on extrinsic factors such as the water source and stress of capture. Other studies indicate that pH values increases after about 6 days which corresponds to the time required for microbial growth to enter the exponential phase (Gram and Huss 1996; Tiecco 2000).–

Microbiological analyses

Fig1. shows the presence of natural microflora comparable to that reported in the literature Gram and Huss 1996;Tiecco 2000; Austin 2002).

At D0, the bacterial load on skin and gills (Fig1.) was between 10^1 and 10^5 cfu/g and 10^1 and 10^6 cfu/g respectively for microbial spoilage components. The highest values were measured for market trout. Conversely, the load on samples of fresh caught fish and market samples was only between 10^1 and 10^2 cfu/g for contaminating flora belonging to the *Enterobacteriaceae* family.

Furthermore, samples from Prata at D0 showed higher microbial values than those of Cesinali and Serino samples. This could be attributed to the microbiological quality of the source water as microflora isolated from the gills and skin of fish reflect the microbiological quality of their aquatic environment (Gram and Huss 1996; Tiecco 2000).

Analysis of D3 F and VP samples stored at 4°C showed a decreased microbial load compared to D0, probably as a result of the fish cleaning and washing processes prior to storage. It is known that storage temperature and manipulation are the main factors that influence shelf-life. Moreover, storage temperatures below 10°C inhibits microbial growth, particularly that of the psychrotropic bacteria. It is likely therefore that at 4°C, residual microflora on trout require a longer adaptation phase before entering the exponential growth phase (Chantarachoti *et al.* 2007).

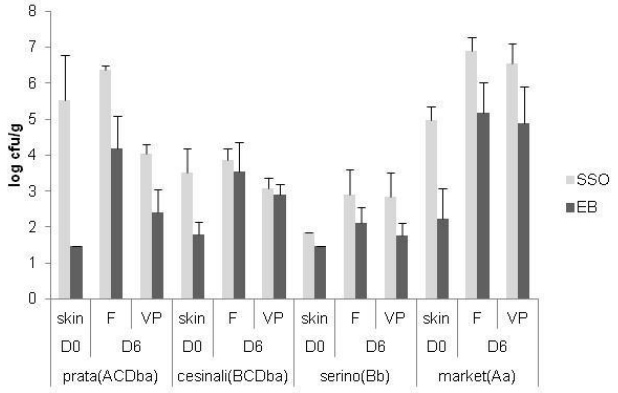
Fig1. shows microbial populations on D6. In fresh caught trout, specific spoilage organisms (SSO) increase up to 10^5 cfu/g and total coliforms (EB) are between $10^1$and 10^3 cfu/g in F samples. Samples stored in VP mode on the other hand have a SSO between 10^1 and 10^5 cfu/g and an increase of EB between 10^1 and 10^2 cfu/g. Purchased supermarket samples are different. In VP and F samples the SSO bacteria increases to 10^4 - 10^6 cfu/g, while EB bacteria are between 10^2 and 10^4 cfu/g suggesting that these samples had a higher microbial population already adapted to the substrate.

While we know that trout caught from the lakes were stored at low temperature within 2h , the time between the catch and sale of the fish purchased at the supermarket is unknown. The occurrence of lactic acid bacteria especially in vacuum packaged fish products has been reported (Gram and Dalgaard 2002) but these were not detected in this study, probably because of the relatively short time to conservation examined. Additionally, a sulphite-reducing bacteria load greater than 10^2 cfu/g was only seen after 6 days of storage where the population reached 10^5 cfu/g in samples stored in both F and VP modes. Market trout stored in both F and VP mode, had a D6 population of *Micrococcaceae* equal to 10^5 cfu/g which suggests contamination by manipulation (Tiecco 2000).

Market samples (M) stored under both F and VP methods had a *Pseudomonas* load of only between 10^1 and 10^2 cfu/g on D6. The fecal coliform load was also far below values reported in the literature as dangerous to the consumer (Tiecco 2000). Finally all samples stored under the two different conditions had SSO values less than 10^7 cfu/g which is considered the upper limit of acceptability for saltwater and freshwater species (ICMSF 1986; Leroy *et al.* 2009).

Fig1. Microbiological analysis (log cfu/g) of total viable counts Specific Spoilage Organism (SSO) and EnteroBacteriaceae (EB), for rainbow trout from sport fishing lakes and from market stored at 4 °C in F (Fridge) and VP (Vacuum Packed) at D0 (skin) and at D6 (meat and skin). Different letters (capital letters for SSO and lowercase letters for EB) are significantly different ($P<0.05$) for proveniences.

Moreover, there were no substantial differences (P> 0.05) between the storage conditions.



Fatty Acids

Table 2. compares the fatty acid (FA) content (% of total fatty acids) of the trout samples under storage conditions F and VP at D0 and D6. The D0 data confirm that the most abundant saturated fatty acid (SFA) is palmitic acid (C16:0) as indicated in other freshwater fish studies (Turchini *et al.* 2004, Jabeen and Chaudhry 2011).

The most abundant monounsaturated fatty acids (MUFA) in all samples are palmitoleic (C16:1ω6) and oleic (C18:1ω9) acids. Their sum is lower, particularly the amount of the oleic acid, in fish samples purchased in local supermarkets compared to those from the lake sampling sites. The most abundant polyunsaturated fatty acids (PUFAs) are linoleic (C18:2ω6), eicosapentaenoic acid (EPA; C20:5ω3) and docosahexaenoic acid (DHA; C22:6ω3). Linoleic acid has a higher concentration in trout from Cesinali Lake, while both the EPA and DHA acids are more abundant in control samples, probably, this is given by the feed. At D0, the ratio ω3/ω6 of our samples is 1.1 for trout from Cesinali Lake and 3.27 for trout from Prata Lake. The ratio DHA/EPA, which is 1.94 and 3.22 for fish samples from Serino and Cesinali Lakes, respectively, showed that DHA is the more copious ω3 fatty acid in all the analyzed trout samples. As it is not synthesized by humans, the amount of linoleic acid (C18:2ω6) is very relevant because it is an essential fatty acid for human nutrition. In the typical human diet the ratio ω3/ω6 is in favor of ω6 fatty acids (1 to 16.7) and it is beneficial to rebalance this fraction by dietary introduction ω3 FA rich food such as fish (Jabeen and Chaudhry 2011; Simopoulos 2002; Hibbeln *et al.* 2006). It has for example, been reported that a high ω3/ω6 ratio helps in reducing the risk of cardiovascular diseases (Pike 1999).

Other fatty acids are also beneficial in human health. DHA plays an important role in relieving muscle pain, inflammatory states and in the development of fetal brain and retina. Together with EPA, it can help to prevent cardiovascular disease in particular by acting directly or indirectly to inhibit platelet aggregation (Jabeen and Chaudhry 2011; Hibbeln *et al.* 2006).

PUFAs of ω3 series have also an anti-thrombogenic effect by reducing platelet activity. They also play a role against inflammatory diseases through participation in prostaglandin, thromboxane and leukotriene synthesis pathways (Ulbricht and Southgate 1991). These authors have proposed the calculation of an atherogenicity index (IA) and a thrombogenicity index (IT) for the qualitative evaluation of the lipid component of foods of different origin. The IA allows assessment the platelet aggregation risk, while IT assesses the anti-thrombogenic effect. Food quality is high when atherogenic and thrombogenic indices are low and this happens when food has a low content of saturated FA and a high amount of unsaturated FA.

In this context, IA values of trout from Cesinali and Prata Lakes are 0.32 and 0.56, respectively, while IT values obtained for fish samples from Cesinali and Prata Lakes are 0.44 and 0.70, respectively. These are relatively low IA values compared to those of beef (0.57 ± 0.06) and very low IT values in relation to those of beef (1.63 ± 0.13) (Simopoulos 2002).

At D6, the fatty acid profile of rainbow trout samples stored at 4 °C under both F and VP conditions show that the quantities of palmitic (C16:0), palmitoleic (C16:1ω6), oleic (C18:1ω9), linoleic (C18:2ω6), EPA (C20:5ω3) and DHA (C22:6ω3) acids and the sums of the SFA, MUFA and PUFA do not significantly vary during preservation regardless the storage conditions. This fact, probably due to the relatively short storage time, allows us to state that the experimental conditions used here did not cause any changes (i. e. oxidation) the trout FA profile.

Table 2. Proportions (Area %) of the Fatty Acids in rainbow trout (mean± standard deviation) stored in Vacuum Packed (VP) and at 4 °C (F)

	PRATA			SERINO		
	D0	D6 VP	D6 F	D0	D6 VP	D6 F
C14	5,31±0,10	5,43±0,70	4,05±0,01	4,81±0,39	4,07±0,44	4,28±0,16
C15	0,45±0,01	0,46±0,04	0,33±0,01	0,33±0,01	0,31±0,02	0,29±0,02
C16	18,87±0,32	19,27±1,40	16,17±0,32	15,03±3,57	15,11±1,05	15,21±0,45
C17	0,28±0,01	0,28±0,04	0,24±0,01	0,29±0,04	0,24±0,07	0,20±0,04
C18	3,12±0,12	3,21±0,27	2,68±0,01	5,61±2,87	5,85±2,82	5,16±1,92
Σ SFA	28,03±0,35	28,66±2,33	23,46±0,32	26,08±0,35	25,58±3,98	25,14±2,22
C16:1ω9	ND	ND	ND	ND	ND	ND
C16:1ω6	6,50±0,38	6,81±0,68	6,31±0,41	7,87±0,67	6,55±0,11	8,39±0,87
C18:1ω9	20,38±0,39	21,73±2,52	22,35±0,05	29,61±2,33	26,17±6,31	29,54±2,15
C18:1	3,26±0,18	3,26±0,41	2,99±0,23	2,30±0,44	1,81±0,45	2,57±0,56
C20:1ω9	2,12±0,17	2,45±0,47	2,64±0,22	3,30±0,24	3,07±0,32	2,77±0,64
C22:1ω9	2,23±0,41	2,04±0,18	1,79±0,02	2,50±0,21	2,30±0,28	1,38±0,18
Σ MUFA	34,50±0,98	36,28±5,36	36,08±0,02	45,58±3,89	39,90±7,47	44,65±6,40
C18:2ω6	7,37±0,40	7,45±0,65	9,75±0,27	10,63±0,71	8,78±1,22	8,94±0,38
C18:3ω3	1,97±0,09	1,98±0,18	2,13±0,02	2,20±0,12	1,80±0,24	1,75±0,13
C16:2ω3	0,39±0,05	0,36±0,10	0,39±0,01	0,44±0,05	0,34±0,09	0,30±0,06
C20:2ω6	0,47±0,03	0,55±0,07	0,52±0,01	0,45±0,02	0,44±0,05	0,45±0,01
C20:4ω6	0,81±0,0.	0,94±0,23	0,76±0,02	0,57±0,03	0,55±0,03	0,62±0,06
C20:5ω3	7,53±0,14	7,38±0,52	4,87±0,08	5,71±0,45	5,08±0,48	5,28±0,23
C22:6ω3	18,36±0,89	15,86±2,76	21,34±0,01	11,07±2,66	17,55±1,87	12,86±1,04
Σ w6	8,65±0,43	8,95±0,71	11,04±0,26	11,65±0,75	9,77±1,27	10,01±0,32
Σ w3	28,25±0,82	25,57±2,02	28,74±0,05	19,42±2,18	24,77±2,07	20,19±1,02
Σ PUFA	36,90±0,75	34,52±1,38	39,78±0,31	31,07±4,04	34,54±3,98	30,20±1,91
IA	0,56±0,01	0,58±0,08	0,43±0,01	0,45±0,05	0,42±0,06	0,43±0,14
IT	0,70±0,11	0,73±0,11	0,56±0,11	0,64±0,11	0,63±0,10	0,62±0,11
DHA/EPA	2,44±0,13	2,15±0,27	4,38±0,08	1,94±0,11	3,45±0,10	2,44±0,10
w3/w6	3,27±0,22	2,86±1,05	2,60±0,06	1,67±0,27	2,54±0,27	2,02±0,12
	CESINALI			MARKET		
	D0	D6 VP	D6 F	D0	D6 VP	D6 F
C14	3,29±0,27	3,17±0,24	3,22±0,07	3,54±0,09	3,80±0,37	3,73±0,08
C15	0,24±0,01	0,22±0,02	0,25±0,01	0,36±0,03	0,35±0,03	0,35±0,01
C16	12,45±0,48	12,01±0,65	13,84±1,03	18,33±0,13	18,36±0,06	17,81±0,60
C17	0,38±0,03	0,37±0,04	0,31±0,04	0,18±0,01	0,18±0,04	0,19±0,01
C18	2,49±0,09	2,38±0,13	2,72±0,10	3,61±0,29	3,42±0,46	3,64±0,06
Σ SFA	18,84±0,70	18,16±1,08	20,34±1,14	26,02±0,55	26,11±0,96	25,73±0,72
C16:1ω9	ND	ND	ND	0,11±0,01	0,10±0,01	0,12±0,02
C16:1ω6	5,40±0,34	5,89±0,37	5,69±0,51	5,15±0,07	5,42±0,15	5,56±0,03
C18:1ω9	21,51±1,23	23,78±1,01	20,50±0,91	12,73±0,17	15,28±0,23	15,24±0,24
C18:1	2,36±0,09	2,03±0,21	2,85±0,25	3,13±0,04	2,99±0,13	3,29±0,03
C20:1ω9	1,03±0,07	1,33±0,05	1,09±0,04	0,56±0,01	0,69±0,19	0,62±0,02
C22:1ω9	0,50±0,08	0,64±0,02	0,56±0,02	0,25±0,01	0,34±0,08	0,22±0,01
Σ MUFA	30,80±1,82	33,67±1,66	30,69±1,73	21,95±0,32	24,82±0,79	25,05±0,35
C18:2ω6	22,63±1,08	22,65±1,11	18,39±0,87	16,19±0,22	17,84±0,23	18,58±0,54
C18:3ω3	2,91±0,14	2,74±0,25	2,26±0,06	2,30±0,26	2,12±0,03	2,31±0,07
C16:2ω3	0,42±0,03	0,41±0,02	0,35±0,04	0,14±0,01	0,43±0,04	0,45±0,01
C20:2ω6	0,57±0,02	0,56±0,01	0,48±0,01	0,60±0,01	0,68±0,11	0,74±0,03
C20:4ω6	0,76±0,22	0,64±0,03	0,81±0,07	0,98±0,02	0,82±0,06	0,98±0,01
C20:5ω3	5,47±0,26	4,98±0,20	5,13±0,35	9,37±0,59	7,86±0,15	8,13±0,04
C22:6ω3	17,61±3,33	16,21±4,34	21,51±2,79	26,46±0,36	20,80±0,98	21,54±0,17
Σ w6	23,96±1,16	23,85±1,14	19,68±0,89	17,77±0,20	19,34±0,39	20,30±0,57
Σ w3	26,41±3,05	24,34±3,87	29,25±2,42	38,27±0,49	31,20±1,20	32,43±0,05
Σ PUFA	50,37±5,08	48,19±5,96	48,93±4,19	56,04±0,28	50,55±1,59	52,74±0,51
IA	0,32±00,06	0,30±0,04	0,34±0,06	0,42±0,01	0,45±0,01	0,42±0,01
IT	0,44±0,10	0,42±0,11	0,48±0,10	0,62±0,0	0,65±0,01	0,62±0,02
DHA/EPA	3,22±0,11	3,26±0,12	4,19±0,11	2,82±0,22	2,65±0,07	2,65±0,03
w3/w6	1,10±0,18	1,02±0,21	1,49±0,18	2,15±0,05	1,61±0,03	1,60±0,05

Volatile compounds

The volatile organic molecule (VOMs) profile determination of trout muscle was performed on D0 and D6 samples from both F and VP storage conditions. Table 3 show the main volatile compounds detected by SPME GC/MS. In order to define the sensory quality of rainbow trout, we have identified those volatile substances, particularly the lipid component, considered as indicators of fresh fish smell, of microbial spoilage and of the oxidation processes as described by Òlafsdottir and Fleurence (1997).

The volatile substances responsible for flavors and odors of fish are mainly small molecules - aldehydes, ketones, alcohols and esters (C2-C9). VOM analysis to identify these was conducted on D3 and on D6 of both F and VP storage to determine changes of their profile and/or formation of new volatile molecules.

Compounds characteristic of fresh fish odor such as hexanal, octanal and 1,5-octadien-3-ol decrease for all the fish samples regardless of storage condition. The octanal concentration decrease is more pronounced for the F trout samples purchased at the market. All these compounds have been identified as those with the greatest impact on fresh fish flavor (Josephson *et al.* 1984). Octanal in fact, is considered as a marker of rainbow trout freshness (Leduc *et al.* 2012; Òlafsdottir and Fleurence 1997).

Ethanol, acetic acid, acetone, acetoin, propanal, 3-methyl-butanal, 2-methyl-butanal and Trimethylamine (TMA) are among the volatile compounds originating from microbial spoilage (Òlafsdottir *et al.* 2005). In the context of this study, small quantities of TMA were found at D6 only in the market fish samples under both storage conditions. Other compounds undergo a slow increase during the storage period in both storage modes.

The presence of TMA in trout purchased at the supermarket may be due to the time between the capture and purchase is much longer and generally unknown compared to that of the trout from sport fishing lakes. We also found the amount of acetoin to be higher in the samples from the supermarket stored in VP condition compared to the F mode. This may be due to an increase in microbial growth of SSO probably owing to the lower concentration of oxygen in the VP samples.

Hexanal, 4-cis-heptanal, 1-penten-3-ol, 1-octen-3-ol compounds indicative of PUFA oxidative processes (Josephson *et al.* 1984). Hexanal and propanal were the more abundant aldehydes seen in all samples and this result agrees with literature reports specifying an increase in these compounds after 6 days of storage (Iglesias *et al.* 2009). Propanal may come from the 16-hydroperoxide released by oxidation of linolenic acid. The high levels of hexanal can likely be ascribed to the oxidation of linoleic acid, the most abundant fatty acid of farmed fish muscle. These aldehydes, come from the oxidation of fats while saturated forms, responsible for ‘‘vegetable, green, rancid or fruity’’ quality characteristics are mainly derived from the catabolism of unsaturated fatty acids. Both hexanal and octanal belong to this group (Yasuhura and Shibamoto 1995).

The importance of aldehydes is due to the fact that their perception threshold is very low. In consequence, they strongly influence fish flavor (Turchini *et al.* 2004). PUFAs, in particular contribute to the volatile compounds resulting from oxidative processes, that are recognized as off-flavors and off-odors. The major amount of aldehydes which increase their concentration during storage, is in trout preserved in F condition compared to the VP storage mode.

Table 3. Change of volatile compounds in rainbow trout stored in Vacuum Packed (VP) and at 4 °C (F). Each value shows the mean value (n = 3) of the log 10 transformed peak areas for all samples.

ND Not Detected

		PRATA					SERINO				
		D0	D3VP	D6VP	D3 F	D6 F	D0	D3VP	D6VP	D3 F	D6 F
Aldehydes	acetaldehyde	6.6	6.4	6.5	6.6	6.8	6.4	6.5	6.8	6.3	6.4
	propanal	7.2	7.3	7.3	7.7	7.7	7.3	7.5	7.5	7.3	7.4
	butanal	6.4	6.4	6.4	6.8	6.8	5.8	6.7	7.0	6.4	6.9
	butanal 2-	5.9	6.4	6.1	6.0	6.0	5.5	5.5	5.3	6.2	6.1
	butanal 3-	6.5	6.8	6.7	6.5	6.2	6.3	6.1	6.0	6.5	6.6
	pentanal	7.1	7.2	7.2	7.6	7.6	7.3	7.5	7.3	7.3	7.3
	hexanal	7.8	7.5	7.7	7.8	7.4	7.4	7.5	7.5	7.4	7.4
	2-pentenal	6.5	6.7	6.6	7.2	7.1	6.6	6.9	6.9	6.6	6.7
	heptanal	6.4	6.6	6.5	6.7	6.9	6.5	6.7	6.7	6.5	6.6
	2-hexenal	6.8	6.4	6.6	6.7	6.1	6.3	6.7	6.5	6.4	6.4
	4-heptenal cis	6.1	6.4	6.2	6.8	6.9	6.3	6.6	6.5	6.5	6.5
	octanal	6.8	6.1	5.8	6.5	6.5	6.4	6.4	6.5	6.5	6.4
	nonanal	ND	ND	ND	ND	6.6	ND	ND	6.5	ND	ND
	benzaldehyde	6.8	6.8	6.8	7.0	7.0	6.7	6.9	6.9	6.7	6.8
Ketones	acetone	7.1	6.9	7.0	6.9	7.0	7.2	7.2	7.1	7.2	7.1
	2-butanone	6.6	6.5	6.6	6.5	6.5	6.5	6.5	6.6	6.5	6.5
	2-pentanone	6.6	6.7	6.7	6.7	6.8	7.0	6.7	6.4	6.5	6.5
	2,3-	7.1	7.2	7.2	7.6	7.5	7.3	7.4	7.2	7.5	7.4
	3-octanone	7.1	6.6	6.8	7.1	6.7	7.4	6.7	5.8	6.7	ND
	acetoin	7.2	7.2	7.2	7.4	7.6	6.9	6.9	6.9	6.9	6.9
	2,3-	5.9	6.1	6.0	6.5	6.7	6.1	6.4	6.3	6.0	6.4
Alcohols	ethanol	8.9	8.8	8.9	8.7	8.9	8.8	8.8	8.7	8.8	8.7
	1-propanol	5.9	6.3	6.1	6.6	6.5	6.1	6.3	6.4	6.3	6.2
	1-butanol	5.7	5.9	5.8	6.1	6.0	5.8	6.0	5.9	6.1	5.9
	1-penten-3-ol	7.5	7.8	7.7	8.2	8.2	7.7	8.0	7.9	8.0	7.9
	isobutanol	6.2	6.5	6.3	7.3	6.7	6.4	6.4	6.4	6.3	6.2
	1-pentanol	6.4	6.5	6.5	6.2	6.9	6.6	6.8	6.7	6.6	6.6
	2-penten-1-ol	6.2	6.5	6.4	6.8	7.1	6.4	6.7	6.5	6.9	6.5
	2-penten-1-ol	6.6	6.9	6.8	7.2	7.0	6.8	7.1	6.9	6.8	6.8
	1-hexanol	6.1	6.1	6.1	6.3	6.5	6.1	6.2	6.4	6.3	6.4
	1-octen-3-ol	6.6	6.6	6.6	6.8	7.0	6.4	6.7	6.6	6.6	6.6
	1-heptanol	6.1	6.3	6.2	6.3	6.3	6.0	6.3	6.2	6.1	6.2
	1,5-octadien-	6.8	6.6	6.7	6.8	7.1	6.7	6.9	6.7	6.8	6.8
	2-ethyl-1-	6.1	6.1	6.1	6.2	6.2	6.0	6.1	6.2	7.9	8.1
Other compounds	carbon	7.3	4.9	6.1	7.3	ND	6.9	6.0	ND	ND	ND
	2-ethylfuran	ND	7.0	7.0	7.2	7.3	ND	7.1	7.0	6.7	6.9
	α-pinene	6.2	5.8	6.0	5.7	5.4	6.1	5.1	5.5	5.6	5.5
	chloroform	6.7	6.6	6.6	6.8	6.8	6.6	6.8	6.7	6.6	6.6
	limonene	6.6	6.3	6.4	6.2	ND	6.0	5.9	6.1	ND	6.0
	acetic acid	6.3	6.4	6.6	6.3	6.4	6.0	6.2	6.2	6.2	6.2
	trimethylamine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

		CESINALI					MARKET				
		D0	D3VP	D6VP	D3 F	D6 F	D0	D3VP	D6VP	D3 F	D6 F
Aldehydes	acetaldehyde	7.0	6.8	6.5	6.8	6.6	6.8	6.7	6.8	6.9	6.2
	propanal	7.6	7.2	7.0	7.5	7.2	7.6	7.5	7.3	7.6	6.7
	butanal	7.2	7.0	6.0	7.2	6.5	6.8	6.8	6.4	6.8	ND
	butanal 2-	5.6	5.5	5.9	5.8	6.1	6.0	6.1	6.2	6.2	6.4
	butanal 3-	6.0	5.9	6.1	6.3	6.5	6.0	6.2	6.5	6.5	7.0
	pentanal	7.4	7.2	6.4	7.4	6.3	7.2	7.3	6.6	7.3	ND
	hexanal	7.9	7.7	7.4	7.8	7.5	7.7	7.5	7.3	7.4	6.7
	2-pentenal	6.7	6.4	5.0	6.5	6.4	6.5	6.5	6.4	ND	ND
	heptanal	6.7	6.4	6.2	6.6	6.5	6.6	6.7	6.7	6.5	5.8
	2-hexenal	6.4	6.1	5.5	6.4	6.2	6.4	6.5	6.1	6.2	5.8
	4-heptenal cis	6.3	6.5	6.4	6.2	6.4	6.0	6.5	6.2	6.1	6.3
	octanal	6.3	6.2	5.4	6.3	6.3	6.1	6.5	5.9	6.0	2.3
	nonanal	ND	ND	ND	ND	6.8	6.7	6.9	ND	ND	ND
	benzaldehyde	7.0	6.7	6.5	6.7	6.4	6.6	6.7	6.5	6.4	ND
Ketones	acetone	7.0	7.2	7.0	7.1	7.0	7.1	7.1	7.5	7.1	7.3
	2-butanone	6.7	6.8	6.4	6.6	6.3	6.4	6.5	6.4	6.5	6.2
	2-pentanone	6.8	6.5	7.1	6.8	7.2	ND	6.4	6.7	6.5	ND
	2,3-	7.7	7.4	6.9	7.5	7.1	6.4	7.5	7.0	7.3	6.3
	3-octanone	6.4	6.3	6.4	6.0	6.2	6.0	6.1	6.8	5.7	6.1
	acetoin	7.0	7.2	7.4	7.0	7.3	7.6	7.8	8.8	7.8	8.2
	2,3-	5.9	6.3	6.0	6.4	6.0	6.1	6.2	5.9	6.1	ND
Alcohols	ethanol	9.0	8.9	9.0	8.9	8.8	8.9	9.0	8.8	9.0	8.8
	1-propanol	6.2	6.0	6.0	6.2	6.1	6.2	6.2	6.4	6.6	ND
	1-butanol	5.8	6.1	6.0	6.1	5.9	5.8	6.0	6.1	5.9	5.9
	1-penten-3-ol	7.5	7.7	7.7	7.6	7.9	7.8	8.0	8.0	7.9	8.1
	isobutanol	6.7	6.7	6.5	6.7	7.7	7.4	6.4	6.8	6.5	6.3
	1-pentanol	6.9	6.7	6.3	6.9	7.7	6.6	6.8	6.6	6.9	ND
	2-penten-1-ol	6.5	6.2	5.8	6.4	6.2	6.4	6.6	6.4	6.5	5.6
	2-penten-1-ol	7.0	6.7	6.4	6.9	6.6	6.7	7.1	6.7	6.8	5.9
	1-hexanol	6.4	6.0	5.9	6.2	6.1	6.1	6.2	6.4	6.0	5.7
	1-octen-3-ol	6.3	6.5	6.6	6.2	6.7	6.1	7.1	6.4	6.3	6.4
	1-heptanol	6.0	6.0	6.0	6.0	6.1	5.8	6.2	5.9	6.0	6.1
	1,5-octadien-	6.9	6.7	6.2	6.7	6.5	6.4	6.7	6.2	6.4	2.3
	2-ethyl-1-	6.0	6.1	6.1	6.2	6.5	6.0	6.2	6.4	6.1	6.3
Other compounds	carbon	7.3	7.2	ND	7.0	ND	7.1	6.3	6.4	6.2	6.9
	2-ethylfuran	7.0	6.9	ND	6.8	6.9	ND	6.9	6.5	6.3	ND
	α-pinene	6.3	5.4	5.4	5.2	5.4	ND	5.9	5.6	6.3	ND
	chloroform	7.1	6.5	6.4	6.7	6.5	7.0	6.7	6.5	6.8	6.1
	limonene	6.0	ND	ND	ND	ND	5.8	ND	6.1	6.0	ND
	acetic acid	6.2	6.4	6.4	6.2	6.3	6.6	6.8	7.1	6.7	7.0
	trimethylamine	ND	ND	ND	ND	ND	ND	ND	5.7	ND	6.5

Principal Component Analysis

PCA results for fatty acids at D0 and D6 in trout samples under different storage conditions (F, VP) are shown in Fig2. The ratios of contribution for the principal components 1 (PC1) and 2 (PC2) were 44.83% and 31.43%, respectively. From Fig2., the PCA analysis gives four clearly defined groups, each belonging to a specific collection area.

PCA results for volatile compounds at D0 and D6 in trout samples

under different storage conditions (F, VP) are shown in Fig3. The ratios of contribution for principal components 1 (PC1) and 2 (PC2) were 24.02% and 13.79%, respectively. Fig3, the cluster shows that there is a high correlation among samples during the whole period of preservation. There is a major difference noted for both F and VP trout from the market and Lake Cesinali after six days.

The market samples showed the highest microbial load, a major presence of volatile compounds related to microbial activity and to the more advanced oxidation processes and this is reflected in the gap of the F samples in Fig3. This is likely due to the fact that, unlike freshly caught trout, the time between capture and death are not know. The deviation of Cesinali trout is probably due to their diet and not to the microbial load, as is the case with market samples, since the microbial counts were lower compared those from the market.

Fig2. PCA results for fatty acids. White symbol D0; Grey symbol D6F; Black symbol D6VP

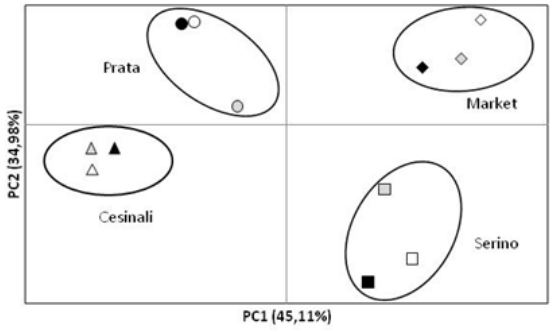
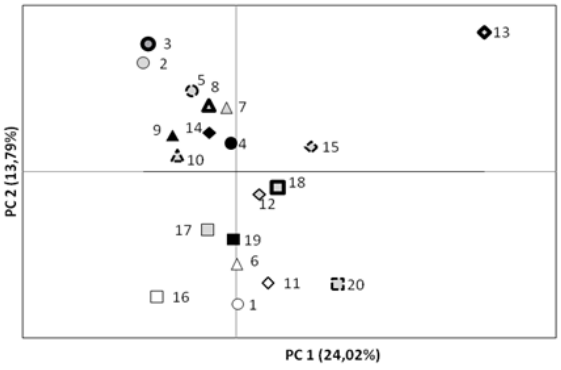


Fig3. PCA results for Volatile compounds. 1: Prata D0; 2: Prata D3F; 3: Prata D6F; 4: Prata D3VP; 5: Prata D6VP; 6:Serino D0; 7: Serino D3F; 8: Serino D6F; 9: Serino D3VP; 10: Serino D6VP; 11: Market D0; 12: Market D3F; 13: Market D6F; 14: Market D3VP; 15: Market D6VP; 16: Cesinali D0; 17: Cesinali D3F; 18: Cesinali D6F; 19: Cesinali D3VP; 20: Cesinali D6



CONCLUSION

In this paper we have studied and characterized some aspects of domestic storage of rainbow trout sold for consumption which may be applicable to fish products in general. Vacuum tools have been used and some quality characteristics during an interval of six days at refrigerator temperature (4°C) have been compared to plastic tray wrapped products. Trout, kept under vacuum, showed a better skin and meat color, firmer muscle texture and limited formation of off-odors and off-flavors related to spoilage and oxidative processes. These data have proved the efficiency of this system in preserving the quality characteristics of the trout. Furthermore, it has been possible to verify the presence of octanal and 1,5-octadien-3-ol, proposed as a marker of freshness of the fish by several authors (Leduc *et al.* 2012; Òlafsdottir and Fleurence 1997).

The study has also allowed us to extrapolate some information on the characteristics of trout samples coming from the sports fishing lakes of the Irpinia region. In particular, the results demonstrate the presence of an autochthonous microflora with absence of potentially dangerous bacteria. This feature is certainly correlated with the quality of water in these lakes which is from natural sources located far from urban areas.

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