“Piloting of eco-innovative fishery supply–chains to market added–value Adriatic fish products”
Priority Axis: Blue innovation
1.1 - Enhance the framework conditions for innovation in the relevant sectors of the blue economy within the cooperation area

D4.3.1 Report on new product analyses and description

WP4 - INNOVATING TOOLS AND PROCESSES FOR ADDED-VALUE ADRIATIC FISHERY PRODUCTS/ A 4.3. DEVELOPMENT AND QUALITY CONTROL OF ECO-INNOVATIVE FISHERY PRODUCTS

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Contents

Document description

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Besides the detection of listed possibilities in field of creation of an innovative product described in previous report D 4.2.4. it is important for the producer organizations to finally pilot the selected option, as well as to conduct all the necessary tests and analysis in order to prove its added value. This added value will widen the market reach and satisfy consumer needs.

This document is divided into 3 separate cases (OP “Omega 3”, OP Istria and O.P. Bivalvia), in order to make it easier for readers to follow each and specific piloting actions which internal PO’s have conducted in cooperation with the external service providers.

1. OP “OMEGA 3” CASE – SARDINE FILETS IN MAP

In this study, different way of preserving Sardina pilchardus fillets have been examined, with the aim to extend the maximum commercial shelf life, by comparison with the product in refrigerated box storage.

It is important to clarify the difference between two terms, often used in a confusing way:
Shelf life (maximum commercial life): this is the maximum time within food preserves unchanged organoleptic, microbial chemical-physical characteristics, by comparison with the human sensory.
Durability (real commercial life): this is the “prudential” time determined by the manufacturer (in Italy the correct term is Food Industry Operator) inside the maximum commercial life, at the end of which still some days last before the actual shelf life.

Sardina pilchardus morphological characteristics

Sardina (Sardina pilchardus) is a bone seafood belonging to Clupeidi family. It’s a hard animal able to create dense herds, sometimes with other fish species having similar size. Sardina eats exclusively plankton, lives in Adriatic Sea and in Mediterranean Sea with an average life of 5 years. Morphological speaking, Sardina has an oval and tapered body, covered with stiff and pointed ventral scales, a big eye and a mouth facing up. The ventral pins are located close to the caudal tract, while the pectoral pins are low on the belly.

The caudal fin has a very evident and flat bifurcation. (Figure 1).

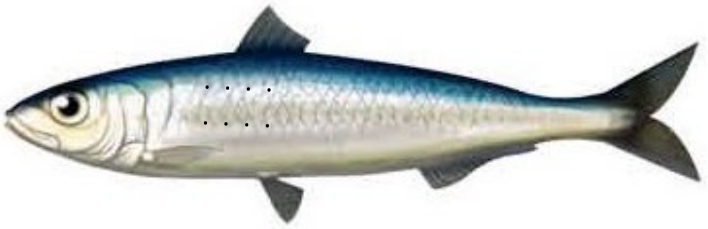


Figure 1. Sardina pilchandus

The dorsal livery presents a green-blue color while the lateral portion and the belly are silvery. There are also small dark spots along the lateral line. About nutritional characteristics, Sardina has a good protein and an excellent unsaturated fats content.

Below a table with nutritional values:

Nutrient	Value for 100 g
Water (g)	62,7
Energy (kcal)	225
Energy (kJ)	943
Proteins (g)	20,3
Lipids (g)	15,4
Cholesterol (mg)	65
Available Carbohydrates (g)	1,5
Soluble sugars (g)	1,5

1.1. Fish products conservation

Fish products are very sensitive to conditions of their storage, processing and conservation areas, as they directly depend on the microbiological, chemical and biotoxicological characteristics (Croci and Suffredini, 2003). From the chemical point of view, factors influencing fish products degradation are: insufficient postmortem acidification, scarce connective tissue presence and high humidity. Therefore, to prolong shelf life it's fundamental to reduce the product temperature as quickly as possible, lowering it to 0° C, immediately after the catching. The lack of meat acidification, due to the low glycogen content, allows the proteolytic enzymes – such as cathepsins and peptidases – to degrade the organic molecules present in the meat. Thus, endogenous and exogenous microorganisms – such as those present in the gills in the skin and buccal apparatus – activity is stimulated.

Let consider that it's possible to measure in the gill's total bacterial loads between 103 e 107 ufc/cm2 Conditions worsens when not eviscerated fish is stored: in the intestinal tract the microbial loads can range between 103 e 109 ufc/g, depending on the type of fish, its eating habits and its biological phase (Giaccone 2001, Gram and Huss, 2000.).

Histamine

Biogenic amines – group where you can find histamine, putrescine, cadaverine and tyramine - are an important parameter to evaluate the good meat conservation. Histamine is produced by decarboxylation of the L-histidine ammino acid, normally present in the musculature of sed fish products. This reaction is mostly attributable to the action of Gram-negative microorganisms, such as Enterobacteria and Cytobacteria. It's important to underline, for processing activities, that histamine is highly thermostable and therefore cannot be denatured with traditional cooking systems.

Total volatile basic nitrogen

Nitrogen present in fish products is mainly of protein origin. Immediately after catching and especially during storage, enzymatic reactions – of bacterial origin – take place in fish products. These reactions increase the volatile nitrogen compounds, such as ammonia and trimethylamine, responsible of product bed smell.

1.2. Materials and Methods

Material

This experimental study has been carried out on a sample of 16 kg of sardines (Sardina pilchardus). Their catching took place the day before, in the sea area in front of Porto Tolle, over 3 miles from the coast.
Sardines have been delivered to the laboratory immediately after the port entry.
Flying trawl is the fishing system used. Immediately after catching, sardines have been baited, stored in polystyrene crates, conserved under a flaky ice layer and kept in a 3° C refrigerated room until the unload.

Sampling and samples preparation

For this study, 6 theses have been identified:

- TQ: Product washed with drinking water and packaged in trays with air
- TQ O3: Product washed with ozonized drinking water and packaged in trays with air
- Thesis 1: Product washed with drinking water, packaged in trays with modified atmosphere Mix1 (60 % Nitrogen – 40 % Carbon Dioxide)
- Thesis 2: Product washed with ozonized drinking water, packaged in trays with modified atmosphere Mix1 (60 % Nitrogen – 40 % Carbon Dioxide)
- Thesis 3: Product washed with drinking water, packaged in trays with modified atmosphere Mix2 (60 % Argon – 40 % Carbon Dioxide)

• Thesis 4: Product washed with ozonized drinking water, packaged in trays with modified atmosphere Mix2 (60 % Argon – 40 % Carbon Dioxide)

The product arrived in the laboratory has been eviscerated and manually threaded. For every thesis, 18 packs – 150 g of sardine fillets each – have been prepared. The fillets of TQ, T1 and T3 thesis have been washed with drinking water for few seconds - in order to eliminate any processing scraps – and then placed for 30 seconds on a grid – in order to reduce the excess water formed on the surface.

The fillets of TQ O3, T2 and T4 have been immersed in ozonized drinking water – with zone content ranging between 4,5 and 5,5 ppm (analysis carried out with WTW photoLab®S12 photometer) and then placed for 30 second on a grid, in order to reduce the excess water formed on the surface. Packaging has been carried out with a modified atmosphere and vacuum heat sealer VGP 60, produced by Orved S.p.A. – Musile di Piave – Italy. During packaging phase, in the trays containing the fish products, first vacuum has been created and then modified atmosphere has been introduced. Both trays and top are made by HDPE (high-density polyethylene) with high impermeability to gases. Packed products have been stored in a + 4° C refrigerated room.

Ozone

Ozone is a triatomic molecule with 3 Oxygen atoms (chemical formula: O3); it's an allotropic form of Oxygen with a characteristic pungent odor and a purplish color.

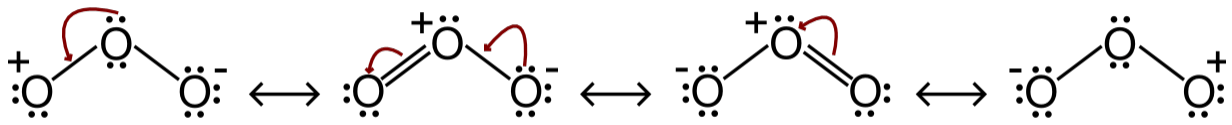


Figure 2. Allotropic Oxygen form

Ozone is a natural component of the earth's atmosphere; it's mainly produced in the stratosphere - at about 30 km from the earth's soil - by a chemical reaction between Oxygen and ultraviolet sun rays.

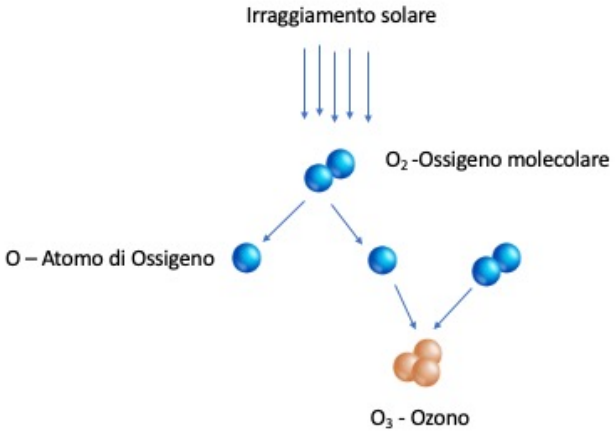


Figure 3. Ozone formation – Oxygen – sun rays' reaction

For the industrial application, ozone is produced flowing oxygen through a high electrical voltage. It's an unstable gas that quickly reacts, returning in its original diatomic Oxygen molecule, without any harmful residues. It's very effective with a very high oxidation potential (2,07 V): it's about 5 times more oxidizing than oxygen and 2 times more oxidizing than chlorine. It's a perfect environmentally friendly substitute for chlorine and many other chemicals products used for antibacterial treatments.

Ozone characteristics as a function of the environmental factors

The Ozone antimicrobial action and its characteristics vary according to the following environmental parameters:

- Substrate pH
- Organic compound present
- Temperature
- Chemical compounds present
- Substate humidity

Specifically:

- The lower the pH values the greater the Ozone stability.
- The grater the substrate humidity the better antimicrobial Ozone effect
- The grater the Temperature the better antimicrobial Ozone effect, considering that Ozone degrades faster at high temperature, while below 5° C Ozone is more stable, significantly reducing the bactericidal activity.

Ozone application in food industry

The Ministry of Health – with 24482 protocol, dated the 31st July 1996 - recognized Ozone as natural device for bacteria, viruses, spores, molds and mite's sterilization and destruction. About food safety, Ozone is one of the best treatments, leaving no residues and, if properly dosed, it doesn't alter the organoleptic food product properties: it can be used also in Biological food.

Ozone for fish industry

In fish industry, Ozone is mainly used in packaging and processing activities of raw materials. During shellfish relaying, for example, ozone is used to destroy the microbial load: the same for fish eviscerating.

The use of ozonized water in fish washing increases shelf life of products. The use of Ozone guarantees:

- Smell reduction
- Maintenance of organoleptic qualities
- Microorganism development limitation, especially on the gills, skin and intestines
- Enzymes release limitation and, as a consequence, the limitation of degradation reactions
- Color retaining
- Delaying of the spread of bed smell generated by the release of trimethylamine – in sed fish – and release of piperidine in freshwater fish.

Sampling and Analysis

All analyses have been carried out over a period of 13 days: in this period, all the 6 theses have been compared. Below the time schedule of all the activities carried out and the sampling plan.

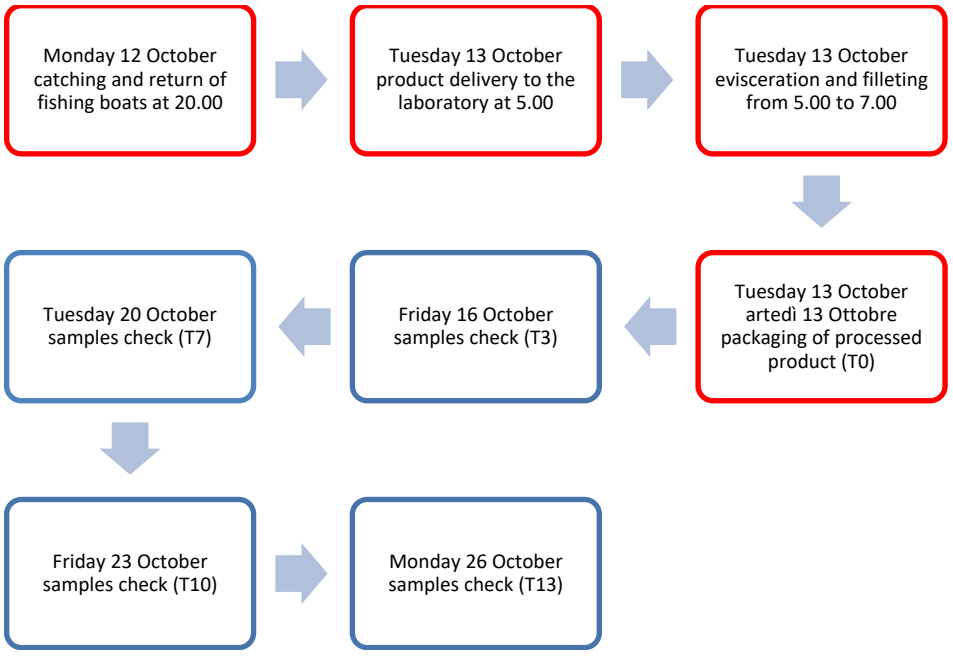


Figure 4. Experimentation Plan

With reference to figure specific analysis have been carried out I order to check freshness also with reference to EC Regulation n. 2406/96, containing the guidelines for classification in categories of freshness for fish products. In particular, the following analysis have been carried out:

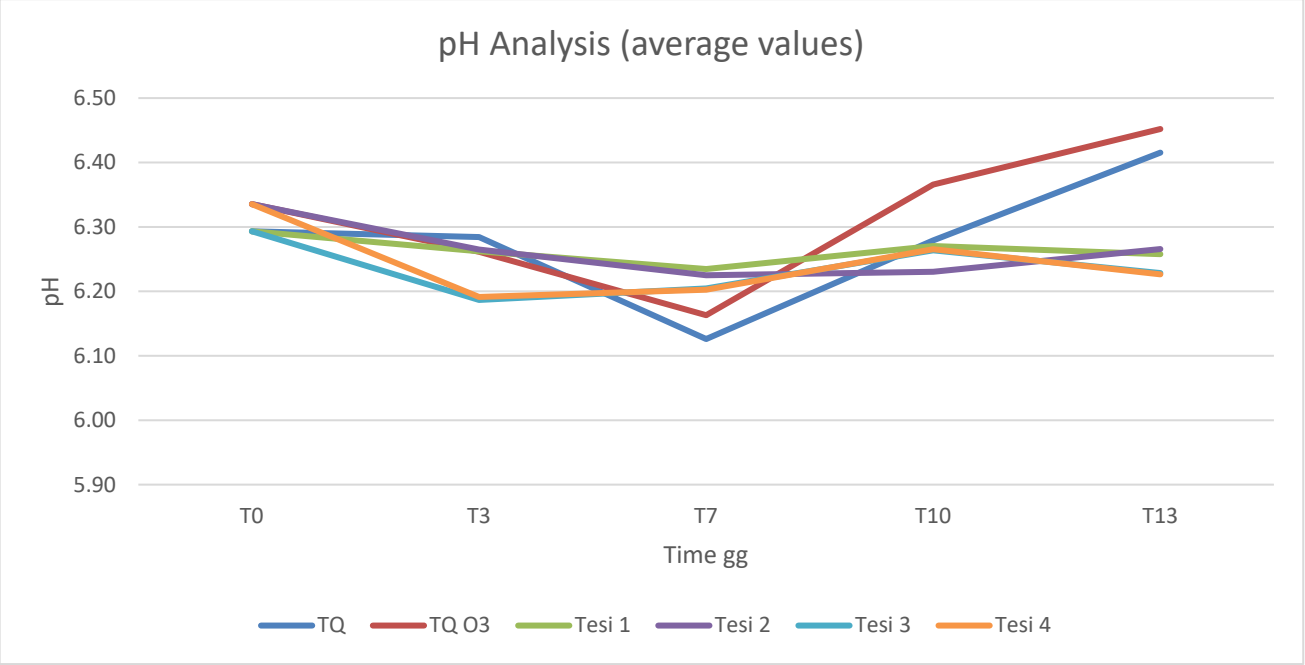
- pH
- weight loss
- Modified Atmosphere analysis
- Chemical Analysis
  - TVBN
  - Trimethylamine
  - Histamine
- Colorimetric Analysis
- Sensory Analysis
- Microbiological Analysis
  - Microorganisms counting at 30°C
  - Enterobacteriaceae Counting at 37°C
  - Thermotolerant Coliform Courting at 44°C
  - Pseudomonas spp Counting
- Texture

1.3. Results

The packaged products have been analyzed following the above experimentation plan (figure 4). Below, results parameter by parameter.

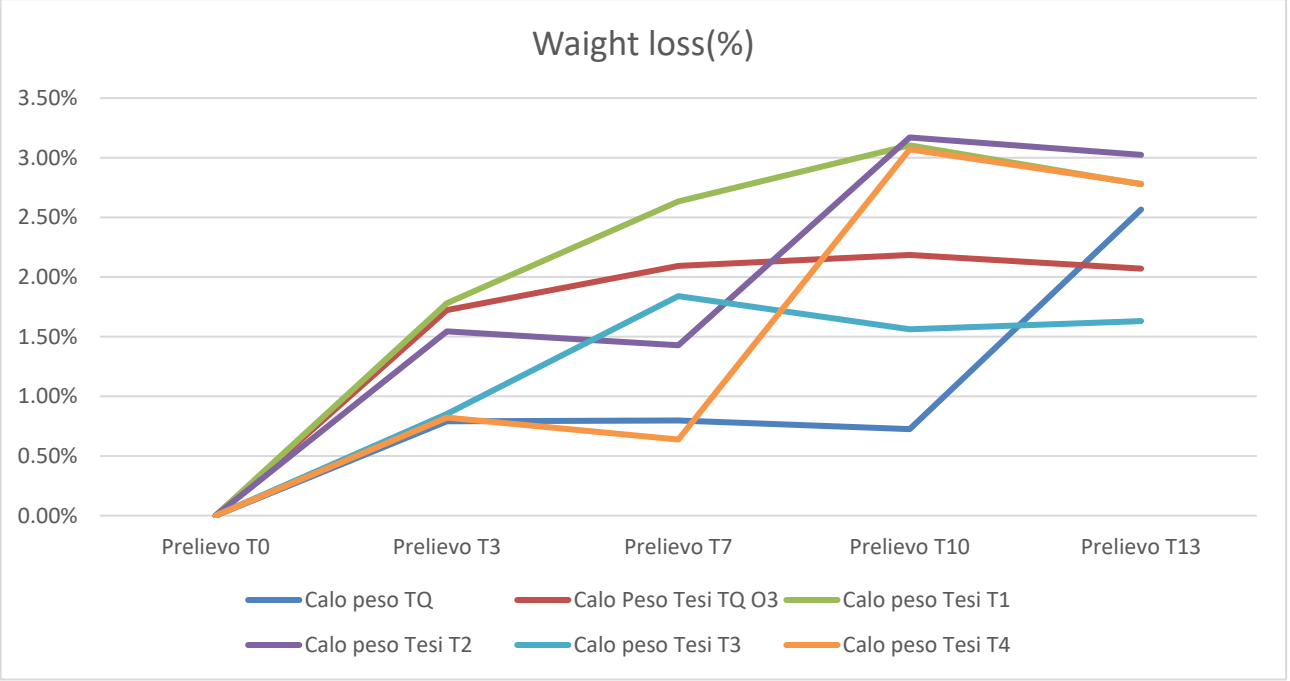
pH Analysis

The pH values during the shelf-life period have been analyzed with pH meter WTW inolab pH7110. From the results, it can be seen that pH variations are modest, even if the products packed with air show a slightly more marked variation than the fresh ones. This is due to the scarce presence of carbohydrates that are usually very low in most of the fish species. In fishes, pH variation is minimal if compared with terrestrial animals in which anaerobic glycolysis degrades the glycogen in the muscle, producing lactic acid which, in turn, lowers the muscle pH.



Weight loss

The weight loss during the shelf-life period has been analyzed with a technical scale Sartorius mod. BL1500. The results show that samples washed with ozonized drinking water have a slightly higher weight loss than samples washed with drinking water. This is due to the different washing methods before packaging. The samples washed with ozonized drinking water has undergone a slightly inhibition than the sardines washed with drinking water. Therefore, minimal exudation phenomena occurred during conservation. Graph n. 1, weight losses in % are showed.



Graph 1. Weight Loss (%)

The graph shows that products packaged with the argon-based mixture (Thesis T3 and T4) have undergone a lower weight loss compared to products preserved with the nitrogen-based mixture (Thesis T1 and T2). This phenomenon most likely originates from the presence of carbon dioxide which interacts with the substrate in different ways depending on the presence of argon or nitrogen used for the composition of the modified atmosphere. For more information, see the paragraph Carbon dioxide.

1.4. Modified atmosphere Analysis

Nitrogen

Nitrogen is an odorless and colorless gas and is used as inert. It is less dense than air; it is not flammable and unlike carbon dioxide it is not very soluble in water (0.018 g / kg at 100 kPa and 20 ° C) and does not reach the isoelectric point of proteins. Nitrogen exerts an inhibitory action on some lipases, decarboxylases and proteolytic enzymes, which reduces the loss of exudate in meat.

Argon

Argon is an odorless, colorless gas, it is not flammable, it has a solubility comparable to that of oxygen about 2.5 times more soluble than nitrogen. At the level of chemical and enzymatic reactions, argon competes with oxygen.

Carbon Dioxide

Unlike the other gases used for packaging in modified atmosphere, Carbon Dioxide is a gas with a pungent odor, perceptible to humans at concentrations above 3%. This gas is used in different percentages, as a component of the modified atmosphere, for food packaging and has a microbiological and chemical relevance. Depending on the gas concentrations used and the organoleptic composition of the product, there will be different levels of acidification of the substrate. From a microbiological point of view, bacteria that are most affected by its presence are narrow aerobes, molds and yeasts. Generally, it can be said that carbon dioxide exerts bacteriostatic activity as its effects increase the Lag phase (Figure 5).

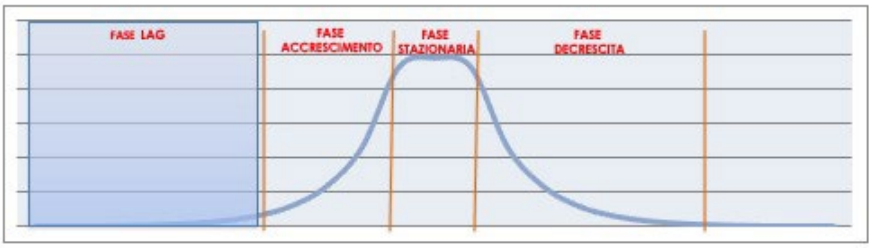
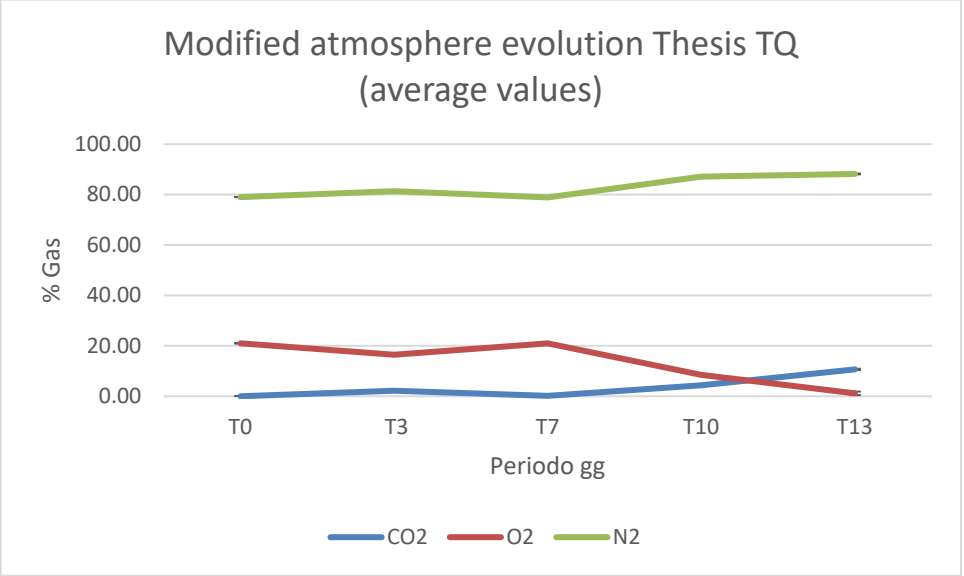


Figure 5 Microbial load development phases

From a chemical and enzymatic point of view, Carbon Dioxide is able to solubilize, as a function of the temperature, in water (1.688 g / L at 20 ° C), in lipids and in other organic solvents. For this reason, it is recognized as an acidifier. In some cases, the effect of Carbon Dioxide denatures the enzymes that can cause organoleptic changes. The concentration of Carbon Dioxide must be carefully evaluated otherwise it can happen, as for example in meat, that dehydration phenomena occur due to a reduction of the adsorption of water by the proteins, making actin and myosin reach their isoelectric point. For each sample the gaseous parameters, Nitrogen, Argon, Oxygen and carbon dioxide were analyzed. The Oxy-Baby (Witt Germany) was used for the gas analysis (Figure 6 Gas analysis inside the package).

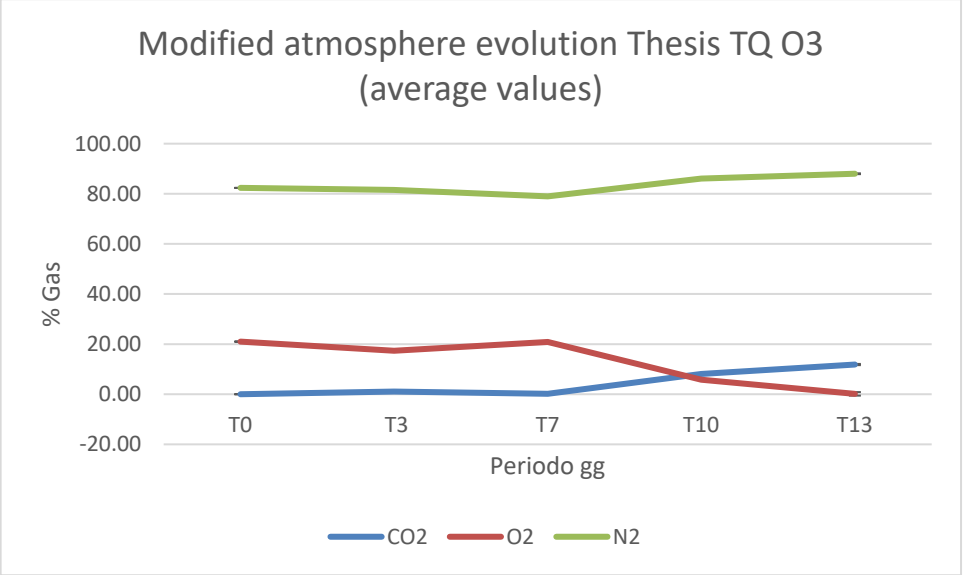
Figure 6. Gas content analysis

Results obtained show that the products stored in a modified atmosphere kept the gaseous composition more stable, unlike the products stored in the air. In the TQ and TQ O3 theses after the seventh day there is a variation in the oxygen and carbon dioxide levels (Graph 2, Graph 3), this phenomenon is generally due to the development of the microbial load, also confirmed by the graphs relating to the growth curves of the charges microbial (Chart 31, Chart 32, Chart 33, Chart 34, Chart 35, Chart 36). Below are the graphs differentiated by Thesis relating to the evolution of the protective atmosphere inside the packages.

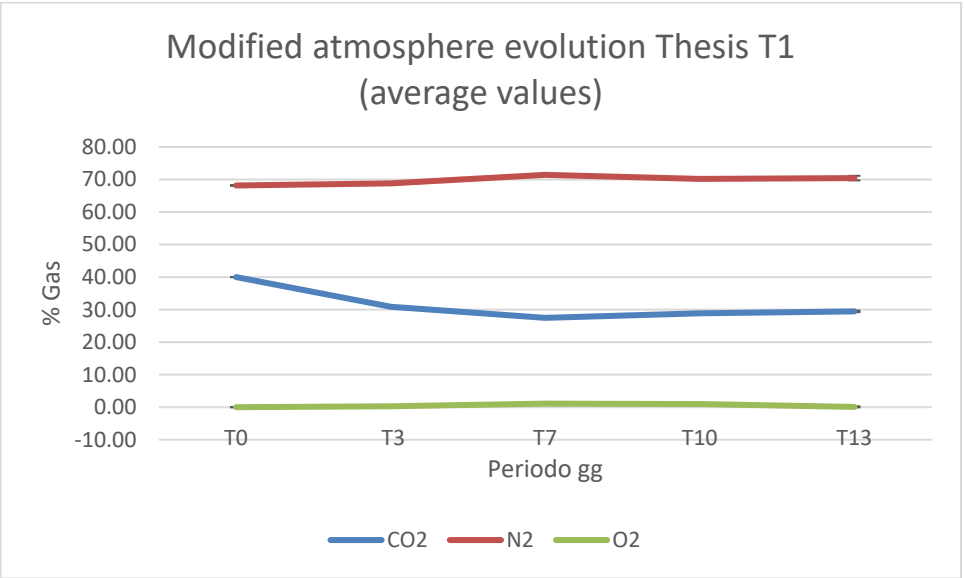


Graph 2. Modified Atmosphere Evolution Thesis TQ

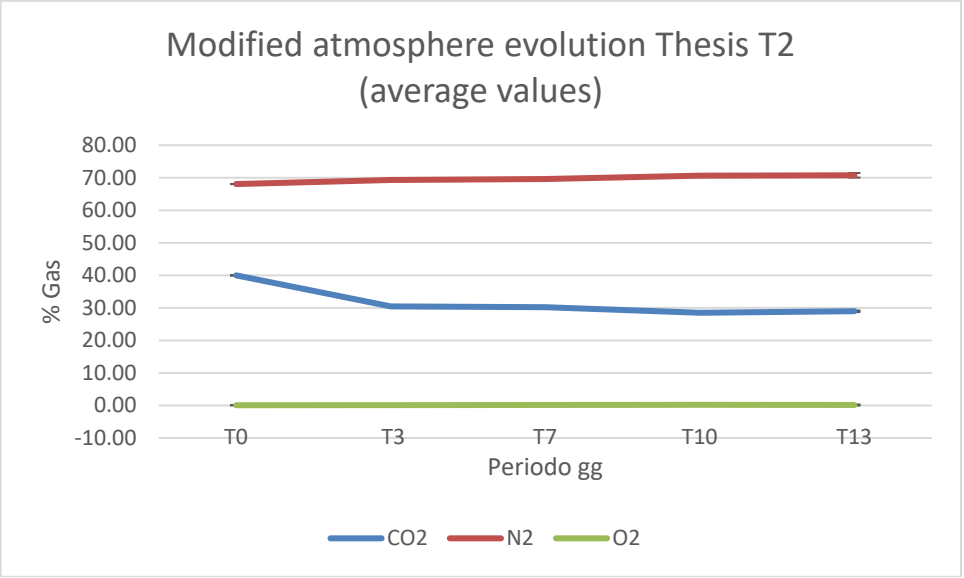
In this case, the reduction in the oxygen content is essentially due to 2 aspects: the solubility of the gas in the aqueous and lipid fraction of the product and the growth of most of the deteriorating bacteria and molds that need oxygen for their metabolism.



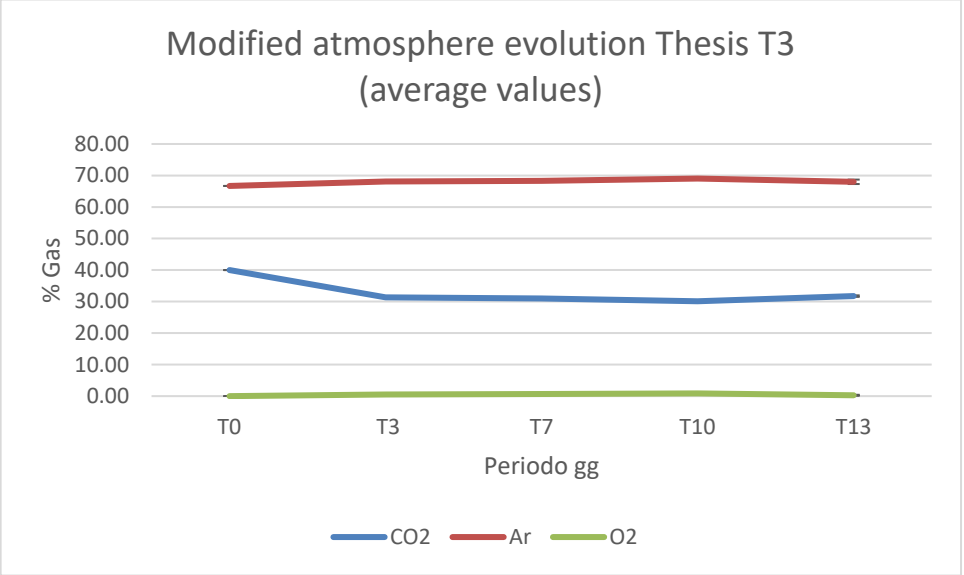
Graph 3. Modified Atmosphere Evolution Thesis TQ O3



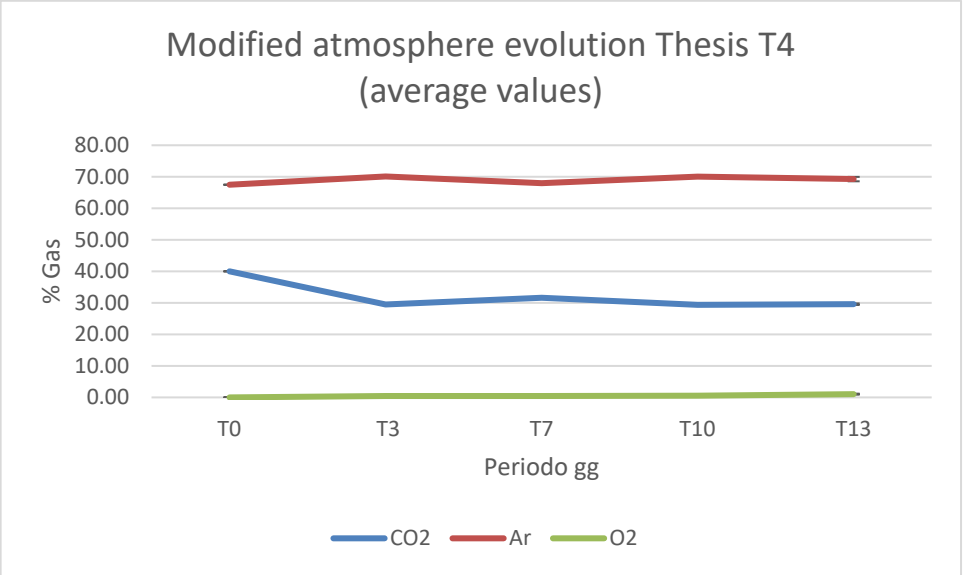
Graph 4 Modified Atmosphere Evolution Thesis T1



Graph 5. Modified Atmosphere Evolution Thesis T2



Graph 6. Modified Atmosphere Evolution Thesis T3



Graph 7. Modified Atmosphere Evolution Thesis T4

1.5. Chemical Analysis

Total volatile basic nitrogen

Total volatile basic nitrogen (TVBN) is formed by the degradation of nitrogenous compounds by tissue and bacterial enzymes, as a result of which ammonia and volatile amines develop. TVBN can express different biochemical and bacteriological realities (different amines, different microorganisms) responsible for organoleptic alterations and the variety of odors found in decaying fish. In agreement to EC Regulation 2074/2005 and subsequent amendments, Section II Chapter I, AFSSA 2007, EU Regulation no. 1022/2008 / EU is considered:

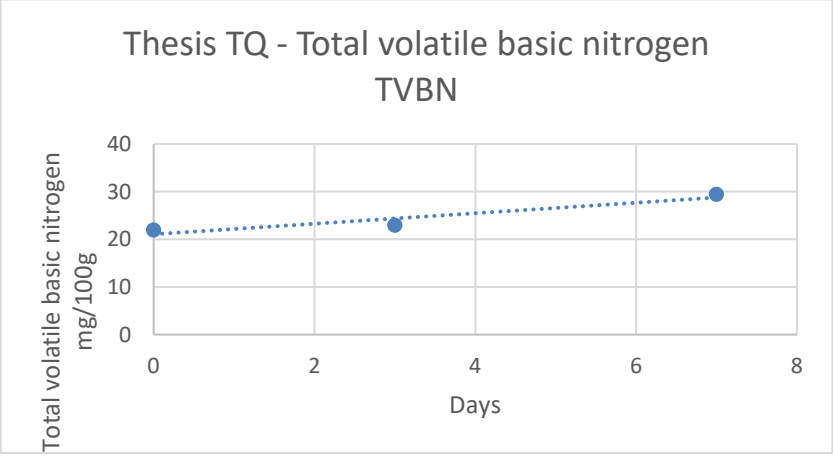
- Very fresh fish: TVBN <12 mg / 100g
- Fresh fish: 12 mg / 100g> TVBN <30-35 mg / 100g
- Deteriorated fish: TVBN> 30-35 mg / 100g

In our case, the most restrictive limit for blue fish was considered: 25 mg of TVBN on 100. From the results measure, it can be seen that the products washed with ozonated water show a lower concentration of total volatile basic nitrogen than those that have not undergone this treatment. Observing the regression lines, it can be seen that the product packaged in air obtained a strong increase in the TVBN values unlike the T2 and T4 theses which kept the values low and within the most restrictive limits.

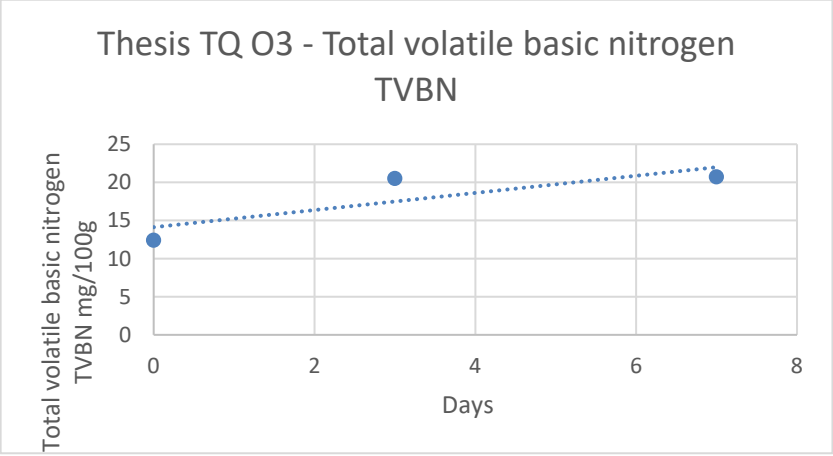
Total volatile basic nitrogen

Total volatile basic nitrogen TVBN mg/100g				
	Period			
	T0	T3	T7	T13
Thesis TQ	21,9	22,9	29,4	
Thesis TQ O3	12,4	20,5	20,7	
Thesis T1	21,9	21,5	33,8	22,6
Thesis T2	12,4	0	25,00	19,8
Thesis T3	21,9	38,2	33,9	40,40
Thesis T4	12,4	0	17,9	26,7

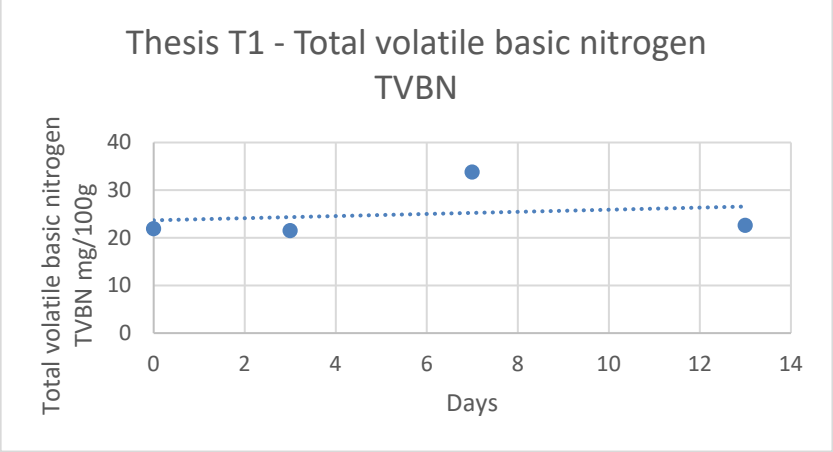
Table 2. Total volatile basic nitrogen values (TVBN)



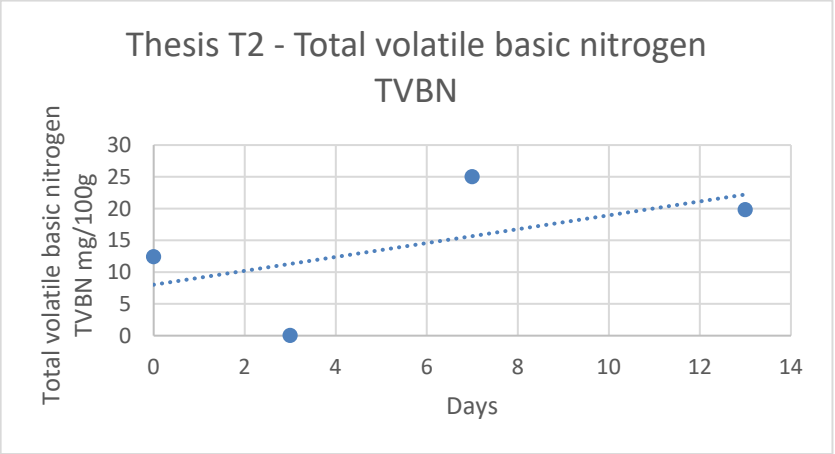
Graph 8. Thesis TQ – Total volatile basic nitrogen TVBN



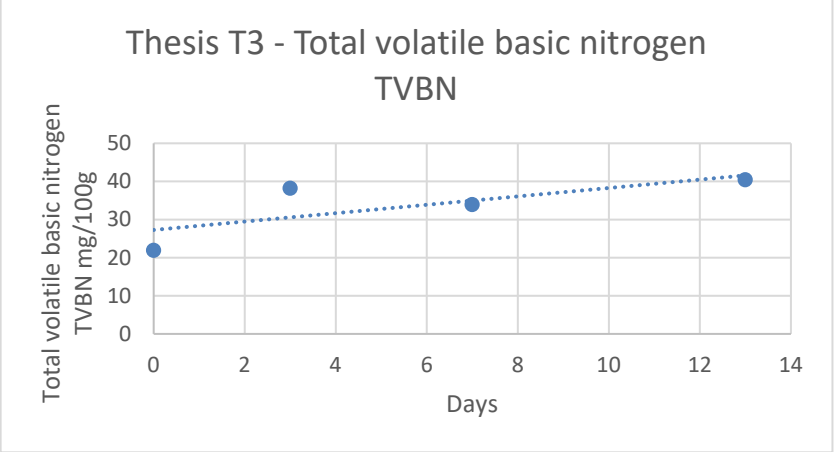
Graph 9. Thesis TQ O3 Total volatile basic nitrogen TVBN



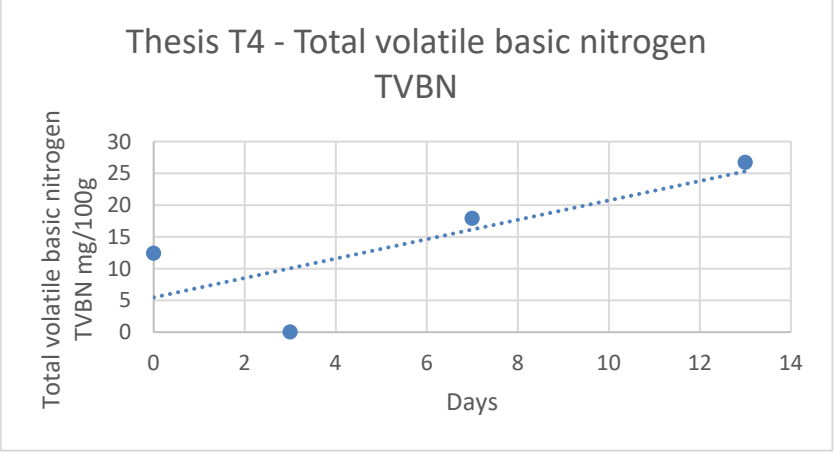
Graph 10. Thesis T1 Total volatile basic nitrogen TVBN



Graph 11. Thesis T2 - Total volatile basic nitrogen TVBN



Graph 12. Thesis T3 Total volatile basic nitrogen TVBN



Graph 13. Thesis T4 - Total volatile basic nitrogen TVBN

Histamine

Current regulations (EC Reg. 854/04 and EC Reg. 2073/05) set a maximum histamine content of 100 milligrams per kilo (mg / kg) for fresh fish and 200 mg / kg for preserved products. The National Health Service has the task of supervising to verify compliance with these limits and compliance with adequate storage and transport temperatures. Below are the values of histamine in relation to the potential harmful to humans:

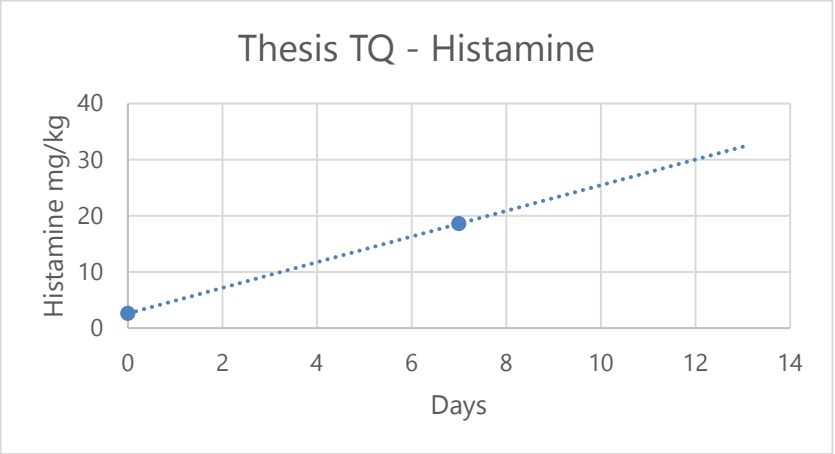
- Mild> 5 - 40 mg / 100 g
- Moderate> 40 mg / 100 g
- Severe> 100 mg / 100 g

The histamine analyzes of theses T1, T2, T3 and T4 were carried out on the freshly packaged product (0) on the seventh day (7) and on the thirteenth day (13). In the TQ and TQ O3 theses the analyzes were carried out on the freshly packaged product (0) and on the seventh day (7) as on the thirteenth day the sardine fillets were no longer marketable.

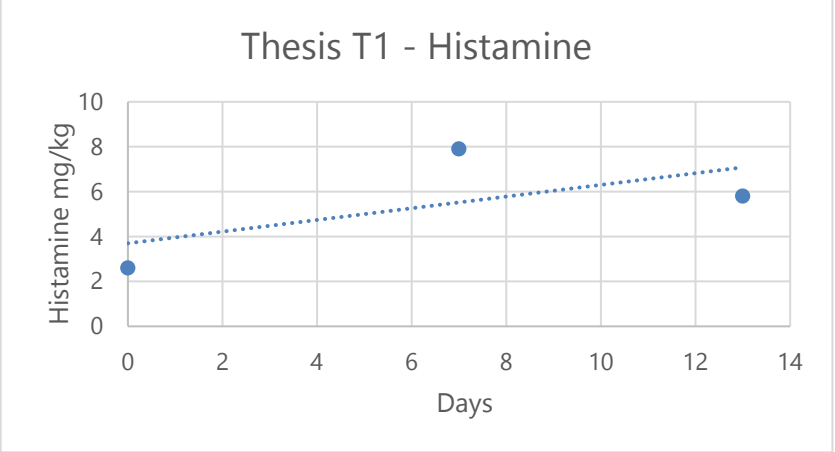
Histamine mg/kg	Period gg		
	0	7	13
Thesis TQ	2,6	18,6	-
Thesis TQ O3	3,6	0	-
Thesis T1	2,6	7,9	5,8
Thesis T2	3,6	0	6,3
Thesis T3	2,6	4,8	4
Thesis T4	3,6	0	6,7

Table 3. Histamine values

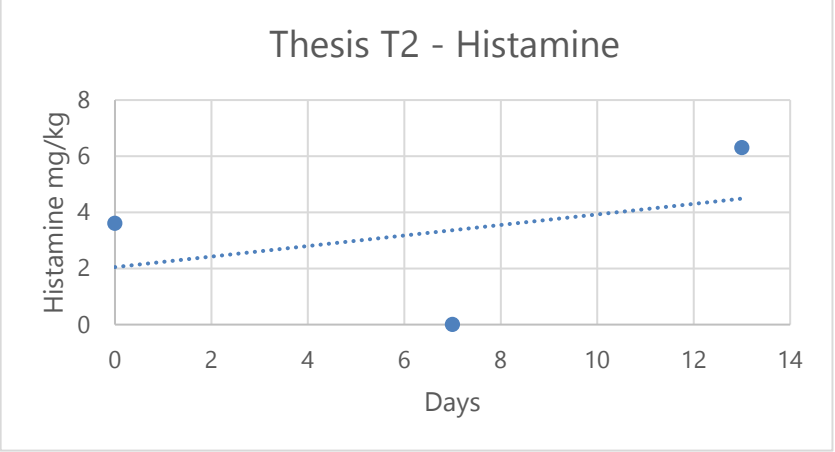
From the results obtained, it can be seen that the products packaged in a modified atmosphere and washed with ozonated water, kept the histamine concentrations lower than the products packed in the air. The data are confirmed by the regression lines obtained in the graphs Graph 14, Graph 15, Graph 16, Graph 17, Graph 18 through which we can observe that the histamine present in the samples relating to the QT thesis increases, while the theses for products with ozonated keep the histamine values lower. Samples from Thesis T4 obtained the lowest histamine values.



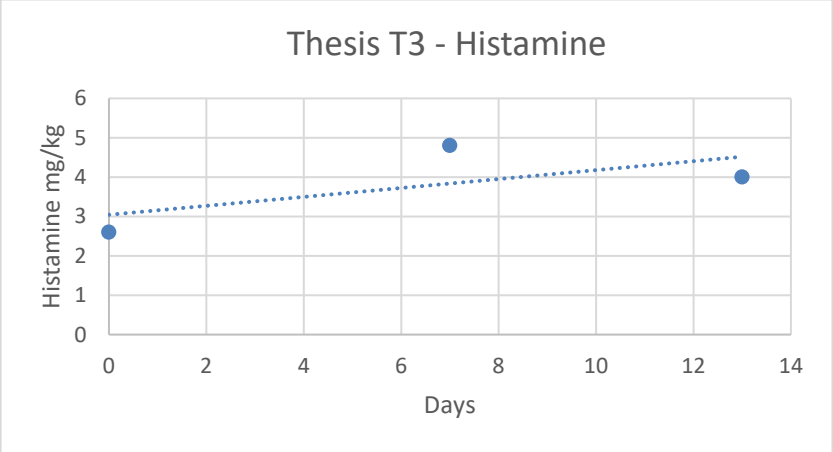
Graph 14. Thesis TQ – Histamine



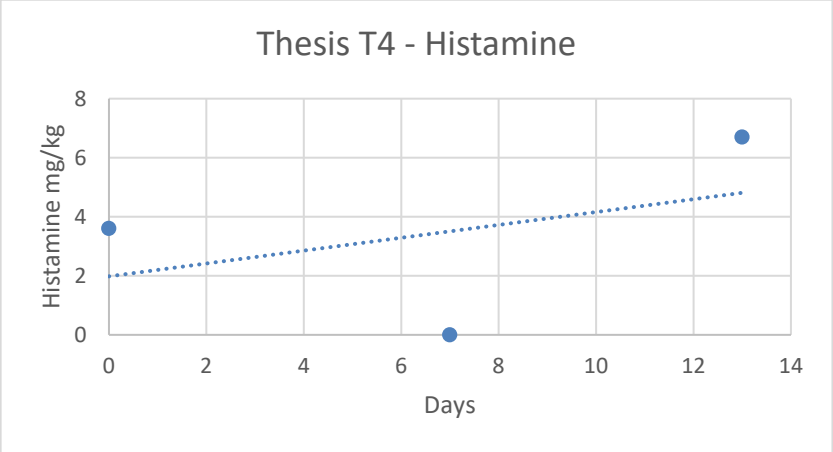
Graph 15. Thesis T1 – Histamine



Graph 16. Thesis T2 – Histamine



Graph 17. Thesis T3 – Histamine



Graph 18. Thesis T4 – Histamine

1.6. Colorimetric Analysis

For Colorimetric Analysis, a tristimulus spectrophotometer ns800 (3nh - Noida Uttar Pradesh – INDIA) has been used. Results are expressed as L* (brightness), a* (red/green index) and b* (yellow/blue index) of CIELab scale Figure 7 Cielab Scale.

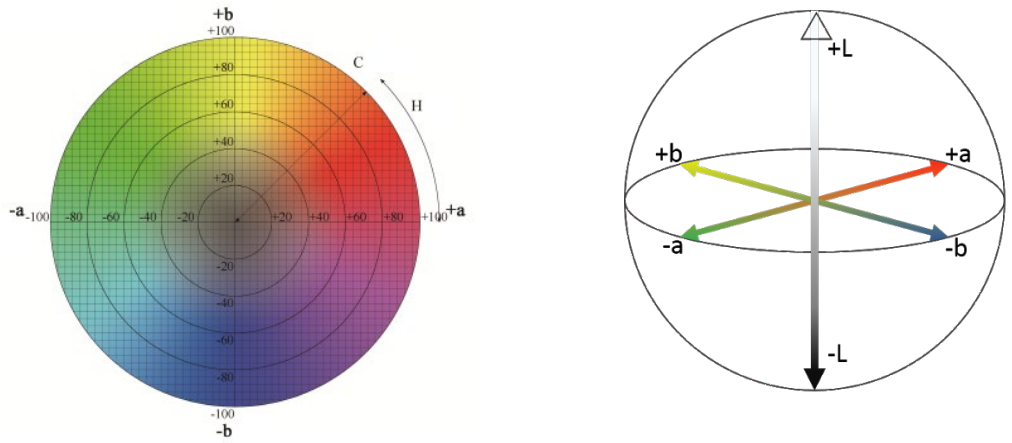
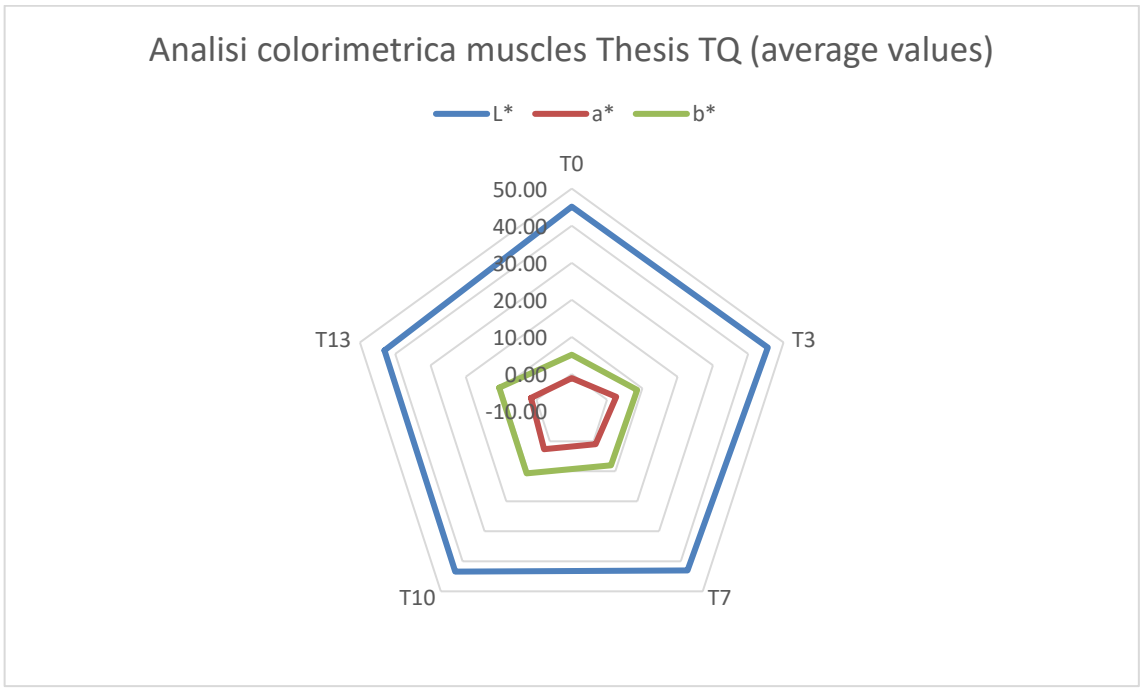


Figure 7. Cielab scale

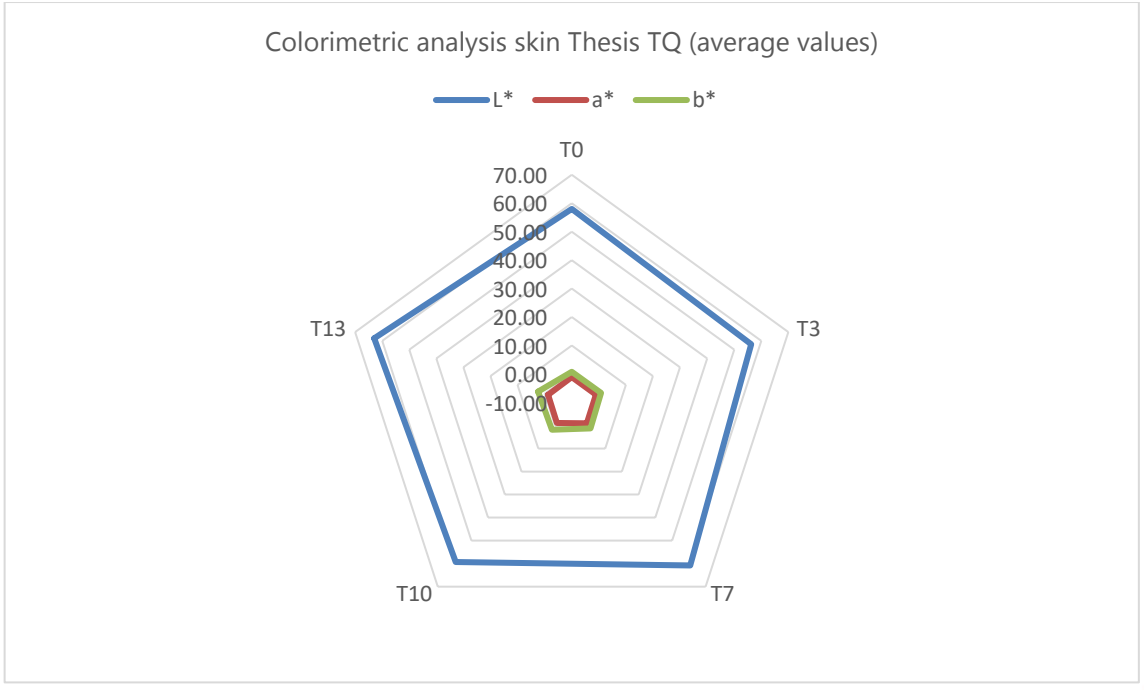
The colorimetric analysis has been carried out on six points, three on the skin surface and three on the muscular one. The colorimetric analysis of the skin surface has been carried out taking three measures for each point: left abdominal (1) - dorsal (2) - right abdominal (3). The colorimetric analysis of the muscle bundles has been carried out taking three measures for each point: left caudal section surface (1) - left abdominal section surface (2) - abdominal section surface (3).

Figure 8. Colorimetric analysis measurement

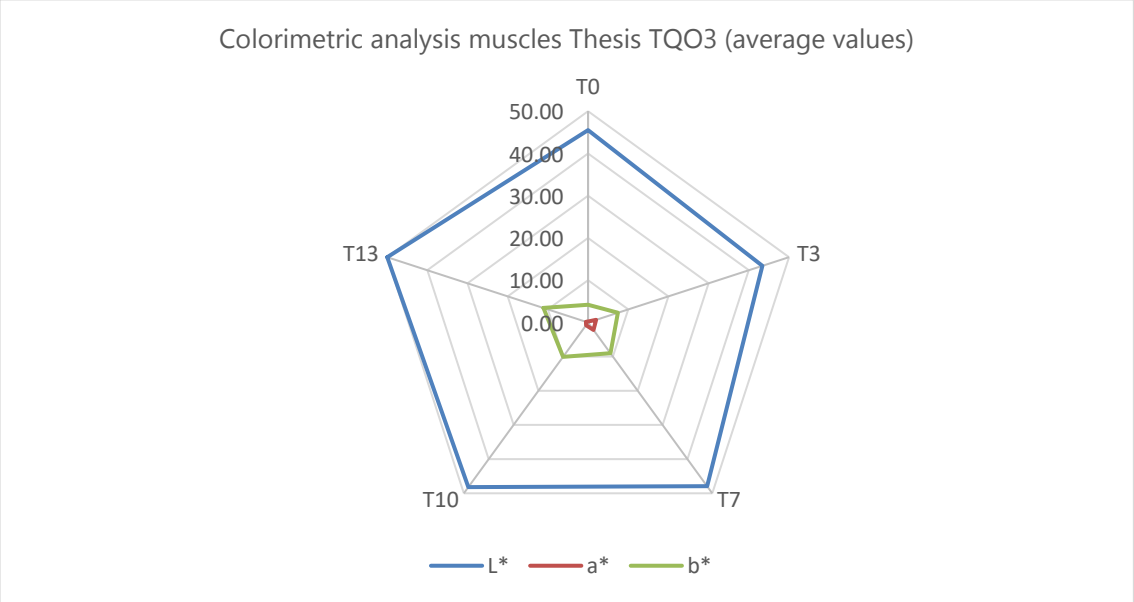
The average of the three points measured has been calculated for each surface. Below are the graphs for each thesis with the results relating to the colorimetric analyzes distributed over the shelf-life period.



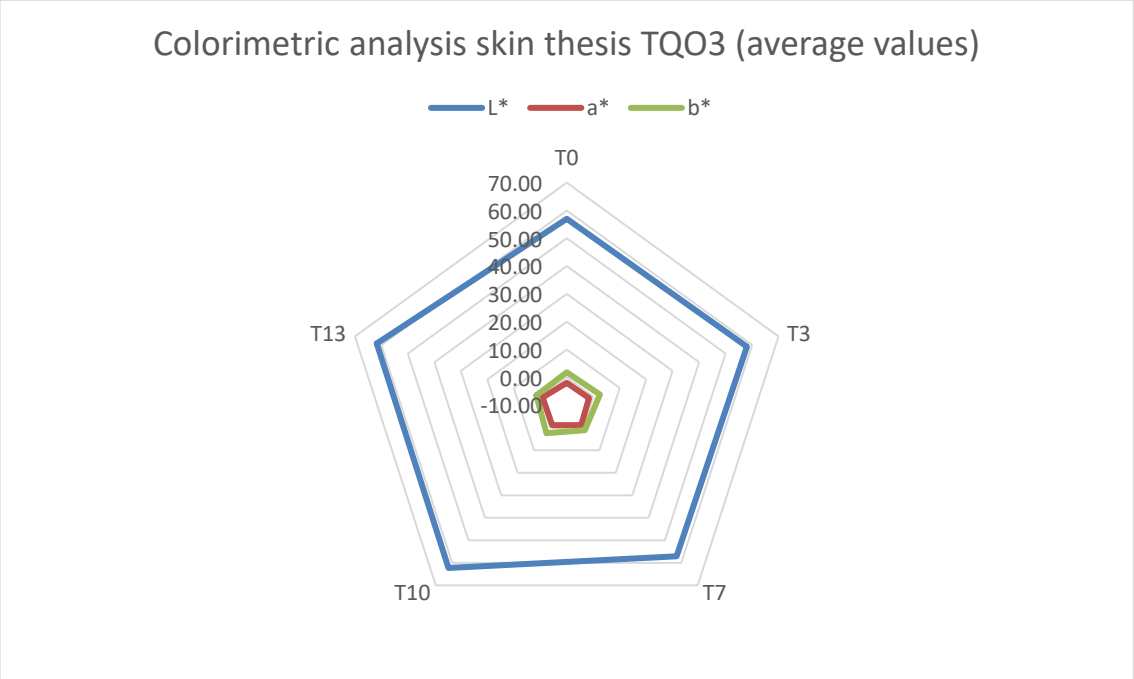
Graph 19. Colorimetric analysis muscles Thesis TQ



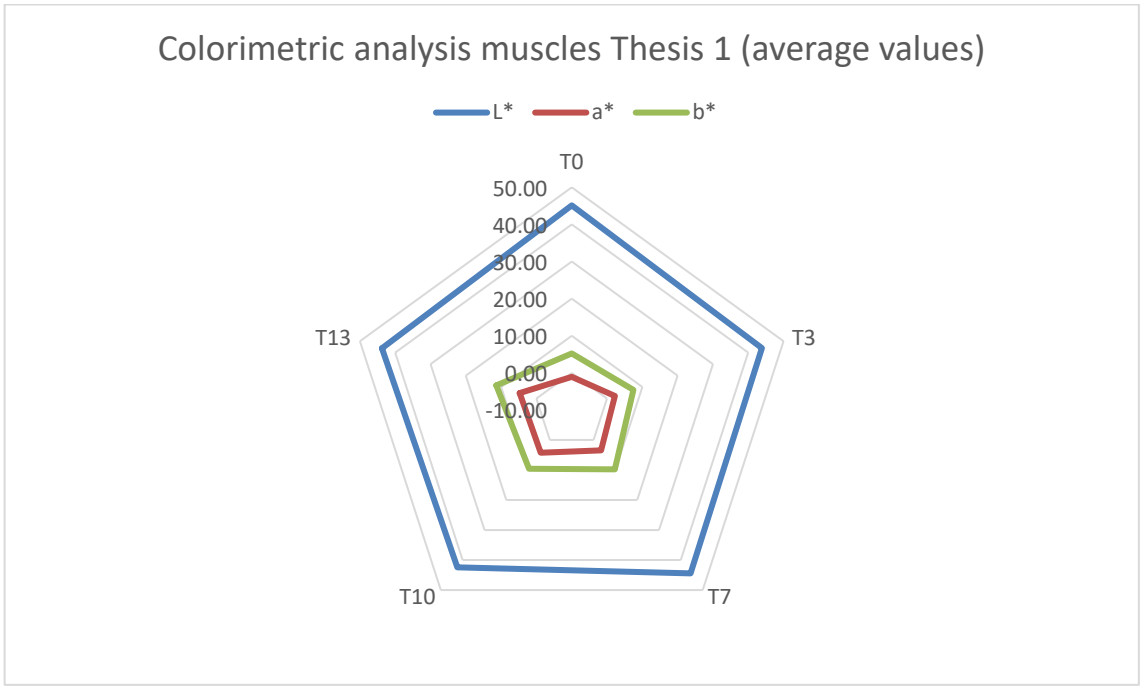
Graph 20. Colorimetric analysis skin Thesis TQ



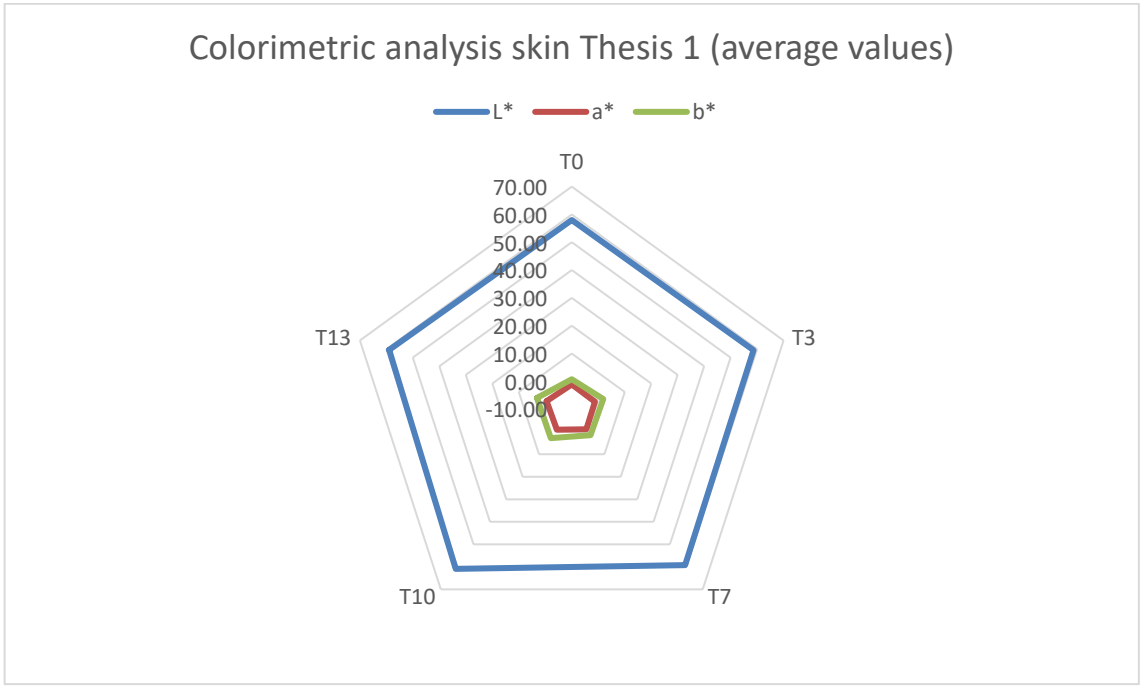
Graph 21. Colorimetric analysis muscles Thesis TQ3



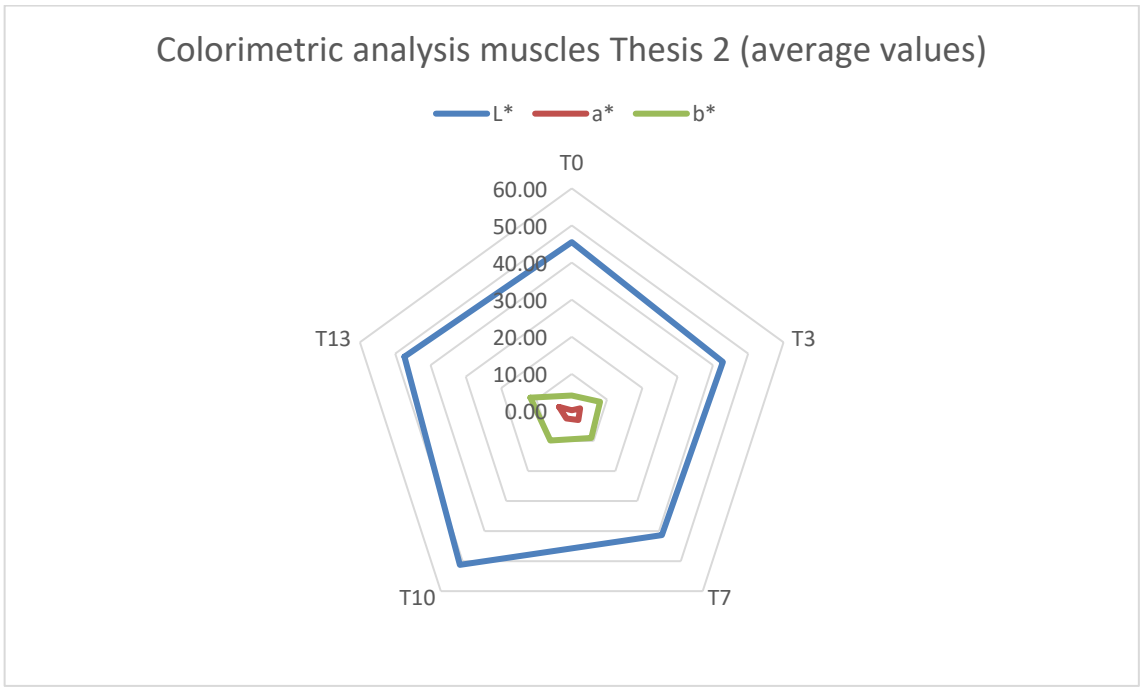
Graph 22. Colorimetric analysis skin Thesis TQO3



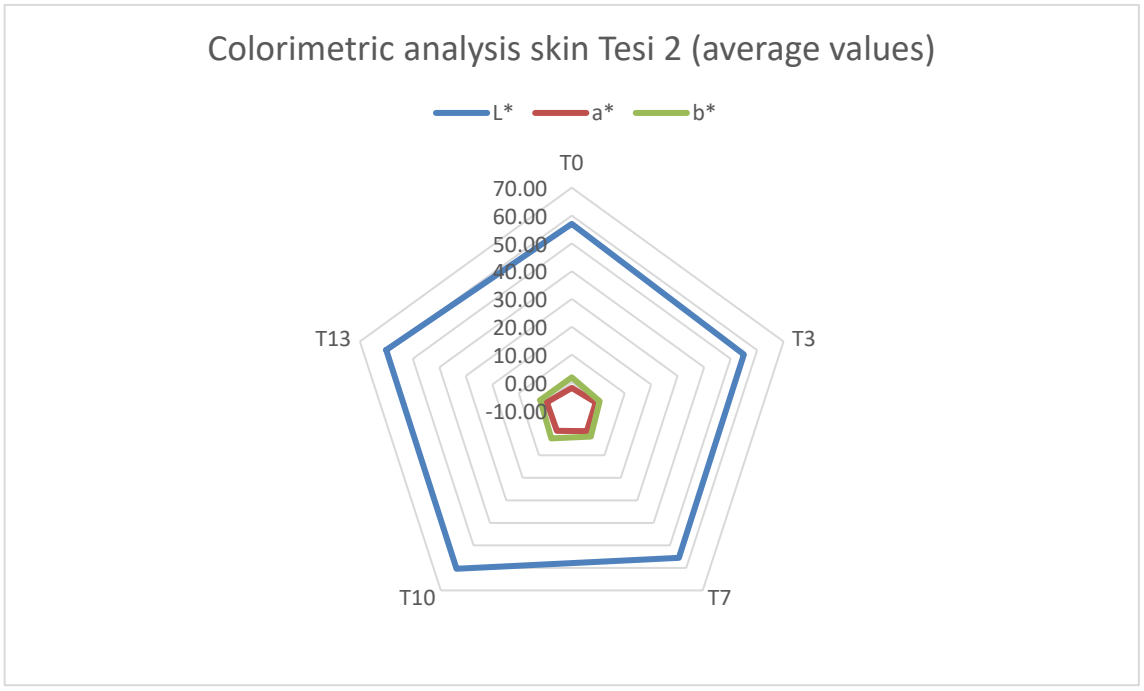
Graph 23. Colorimetric analysis muscles Thesis T1



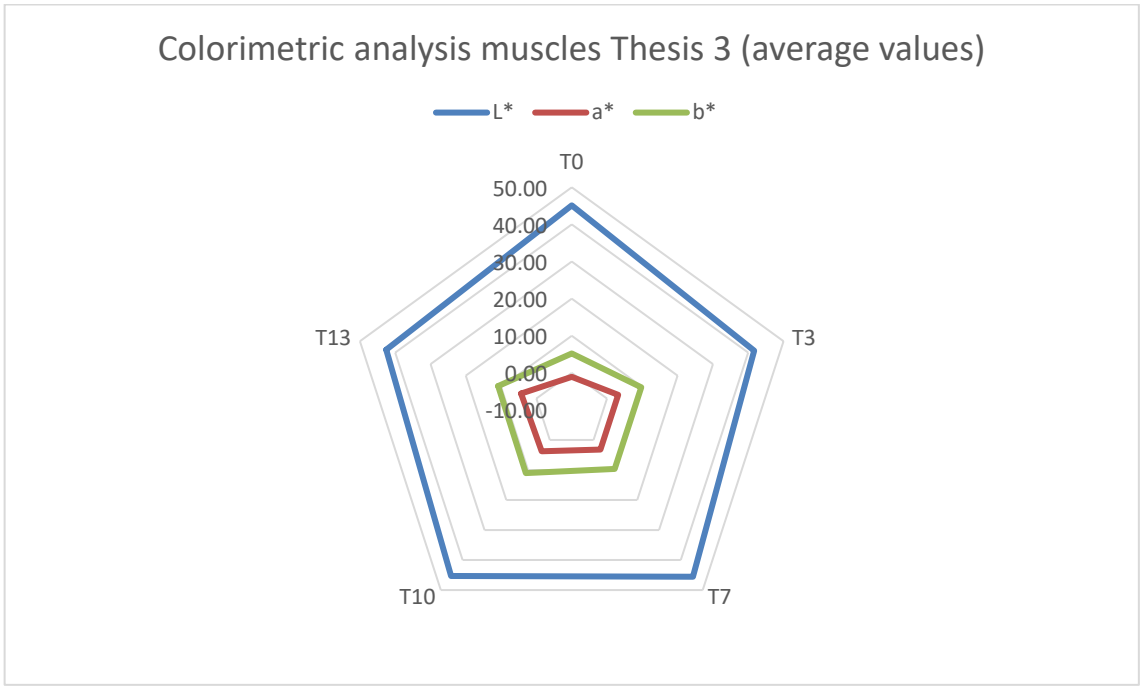
Graph 24. Colorimetric analysis skin Thesis T1



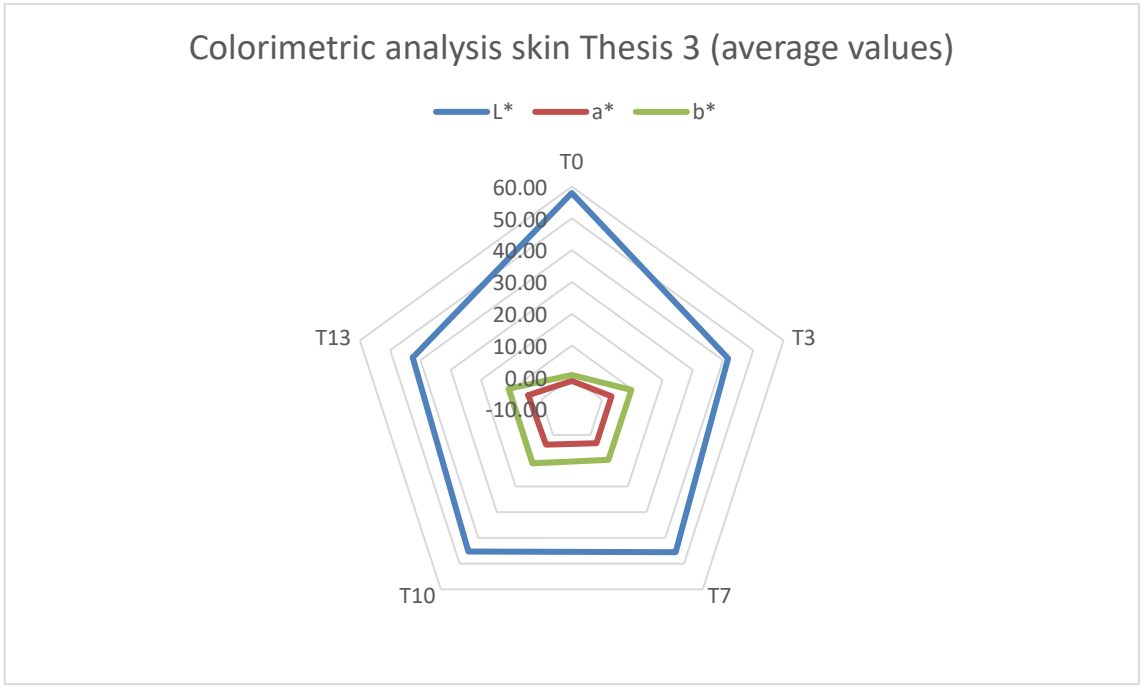
Graph 25. Colorimetric analysis muscles Thesis T2



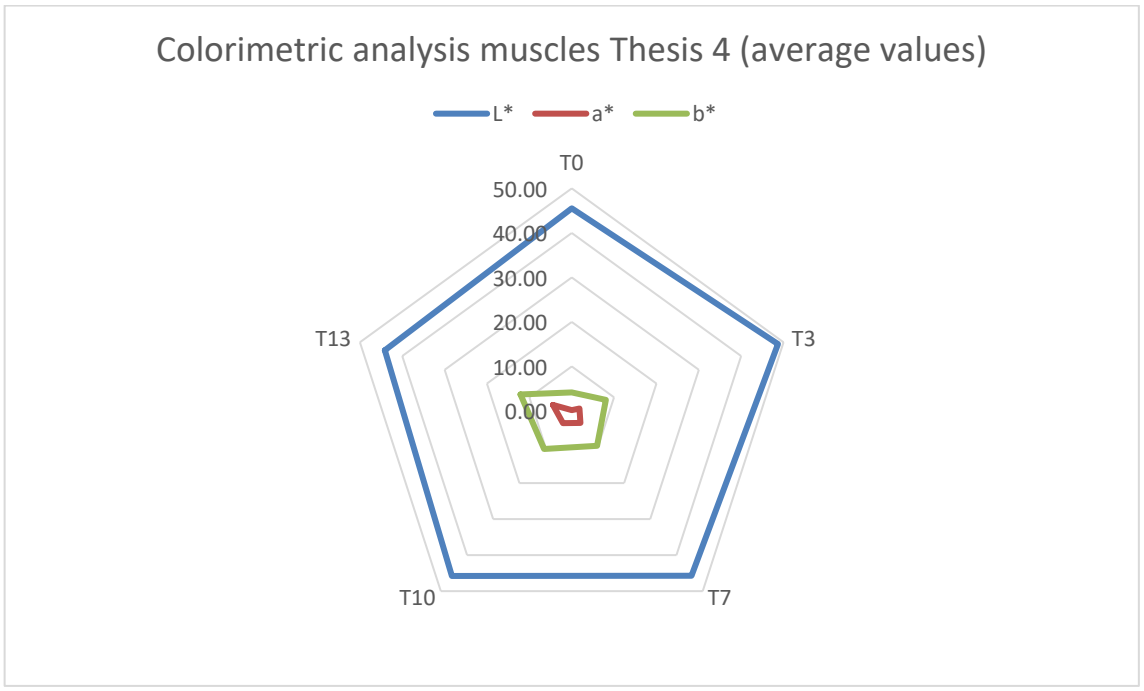
Graph 26. Colorimetric analysis skin Thesis T2



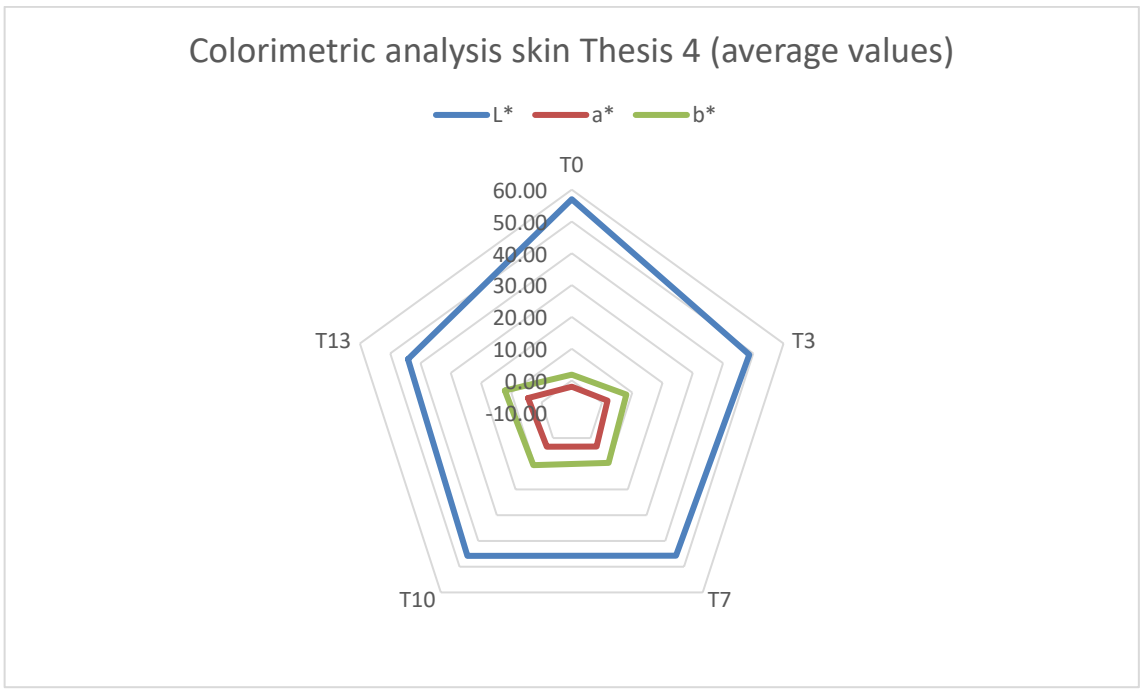
Graph 27. Colorimetric analysis muscles Thesis T3



Graph 28. Colorimetric analysis skin Thesis T3



Graph 29. Colorimetric analysis muscles Thesis T4



Graph 30. Colorimetric analysis skin Thesis T4

The results confirm that the degree of brightness of the muscular tract, during shelf life, remained almost constant for all the theses unlike the skin, which varies according to the treatments performed. With regard to the parameter a * (red-green index), an increase in the value tending to red is observed, especially in products packaged in a modified atmosphere compared to those packaged in air. As regards the parameter b * (yellow-blue index), a generalized increase in the value tending to yellow is more marked in thesis T4 in both surfaces subjected to analysis (muscle bundles, skin). The data obtained show that products packaged in a modified atmosphere have more intense colors than those packaged in air. Treatment with ozonated water has improved the appearance of the skin, making it brighter than that product washed with non-ozonated water. Below you can see the photo of the theses after thirteen days.

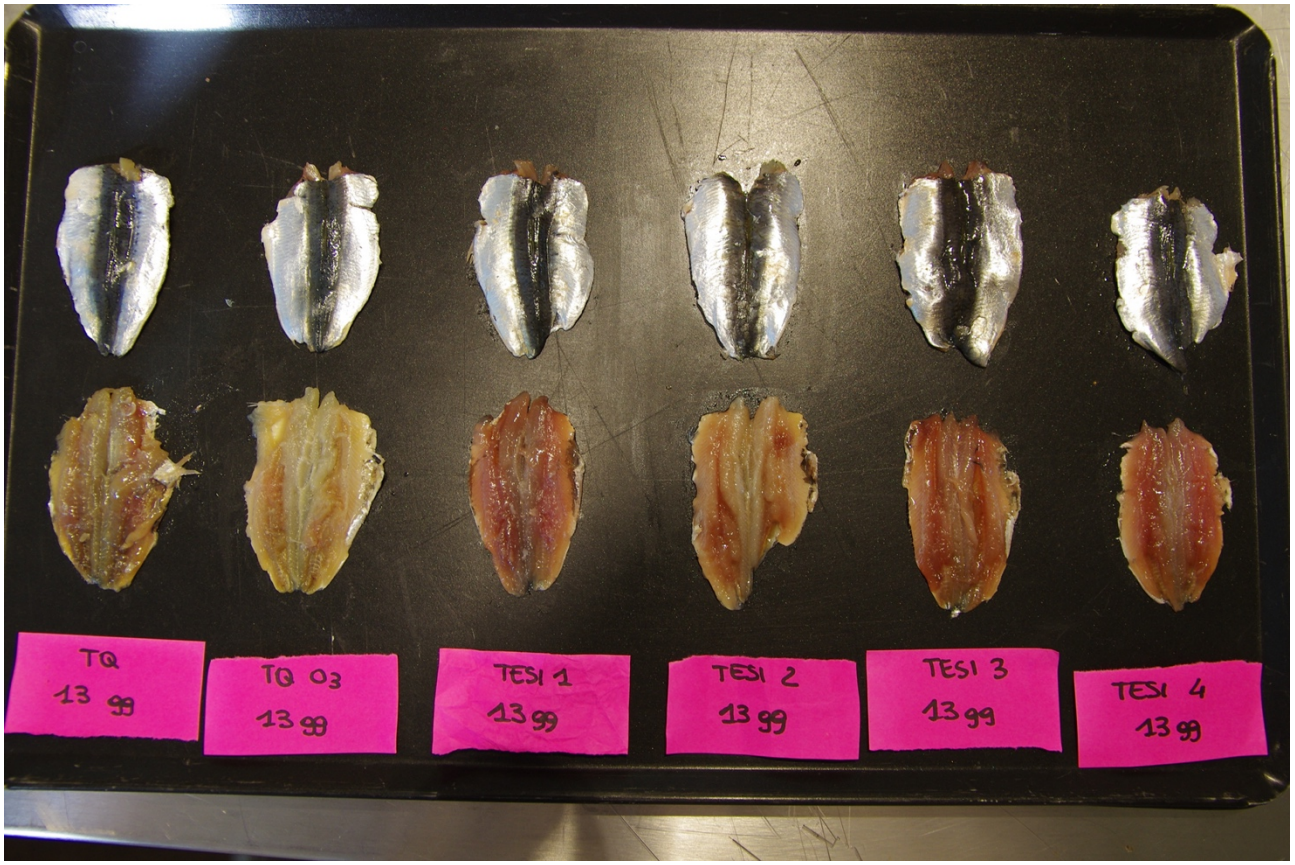
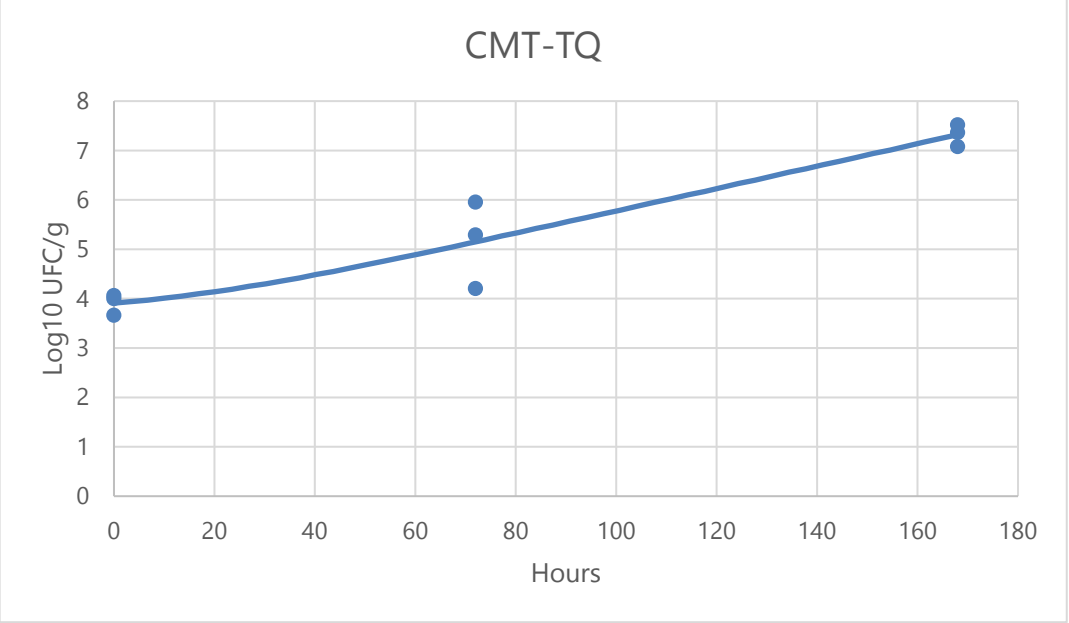


Figure 9. Thesis pictures after 13 days

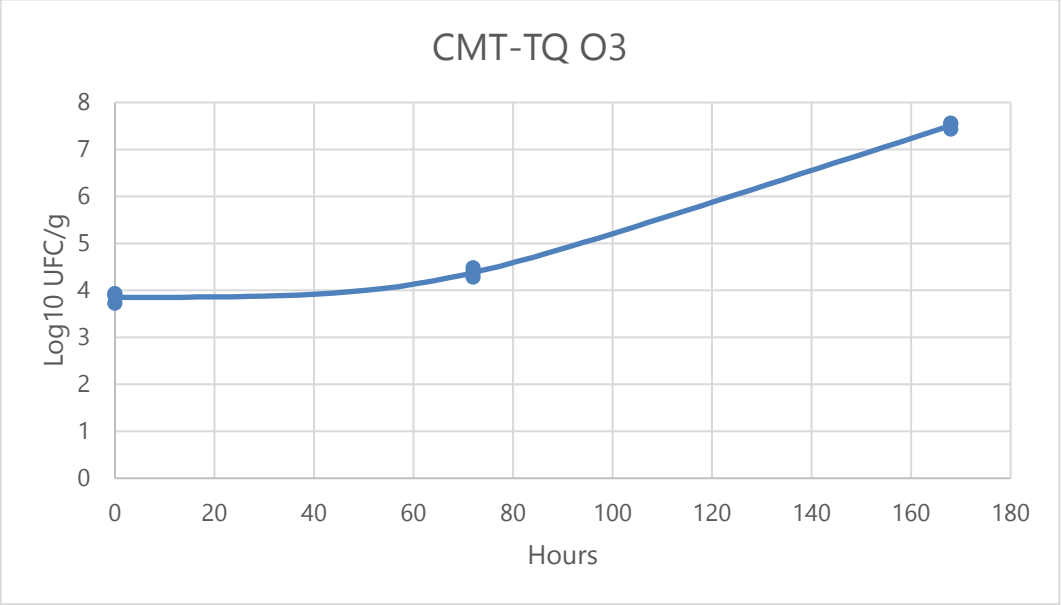
1.7. Microbiological analysis

From the results obtained it can be deduced that the products washed with ozonated drinking water have a lower microbial load than those washed with drinking water only. In addition, the sardine fillets packaged in a modified atmosphere have maintained a lower microbial load over time due to the carbon dioxide present. This gas carries out an important activity from the point of view of the product acidification as carbon dioxide, coming into contact with water, develops carbonic acid according to the following reaction: $H_2O + CO_2 \rightarrow H_2CO_3$

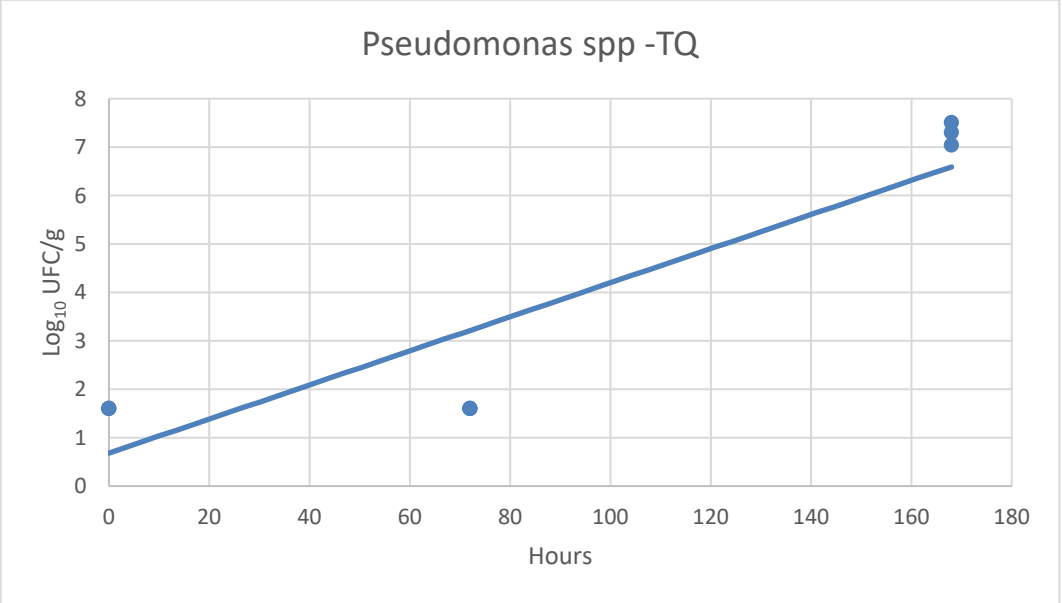
Carbonic acid is a water-soluble molecule therefore it will tend to bind to the substrate making it moderately acidic. The graphs relating to microbial loads are shown below.



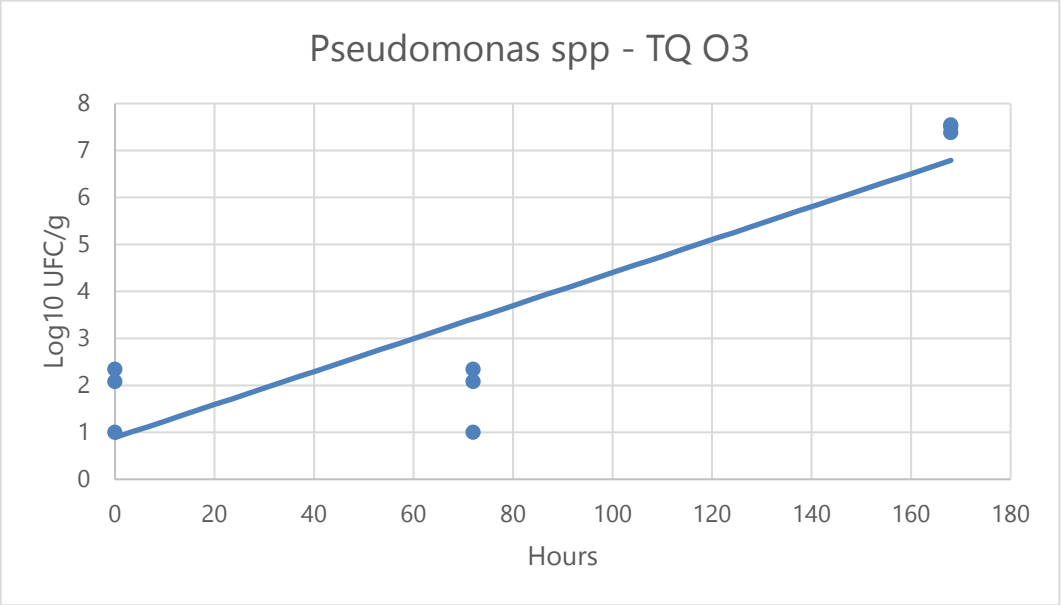
Graph 31. Thesis TQ – Total Mesofila growth curve at 30°C



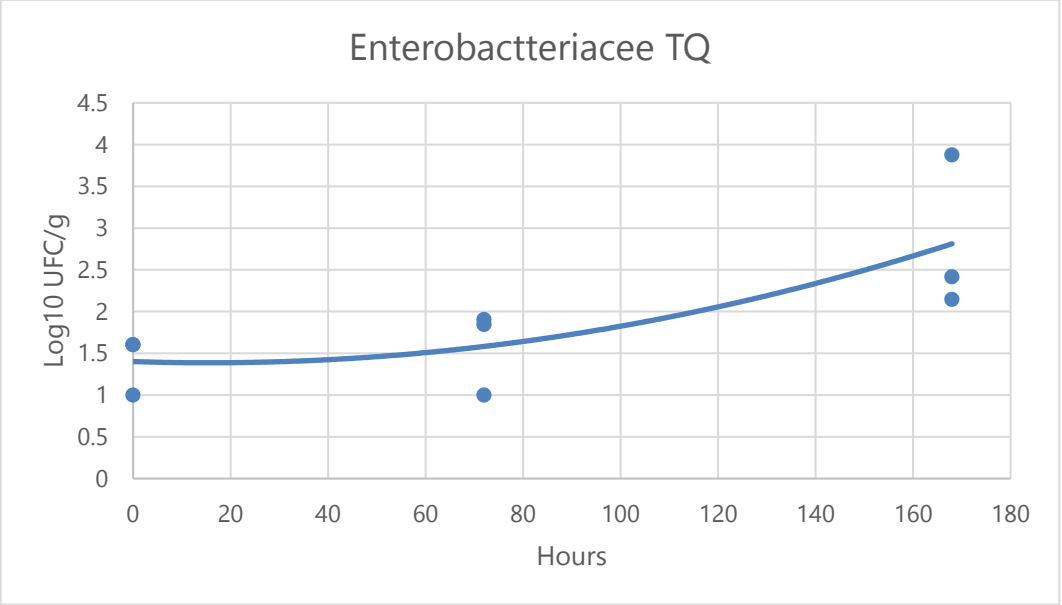
Graph 32. Thesis TQ O3 - Total Mesofila growth curve at 30°C



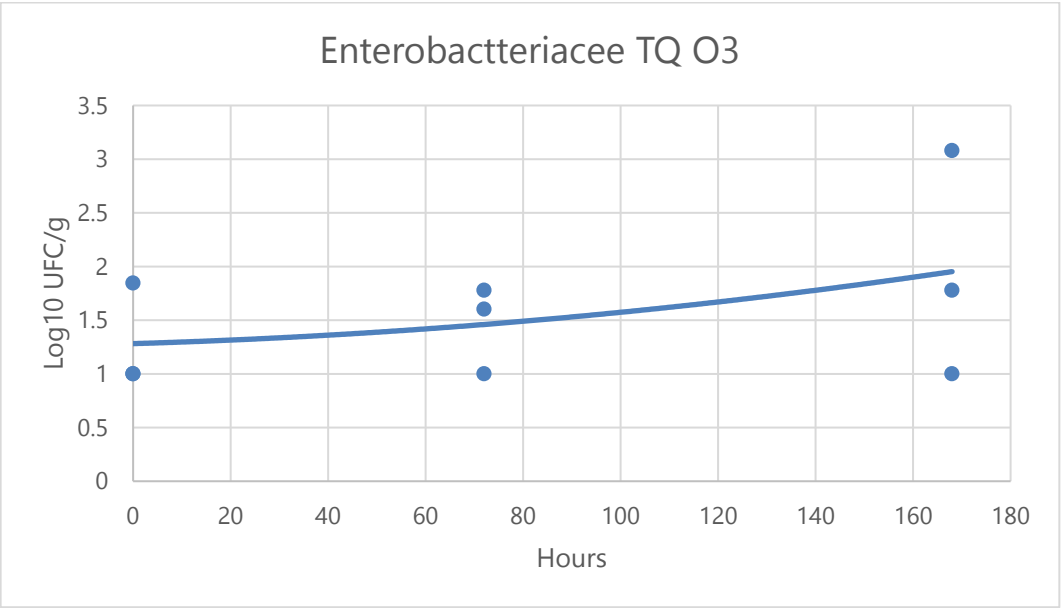
Graph 33. Thesis TQ - Pseudomonas spp growth curve



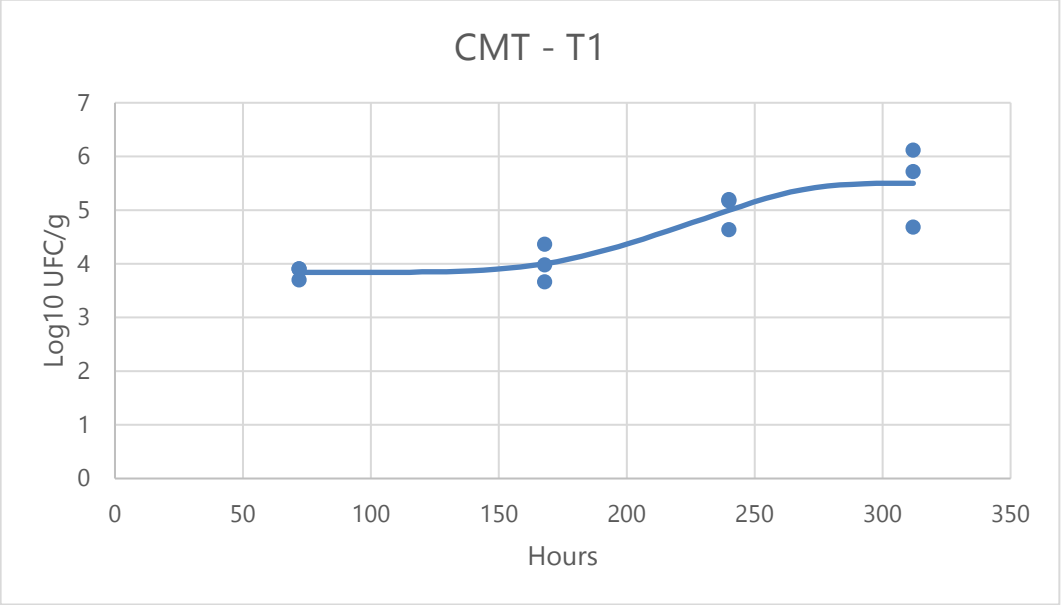
Graph 34. Thesis TQ O3 - Pseudomonas spp growth curve



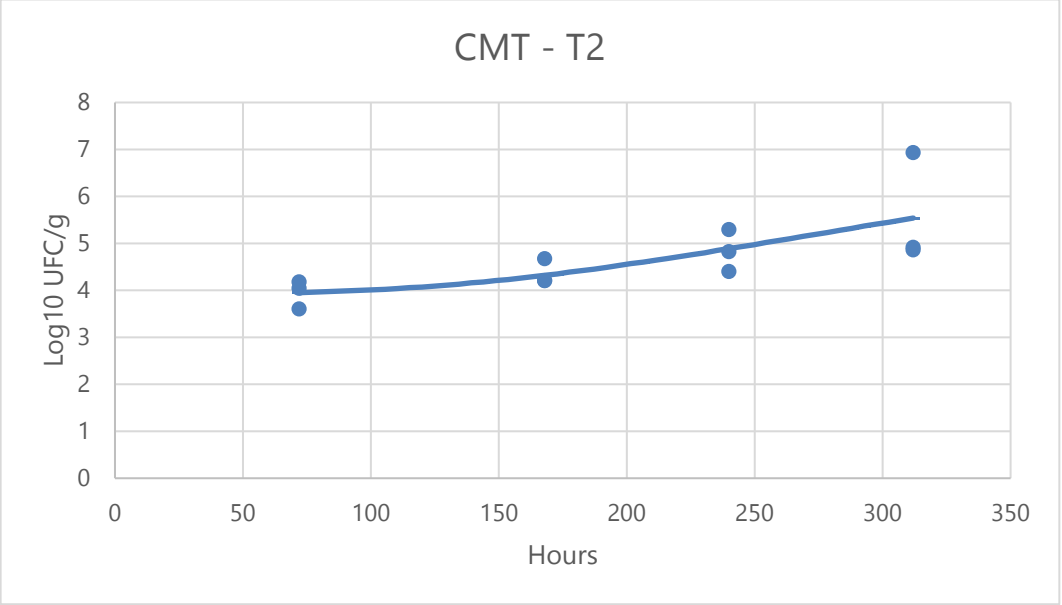
Graph 35. Thesis TQ - Enterobactteriacee growth curve at 37°C



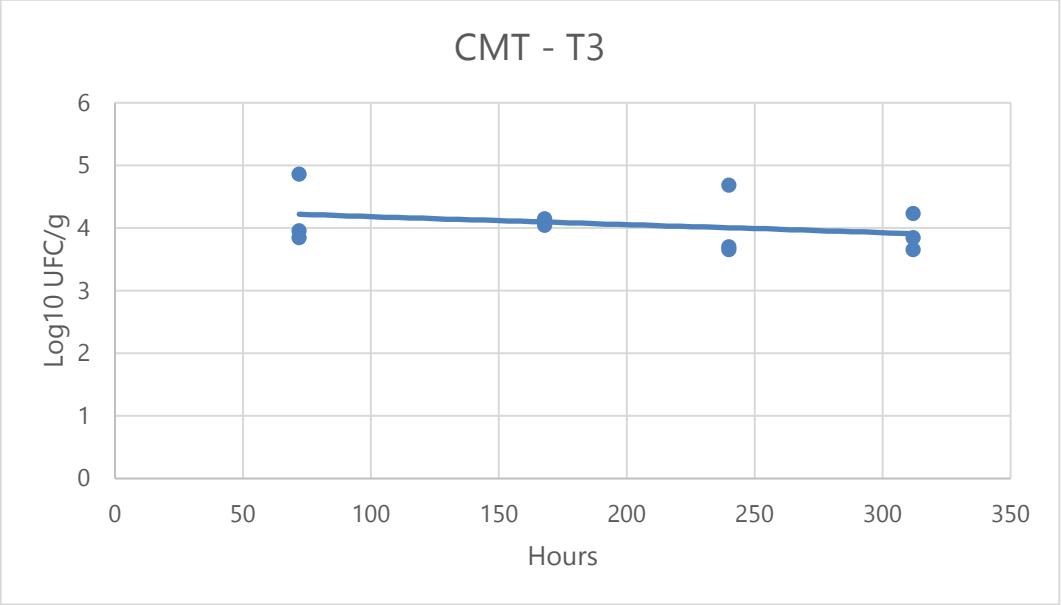
Graph 36. Thesis TQ O3 - Enterobactteriacee growth curve at 37°C



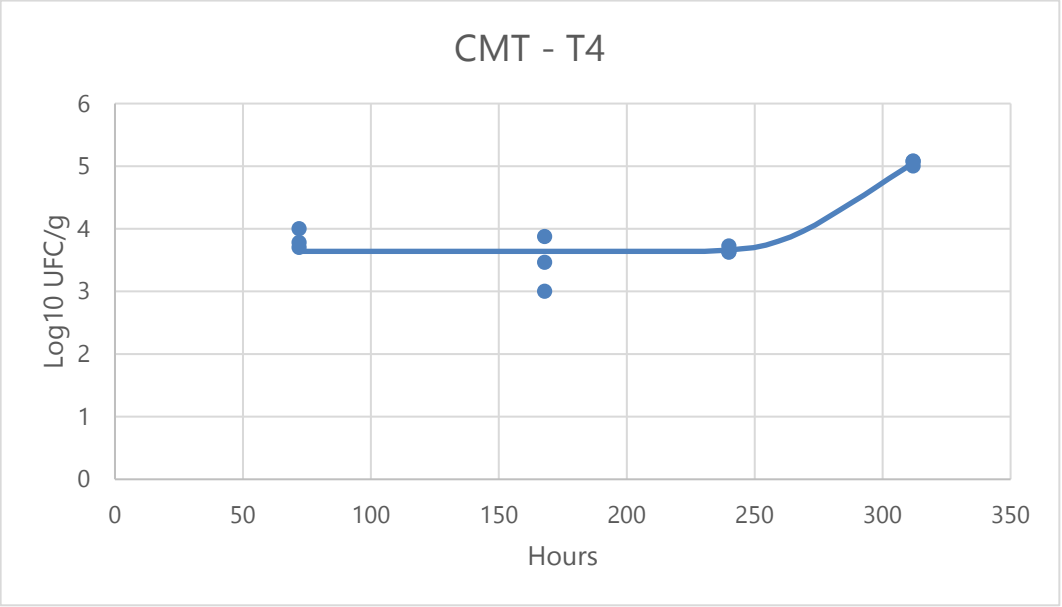
Graph 37. Thesis T1 - Total Mesofila growth curve at 37°C



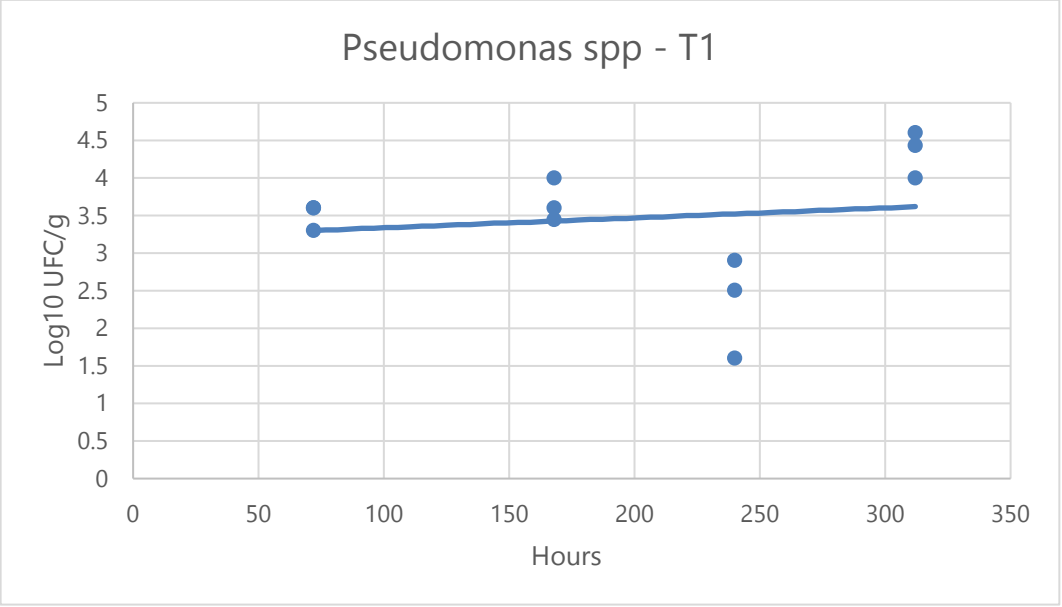
Graph 38. Thesis T2 - Total Mesofila growth curve at 37°C



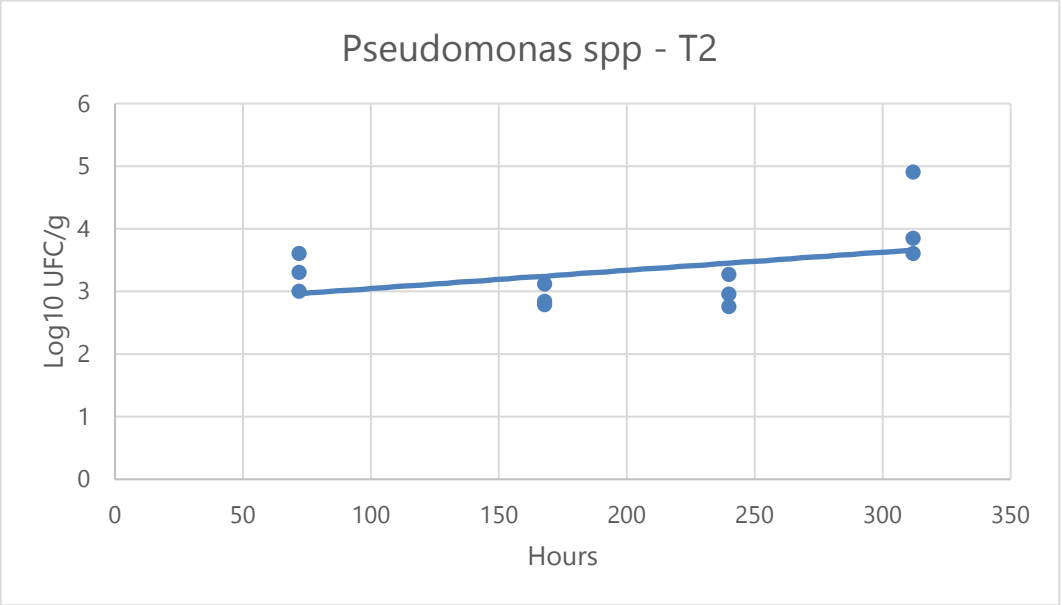
Graph 39. Thesis T3 - Total Mesofila growth curve at 30°C



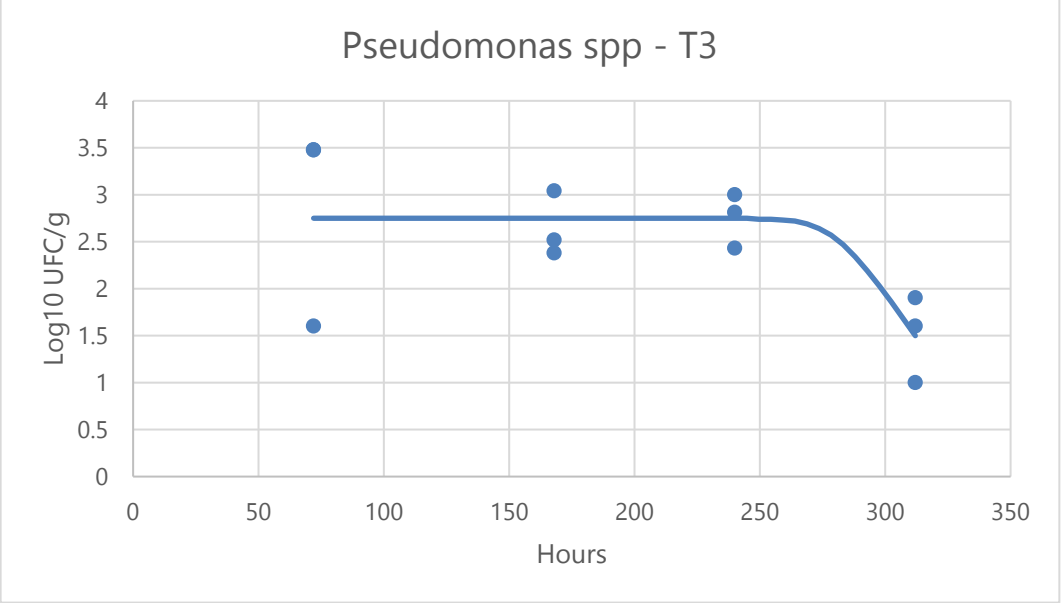
Graph 40. - Thesis T4 Cu Total Mesofila growth curve at 30°C



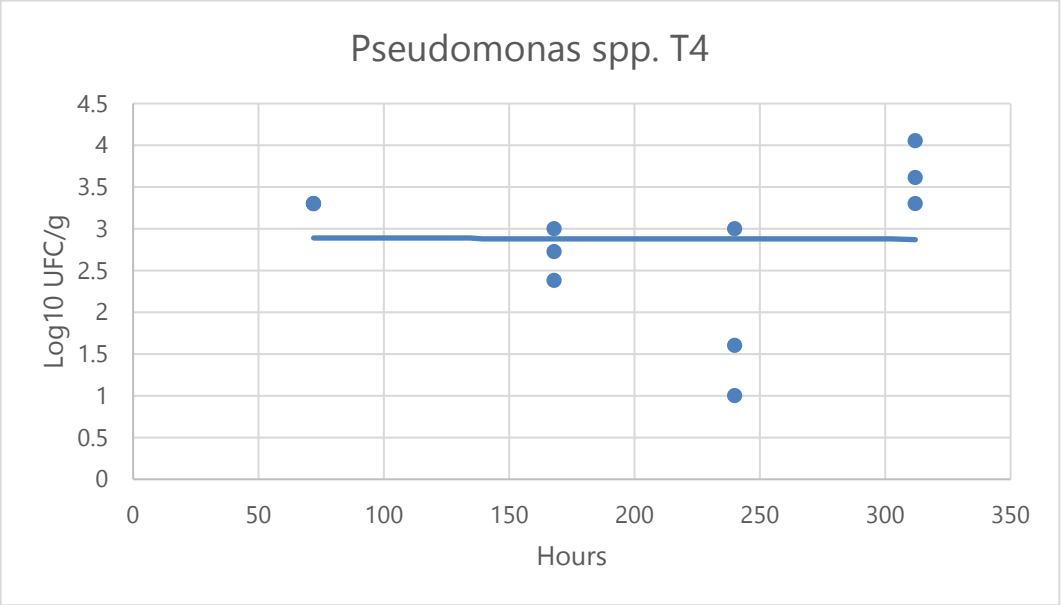
Graph 41. Thesis T1 - Pseudomonas spp. Growth curve



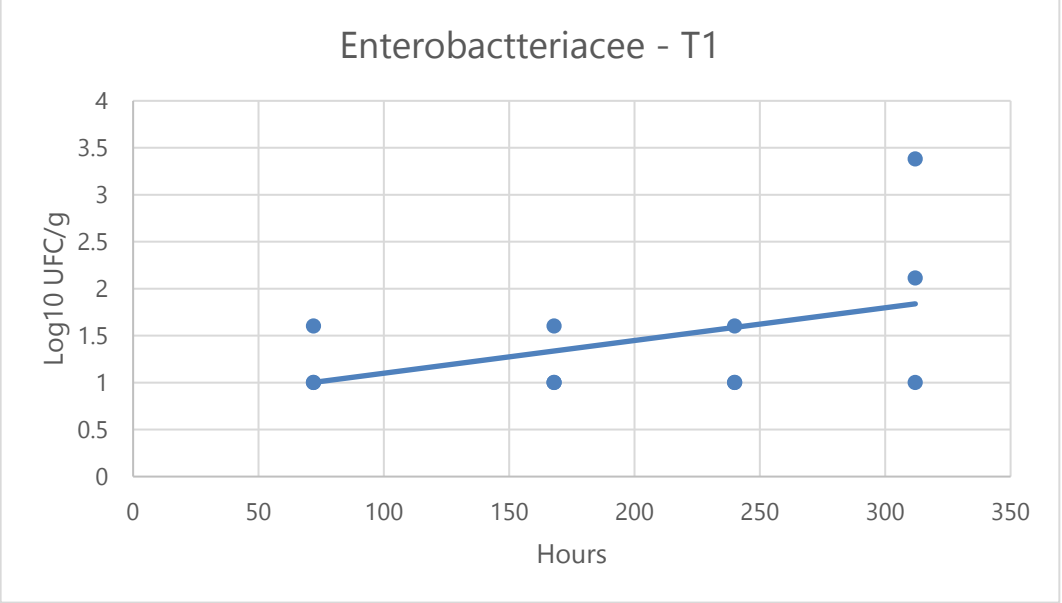
Graph 42. Thesis T2 - Pseudomonas spp. Growth curve



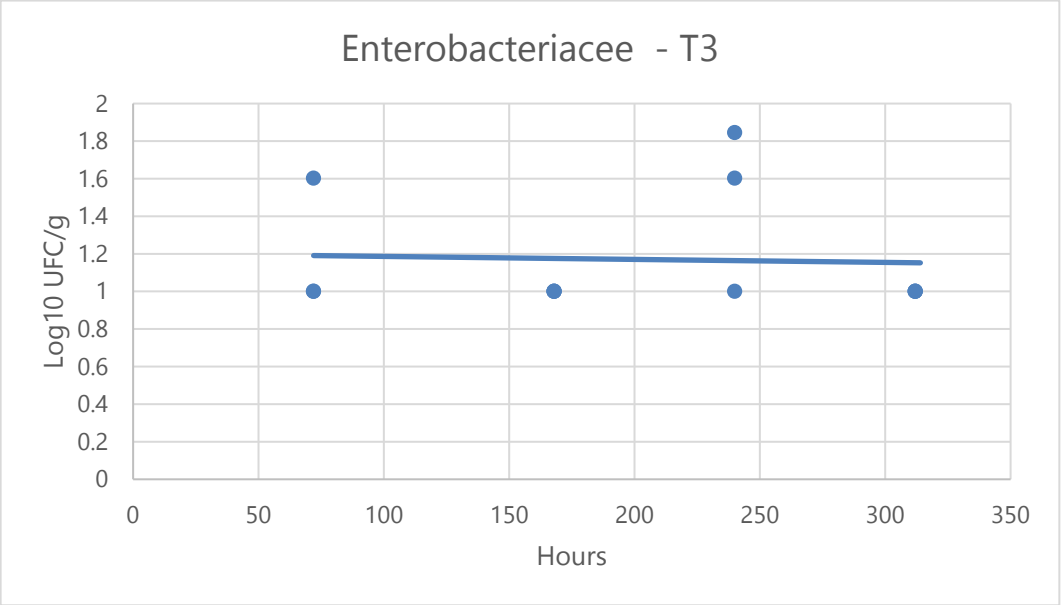
Graph 43. Thesis T3 - Pseudomonas spp. Growth curve



Graph 44. Thesis T4 - Pseudomonas spp. Growth curve



Graph 45. Thesis T1 - Enterobactteriacee growth curve at 37°C



Graph 46. - Thesis T3 - Enterobactteriacee growth curve at 37°C

The graphs relating to the analysis of Enterobactteriacee at 37 ° C for T2 and T4 theses have not been included as they are not significant.

1.8. Texture

Hardness was measured using a dynamometer mod. HDi 500 Texture Analyzer (Stable Micro System, Surray, UK) equipped with a 25 kg load cell. A cutting test was performed using a steel Kramer 5 blade (Figure 10). Approximately 50 g of sardine fillets were used for each analysis. The data obtained were expressed in terms of hardness evaluated with the maximum force peak recorded (g) during cutting, in the direction transversal to the muscle fibers of the analyzed threads.

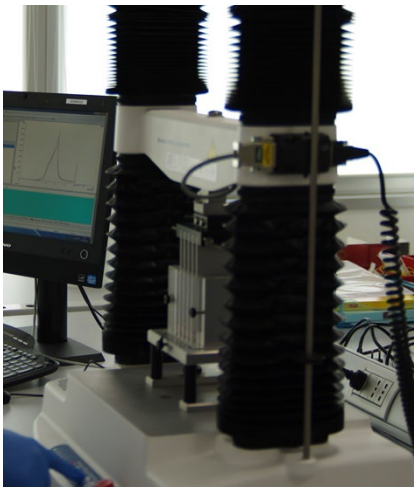
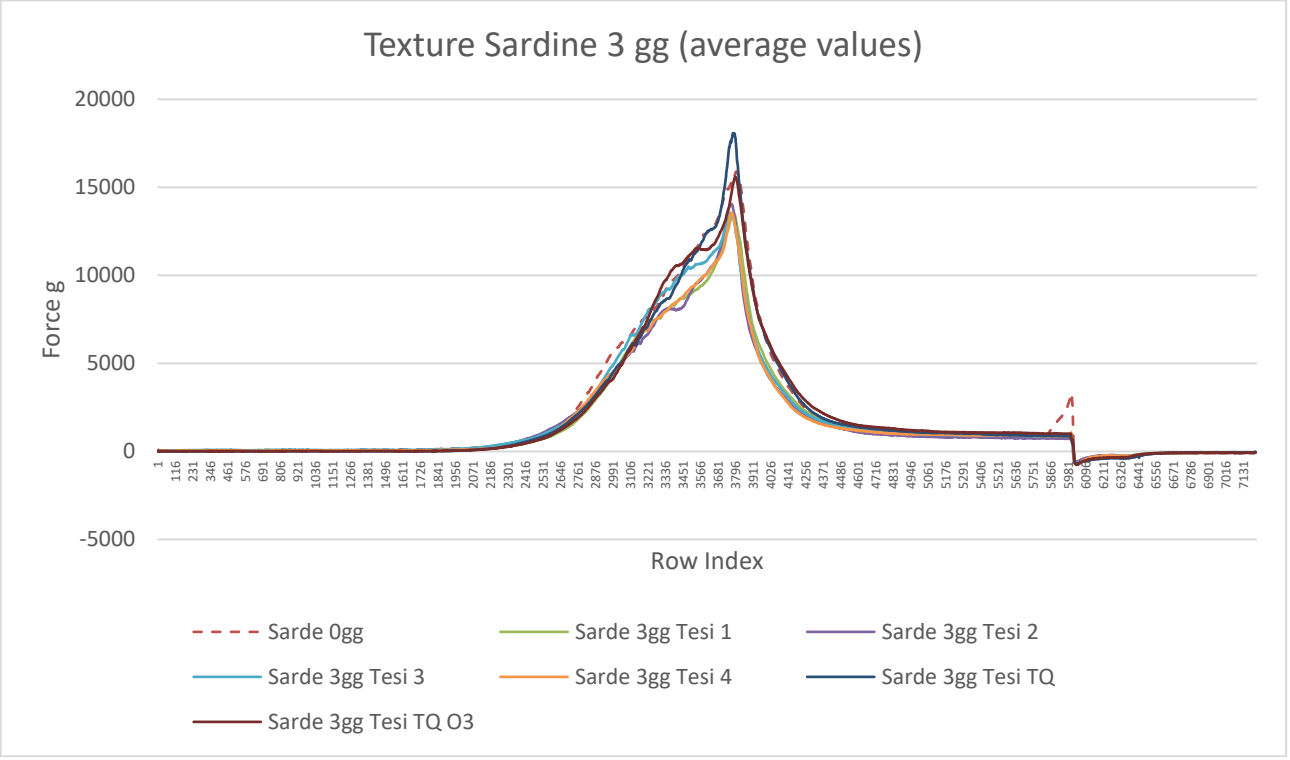
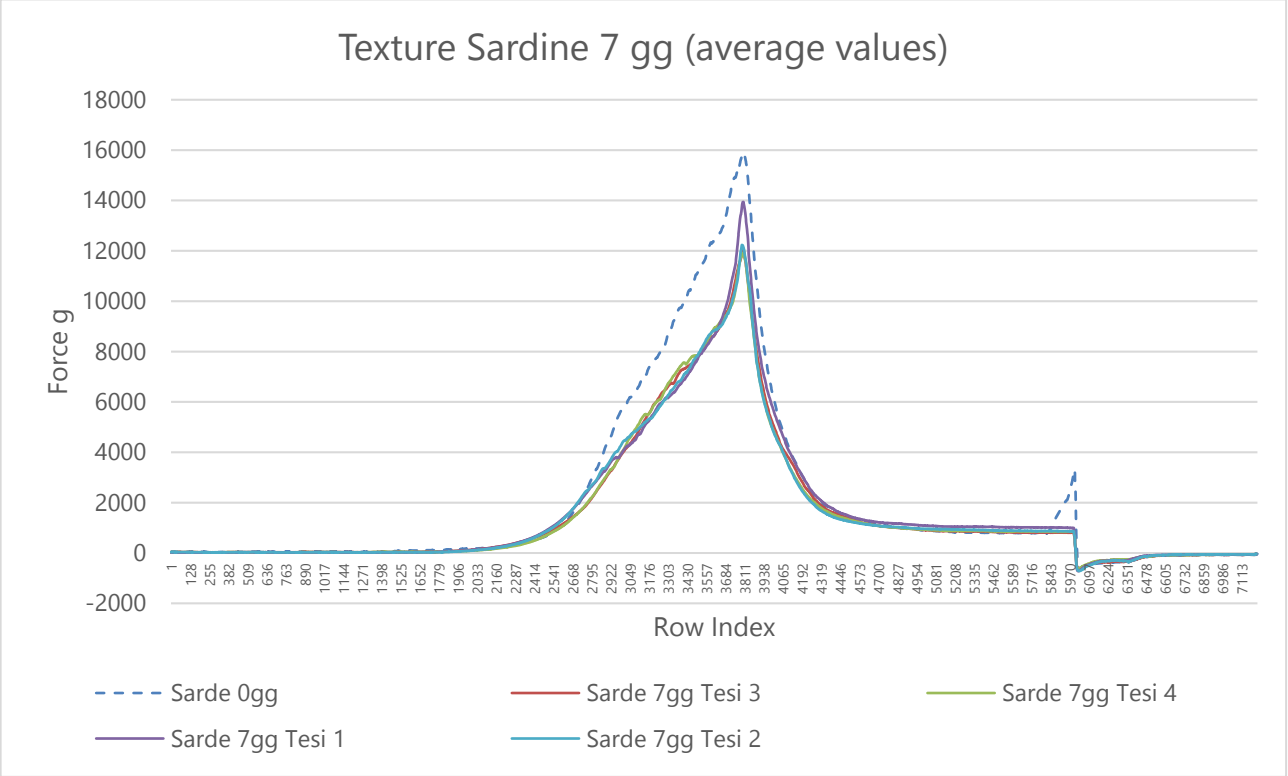


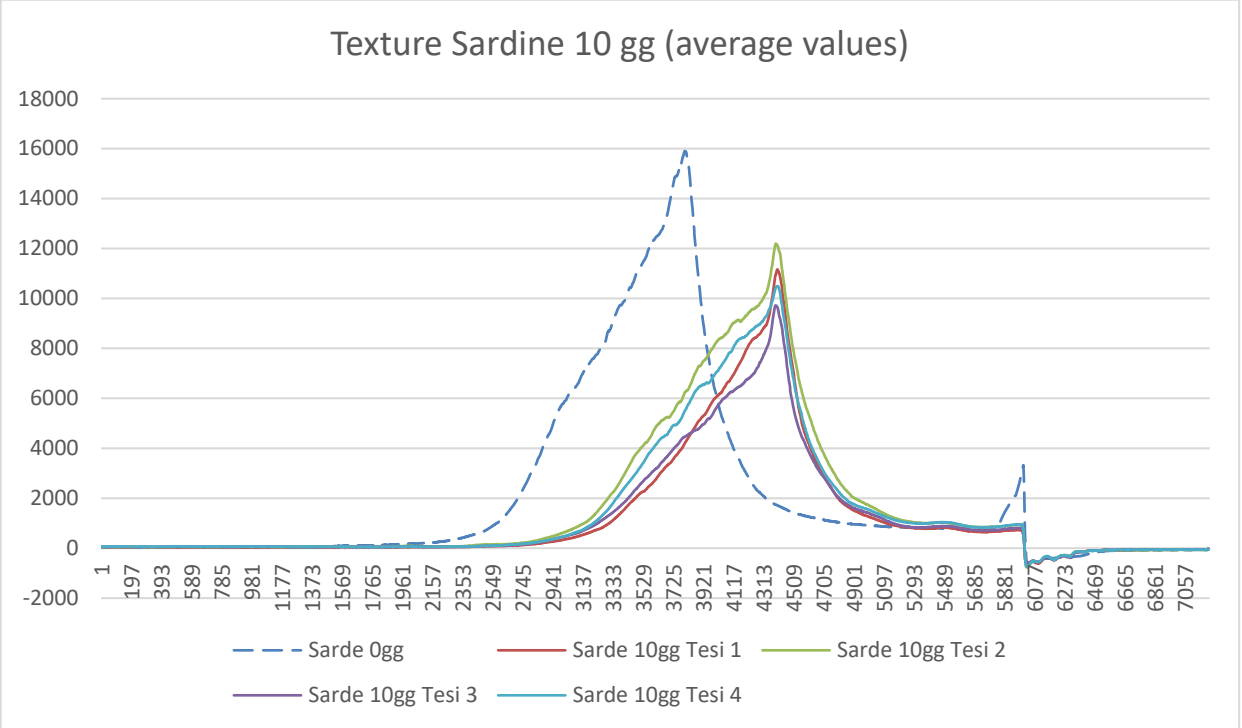
Figure 10. Kramer Cell



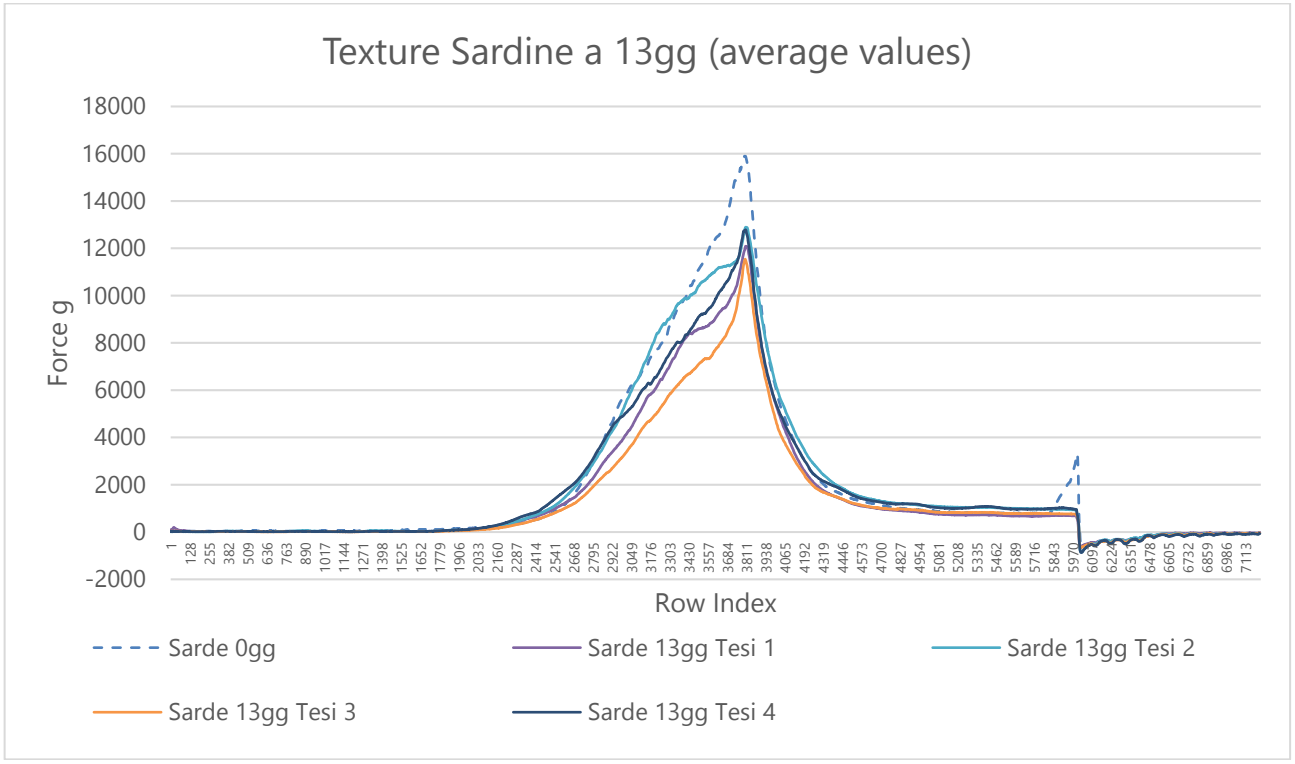
Graph 47. Sardines Texture after 3 days (average values)



Graph 48. Sardines Texture after 7 days (average values)



Graph 49. Sardines Texture after 10 days



Graph 50. Sardines Texture after 13 days

The results show that freshly packaged sardine fillets reach a maximum cutting effort of 16,000 g. On the third day, the product packaged in air (Thesis TQ) shows an increase in the cutting effort of 18,000 g, in contrast to the products of the other theses. On the seventh day, the TQ and TQ O3 Theses were not examined as they are no longer marketable. In the subsequent periods of the shelf life, the T2 and T4 theses maintained a good consistency compared to the T1 and T3 theses. On the thirteenth day, the theses that have maintained the best consistency are T2 and T4.

1.9. Sensory analysis

Sensory analysis is important to perceive any variations in the food organoleptic characteristics. The evaluation of a panel on the degree of acceptability of a food product is subject to the sensory deterioration of the same. Sensory evaluation is accompanied by sensory analyzes as they are closely related to each other. It is very important, for the purposes of a greater reliability of the shelf-life estimate, to obtain repeatable analytical evidence that must be integrated with the sensory investigations. To evaluate the product sensory decay, organoleptic analyzes were carried out according to five descriptors and a global satisfaction value or Sensory Index. The descriptors considered are odor, meat appearance, exudate, skin appearance, meat texture. These analyzes have been carried out on the samples of the 6 experimental theses for the 5 analysis periods and then statistically processed.

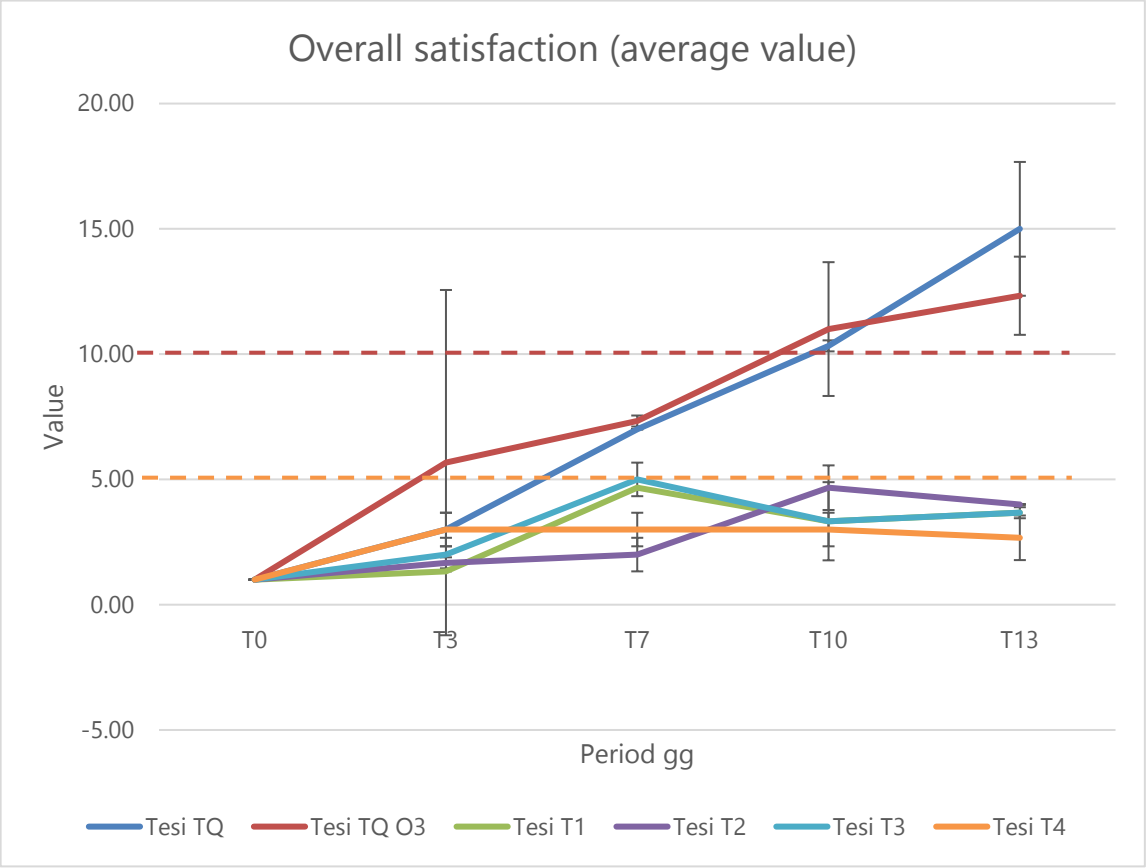
Pannel Test filetto di sarda
(Sardina pilchardus)

Campione Tesi:_____

Nome Panelista:_____ Data analisi:_____/_____/_____

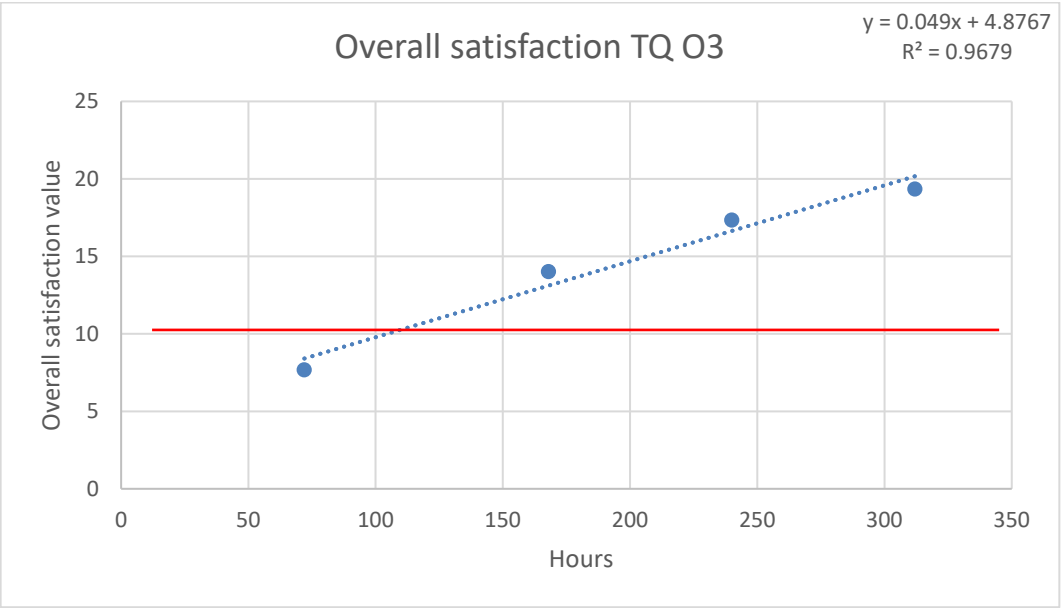
Parametri	1	2	3	4	5
Odore	Alghe marine, salso	Bassa percezione alghe marine	Neutro	Fermentato Lievemente solforoso	Putrido, acre
Aspetto carne	Brillante traslucida	Perdita brillantezza e lucidità	Lievemente opaco	Opaco disomogeneo	Opaco e variazioni colore
Essudato	Acquoso e trasparente	Acquoso leggermente opaco	Leggermente lattiginoso	Lattiginoso	Fortemente lattiginoso
Aspetto cute	Viva, cangiante, iridescente	Pigmento vivo poco cangiamnte	Lievemente opaco	Opalescente	Pigmentazione spenta
Texture Carne	Molto soda, rigida	Solida, rigida	Elastica	Molle	Flaccido
Gradimento Globale	1 2 3 4 5 6	7 8 9	10 11 12	13 14 15	
	1-5 Eccellente			11-15 Non Accettabile	

Figure 11. Detection of sensory descriptors data sheet

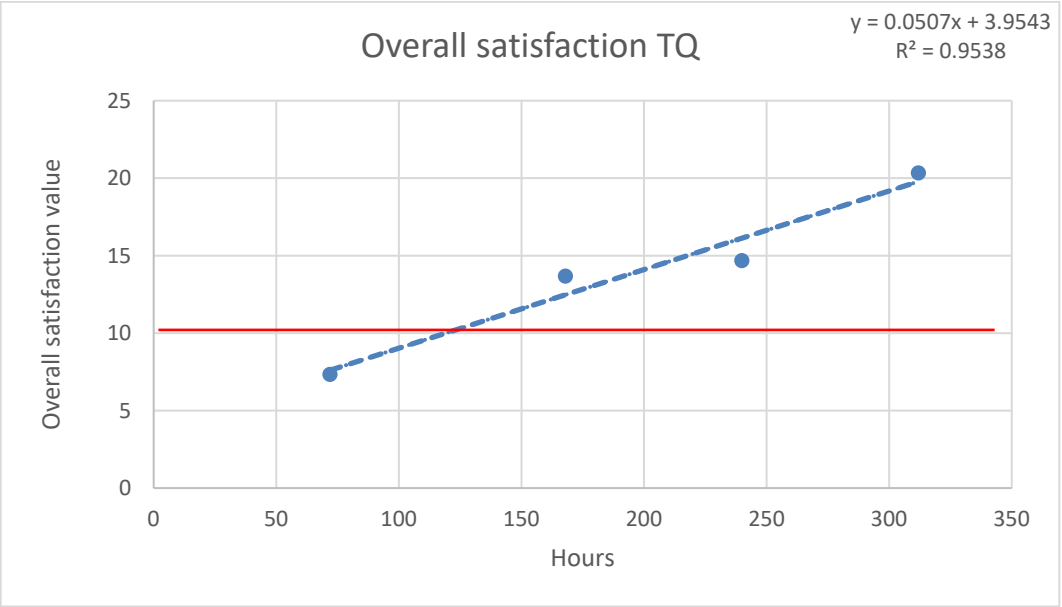


Graph 51. Overall satisfaction average values comparison

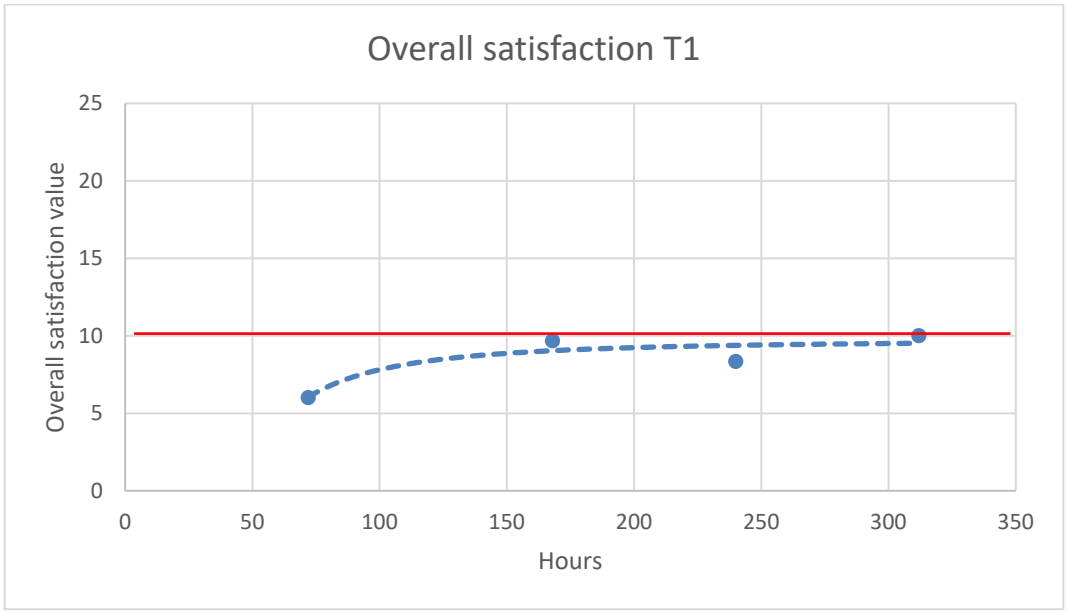
Graph 47. indicates the overall satisfaction with the quality of the products subjected to panel testing. The green dotted line - corresponding to the value 5 - indicates the limit within which the product is considered to be of good quality while the red dotted line - corresponding to the value 10 - indicates the limit beyond which the product is no longer acceptable. The results obtained show that the sardine fillets packaged in a modified atmosphere, on average, maintain a higher organoleptic quality than those packaged in the air. The next graphs will show the statistical analyzes relating to sensory data. Each graph will describe a single thesis in which the regression line or curve applied to estimate the sensory shelf life can be observed.



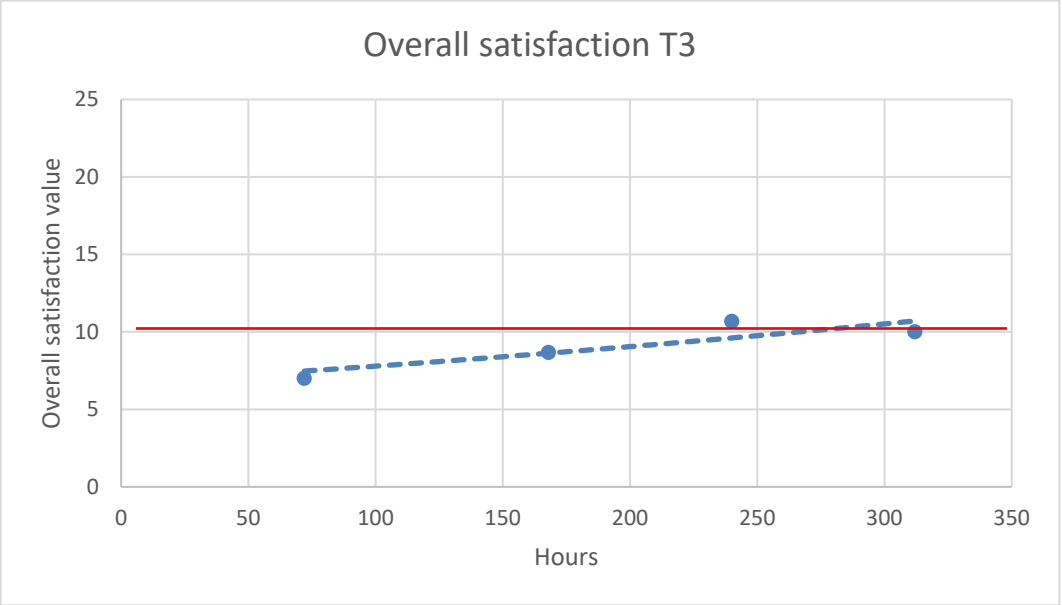
Graph 52. Overall satisfaction Thesis TQ O3



Graph 53. Overall satisfaction Thesis TQ


Overall satisfaction T1
Overall satisfaction value
25
20
15
10
5
0
0
50
100
150
200
250
300
350
Hours


Overall satisfaction T2
Overall satisfaction value
25.00
20.00
15.00
10.00
5.00
0.00
0
50
100
150
200
250
300
350
Hours



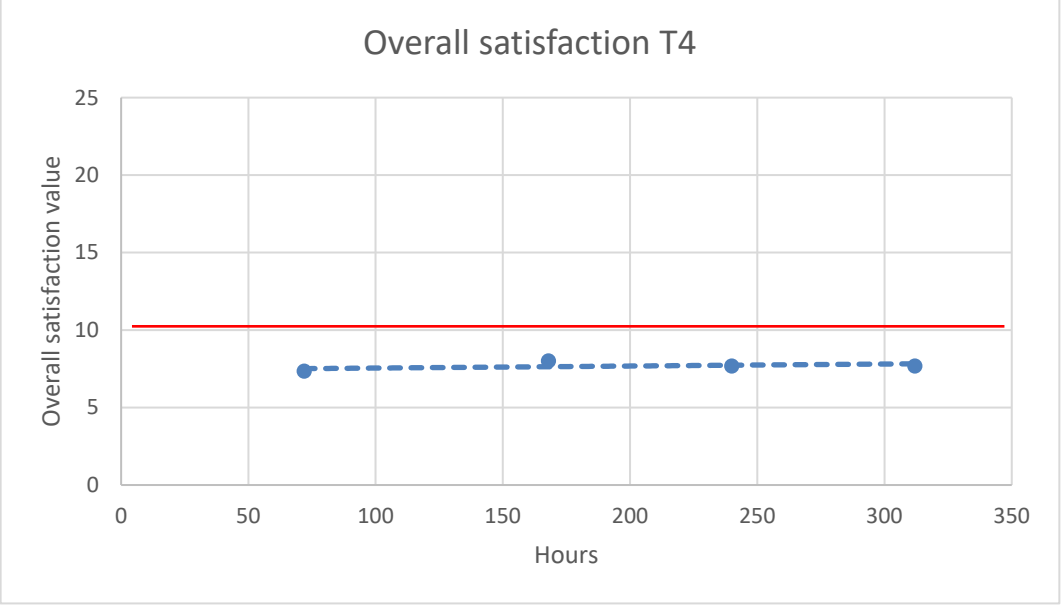


Table 4 shows the estimated sensory shelf-life duration, based on the regression line or curve estimated in the different models. The results obtained show that Thesis T4 is the only one to maintain the organoleptic characteristics almost unchanged beyond 13 days.

Thesis	Hours	Days
TQ	119,2	5,0
TQ O3	104,6	4,4
T1	312	13
T2	228,0	9,5
T3	266,0	11,1
T4	>312	>13

Table 4. Thesis Shelf-life assessment – hours and days

1.10. Conclusions

The study shows that the use of ozone reduces the microbial load making the product much more stable during the storage period. Washing with ozonated water made it possible to keep the values of histamine (Table 3) and total volatile basic nitrogen (TVBN) low (Table 2). The results obtained are in line with the results of the analyzes of the microbial load which indicate a reduction of the same in the T2 and T4 theses compared to the other theses (Graph 38, Graph 40, Graph 42, Graph 44). Furthermore, products packaged in a modified atmosphere attest to an increase in stability from the point of view of microbial load and color. The products packaged with the Argon - Carbon Dioxide mixture (Theses T3 and T4) on the thirteenth day underwent a lower weight loss reduction than the products preserved with the Nitrogen - Carbon Dioxide blend (Theses T1 and T2).

The analysis of the texture of the sardine fillets showed that the products packaged according to the T2 and T4 theses maintained a consistency very similar to the fillets just at time zero on the thirteenth day. The results obtained with the panel test show that sardine fillets packaged in a modified atmosphere, on average, maintain a higher organoleptic quality than those packaged in air. In particular, the T4 thesis attests a much higher quality than the T1, T2 and T3 theses in which modified atmospheres are applied. The best result was obtained with the T4 thesis: product washed in ozonated water and packaged with a mixture consisting of 60% argon and 40% carbon dioxide.
In Thesis T4 the color at the level of the pulp and skin remains more vivid until the thirteenth day (Figure 12 Thesis T4 product at 13 days of shelf life). The results are supported by the colorimetric analysis (Graph 29 Colorimetric analysis of muscle bundles Thesis T4), (Graph 30 Skin colorimetric analysis Thesis T4).

Figure 12. Thesis T4 with 13 days shelf-life

2. OP ISTRIA CASE

With this study, the preparation and packaging of fish burgers has been examined with the aim of extending their maximum shelf-life compared to packaging the product in the air under refrigeration. In this regard, it is important to clarify the difference between two terms often confused with each other:

Shelf life (maximum commercial life): is that maximum period of time within which a food maintains its safety and quality characteristics (organoleptic, microbial and chemical-physical) above a minimum level of acceptability.

Durability (effective commercial life): it is the "prudential" period of time set by the manufacturer (or rather by the Food Sector Operator OSA) within the maximum commercial life, after which a few days remain before reaching the true shelf life.

The project is made by two phases.
In the first phase, the composition and preparation of the burger based on mullet and pink shrimp has been studied. In the second phase, a part of the finished product has been subjected to High Pressure Processing (HPP) treatment while another portion of the sample was not subjected to treatment.

2.1. Fish catching description

The mullet (Mugil cephalus)

The mullet is a fish belonging to the Mugilidae family and is characterized by a fusiform body with large scales. The dorsal tract is gray-blue while the ventral tract has a white-silver color. The eye is covered with an adipose membrane, a trait that distinguishes it from other fish belonging to the Mugilidae family. It can reach an average length of 20 cm and a weight of about 1 kg. It feeds mainly on benthic invertebrates, decaying organic substances and small organisms.

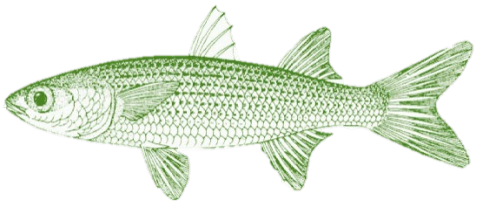


Figure 1 Mugil cephalus or Mullet

From a nutritional point of view, the meat is firm and digestible with a protein content of 19.35% and fat equal to 3.79%.

The pink shrimp (Parapenaeus longirostris).

Parapenaeus longirostris is a crustacean belonging to the Penaeidae family; it is a benthic species with a gregarious behavior; it has an average length of 6-12 cm and an average weight of 100 g. The external surface of the carapace is smooth and devoid of bristles. This shrimp is made up in the cephalothoracic section of a carapace from which 13 pairs of appendages branch off while the abdominal section is characterized by lateral compression. In the carapace at the level of the gastric region, there is a particular tooth that allows its recognition and distinction from the other Peneids.

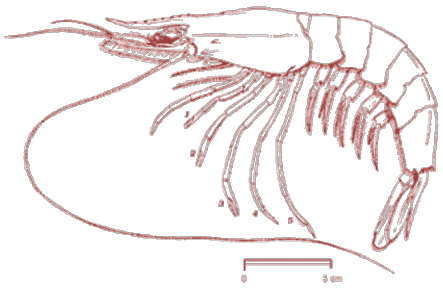


Figure 2 Parapenaeus Longirostris or Pink shrimp

At the level of the head a rostrum is visible consisting of a number of spines varying between 5 and 9; moreover, there are two pairs of antennas of different lengths that are used to capture the movements of marine organisms that are nearby. Their diet is mainly characterized by live plankton, decaying animals and small plant organisms. The catch is mainly done by trawling. From a nutritional point of view, it has a very low lipid content (0.6%) and a high protein intake of about 13.6%.

Basic ingredients characteristics

The mullet has been delivered to the frozen laboratory in fillets with skin, wrapped in a plastic film and stored in a polystyrene box. Before processing, the fillets have been subjected to defrosting in a cold room at + 2 ° C for 12 hours. At the end of defrosting, the fillets showed a sufficiently compact texture to be processed in the subsequent phases. The pink shrimp pulp has been delivered to the laboratory frozen and packaged in resealable plastic trays. The pulp was extracted from the carapace in the customer's plant with the aid of a Baader 601 separating machine (Figure 3).

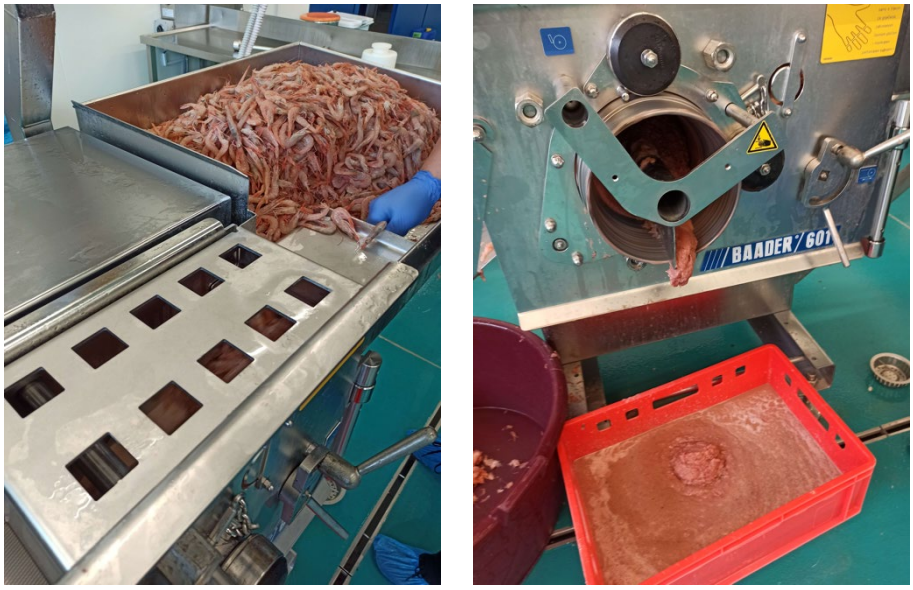


Figure 3 Separating machine Baader 601

Before processing, the product has been subjected to defrosting in a cold room at + 2 ° C for 12 hours. The defrosted product was characterized by a very low consistency due to the presence of a high quantity of intra / extra cellular liquid, released during the separation phase of the pulp from the carapace, too high to obtain a finished product without adding ingredients thickeners and / or structuring agents. Both products have been subjected to analysis in order to evaluate their dry matter, water mobility (Aw) and pH before subsequent processing.

2.2. Technologies

Skin packaging

The skin packaging technology is characterized by the application of a heat-shrink plastic film in direct contact with the product, at the base of which a plastic tray is placed. Vacuum and heat sealing are used to seal the product: at this point the film adheres perfectly to the food, thus maintaining the organoleptic characteristics of the product unaltered for a certain period. The intimate adhesion of the plastic film with the food creates a condition of anaerobiosis, thus preventing the development of aerobic pathogenic bacteria that would risk altering the organoleptic characteristics of the product. This type of packaging allows an increase in shelf life up to 15 days. This technology can be applied to meat, fish, fruit and other foods such as ready meals and deli dishes.

HPP technology

High hydrostatic pressure, HPP (High Pressure Processing) is a technology able to microbiologically stabilize foods with liquid, semi-solid and solid matrix, extending their shelf-life. HPP technology uses pressures of up to 6,000 atmospheres on the food at refrigeration or ambient temperatures, thus achieving the deactivation of unwanted microorganisms, such as bacteria, viruses, molds, yeasts and parasites, present in the product. For this study, the burgers have been packed in a flexible plastic skin packaging and loaded into the high-pressure chamber. The chamber is then filled with water in order to uniformly transmit the pressure to the product, thus preventing any deformations. High pressure is applied for a few minutes depending on the characteristics of the product. This technology has long been widespread in the United States, Japan and Australia.

Crusting

Cryogenic crusting or crusting is a process through which a very fast surface freezing of the product is obtained. This process is very often used in production processes in order to obtain a stabilization of the three-dimensional shape of the food, especially when the product is subjected to vacuum packaging. MedicAir Industry Cryogen™ technology is used for this application. Cryogen™ technology allows to reach -90 ° C in the freezing chamber in a few seconds, drastically reducing the growth of ice crystals, favoring the nucleation phase of intra and extra cellular fluids. In this way, fractures in the muscle fibers are avoided: these fractures are responsible for weight loss due to the loss of liquids, visible in the package during the shelf life or during the defrosting or regeneration phase of the food. With Cryogen™ technology there is not this problem.

Carbon snow

Carbonic snow is a particular physical state of dry ice as it is very soft and able to absorb heat much more quickly than classic dry ice pellets. Carbonic snow and dry ice are obtained when CO2 (carbon dioxide) reaches the solid state at a temperature of -78 ° C. Carbonic snow under standard pressure conditions transits from the solid to the gaseous state without passing through the liquid one. In this way the product subjected to abatement does not risk increasing the liquid fraction, a phenomenon that occurs when the classic ice produced with water is used. In addition, during the dough processing, the use of carbonic snow allows a rapid reduction of temperatures with the relative formation of carbonic acid and a potential decrease in pH, which amplifies the bacteriostatic effect.

2.3. Physico-chemical characterization of products

Before proceeding with the tests, the products have been analyzed with the aim of obtaining an adequate analytical framework to be able to carry out the test in a specific manner. The parameters analyzed with the relative results are shown below.

Mullet
- pH
- water activity (Aw)
- dry matter

Pink shrimp
- pH
- water activity (Aw)
- dry matter

Processes description

The study has been divided into several phases, the diagram relating to each individual process stage and relative description is shown below (Figure 4).

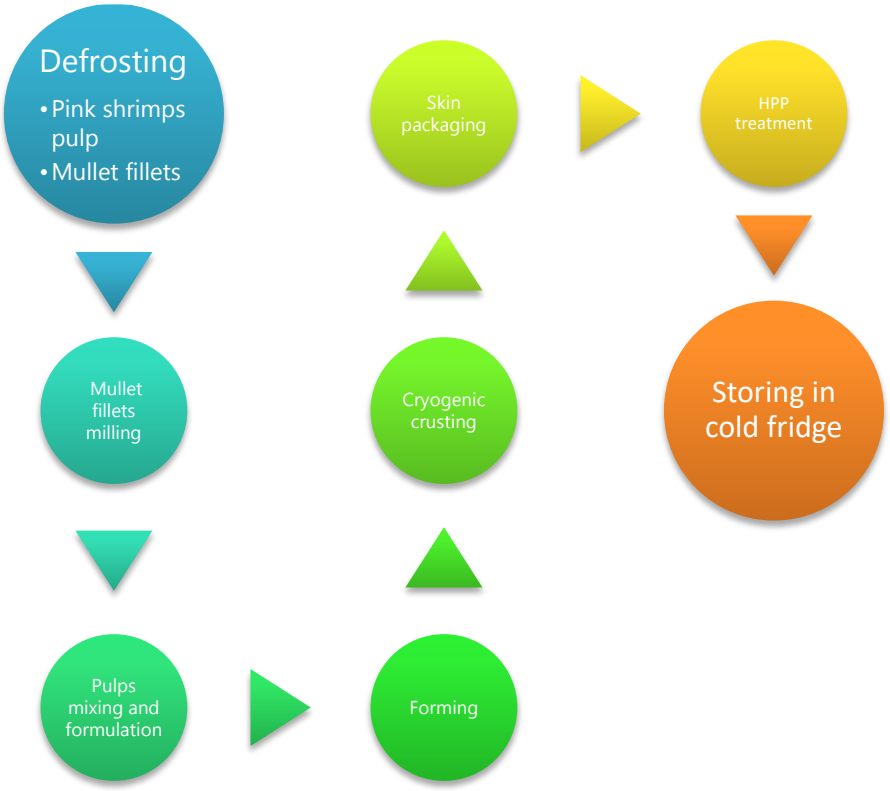


Figure 4 Process phases

Milling

After defrosting, the mullet has been separated from the skin and milled with an electric meat grinder, having a multi-perforated plate with 5 mm diameter holes.

Mixing

After milling, the mullet pulp obtained has been mixed with pink shrimp pulp and with two types of thickener and flavorings.

Forming

In this phase, the compound obtained from the mixing, added with the other ingredients, has been subjected to shaping using a manual hamburger press to obtain burgers with a diameter of 12 cm, weighing 150 g.

Cryogenic crusting

In this phase the formed product is subjected to crusting at a temperature of -90 ° C.

Figure 5 Crusting phase

With this process, the product will increase the surface resistance which will prevent its deformation during the packaging phase. Furthermore, thanks to the low surface temperature (about -60 ° C), the shrink film will be prevented from overheating the product during the sealing phase.

Skin packaging

At the end of the crusting phase, the product was packaged in skin with a packaging machine by the company Orved di Musile di Piave mod. (Figure 6). For packaging, Prima packaging solution 229mm x 143mm x 27mm weight peso 19,50 g trays have been used. Cryovac VST0250 Skin top brand film (Table 1).

Property	Unit	Nominal values
OTR at 23°C, 0% RH	cm^3/m^2, 24 h, bar	1,5
MVTR at 38°C, 90% RH	g/24h, m^2	6

Table 1 Film Cryovac VST0250 Skin technical characteristics

Figure 6 Orved skin packaging machine

Cold Fridge conservation
The final product has been stored in the cold fridge at 5 ° C.

Burger preparation

The basic ingredients for burger preparation are mullet pulp and pink shrimp. In addition, other ingredients have been provided to promote pulp cohesion, thus increasing the texture of the finished product. During the preparation phases, the product was kept at + 5 ° C with the help of carbonic snow. The ingredients used for the tests are listed below.

Ingredients	*Quantity [g for kg]*
Mullet pulp	547,5
Pink shrimp pulp	347,5
FAST FH 67002 thickener	50
MIX DRY FH 67001 thickener	50
NAT FIT 44751 aroma	5

Thickener characteristics

The Fast FH 67002 product is a creamy white semi-finished product for food, containing dietary vegetable fiber (pea), starch, natural flavors (aromatic preparations).
Fast Dry FH 67001 is a creamy white semi-finished product for food, containing dietary vegetable fiber (pea; chicory), potato starch, natural flavors (aromatic preparations), dehydrated potato flakes.

Procedure

The pulps were mixed with the thickeners, the aromas and carbonic snow for about a minute. The obtained product has been then left to rest for about five minutes before forming. At the end of the five minutes, the product was divided into portions of 150 g each subsequently formed (Figure 7).

Figure 7 Burger forming and positioning in the tray

The product thus obtained showed the following estimated nutritional values

Nutrient	Value for 100 g
Water (g)	69,4
Energy (kcal)	127,4
Energy (kJ)	532,2
Proteins (g)	14,5
Lipids (g)	4,0
Cholesterol (mg)	81,1
Available carbohydrates	8,3
Soluble sugars (g)	2,0

2.4. Cooking trials

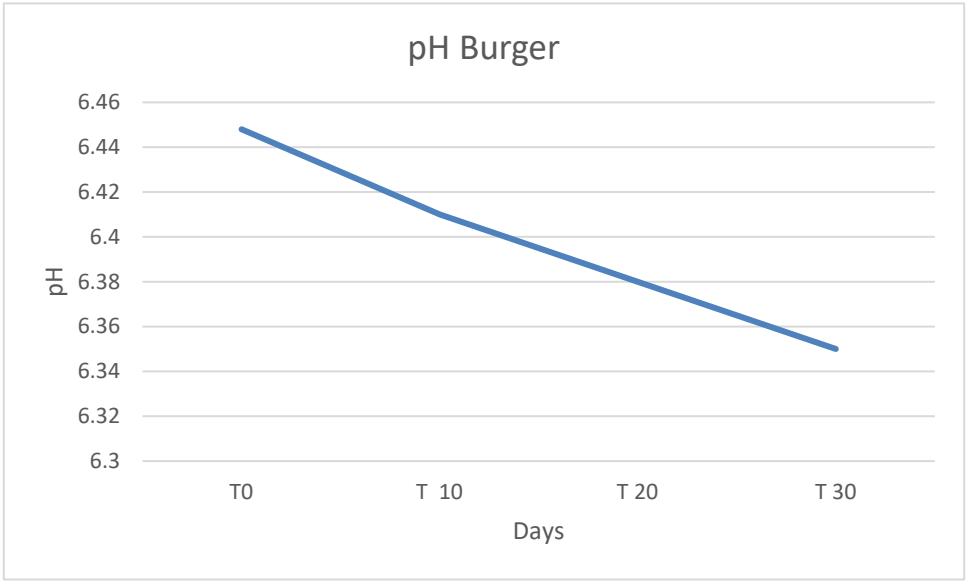
Some burgers have been cooked to evaluate their structural integrity according to the thermal profiles adopted.

Figure 8 Cooking trial
The image shows that the product has perfectly maintained its structure characteristics and shape.

2.5. Analyses

pH analysis

The pH values during the shelf-life study period have been analyzed with WTW inolab® pH7110 pH meter. From the obtained results, it can be seen that the variations in pH are modest even if the products stored in air show a slightly more marked variation in pH than the fresh product. This condition is determined by the scarce presence of carbohydrates which in most fish is present in very low quantities. In fishes, the pH variation is minimal unlike in terrestrial animals in which anaerobic glycolysis degrades glycogen in the muscle, producing lactic acid which in turn causes a lowering of the muscle pH.



Graph 1 pH burger

Water activity analysis (Aw)

The Aw indicates the ratio between the vapor pressure of the water present in the food and the vapor pressure of pure water. It is a very important parameter as it indicates the level of mobility of water present in a food product: the water that can actually participate in chemical, physical and enzymatic degradation reactions.

The Aw is expressed in a dimensionless value ranging from 0 to 1. The more the value approaches 1, the greater the mobility of water in the system, therefore the more the product could be subject to deterioration. Aw values have been analyzed in mullet, shrimp and burger pulp. The obtained results show that all the products have a value above 0.90, the limit above which the growth of most microorganisms is not inhibited. Table 1 shows the values as a function of microbiological stability.

Water activity	Organisms
aw = 0.91-0.95	Lots of bacteria
aw = 0.88	Lots of yeasts
aw = 0.80	Lots of molds
aw = 0.75	Halophilic bacteria
aw = 0.70	Osmophilic yeasts
aw = 0.65	Xerophilic molds

Table 2 Free water values as a function of microbiological stability

The water activity values have been determined with AwTherm ROTRONIC; the table with the average values is attached below (Table 2).

	Aw
Mullet pulp	0,9288
Pink shrimp pulp	0,9215
Burger	0,9227

Table 3 Aw values

Dry matter

Dry matter is the percentage of solids present in a product. The higher the percentage, the drier the food is. From the results obtained (Table 3), it can be seen that shrimp pulp has a low dry matter content compared to mullet pulp. In the burger, it is possible to notice an increase in dry matter as it has been added with thickeners based on potato starch. The dry matter has been determined with a thermo balance mod. DBN 60.

	D%
Mullet pulp	30,623
Shrimp pulp	22,822
Burger	37,543

Table 4 Dry matter analysis

Colorimetric analysis

The tristimulus model ns800 spectro-photocolorimeter (3nh - Noida Uttar Pradesh - INDIA) has been used for the colorimetric analysis. The results have been expressed as L * (brightness), a * (red / green index) and b * (yellow / blue index) of the CIELab scale (Figure 9).

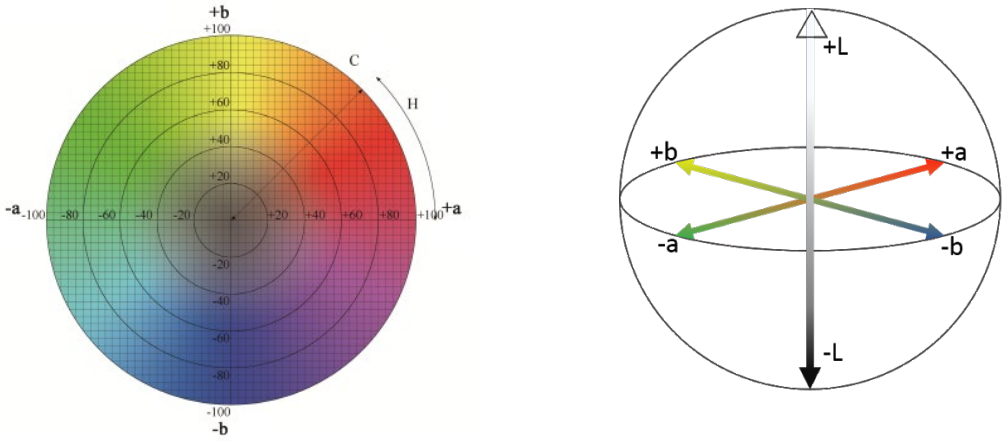


Figure 9 Cielab scale

The colorimetric analysis has been carried out on 6 points in triplicate (Figure 10).

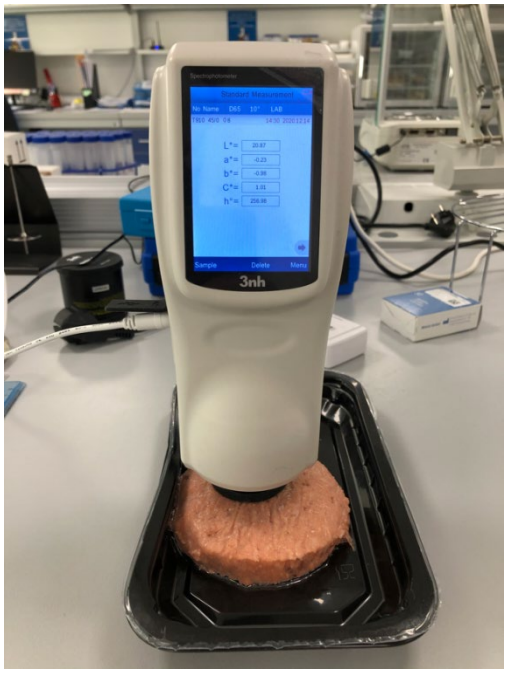
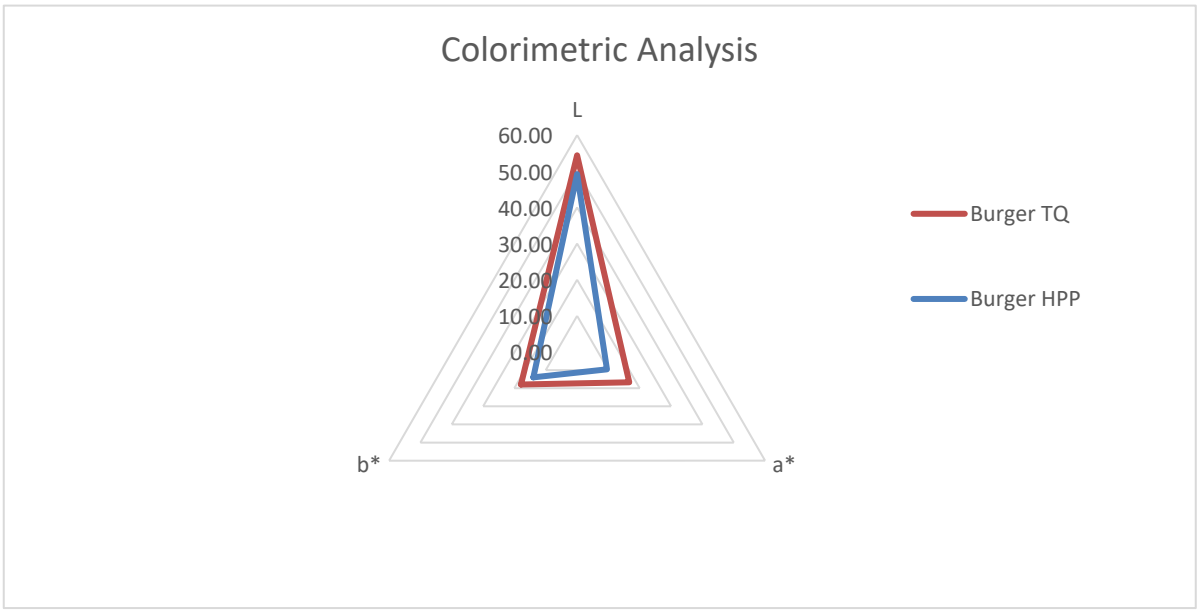


Figure 10 Colorimetric Analysis

For each surface, the average of the three values analyzed for each point has been calculated. Below are the graphs for each thesis with the results relating to the colorimetric analysis of the product treated in HPP with respect to the untreated product. From the obtained results, it can be confirmed that the degree of brightness of the skin packaged and treated with HPP product has undergone a slight reduction in intensity. With regard to the parameter a * (red-green index), a reduction in the red value is observed in the skin packaged and subjected to HPP burger compared to the packaged one not subjected to HPP. As regards the b * parameter (yellow-blue index), a reduction in the yellow value is observed in the burger subjected to HPP with respect to the untreated product. The obtained values attest that the untreated product packaged in skin has slightly more intense colors than the one packaged in skin and subjected to HPP. The table with the average values (Table 4) and relative graph (Graph 2) are shown below.

PARAMETER	BURGER TQ	BURGER HPP
L	54,41	49,21
A*	16,65	9,535
B*	17,90	13,955

Table 5 Colorimetric analysis results (average values)



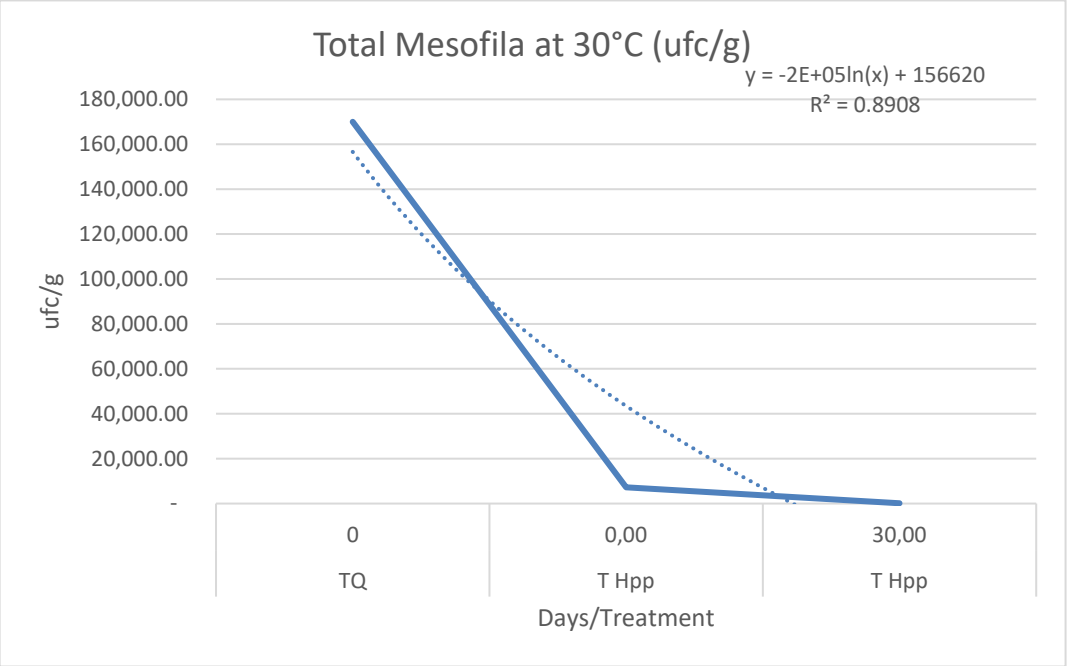
Graph 2 Colorimetric analysis results

Microbiological analyses

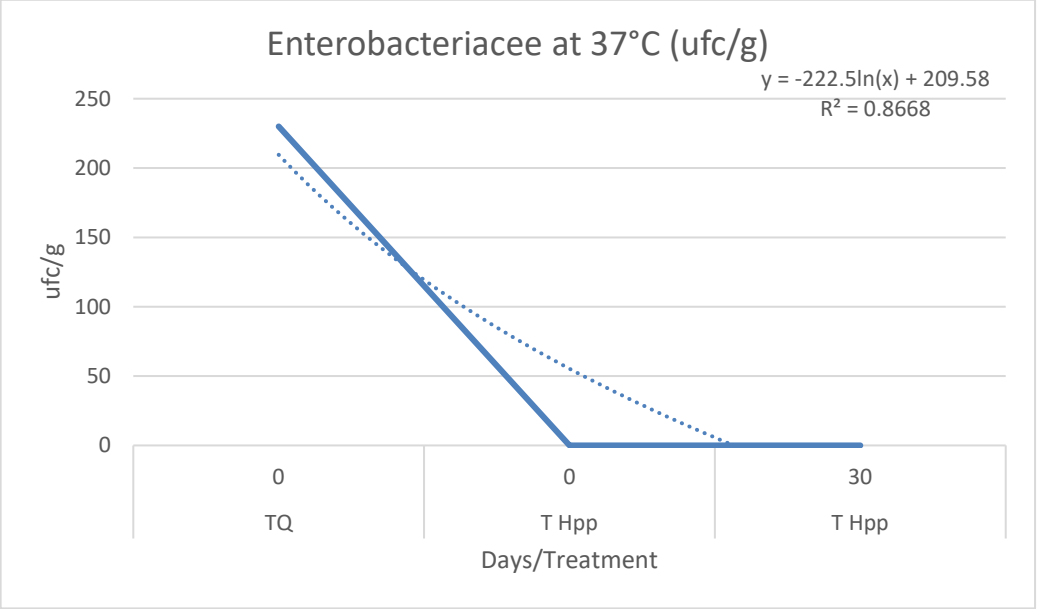
From the obtained results, it can be deduced that the products treated with HPP have a microbial load tending to zero compared to the untreated ones. Moreover, the burgers made with carbonic snow have maintained a lower microbial load over time. This gas carries out an important activity from the product acidification point of view, as carbon dioxide, due to the contact with water, develops carbonic acid according to the following reaction: $H_2O + CO_2 \rightarrow H_2CO_3$. Carbonic acid is a water-soluble molecule therefore it will tend to bind to the substrate making it moderately acidic. The table and graphs relating to microbial loads are shown below.

Treatment	*TQ*	*T HPP*	*T HPP*
Days	0	0	30
Total Mesofila counts ay 30°C (ufc/g)	170,000.00	7,200.00	130.00
Enterobacteriacee counts at 37°C (ufc/g)	230	0	0
E. coli counts (ufc/g)	0	0	0
Positive Stafilococchi coagulase counts (ufc/g)	0	0	0
Anaerobes sulphite-reducing counts (ufc/g)	120	0	0
Salmonella search (ufc/g)	absent	absent	absent
Listeria monocitogenes search(ufc/g)	absent	absent	absent

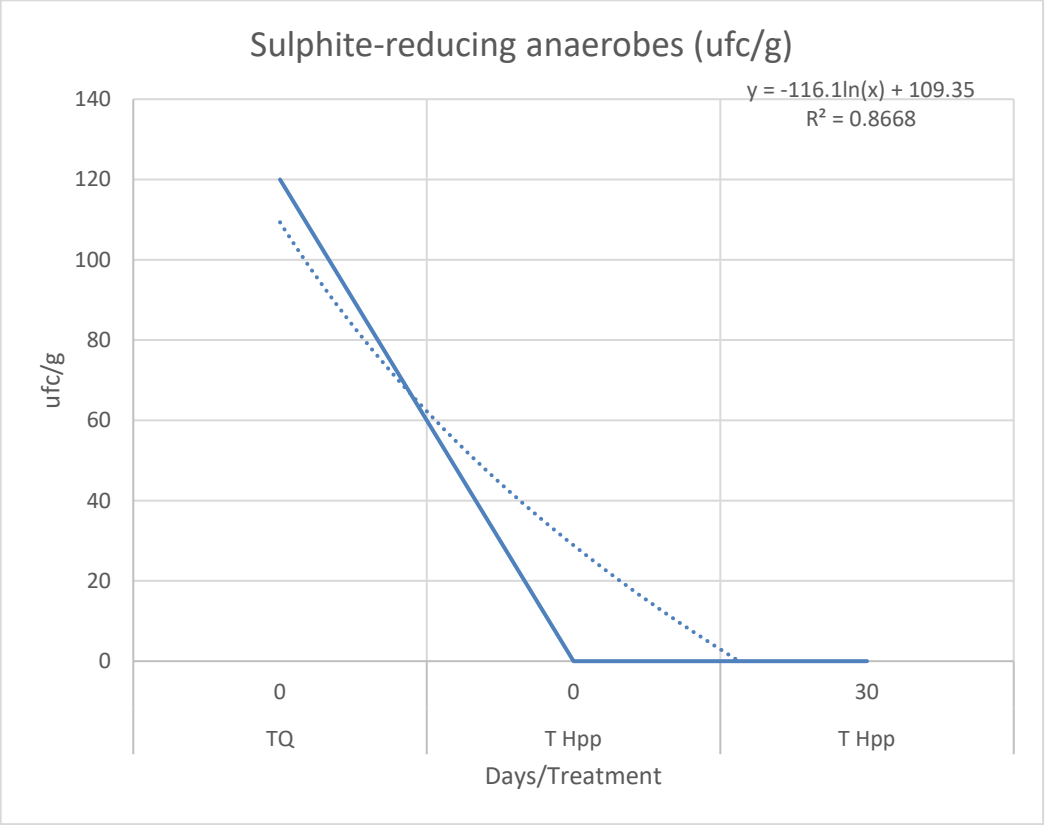
Table 6 Microbiological charges analysis ufc/g



Graph 3 Total mesophilic growth curve at 30 ° C



Graph 4 Enterobacteriaceae growth curve at 30 ° C



Graph 5 Growth curve Anaerobes Sulfite reducers

Total Volatile Basic Nitrogen TVBN

Total volatile basic nitrogen (TVBN) is formed from the degradation of nitrogenous compounds by tissue and bacterial enzymes, as a result of which ammonia and volatile amines develop. TVBN can express different biochemical and bacteriological realities (different amines, different microorganisms) responsible for organoleptic alterations and for the variety of odors generated in decaying fish. Pursuant to EC Regulation 2074/2005 and subsequent amendments, Section II Cap. I, AFSSA 2007, Regulation UE n. 1022/2008/UE is considered:

- Very fresh fish: TVBN < 12 mg/100g
- Fresh fish: 12 mg/100g < TVBN < 30-35 mg/100g
- Deteriorated fish: TVBN > 30-35 mg/100g

In our case, the most restrictive limit for blue fish has been considered: 25 mg of TVBN on 100 g.

From the obtained results, it can be seen that the freshly produced burger has a very high value which increases considerably on the thirtieth day. In both analyzes the TVBN values are above the limit. This result was most likely caused by the characteristics of the starting raw materials. In particular, with excessive mechanical pulping and / or when processing temperatures and the first stage abatement are too high, the endogenous and exogenous reactions responsible for the increase in TVBN are more favored. Even if the treatment with HPP allowed the achievement of a shelf-life of about 30 days under refrigeration, it cannot be considered decisive in the case of a raw material with too high TVBN values.

Total Volatile Basic Nitrogen

Total Volatile Basic Nitrogen TVBN mg/100g		
	Period	
	T0	T30
HPP treated burger	39,9	92,2

Texture

Hardness has been measured using a dynamometer mod. Texture Analyser HDi 500 (Stable Micro System, Surray, UK), equipped with a 25 kg feed cell. An extrusion test has been performed using a steel Kramer 5 blade (Figure 9). Approximately 80 g of burgers measuring 6 cm x 82 cm x 2 cm have been used for each analysis. The obtained data have been expressed in terms of hardness evaluated with the maximum force peak recorded (g) during cutting.

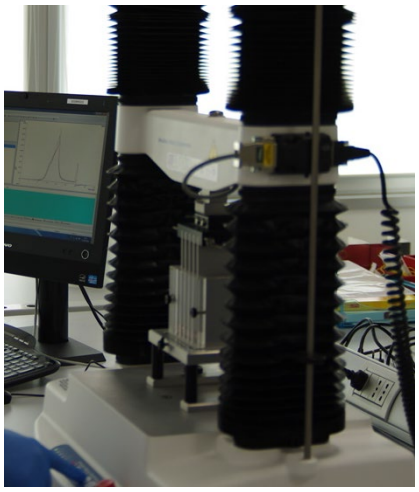
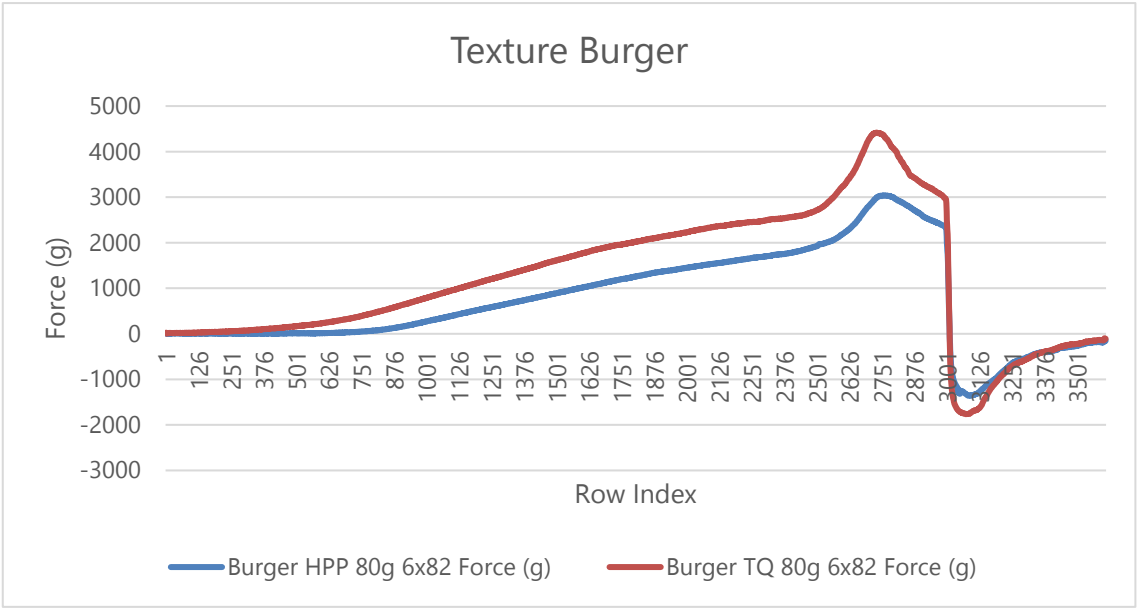


Figure 11 Kramer Cell

The obtained results show that the product had an adequate structure, confirming that the formulation adopted made it possible to obtain a product suitable for final preparation and consumption. After the HPP treatments, the product has been characterized by a reduced softening, however irrelevant from the point of view of the overall sensory quality.



Graph 6 Texture burger

2.6. Conclusions

The burger subjected to HPP has kept its organoleptic characteristics almost unaltered, both in terms of texture and color. In particular, the HPP treatment has slightly changed the color of the product, much less than what happens with raw red meats, subjected to the same treatment. Probably, in our testing conditions, this phenomenon is attributable to the presence of astaxanthin in the pulp of the shrimp. This molecule is an antioxidant belonging to the carotenoid family which would appear less susceptible to high treatment pressures. From a microbiological point of view, the quantities have undergone a considerable decrease following the HPP treatment: in fact, the total mesophilic charge at 30 ° C, for example, has gone from 170,000 cfu / g to 7,200 cfu / g, allowing the achievement of a burger with a shelf life of about 30 days.

Regarding the texture, a value between 3,000 g and 4,500 g of peak was determined. Shrimp pulp greatly affected due to its high-water content, probably due to a too thorough mechanical separation process. To overcome this drawback, it was necessary to add thickeners during the preparation phase of the burger. The TVBN showed very high values already in the preparation phase of the burger. This phenomenon may be due to many factors, which must be carefully considered during the preparation of semi-finished products based on mullet and shrimp.

In particular, in conditions of excessive mechanical pulping and / or processing temperatures and the first stages of abatement too high, the endogenous and exogenous reactions responsible for the increase in TVBN are more favored. Even if the treatment with HPP has allowed the achievement of a shelf-life of about 30 days under refrigeration, it cannot be considered decisive in the case of a raw material with too high TVBN values.

3. OP BIVALVIA CASE

3.1. Piloting

The object of this test was to develop an innovative product of stripped venus and obtain a longer shelf-life in order to reach new customers and markets.
The study on products based on the stripped venus clam products (*Chamelea gallina*) has been carried using vacuum skin packed plastic trays, containing approximately 400 grams of shellfish. The clams we caught on 22nd October 2020. A standard sorting procedure was adopted on the board fishing vessel and in the production facility.
Two type of products were prepared. The first were the stripped venus packed in the plastic tray and skin vacuumed. The second product was prepared using the clams and a tomato sauce, filling completely the tray and then vacuum skinned. For each product three treatments were conducted; a control without applying HPP and HPP treatments at 6000 bar for 3 minutes and at 4000 bar for 2 minutes. The products were prepared at 22nd October 2020 and were scheduled to be tested at 1, 8, 15, 22 and 29 days of shelf-life (Table 1).

Table 1: Experiment plan

Production data	Data analysed	Shelf-life	no HPP treatment	HPP treatment 3 min @ 6000 bar	HPP treatment 2 min @ 4000 bar
22/10/2020	23/10/2020	1	Product samples “natural clams” and “clams wih tomato souce”		
22/10/2020	30/10/2020	8			
22/10/2020	06/11/2020	15			
22/10/2020	13/11/2020	22			
22/10/2020	20/11/2020	29			

Figure 1. Samples of two different products based on the stipped venus (Chamelea gallina) placed in the cylindrical tray prior the HPP treatment.

Figure 2. Detail of the striped venus (*Chamelea gallina*) product with souce.

The laboratory analysis we carried in the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) in San Donà di Piave (Italy) and it was selected a set of standard tests already used for mollusc bivalves:

- Number of *Escherichia coli* beta-glucuronidase positive at 44 °C,
- Number of psycrophyll bacteria at 15 °C,
- Determination of the Total Volatile Basic Nitrogen (TVBN)

Escherichia coli is a bacterium composing the normal gastric flora of animals and therefore used as an indicator to measure faecal pollution. It is a food safety criterion for seafood that has to be met to guarantee the quality of the products, and is included in the Appendix I of Regulation Reg. CE 2073/2005 and further integrations. Usually, the depuration process is enough for reducing the concentration of *E. coli* present in the shellfish. The legal limit is 230 MPN/100 g and should be determined according to the method UNI EN ISO 16649-3:2015/EC 1:2017 (MPN method). In this study it was decided to use the method of counting bacteria colonies on the plate (UNI EN ISO 16649-2:2001) instead of the MPN method (UNI EN ISO 16649-3:2015/EC 1:2017), being as reliable as the MPN method but simpler and faster as there is a considerable number of samples to analyse.
The psycrophyll bacteria at 15 °C represent a saprophytic bacteria flora with low metabolic activity, whose even high numbers do not pose usually a danger for consumer's health or the sensorial perception of the product. However, these bacteria represent an indicator of the product's hygiene and food conservation in time. The temperature of 15 °C is selected as the most similar to the temperature where the shellfish are grown or cultured. The selected method is specified in ISO 4833-1:2013 (counting the mesophyllic bacteria on a plate cultured at 30 °C), but modifying the cultivation temperature from 30 to 15 °C as more suitable for the psycrophyll bacteric flora found in seafood products. There are no regulated limits by law for this parameter.
The total volatile basic nitrogen (TVBN) is among the most common indicators used for evaluating the level of freshness or degradation of seafood. The reference regulation (Reg. CE 2074/2005) gives upper limits for some seafood products, between 25 and 35 mg of nitrogen for 100 of meat, however, there is not an indication for shellfish. The method used in the analysis was according the regulation UE 2019/627 15/03/19 GU UE L131 17/05/19.

3.2. Results

The samples of product with the stripped venus without sauce were discarded from further analysis because the plastic film was ruptured during the HPP treatment, therefore the product was not sealed anymore.
The stripped venus without HPP treatment lasted until day 8, while on the day 15 the package although still under vacuum but not anymore skin and there were specimens with open valves and with a decaying scent. The tests for the day 22 and 29 were cancelled.
On the other hand, the stipped venus with the tomato sauce had their packaging regular, under vacuum and skin in the days 1, 8 and 15, while at day 22 the package was under vacuum, but not skin and with a decaying scent. The test for day 29 was cancelled.

The analysis of the psycrophyll bacteria indicated a distinctive improvement by applying the HPP treatment, reducing the number of bacteria up to 10 000 by the 15th day of shelf-life (Figure 3). The non-treated clams had low levels of bacteria only at the 1st day, just 10 higher than the treated samples of the clams with the tomato sauce. By the 8th day, the untreated sample had already a significant increase of bacteria, reaching close to the maximum value observed on day 15. The samples of clams with tomato sauce treated with HPP continued to have low levels of bacteria on day 8 and only on day 15 a slight increase, less than an order of magnitude. This indicates how the HPP treatment allowed to create a new product with an extended shelf-life.

However, in the samples treated with HPP it was observed that some shells had cracks or were broken, which is an undesirable characteristic for the consumer and the market. This could be due to the rapid change of pressure, where a rapid decompression of the air within the microscopic structure of the shell creates additional pressure and crack the shells. Additional testing should be performed to investigate this phenomenon.

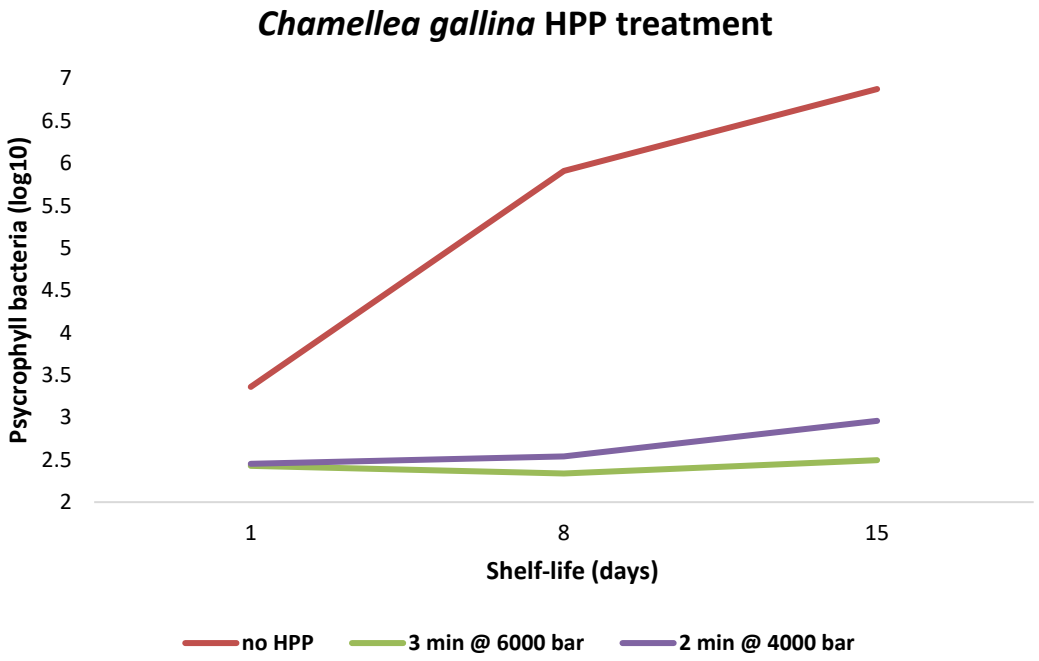


Figure 3. Detail of the striped venus (*Chamelea gallina*) product with souce.