CONTENTS

GENERAL INTRODUCTION

Opportunities for innovation in the mussels supply chain

The development of the mussel culture has so far been mainly related to innovations of primary production. Despite the quality of the product, the marketing of mussels has not yet been supported by a real valorization, and it is mainly limited to the distribution of the fresh product in traditional packaging represented by plastic mesh.

There are numerous strengths that characterize this product. Mussels are characterized by:

- high nutritional value;
- low cost;
- monitoring and control during cultivation;
- production sustainability with a low environmental impact;
- appreciation by the consumer;
- short cooking time.

But also numerous weaknesses related to the intrinsic characteristics of the product as:

- high perishability due to high content in water;
- nutritional characteristics variable according seasons, site of cultivation and physiological status;

weaknesses related to the production as :

- poor training for operators in this sector;
- scarce disciplinary for production;
- scarce organization-synergy between producers;
- scarce tecnological improvement (plant engineering and equipment);

weaknesses related to health controls as:

- response and analysis time are often incompatible with the commercial needs;
- limit and methods proposed by EU EFSA and *Codex alimentarius* eterogeneus;
- monitoring of norovirus not contemplated by law.

weaknesses related to commercial aspects as:

- scarce diversification and processing of the product;

- scarce divulgation and promotion of the nutritional mussels properties of the mussels.

In recent years on the market are widely sold mussels from Spain or New Zealand, processed and frozen then boiled with or without shells, loose or packaged in different ways.

Are found in commerce small quantities of shelled and boiled mussels stored in brine or oil in jars or cans. This processes can ensure extension of the shelf-life of the product to several months even if product undergoes substantial structural and sensory changes compared to fresh and cooked at home.

The ability to offer products with extended shelf life coupled with high quality characteristics represents an opportunity for innovation in the mussels supply chain.

Aim and outline of the thesis

This PhD thesis aims to provide practical suggestions for the evaluation of nutritional properties, freshness and quality, and the development of innovative products, able to preserve the quality of the raw material and able to meet new consumer needs and ease of use.

The experimental project was funded by Regione Friuli Venezia Giulia (L.R.n°26 del 2005 art.17), concerning the application of innovative techniques for the valorization of shellfish products with methods of processing and control.

Trying to use a multidisciplinary approach, experimental activity has been organized as follows:

Chapter 1 *"Description of mussels and their supply chain"* provides to give information on physiology and anatomy of mussels and an overview of the production chain, considering cultivation techniques, distribution-trade and related aspects of food safety.

Chapter 2 "*Nutritional and merchantable traits of M. galloprovincialis relative to the physiological condition"*, was aimed to evaluate the attitude of *M.galloprovincialis* to processing in relation to the physiological condition, characterizing the nutritional and commercial traits of the product;

Chapter 3 *"Application of sous vide techonology to preserve live mussels"*, was aimed to study new storage methods to extend the shelf life of live mussels; and identify quality attibutes to develop a practical scheme for evaluation of live mussels *Mytilus galloprovincialis* freshness and quality during storage.

Chapter 4 *"Application of heat treatment sous vide cook chill to mussels M. galloprovincialis*" was aimed to to test *sous vide* technology associated with the heat treatment of pasteurization and their stability during storage.

Chapter 5 *"General conclusion"* summarize the main achievements of this thesis.

1. MUSSELS: BIOLOGICAL ASPECTS AND PRODUCTION

1.1 Functional morfology of mussels

The mussel is a mollusk equipped with a hard, protective outer shell: composed of two convex valves of similar size. Similarly to other bivalves three layers make up the shell 1) a thin outer periostracum of horny conchiolin, 2) a middle prismatic layer of aragonite or calcite, a cristalline form of calcium carbonate and 3) an inner calcareus layer, that is either of dull texture or iridescent mother-of-pearl. The outer mantle fold secretes the periostracum and prismatic layers, while the inner layer is secreted by the general mantle surface. The shell grows in circumference and in thickness. Calcium for shell growth is obtained from the diet or taken up from seawater and the energy required for shell growth is not an insignificant portion of a bivalve's total energy budget (Hawkins & Bayne, 1992). The valves are hinged together at the anterior by means of ligament. This area of the shell is called umbo, while the part along which the mantle is attached is called pallial line. On the inside of each valve are two posterior and anterior muscle adductor that act in antagonism with the ligament and, contracting, determine the closing of the valves even for long periods of time.
The mussels are equipped with an appendix muscular call foot inturn secretes a byssus, a bundle of tough threads of tanned protein that comes out through ventral part of the shell and serve as mooring lines for attachment of the mussels to the substrate (Sun *et al.*, 2002)(fig. 1 and 2).
The mantle consists of two lobes of tissue which completely enclose the animal within the shell. Between the mantle and internal organs there is a mantle cavity. The mantle consists of connective tissue with haemolymph vessel, nerves and muscles that are particularly well developed near the mantle margins. A large opening in the inhalant mantle allows entry of water while an exhaling orifice allows the expulsion of the water after the uptake of dissolved oxygen and particles of food. The gills are composed of two ctenidi or emibranchie, each in turn composed of two blades with one ascending and descending, held together by connective junctions (W-shape). The gill are coated by a ciliated epithelium very vascularized that promotes the correct circulation of the water and that it is important both for the filtration and for gas exchange related to respiration.

The particles in suspension in the water are retained by the cilia and conveyed, via ciliary beat along a branchial groove, towards the labial palps and mouth. From there, the particles arrive in the stomach, of the stylus and, within it, of the stylus crystalline, where contents are digestive enzymes such as amylase, lipase and cellulase. The partially digested food particles are conveyed to the digestive gland, constituted by a series of diverticula communicating with the stomach. Finally, the digested material is conveyed in the intestines until it emerges, through the anus into the cavity palleale near to the current exhaling.

The excretory system consists of two nephridia type metanefridiale, placed under the pericardial cavity.

The circulatory system is open-ended circulation incomplete and mixed, the heart is positioned dorsally and consists of a ventricle, the main propeller of hemolymph, and two atria.

The mussels generally have separate sexes, although there are cases of hermaphroditism, and external fertilization although sometimes it can take place within the palleal cavity of females.

The gonads are composed of glandular tissue located between the digestive organs and the mantle. Less abundant is the information on the sex cycle of *M. galloprovincialis* (Boucart & Lubet, 1965; Valli, 1971; Renzoni & Giusti, 1972; Renzoni, 1973; Valli *et al.*, 1975). The conclusions of the various studies sometimes disagree on the time and duration of the reproduction cycle stages, especially about spawning, and this is probably a reflection of the variety of local ecological conditions, by the contraction of the follicular epithelium generally coincided with the warmer months of June, July and August.

In the mussels cultured in the Venice Lagoon the highest percentage of individuals showed gametogenetic inactivity in August when the water reach its highest temperature (25-26°C). At the end of summer when the temperature dropped to 20°C, the germinal epithelium resume activity, producing gametocytes which became mature gametes in two or three weeks. By the beginning of October it was possible to find individuals which were in the spawning stage or had alredy spawned already and were forming a second set of gametes.

The period of spawning extends from the end of September through the minter to the beginning of June (Da Ros *et al.*, 1985). Fertilized oocyte originates the first larval stage, larva trocofora which has a central band and a terminal ciliated ridge. After a few days trocofora is transformed into veliger larva, the pelagic larval stage ensures the colonization of new areas and the genetic recombination (Gilg & Hilbish 2003).

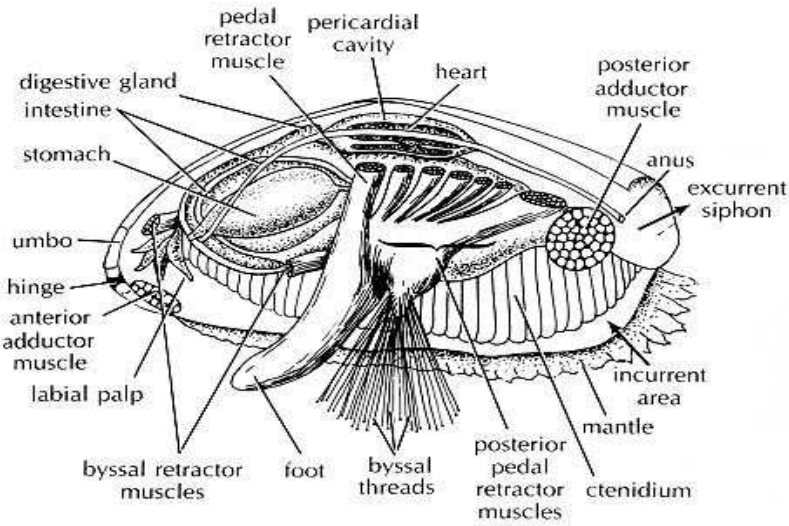

Figure 1 Graphic representation of the Internal anatomy of Mytilus (Source http//.manandmollusc.net)

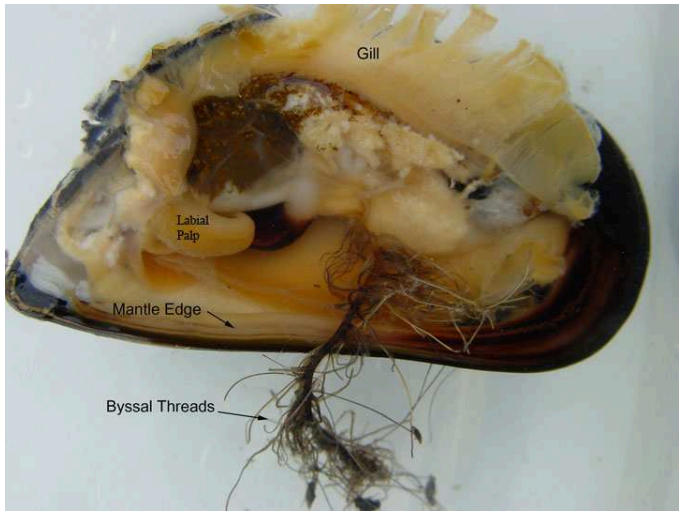

Figure 2 Mussel internal anatomy (http://www.bigelow.org/mitzi/mid_9a.html)

The larva reaches a size of 5 mm in about five weeks turning into a young mussel (fig. 3). After this metamorphosis it attaches itself, with the byssus threads, to hard substrates, probably in response to the presence of a biofilm surface (Bao *et al*., 2007), after a few weeks, doubled in size and assume all the characteristics of an adult mussel.

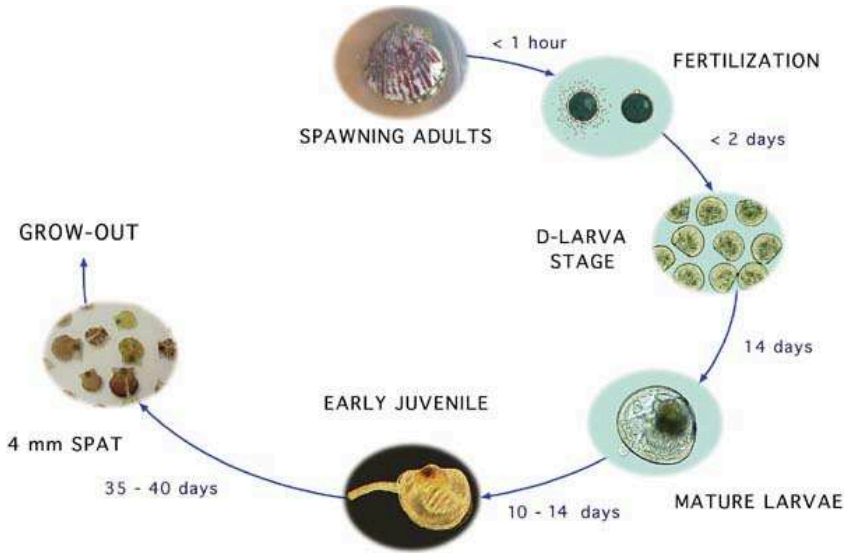

Figure 3 Development stage of bivalvia
(**http://www.fao.org/docrep/007/y5720e/y5720e07.htm**)

The growth of the mussel has been extensively studied for both the ecological and commercial importance. It is known that the optimal growth temperature for *M. galloprovincialis* is between 23-28°C. Besides these temperatures growth rate declines significantly but within this range are not noticed macroscopic variations of the conditions of the organism that shows good adaptability to seasonal fluctuations (Almada-Villela *et al*., 1982). The main factor that influence mussels growth is the food availability in the environment, as a primary energy source. The mussels are filter feeders that hold a particle size of less than a 2-3 μm with an efficiency of 80 to 100% (Møhlenberg and Riisgard, 1977). The material present in suspension consists of bacteria, phytoplankton, organic debris and residues of inorganic origin, with variations depending on the site and season. Under

conditions of emergence at low tide, mussels are unable to adequate nutrition but then quickly restore normal metabolism and growth.
Among the factors that influence negatively the mussels growth there is the prolonged exposure to light, the presence of strong waves and intraspecific competition for food and space that is found in populations overly crowded (Gosling, 1992). The weakening of the mussel and increased mortality are also affected by the presence of predators, parasites, pathogens and natural competitors.

1.2 Bivalve mollusc production

Global production by capture and aquaculture in 2010 was 148 million tonnes of which 128 million tonnes were used for food. Bivalve molluscs represent almost 10% of the total world fishery production, but 26% in volume and 14% in value of the total world aquaculture production. World bivalve molluscs production (capture and aquaculture) has increased substantially in the last fifty years, going from nearly 1 million tons in 1950 to about 14.6 million tons in 2010. While production by capture has marginally declined from about 1.9 million tonnes to about 1.7 million tons in 2010, production by aquaculture increased from 8.3 million tons in 2000 to 12.9 million tons in 2010 (Karunasagar 2012) (Fig. 4).

1.2.1 Major bivalve producing Countries

China is by far the leading producer of bivalve molluscs, with 10.35 million t in 2010, representing 70.8% of the global molluscan shellfish production and 80% of the global bivalve aquaculture production. The whole the Chinese bivalve production is cultured. Other major bivalve producers in 2010 were Japan (819 131 t), USA (676 755 t), Republic of Korea (418 608 t), Thailand (285 625 t), France (216 811 t) and Spain (206003t). Concerning mussels, China, Chile and Spain, resulted in 2010 major produces followed by Italy (Sfetcovici & Tribiluskova, 2012)(Table 1).

By species, the bivalve mollusc production by aquaculture in 2010 consisted of 38.0% clams, cockles and arkshells, 35.0% oysters, 14.0% mussels and 13.0% scallops and pectens (Fig.5).

The mussels production by aquaculture consisted of 6 species in relation to geographic cultured site, Chilean mussel, green mussel, blue mussel (*M. edulis*), Mediterranean mussel (*M. galloprovincialis*), New Zealand mussel and Korean mussel (Sfetcovici & Tribiluskova, 2012) (Fig. 6).

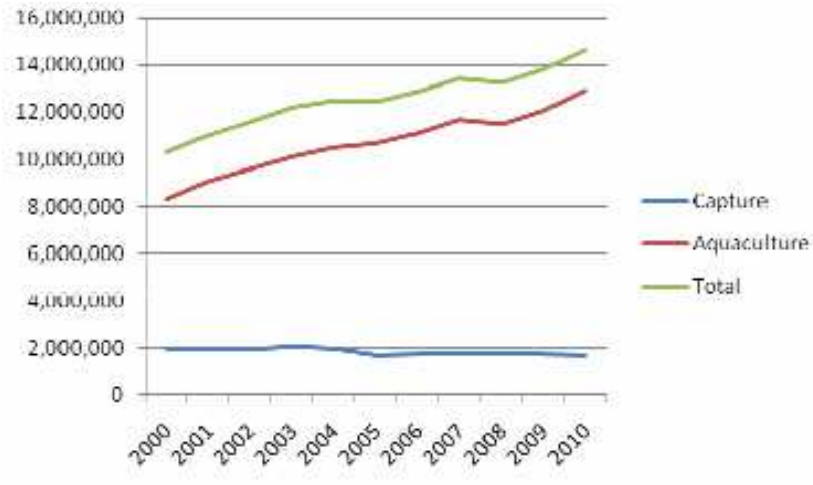

Figure 4 Global bivalve production (FAO, 2012)

Table 1 Major global mussels producer Countries (capture and culture), in 1000 tonnes (Souce: Fishstat Plus, 2012)

Country	2005	2006	2007	2008	2009	2010
China	675.4	652.4	448.7	479.9	637.4	702.2
Chile	91.2	131.9	166.6	193.9	170.5	228.6
Spain	158.2	228.8	209.7	180.3	198.8	189.3
Thailand	270.7	229.7	228.3	203.2	193.6	166.9
New-Zealand	95.5	97.4	99.7	100.3	90.0	95.3
France	76.3	78.7	76.0	81.7	79.2	78.0
Korea Rep.	48.1	88.2	107.6	75.4	65.8	67.9
Italy	63.6	61.9	58.5	67.2	76.8	64.3
Netherlands	59.5	31.3	43.7	36.1	45.6	56.2

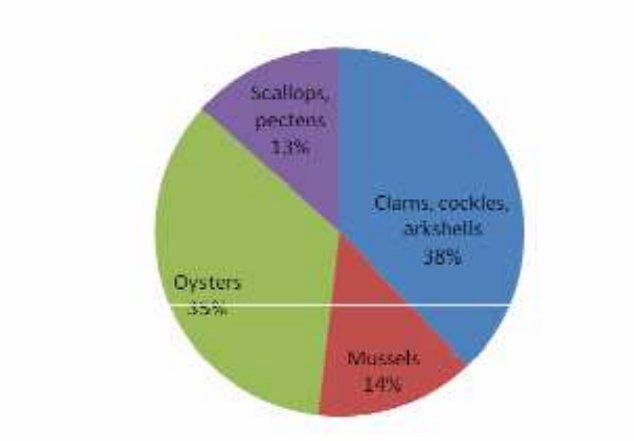

Figure 5 Bivalves mollusc production by species
(source: Fishstat Plus, 2012)

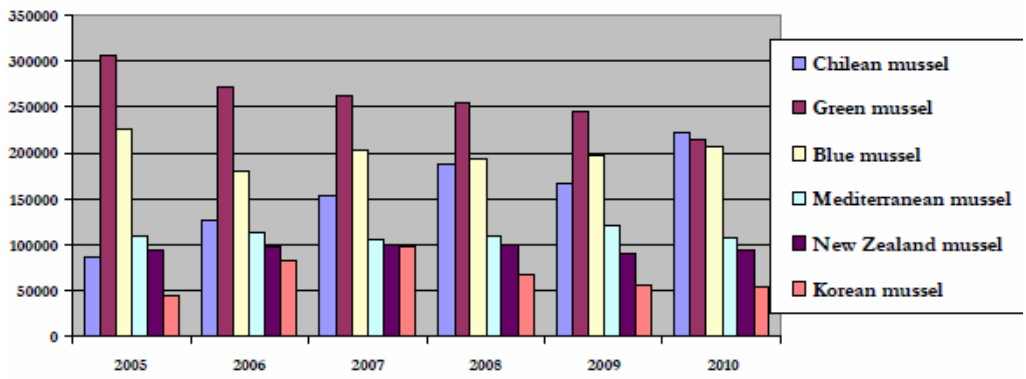

Figure 6 Mussels production by species in tonnes (source: Fishstat Plus, 2012)

The increase of bivalve mollusc production was driven by international demand since the early 1990s.

Total bivalve trade has expanded continuously during the past three decades to reach US$ 2.1 billion in 2009. In terms of quantity, scallops accounted for 24% of export, while mussels contributed to 48%, oyster 11%. In terms of value, scallops are the most important species with 46% of value, followed closely by mussels (26%) (fig 7).

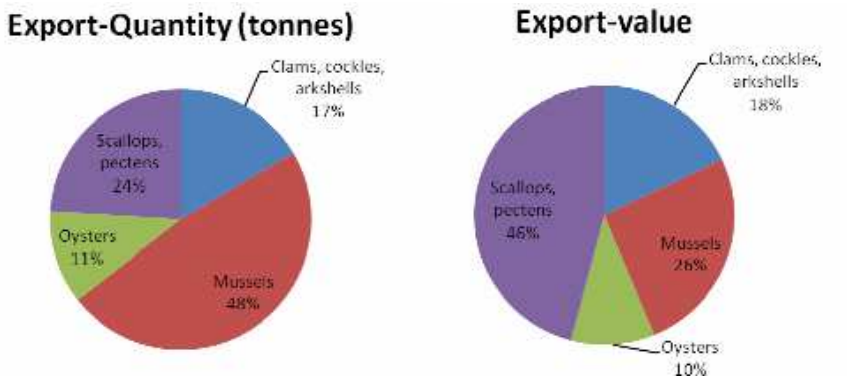

Figure 7 Bivalve mussel Export-Quantity (left) and Export-Value (right) 2009 (Source: Fishstat Plus, 2012)

1.2.2 Italian production of bivalve molluscs

Italian mollusk production is about 100.000 tons that place the Country around 10th position among the world bivalve producers, preceded in Europe only by Spain and France.

The national shellfish production is exclusively based on aquaculture and is related to two main species of bivalve: mussel (*Mytilus galloprovincialis*) and clam (*Ruditapes philippinarum*). Followed by timid attempts to reintroduce oyster culture for the species: *Crassostrea gigas* and *Ostrea edulis*.

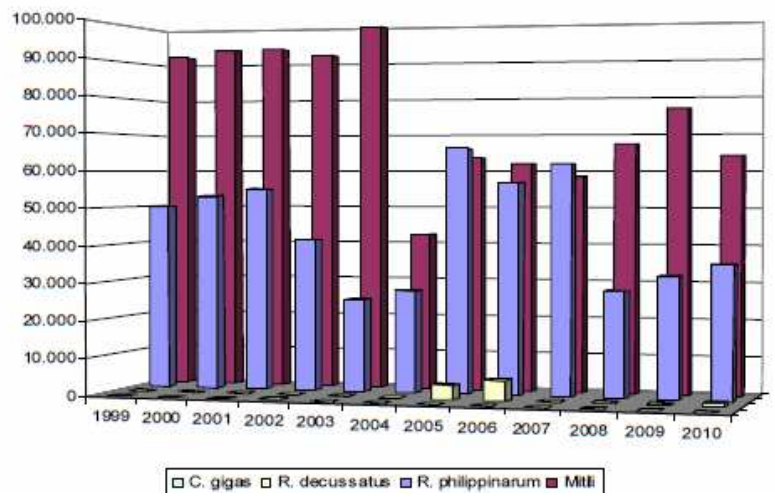

Figure 8 Italian production (tonnes) of bivalve molluscs (Source: Fishstat Plus, 2012)

Mussels culture, although diffused along the Italian coast, has production centers located in geographic areas along the Adriatic coast, in the Gulf of Taranto, in the Lake Ganzirri in Sicily, along the coast Flegreo, in the Gulf of Olbia and other areas of the lagoon placed along the Sardinian coast and in the Gulf of La Spezia. Farming clams is instead confined to three principal production areas: Po delta river, the lagoon of Venice and the lagoon of Grado and Marano while the production of oysters are present in Sardinia and Tuscany.

According to a recognition carried out in 2010, 332 companies resulted involved in the shellfish industry, with about 472 active plants with a total production of around 103,700 tonnes, of which 67.133t of mussels, clams 36,490 t, 78 t of oysters (Prioli, 2008; 2012).

Although the quantities of national production of mussels is not able to satisfy demand also in relation to seasonality of the offer that characterizes the national product, in 2010, the amount of mussels imported in Italy, was about 48,000 t (source Globefish, 2012) represented mainly by live mussels fresh or chilled (fig.9).

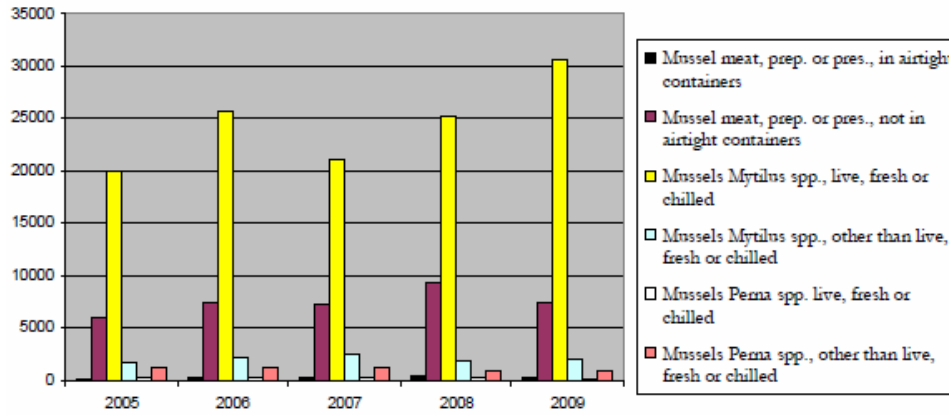

Figure 9 Italian mussels importation in tonnes (Source, FAO- Fishstat plus, 2012)

1.3 Mussels cultured: longline system

For rearing mussels three main systems are used, in relation to the characteristics of rearing sites: fixed poles systems (fig. 10), or the suspended ones; single long-line (fig. 11) or multiple long-line superficial or submerged (fig.12).
This latter technology evolved directly by the scallops and oyster culture from Japan. The system consists of a series of floats connected together by horizontal lines supporting a large number of vertical ropes or mesh stockings on or in which the mussels are grown (Hickman, 1992).
Each module is constituted by a rope called rafter or "*Ventia*" which made of polypropylene, polyester or polyamide, of 100-300 meters total length, at whose two ends is coupled a sinker (concrete, stone or metal) which has anchoring function.
The rope has the function of supporting the vertical container of mussels that descend in water perpendicularly.
Each vertical substrate consists of a polypropylene reticulated envelope between 2 to 4 meters length, with a mesh size appropriate to mussels inserted. Vertical container are hunged up to the ropes and spaced from each by approximately 50 centimeters (Gosling, 2003; Rossi *et al.*, 2001).
The entire module maintains the hydrostatic trim thanks to a series of special polyethylene buoys, positioned on rope and the number varies according to the product weight. Thirteen or fourteen vertical substrate are hung between each flotation drum, other buoys are submerged and positioned close to the anchors.
The minimum distance between two parallel modules is about 20 meters, long-lines are generally cheaper, easier to construct and maintain, and give slightly higher mussel growth rayes than equivalent raft systems. Variation on the single or double or triple long-line system have been developed (Hickman, 1992).

Figure 10 Fixed poled system (photo by G. Prioli)

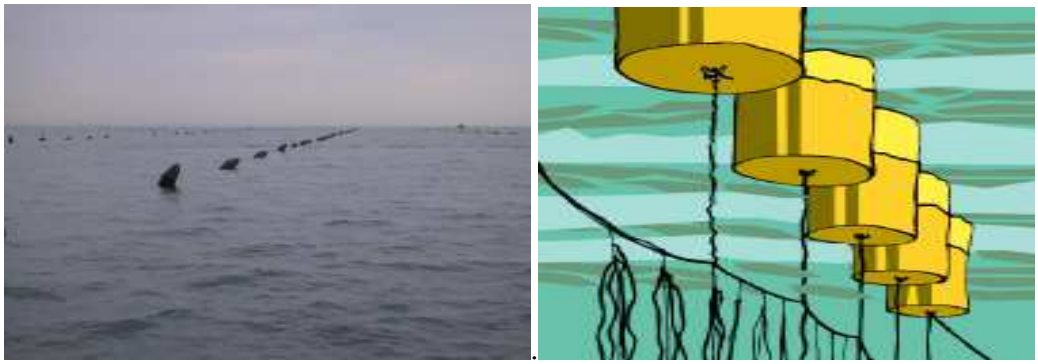

Figure 11 Single long-line system (photo by G. Prioli)

Figure 12 Double longline system (photo by G. Prioli)

1.4 Production mussels chain

Mussels production is characterized by the following steps (fig. 20 and 21) detailed below.

1.4.1 Collection and preparation of the seed

After fertilization, the planctonic larvae develop into the sea and are attached by the byssus to hard substrates specially placed (poles, ropes). The seed, very small (2-3 cm) is recovered between April and July on specific structures that generally are part of the farm (ropes) or collected from other substrates such as reefs wooden poles etc. The separation of the seed from the substrate is performed manually using scrapers. Also Mussels that resulted under size during final collection are used as seed.

1.4.2 Preparation of mesh stockings with the seed

This operation consists in inserting the seed, previously collected, in polypropylene bags with a mesh diameter appropriate to the size of the inserted

Figure 13 Collection of mussel seed on rope and mesh stocking preparation (photo by G. Prioli and ISPESL, 2004)

seed. The operation is performed manually, and usually in ground systems.
To facilitate insertion of the seed in the mesh stocking, a mechanical filling machines (hoppers) or plastic tubes with appropriate diameter can be used (fig. 13).

1.4.3 Placement of vertical container

To place the seed at the plant in the open sea, the vertical container (or mesh stockings) are transported with a motorboat and, with a special equipment, the rope is lifted from the water and recovered onboard (fig. 14). Mesh stockings, with the seed are then hooked manually (knotting). To allow the waterline in the rope, with the mesh stockings hung, are placed the flotation system.

Figure 14 Placement of vertical substrate with seed (source ISPESL, 2004)

1.4.4 Breakdown of mussels in new mesh stockings

To prevent the detachment of vertical substrate, due to mussels growth and weight gain, after 2 to 3 months from the first dive, the mesh stockings with mussels are collected and mussels are harvested mechanically or manually and divided into aliquots that are placed in new propylene bags (mesh stockings) with

larger mesh (fig.15). Generally this process is carried out one or two times during the entire production cycle (8-12 months).

1.4.5 Cleaning of mesh stockings

This operation consists in fouling removal of from the mesh stockings. The accumulation of fouling is caused by the microrganisms colonization with deposition of minerals. An excess causes a reduction in the mussels growth and in severe cases, anoxia and phenomena of detachment of mussels from the substrate and/or mortality. The fouling also causes a considerable increase of weight with increasing risks of detachment of vertical substrate from the ropes. To perform the cleaning operation, the vertical substrate are extracted from the water, placed on the boat and washed with jets of water under pressure and repositioned on the beam. During the production cycle, the mesh stockings are washed several times in relation to the amount of fouling present according to season.


Figure 15 Manual separation of mussels (left) and hilling and repositioning of mesh stockings (right) (Source ISPESL 2004)

1.4.6 Final collection of the mussels

Final collection of the product takes place using a motorboat which proceeds parallel to the rope and with a special equipment, the mesh stockings are extracted from the water, collected and stored on the motorboat (fig.16).

Figure 16 Collection of the product (photo by G. Prioli)

1.4.7 Selection and processing product

After collection the mesh stocking with mussels are subjected to processing in the boat during navigation and / or in plant (fig. 17). The separation of mussels occurs with a special machine that detaches the mussels from the stocking. Separated mussels, are subjected to selection size through a vibrating screen. Mussels under commercial size are used for filling additional mesh stockings that are hung on the rope at sea.

The product is screened and if coming from waters classified as Type A according to EU Rg 854/04, is packaged in bags by weight variable between 3 and 20 kg and cold storage at 3°C. When mussels come from waters classified as type B, they are placed in containers and sent, under refrigeration condition, to the depuration center (CDM) as stated in EU reg 853/04.

1.4.8 Depuration

The depuration is the process of microbiological remediation that consists in the placement of the product in containers immersed in filtered and sterilized water and the period is determined according to the purification capacity plant and the microbiological quality of the product.
In so-called vertical purification, the product is placed in large containers which are stacked vertically perforated and subjected to jets of water from above. The depuration called "horizontal" is realized by placing the containers in rectangular tanks where water flows for the depuration (fig. 18).

Figure 17 Ginning and selection of mussels after harvest (source ISPESL, 2004)

The depuration of mussels must be performed in centers approved by the Ministry of Health to guarantee that the mussels have to find the optimal conditions to restore their nutrition quickly and maintain vitality. During depuration clean sea or treated water must be used. Moreover, the process must ensure that different batches of molluscs are not placed in same tanks at the same time. Purification centers must ensure compliance with good manufacturing practices and provide analytical checks on the characteristics of the shellfish sanitation in a internal or external laboratory, approved by the Ministry of Health. Limitations of depuration are:

- Viruses are removed slowly. About one third of viral levels may remain after 2 days at 8°C. At higher temperatures, eg 18-21°C, though most viruses are removed in 5-7 days, some residual viral contamination persists even when moderately contaminated shellfish are depurated.
- Depuration is ineffective to remove pathogenic *Vibrio* spp, eg. *Vibrio parahaemolyticus, Vibrio vulnificus*.
- Depuration is not considered viable option for removal of biotoxins, heavy metals and chemical contaminants.
-

Figure 18 Vertical (left) and horizontal (righ) purification center (CDM)

1.4.9 Packaging and shipment

Product is packed in labelled plastic mesh bags of a variable weight between 1 and 20 kg. Containers with packs of mussels are shipped within a few hours by a dispatch centre (MSC) using refrigerated trucks (fig.19). Bivalve mollusc can be commercialized until alive (Reg. EC 853/2004) .

1.4.10 Commercialization

After the shipment, the product will follow three main routes to market:

- restaurants;
- wholesalers - distribution (GDO or traditional) or traditional retailers;

- fish markets follows traditional retails and / or restaurants (ISPESL 2004).

Figure 19 Packaging and packed of Mediterranean mussels (source FAO)

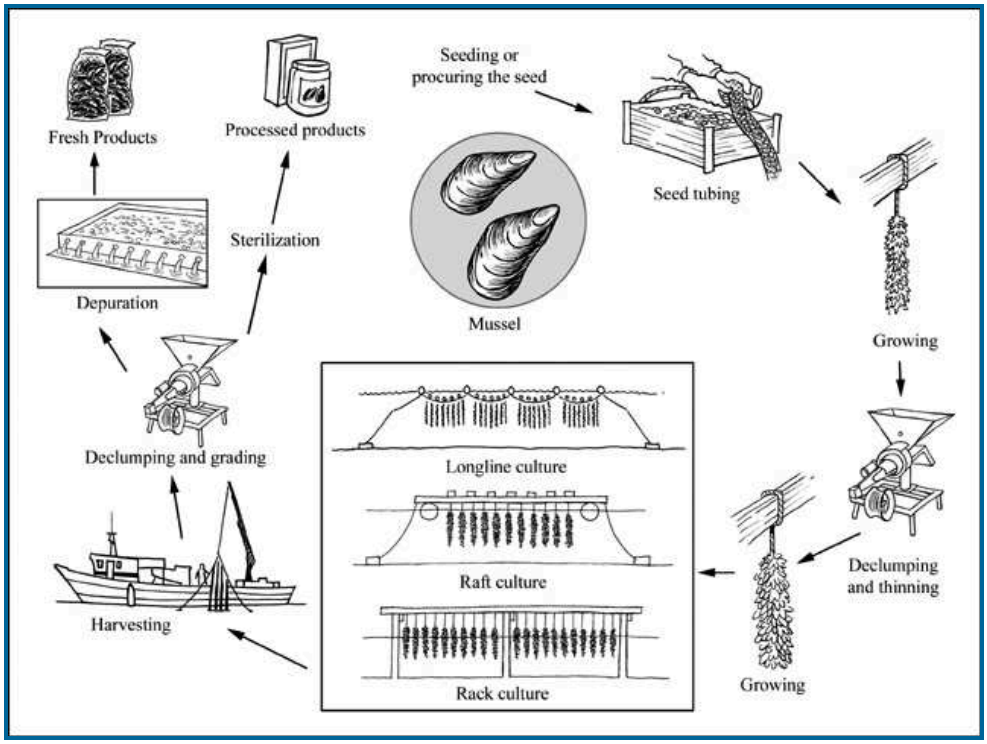

Figure 20 Graphic representation of mussels production chain (source FAO)

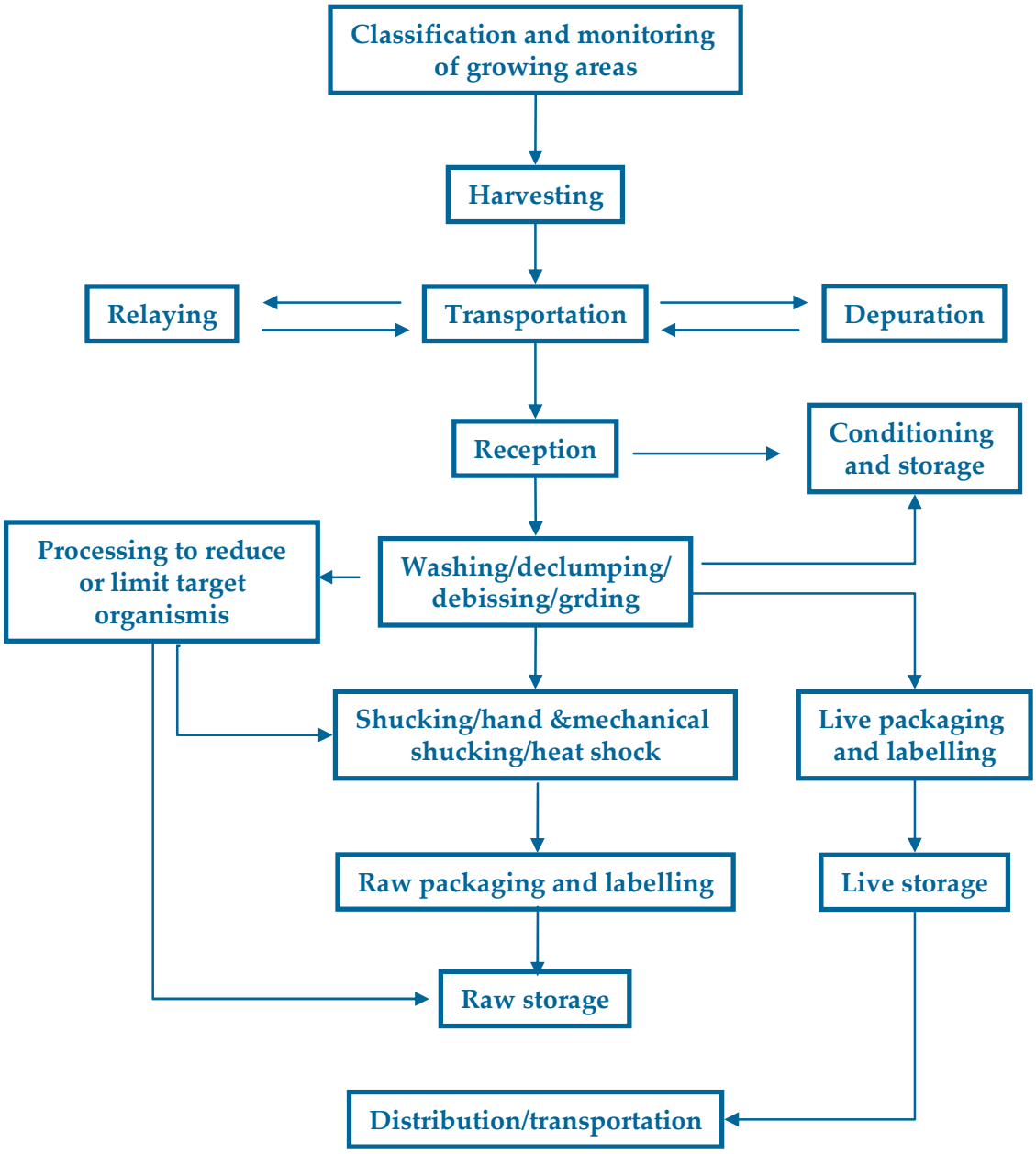

Figure 21 Flow Diagram of mussels chain (source FAO 2012)

1.5 Aspects of food safety

1.5.1 Bivalve mollucs hazards

There have been 52 rapid alerts for bivalve mollusks in the EU Rapid Alert System for Foods and Feeds (RASFF) during 2009, 78 in 2010 and 68 in 2011. The major causes were related to hygiene (*Escherichia coli* exceeding limits), biotoxins, enteric viral pathogens (Norovirus), enteric bacterial pathogen (Salmonella *spp*), chemical contaminants and other causes (labelling, organoleptic etc) (Croci 2012, Karunasagar 2012).

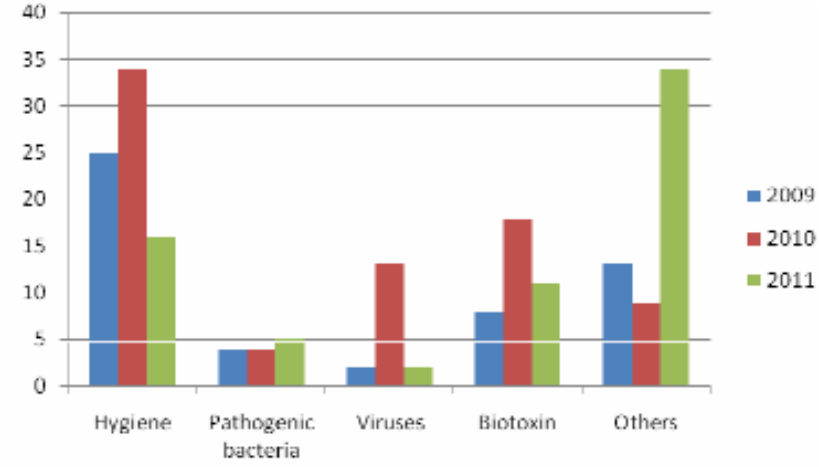

Figure 22 Causes of non-compliance in trade-EU

The safety record of bivalve molluscs that are managed by sanitary surveys has been fairly good. According to database of the US Center for Science in Public Interest (CSPI), during the decade of 1999 -2008, there were 792 seafood associated outbreaks involving 6337 cases. Of these, 118 outbreaks involving 1444 cases were due to bivalve molluscs. The causes of illnesses range from bacteria (*V. parahaemolyticus*) to viruses (norovirus), biotoxins.
In the EU, during 2009, out of 977 food borne outbreaks for which a cause could be verified, 35 (3.6%) were linked to shellfish including crustaceans and molluscs.

1.5.2 Process to limit microbiological hazard

Heat Treatment: Heat treatments of bivalve molluscs should be validated for their ability to inactivate viruses. An internal temperature of 85 to 90 °C for at least 90 seconds is considered to be a virucidal treatment.
However, this degree of cooking would probably render specific bivalve molluscs, such as oysters, unpalatable to consumers. Even though cooking temperatures typically used by consumers may not achieve 90 °C for at least 90 seconds and thus ensure inactivation of viruses, any cooking would reduce viral levels and depending on the initial level of contamination possibly would reduce the risk of causing foodborne infection.
High Hydrostatic Pressure (HHP): A technology applicable for microbiological stabilization is represented by high hydrostatic pressures which involves placing the food in sealed flexible containers (trays, dishes) at pressures of 500-600 MPa for several minutes. The high pressure processing, is ultimately taking in importance in the fisheries sector and already led to several industrial facilities in Europe, but especially in the U.S.A. The industrial presses operate at pressures less than 700 Mpa.
The HHP conditions for inactivation depend upon pressure as well as time, temperature and the salinity of the water. HHP may reduce virus titers in bivalve molluscs with relatively small effects on the character of the meat. e.g. a pressure of 600 MPa applied at 6 ºC for five minutes can completely inactivate NoV in oysters. The treatment at 205-275 MPa (tipical process) and 10-30°C for a time 1-3 minutes do not allows 5 log reduction of *V. parahemoliticus* as recommended from Interstate Shellfish Sanitation Conference. To achieve this is required a pressure treatment higher than 350 MPa at 1- 35°C for 2 minutes, or higher than 300 MPa at 40°C for 2 minutes. Such treatment also allows 5 log reduction of V. *vulnificus.* The duration of treatment has to be more than 2 minutes to obtain 5 log reduction at lower pressures (Kural and Chen 2008; Kural *et al.*, 2008) but affect the composition of oysters and cause the shucking and changes of colour increase as pressure increases. Pressures higher than 300 MPa accelerate the protein denaturation and the shelf life achieved is 27 days at t < 4°C. Pressures higher

than 300 MPa provide a product completely detached from the shell (HE. *et al*., 2002). Use of HHP alone or in combination with other inactivation procedures should be validated for the virus of concern in the specific bivalve mollusc species prior to its application.

1.5.3 Legislation for mussels commercialization

The mussels are non-selective filter that tend to accumulate substances that can make it unfit for human consumption, so chemical, physical, microbiological and biological mussel quality depends primarily on the rearing water quality. According to the classification criteria defined by Regulation (EC) 854/2004, after the collection, mussels fate depends of the 'area of origin can be of type A, B or C.
Zone A: water in which shellfish can be collected and used for human consumption. The molluscs collected in these areas must satisfy the health requirements: contain less than 300 faecal coliforms or less than 230 *E. coli* per 100g of flesh and intravalvular liquid, not contain salmonella in 25 g of flesh and do not contain toxic or harmful naturally present in the environment or in an amount such that the intake through food does not exceed the acceptable daily intake for humans. The legislation also provides that the maximum radioactive nuclides in mussels does not exceeding the limits fixed by the European Union.
They must not contain total quantities of marine biotoxins (Reg (CE) 853/2004) (measured in the whole body or any part edible separately) exceed the following limits:
(a) paralytic shellfish poison (PSP), 800 micrograms per kilogram;
(b) amnesic shellfish poison (ASP), 20 milligrams of domoic acid per kilogram;
(c) okadaic acid, dinophysistoxins and pectenotoxins together, 160 micrograms of okadaic acid equivalents per kilogram;
(d) yessotoxins, 1 milligram of yessotoxin equivalent per kilogram;
(e) azaspiracids, 160 micrograms of azaspiracid equivalents per kilogram.
Zone B. Mussels come from waters classified B may be intended for direct human consumption only after treatment in a depuration center or after relaying in an area with microbiological, biological, chemical and physical previously prescribed

for the area A. The molluscs collected in areas type B should not exceed 6000 levels of fecal coliform or E. 4600 *E.coli* per 100g of flesh and intravalvular liquid in 90% of samples analyzed.

Zone C. The mussels come from waters classified C may be intended for direct human consumption only after treatment in a purification center or after relaying in an area with microbiological, biological, chemical and physical previously prescribed for the area A. The molluscs collected in areas type C should not exceed 60000 levels of fecal coliform

Regarding the accumulation of heavy metals is referred to Regulation (EC) 221/2002 setting maximum levels of heavy metals in fish and shellfish, particularly by establishing the limits for lead, cadmium and mercury.

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2. NUTRITIONAL AND MERCHANTABLE TRAITS OF *M. GALLOPROVINCIALIS* REARED IN (SUSPENDED) LONG-LINE CULTURE IN THE GULF OF TRIESTE

2.1 Introduction

Mussels are important to marine ecology, but also represent an important source of nutrients for human consumption. Bivalve molluscs provide high biological value proteins and essential vitamins and minerals as well as polyunsaturated fatty acids known to have beneficial effects on the human organism (Fuentes *et al.,* 2009). Mussels are also very appreciated by the consumers for their sensorial characteristics and their competitive price compared with other bivalves (Orban, 2002).

An European study (Seafood Plus) regarding the reduction of health problems, the prevention of major diseases and the increase of well being among European consumers through the promotion of health issues and the consumption of high quality seafood products, shows that European consumers are very sensitive to intrinsic and nutritional product properties, the simplicity of its preparation and its commercial availability (Børresen *et al.,* 2008; Morrissey, 2008)

However recent changes in consumer's habits suggest the need for processed products with longer shelf-life and friendly/ easier preparation also for traditional seafood like mussels so seafood processing industries are implementing the range of products including ready to eat and ready to cook mussels. But to ensure product retains the optimal characteristics during storage time, the raw material used must meet high quality requirements.

Several factors, quality and temperature of water, nutrient availability and reproductive cycle can affect the overall quality of mussels in terms of microbiological, nutritional, technological and sensory quality. According to season the physiological condition and the reproductive cycle significantly modifies mussel's characteristics (Okumuş and Stirling, 1998; Orban *et al.,* 2002; Vernocchi *et al.,* 2007; Parisi *et al.,* 2005) thus possibly affecting the aptitude of mussels to processing.

2.2 Main goals of the research

The aim of this trial was to describe the seasonal variations of biometric and physico-chemical parameters related to quality of suspendend–cultivated *Mytilus galloprovincialis* in the Gulf of Trieste (North Adriatic Sea) and define the period in which the mussels, in relation to their physiological condition, are more suitable for processing.

2.3 Materials and methods

2.3.1 Animals and environmental conditions

Commercial-size mussels (individual weight: 17.0±2.63g; length: 60.6±6.06mm), cultured in an coastal longline system in the Gulf of Trieste, were monthly collected and used as raw material between December 2009 and November 2010. During the sampling period the water temperature fluctuated between a minimum of 7.4°C in February to a maximum of 22.4°C in July while dissolved oxygen (DO) and water salinity ranged from 87 to 116 mg/L and 7.14 to 38.3 g/L, respectively (fig.1). The samples were immediately transported to the laboratory of the University of Udine under refrigerated conditions (3±1°C). Mussels were brushed, washed and processed in the same day.

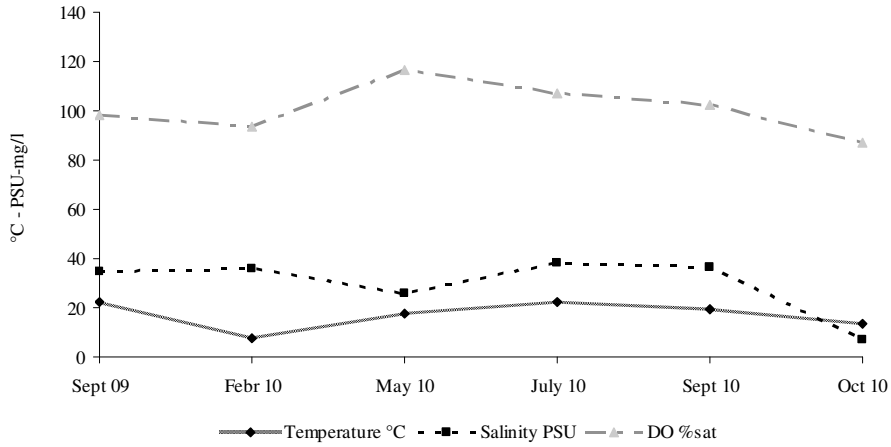

Figure 1 Water temperature (°C), salinity (psu) and dissolved oxygen(% sat) in the mussels rearing area during the experimental period

2.3.2 Biometric parameters

Shell hardness, Condition Index and Meat yield

Animal length (maximum anterior-posterior axis), width (maximum lateral axis) and height (maximum dorso-ventral axis) were measured on 50 randomly mussels, each month (table 2), using a 0.05 mm precision calliper (Storm 0-25mm inox).

Thickness was detected at the margin (TM) and at the point of maximum curvature (TC) of the right and left valves using a micrometer (Maurer 1/20 cm inox).

Shell hardness was measured on the right and left valves of each specimen by the stress rupture test operated by INSTRON 4301 according Parisi *et al*. (2008). The load of 1 kN and a speed of 50 mm/min was applied to maximum shell height point.

After the determination of total weight, meat was spun off from its shell, and both were weighed before and after driing (105°C) using a precision analytical balance (± 1 mg). Condition Index (CI) was calculated according to Orban *et al.* (2002) as follows:

$$CI_{dry}=[\text{ meat dry weight (g) / shell dry weight (g)}] \times 100$$

Meat yield (MY) was calculated according Okumuş and Stirling (1998) as follows:

$$MY_{wet}=[\text{Wet Meat Weight(g)/ live Weight (g)}] \times 100$$

Cooked meat yield was also calculated according to Parisi *et al.* (2008) and Vernocchi *et al.* (2007), after 7 minutes of boiling and 5 minutes of cooling, as follows:

$$MY_{cook}=[\text{Meat Weight after cooking (g)/ live Weight (g)}] \times 100$$

2.3.3 Water holding capacity

Water holding Capacity (WHC) was determined on six pooled samples per month (table 2) from further randomly chosen specimens (about 30 individuals) after byssus cutting, by means of a scalpel and gently removal of the soft part. Mussels pools (15g) were centrifuged at 210 x *g* for 15 minutes at 5°C according the method proposed by Olsson *et al.* (2003) and adapted to mussels. The WHC was determined as liquid loss (LL) and expressed as a percentage of liquid released (Ofstad *et al.*, 1993) as follows:

WHC (%) =[liquid released (g) / total weight (g)] x100.

2.3.4 Chemical analysis

Protein, ash and mineral content

Each month 3 pools of 60 g of mussels edible portion were sampled (table 2), homogenates and freeze dried.

Moisture, protein and ash contents were determined on a aliquot of freeze dried pooled samples according to AOAC (1997): dry matter (DM) (method 950.46), crude protein as Kjeldahl N *6·25 after acid digestion (method 928.08) and ash content (method 920.153).

Mineral content was determined after samples digestion with nitric acid suprapur (65%) and hydrogen peroxide suprapur (90%) in high performance microwave digestion unit (MLS 1200 MEGA) that increased from 200 to 700 watt in 20 minutes. There the samples were carried to a volume of 100 mL with deionized water mQ. Macroelements (Na, K, P, Ca) were analyzed using kit spectroquant (MERCK) mesured by spectrophotometer (VARIAN CARY 50BIO) and quantified by software Cary Win UV. Microelements (Cr, Mn, Ni, Mg, Fe, Cu, Zn, Se) were quantified by inductively coupled plasma mass (ICP-MS). The spectrometric measurements were perfomed by a Spectromass 2000 Type MSDIA10B (Spectro Analytical Instruments, D) equipped with a standard torch and High Matrix

Content (HMC) Ni sampler. Pure argon was used for all determinations and the operating condition are given in table 1.

Table 1 Operating conditions of ICP-MS

Plasma conditions		**Ion Optic Ensemble**	
Frequency	27.12MHz	Extraction lens (LO)	(-460)-(-500) V
Rf power	1200-1350 W	Optic lens (LA)	-60 V
Auxiliary gas flow	1.2 L/min	Photon stop lens (LB)	0 V
Coolant gas flow	18 L/min	Optic lens C (LC)	-80V
Sampler	Nichel (HMC) 1.0 mm diameter orifice	Quadrupole entrance lens (LD)	(-60)-(-70) V
Skimmer	Nichel (HMC) 0.8 mm diameter orifice	Field axis (FA)	0-9.0 V
		Deflexion in	-500 V
		Deflexion out	-200 V
Vacuum parameters		**Mass Spectrometer settings**	
Interface	2.04 mbar	Secondary electron multiplier	1900 V
Quadrupole	8.9 10-6 mbar	Peak resolution	0.75 a.m.u.
Turbo voltage	10 V	Pause calibration factor	3.0

Lipid content and fatty acids analysis

Each monts 3 pools of 120 g of mussels edible portion were sampled and homogenates (table 2). An aliquote for each pooled mussel samples were extracted by homogenization in chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) according to Folch *et al.* (1957).

A 5% solution of HCl in methanol was used to prepare fatty acid methyl esters (FAMEs) according to Christie (2003). FAMEs were separated and quantified by gas chromatography (TRACE GC2000, Fisher Scientific SAS - F67403 Illkirch

Cedex – France) using a 30 m×0.32mm i.d. Omegawax™ Capillary GC Column (Supelco Bellefonte, PA, USA) using a flame ionization detection at 250°C. Carrier gas (H_2) flow rate was 1 mL/min and samples (1 mL) were directly injected in a split ratio of 1:30. Oven temperature was programmed from 180 °C for 6 min, then at 3°C/min till 225°C and held for 10 min. The quantification of FA of mussel sample was perfomed using nonadecanoic acid (C19:0) as internal standard. The chromatograms were acquired and processed using ChromQuest integration software (Thermo Quest Italia, Rodano (MI), Italy) and individual methyl esters were identified by comparison to known standards (Ackman, 1980). Repeated injections of standard solutions were carried out to test the analytical precision. The relative standard deviations were less than 5% for all the FA, both considering the intradie precision, calculated on four repeated injections, and the interdie precision, evaluated over six days. All solvents and reagents were of analytical grade and were purchased from Sigma-Aldrich (Milan, Italy).

Amino acids analysis (AA)

Amino acids analysis was performed using a LC 200 Perkin Elmer pump fitted with an ISS-100 auto sampler (20 μL loop) and a fluorimetric detector (Perkin Elmer, Norwalk, Connecticut, USA), λ excitation 250 nm and λ emission 395 nm. Separation was achieved by using one AccQ.Tag Amino Acid Analysis column (Waters Corporation Milford, MA, USA) and one Waters pre-column filter. The column was thermostatted at 37°C and the flow rate was 0.8 mL/min (Liu et al., 1995). Mobile phase A consisted of acetate-phosphate aqueous buffer, mobile phase B was acetonitrile 100% and C was UHQ water.

Acid hydrolysis with HCl 6 M at 110°C for 22-24h was used for all amino acids (but under these conditions cysteine (Cys), methionine (Met) and tryptophane (Trp) have been destroyed). After addition of borate buffer, filtered hydrolysated samples were derivatized at 55°C for 10 min with 20 μl of AccQ.Fluor reagent (6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate) and injected in HPLC (Bosch, *et al.*, 2006).

Table 2 Sampling

Analisys	Mussels/pools (*n*)	Sampling (months) (*n*)
Length, height, width, TM, TC and shell hardness	50 (both left and right side)	11
Shell incidence, Intervalvar Fluid, MYwet CIdry	50	
MYcook	50	11
WHC	6 pools	11
Protein, ash and mineral content, amino acids analysis (AA)	3 pools of 60g of edible portion	11
Lipid content and fatty acids analysis	3 pools of 120g of edible portion	11

2.3.5 Statistics

Results are presented as means ± standard deviations. Data were subjected to one-way ANOVA to test significant seasonal differences for each parameter (SPSS-PC Release 17.0, SPSS inc., IL, USA) and if appropriate statistical differences were subjected to Tukey's test ($P<0.05$). Non linear regression analyis was applied to Water Holding Capacity data.

2.4 Results and discussion

2.4.1 Biometric parameters and merchantable traits

The biometric characteristics of the mussels sampled throughout the experimental period did not exhibit differences in terms of total weight, length, width and height (Weight: 17.0±2.63 g; Length: 60.6±6.06mm; Height: 21.4±1.31 mm; Width: 30.6±2.34mm) (table 2).

Shell thickness at maximum curvature (TC) and shell margin (TM) are presented in Table 3 and fig. 2A coupled with rupture resistance. No evident variation in

shell thickness due to season were observed. Instead shell hardness exhibited significant variation (<P0.005) among the year with minimal values in March (59.2±36.6 N/cm²) and maximum value in June (133.0±65.1 N/cm²).

Montly variation in Condition Index are reported in fig 3, its value varied significantly according to season (P< 0.05) and showed very similar patterns according to food availability and physiological status of the animals as evidenced by Okumus and Stirling (1998). The lowest values occurring during winter months (January 7.4±1.9) and maximum values from late spring (July 18.19±2.9). Similarly MYwet and MYcook (table 4), ranged, respectively, from and 17.9±4.8 % and 12.5±2.7% in January to 32.7±1.2% and 26.3 ±4.3% in May.

Table 3 Biometric measurement, shell hardness of the mussels in the different months harvested in the Gulf of Trieste

	Lenght (mm)	Width (mm)	Height (mm)	TM (mm)	TC (mm)	Shell hardness (N/cm²)
Dec	61.6±6.6	32.5±2.4	22.4±2.2	0.69±0.1^{a}	1.02±0.1^{bc}	108.0±51.1^{b}
Jan	70.4±7.3	32.0±3.3	22.3±3.2	0.66±0.1^{ab}	0.99±0.2^{a}	69.8±35.6^{ef}
Feb	64.8±5.5	31.9±2.2	23.0±3.6	0.58±0.1^{de}	0.95±0.2^{ab}	83.0±38.1^{cd}
Mar	56.3±4.0	28.0±2.3	19.4±1.9	0.69±0.1^{a}	0.90±0.1^{cd}	59.2±36.6^{f}
Apr	64.2±6.0	32.6±5.4	21.8±3.3	0.56±0.0^{e}	0.76±0.1^{e}	73.2±45.9^{de}
May	62.7±7.1	33.1±5.6	23.02±2.8	0.59±0.1^{de}	0.80±0.1^{e}	94.1±65.9^{c}
Jun	62.4±4.2	31.2±2.8	21.9±1.6	0.59±0.1^{e}	0.77±0.1^{e}	133.0±65.1^{a}
Jul	59.6±4.1	28.2±2.5	20.4±2.1	0.64±0.1^{bc}	0.87±0.1^{d}	89.7±43.9^{c}
Sep	58.1±2.9	28.0±1.9	20.2±2.4	0.56±0.0^{e}	0.78±0.0^{e}	88.3±42.2^{cd}
Oct	59.4±3.7	32.7±2.9	21.2±2.4	0.62±0.1^{cd}	0.79±0.1^{e}	82.3±39.4^{cde}
Nov	57.3±6.4	27.0±2.3	19.5±1.1	0.48±0.0^{f}	0.77±0.1^{e}	59.9±25.4^{f}

Data are reported as average ± standard deviation of 50 individuals (right and left valves measurements).

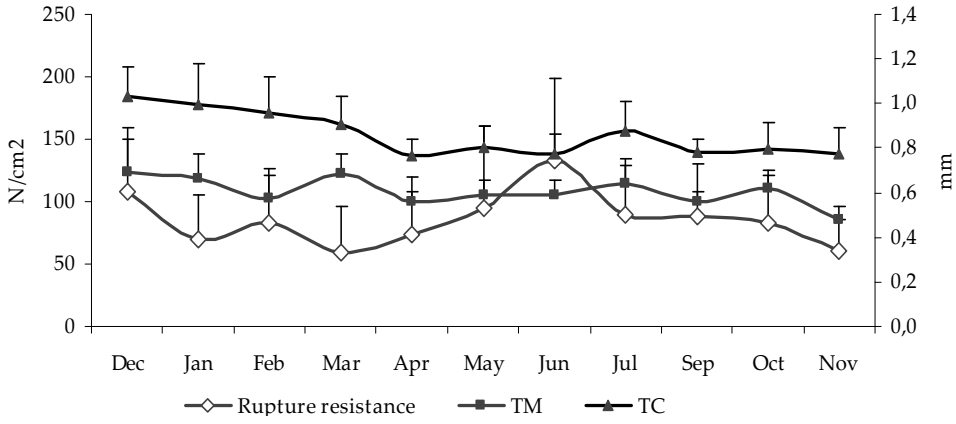

Figure 2 Seasonal change of shell characteristics thickness at margin (TM) and at maximum curvature point (TC) and rupture resistence of shell mussels reared in the Gulf of Trieste. Data are reported as average ± standard deviation of 50 individuals (right and left valves)

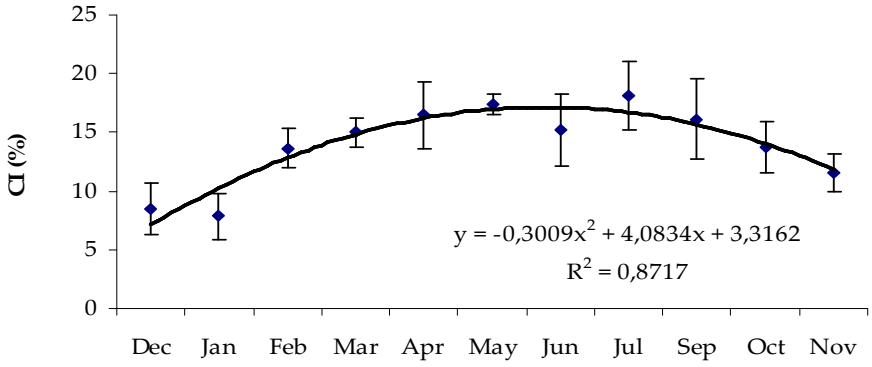

Figure 3 Seasonal change of Condition Index CI of mussels reared in the Gulf of Trieste. Data are reported as average ± standard deviation of 50 individuals

Consequently shell incidence (table 4) ranged from 32.3% (March) and 39.9% (December) and the highest percentage of intervalval fluid occurred during the winter period (44.6% in Jan).

As well known the meat yield in mussels are affected by a variety of factors, such as water temperature and salinity, food availability, good state of health of mussels and gamethogenic cycle of animals that may influence the meat content and biochemical composition (Fernandez-Reiriz, Labarta & Babarro, 1996; Okumuş and Stirling, 1998; Orban et al., 2002; Parisi et al., 2008). This parameter, calculated on wet basis, for the mussels cultivated in the Gulf of Trieste (North Adriatic Sea) resulted during the summer months (32.7%) comparable to the values reported by Fuentes *et al.* (2009) for mussels cultivated in Galicia (31%) and Valencia (34%) in the same period.

Table 4 Seasonal change of merchantable traits, meat yield (wet and cooked basis), Liquid loss in mussels reared in the Gulf of Trieste

	Shell Incidence (%)	Intervalvar Fluid (%)	MYwet (%)	MYcook (%)	Liquid loss (%)
Dec	39.9±10.7^{a}	39.9±14.3^{bcd}	19.6±3.8^{d}	14.4±3.2^{e}	12.4±2.4^{c}
Jan	37.4±7.8^{abc}	44.6±12.2^{ab}	17.9±4.8^{d}	12.5±2.7^{f}	15.0±1.9^{b}
Feb	34.6±6.9^{bcd}	39.8±11.5^{bcd}	25.7±4.9^{b}	22.1±4.1^{a}	17.0±1.5^{f}
Mar	32.3±3.9^{d}	40.5±5.9^{bcd}	27.1±1.4^{d}	21.1±1.8^{f}	19.6±2.2^{a}
Apr	38.2±8.2^{ab}	30.0±13.6^{de}	31.4±5.7^{a}	20.1±4.2^{b}	13.4±3.4^{de}
May	35.0±7.8^{bcd}	32.3±15.6^{de}	32.7±1.2^{a}	26.3±4.7^{a}	9.9±1.1^{d}
Jun	36.3±5.7^{abc}	36.2±10.2^{de}	27.5±4.8^{b}	18.3±3.9^{c}	9.8±2.5^{d}
Jul	33.4±4.0^{cd}	40.8±7.1^{bcd}	25.6±3.2^{b}	23.2±4.3^{a}	5.2±0.3^{f}
Sep	35.8±5.0^{bcd}	37.9±8.9^{cde}	26.3±4.4^{b}	18.1±3.6^{c}	5.5±0.8^{f}
Oct	37.5±6.1^{abc}	36.4±9.4^{de}	25.9±3.5^{b}	16.7±3.2^{d}	6.3±1.2^{ef}
Nov	33.9±3.7^{cd}	43.7±6.6^{abc}	22.2±3.1^{c}	16.9±3.0^{c}	9.7±2.3^{d}
SEM	43.560	108.765	18.036	11.941	3921
d.f.	494	495	465	444	55

Data are reported as average ± standard deviation of 50 individuals, while liquid loss data represent average value of six mussel pools. Whitin each parameter, different letters in the columns indicate significant differences ($P<0.05$)

The meat yield calculated on cooked mussels (MYcook) was used to compare results with the ones reported by Vernocchi *et al.* (2007) for long-line off-shore mussels cultivated in the Adriatic Sea adjacent to Cattolica (Rimini, Italy).
High meat yield were observed from February to July (22.1-23.2%) and a maximum value of 26.3% in May, with a single decrease after the gametes emission in late June (18.3%) in the North Adriatic area, while Vernocchi *et al.* (2007) registered in their study area located under the influence of Po river delta maximum yield in January (25.2%) and lower yield (13.4-16.2%) during the summer months (June-July) in correspondence of the highest counts of *E. coli*.

2.4.2 Water Holding Capacity

The Water Holding Capacity is a very important parameter of the quality of seafood since it influences acting on the texture and softness on juiciness, generally in the fish WHC is evaluated considering the muscle tissue, in our case, for assessing the quality of the mussels, we considered the whole tissues and the analyzes were carried out considering the entire edible portion and expressed as liquid loss.
WHC ranged from a minimum value of 5.2±0.3 in July to a maximum value of 19.6±2.2 in March (Table 3). In fact the mantle tissue of the animal where the gonad follicules are located, during the seasons, is subjected to continuous variations of structure related to gametogenesis as described and reported by Thorarinsdòttir (1993) and Gosling (2003a).
During stage I (maturing recovering) the gonads contain a lot of free water, subsequently during stage II (maturing-filling) and III (maturing half-full), the free water, into the gonads decreases progressively until the stage IVA when gonads reach their maximum size and do not contain free water (Thorarinsdòtt ,1993 and Gosling, 2003a). Following the deposition (stage IVB), the tissue becoming dull flabby and containing much free water as well as in stage IVC (spent). In our study, liquid loss exibited the highest figures in March (19.6±2.2%) which correspond to the stage IVC ”spent”, then the WHC decreased progressively, reaching the lowest values in July (5.2±0.3%) and September

(5.5±0.8%), this period corresponding to the stages of matur-full IVA, the WHC values increased, probably, in relation to the beginning of the deposition (stage IVB).

2.4.3 Biochemical composition and mineral content

Moisture, crude protein, total lipid, ash and mineral content of the mussels harvested and collected in the different months in the Gulf of Trieste are shown in Table 5. Variations in water, protein and lipid content of the animals during the seasonal cycle reflected the food availability and the reproductive stage of the animals with alternation of phases of energy accumulation and depletion.

During the experimental period the water content of the mussel tissues showed fluctuations between 78.7-88.8% with minimum values during spring and summer months coinciding with the high values in Condition Index described before. Mussel tissue protein content ranged from 7.5 to 11.6 g/100g, with higher values from May to September. The total lipid content of the mussel tissues fluctuated from 1.0 g/100g in January to 2.2 g/100g in July without marked seasonal trend.

The concentration of the macroelements analysed were similar to those found in other studies on mussels (Fuentes *et al*., 2009; INRAN 2002); Na content ranged from 225.3±3.0 mg/100g (December) to 45.7±8.3 mg/100g (May); K content ranged from 80.9±1.1 mg/100g (February) to 25.0±16.6 mg/100g (January and November); P content ranged from 55.0±0,0 mg/100g (February) to 22.7±0.6 mg/100g (March); Ca content ranged from 11.5±0.7 mg/100g (April) to 2.6±0.6 mg/100g (February). Trace metal concentrations (Fe, Zn, Mn, Ni, Cu, Fe and Se) were similar to those detected in other Italian coastal zones (Locatelli, 2003), and indicate that the seafood under investigation poses no hazard to human health because metal content always resulted within the permissible range established for safe consumption by humans.

Seasonal changes in trace metal concentrations were observed with maximum values in later winter–early spring, followed by a progressive decrease during the

summer. In particular the maximum Fe, Zn, Mn, Ni, Cu, Co and Se concentrations in the mussel tissues were 1.9, 0.7, 0.11, 0.26, 0.076, 1.9, 0.075 mg/100g, respectively. They resulted much lower than the values reported for *M. galloprovincialis* collected in other coastal Mediterranean areas such as along the Moroccan coast (Maanan, 2008).

Table 5 Seasonal change in moisture, crude protein, total lipid, ash, (g/100g), and mineral content (mg/100g) of mussels reared in the Gulf of Trieste

	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Sep	Oct	Nov
Moisture	87.3 ±0.2	85.3±0.7	83.3±0.8	84.2±1.8	82.8±0.3	81.4±0.4	81.6±1.2	78.7±0.8	80.7±3	81.7±0.5	84.9±0.2
Protein	7.5±0.2	8.9±0.6	10.9±0.7	9.7±1.4	8.8±0.1	10.4±0.2	9.7±0.5	11.6±0.5	10.6±0.8	10.7±0.0	9.4±0.2
Total lipid	1.1±0.1	1.0±0.1	1.3±0.2	1.2±0.1	1.5±0.5	1.3±0.4	1.6±0.1	2.2±0.2	1.7±0.0	1.6±0.2	1.2±0.1
Ash	3.0 ±0.03	3.3±0.02	3.2±0.1	3.2±0.03	3.3±0.2	2.2±0.03	2.6±0.1	2.8±0.05	2.9±0.1	2.9±0.0	3.2±0.04
Minerals											
Na	225.3±3.0	139.8±95.4	156.3±28.9	177.0±52.4	151.3±11.1	45.7±8.3	75.6±4.6	187.3±77.8	210. 1±94.5	156.4±61.4	263. 3±164.1
K	25.9±9.7	25.0±16.6	80.9±1.1	42.0±22.3	37.8±20.9	42.7±9.5	39.0±23.8	39.2±8.0	49.4±17.6	42.4±0.7	25.0±14.4
P	26.1±0.0	26.6±5.5	55.0±0.0	22.7±0.6	32.3±0.8	37.5±0.3	33.7±2.0	37.1±5.9	37.4±0.5	39.3±0.7	36.8±7.2
Ca	4.8±1.6	6.8±1.2	2.6±0.6	3.4±0.6	11.5±0.7	4.0±1.3	3.9±1.7	3.1±1.3	3.3±2.6	5.5±2.2	4.7±1.2
Cr	0.039±0.01	0.022±0.004	0.024±0.005	0.025±0.01	0.033±0.01	0.032±0.005	0.024±0.004	0.028±0.04	0.019±0.03	0.018±0.002	0.021±0.01
Mn	0.079±0.01	0.11±0.003	0.076±0.01	0.041±0.01	0.06±0.003	0.054±0.007	0.049±0.004	0.061±0.01	0.087±0.002	0.084±0.005	0.087±0.01
Ni	0.14±0.01	0.18±0.01	0.12±0.03	0.13±0.01	0.26±0.05	0.26±0.09	0.13±0.02	0.17±0.06	0.13±0.00	0.13±0.01	0.14±0.02
Mg	14.2±0.1	14.5±0.1	14.3±0.2	14.5±0.0	14.3±0.1	9.3±0.1	12.3±0.0	13.8±0.7	15.2±0.1	14.1±0.3	15.0±0.2
Fe	1.5±0.5	1.9±0.3	1.6±0.4	0.9±0.1	1. 2±0.2	0.9±0.1	0.7±0.2	0.9±0.1	0.9±0.1	1.2±0.1	1.4±0.0
Cu	tr	0.041±0.001	0.076±0.001	tr	tr	tr	tr	tr	tr	0.046±0.001	0.045±0.001
Zn	0.64±0.05	0.62±0.02	0.45±0.07	0.56±0.02	0.42±0.05	0.66±0.08	0.54±0.03	0.61±0.03	0.70±0.20	0.70±0.01	0.62±0.01
Se	0.075±0.01	0.038±0.01	0.029±0.01	0.07±0.01	0.039±0.01	0.060±0.01	0.059±0.03	0.068±0.01	0.056±0.01	tr	0.058±0.02

Data are reported as average ± standard deviation of 3 pooled samplesfor proximate composition and for mineral content

2.4.4 Fatty acids content

The fatty acid profile of mussels (*M. galloprovincialis*) from the Gulf of Trieste studied monthly from December to November is reported in table 6.
Satured fatty acids, MUFA and PUFAs underwent statistically significant change during the period under study (P<0,05). Satured fatty acids (fig. 4A) ranged from 18.7 % (may) to 30% (July) and in according with many authors (Fuentes *et al*., 2009; Vernocchi *et al*., 2007; Alkanano *et al*.,2007; Otles & Sengor, 2005; Orban *et al*., 2002; Freites *et al*., 2002a 2002b; Karakoltsidis *et al*., 1995) predominat satured fatty acid was palmitic (C16:0) acid, its value ranging from 11.9% (April) to 17.9 % (September).
MUFA ranged from 14.5 % in November to 23 % in April and palmitoleic acid (C16:1n-7) was the most aboundant monoinsatured fatty acid and their value oscilled from 3.0% (October) to 11.2% (April). Similarly to offer data were found in the literature (Fuentes *et al*., 2009; Vernocchi et al., 2007; Alkanano *et al*., 2007; Otles & Sengor, 2005; Orban *et al*., 2002; Karakoltsidis *et al*., 1995) (fig. 4B).
PUFA ranged from 52.4% (April) to 60.2% (January) (fig. 5A) and eicosapentaenoic (C20:5n-3) and docosahexaenoic (C22:6n-3) acids resulted the most abundant polyunsatured fatty acids, their values ranging from 9.1% (June) to 16.5% (February) and from 19.0% (April) to 23.2% (June) respectively and similar values were reported in other studies (Fuentes *et al*., 2009, Vernocchi *et al*., 2007, Orban *et al*., 2002; Fernandez-Reiriz e*t al*., 1996.) The seasonal fluctuation in eicosapentaenoin C20:5n-3 and decosahexaenoic acid C22:6n-3 content may be related to the type of food ingested by the molluscs during the year and to their reproductive cycles.
High level in total n-3 (from 36.2% in September to 44.2% in Febrary) and low levels in total n-6 (from 3.5% in April to 7.4% in November) were detected in all samples, high n-3/n-6 ratio values (5.2-10.7) characterised mussels from Gulf of Trieste (fig. 5B). The intake of n-3 PUFA from natural source are widely accepted as a part of modern nutrition because of their beneficial effects on human health. Most significantly, the reported protective effect of the n-3 omega fatty acids in

relation to cardiovascular inflammatory diseases and cancer the effect in medical treatment of important diseases such as Alzheimer's multiple sclerosis led people to consider these fatty acids as the most beneficial (Gogus and Smith, 2010, Shahidi & Miraliakbari, 2004).

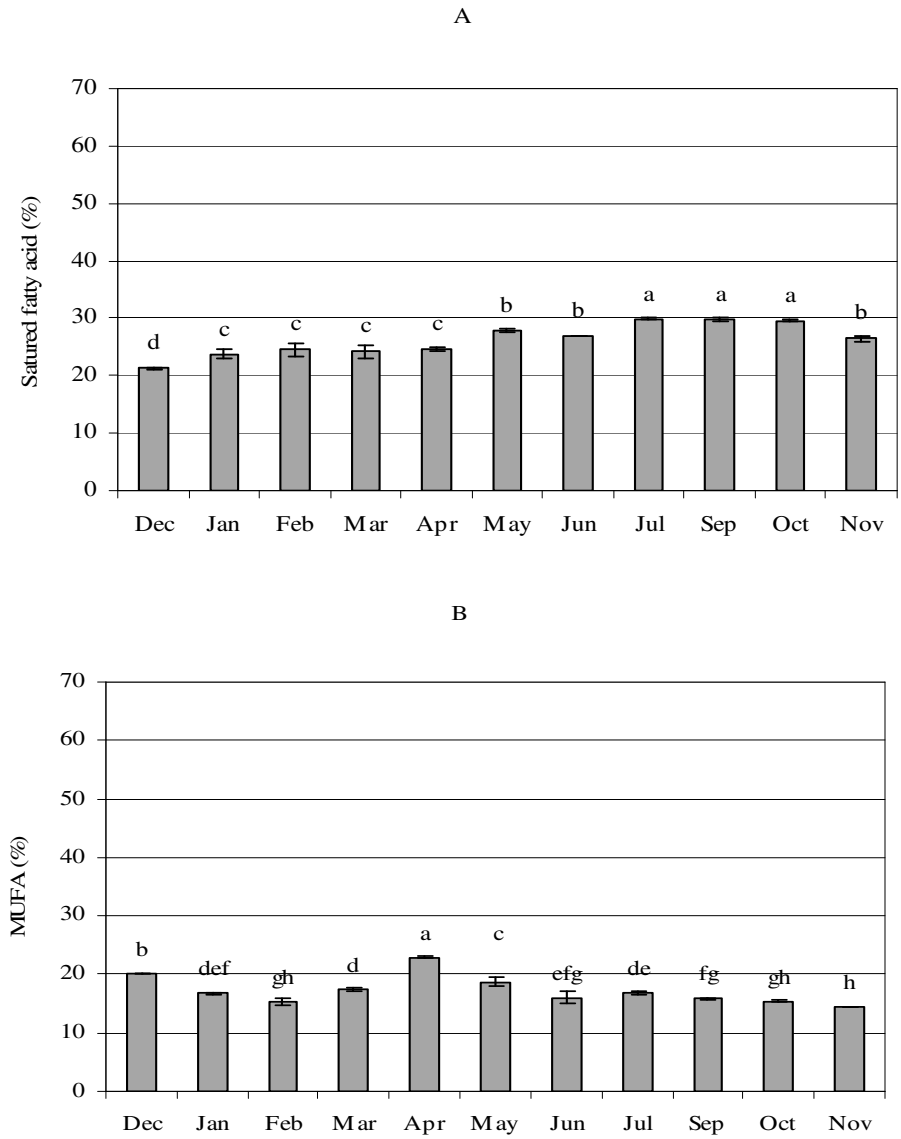

Figure 4 Seasonal changes in fatty acid composition of mussels reared in the Gulf of Trieste A) saturated fatty acids, B) monoinsaturated fatty acids (MUFA). Data are reported as average ± standard deviation of 3 pooled samples

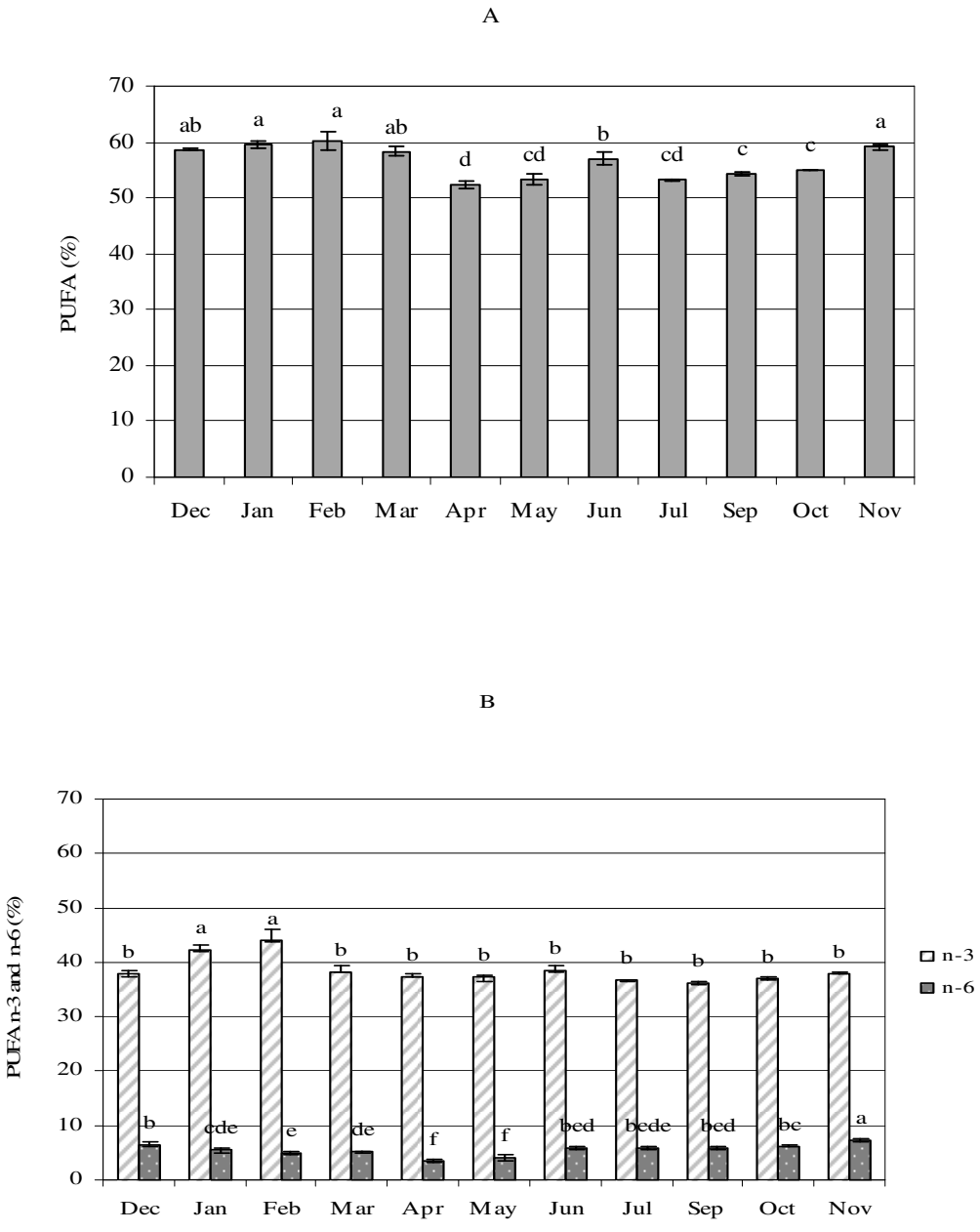

Figure 5 Seasonal changes in fatty acid composition of mussels reared in the Gulf of Trieste C) PUFA D) PUFA n-3 and n-6. Data are reported as average ± standard deviation of 3 pooled samples

mg/100g ed. por.	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Sep	Oct	Nov
C14:0	19.7±2.3	12.4±0.9	21.3±4.6	13.6±2.2	42.9±6.6	31.5±2	18.2±1	28.2±4.4	19.7±0.8	15.0±1.7	11.6±1.8
C14:1	11.3±1.1	5.3±0.1	5.4±0.9	6.5±1	8.2±1.3	7.4±0.6	3.6±0.3	4.3±0.7	3.3±0.2	2.7±0.3	3.6±0.6
C15:0	4.0±0.3	3.5±0.1	5.0±0.8	2.7±0.5	3.7±0.6	4.8±0.2	5.3±0.1	8.3±1.2	6.4±0.2	6.0±0.8	4.5±0.6
C16:0	66.8±1.9	79.2±9	125.5±25.1	70.0±10.9	95.5±17.4	155.7±5.3	115.4±4.9	220.1±25.7	163.2±3.5	145.9±17.2	100.4±11.1
C16:1w7	37.7±4.1	20.3±2.1	35.0±9.2	21.3±2.9	90.1±14.8	61.6±2.8	26.2±1.6	54.6±9.1	30.8±1.0	25.1±2.4	15.6±2.4
C17:0	6.8±0.4	5.5±0.4	8.3±1.1	5.0±0.7	5.7±0.8	8.0±0.4	8.7±0.7	12.6±1.3	9.8±0.2	9.9±1.1	7.7±0.6
C16:3n4	6.3±0.4	4.0±0.2	5.1±0.6	4.2±0.6	4.4±0.6	2.9±0.3	4.6±0.2	5.0±0.2	5.6±0.1	5.2±0.5	4.9±0.6
C16:4n1	51.9±3.3	30.2±5	52.3±7.6	40.1±4.6	28.0±1.7	50.2±7.7	52.1±2.1	68.0±3.1	61.2±0.6	54.1±4.6	46.8±5.4
C18:0	23.8±1.2	18.9±1.4	28.7±9.2	18.5±2.3	22.8±2.7	27.6±1.5	25.3±2.3	39.8±3.6	28.6±0.5	27.6±3.1	21.6±1.5
C18:1n9	14.6±0.1	8.6±0.3	8.6±0.6	6.8±0.9	17.8±3.2	25.2±1.5	19.9±3.1	26.2±3.4	21.2±0.4	19.1±1.7	9.6±1.2
C18:1n7	14.1±1.5	11.2±1	16.9±2.7	9.3±1.2	21.2±3.2	22.7±1.1	16.9±1.3	28.9±3.4	19.6±0.5	17.4±1.7	11.1±1.2
C18:2n6	11.1±0.7	7.1±0.2	8.7±0.6	7.3±0.9	15.2±2.2	20.8±0.4	20.1±2.1	30.8±2.4	23.2±0.9	19.4±1.7	10.2±1.1
C18:3 n-3	8.8±0.8	8.8±0.1	12.0±2.5	8.7±1.2	17.8±2.8	18.6±1.2	15.3±2.1	19.5±2.8	16.9±0.9	15.4±1.4	11.2±1.8
C18:4n3	11.6±1.4	18.9±0.3	26.7±6.5	16.8±0.9	36.0±5.8	30.8±0.7	18.0±3.1	22.6±2.1	17.5±1	13.8±1.1	11.8±2.4
C20:0	9.3±1	5.0±0.5	8.6±0.6	5.3±1	7.6±0.6	8.1±3.0	11.7±0.8	19.5±2.1	15.7±0.7	14.8±1.0	9.2±1.2
C20:1n11	22.0±0.1	19.5±1.2	26.2±3.1	16.1±1.9	16.3±1.8	25.8±5.8	20.4±7.2	34.4±3.1	27.3±0.5	25.7±3.0	20.0±1.9
C20:1n9	5.5±0.5	4.3±0.3	9.3±1.1	4.3±0.6	5.3±0.6	6.6±0.7	5.8±0.3	10.7±1.4	7.3±0.1	5.4±1.4	4.4±0.4
C20:3n9	23.3±1.7	14.1±1.2	14.7±1.7	14.4±2	14.3±1.3	23.2±2.5	23.9±1.4	32.9±2.9	27.8±0.2	23.4±4.7	18.9±2.2
C20:2 n-6	5.1±0.1	3.1±0.2	4.7±0.0	3.3±0.7	4.9±0.1	6.8±1.4	6.2±0.5	10.6±1.8	7.1±0.2	6.6±1.1	4.9±0.9
C20:3 n-6	3.7±0.1	1.0±0.1	2.9±0.2	2.1±1.6	4.0±0.4	5.1±0.6	4.8±0.5	6.7±1	4.4±0.1	4.4±0.7	3.4±0.5
C20:4 n-6	24.5±0.4	18.1±1.5	21.1±1.1	15.2±0.5	11.1±1.3	14.4±1.3	18.3±1.6	30.9±2.6	24.6±0.6	25.2±3.1	21.9±2.4
C20:3n3	1.3±.0	0.8±0.2	1.7±04	0.6±1.4	1.8±0.	2.0±0.1	2.1±0.4	2.7±0.5	1.5±0.0	1.5±0.5	1.2±0.3
C20:4n3	2.5±0.5	3.0±1.2	4.9±0	1.9±2.1	4.1±0.3	3.4±0.5	2.8±0.6	4.5±1	2.4±0.1	2.5±0.7	2.7±0.8
C20:5n3	98.5±8.4	84.4±4.5	145.3±23.8	59.8±0.1	100.1±14.2	100.6±8	72.1±5.6	128.9±14.2	88.1±2.4	79.6±8.4	69.0±9.0
C22:1n9	6.2±0.8	4.5±0.6	5.1±0.2	3.7±1	2.8±0.2	3.8±1.3	7.0±0.4	9.4±1.2	7.2±0.0	7.0±1.3	5.8±1
C22:1n11	22.7±1.4	16.2±0.8	21.9±2.2	20.6±3.1	18.2±1	20.4±3.4	22.6±1.6	32.2±2.9	23.5±23.5	22.1±2.8	18.4±3.0
C22:2	1.7±0.2	1.6±0.8	1.5±0.2	2.1±1.4	1.1±0.1	1.2±0.3	2.3±0.3	2.9±0.5	1.4±0.0	0.9±0.1	1.9±0.6
C21:5n3	13.7±0.8	12.0±1.9	27.0±12.4	11.7±2.6	12.1±1	14.2±1.6	14.0±1.1	18.9±2.1	13.4±0.3	12.8±2.1	10.8±1.8
C22:4 n-6	12.5±2	8.1±0.5	15.1±1.9	6.3±1.9	6.8±1.8	11.9±3.9	17.2±1.5	24.2±6.3	18.0±2.6	15.2±3.6	17.1±4.3
C22:5n3	13.3±2.4	11.4±0.8	16.9±2.2	8.0±2.2	8.9±0.9	10.5±1.3	12.0±0.9	18.0±2.1	11.9±0.8	11.6±2.0	11.1±2.1
C22:6 n-3	126.2±6.8	116.5±4.4	175.9±15.2	108.8±15.2	152.3±20.7	200.9±22.8	185.2±22	255.5±26.5	194.6±7.2	181.7±19.9	135.3±16.7
C24:1	3.4±0.4	3.5±0.9	6.0±0.6	1.8±0.6	2.4±0.5	1.9±0.1	2.4±1.4	4.0±0.0	2.4±0.4	2.7±0.6	2.5±0.5

Table 6 Seasonal change in fatty acid content of mussels reared in the Gulf of Trieste. Data are reported as everage± standard deviation of 3 pooled samples. Fatty acid in percentage <0.05% of total Fatty acids were omitted

Table 7 Seasonal change in Satured, PUFA n-3 and PUFA n-6 content in mussels reared in the Gulf of Trieste

mg/100g of edible portion	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Sep	Oct	Nov
MUFA	139.5±9.7^{cd}	89.50±9.5 ^{f}	135.84±24.1^{cde}	91.7±13.3^{ef}	183.9±26.9^{ab}	177.2±24.8^{abc}	126.71±15^{def}	207.8±25.6^{a}	144.4±3.2^{bcd}	128.9±15.7^{def}	92.7±12.5^{ef}
PUFA	411.5±26.5^{bcde}	319.33±33.7^{de}	532.47±60.5^{ab}	305.9±43.1^{e}	418.9±49.7^{bcde}	504.81±54.2^{bc}	457.44±42^{bcd}	656.8±72.1^{a}	499.2±16.9^{bc}	457.0±55.3^{bcd}	378.7 ±52.5^{cde}
Saturated	147.2±9.6^{de}	127.34±20^{e}	212.84±39^{bcd}	125.8±18.6^{e}	196.8±31.7^{cd}	261.2±8.3^{bc}	210.35±11.1^{bcd}	367.9±41.4^{a}	271.2±5.5^{b}	243.2±28.2^{bc}	168.8±18.7^{de}
PUFAw3	276±21^{cde}	241.50±25.2^{de}	410.28±44.9^{ab}	216.2±32.5^{e}	333.0±45.6^{bcd}	381.0±36.3^{abc}	321.63±33.5^{bcde}	470.6±52.4^{a}	346.3±12.7^{bcd}	319±36.2^{bcde}	253.1±34.8^{de}
PUFAw6	45.8±1.2^{bc}	29.34±2.4^{c}	43.87±2.6^{bc}	26.9±3.5^{c}	28.3±2.3^{c}	39.3±6.5^{bc}	46.54±3.4^{bc}	72.8±11.9^{bc}	54.2±36^{b}	51.7±8.3^{b}	47.3±8.1^{bc}

Counted on total determined FAMEs

2.4.5 Amino acids analysis (AA)

The seasonal changes in the amino acid content of mussels reared in the Gulf of Trieste is presented in Table 8. Glutamic acid glycine and aspartic acid resulted the major amino acids and accounted around 40% of total amino acids in mussels, irrespectively of the sampling period. Arginine and lysine were the most abundant among the Essential Amino Acids, representing 7-8 % of total amino acid content.

Seasonal variations in amino acid content could be appreciated; with higher values from May to October according to favourable environmental and nutritional conditions that are well documented to affect nitrogen related-compounds in other bivalves (Hwang *et al.*, 2000).

Table 8 Seasonal change in total amino acid (TAA) concentrations (mg/100g of edible portion) of mussels reared in the Gulf of Trieste

	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Sep	Oct	Nov
Asp	637.8±1.3	785.5±15.3	766.9±14.8	695.7±16.7	662.4±13.7	873.3±12.4	821.8±24.1	895.7±16.2	829.6±50.5	820.1±42.3	686.0±75.3
Ser	273.8±0.7	322.1±8.9	368.3±22.0	238.4±39.1	295.8±10.1	388.5±10.7	340.5±13.3	335.6±9.6	313.2±19.5	315.1±14.9	352.9±26.9
Glu	796.2±3.4	985.4±16.6	900.8±4.8	854.8±63.3	859.1±20.4	1148.3±11.8	982.5±40.8	1039.4±21.9	1002.1±56.4	1008.7±53.0	914.7±8.8
Gly	676.1±9.7	874.2±1.2	1167.9±19.9	842.4±17.1	769.3±7.4	924.2±28.7	776.9±37.4	819.3±27.4	770.6±35.1	806.2±21.1	808.6±45.2
His	99.7±11.2	103.1±16.3	144.5±29.3	79.9±4.1	120.3±31.4	149.2±21.2	159.7±18.3	179.9±21.2	163.8±7.2	147.3±15.1	114.4±14.0
Arg	437.4±19.4	502.7±12.7	662.8±9.7	423.5±31.8	456.4±12.1	543.2±17.9	561.1±42.2	544.7±19.6	568±45.4	575.8±59.4	588.2±67.2
Thr	274.3±1.2	326.7±5.4	333.8±12.7	259.3±57.4	292.6±6.4	410.1±5.2	368.5±16.9	433.2±4.9	377.7±11.6	371.5±12.2	331.1±2.5
Ala	302.6±3.7	384.3±5.0	434.4±27.5	332.6±41.6	357.3±4.6	476.3±10.8	389.8±2.9	422.0±0.2	393.4±1.8	388.0±13.0	388±5.6
Pro	270.0±0.3	343.9±2.3	314.9±2.5	266.2±40.2	276.2±4.9	375.1±8.1	331.14±20.2	510.4±11.9	357±7.8	341.6±16.7	313.6±21.2
Tyr	198.9±0.2	179.3±4.3	189.8±3.1	170.0±7.4	185.3±0.2	247.8±0.4	240.8±10.0	275.8±0.4	229.8±8.5	202.6±11.1	256.5±30.9
Val	310.5±1.1	380.8±2.9	379.8±3.8	328.9±6.6	325.3±2.4	431.3±7.1	385.7±14.6	469.2±6.0	421.6±11.8	416.2±13.9	376.8±23.2
Lys	398.2±8.9	440.6±5.2	694.5±16.7	347.2±0.9	415±1.4	525.0±13.8	529.0±3.2	635.6±3.9	573.9±22.2	569±28.7	611.1±42.1
Ile	267.7±1.6	328.1±5.0	317.8±4.7	267.5±31.0	277.4±3.2	369.3±10.7	318.4±24.9	377.2±13.8	342.6±17.3	342.2±17.9	310.0±13.7
Leu	375.2±6.7	460.0±3.7	446.4±8.4	372.1±26.7	389.5±8.1	505.9±16.1	454.8±37.0	521.4±18.7	485.6±21.8	486.8±27.2	468.0±23.8
Phe	205.1±4.7	231.4±5.2	240.0±8.5	190.7±9.0	216.5±3.3	291.6±12.6	283.4±27.9	350.9±17.9	320.0±20.2	315.5±28.0	280.2±38.1

Data are reported as average ± standard deviation of 3 pooled samples

2.5 Conclusions

Changes in consumer's habits suggest the need for processed products with longer shelf-life and more simple to cook also for traditional seafood like mussels and various of seafood processing companies are implementing the range of products ready to eat and ready to cook also for mussels.
For the success of this process high quality raw material and optimal processing and storage conditions are needed to meet the quality requirements.
The data obtained from the present research give an overview of the changes in the commercial and nutritional characteristics of *M. galloprovincialis* cultivated in the North Adriatic Sea during year around
Mytilus galloprovincialis exhibited optimal nutritional quality as well as valuable merchantable traits from April to October, thus identifying this period as ideal for their direct human consumption and for their processing.

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3. APPLICATION OF *SOUS VIDE* TECHNOLOGY TO PRESERVE LIVE MUSSELS (*M. GALLOPROVINCIALIS*)

3.1 Introduction

The requirements for food marketing are defined by the EC Regulations 852, 853/04. In chapter IX of EC Reg 852/04 the need to protect food during all stages of production, processing and distribution from any form of contamination which could maker the food unsuitable for human consumption, is stated. However, in the section VII of the EC Reg 853/04 dedicated to live bivalve molluscs, there is no reference to the most suitable type of package for their storage. Chapter V, of the same Regulation, also indicated that, in order to meet both health and hygiene requirements, mussels must have typical organoleptic features of freshness and vitality, respond appropriately to percussion and maintain a normal level of intervalvar liquid.

Mussels and live bivalve molluscs are now marketed in plastic net packs, closed by metal clips with a label containing all information required by the law. The limits of this type of packaging are numerous:

- expose the product to risk of cross-contamination and the effect of air;
- cause the dispersion of organic liquids and flavours during storage;
- the round shape, that assumes the packed product, requires large storage volumes;
- the sale can be made only at fish markets and supermarkets equipped with refrigerated exhibitors with drainage system;
- the plastic net does not allow graphical presentations of particular communicative and informative efficacy.

In optimal storage condition, the shelf-life of products packaged in plastic nets is limited to around 2-5 storage days and, during storage period, there is a loss of weight due to intervalvar liquid leakage that causes a considerable economic loss for the whole chain (Cattaneo Bernardi, 2010), and dehydration of the mussels tissues with consequences on quality and freshness parameters.

Recently the packaging methods that use Modified Atmospheric Packaging (MAP) and Vacuum Packaging (VP) technology have been applied in the fisheries sector.

The application of MAP to preserve fishery products has been widely studied (Sivertsvik *et al.,* 2002), the gas composition is dependent on the species and it has been reported an extention of shelf life of fish and shellfish products (Özogul *et al.,* 2000; Goulas *et al.,* 2005, 2008; Erkan, 2005; Pantazi *et al.,* 2008 Gonçalves *et al.,* 2003; 2009). However so far, the application of MAP to preserve live bivalve molluscs like clams (*Ruditapes decussates, Chamelea gallina*) and mussels (*Mytilus galloprovincialis*), show advantage in keeping mollusks alive, thereby extending the marketable period compared to conventional packaging (Gonçalves *et al.,* 2009; Pastoriza *et al.,*2004; 2011 Maffei *et al.,*2010).

The products under vacuum (VP), are defined as "chilled products of new generation" also by the National Food Processors Association (Juneja, 2003) and then placed among the Refrigerated Processed Food of Extended Durability (REPFEDs).

These products are popular in Europe since the 90's following the growing demand from consumers for products ready to cook or to eat, with few preservatives and high organoleptic characteristics similar to fresh products (Armstrong, 2000).

The process is based on removing as much air as possible from the pack reducing the pressure at values lower than 1 mbar.

The absence of air in contact with the product reduces the development of aerobic microbial flora responsible for the rapid spoilage of fish or shellfish. This technique can be combined with heat treatment of pasteurization, sterilization, cooking (*sous vide* cooking or vacuum cooking), freezing and non-thermal treatment, such as high pressures using proper materials.

The VP technology, also named in french language "*sous vide* packaging", has been recently tested on a variety of fish and seafood (Ozogul *et al.,* 2000; Patanzi *et al.,* 2008; Goulas *et al.,* 2005; Goulas 2008; Paleari *et al.;* 2009, Maffei *et al.,* 2010). Comparative tests on traditional packaging (plastic net), MAP and VP packaging, applied to mussels (*Mytilus galloprovincialis*), clams (*Tapes philippinarum*) and lupines (*Chamelea gallina*) under refrigerated conditions (+4° C) revealed that the MAP packaging resulted in several advantages compared to conventional methods such as the reduction of the microbial degradative process and the

crushing of the valve, but resulted not able to prevent intervalvar liquid loss (Maffei *et al*., 2010).

Compared to MAP, vacuum packaging limits the development of microbial population, allows mussels to maintain the shells well-closed minimizing losses of intervalvar liquid reducing product's weight loss during storage.

The effect of Vacuum Packaging technology on live mussels quality has been poorly studied especially in reference to the freshness and sensorial quality of the raw product, probably because it is not yet predisposed a scheme for the evaluation of specific sensory attributes for mussels analogous to those developed since 1985 (Bremner 1985) to assess the freshness of wide raw fish, crustaceans and cephalopods species (see tables reported by the authors Barbosa *et al.,* 2004 and Sant'Ana *et al*., 2011). According to Quality Index Method is can be carried out on whole or processed individuals representing a simple and useful tool for industry technical research and consumer (Hildig *et al.,* 2010). Another aspect is related to the lack of reference on the effect of the byssus removal on the mussels' vitality.

3.2 Main goals of the research

The application of *sous vide* technology to live mussels (*Mytilus galloprovincialis*) was evaluated in two sparate trials.

The first trial (paragraph 3.3) was aimed to assessing the stability of live mussels packaged by *sous vide* technology compared to traditional packaging during storage at 3 ±1°C, physical chemical, microbiological and sensorial aspect have been considered and a practical scheme for evaluation of fresness and quality of raw mussels has been developed to determining their shelf life.

The second trial (paragraph 3.4) was aimed to evaluate the effects of live mussels debyssing and packaged with *sous vide* tecnology on mortality, microbiological properties and the percentage of intervalvar liquid released in the pack.

3.3 Effect of *sous vide* packging on live mussels *M. galloprovincialis* shelf-life

3.3.1 Materials and methods

Raw materials and thesis compared

Live mussels (40 kg) reared in mussel farms located in the Gulf of Trieste (Friuli Venezia Giulia-Italy) were maintained in sea water (12°C, 24 psu salinity) for 24 h in a purifing station before transfering to the processing plant.

Mussels were individually selected: deformed or undersized individuals (<5 cm) were discarded. The selected ones were brushed and immersed in refrigerated water (+2°C) to reduce the handling stress.

According to the experimental design shown in table 1 Two packaging technology were compared: live mussels packaged *sous vide* with byssus (SVBS) and live mussels packed conventionally with byssus (RTBS) for 16 storage days at 3°C ± 1°C to evaluate the effect of *sous vide* on live mussels (*M. galloprovincialis*) shelf-life.

Table1 Experimental design

Factors	Low	High	Levels	Units/abbr.
Storage	1	16	6	days
Treatment	1	2	2	SVBS RTBS

Packaging and treatments

Conventional packaging

RTBS mussels (fig. 1 A) were packed in packs of 15 individuals in traditional plastic nets closed and placed on a support to collect liquid released and maintained at 3°C ± 1°C for 16 days.

Sous vide packaging

SVBS mussels (fig. 1 B) were packed in groups of 15 individuals, in Polyammide/Polyethylene bags (140 µm of thickness, Orved S.p.A., Musile di piave, VE); vacuum condition was obtained with a vacuum machine VM 53 (Orved S.p.A., Musile di Piave, VE). After packaging mussels were maintained at 3°C ± 1°C for 16 days.

A B

Figure 1 Mussels in conventional packaging (A RTBS) and *sous vide* (B SVBS)

Sampling

Mussels were analysed for mortality, physico-chemical, sensory, microbiological and histological characteristics for each treatment and for each sampling time (1, 3, 6, 9, 12, 16 days). The packaging technology was evaluated in duplicate according to table 2.

Table 2 Sampling

Analysis	Packs (*n*)	Treatments (*n*)	Sampling (*n*)
Biometric indices	2	2	6
Mortality evaluation	2	2	6
Liquid release	2	2	6
pH	2	2	6
W H C	4 pools of 15g	2	6
Microbiological analysis	2	2	6
Sensory Analysis	6 inividuals	2	6

Biometric indices

Biometric indices were evaluated on 30 mussels (2 packs) per thesis. After the determination of total weight, meat was spun off from its shells, and both shell and meat were weighed before and after drying (105° C until constant eight), using a precision analytical balance (± 1 mg). Condition Index (CI) was calculated according Okumuş and Stirling (1998) and Orban *et al*. (2002) as follows:

$$CIdry(\%)=[\text{ meat dry weight (g) / shell dry weight (g)}] \times 100$$

Intervalvar liquid (IL) was calculated as follows:

$$IL(\%)=[\text{total weigth(g)-(meat fresh weigth(g)+shell wet weight(g)]/ total weigth(g)} \times 100$$

Meat yield (MYwet), was calculated according Okumuş and Stirling (1998) as follows:

$$MYwet=[\text{wet meat weight(g)/live weight (g)}] \times 100$$

Mortality evaluation

Mussels mortality was evaluated by tapping on gaping bivalve shells, and any shells remaining open were considered dead according to Bernardez & Pastoriza (2011) and Goncalves *et al.*,(2009).

Liquid release

Liquid released was evaluated in terms of percentage of intervalvar liquid lost in the package compared to the total initial mussels weight, according to Bernardez & Pastoriza (2011), and in terms of sensory characteristics perceived: changes of odour and colour of the liquid during the storage period were described.

Chemical Analysis

pH value was measured by dipping the pH electrode into a mixture in 15 mL of homogenized mussels with digital pH-meter (model basic 20 Crison instruments S.A., Barcelona, Espaňa).

Water Holding Capacity measurement

The WHC was measured on mussel pool (15 g) adapting the method proposed of Olsson *et al.* (2003b) for Atlantic halibut muscle. The samples were transferred to centrifugation tubes and centrifuged at 5°C (210 x g for 15 min). The WHC was determined as liquid loss (LL) and expressed as percentage of weight of liquid released (Ofstad et al., 1993) as following:

$$WHC\ (\%) = [\text{weight liquid released (g)} / \text{total weight (g)}] \times 100$$

Microbiological analysis

Samples were analyzed according to official microbiological methods, to determine mussels main microbial population and their hygienic quality. Each specimen has been opened using a sterile cutter to recovery the edible portion and intervalvar liquid.

Ten grams of sample were transferred into a sterile stomacher bag and 90 mL of saline-peptone water (8 g/L NaCl, 1 g/L bacteriological peptone, Oxoid, Milan, Italy) were added and mixed for 1.5 min in a Stomacher machine (PBI, Milan, Italy). Further decimal dilutions were made in the same solution and microbial analyses were performed in duplicate agar plates. Total aerobic bacterial count (TBC) was determined on Plate Count Agar (PCA, Oxoid, Milan, Italy) following the pour plate method and incubated for 48 -72h at 30°C ;

Pseudomonas were enumerated on Pseudomonas Agar Base (PAB, Oxoid) by spread plating technique after incubation at +30° C for 48 h.

Lactic acid bacteria (LAB) were determined on double layer of Man Rogosa Sharpe lactobacillus agar (MRS, Oxoid, Milan, Italy) and incubated for 48h at 30°C. Anaerobic Sulphite-reducing clostridia were enumerated in double layer SPS agar (Oxoid, Milan, Italy) incubated for 48h at 37° C under anaerobiosis.

Enterobacteriaceae were determined on a double layer of Violet Red Bile Glucose Agar (VR-BA Oxoid, Milan, Italy) incubated for 48h at 37 °C.

Sensory Analysis

Two preliminary trials of sensorial analysis were used for train assessors and to define specific attributes for mussels to be included into the scheme of the quality attributes and descriptors for mussel. Scores were given for each attribute according to description, ranging from 0 to 3 and the sum of each score resulted in an total demerit point that ranging from 0 to 19.

In the last trial, five selected assessors, evaluated 6 mussels for different packaging (RTBS and SVBS) after 1, 3, 6, 9, 13 and 16 storage days at 3±1°C.

Hystological analysis

Mussel samples were fixed in 4% buffered formaldehyde and processed with standard histological techniques. 5 micron sections, obtained by microtome, were stained with hematoxylin-eosin and the specimens were observed under a light microscope (Leitz DMRB). Nikon System was used to document the specimen.

Statistics

Experimental data were subjected to statistical analysis (analysis of variance, ANOVA) according to completely random experimental design. Average values were compared using the Duncan's multiple range test for $P<0.05$. All data were processed with SPSS-PC release 17 statistical software (SPSS Inc. Chicago, IL, USA).

3.3.2 Results and discussion

Condition Index, Intervalvar liquid and meat yield

The results of Condition Index, Intervalvar liquid and meat yield is showed in table 3.

CI ranged from 12.8±5.3% to 17.5±0.9% in RTBS and from 12.9±3.3% to 17.3±2.1% in SVBS and was not altered by the packaging method (P>0,05). The incidence of Intervalvar liquid (IL) decreased during storage period in both groups RTBS and SVBS (from 32.3±1.5% to 4.6±1.2% and 40.8±4.0 to 14.6±9.9% respectively) and the differences were statistically significant on 13 and 16 storage days.

MY wet (%) increased over the sampling time during the storage period despite the differences in packaging (P>0.05). In this case anyway the MY increase was coupled with a corresponding decrease in IL and doesn't indicate an improvement in product's quality but a deterioration.

Table 3 Effect of packaging technique (RTBS, SVBS) on Condition Index (CI), intervalvar liquid (IL) and edible meat yield (MY) in Adriatic mussels stored at 3±1°C

Storage days	Treatment	CIdry (%)	IL(%)	MYwet (%)
1	**SVBS**	17.3±2.1	40.8±4.0	27.8±2.3
	RTBS	17.5±0.9	32.3±1.5	32.7±8
3	**SVBS**	15.0±2.5	42.5±4.3	26.4±2.9
	RTBS	13.4±5.1	41.7±8.3	24.1±6.2
6	**SVBS**	15.0±2.8	29.8±5.2	31.9±4.2
	RTBS	16.8±1.0	26.2±14.8	35.1±6.2
9	**SVBS**	12.9±3.3	32.2±18.7	30.6±10
	RTBS	12.8±5.3	36.4±11.6	28.4±5.6
13	**SVBS**	14.5±2.2	45.0±2.2^{a}	25.2±1.8
	RTBS	12.4±7.7	25.4±19.1^{b}	34.3±8.2
16	**SVBS**	14.2±3.8	14.6±9.9^{a}	34.4±7.9
	RTBS	14.8±3.4	4.6±1.2^{b}	40.5±4.8

Data are reported as average ± standard deviation

Mortality

The effect of packaging on mortality is reported in fig. 2, in both groups RTBS and SVBS mortality increased during the storage period. Significant differences in mortality rate between the experimental groups were observed at 6 and 9 storage days ($P<0.05$).

In conventionally packaged mussels (RTBS) mortality started from 6 storage days (6.7±0.0%) and similar values (9.8% and 10.9±4 %) were reported by Maffei *et al.*, (2010) in live mussels, after 4 storage days and by Goncalves *et al.,* (2009) in clams (*Ruditapes decussatus*) after 6 storage days in air in usual mesh bags.

In sous vide packaged mussels (SVBS) mortality started from 9 storage days (20±0.0%). Similar values were reported by Bernardez *et al.*, (2011) in mussels (*Mytilus galloprovincialis*) of larger size, packaged in MAP with different initial concentration of oxygen (20%, 75% and 85%) on 9th storage day, and by the result reported by Maffei *et al., (*2010) in mussels after 10 storage days (mortality 18.4%) in *sous vide* condition added with a citric acid, potassium nitrite and sodium ascorbate solution (6 mL of citric acid solution 1g/L, 1mL of potassium nitrite solution of 25 g/L, 1 mL di sodium ascorbate solution 100 g/L).

In both groups tested in this study, mortality reached 96.7±4.7% after 16 storage days.

Mytilidae belong to the facultative anaerobes group, as they can live either aerobically or anaerobically for short periods. For this reason Mytilidae are also called euryoxic because they tolerate a wide range of oxygen concentration, including extreme condition such as zero oxygen concentration (Zwaan & Michel, 1992).

The ability to withstand adverse environmental factors (large fluctuations in temperature, salinity and oxygen availability) is a peculiarity of intertidal species that are able to implement physiological responses to prevent desiccation during low tide. Bivalves are able to completely close their shells and remain inactive for days or to breath anaerobically while they are closed (Shick, 1986; Mitchell *et al.*, 1988). Metabolic depression and anaerobiosis are clearly implicated as key factors in survival at freezing temperature. Low temperature induces a switch to anaerobic metabolism (Zwaan & Michel 1992) and determine a complete reduction

in the rates of energetic processes such as digestion and absorption of food, growth, and muscular activity (heart, foot, byssus gland, shell) and whereas the adductor muscle, once contracted, is not in an active state as a result of the so called "catch" phenomenon. Consequently, in sessile bivalves, the ATP turnover is less than 10% of the resting aerobic rates (Zwaan & Michel, 1992).

In our study it is interesting to note that mortality rates in low temperature conditions (+3°C) were lower in SVBS compared with RTBS until 13 storage days. The highest resistance to stress of conservation of SVBS mussels could be due to the presence of the plastic wrapping that could help mussels to keep the intervalvar liquid, keeping tissues hydrated and extending the breathing time longer than for mussel stored in plastic nets, where the liquid is dispersed as soon as the valves are opened.

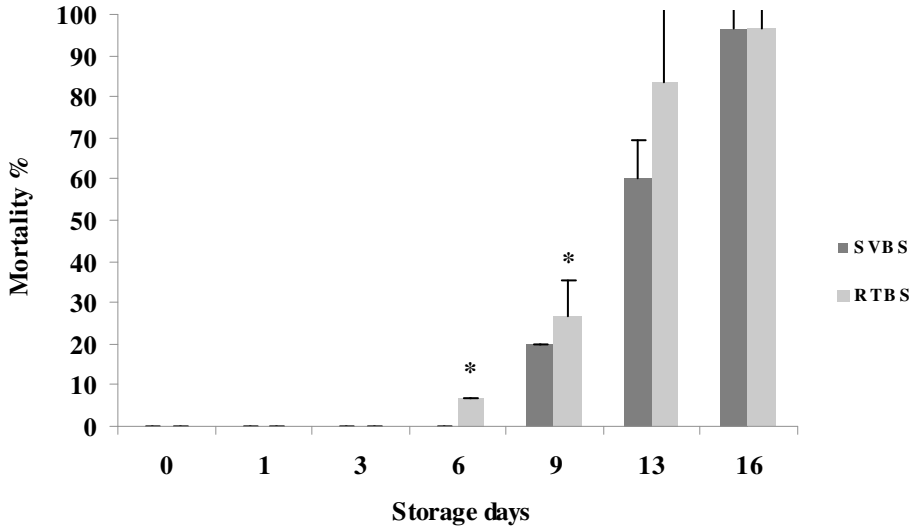


Figure 2 Effect of packaging technique (RTBS, SVBS) on mortality of mussels stored in conventional (RTBS) and *sous vide* (SVBS) packaging at 3±1°C. Data are reported as average ± standard deviation, P<0,05

Changes in liquid release

The effect of the packaging conditions on the weight loss represented by the liquid released is showed in fig. 3.

During the storage period mussels tended to loose intervalvar liquid with both storage methods, but in the RTBS group liquid released was higher respect SVBS group and this difference was significant after 6 storage days (6.7±0.0% and 0.0±0.0% respectively) and 9 days (26.7±8.9% and 20.0±0.0% respectively).

This trend is in accordance to the mortality data recorded in RTBS group compared SVBS group (fig. 2).

In *post-mortem* phase, the mussel relaxes its adductor muscles and the consequent opening of the valve determines the leakage of intervalvar liquid. Table 4 shows the change of sensory properties, as odour and colour, in liquid release.

Negative connotation in both groups have been observed after 6 (RTBS) and 9 (SVBS) storage days to indicate a deterioration of sensory quality of the product.

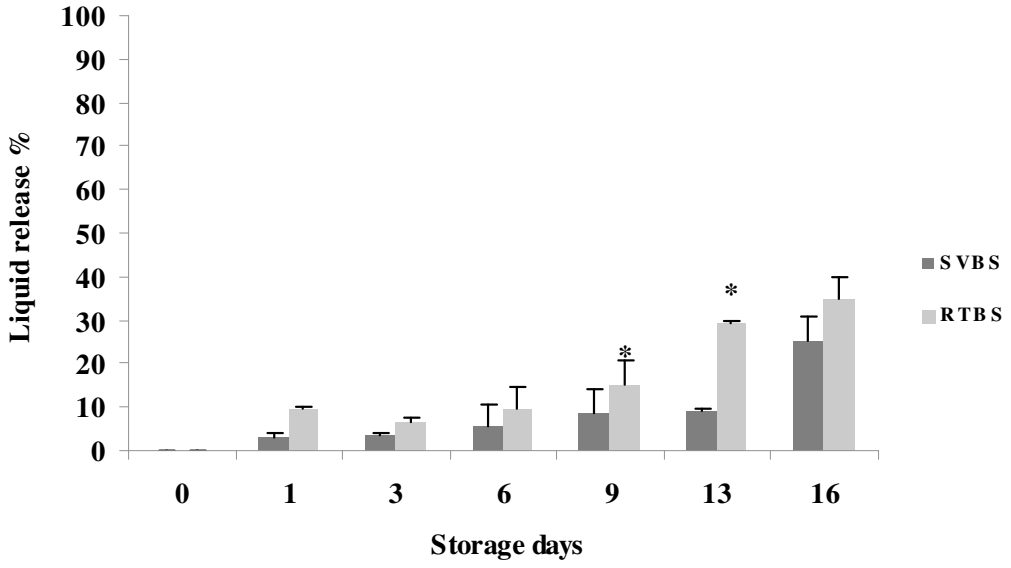


Figure 3 Effect of packaging technique (RTBS, SVBS) on Liquid released by live mussels stored at 3±1°C in conventional (RTBS) and *sous vide* (SVBS) packaging. Data are reported as average ± standard deviation, P<0,05

Table 4 Changes in odour and colour proprerties of intervalvar liquid released in live mussels stored at 3±1°C in conventional (RTBS) and *sous vide* (SVBS) packaging

Storage days		1	3	6	9	13	16
RT	Odour	Marine	Mild	Stagnant	Stagnant	Putrid algae	Putrid algae
SV		Marine	Mild	Mild	Pungent	Putrid algae	Putrid algae
RT	Colour	Clear	Clear	Opaque pink	Opaque pink	Opaque brown	Opaque brown
SV		Clear	Clear	Clear	Opaque greenesh	Opaque brown	Opaque brown

Microbiological evaluations

The changes in the average total bacterial count in different group of live mussels during chilled storage are shown in Fig 5. The initial TBC in RTBS was 3.9±0.5log CFU/g; this value increased to 6.7± 0.2log CFU/g after 16 storage days.

In SVBS the initial value in TBC was 3.6±0.5 log CFU /g and this value increased to 4.8± 0.0 log CFU/g after 16 storage days, according to studies on mussels stored in MAP condition for 13 days in refrigerated condition (Pastoriza and Bernardez, 2011). Statistically significant differences were observed only after 13 and 16 storage days.

The limit for rejection of live molluscs 5 log CFU/g (Anonymous, 1992) was reached only in mussels stored in traditional packaging (RTBS) after 6 storage days. The increase in bacterial numbers may indicate an alteration of tissues in weaker mussels.

Lactic acid bacteria are capable of growing in microaerophilic/anaerobic environments and could be associated with the spoilage of *sous vide* products, involving the swelling of the packs and/or the development of off-flavors and off-odours (Carlin *et al.*, 1999), because vacuum produces favourable conditions for their growth (Zurera-Cosano *et al.*, 1988). In this study, LAB (fig. 6) presented

similar values in both groups RTBS and SVBS during the storage period (2.3±0.2 log CFU/g and 2.1±0.3 log CFU/g).

Pseudomonas spp. (fig. 6) remained constant and similar values in both groups (2±1 log CFU/g and 1.9±0.6 log CFU/g in RTBS and SVBS respectively) were observed.

Enterobacteria (fig. 6) increased during storage in SVBS group (from 1.5±0.0 log CFU/g to 2.5±0.3 log CFU/g at 16 storage days) and decreased in RTBS group (from 1.8±0.3 log CFU/g to 1.1±0.4 log CFU/g at 16 storge days).

Sulphite reducing bacteria resulted always and in both groups RTBS and SVBS below the detection limits suggested by the method (1log CFU /g).

Concerning the igyenic-sanitary aspect, the presence of *Vibrio* and other potential bacterial pathogens such as *Escherichia coli*, *Salmonella* and *Shighella* have been detected in cultivated mussels at retail markets (Ripabelli *et al.,* 1999; Maugeri *et al.,* 2000).

Some of these bacteria are present in the natural marine microflora and may be accumulated by shellfish during feeding (Toti *et al.,* 1996). *Salmonella* spp is among the most important group of global food pathogens. These species are often agents of gastroenteritis, commonly associated with consumption of contaminated shellfish, particularly oyster and live mussels.

In this study, mussels were purified and health aspects of the original batch, were verified by the CDM (Mollusk Depuration Center) and the Competent Authority for sanitary control, so we considered only the bacteria responsible for product deterioration.

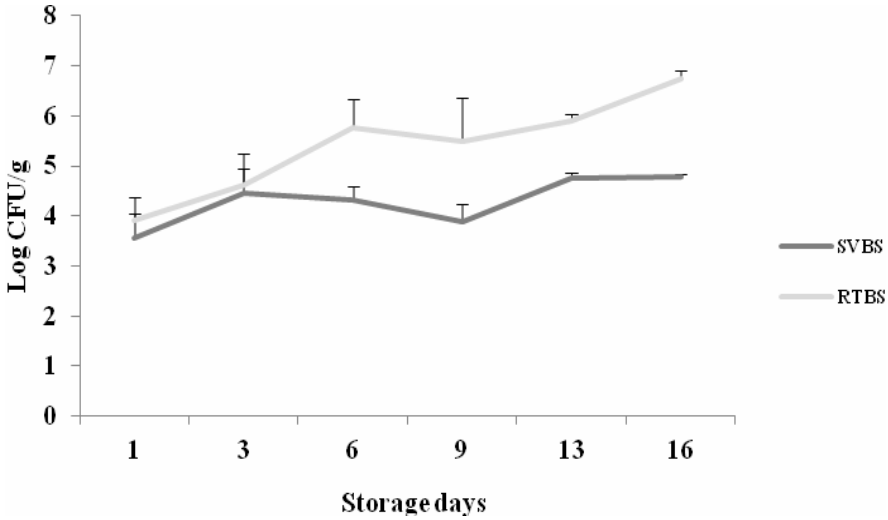
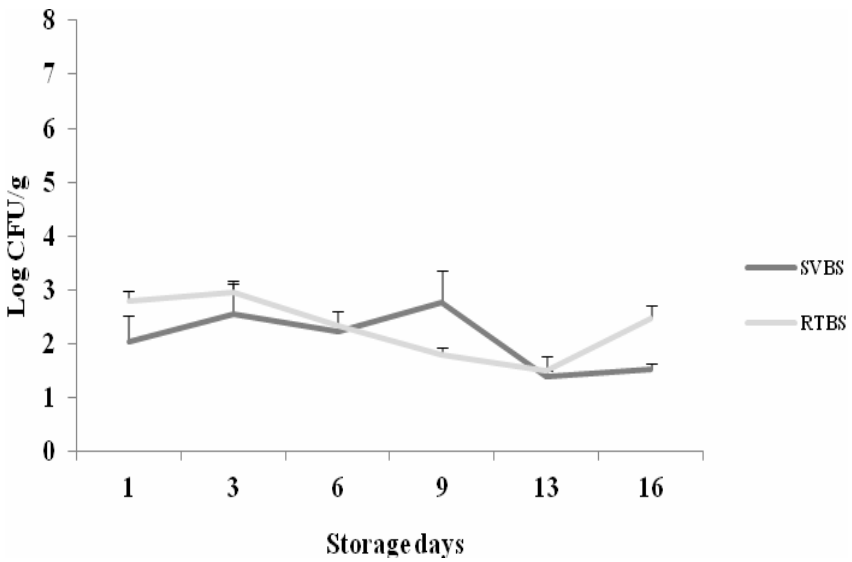


Figure 5 Changes in total bacterial count in live mussels stored at 3±1°C in conventional (RTBS) and *sous vide* (SVBS) packaging. Data are reported as average ± standard deviation, P<0,05

A



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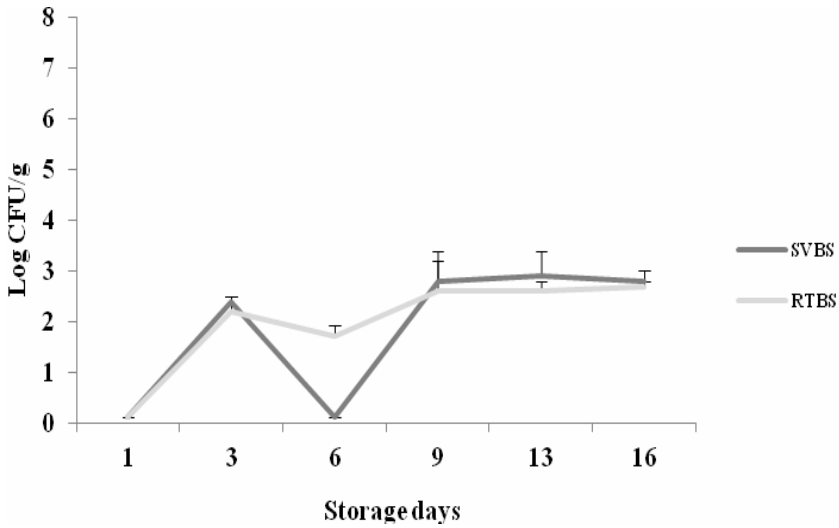

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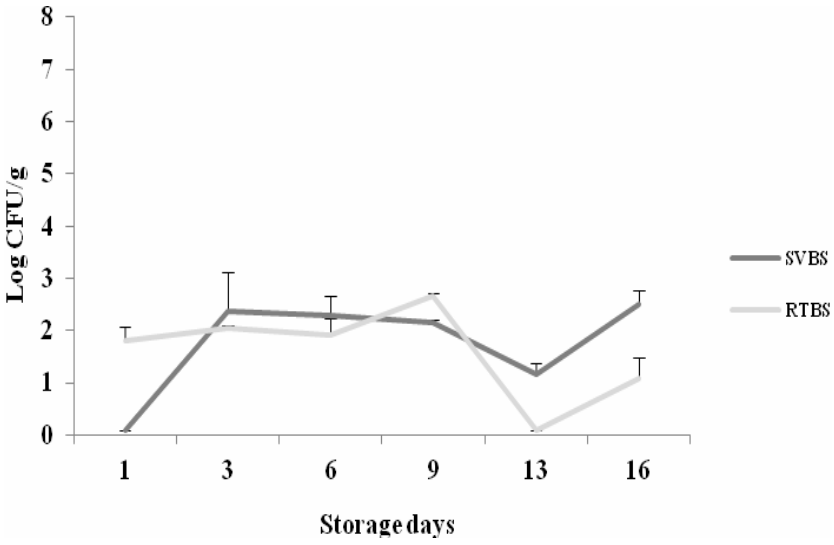

Figure 6 Changes in *Pseudomonas* spp (A), LAB (B) and enterobacteriaceae (C) in live mussels stored at 3±1°C in convetional (RTBS) and *sous vide* (SVBS) packaging. Data are reported as average ± standard deviation

Changes in pH measurement

The changes in the pH value of the flesh of live bivalves are used as a stress indicator (Hummel *et al.*, 1989; Pastoriza *et al.*, 2004) reflecting if the organisms shift their metabolism to anaerobiosis (by closing their valves) resulting in the production of acids compounds. During storage at low temperatures, the mussels, while slowing their metabolism, keep breathing, producing catabolites; later, when mortality occurs, alternative metabolic pathways are activated, determining lactic acid accumulation and decrease of pH values. This parameter has been considered by several authors as a good indicator of quality in shellfish and values below 6 are related to an altered product (Hunter *et al.*, 1928; Pottinger 1948).

In this study, RTBS and SVBS presented similar pH values: in both groups decreased during storage period from 7 to 6.1 in (RTBS) and 6.4 (SVBS) (fig. 7), but after 13 days of storage the pH value of the SVBS mussels tended to higher values than RTBS group ($P>0.05$). This is not due to a decreased glycolitic activity, but to the development of *Enterobacteria* in SVBS group, producing ammonia, which maintains the pH at higher values.

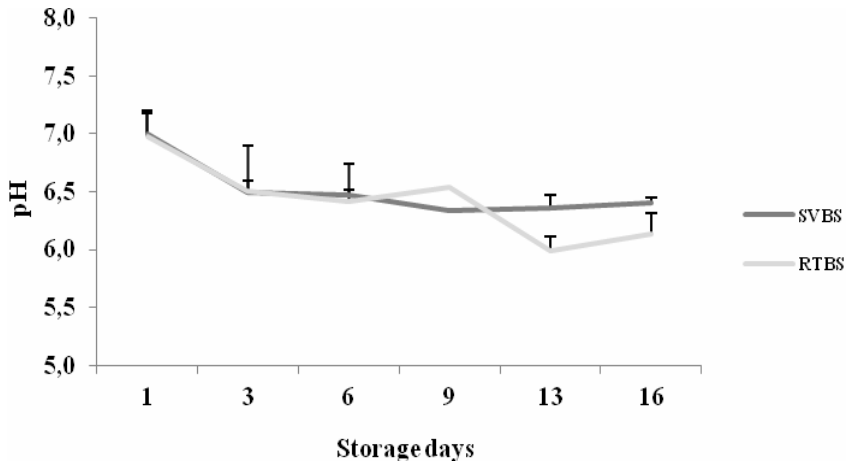

Figure 7 pH of live mussels stored in conventional packaging (RTBS) and *sous vide* (SVBS) at 3±1°C. Data are reported as average ± standard deviation

*Changes in Water Holding Capacity (WHC***)**

As showed in fig. 4, the initial value of WHC was similar in both groups SVBS and RTBS (9.7±1.2% and 9.2±1.3% respectively).

After 6 storage days, this value decreased to minimal value of 4.6±1.6% in RTBS group, while in group SVBS, the same parameter begins to decrease after 13 storage days to 8.4±1,5%. In both cases, after reaching a minimum value, an increase in the WHC was observed: in RTBS it reached a value of 10.3±1.7% (9 storage days) and in SVBS 10±1.3% (16 storage days).

This could be linked to the the pH decrease in post-mortem condition. Consequently, autolytic enzymes are actived and membrane protein surface net charge is reducted, contributing to the denaturation and reduction of tissue's water retention capacity (Olsson *et al.*, 2003b).

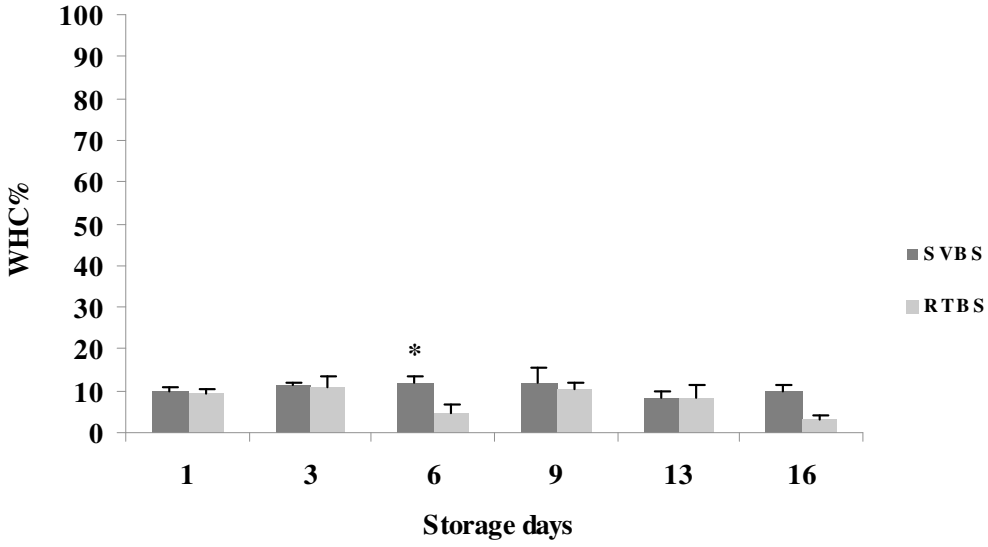

Figure 4 Changes in WHC of live mussels stored in conventional packaging (RTBS) and *sous vide* (SVBS) at 3±1°C. Data are reported as average ± standard deviation, P<0,05

Changes of the sensory properties

Sensory analysis plays a key role assessing the quality of fresh seafood products (Botta, 1995; Bonilla *et al.*, 2007; Huidobro et al., 2000).

The designed sensory scheme for raw mussels included 10 parameters : reactivity to open shell, intervalvar liquid aspect and presence, odour of mollusc and interalvar liquid, gills aspect, gill idratation and gill margin and mantle aspect, mantle idratation and mantle margin (Table 5).

Table 5 Scheme developed for whole raw mussels (*Mytilus galloprovincialis*)

Fresness quality parameters	**Description**	**Score**					
		M1	**M2**	**M3**	**M4**	**M5**	**M6**
Reactivity to open	Good resistance and reactivity, rapid closing movements	0	0	0	0	0	0
	Sufficient resistance and reactivity	1	1	1	1	1	1
	Poor resistance and reactivity slower closing movements	2	2	2	2	2	2
Inervalvar liquid aspect	Trasparent/rosè, brilliant	0	0	0	0	0	0
	Opalescent/brown, moderatly	1	1	1	1	1	1
	Absent	2	2	2	2	2	2
Odour mollusc and liquid	Marine, fresh	0	0	0	0	0	0
	Brackish/, neutral	1	1	1	1	1	1
	Stagnant water	2	2	2	2	2	2
	Putrified seaweed, rotten egg	3	3	3	3	3	3
Gills Aspect	Bright, brilliant	0	0	0	0	0	0
	Opalescent	1	1	1	1	1	1
	Opaque	2	2	2	2	2	2
Gills Idratation	Hydrated, turgid	0	0	0	0	0	0
	Hydrated but little turgid	1	1	1	1	1	1
	Dehydrated	2	2	2	2	2	2
Gill Margin	Lying flat	0	0	0	0	0	0
	Slight folded up	1	1	1	1	1	1
	Folded up or frayed	2	2	2	2	2	2
Mantle Idratation	Hydrated	0	0	0	0	0	0
	Sufficiently hydrated	1	1	1	1	1	1
	Dehydrated	2	2	2	2	2	2
Mantle aspect	Turgid adherent to the shell	0	0	0	0	0	0
	Not adherent to the shell	1	1	1	1	1	1
Mantle Margin	Lying flat to the margin shell	0	0	0	0	0	0
	Slightly inside retracted	1	1	1	1	1	1
	Iinside retracted/flattened	2	2	2	2	2	2
Tissue mantle margin	Turgid	0	0	0	0	0	0
	Flattened	1	1	1	1	1	1
Total demerit point							
Range of QIM score		**0-19**					

During storage period mussels showed gradual changes for all the parameters of sensory evaluation, reaching a total score of 19 demerit points. Rejection, based on acceptability of external sensorial attributes of raw mussels packaged traditionally occurred ca. 13 days of storage, but considering the increase of mortality incidence (>20%) was considered a score of 6 demerit point corresponding at 3 (RTBS) and 9 (SVBS) storage days.

The proposed scheme was able to describe *M. galloprovincialis* freshness decline associated to microbiological and physico-chemical parameters. The evaluation of few sensory attributes highlighted differences between the two methods of packaging (fig. 8).

Differences between mussels RTBS and SVBS in terms of reactivity and intervalvar liquid quality (fig. 9 A-B), odour (fig. 9 C), gills quality (fig.9-D) and mantle quality (fig. 9 E) were highlighted especially between 3rd and 9th storage days. Probably the low hydration due to the loss of intervalvar liquid determined degeneration of mussels tissues and a decline of the sensory characteristics and freshness in the group RTBS. In SVBS group the presence of intervalvar liquid provides tissues hydration and good sensory quality and freshness of the product over 9 days of storage.

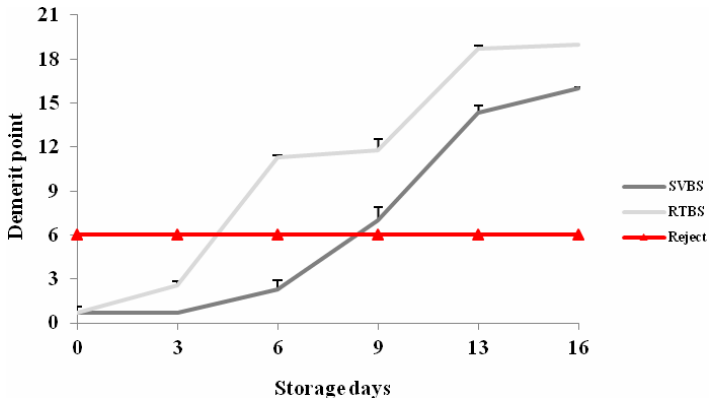

Figure 8 Changes in the overall sensory scores of live mussels stored at 3±1°C in conventional (RTBS) and *sous vide* (SVBS) packaging. Data are reported as average ± standard deviation of 6 individual samples

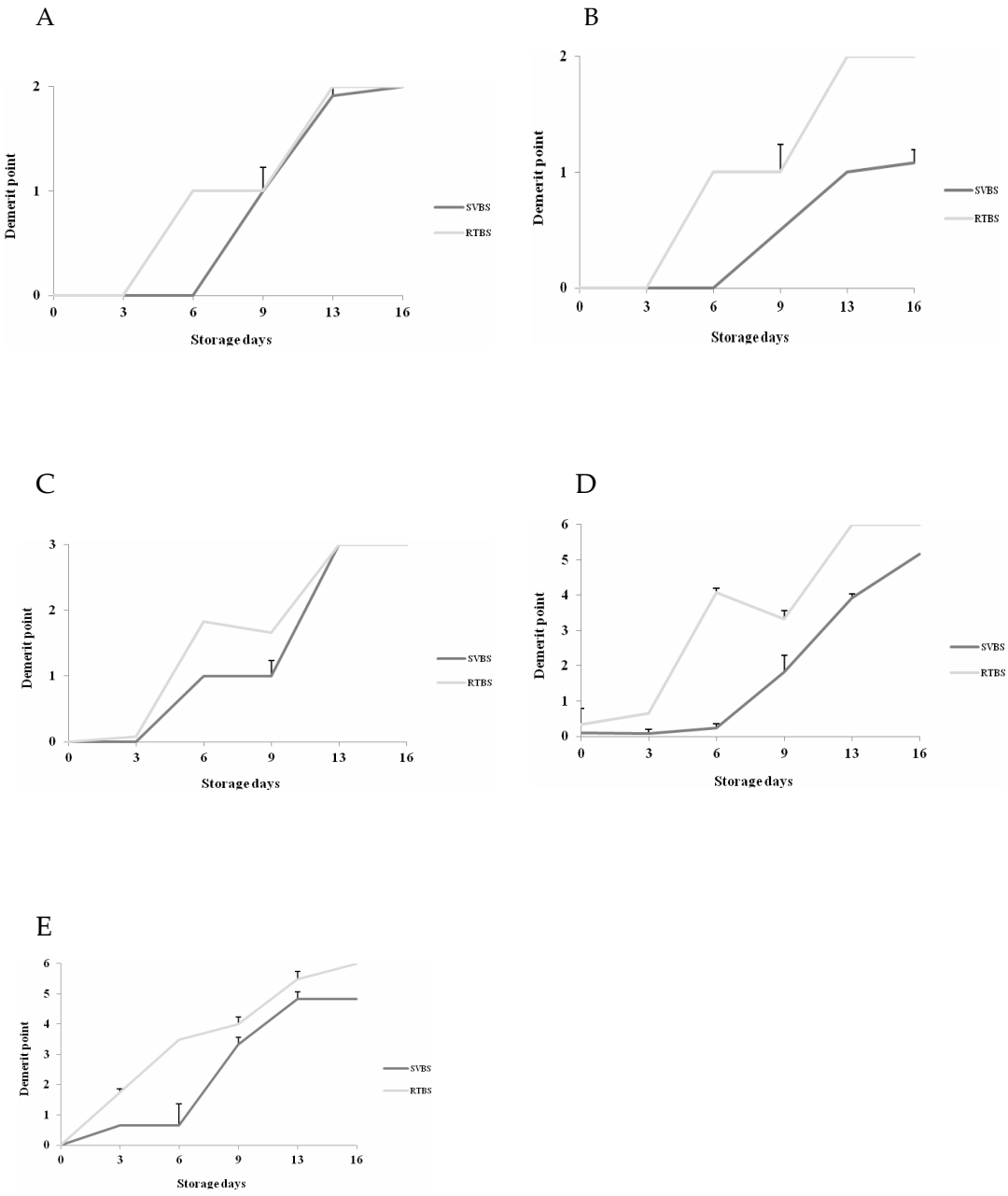

Figure 9 Attribution of demerit points for each parameter of live mussels stored at 3±1°C in conventional (RTBS) and *sous vide* (SVBS) packaging. Reactivity (A), intevalvar liquid aspect (B), odour (C) gills quality (D) and mantle quality (E). Data are reported as average ± standard deviation.

According to the number of days elapsed until the mesophiles reached a population above 5 log CFU/g, mortality below 20% and the mussels obtained an demerit point over 9, it was inferred that live mussels packaged traditionally (with byssus) had shelf –life of ca. 3-6 days while live mussels packaged *sous vide* had an estimated shelf –life of ca. 9 days (tab. 6).

Histological evaluation

Ctenidia hystological sections of *M. galloprovincialis* stored in conventional (RTBS) and *sous vide* (SVBS) packaging are showed in fig. 10. After 1 storage days ctenidia presented normal tissues configuration in both groups.

After 6 storage days in RTBS mussels showed degeneration and loss of cellular boundaries of ctenidia compared to SVBS mussels confirming what was observed with sensory analysis and the prominent role of intervalvar liquid for hydration of the mussels tissues.

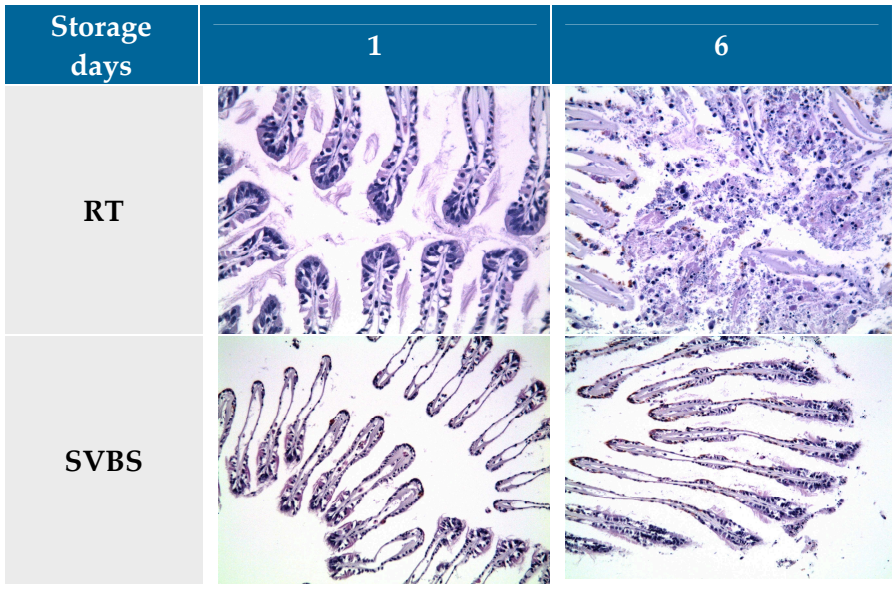

Figure 10 Ctenidia hystological sections of *M. galloprovincialis* stored at 3±1°C in conventional (RTBS) and *sous vide* (SVBS) packaging, after 1 and 6 storage days

Table 6 Established shelf life (ES) of mussels stored at 3±1°C in conventional (RTBS) and *sous vide* (SVBS) packaging, after 1 and 6 storage days

Thesys	Storage days to reach total mesophiles ≥5 log CFU/g (days)	Mortality ≤20% (days)	Days to reach demerit point ≥9 (days)	ES (days)
SVBS	>13	9	9-13	9
RTBS	3-6	6	3-6	3-6

3.3.3 Conclusions

In refrigerated condition (+3° C) and maintaining the cold chain, the use of *sous vide* tecnology to preserve live mussels determined:

1. maintenance of initial product weight;
2. maintenance of the quality attributes;
3. containment-reduction of the microbial population;

That resulted in an extension of shelf life of live mussels from 3 to 9 storage days;
The evaluation of the freshness and sensory attributes, in addition to the chemical-physical and microbiological characterizations, is essential for the assessment of this type of product and the developed scheme can be further tested by future trials.
Histological analysis for the assessment of freshness of shellfish products therefore appears to be a good method, especially if associated with other analysis.

3.4 Effect of byssus removal on live mussels *M.galloprovincialis* stored with *sous vide*

3.4.1 Materials and methods

Raw materials and thesis compared

Live mussels (for total weight of 50 kg) reared mussel farms located in the Gulf of Trieste (Friuli Venezia Giulia-Italy), were maintained in sea water (12°C, 24 ‰ salinity) for 24 h in a purifier station before transfer to the processing plant. Mussels were selected: deformed or undersized individuals (<5 cm) were not considered. After the selected ones were brushed and immersed in refrigerated water (+2°C) to reduce stress.

According to the experimental design showed in table 7, the effect of the mechanical byssus asportation was evaluated: *sous vide* live mussels with byssus (SVBS) were compared to live mussels debissed (SVDB) for 13 storage days at 3°C ± 1°C to evaluate the effect of mechanical byssus asportation on live mussels (*M. galloprovincialis*)

Table 7 Experimental design

Factors	Low	High	Levels	Units/abbr.
Storage	1	13	13	days
Treatment	1	2	2	SVBS, SVDB

Debissing

For SVDB sample preparation, 25 kg of mussels were subjected to mechanical debissing, before packaging, with a dedicated machine equipped by rollers with blades, mounted on an oscillating frame able to pull and cut the external part of the byssus of the mussel.

Sous-vide packaging

Both SVBS and SVDB were packaged, in groups of 15 individuals, in Polyamide/Polyethylene bags (140 μm of thickness, Orved S.p.A., Musile di Piave, VE) and vacuum condition was obtained with a vacuum machine VM 53 (Orved S.p.A., Musile di Piave, VE). Packaged mussels were kept at 3°C ± 1°C for 13 days.

Sampling

According the table 8, four mussels packs for each treatment were analysed in quadrupled daily (from 1 to 13 storage days) for mortality, sensory properties and percentage of intervalvar liquid realeased, while were analysed in duplicate microbiological and pH analysis on days 2, 6 and 12.

Mortality evaluation

Mussels mortality was evaluated by tapping on gaping bivalve shells and any shells remaining open were considered dead according to Bernardez & Pastoriza, (2011) and Goncalves *et al.* (2009).

Table 8 Sampling

Analysis	Packs (*n*)	Treatments (*n*)	Sampling (*n*)
Mortality evaluation	4	2	13
Liquid release	4	2	13
pH	2	2	3
Microbiological analysis	2	2	3

Liquid Release in the pack

Liquid released was evaluated in terms of percentage of intervalvar liquid released in pack respect to total initial mussel weight according to Bernardez & Pastoriza (2011).

Microbiological analysis

Samples were analyzed according to official microbiological methods, to determine the main microbial population and the hygienic quality of the mussels as previously described.

Each specimen has been opened using a sterile cutter to recovery the soft parts and intervalvar liquid. Ten grams of each sample were transferred into a sterile stomacher bag, 90 mL of saline-peptone water (8 g/L NaCl, 1 g/L bacteriological peptone, Oxoid, Milan, Italy) were added and mixed for 1.5 min in a Stomacher machine (PBI, Milan, Italy). Further decimal dilution were made in the same solution and the following microbial analyses were performed in duplicate agar plates: The total aerobic bacterial count (TBC) was determined on Plate Count Agar (PCA, Oxoid, Milan, Italy) following the pour plate method and incubated for 48 -72h at 30°C ;

Lactic acid bacteria (LAB) were determined on double layer of Man Rogosa Sharpe lactobacillus agar (MRS, Oxoid, Milan, Italy) and incubated for 48h at 30°C. Anaerobic Sulphite-reducing clostridia were enumerated in double layer SPS agar (Oxoid, Milan, Italy) incubated for 48h at 37 °C under anaerobiosis.

Enterobacteriaceae were determined were determined on double layer of Violet Red Bile Glucose Agar (VR-BA Oxoid, Milan, Italy) incubated for 48h at 37 °C.

Statistics

Experimental data were subjected to analysis of variance, ANOVA according to completely random experimental design, and average values were compared used the Duncan's test for $P<0.05$ using the SPSS-PC release 17 statistical software (SPSS Inc. Chicago, IL, USA).

3.4.2 Results and discussion

Mortality

The effect of byssus asportation on the mortality is reported in fig. 11. In both groups, SVBS and SVDB, low mortality was observed till 3 storage days (1.7%) and tended to increase during subsequent storage period.

The group SVBS presented lower mortality incidence during the entire period storage compared SVDB and significant differences, among the groups are observed at 6, 8, 9 and 11 storage days. Byssus consists of extracellular threads with mainly proteinaceous components, as collagen/elastin protein (Gosling, 2004, Hagenau *et al*., 2009) secrete by byssus gland placed in the foot of Mytilus spp. Mechanical removal byssus could cause injuries to the foot and other organs connected, compromising mussels vital functions.

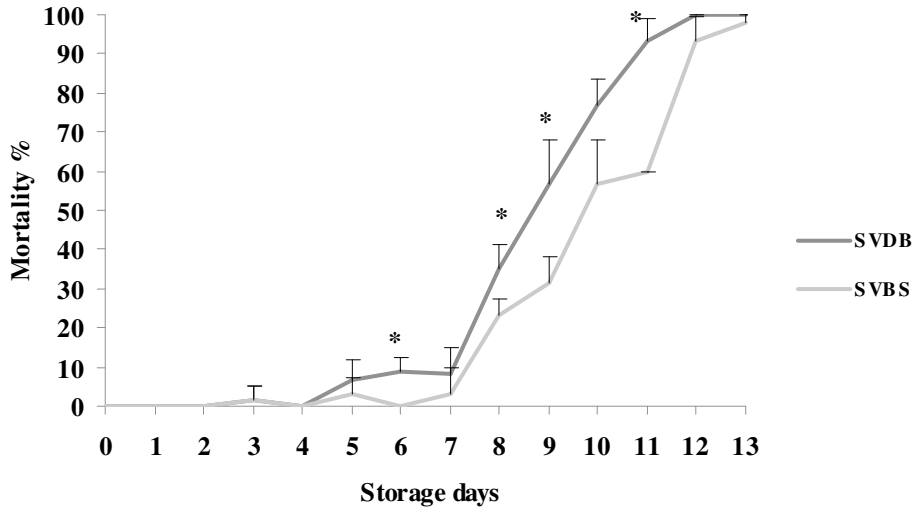

Figure 11 Percentage mortality of Adriatic mussels stored at 3±1°C in *sous vide* packaging with byssus (SVBS) and debissed (SVDB). Data are reported as average ± standard deviation, P<0,05

Intervalvar Liquid release

The effect of byssus removal on percentage of liquid released of the mussels storage *sous vide* pack is showed in fig.12.

In both groups intervalvar liquid release in the pack was mantained constant during the first 8 days of storage (around 5-8%), then it rapidly increased.

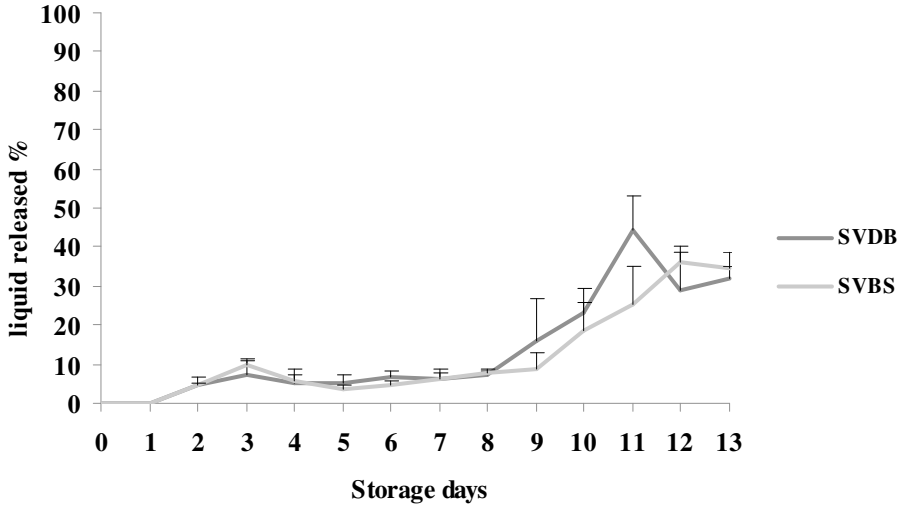

Figure 12 Liquid released of live mussels stored to 3±1°C in *sous vide* packaging with byssus (SVBS) and debissed (SVDB). Data are reported as average ± standard deviation, P<0,05

Microbiological and pH analysis

The changes in the average total bacterial count in SVBS and SVDB live mussels during chilled storage at 3±1°C are shown in Fig.13. The byssus asportation did not influenced the TBC (P>0.05).

The SVBS had initial TBC of 3.4±0.5log CFU/g and this values increased until to 4.0± 0.6log CFU/g after 13 storage days.

In SVDB the initial value in TBC was 2.6±0.5 log CFU /g and this values increased to 3.5± 0.3 log CFU/g after 13 storage days.

Similar values in *Lactic acid bacteria* were observed in both groups SVDB and SVBS during the storage period (2.3±0.2 log CFU/g and 2.1±0.3 log CFU/g).

Sulphite reducing bacteria and *Enterobacteriacae* was always and in both groups below the detection limit of the method (1 log CFU /g) .

The pH of the soft parts homogenate, although modified during storage, does not reveal significant differences between groups SVBS and SVDB (data not shown).

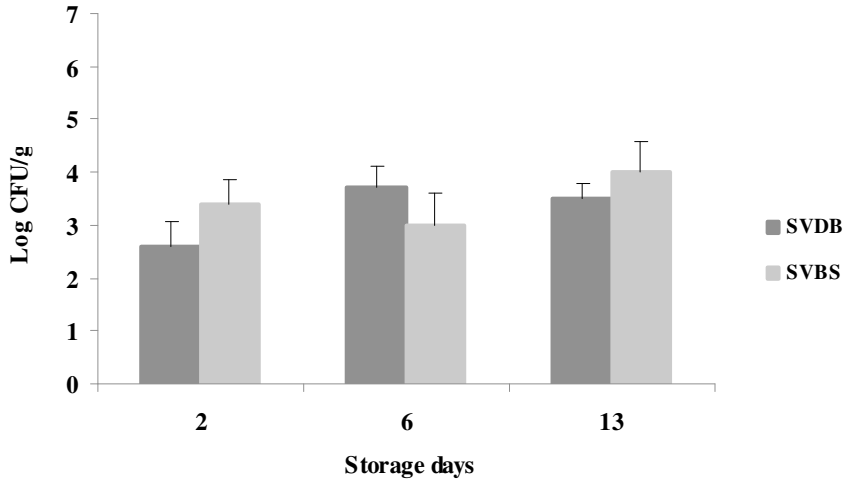

Figure 13 Total bacterial count in Adriatic live mussels stored to 3±1°C in *sous vide* packaging with byssus (SVBS) and debissed (SVDB) (n= 2). Data are reported as average ± standard deviation

Conclusions

Byssus removal did not affect microbiological caracteristics, pH of edible portion and percentage of intervalval liquid released, but increased mortality after 8 storage days.

The application of alternative methods of debissing such as cutting, instead of the mechanical snatch, could prevent alteration of anatomical structures of mussels and ensure the convenience of use of the product.

3.5 References

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4. APPLICATION OF HEAT TREATMENT "*SOUS VIDE* COOK AND CHILL" TO MUSSELS (*M.GALLOPROVINCIALIS*)

4.1 Introduction

4.1.1 Ready meals: evolutionary scenarios

Ready meals are packaged food product already prepared to be consumedng with minimum handling. Ready meals can be defined as already complete meals that require few or no extra ingredients, prepared by external procedures and designed to fully and speedily replace- at home- the main course of a home-made meal (Costa *et al.,* 2001; Calderon *et al.,* 2010; Mahon *et al.,* 2006).
Generally, this product require only heating or hydratation and they shoud be distinguished from ready-to- eat take away foods, as the former type of home meal replacement still requires some cooking or re-heating prior to serving whereas with take away foods no cooking or heating is needed anymore (Verlegh & Candel 1999).
The ready meals can be divided into 4 categories: frozen, chilled, dried, canned (Cellie, 2011).The demand for ready meals is now increasing (Hospido *et al.,* 2006). The table 1 shows that have increased production volumes of ready meals on the market from 2004 in the G8 countries, from 8 million kg in 2004 to 8.8 million kg in 2009, it is estimated that in 2014 will increase to 9.7 million kg.
Also in Italy we observe a growing interest in the ready meals production, in fact, the production volume has increased from 0.164 million of kg in 2004 to 0.209 million kg in 2009, it is estimated that in 2014 our country will produce 255 million of kg of ready meals with an Compound Annual Growth Rate as 5%, and an increasing value (euro m) for all categories of ready meals (table 1 and fig.1).
The increase in volume is determined by the increase in consumer demand. Several socio-economic evolution and lifestyle, such as increased female participation in the workforce, increasing time pressure brought about by job and leisure related activities (Browers, 2000; Calderon *et al.,* 2010), a growing number of single-person and small households (Byrne, 1998), and lack of abilities and experience with preparing meals at home (Gofton, 1995) have boosted the demand for convenience in meal preparation (Costa *et al.,* 2007).

The added value, that the product obtained after transforming, represents an interesting aspect for companies in when the sale of raw product gives a low profit margin.

Today's food industry and retailers have readily reacted to this growing demand for convenience by considerably expanding their assortment of pre-prepared meal solution, including ready meals (Nele *et al.*, 2008).

Table 1 Ready meals production (kg, million) in G8 countries (Source Datmonitor)

Country	2004	2009	2014	CAGR 04-09	CAGR 09-14	CAGR 04-14
US	3,493.3	3,704.6	4,022.9	1.2%	1.7%	1.4%
Japan	1,867.7	1,947.1	2,072.6	0.8%	1.3%	1.0%
UK	636.6	734.6	806.8	2.9%	1.9%	2.4%
Russia	474.8	653.3	798.9	6.6%	4.1%	5.3%
France	572.0	605.0	648.5	1.1%	1.4%	1.3%
Germany	499.0	593.3	692.8	3.5%	3.2%	3.3%
Canada	356.8	370.9	386.0	0.8%	0.8%	0.8%
Italy	164.2	209.1	255.0	5.0%	4.1%	4.5%
Total	**8,064.3**	**8,817.9**	**9,683.6**	**1.8%**	**1.9%**	**1.8%**

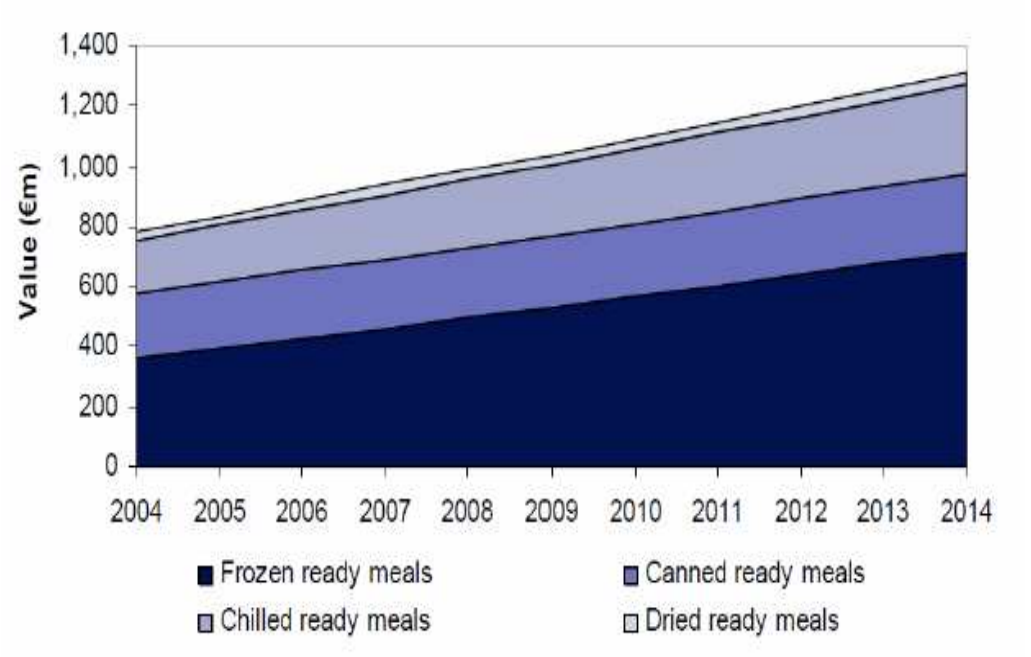


Figure 1 Italy ready meals, value by category (euro m), 2004-14 (Source Datamonitor)

In the sector of fish products can be observed a growing interest towards products ready-to eat and fast to prepare, easy to store and the market offers new ready meals with fish and seafood (Scano 2009, Kennedy *et al.*, 2007). Although the growing demand from consumers and the high quality and quantity of mussels produced in Italy, the market of *M. galloprovincialis* is not supported by development of processing techniques able to maintain product quality and facilitate storage and utilization along the supply chain. For seafood products, the demand for convenience is a trend that needs to be addressed by research and development. The combination of convenience and sensory quality, however, must never compromise food safety.

4.1.2 Heat processing in fish and seafood

Thermal processing is still one of the most common methods for achieving safe convenience foods with an extended shelf life. Designing thermal process for such products, typically in the range of 60-95 °C for 10 to 30 min, is challenging since

the load required for inactivating target microrganism may cause undesiderable quality changes in the lipid and protein fraction. The quality of fish is severely reduced if the thermal process is designed for a shelf life of more than 21 days under chilled condition (Rosnes *et al.,* 2011). New methods focusing on a rapid or minimal heating of fish products, while maintaining the safety level, are therefore vital for future developments. *Sous vide* cook-chilled (SVCC) foods are gaining popularity in institutional catering, fast food trade and supermarkets. This processing and product originated in France in the 1970 when a chef experimented vacuum sealing and slow cooking as a way to extend shelf-life and prevent shrinkage of foie gras. When the foie gras was consumed, it tasted as though it had been freshly prepared (Ghazala *et al.,* 1995). The processing is simple and consists in fresh, raw products placed in a bag or a semi-rigid tray, vacuum sealed, cooked slowly under mild heating conditions as pasteurization, quick immediate cooling and subsequent refrigerated storage at 3±1°C until serving (Rhodehamel, 1992; Hansen *et al.,* 1995).

SVCC processing has many advantages over traditional food processing. The hermetic seal prevent moisture loss, there is no chance for post-process contamination as the heat processing, chilling and storage takes place in packages. Heat treatment is less severe in order to preserve the original flavour / texture and nutritional qualities, vacuum packaging increases the shelf life by inhibiting aerobic spoilage microrganism, oxidative and other chemical spoilers (Rhodehamel, 1992; Ghazala, 1995).

Safety of SVCC products depends on the heat treatment and low temperature storage. Salted fish products are popular in many countries around the world. The addition of NaCl plays an action on water activity used in food product (Lupin 1984) and inhibits microbial growth and the combined use of the salt with the treatment of pasteurization and low storage temperature represents a strategy to hinder bacterial growth and maintain stable characteristics of a food product.

The possibility of producing pasteurized mussels ready to use, with a more extended shelf life and high quality product, could become an innovation for companies in the sector that may give additional value to this aquaculture

product (Venugopal, 2003), but there are many references to the safety of these products.

Bivalve molluscs, as filter feeders, can accumulate microorganisms, including pathogens, from seawater (Cook, 1991) and the number and type of microorganisms present in the water depend on several seasonal, climatic and anthropogenic factors (Šolìć *et al.,* 1999). The microbiota found in shellfishes can be divided into autochtonous and allochtonous microorganisms, and respects the microbial population of the water in which they grow.

As stated before, the production, harvesting and commercialization of live bivalve molluscs is regulated by the EC directive 79/923 (Anonymous, 1979), which defines the classification of the waters in which the mussels grow, and by the EC directive 91/942 (Anonymous, 1991) which states the safety standards for live mollusc sale (Vernocchi *et al.,* 2007).

Bacteria from the environment such as psychrotrophic *C. botulinum* tipe E and *L. monocytogenes* may easily contamine fish and shellfish (Rosnes *et al.,* 2011), pathogenic toxin-producing *Bacillus cereus* is not associated with raw fish materials, but may be a risk factor from the ingredients in mixed or minced fish products or in marinades (Feldhusen 2000).

The UK Advisory Commitee on the Microbiological Safety of Food (AC, MSF 1992) and European Commission (1997) recommends for cooked-chilled products with an extended shelf life of more than 10 days a heat treatment at 90° for 10 min or equivalent lethaly and strict chill conditions to control *Clostridium botulinum.*

Regard to *Lysteria monocytogenes*, ICMSF (1996) recomend a heat treatments at 70°C for 2 min or an equivalent heat process is required.

4.2 Main goals of the research

There are any work about the application of *sous vide* cook-chilled on fish product Shakila *et al.*, 2009, Diaz *et al.*, 2009 tested this treatment on salmon (Salmo salar), and established the shelf life of this product storage at +2°C, Gonzalez-Fandos *et al.*, (2005-2004), Garcia –Linares *et al.*, (2004) tested *sous vide* cook chill on salmon and trout (latin names not given); Fagan and Gormley (2005) reported data of Ghazala *et al.*, (1995) several studies on mussels were made on chilled storage (Gökog˘lu 2002; Vasakou et al. 2003; Erkan 2005; Ziino et al. 2005), modified atmosphere packaging (Pastoriza et al. 2004; Goulas et al. 2005), smoking (Dalgiç and Erkoyuncu 2003; Kyriazi-Papadopoulou et al. 2003) or canning of Mediterranean mussels (Sengör et al. 2003, 2004) but there are still no work on the application of sous-vide cook-chilled treatment with seafood.
The application of the *sous vide* cook and chill technique to mussels was evaluated by means of three trials.

The first trial (paragraph 4.3) was aimed to identify the range time/temperature combination that results in the desidered microbial inactivation (F value) of natural microbial contamination of mussels.

In the second trial (paragraph 4.4) the optimal time/temperature combination identify in previous trial was applyed to mussels experimentally inoculated with *Pseudomonas* spp. to confirm the efficacy of the *sous vide* cook process.

The third trial (paragraph 4.5) was aimed to assess the stability of the product *M.galloprovincialis sous vide* cook and chill, with and without the addition of brine in comparison to conventional cooking during over time, determing their shelf-life. In this ultimate step, to have a complete view of degradation process, were considered microbiological, sensory and chemical-physical aspects.

4.3 Effect of *sous vide* cook tecnology on natural bacterial load of mussels (*M. galloprovincialis*)

4.3.1 Materials and methods

Raw material and treatment

Commercial live mussels from Spanish mussel farms, acquired in local supermarket, were transported in the Department of Food Science of Udine University under controlled temperature conditions. After manual removal of the byssus, they were brushed and processed.

Mussels were packaged, in groups of 15 individuals, in OPA/PP bags (15/65 μm, 250 x 300mm, Orved S.p.A., Musile di piave, VE) and vacuum conditions were obtained with a vacuum machine VM 53 (Orved S.p.A., Musile di Piave, VE).

Mussels were cooked, with trivalent oven HMG061X (Lainox Ali S.p.A., Treviso) and immediately chilled to +3°C using a blast chiller RCM051S (Lainox Ali S.p.A., Treviso) maintained to 3±0.2°C. Temperature condition during process was monitored in the heart of the products with a datalogger sensors Tracksense Pro Val (Fasinternational, Milan, Italy), the management of the probes and processing of time-temperature data were performed with the software Valsuite Basic (Ellab, Hilleroed, Denmark).

The pasteurization value (P0) and Cook value Cg) have been determined about previus trials aimed to optimize thermal process (Benedetti 2011).

Thesis compared

According the experimental design show in table 2, to evaluate the effect of the heat treatment *sous vide* cook on natural bacterial load of mussels (*M. galloprovincialis*), 3 temperature condintion (75, 85 and 95°C) and three cooking time were considered and compared to 1 control RM (raw mussels).

- M75/10 and M75/30 were mussels treated with *sous vide* cooking to 75°C for 10 and 30 min in core respectively and oven condition to 80°C for 15 and 35 min;

- M85/10 M85/20 M85/30 were mussels treated with *sous vide* cooking to 85°C for 10, 20 and 30 min in at the coldest point and oven condition to 90°C for 15, 25 and 35 min.
- M95/10 and M95/30 were mussels treated with *sous vide* cooking to 95° for 10 min and 30 min in core respectively and oven condition to 100°C for 15 and 35 min.

Table 2 Experimental design

Factors	Low	High	Levels	Units
Temperature	75	95	3	°C
Time	10	30	3	Min
Treatment	1	1	1	
Control	1	1	1	RM

Sampling

After each treatments mussels were analysed for microbiological characteristics and pH according to table 3 for each sampling time.

Table 3 Sampling

Analysis	Packs (*n*)	Treatments and control (*n*)	Sampling time (*n*)
Microbiological analysis	2	8	1
pH	2	8	1

Microbiological analysis and pH

Samples were analayzed by official microbiological methods, to determine the main microbial population and the hygienic quality of the mussels.

Ten grams of each sample were transferred into a sterile stomacher bag, 90 mL of saline-peptone water (8 g/L NaCl, 1 g/L bacteriological peptone, Oxoid, Milan, Italy) were added and mixed for 1.5 min in a Stomacher machine (PBI, Milan,

Italy). Further decimal dilution were made in the same solution and the following microbial analyses were performed in duplicate agar plates.
Total aerobic bacterial count (TBC) was determined on Plate Count Agar (PCA, Oxoid, Milan, Italy) following the pour plate method and incubated for 48 -72h at 30°C;
Lactic acid bacteria (LAB) were determined on double layer of Man Rogosa Sharpe lactobacillus agar (MRS, Oxoid, Milan, Italy) and incubated for 48h at 30°C.
Anaerobic Sulphite-reducing clostridia were enumerated in double layer SPS agar (Oxoid, Milan, Italy) incubated for 48h at 37 °C under anaerobiosis.
The pH was measured by dipping the pH electrode into a mixture in 15 mL of homogenized mussel with digital pH-meter (model basic 20 Crison instruments S.A., Barcelona, Espaňa).

4.3.2 Results and discussion

Microbiological quality and pH
The effect of different time-temperature combinations for heat treatment *sous vide* cook on natural bacterial load of mussels (*M. galloprovincialis*) on total viable count (TVC), lactic acid bacteria (LAB) and anerobic sulphite-reducing clostridia, are shown in Fig. 2.
Raw mussels (RM) had initial TVC of 4.8±0.1 log CFU/g, after treatment at 75°C for 10 and 30 min TVC decreased to 1.7±0.9 log CFU/g (-3.1 log) and below the detection limit of the method (-4.8 log), respectively.
After treatment at 85°C for 10, 20 and 30min, TVC decreased to 2.4±0.4 (-2.4 log), 2.1±0.3 (-2.7 log) and 1.3±0.7 (-3.5log) log CFU/g respectivly.
After treatment at 95°C for 10 and 30 min TVC resulted below the detection limit of the method (-4.8 log). The heat treatment had a significant effect betwen M75/10-30, M85/20 and M95/10-30.

The results observed in this study resulted similar to ones conducted on different species of fish such as rainbow trout (*Onchorinchus mikyss*) (Gonzalez-Fandos *et al.,* 2004), salmon (*Salmo salar*) (Gonzalez-Fandos *et al.,* 2005; Garcia-Linares *et al.,* 2004) and carp (*Cyprinus carpio*) (Can, 2011).

Rainbow trout (*Onchorinchus mikyss*) that reported an initial mesophiles count of 4.40±0.5 log CFU/g after process by the *sous vide* tecnology with 3 different combination of time/temperature combination (70°C for 10 min and 90° C for 15 and 5 min) obtained a reduction of bacterial load to about 2.5-3 log CFU/g according to the treatment (Gonzalez-Fandos *et al.,* 2004).

Salmon (*Salmo salar*) slice processed in the same way and with similar combination time/temperature (65° for 10 min and 90° C for 15 an 5 min) before of treatment reported an mesophiles count on raw salmon of 4.7±0.4 log CFU/g that decrease to 3-4 log CFU/g according to the treatment (Gonzalez-Fandos *et al.,* 2005).

Similar values for the same species processed with *sous vide* method at 90° for 10 min, was reported from Garcia-Linares *et al. (*2004).

Shakila *et al.,* (2009) in work on fish cake processed *sous vide* tecnolology at 100°C for 20 min, reported an initial total bacterial count of 3.8±0.4 log CFU/g that decreased after *sous vide* treatment to 2.3log CFU/g.

Finally, recent study on carp fillet processed by *sous vide* method, with or without addition in pack of the sauce, show that the treatment resulted efficacy to reduce the mesophiles count from 3.9log CFU/g, initial in raw carp, to about 2 log CFU/g in carp fillet with sauce and 3log CFU/g in carp fillet without sauce (Can 2011).

LAB in raw mussels were 1.8±0.2log CFU/g and were below the detection limit of the method (1log CFU/g) after all different treatments. These results are in according with data reported by the authors previously cited Gonzalez- Fandos *et al.,* (2004-2005) and Can (2011) that detected 2.4 -2.8, 3.5-4.0 and 2.6 log CFU/g LAB in raw trout, slice salmon and carp respectively, after heat treatment their levels resulted below the detection limit (<1log CFU/g).

Guerzoni *et al.* (1999) studied the changes over time of the lactic acid bacteria (LAB) in *sous vide* meat products observing that LAB were undetectable immediately after the *sous vide* treatment in meat products, but that they could

recover during storage. However, these authors reported that the lactic acid bacteria growth only occurred sporadically in a few samples.
In according with Gonzalez- Fandos *et al.,* (2004-2005) and Can (2011) anerobic sulphite-reducing clostridia were not found in fresh and processed mussels.
The heat treatments did not influence the pH values of the homogenate compared to the control sample (6.27 vs. 6.18) (P>0,05) (fig. 3).

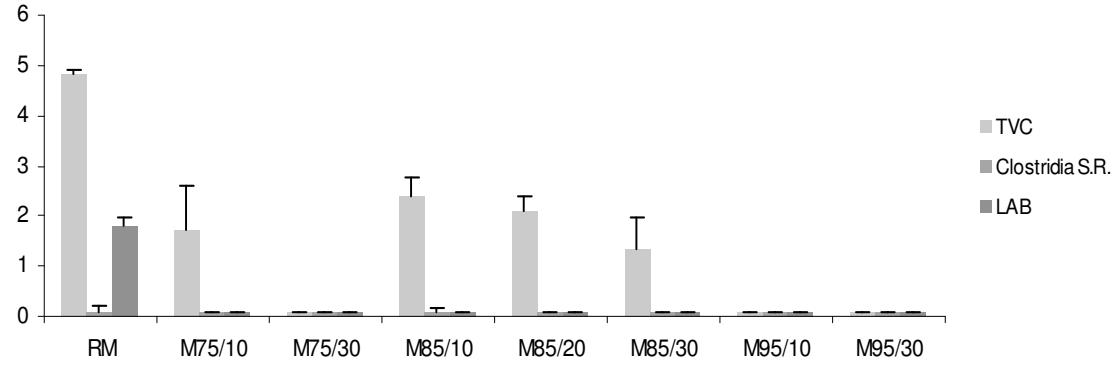


Figure 2 Effect of different time-temperature combinations in *sous vide* cook treatment on natural bacterial load of mussels. Data are reported as average + standard deviation

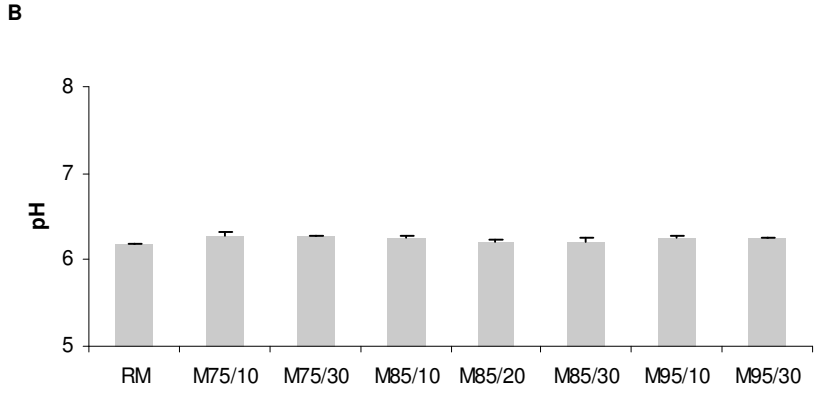


Figure 3 Effect of different time-temperature combinations in *sous vide* cook treatment on pH of mussel homogenate. Data are reported as average + standard deviation

4.3.3 Conclusions

The present study indicates that all time/temperature combination tested with *sous vide* cook treatment where able to reduce the natural microbial population present in raw mussels; other tests will be necessary to evaluate the effect of this heat treatments on products with higher microbial contamination.
Stability of these products can be considered during storage under refrigeration conditions and thermal abuse, taking into account the microbiological, chemical, physical and sensory characteristics.

4.4 Effect of *sous vide* cook tecnology on mussels (*M. galloprovincialis*) inoculated with suspension of *Pseudomonas sp.*

4.4.1 Materials and methods

Preparation of the inoculum

Five strains of *Pseudomonas* were used for the inoculum preparation: *Ps. syringae*, *Ps. fluorescens*, *Ps. libanensis*, *Ps. dessavai*, *Ps. putida*,.

Bacteria were plated on Pseudomonas Agar Base (PAB) and incubated for 48 h at 30°C. A colony for each strain, was sowed into BHI steril broth, and incubated for 48 h. The broth with bacteria was portioned in steril eppendorf tubes (1.5 mL) and centrifuged for 5 min. at 13000 rpm, by Mini spin (model Eppendorf) to remove the BHI, bacteria and precipitates on the bottom of each eppendorf, were suspended in 0.75 mL physiological solution and recovered in one mix. The presumed bacterial load was evaluated measuring the absorbance of the suspension, using spectrophotometric method with NanoDrop 200c (Thermo scientific, USA) The suspension was diluted until reaching a abs equal to 1, corresponding to a bacterial load of about 10^8UFC/mL (according to previous tests on plate). Suspension was further diluted to obtain a suspension with presumed load of 10^7 UFC/mL, after dilutions were seeded on plates to verify the real bacterial load.

Raw material

Commercial live mussels from Spanish mussel farms and acquired in local supermarket, were transported in the Department of Food Science of Udine University, under controlled temperature conditions.

After removal of the byssus, were individually inoculated (fig 4 A), using a 1mL syringe volume, with 100 μL of inoculum, previously prepared, the syringe needle was inserted between the valves of the mussel and inoculum injected in correspondence of the stomach. Mussels were placed in groups of 3 individuals in OPA/PP bags (15/65 μm, Orved S.p.A., Musile di Piave, VE) appropriate for *sous-*

vide cooking, and vacuum conditions were obtained with a vacuum machine VM 53 (Orved S.p.A., Musile di piave, VE). Except the control, mussels were cooked in trivalent oven HMG061X (Lainox Ali S.p.A., Treviso) (fig 4 B) and immediately chilled at +3°C using blast chiller RCM051S (Lainox Ali S.p.A., Treviso) maintained at +3° C. Temperature condition during process was monitored in the heart of the products with datalogger sensors Tracksense Pro Val (Fasinternational, Milan, Italy), the management of the probes and processing of time-temperature data were performed with the software Valsuite Basic (Ellab, Hilleroed, Denmark).

Thesis compared

According the experimental design, show in Table 4, six thesis (3 cooking temperature x 2 cooking time x 1 treatment) were compared respect 2 control (FM raw mussels and IFM inoculated raw mussels).

- IM75/10 and IM75/30 were inoculated mussels packaged and cooking at 75°C for 10 and 30 min in core respectively with oven condition at 80°C for 15 and 35 min;
- IM85/10 and IM85/30 were inoculated mussels packaged and cooking at 85°C for 10 min and 30 min in core respectively with oven condition at 90°C for 15 and 35 min;
- IM95/10 and IM95/30 were inoculated mussels and packaged and cooking at 95°C for 10 min and 30 min in core respectively with oven condition at 100°C for 15 and 35 min;

Table 4 Experimental design

Factors	Low	Hight	Levels	Units
Temperature	75	95	3	°C
Time	10	30	2	Min
Treatment	1	1	1	SVCC
Control	1	2	2	FM, IFM

Sampling

After each treatments and for each sampling time mussels were analysed for microbiological characteristics and pH acconding table 5.

Table 5 Sampling

Analysis	Packs (*n*)	Treatments and control (*n*)	Sampling time (*n*)
Microbiological analysis	3	12	1
pH	2	12	1

Microbiological analysis and pH

Samples were analyzed by traditional microbiological methods, to determine the main microbial population and the hygienic quality of the mussels.

The entire contents, except the shells, of each pack and for each treatment, was transferred into sterile stomacher bag and mixed for 1.5 min stomacher machine (PBI, Milan, Italy). Serial decimal dilutions of each homogenate mussels were carried out using 9 mL saline-peptone water (8 g/L^{-} NaCl, 1/g L^{-} bacteriological peptone, Oxoid, Milan, Italy) (fig 4 C). the following microbial analyses were performed in duplicate agar plates: The total aerobic bacterial count (TBC) was determined on Plate Count Agar (PCA, Oxoid, Milan, Italy) following the pour plate method and incubated for 48 -72h at 30°C ;

Pseudomonas were enumerated on *Pseudomonas* Agar Base (PAB, Oxoid) by spread plating technique after incubation at 30°C for 48 h.

Lactic acid bacteria (LAB) were determined on double layer of Man Rogosa Sharpe lactobacillus agar (MRS, Oxoid, Milan, Italy) and incubated for 48h at 30°C. Anaerobic Sulphite-reducing clostridia were enumerated in double layer SPS agar (Oxoid, Milan, Italy) incubated for 48h at 37 °C under anaerobiosis.

The pH was measured by dipping the pH electrode into a mixture in 15mL of homogenized mussel with digital pH-meter (model basic 20 Crison instruments S.A., Barcelona, Espaňa).

A B C

Figure 4 Some phases of the experiment: mussels inoculation (A), mussels cooking (B), microbiological analysis (C)

4.4.2 Results and discussions

Microbiological evaluation and pH

The effect of different heat treatment *sous vide* cook on mussels (*M. galloprovincialis*) inoculated with suspension of *Pseudomonas sp.* on total bacterial count (TBC), lactic acid bacteria (LAB) and anerobic sulphite-reducing clostridia, are shown in Fig. 5 (A, B,C).

Before of heat tratment 75°C, 85°C and 95°C, FM (raw mussels) had initial TVC of 3.6±0.1, 3.7±0.1 and 4.6± 0.3 log CFU/g respectively, and IFM initial TVC of 5.3 ± 0.7, 5.5±0.0 and 5.2±0.2 log CFU/g .

TVC decreased in IM75/10 and IM75/30 to 1.4±0.1 and 1.1± 0.5 log CFU/g (-3.9 and -4.2 log) respectively (table 5).

Table 5 Microbiological quality in fresh mussels (FM) and inoculated fresh mussels (IFM) and inoculated-treated mussels to 75°C for 10 (IM75/10) and 30 min (IM75/30)

	TBC log CFU/g	*Psedomonas* log CFU/g	C.S.R log CFU/g	LAB log CFU/g
FM	3.6±0.6	4.4±0.2	1.4±0.1	2.6±0.1
IFM	5.3±0.7	5.5±0.2	0.8±0.2	2.5±0.1
IM75/10	1.4±0.1	<1	0.5±0.3	1.4±0.2
IM75/30	1.1±0.5	<1	0.5±0.3	1.1±0.6

Data are reported as average ± standard deviation

In IM85/10 IM85/30 TVC decrease to 2±0.2 and <1 log CFU/g (-3.5 and -5.1 log CFU/g) respectively (table 6).

Table 6 Microbiological quality in fresh mussels (FM) and inoculated fresh mussels (IFM) and inoculated-treatated mussels to 85°C for 10 (IM85/10) and 30 min (IM85/30)

	TBC log CFU/g	*Psedomonas* log CFU/g	C.S.R log CFU/g	LAB log CFU/g
FM	3.7±0.1	5.6±0.8	1.8±0.3	2.4±0.2
IFM	5.5±0.0	6.1±0.1	2.0±0.4	2.6±0.5
IM85/10	2.0±0.2	<1	0.8±0.1	1.8±0.1
IM85/30	<1	<1	0.7±0.1	1.3±0.3

Data are reported as average ± standard deviation

In IM95/10 IM95/30 TVC was below the detection limit of the method (<1log) (table 7).

The results showed in this study resulted similar and confirmed those observed in previous trial this thesis (paragraph 4.3) and are in according with the studies conducted on different species of fish or fish products (Gonzalez-Fandos *et al.*, 2004; Gonzalez-Fandos *et al.*, 2005; Garcia-Linares *et al.*, 2004; Shakila *et al.*, 2009; Can, 2011).

LAB before of heat tratment (75°C, 85°C and 95°C) were respectvely 2.6± 0.1, 2.4± 0.2 and 2.2±0.2log CFU/g and IFM initial LAB were 2.5 ± 0.1, 2.6± 0.5 and 2.0± 0.2 log CFU/g.

After treatment LAB decreased in IM75/10 and IM75/30 to 1.4±0.2 and 1.1±06 log CFU/g respectively (table 5).

In IM85/10 IM85/30 LAB decrease to 1.8±01 and 1.3±0.3 log CFU/g respectively (table 6).

In IM95/10 IM95/30 LAB were not found (table 7).

The results shows in this study are similar and confirm those observed in previous trial this thesis (paragraph 4.3) and are in according with data reported by the authors previously cited Gonzalez- Fandos *et al.* (2004-2005) and Can (2011).

Table 7 Microbiological quality in fresh mussels (FM) and inoculated fresh mussels (IFM) and inoculated-treatated mussels to 95°C for 10 (IM95/10) and 30 min (IM95/30)

	TBC log CFU/g	*Psedomonas* log CFU/g	C.S.R log CFU/g	LAB log CFU/g
FM	4.6±0.3	4.8±0.9	1.4±0.3	2.2±0.2
IFM	5.2±0.2	5.4±0.3	1.5±0.1	2.0±0.2
IM95/10	<1	<1	NF	NF
IM95/30	<1	<1	NF	NF

Data are reported as average ± standard deviation

Anaerobic Sulphite-reducing clostridia before of heat treatment 75°C 85°C and 95°C were respectively 1.4±0.1, 1.8± 0.3and 1.4± 0.3 log CFU/g and in IFM were 0.8 ±0.2, 2.0± 0.4 and 1.5± 0.1 log CFU/g.

After treatment Anaerobic Sulphite-reducing clostridia decreased in IM75/10 and IM75/30 to 0.5±0.3 log CFU/g (table 5).

In IM85/10 IM85/30 Anaerobic Sulphite-reducing clostridia decrease to 0.8±0.1 and 0.7± 0.1log CFU/g respectively (table 6).

In IM95/10 IM95/30 Anaerobic Sulphite-reducing clostridia were not found (table 7).

Finally *Pseudomonas*, the tester bacterium, before of heat tratment (75°C 85°C and 95°C for 10 and 30 minutes) were respectvely 4.4± 0.2, 5.6±0.8 and 4.8±0.9 log CFU/g and in IFM were 5.5± 0.2. 6.1± 0.1 and 5.4±0.3 log CFU/g.

After all treatments *Pseudomonas* decreased in each thesis and was below the detection limit of the method (<1log CFU/g).

The heat treatments did not influence the pH values of the homogenate compared to the control sample (6.27 vs. 6.18), similar values were found by other authors on fresh mussels (Kyriazi-Papadopoulou *et al*., 2003, Turan *et al*., 2008).

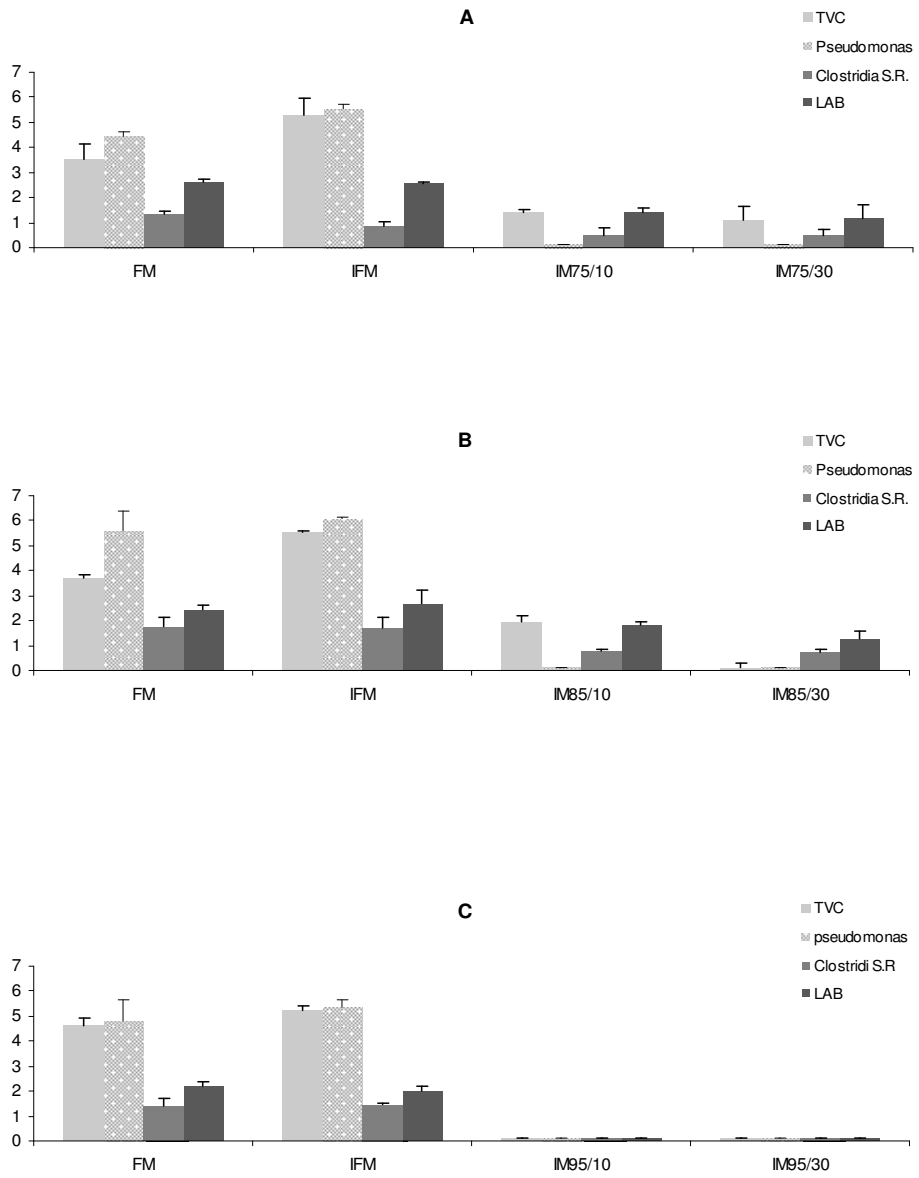


Figure 5 Effect of heat treatment *sous vide* cook 75/10/30 (A), 85/10/30 (B) and 95/10/30 (C) on inoculated mussels (*M. galloprovincialis*). Data are reported as average + standard deviation

4.4.3 Conclusions

The present study indicates that all time/temperature combination tested with *sous vide* cook treatment where able to reduce the microbial population present in the raw mussels including bacterial concentration of *Pseudomonas* spp., inoculated experimentally and confirm the efficacy of *sous vide* cook treatments.
Other tests will be necessary to evaluate the stability of these products during storage in both condition of refrigeration and thermal abuse, taking into account the microbiological, chemical, physical and sensory properties.

4.5 Shelf life of mussels (*Mytilus galloprovincialis*) processed by *sous vide* cook and chill method: evaluation of microbiological, chemical and sensory quality

4.5.1 Materials and methods

Raw material and thesis compared

Live mussels (80 kg) from mussel farms in the gulf of Trieste (Friuli Venezia Giulia-Italy), were maintained in sea water for 24 h in purifier station before transfer to the processing plant. Mussels were selected and deformed or undersize individual was eliminated, after they were brushed and immersed in refrigerated water (+2°C) to reduce stress. After the cutting of the byssus, they were brushed and processed under controlled temperature conditions were transported in the Department of Food Science of Udine University.

According the experimental design shown in table 7, 3 thesis were compared, respect to 2 controls for 50 days: CMC were boiled mussels with conventionally cook and chilled, SVCC were mussels *sous vide* cook-chilled, BSVCC were mussels *sous vide* with brined and cook-chilled.

Treatments

Conventionally cook and chill

For the CMC treatment the clean and selected mussels, with shell, were boiled in water to 100°C for 10 min and were placed in OPA/PP bags (15/65 μm, 250 x 300mm Orved S.p.A., Musile di Piave, VE), in groups of 15 mussels and immediately chilled using blast chiller RCM051S (Lainox Ali S.p.A., Treviso) maintained at 3±0.2°C (fig. 6 C).

Sous vide cook chill

For SVCC treatment, the mussels were packaged *sous vide*, in groups of 15 individuals, in OPA/PP bags (15/65 μm, Orved S.p.A., Musile di Piave, VE) and vacuum conditions were obtained with a vacuum machine VM 53 (Orved S.p.A.,

Musile di piave, VE). Mussels were cooked, at 85°C for 10 min in core, with trivalent oven HMG061X (Lainox Ali S.p.A., Treviso) (fig 7B) and were immediately chilled at +3°C as previously described and maintained to 3±0.2°C (fig 6 C).

Brine sous vide cook chill

In the BSVCC treatment mussels were treated as SVCC mussels plus the addition in the bags was of brine, containig 3g NaCl/100mL with a ratio 1/2 respect total mussels weight (150mL in pack of 300g of mussels) (fig 6B).

Controls

Raw mussels (FM) were analyzed to verify the effect of heat treatments on time 0. Mussels cooked and chilled (CC), under the same conditions of the SVCC and BSVCC mussel, but without vacuum conditions (fig 6 D), were analyzed to verify the effect of vacuum, during heat treatment.

Temperature condition during all process was monitored by datalogger sensors Tracksense Pro Val (Fasinternational, Milan, Italy) (fig. 7 A). The pasteurization value (P0) and Cook value (Cg) was determined in previously test finalized to optimize the thermal process.

Sampling

The mussels for each treatment were analysed in duplicate for physico-chemical, sensory and microbiological characteristics for each sampling time (0 for the control FM, 1-7-14-21-30-50 days treated sample) as sumarizing in table 8. For Total Volatile Basic Nitrogen (TVB-N) content the sample for each sampling time was frozen at -80°C and subsequently analyzed. data are expressed as average value and standard deviations.

Table 7 Experimental design

Factors	Low	Hight	Levels	Units/abbr.
Temperature	85	85	1	°C
Time	10	10	1	Min
Storage	1	50	6	Days
Treatments	1	3	3	CMC, SVCC, BSVCC
Controls	1	2	2	FM, CC

Table 8 Sampling

Analysis	Packs (*n*)	Treatments (*n*)	Sampling time (*n*)
Microbiological analysis	2	5	6
pH	2	5	6
TVB-N	2	5	6
Sensory Analysis	3	5	6
Moisture Content	2 (measurement 30 mussels)	5	6
W H C	6 pools of 15g	5	6

Chemical Analysis

Moisture content was determined according to the Association of Official Analytical Chemists procedures (AOAC 1990). Total volatile basic nitrogen (TVB-N) content was determined according to Pearson (1973).

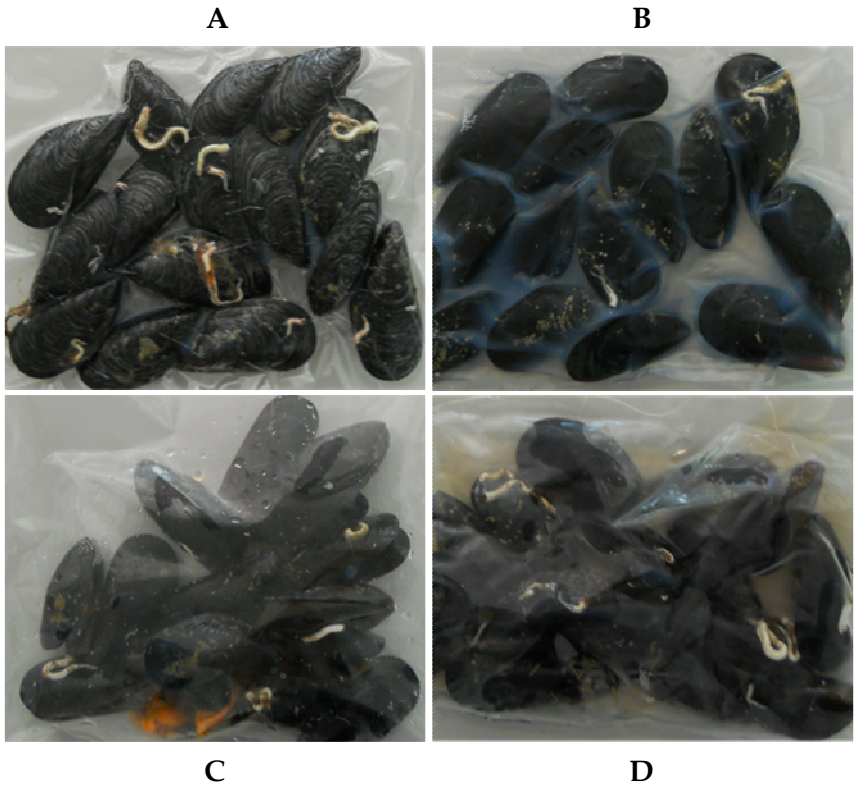
Water Holding Capacity measurement (WHC)

The WHC was measured on mussels pool (15 g) with the adapted method proposed of Ofstad *et al.*, 1993 for Atlantic Halibut. The samples were transferred

to centrifugation tubes and centrifuged at 5°C (210 x g for 15 min). The WHC was determined as liquid loss (LL) and expressed as percentage of weight of liquid released (Hermansson, 1986; Ofstad *et al.*, 1993) as following:

WHC (%)= [weight liquid released (g) / total weight (g)] x100.

Figure 6 Mussels after treatment: SVCC (A) BSVCC (B), CMC (C), CC (D)



Sensory analysis

The sensory analyses were carried out by experienced seven-member panel (fig 7 C), selected and trained under ISO standards (ISO 8586-1, 1993) that evaluated the quality of mussels meat (SVCC, BSVCC, CMC, CC), during processing and chilled storage. In each sampling time, judges rated quality of different mussels using characteristics to describe: color, odor, taste and texture mussel meat. Each

characteristic was scored using a point scale ranging from 1 to 14 according to the scale reported in table 9. If the score was lower than seven, mussels was considered as unacceptable. The sample, 5 mussels for each judge for each thesis, were analysed after heating for 10 min in water-bath at 50°C (mod. MP Julabo 19).

Table 9 Quality scale used in the sensory analysis

Characteristic	Score					
	1	3	6	9	12	14
Color/appearence	Opaque/old					Very bright/fresh
Odor intensity	Rotten seaweed					Fresh
Meat turgidity	Flaccid					Very firm
Flavor	Insipid					Very tasty
Succulence	No juicy					Very juicy
Aftertaste	No persistent					Very persistent

Microbiological analysis and pH

The whole contents, except the shells, of each pack and for each treatment, was transferred into a sterile stomacher bag and mixed for 1.5 min stomacher machine (PBI, Milan, Italy). Serial decimal dilutions of each homogenate mussels were carried out using 9 mL saline-peptone water (8 g/L NaCl, 1/g L bacteriological peptone, Oxoid, Milan, Italy). Following microbiological analyses were perfomed in duplicate agar plates. The total aerobic bacterial count number (TBC) was determined on Plate Count Agar (PCA, Oxoid, Milan, Italy) following the pour plate method and incubated for 48 -72h at 30°C ;

Lactic acid bacteria (LAB) were determined on double layer of Man Rogosa Sharpe lactobacillus agar (MRS, Oxoid, Milan, Italy) and incubated for 48h at 30°C.

Pseudomonas were enumerated on Pseudomonas Agar Base (PAB, Oxoid, Milan, Italy) by spread plating technique and incubated for 48 h at 30°C.

Anaerobic Sulphite-reducing clostridia were enumerated in double layer SPS agar (Oxoid, Milan, Italy) incubated for 48h at 37 °C under anaerobiosis (ICMSF, 1978).

Total enterobacteria were determined on double layer of Violet Red Bile Glucose Agar (VRBGA, Oxoid, Milan, Italy) and incubated for 48 h at +37°C (ICMSF, 1978).

pH was measured by dipping the pH electrode into a mixture in 15 mL of homogenized mussel with digital pH-meter (model basic 20 Crison instruments S.A., Barcelona, Espaňa).

Statistics

Experimental data were subjected to statistical analysis (analysis of variance, ANOVA) according to completely random experimental design, and if appropriate average values were compared used the Duncan's multiple range test for $P<0.05$ using the SPSS-PC release 17 statistical software (SPSS Inc. Chicago, IL, USA).

Figure7 Any steps of experimental activity: datalogger sensors (A), mussels cooking (B), session of sensorial analysis (C)

4.5.2 Results and discussion

Changes in the moisture during process and storage

The moisture content of FM, SVCC, BSVCC, CC and CMC mussels are presented in Fig. 8. Respect to FM (78.9±2.2%) Moisture content significantly decreased after

conventional cooking (CMC 73.5±1.0% on day 1) while increased in BSVCC (81.4±1.3% on day 1) and remain similar in CC (79.9±1.6% on day 1) and SVCC (79.7±1.1% on day 1) to indicate that there are not effect of *sous vide* on moisture content, while would seem to be one effect of heat treatment of pasteurization respect conventional cooking.

Along the 50 days of storage time, the moisture contents were reamained constant in SVCC and BSVCC. Vacuum packaging seem to be able to limit the weight loss respect to conventional cooking.

Concerning salting in fish, Voskresensky (1965), divided this process into three stages. At stage one, the fish is exposed to a high osmotic pressure. The salt diffuses into the fish tissues, but at the same time water moves by osmosis from the fish at a high speed into the surrounding brine and the fish declines in weight. At stage two, salt and water still diffuse as in stage one, but at the same rate so no weight changes takes place. The salt concentration in the surface layer of the fish tissue reaches equilibrium with the surrounding brine. At stage three, minor salt quantities move into the fish tissue and as a result the fish increases slightly in weight.

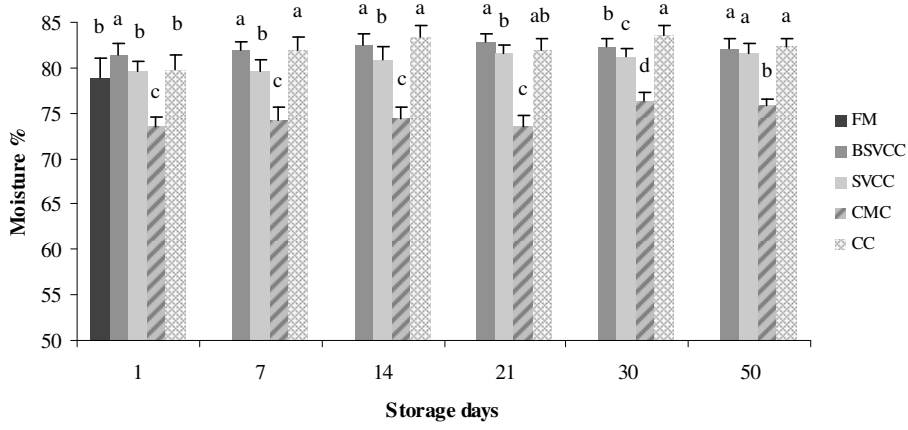


Figure 8 Changes in moisture content of different packs of mussels during processing and chilled storage at 3±1°C. Data are reported as average + standard deviation, different letters indicate significant differences among treatments for each inspection time, P<0,05.

All parts of the fish have reached the same concentration as the surrounding brine. In our case the low content salt in brine could have led the reverse process in mussels BSVCC. Based on this study the pasteurization treatment resulted able to mantained moisture content during storage and the integration of brine have a positive effect on this parameter.
The higher moisture in content in SVCC and in BSVCC, also indicates less weight loss after heat treatment of vacuum cooking tested, therefore beneficial economic.
The TVB-N value decreased after SVCC and BSVCC treatments (12 and 10.35 mg N/100g rexpectively), while dos not vary after CC treatment (15.35 mg N/100g) and increases after CMC treatment (16.70 mg N/100g).

Changes in TVB-N content during process and storage
TVB-N values for all treated and raw mussels are given in Fig. 9.
The TVB-N value of fresh mussels control was determined as 15.25 mg N/100g, and resulted similar to the values reported by other authors for mussels (11.5-14.7, mg N/100g) (Turan *et al.*, 2006; Kyriazy-Papadopouulou *et al.*, 2003), Vasakou *et al.*, 2003; Erkan, 2005).
During storage period the TVB-N value remained constant in mussels SVCC and BSVCC, while increased in CMC and CC respectively from 14 and 21 storage days. At the end of the storage period the TVB-N content in mussels processed and stored in vacuum condition were 12.5 mg N/100g (BSVCC) and 14.00 mg N/100g (SVCC), and exibited the highest values in CC and CMC mussels (44.4 and 32 mg N/100g respectively).
Total volatile basic amine represents all nitrogen fractions that are formed in the tissues during the post-mortem processes, due to bacterial degradation that are the main responsible of seafood spoilage (Olafsdottir and Jonsdottir 2010; Huss, 1995); for this reason TVB-N is one of the most widely used measurements of seafood freshness and quality.
According Ludorff and Meyer (1973) a TVB-N content of 25 mgN/100 g is expected for very goods product, 30 mg N/100 g for a good, 35 mg N/100 g for a marketable while TVB-N values over 35 N/100g are atribuited to spoiled seafood.

Goulas *et al.* (2005) and Erkan (2005) suggest one acceptability limit for mussels of TVB-N content of 22-25 and 15 mg N/100g, respectively. In this study TVB-N content of *sous vide* cook and chill mussels (with or without brine supplementation) remained constant and lower than the acceptability limit suggested in these studies and lower than the acceptability limit of 35 mg N/100 g set by the European Union (EEC1995) for all storage time.

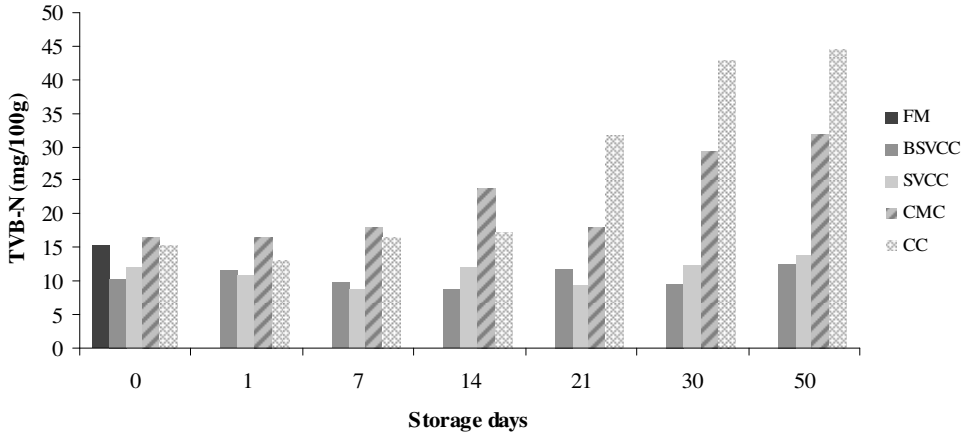


Figure 9 Changes in total volatile basic nitrogen (TVB-N) in mussels meat during processing and chilled storage at 3±1°C

Change in Water Holding Capacity

The WHC of FM, SVCC, BSVCC, CC and CMC mussels are given in fig.10.

The mechanisms involved in the water retention during pasteurization treatment are difficult to describe, according Szerman *et al.* (2006) probably solubilised proteins are able to form a gel that entrap and retain water inside and/or due to the interaction between and added proteins. In particulary there could be a positive effect of sodium cloride on tissue WHC.

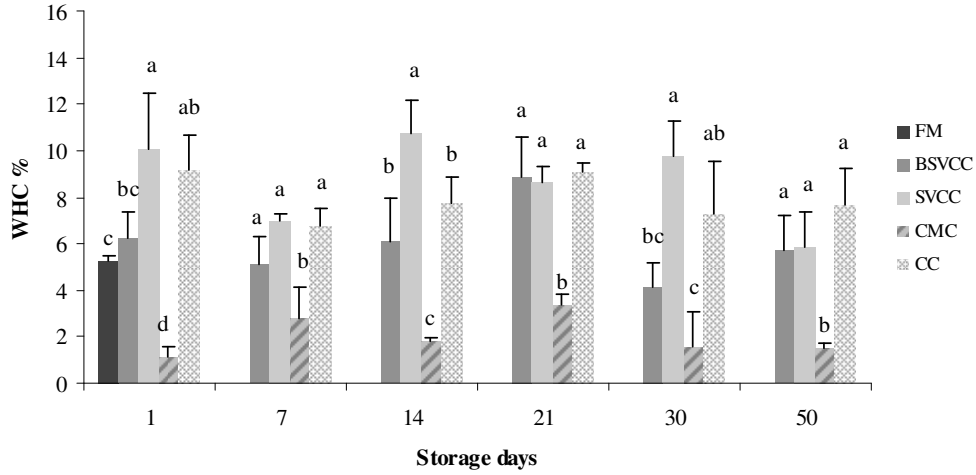


Figure 10 Changes in WHC of different packs of mussels during processing and chilled storage at 3±1°C. Data are reported as average ± standard deviation, different letters indicate significant differences among treatments for each inspection time, P<0,05

Changes in sensory quality during storage

The results obtained in the sensory analysis are shown in Figures 11, 12, 13 and 14.

On day 1 BSVCC, SVCC and CMC mussels obtained a score greater of limit of acceptability but SVCC mussels obtained higher score in terms of odour intensity, succulence and flavour. The control CC resulted unacceptable, for the majority of the attributes considered, from the first tasting according to the panellists; the cooking process of the mussels appeared visibly heterogeneous, the tissues the mantle adhered to the shell, and overall appearance was unpleasant, probably, even if the heat treatment of pasteurization was equal for all theses, the absence of a vacuum in CC pack, resulted in a diversity of cooking mussels.

After 7 storage days the thesis BSVCC, SVCC e CMC obtained one similar score in all attributes considered.

After 14 storage days mussels CMC resulted unacceptable for the majority of the attributes considered, while even if in BSVCC and SVCC mussels decrease their score still resulted acceptable for all the attributes considered.

After 21 storage days the BSVCC mussels obtained a score higher than SVCC ones in terms of colour/appeareance, odour intensity, succulence, aftertaste and flavour, while SVCC mussels resulted unacceptable.
After 30 storage days the score of BSVCC decreased even if it was considered acceptable in terms of odour intensity and meat turgidity and aftertaste.
After 50 days storage the BSVCC mussels resulted unacceptable for the majority of the attributes considered.

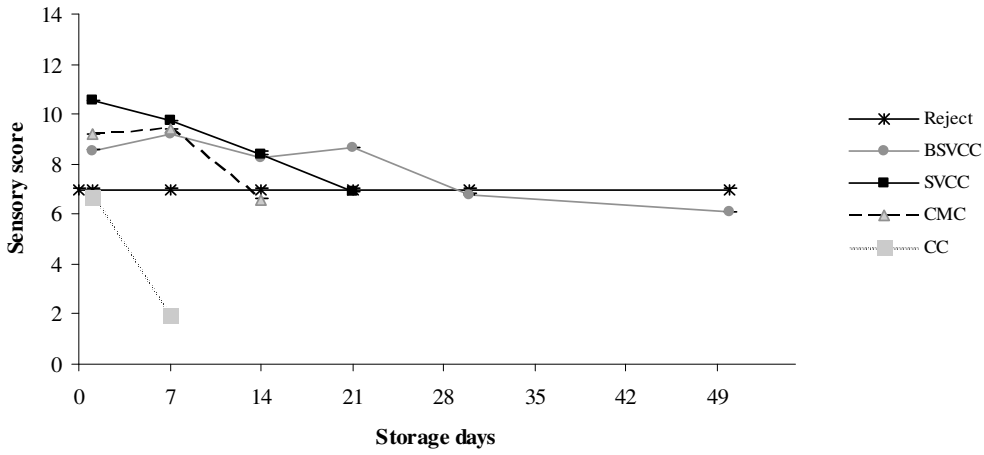


Figure 11 Changes in the overall sensory scores of the mussels differently processed and chilled storage at 3±1°C.

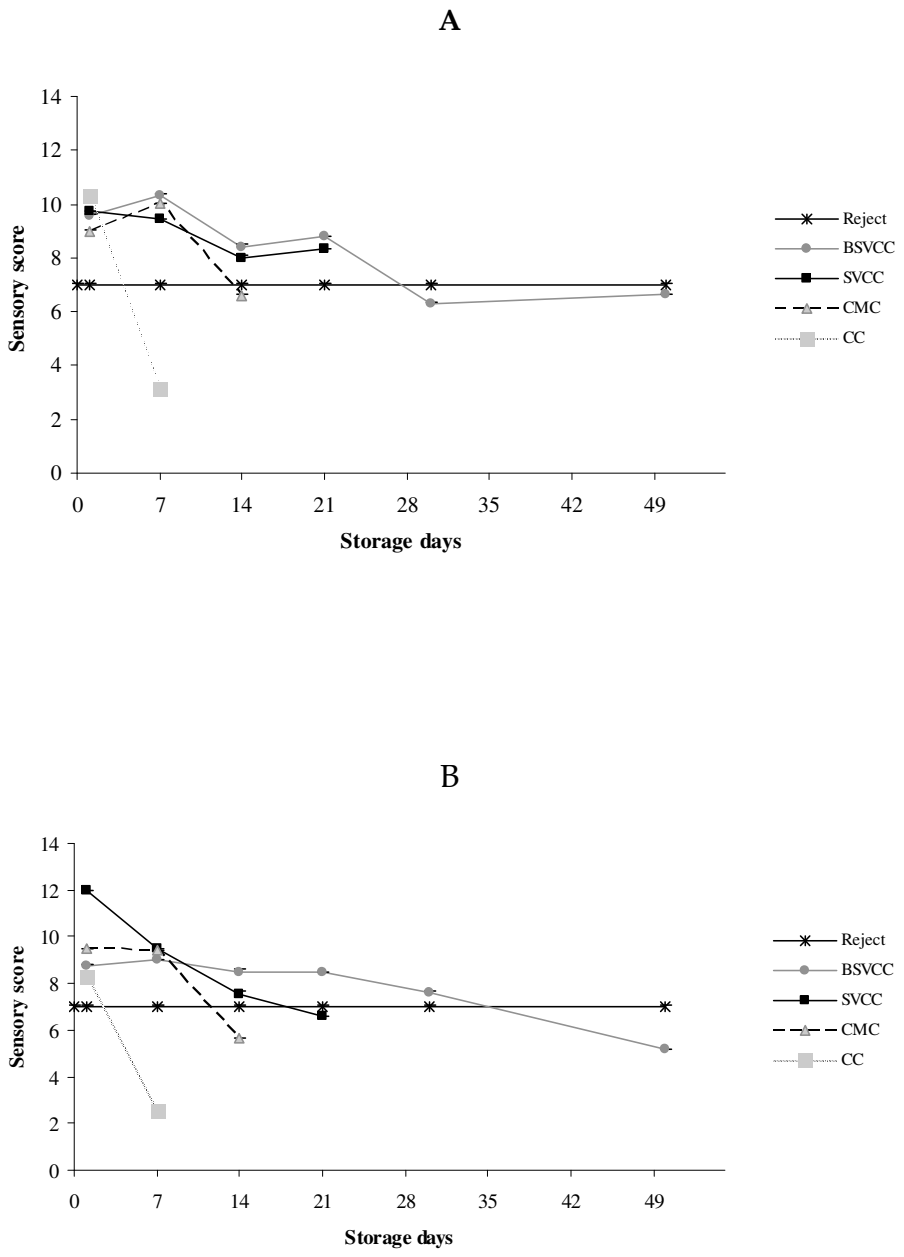
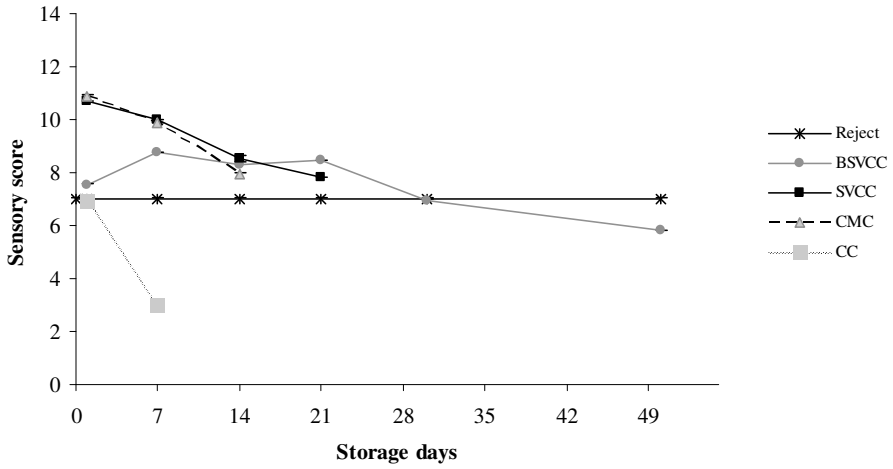


Figure 12 Changes in the color/ appearance (A) odor intensity (B) scores of the mussels during processing and chilled storage at 3±1°C.

A



B

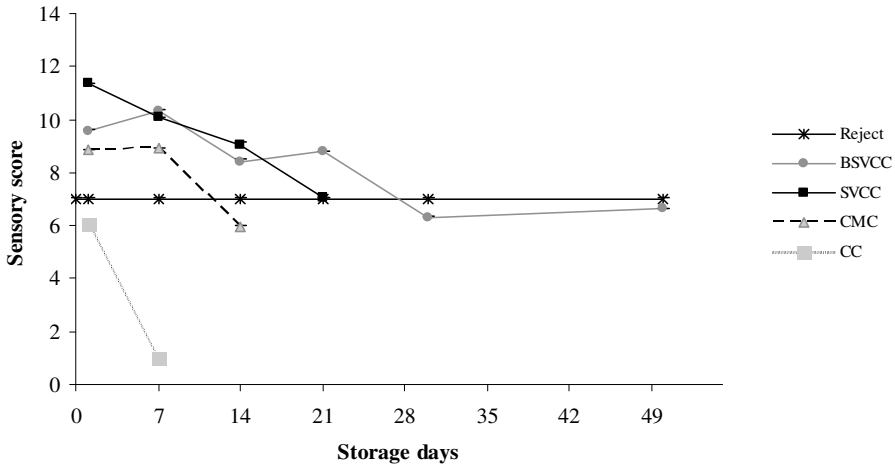


Figure 13 Changes in meat turgidity (A) and flavour (B) scores of the mussels during processing and chilled storage at 3±1°C.

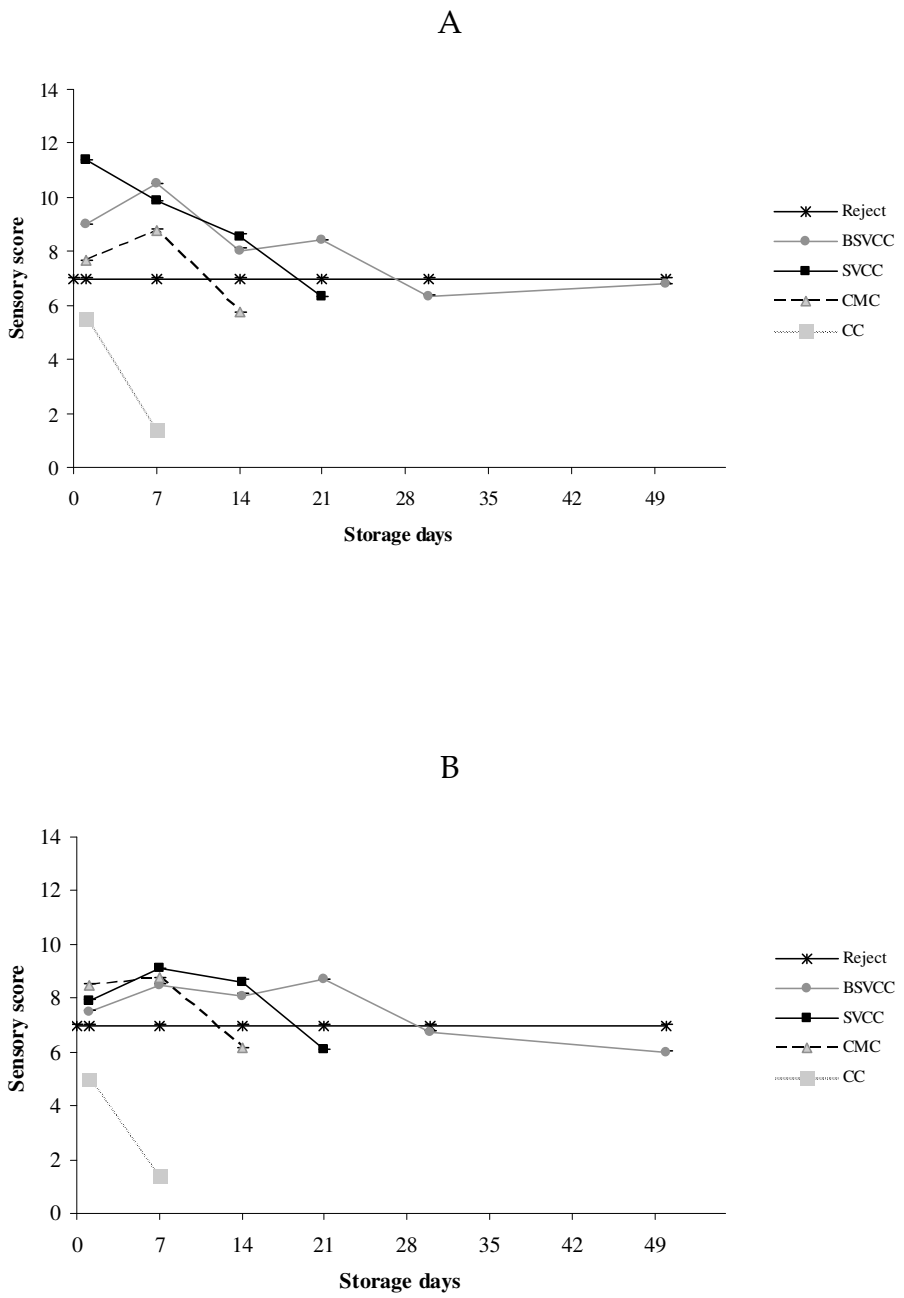


Figure 14 Changes in succulence (A) and aftertaste (B) scores of the mussels during processing and chilled storage at 3±1°C.

Changes in microbial quality during process and storage

The average total bacterial count in different mussels packs after 1 day of storage are shown in table 10, whereas in Fig. 15 the relative changes during the storage period are shown.

The FM (raw mussels) had initial TVC of 2.2±0.33 log CFU/g. After treatments the mean of TVC in CMC SVCC and BSVCC was below the detection limit of the method and remain constant until 7 storage days then increased till 1±0.6 and 0.7±0.01 log CFU/g in SVCC and BSVCC respectively and after 50 storage days was 1.9±0.20 and 3.1± 0.01 log CFU /g respetcively.

In CMC initial low value in TVC remained constant until 14 storage days, after increased significatively respect to other groups till 2.6±0.4 and 4.2±0.08 log CFU /g after 21 and 30 storage days and decreased to 2 log CFU/g after 50 storage days ($p<0.05$).

In CC control the initial value in TVC was 0.9± 0.06 log CFU /g and this values remained constant until 30 storage days, then increased rapidly to 4,5± 0,08 log CFU/g after 50 storage days. Cooked foods are generally judged acceptable, if the total bacterial counts results below 5 log CFU/g (Huss, 1995), in this study SVCC and BSVCC mantained value, always below this limit compared to the CMC and CC control, in which limit values are observed after 30 and 50 days respectively.

Our study is in according with Rybka *et al.,* (1999) that have tested microbiological safety in SVCC foods could be attribuited to severe heat treatment in combination with low storage temperature (0-3°C) and found that the cook-chill fish was good during 54 days of testing.

Microbiological safety and sensorial quality in rainbow trout fillets (*Oncorhynchus mykiss*) and salmon slice processed by *sous vide* method determined a shelf life of 45 days in optimal storage condition (+2°C) (Gonzalez *et al*., 2004; 2005).

Can (2011) in its study on carp fillets with or without sauce processed with *sous vide* method maintained good microbiological quality for 56 storage day at 2°C.

Lactic acid bacteria are able to grow in microaerophilic/anaerobic environments and could be associated to the spoilage of *sous vide* products, involving the swelling and/or development of off-flavors and off-odors (Carlin *et al.,* 1999), as vacuum produce an ecosystem that encourages their growth (Zurera-Cosano *et*

al., 1988). Guerzoni et al., (1999), studied the evolution of the lactic acid bacteria (LAB) in *sous vide* meat products. These authors observed that LAB were undetectable immediately after the *sous vide* treatment in meat products, but that they could recover after storage. However, these authors reported that the lactic acid bacteria growth only occurred sporadically in a few samples.

In this study, LAB was found in FM (2.00± 0.09 log CFU /g) but have maintained always, below the detection limit of the method in mussels SVCC, BSVCC and CC control, while appeared in CMC with 4.1± 0.08 log CFU /g after 30 storage days. Cooking at 85° for 10 min resulted able to reduce these groups of bacteria. Similarly to the results reported by Rosnes *et al.,* (1999) where lactic acid bacteria in *sous vide* fish- based meals were not detected during a storage time of 42 days, differently other authors as Gonzalez- Fandos *et al.*(2004 and 2005) and Can (2011) observed a gradual increase in lactic acid bacteria counts, in this fish, after 3 (trout filets) and 14 (salmon slice and carp filets) storage days at 10°C.

Pseudomonads were enumerated in FM control with 5.32±0.21 log CFU /g. After treatments the values of this bacteria resulted always below the detection limit of the method (1log CFU /g) and appeared only in CMC mussels after 30 storage days. According to Rhodehamel (1992) normal spoilage microrganism, that is *Pseudomonas*, yeast and molds are inhibited by vacuum packaging in *sous vide* foods. Probably the vacuum packaging and cooking had prevented the growth of pseudomonads in mussels.

Enterobacteriaceae were enumerated in FM control with 2.00± 0.23log CFU/g. After the cooking treatment these have remained always, for all treatments and control, below the detection limit of the method. Similar results were reported by Gonzalez-Fandos *et al.*, (2004 and 2005) on raw trout (2.18±0.41 log CFU/g) and salmon (2.66±0.91 log CFU /g) and Diaz *et al.*(2009) that in its work on shelf life of *sous vide* salmon (*Salmo salar*) detected *Enterobacteriaceae* on days 18, 22, and 25 and similarly to our study, they were always below the minimal detection limit (<10 CFU/g).

Differently Can (2011) reported value about 2 log CFU/g *Enterobacteriaceae* after 14 storage days to 10°C.

Anaerobic Sulphite-reducing clostridia were enumerated in FM control with 2.00± 0.17 CFU/g and after treatment resulted always below the detection limit of the method. Shakila *et al.* (2009) reported the presence of anaerobic sulphite-reducing clostridia in conventional pack of fish cakes and in conventionally cook-chilled pack at the end of storage (3MPN g^{-1}), but not in vacuum pack and *sous vide* cook chill pack. Schmidt *et al.*, (1961) reported that the lowest temperature limit established for the growth and toxin production by strains of psychrotrophic *C. botulinum* is 3.3°C. However, recent studies have indicated that they may grow in VP meats at temperatures as low as 2°C (Moorhead & Bell, 2000). Due to the common temperature abuses during the distribution, retailing and consumption, additional hurdles shuld be included (Tolstoy, 1991; Genigeorgis, 1993). Gonzalez-Fandos *et al.*, (2004 and 2005) showed in its studies that, the temperature abuse of 10°C, decreased the shelf life of *sous vide* trout and salmon and allowed the growth of sporeforming bacteria, thus implying a potential risk for the consumer health.

Table 10 Microbiological quality in fresh and treated mussels after 1 storage day at 3±1°C

	FM	CC	BSVCC	SVCC	CMC
Total bacterial count (log CFU g^{-1})	2.21±0.33	0.88±0.68	<1	<1	<1
Total Lactics (log CFU g^{-1})	2.00±0.09	NF	NF	NF	NF
Total Pseudomonads (log CFU g^{-1})	5.32±0.21	<1	<1	<1	<1
Enterobacteriaceae (log CFU g^{-1})	2.00±0.23	NF	NF	NF	NF
Anaerobic sulphite-reducing bacteria (log CFU g^{-1})	2.00±0.17	NF	NF	NF	NF

NF, not found

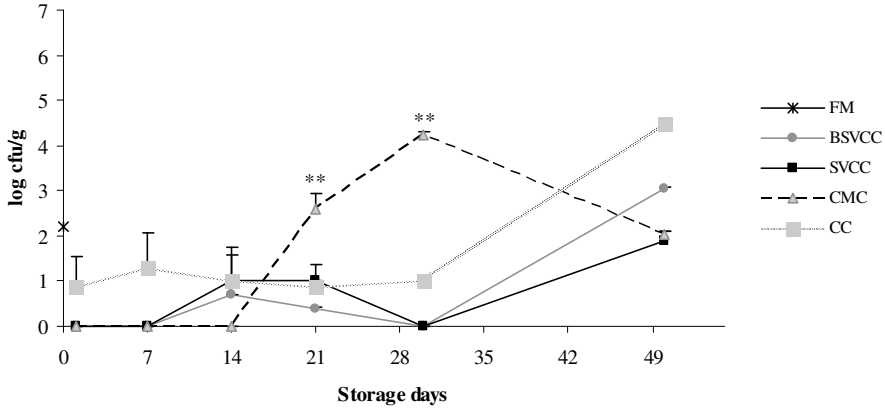


Figure 15 Changes in total bacterial count in mussels meat after processing and chilled storage at 3±1°C. Data are reported as average + standard deviation

Similarly to Kyriazi-Papadopoulou *et al.* (2003) and Turan *et al.* (2008), pH values in FM were 6.2±0.04 and maintain similar values after all treatments (average 6.3±0.02), and increased during chilled storage (fig. 16). Goulas and Kontominas (2005) attributed the increase of pH to the production of volatile basic components, such as ammonia, trimethylamine, etc., by fish-spoiling bacteria.

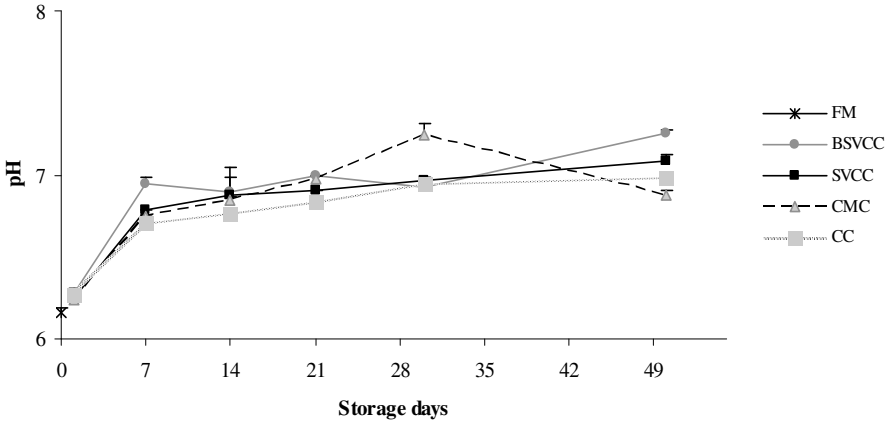


Figure 16 Changes pH in mussels meat after processing and chilled storage at 3±1°C. Data are reported as average + standard deviation

According to the number of days elapsed until the mesophiles reached a population above 5log CFU/g, TVB-N below 35 mg/100g and the mussels obtained scores below 7 and, it was inferred that mussels cooking traditionally (100°C fot 10 min) had shelf –life of ca. 14 days, mussels *sous vide* cook and chill (85°C for 10 min) had shelf –life of ca. 21 days and mussels *sous vide* cook and chill (85°C for 10 min) whid brine integration had shelf –life of ca. 30 days (tab. 11).

Table 11 Establisced shelflife of different treated mussels stored at 3±1°C

Thesys	Storage days to reach total mesophiles ≥4 log CFU/g (days)	TVB-N ≤ 35mg/100g	Days to reach score ≤7 (days)	ES* (days)
BSVCC	>50	>50	30	30
SVCC	>50	>50	21	21
CMC	30	>50	14	14

Established shelf-life

4.5.3 Conclusions

Based on this study, it was concluded that the SVCC and BSVCC process for mussels would provide an extended shelf life in term of technological, sensorial and microbiological quality.

During *sous vide* packaging, live mussels tend to open the valves and the gas content are stripped, when the flaps of the pack are heat-sealed and pressure re-established, the closing of the valves is favored. During heat treatment *sous vide*, the retention of intervalvar liquid, within the valves, ensures a uniform heat trasmission.

In fact in CC control, where mussel was not subject to pressure, packaged gases expanded due to the heat and reducing the thermal transmission (Skipners *et al.*, 2002), also the opening of the valves determines the release of intervalvar liquid.
The integration with salt of the brine in this study had a positive effect on moisture content, on Water Holding Capacity and TVB-N and sensorial quality, the reduction of water activity is another obstacle to the bacterial proliferation, and it is an carrier of aromatic compounds.
Moreover the introduction of the brine, is a technological advantage which facilitates the packaging, in fact, it avoids the direct contact of the cutting edge shells margin with the bag preventing the formation of microlesions.
The storage temperature plays a key role to ensure the quality and safety of *sous vide* product, together with the heat treatment. It must be highligheted that the microbiological shelf life of *sous vide* mussels depends of different factor including the microbiological quality of reared water efficacy of depuration, the process parameters applied and the continuity of the cold chain and the safety of this product can be guaranteed by applying the concept of Hazard Analysis Critical Control Point (HACCP), as well as by means of an adequate combination of different factors.

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5. GENERAL CONCLUSIONS

In this chapater the main achievements of this thesis are summarised and it is reported to what extent the objectives have been met

5.1 Introduction

The development of the mussel culture has so far been mainly related to innovations of primary production. Despite the quality of the product, the marketing of mussels has not yet been supported by a real valorization, and it is mainly limited to the distribution of the fresh product in traditional packaging represented by plastic mesh. The ability to offer products with extended shelf life coupled with high quality characteristics represents an opportunity for innovation in the mussels supply chain.

5.2 Aim and main achievements of this study

This PhD thesis was aimed to provide practical suggestions for the evaluation of nutritional properties, freshness and quality, and the development of innovative products, able to preserve the quality of the raw material and able to meet new consumer needs and ease of use.

Chapter 2 was dedicated to the characterization of *Mytilus galloprovincialis* cultivated in the Gulf of Trieste describing the seasonal variations of biometric and physico-chemical parameters related to quality for define the period in which the mussels, in relation to their physiological condition, are more suitable for processing.

The data obtained from the present research give an overview of the changes in the commercial and nutritional characteristics of this product during seasons, the high nutritional quality and technology found in spring and summer, allowing to define these seasons as the periods in which the mussels are more suitable for processing.

Chapter 3 was dedicated to the study of the "application of *sous vide* tecnology to preserve live mussels" assessing the stability of the product packaged with or

withouth byssus and considering physical chemical and microbiological and sensorial aspect to determine their shelf-life

The use of *sous vide* tecnology on live mussels, resulted able to maintain initial product weight, quality attributes and contain the microbial population with an extension of shelf life from 3 to 9 storage days respect to mussels preserved traditionally,

Byssus removal not influenced microbiological caracteristics, pH and percentage of intervalval liquid released of live mussels packaged by *sous vide* tecnology, but increased mortality after 8 storage days. The application of alternative methods of debissing, such as cutting, is suggest to prevent alteration of anatomical structures of mussels and ensure the convenience of use of the product.

Evaluation of the freshness and sensory attributes, in addition to the chemical-physical and microbiological characterizations, resulted essential for this product and for this reason was developed a practical scheme for evaluation of fresness and quality. while histological analysis appears to be a good method the support for other analysis.

Chapter 4 titled " Application of heat treatment *sous vide* cook and chill to mussels *M. galloprovinlcialis*" was focused to test *sous vide* technology associated with heat treatment of pasteurization and assessing their stability during storage period determing the shelf-life of this product.

Different time/temperature combination results in the desidered microbial inactivation of natural and inoculated microbial population in raw mussels.

The use of *sous vide* cook and chill on mussels, resulted able to to extended shelf life to until 30 storage days.

The addition of the brine in this study had a positive effect on several quality parameters, and determining an reduction of water activity provides an other obstacle to the bacterial proliferation, and it is an carrier of aromatic compounds.

Moreover the introduction of the brine, is a technological advantage which facilitates the packaging, in fact, it avoids the direct contact of the cutting edge shells margin with the bag preventing the formation of microlesions.

Other tests will be necessary to evaluate the stability of these products during storage in conditions of thermal abuse, taking into account the microbiological, chemical, physical and sensory properties.

About the author

Tiziana Bongiorno was born in Mazara del Vallo (TP), Italy, on the 23th of January 1981. She received her high-school diploma at the Lyceum "Giovanni Gentile" of Castelvetrano, Italy, in 1999, with mark 100/100. In the same year she started her BSc in Production Sanity and Storage of Acquatic Organisms at University of Camerino, Faculty of Veterinary Medicine, from which she graduated in 2003, with mark 110/110 cum laude. Her BSc thesis, titled "Influence of rearing conditions on the morphometric characteristics of sea bream (*Sparus aurata* L. 1758)". In 2004 she started her MSc in Aquaculture at University of Udine, Faculty of Veterinary Medicine. During 2006 she has partecipated to Professional Training and Erasmus Project at University of Gran Canaria, Faculty of Veterinary Medicine and at Istituto Canario de Ciencias Marinas, Talliarte-Gran Canaria island.

In 2007 she received her MSc degree with mark 110/110. Her MSc thesis titled "Changes of the skeletal structure during the recovery process of the opercular anomalies in sea bream (*Sparus aurata* L.1758)", was done again at the University of Udine. In the same year receives the First Prize as "best thesis on ictiopathology" during the "XIV National congess of ictiopathology"of Ictiopathology Italian Society (S.I.P.I 15-16 November at Castiglione della Pescaia, Italy). In May 2007 he started research at the department of Animal Science by a fellowship on meat quality.

In January 2009 she started her PhD course at the Department of Food Science, University of Udine. The topic of her research was "Quality of Mediterranean mussels (Mytilus galloprovincialis) and valorization of the product throgh sous vide tecnologies".

During her studies she collaborated with CNR (National Research Center) of Mazara del Vallo and carried out seasonal working experiences as promoter and consultant for pet food and health care products in pet shop, she carried out activities tutoring and orientation for students of Udine University. Currently carries out research in the Department of Food Science at the section of Aquaculture and she collaborates with a company as responsible for the marketing of products and services for aquaculture and ecology.

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