SEAFOOD^TOMORROW

Nutritious, safe and sustainable seafood for consumers of tomorrow

Grant agreement no: 773400

Deliverable D4.1

Specifications and requirements from enduser's

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Dissemination Level	
PU Public	X
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	
CO Confidential, only for members of the consortium (including the Commission Services)	

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1. Summary

Deliverable 4.1. is based on the responses from the task leaders, the focus group discussions, personal interviews with end users and 24 responses obtained through an online questionnaire designed to catch the most relevant demands from the seafood industry with regards the control of safety and authenticity, and the implementation of rapid detection kits. The information gathered will help SEAFOOD^TOMORROW to design new tools in accordance with these demands and, therefore, have new products closer to the market at the end of the project. In this sense, regulation, improvement of the results from already existing technologies and cost have been pointed out as major characteristics to consider. In addition, the need for adequate communication of the benefits to consumers is highlighted.

2. Objective

The purpose of this deliverable is to determine the stakeholders' requirements and real conditions in which the different innovative solutions should operate. The design and characteristics of the new solutions should be adapted to the particular needs of the end users. The project will work in providing really innovative solutions, but what works well in a laboratory or in a controlled operating environment may not be suitable for aquaculture facilities or for the seafood processing industry. This fact will finally impact on the applicability of the different solutions and their marketability in the seafood sector. Therefore, the sooner the demands of the end users are taken into consideration, the closer to the market the different solutions will be.

3. Background

Similar studies as the one described in this deliverable have been made in the past in order to assess the market demands of new devices or biosensors or rapid detection kits. For example, in the frame of the AWACSS project (Tschmelak et al, 2005), real-time sensing, improved regulatory and public acceptance and less expensive monitoring were found to be prime end user requirements. More recently, the FP7 Enviguard project (http://enviguard.eu/index.php/news/item/15-results-enviguard-survey) launched a survey to monitor recent monitoring practices, their associated costs, related problems and losses due to shortcomings, previous problems and respective patterns in the Mediterranean fish farmers. This survey is still open, but first analysis of responses showed a concern about price and usefulness of the different solutions.

SEAFOOD^TOMORROW project has its own particularities addressing food safety and authenticity along the whole seafood value chain. The seafood industry is evolving rapidly, and new technologies are being implemented to improve not only productivity but also safety, authenticity and sustainability of seafood products. Therefore, the demands from potential end users might be different from other surveys.

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4. Experimental design

In order to gather the end users' requirements personal interviews with task leaders have been made. All of them have been asked to answer some questions related to the current technologies used, the interest of the sector in new solutions and the characteristics that the new solutions should have to favour their implementation. In addition, the results of the focus group discussions have been analysed to identify requirements from consumers.

In the case of task 5.2, personal interviews with seafood companies have been made and an online questionnaire was designed specifically for task 1.4 and 5.1 (biosensors for safety and authenticity). This questionnaire has been designed in four different sections:

- Introduction. It contains a brief explanation of the project and its objectives as well as the aim of the questionnaire. Some definitions (rapid detection kits, marine toxins, chemical contaminants and authenticity) have been included so that all respondents have the same idea about what these terms mean.
- Contact information. This section records some information of respondents and personal details. The latter are optional and if provided this information will be uploaded to the stakeholder database as explained in the introductory paragraph of this section.
- Control of seafood integrity. It comprises only 3 questions to assess the importance of safety and authenticity in the routine operating procedures of companies. If the company does not perform routine analysis, the respondent will follow to section 4, whereas if these analyses are performed a set of 13 additional questions about the main characteristics of these analyses have to be answered.
- Assessment of rapid detection kits. 7 questions are included to ascertain the degree of interest of the respondent in the implementation of rapid detection kits. These questions will help to establish some important parameters that should be considered in the design of rapid detection kits.

The questionnaire has been done in four different languages (Spanish, English, Portuguese and Italian) and the online version has been developed using SurveyMonkey® platform. The following links were created (05/07/2018):

https://es.surveymonkey.com/r/TT8CYHS (Spanish)

https://es.surveymonkey.com/r/YCDVXWK (English)

https://it.surveymonkey.com/r/JBL5T7Q (Italian)

https://pt.surveymonkey.com/r/PJM7638 (Portuguese, in this case it was created the 10/07/2018)

In **Annex 1** the quesationnaire in English is shown.

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5. Results and Discussion

5.1. Novel sustainable feed materials

Aquaculture production has been growing very fast in the last decade and will supply more than half of the world's seafood for human consumption. Therefore, technological and methodological innovations are needed not only to optimize the production, but also to assure its sustainability. In this sense the availability of suitable aquafeed is an important factor in the aquaculture development.

The uncertainty concerning the availability of traditional fishmeal and fish oil, and rising prices have required major industrial aquafeed manufactures to identify and evaluate alternative protein and oil sources and considerable progress has been made in recent years to replace fish protein and oil with proteins and oils of plant origin (Rana et al., 2009). However, this trend in aquafeeds can interfere with the fish nutritional profile. For instance, vegetable ingredients are often characterized by low amounts of n-3 LCPUFAs. Although conditioned by the elemental soil content of cultivars, cereals, pulses and oilseeds are also poorer sources of iodine and selenium when compared to marine protein ingredients.

In task 1.1. the objective is the development of “finishing feeds” (to be used close to fish slaughter time) enriched with selected nutrients, such as iodine, selenium and DHA, that would allow the fortification of fish with these health valuable nutrients and enhance its nutrition contribution to the daily recommended intake. Ingredients used to supplement the new feeds are natural sources, including a selenised-yeast (for selenium), macroalgae *Laminaria digitata* and *Saccharina latissima* (for iodine) and *Schizochytrium* algae biomass (for DHA). All these supplements are commercially available and industrially produced.

To our knowledge, there is no similar product in the market and no technical problems are foreseeable on its implementation on the current industrial process of manufacturing aquafeeds. However, there is a cost constraint. At this stage various scenarios are being tested (combinations of doses) to check their efficacy. The increase of formulation cost ranges from 1 to 20% (depending on the scenario). If the lowest cost scenario proves to be effective, its industrial application should be straightforward. At higher feed costs, the farmer would have to find a marketing strategy of these fish to compensate it. The interest of the sector is high, but clearly conditioned by the cost-scenario.

The new solution being developed should go with the promotion of farmed fish as part of an healthy eating lifestyle and of the use of natural sources for the fortification strategy (yeast and algae) that will help the sustainability of the environment.

5.2. Integrated Multi-Trophic Aquaculture (IMTA)

Integrated multitrophic aquaculture is a concept where organisms of different trophic levels are farmed together so that waste products form organisms at one trophic level can serve as nutrients for organisms at a lower level. In our case, the macroalgae *Saccharina latissima* is grown on ropes nearby a conventional salmon farm consisting of open cages with continuous water exchange with surrounding waters. The three fish farms participating in SEAFOOD^TOMORROW are at three different locations with distinct hydrodynamic qualities with respect to water depth and exposure to wind and wave generation. This is expected to have impact on the interaction between the fish and the seaweed, nutrient dynamics and biomass transport. In the project hydrodynamic and water quality parameters will be monitored and related to biomass production. Vertical and horizontal placement of seaweed relative to the fish cages, deployment and harvesting times, methods and equipment are expected to vary between fish farming sites and will be taken into consideration for validation and optimization of IMTA production. The direct and indirect relationships between the final fish product quality and fish welfare as well as seaweed quality as food product and environmental health will be validated.

Norwegian salmon aquaculture has been gradually increasing over the last decades and in 2017 Norway exported salmon worth NOK 64.7 billion. This is an increase of 5 per cent or NOK 3.4 billion compared with 2016, and it is the highest export value ever for salmon. Measured in product weight, Norway exported 1 million tonnes of salmon in 2017, which is 2.8 per cent or 27,000 tonnes more than in 2016. According to the Norwegian Seafood Council the export growth for salmon flattens out in 2017 after a very strong year in 2016. The most important reason is a stable EU market.

At present, the main obstacle for further increase in production is related to challenges with sea lice. A lot of effort is made in keeping the number of parasites low and as a result the production costs increase, both because of cost related to lice counting and treatments as well as the negative influence of treatments on animal welfare. For a number of years, until 2013, the aquaculture industry has shown an annual increased productivity but has since then been decreasing. The cost of production has been increasing, to a large extent due to problems related to salmon lice. From 2015 to 2016 the average production cost increased from NOK 26,15/kg to 30,60 and continues to increase. Nearly 50% of the cost is feed and the cost related to controlling lice and maintaining good welfare is 28%. A premise for further growth in salmon production is the access to enough feed raw materials of needed nutritional value and qualities, and sustainable solution for developing new raw materials are needed. Utilization of seaweed as feed component is one possibility, where development of efficient cultivation systems is important.

There is generally a great interest in the sector for new solutions that can contribute to more efficient production, good animal welfare and environmental sustainability. Non-medical treatments for sea lice is favoured and a number of different systems using steam, fresh water and specialized feed is being used with great success.

Production of seaweed is generally encouraged, and a number of licenses have been given over the last few years. Currently the biggest obstacle for seaweed production is the processing and market.

There are a few IMTA facilities in commercial production. These are currently in a pilot scale, while the effects on salmon and the environment are being tested. The new solution should have no negative impact on the fish in the IMTA system. It should contribute to added biomass production on the sites and the solution should contribute to increase the environmental sustainability of the productions. We expect nutrients to be circulated in the system as dissolved nitrogen compounds released as waste from salmon production should be taken up by the seaweed and utilized for growth. Ultimately, the seaweed can be utilized as a component in fish feed. Also, seaweed can contribute to a better environment in the salmon cages for example by the production of oxygen during daylight. Good cage environment is important for fish welfare and health. In order for the new solution to be implemented it is also important to have efficient harvesting methods, processing technologies, markets and distribution channels for the seaweed in place.

5.3. Technologies for the reduction of risks associated with Norovirus and biotoxins in shellfish

In this section, two different technologies are included: one for shellfish production (task 1.3) and one for shellfish processing (task 2.3).

Marine biotoxins and human norovirus (NoV) represent long standing and internationally recognised human health hazards associated with the consumption of bivalve shellfish (oysters, mussels, clams) worldwide. In the European Union, there is legislation with requirements for monitoring and classification of shellfish harvesting areas, post-harvest treatments and end-product standards for marketed products intended to ensure that shellfish products are safe to eat. It is accepted by food safety agencies and the shellfish farming sector that these controls have contributed significantly to reduce the burden of illness associated with bacterial pathogens, but have not adequately controlled the risks from NoV. The epidemiological data available indicate that most outbreaks are associated with shellfish harvested from class B production areas usually located in estuarine areas close to urban settlements. Furthermore, proliferations of toxin-producing phytoplankton are common in nearshore areas and are expected to increase in some geographical areas because of warmer water temperatures linked to climate change. These problems have significant impacts on the sustainability of the shellfish industry because of harvesting area classification downgrades/closures, additional costs associated with loss of sales/product recalls and also impacts on consumers' confidence on shellfish products. In recent years, the European Food Safety Authority has published several scientific opinions recommending the consideration of additional control measures to better manage risks from marine biotoxins and NoV. In considering possible risk management options for NoV, the EU Reference Laboratory for monitoring bacterial and viral contamination of bivalve molluscs identified, among others, the development of specific criteria for buffer zones (areas around pollution sources where harvesting is not permitted to allow sufficient contaminant dilution) and improvement of depuration (process by which shellfish are held in tanks of clean seawater to maximise the natural filtering activity and expulsion of intestinal contents) for virus removal. The main advantage of these measures is that, if successful, they would be relatively easy to implement as part of shellfish safety programmes and would not require thorough cooking thus allowing marketing of raw shellfish as this is generally preferred by consumers in Europe.

In SEAFOOD^TOMORROW, we test the premise that a combination of buffer zones in production areas and enhanced shellfish depuration would further reduce risks associated with marine biotoxins and NoV thus improving the safety and quality of shellfish products to levels that are considered acceptable by the industry and consumers.

5.3.1. Buffer zones for sustainable management of shellfish production areas

At harvesting area level, we are developing mathematical models for the prediction of NoV and toxin-producing phytoplankton to help inform site-specific criteria for delineation of buffer zones. In practice, this would allow greater stability in the classification status of production areas, fewer harvesting closures and development of new farming operations in low risk areas. It should also help identify more effective and potentially cheaper pollution reduction measures to ensure that harvesting areas consistently meet water quality standards. The key environmental parameters considered in predictive models for NoV are sewage dispersion and dilution, virus prevalence in human populations, water temperature and salinity; for toxin-producing phytoplankton, the key parameters are water circulation, meteorological factors, nutrient pollution and ecological aspects of algal blooms.

The factors that favour the implementation of buffer zones in Europe are primarily of a regulatory nature. In the USA, the National Shellfish Sanitation Program (NSSP) contains specific requirements for the establishment of buffer zones for approved, restricted and conditionally managed areas. These requirements are essentially based on specific sewage dilution requirements. Countries that have a Memorandum of Understanding with the US to allow the import of bivalve shellfish into that country have to follow the requirements of the NSSP. Currently, the EU Food Hygiene Regulations do not contain equivalent requirements. However, the EURL has drafted guidance on the determination of buffer zones for EU harvesting areas whenever the product is to be exported to the US. The EURL Good Practice Guide for the Microbiological Monitoring of Bivalve Mollusc Harvesting Areas contains recommendations with regard to buffer zones around outfalls, harbour and marinas. However, the EURL is not aware of this section of the GPG being applied, as written, to date in any EU Member State. Some EU countries presently prohibit harvesting of shellfish around point source discharges and/or the mouths of large rivers. These requirements have been based on general pollution grounds, rather than specifically targeting viruses. The criteria do not generally seem to have been based on an assessment of the discharges and their receiving waters but are based on a simple definition of distance from the point source. Those distances vary between, and sometimes within, the countries. The prohibitions are not undertaken under food safety legislation but rather under environmental or fisheries legislation. Therefore, to estimate the social and economic impacts of implementing buffer zones in the EU, consideration needs to be given to differences in shellfish farming and harvesting operations, environmental conditions, NoV prevalence associated with different types and treatment levels of sewage discharge and growing waters, monitoring practices and other risk management approaches, etc., between individual Member States.

5.3.2. Post harvest strategies to reduce the risk of Norovirus and marine toxins in shellfish

In the case of Norovirus, at post-harvesting level (depuration), we are conducting laboratory experiments to explore ways to optimise the health/fitness of the shellfish and promote optimal digestion and contaminant elimination while the animals remain in the tanks. We are quantifying NoV removal at a range of water temperatures and feeding regimes using algal cultures. As measures of stress and condition levels, we are monitoring heart rate and valve gap using sensors. In the final stages of the project, we will validate optimal depuration practices using commercial depuration tanks in collaboration with our industrial partners. The studies focus on Pacific oysters (*C. gigas*), a species that dominates the oyster trade in Europe and is implicated in most NoV outbreaks.

Preliminary studies conducted at Cefas demonstrated that depuration at higher temperatures and over longer time periods increases NoV reduction although marginally. With increasing access to norovirus testing, and further understanding of NoV levels which are associated with illness, there is scope for depuration plant operators to improve their risk assessments. In fact, some operators who have had problems with NoV contaminated product, already increase the depuration time and the temperature of the depuration water. However, this requires experimental trials to optimise the process and these trials are expensive. After undertaking costly trials, operators may not wish to share knowledge gained since this may give them a competitive advantage. The report by Neish (2013) provides some cost estimates for trials to investigate increased time and temperature and testing for NoV over an extended period.

Oysters held in commercial depuration tanks can be considered in a non-feeding state. It has been suggested on multiple occasions that this may influence the digestive processes and could be a factor in slow depuration of viruses. Studies conducted at Ifremer reported that feeding algae, in combination with evaluated temperature, was the most effective regime for removal of FRNA bacteriophage in a commercial scale system. However, it has also been reported that the presence of food alone does not have an effect on the depuration of other microbial contaminants. Thus far, no work has convincingly shown that feeding oysters during depuration will facilitate faster NoV reductions. Some limited experiments at Cefas have explored feeding algae during the depuration run but these did not show any increased virus reductions over non-feed controls. Unfortunately, feeding of bivalves is in itself a quite complex question and it is quite possible that the feeding regimes used were not optimal in our experiments.

In both elements of the studies (buffer zones and improved depuration), we test shellfish samples for *E. coli* (indicator of faecal contamination required by legislation) with standardised culture methods, NoV by PCR with a standardised method, and bacteriophage by both PCR and culture methods developed at Cefas laboratory. Results of bacteriophage testing provide an indirect measure of the proportion of infectious NoV in the samples since there are no standardised methods for this purpose. This also addresses a concern expressed by industry members that current PCR-based methods for NoV testing do not measure virus infectivity.

In summary, we expect that the new processes and products generated by SEAFOOD^TOMORROW will provide greater confidence to shellfish businesses by encouraging production in low risk areas and addressing regulatory concerns, as well as increasing availability of safer and healthier products to consumers in Europe.

5.4. Biosensors for safety and authenticity

In this section two tasks related to the development of biosensors are included: task 1.4 (marine toxins and xenobiotics) and task 5.1 (authenticity). With this objective an online questionnaire was developed and results are shown below.

5.4.1. General assessment of responses

24 responses have been obtained until 28/10/2018. Only 5 of them did not complete the whole questionnaire, but their input has been also considered.

In 14 cases the name of the company is provided and in 11 also the personal details. Figure 1 shows the distribution among countries and the size of the industry.

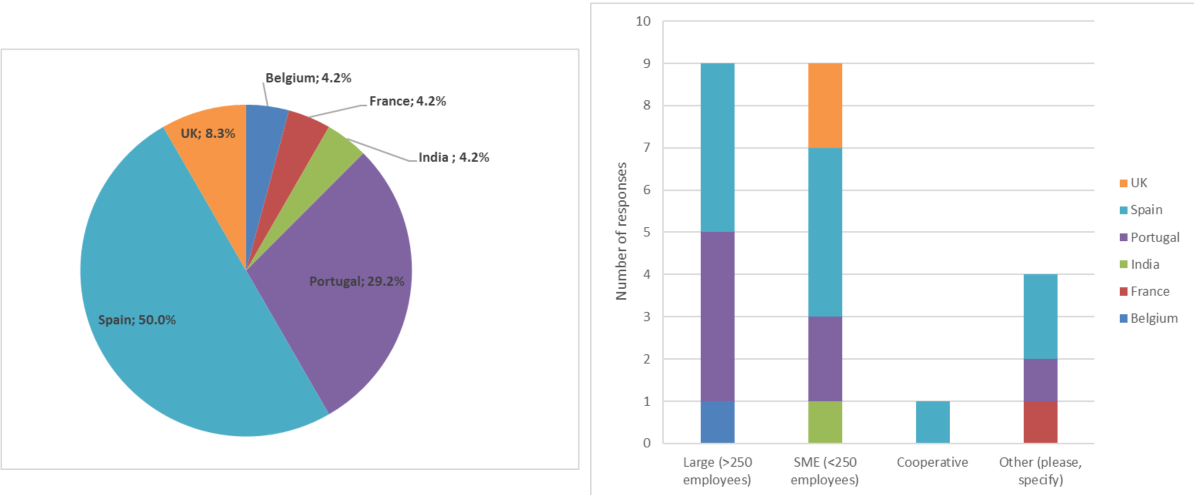

Figure 1. Distribution among countries and type of industry.

Most companies are located in Spain (50%) and Portugal (29.2%), and there is no difference between large companies and SMEs. Also 2 research centers, 1 state organism and 1 industrial association have answered the questionnaire. With regards to the type of activity 6 respondents are retailers, 5 seafood processors and 3 seafood suppliers. The other respondents are devoted to the development of aquaculture cages, official controls, consumer protection, physico-chemical analyses, bivalve treatment plants and a food diagnostic company.

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A variety of seafood species are mentioned in the responses: bivalves (16), cephalopodes (11), crustaceans (9) and fish (hake, mackerel and salmon are the most cited fish species).

5.4.2. Control of seafood integrity

In this section there are questions about the controls already implemented in the companies.

First, 6 topics were assessed by the respondents: seafood safety, seafood species authenticity, marine toxins, chemical contaminants, species sustainability and species diversity. All of them were considered important or very important (see Figure 2). There are almost no significant differences between them. Only sustainability and diversity obtained a slightly lower score in comparison with seafood safety.

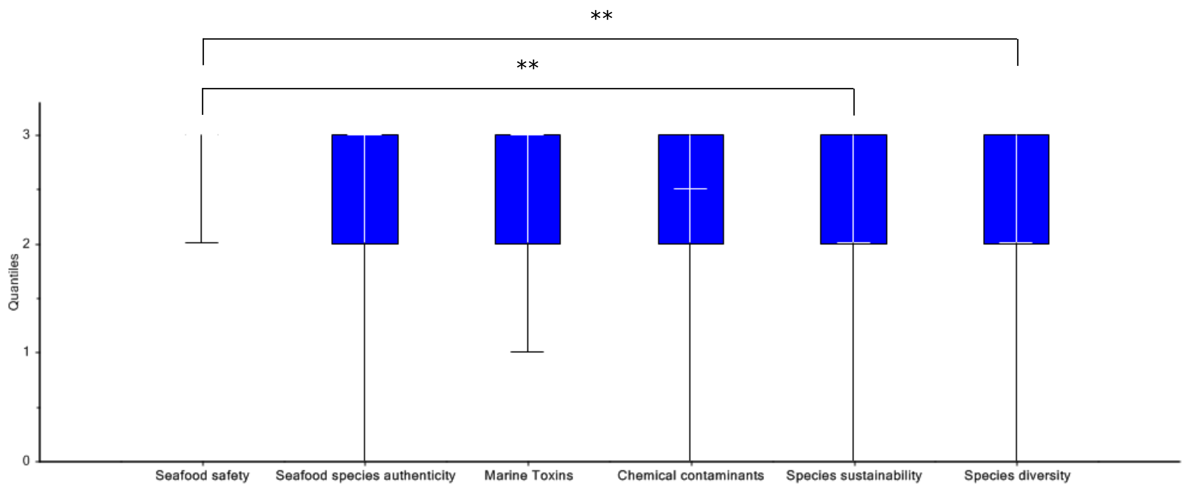

Figure 2. Box and whisker plot of the assessment of 6 different topics: seafood safety, seafood species authenticity, marine toxins, chemical contaminants, species sustainability and species diversity. Statistical differences are labeled with asterisks. ** ($p<0.01$).

When some specific information about main toxins, contaminants or authenticity issues was asked, all respondents referred to regulatory aspects. In this sense, almosts all regulated contaminants are mentioned. In the case of marine toxins PSP, ASP and DSP are cited whereas in the case of chemical contaminants, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins and toxic metals (mercury, lead and cadmium) are mostly highlighted. Also, some companies cited histamine or biogenic amines, pesticides, mineral oils and radioisotopes. Only one respondent said that they do not perform any analysis because their suppliers do. In this sense, the trust in suppliers is also mentioned with regards to authenticity issues. It is worth mentioning that the determination of authenticity in processed food is important for several companies (50% of those who specified some authenticity issues) and that the cost of analysis seems to be a hindrance to the implementation of measurement controls.

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There was a third question about wether they perform routine analysis or not. Only 4 respondents (18.2%) do not perform routine control analysis and therefore did not answered the following 13 questions. In addition, there were also other 4 respondents who did not answered to these questions. Therefore, the following results are based on 16 respondents.

Figure 3 shows the periodicity of routine controls. Very few (13.3%) perform these controls in a daily basis. Most companies do it monthly or less frequently. Indeed, the majority of responses selected the option ”only in case of suspicious”. These controls are usually performed by external laboratories (66.7%).

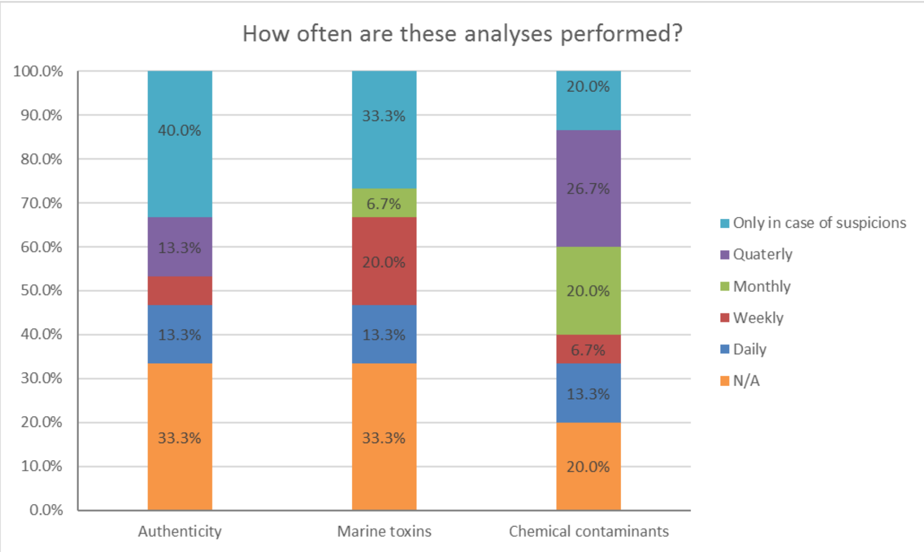

Figure 3. Periodicity of routine controls of authenticity, marine toxins and chemical contaminants

After these questions there were two about the type of analysis performed. If the analysis is done at their facilities, PCR is the technique used for authenticity, whereas chromatographic techniques are used for chemical contaminants and marine toxins. ELISA is mentioned by two respondents for the analysis of marine toxins and in the case of heavy metals atomic absorbance spectroscopy is used. Similar results are obtained when the analysis are perfomed by external laboratories. Only Next Generation Sequencing (NGS) is added for authenticity and the mouse bioassay for marine toxins.

The degree of satisfaction on the results from these techniques is not too high (2.38±0.96 over 4). Positive assessment of results are mainly based on their confidence in the external laboratory and in oficial methods. However, the price and the time of response seems to be a drawback. There are also some doubts about the consistency of the results obtained from different methodologies, in particular with regards to marine toxins and authenticity.

In Figure 4 the results from the costs of analysis are shown.

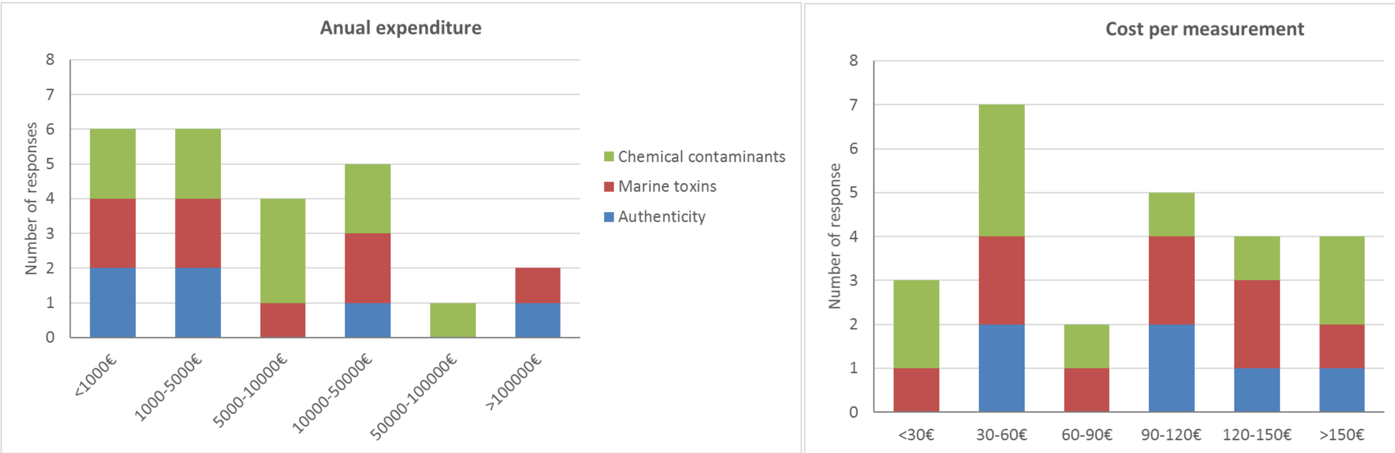

Figure 4. Budget allocated to routine controls and cost per measurement.

It should be taken into account that less than half of the respondents (23) provided specific data about this issue. Most companies spend up to 10,000€ annually in safety and authenticity of seafood products. Although the cost per measurement is more frequently in the range 30-60€, a great variety of prices can be found for the three target topics: chemical contaminants, authenticity and marine toxins.

In a previous question the time of response was pointed out as a reason for disatisfaction. This is an important feature of analytical methods and three questions were raised to know the current situation. In Figure 5 it can be observed that nowadays more than one day is needed to get the results and the industry is demanding for results in less than one day. A high variety of response time values can be found that might depend on the type of analysis.

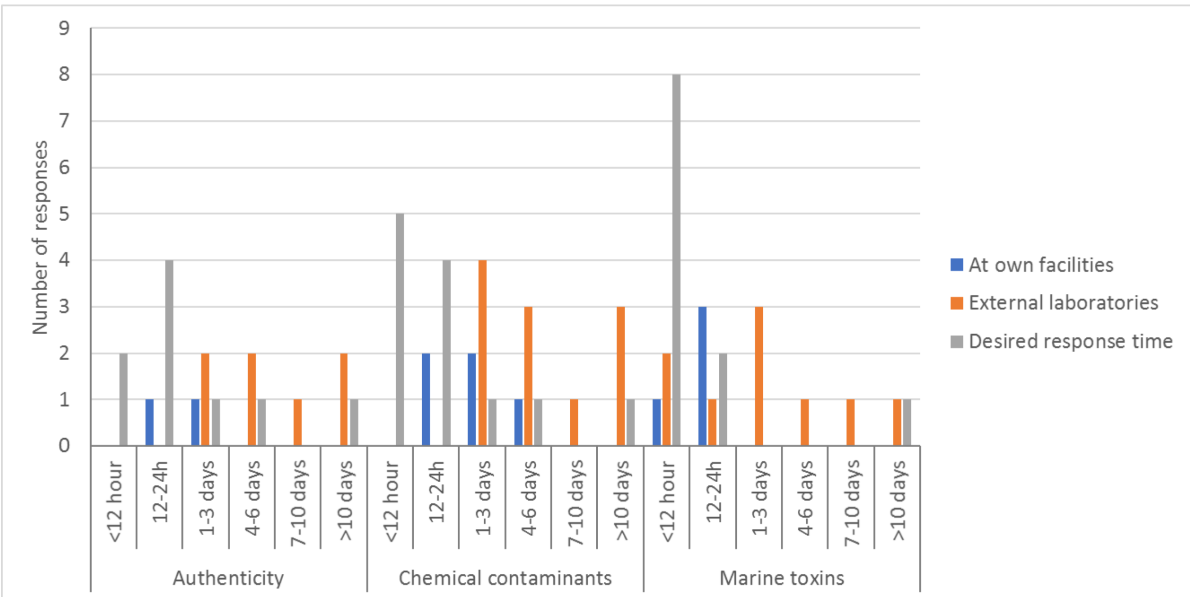

Figure 5. Current and desired time of response

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To finish this section two more questions about some aspects of the methods and the willingness to change or not are made. As it is shown in Figure 6 all aspects (response time, accreditation, cost and reference method) are considered important and the most valuable parameter is the response time. This correlates well with previous answers where this fact was also considered a critical parameter. Most respondents (75%) are willing to change analytical methods if response time and cost are improved, but always if the new methodologies are finally accredited and considered as reference methods.

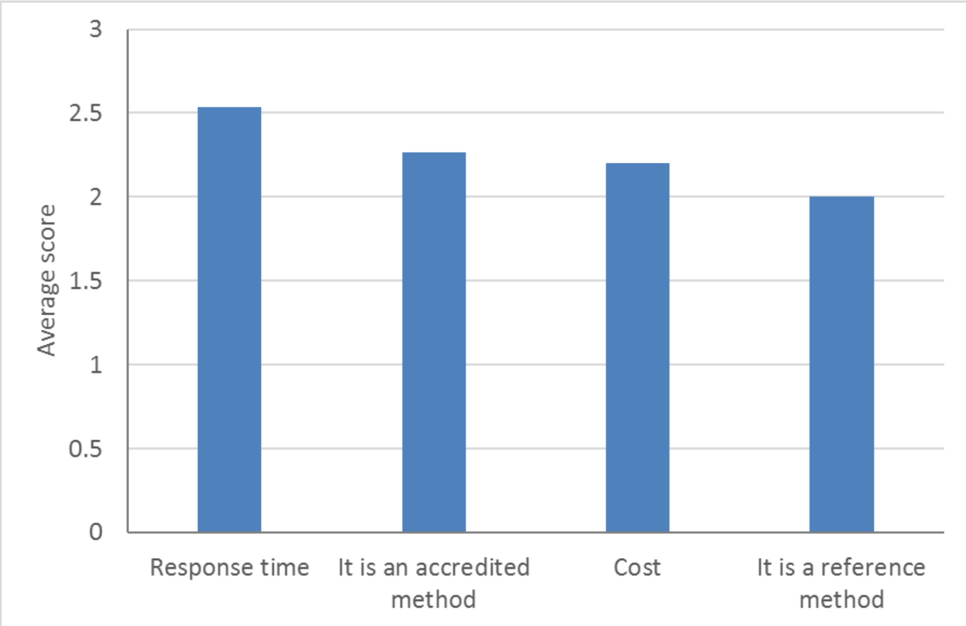

Figure 6. Assessment of several characteristics of analytical methods. 0= not important; 1=Somewhat important; 2= important; 3=Very important

5.4.3. Assessment of rapid detection kits

In this last section of the questionnaire, first their degree of interest in rapid detection kits is asked. 72% of respondents showed high interest or moderate interest (22%) in rapid detection kits. The main reasons for this interest are to guarantee food safety, enhance the response time and implement corrective measures (see Figure 7).

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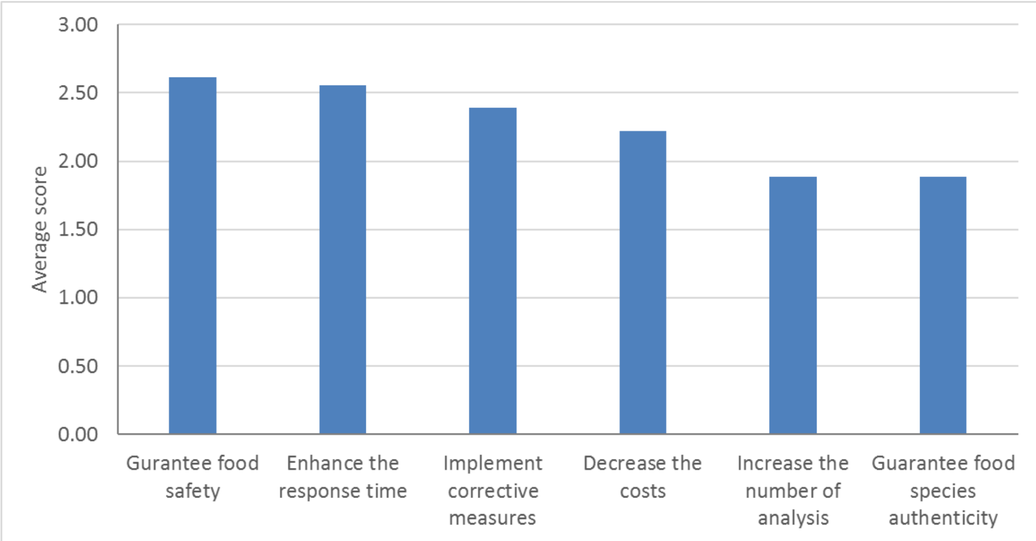

Figure 7. Motivation for the interest in rapid detection kits. 0= not important; 1=Somewhat important; 2= important; 3=Very important

With regard to certain attributes of rapid detection kits all of them have been considered important or very important (see Figure 8).

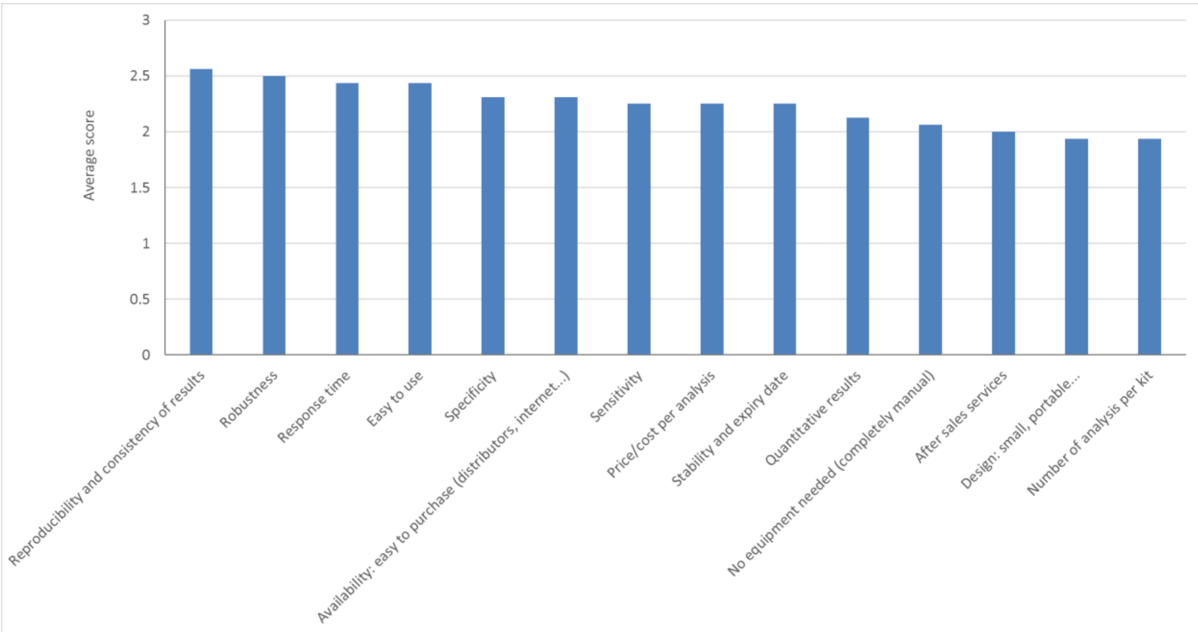

Figure 8. Assessment of attribute of rapid detection kits. 0= not important; 1=Somewhat important; 2= important; 3=Very important

It was asked to establish a price for a rapid detection kit with the capacity to perform 10 analysis. In figure 9 an histogram shows the results obtained. An average value of 230€ was calculated from this data.

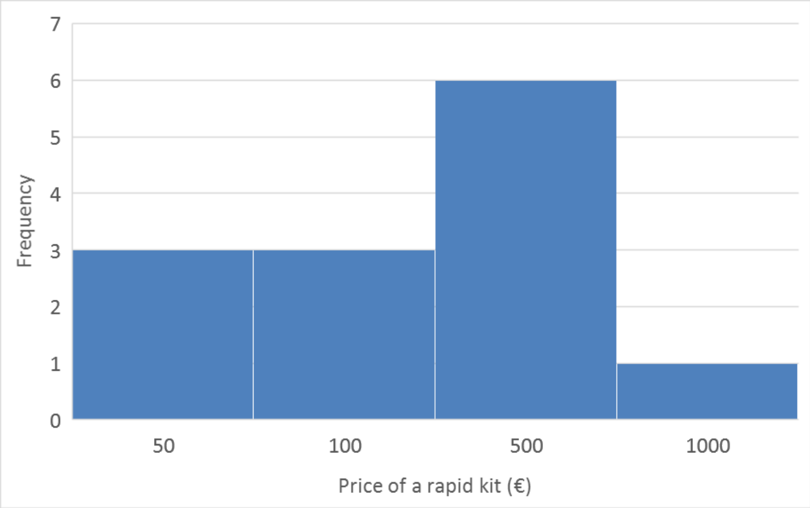

Figure 9. Histogram of the maximum price of a rapid kit

When the periodicity of use was asked, a variety of responses have been obtained. Most respondents selected a few times a week (39%) and less than once a month (28%). Potential end users would perform between 50 and 100 analysis per year (56% of responses) with these kits and they would accept up to one year (72%) of lifetime.

5.5. Sodium reduction in seafood products

The solution proposed to be implemented to reduce Na content on seafood is its replacement with other additives without loosing the quality and safety properties of NaCl.

Salt plays a vital role to protect fish from spoilage in cold-smoked products. However, high salt levels go against current trends to reduce the amount of sodium in processed food in line with public health demands. For this reason, some manufacturers are reformulating recipes to reduce the salt content of their products, and many studies focus on salt reduction and salt replacement in different food types (Rizo et al, 2017). Some options to replace sodium include the use of other salts such as KCl, MgCl2, CaCl2, potassium lactate...

The functional properties of NaCl on protein solubility and water holding capacity are basically due to the chloride ion, therefore it is possible to use other salts without loosing functionality. However, changes in the safety and sensory properties might occur and should be checked.

In this case, the requirements have been assessed through the information obtained from the focus group discussions, as consumers are the end users of these products.

It seems that the salt content does not play a crucial role during seafood purchasing except for the high frequency consumer group or for those with health problems. Indeed, some consumers think that this is only ”fashionable”. In all countries (Belgium, Hungary and Italy) there are mixed opinions about the willingness to buy seafood products with reduced salt. Most concerns are about taste, the manipulation of the product, its preservation and the ingredients used for the replacement of salt.

The nutrional quality of seafood can be improved by means of the partial replacement of NaCl by other salts. However, the effect on the physicochemical and sensory characteristics, as well as on the quality and shelf-life during storage should be evaluated.

5.6. Healthier and adapted food to specific population groups

As in the case of the previous product (section 5.5) the main requirements from end users have been identified through the focus groups discussion. 3 diffferent types of products are foreseen:

5.6.1. Seafood products for children

In all groups and regions seafood products are perceived as healthy for children. These are products considered to be included in the children's diet. Only two concerns were highlighted: the high price of seafood and the vulnerability of children regarding pollutants coming from the ocean and the environment.

In Belgium fresh fish, steamed fish, shellfish, sandwiches with fish or fish fillets are perceived healthier than fish sticks, deep-fried fish, fish spreads and fish burgers. Crustaceans and molluscs were mentioned in Italy as a potential source of allergies. *Pangasius* was also mentioned as a fish which is not healthy at all.

An important requirement highlighted by the focus groups is the presences of bones. Products for children should be boneless. In Hungary it was also prefered a less intense fish smell.

5.6.2. Seafood products for pregnants

Several national food safety authorities have established some recommendations to avoid or reduce the consumption of certain seafood species due to the presence of persistant contaminants (mainly mercury) that might affect negatively the development of the foetus. However, in the focus groups the knowledge of products which should be avoided by pregnant women is rather limited in all regions, but they mention to avoid raw and smoked seafood products while being pregnant. As in the case of the products for children in Italy they would avoid molluscs and crustaceans for allergies issues. In Hungary the origin of seafood would be also important because it is considered to be a factor that might affect the presence of pollutants.

Despite these considerations, in general seafood is recommended for pregnants due to its high nutritonal quality, such as source of minerals, omega-3 and proteins.

5.6.3. Seafood products for elderly

In general, the nutritional quality of seafood is claimed as an important factor for elderly and should be included in their diet. However, depending on the disease, their diet should be adjusted although there are some doubts (in Hungary) about the possibility of elderly to eat as much as they can cover their necessary intake.

The digestibility is another characteristic that should be taken into account when preparing seafood products for elderly, as well as the esasiness to prepare and the price.

5.7. Strategies to reduce contaminants (*Listeria* and marine biotoxins) from seafood products

In this section 2 strategies are discussed: the reduction of *Listeria* and *marine biotoxins*. In task 2.3 there is also the reduction of norovirus, but that has been explained in section 5.3.

5.7.1. *Listeria* decontamination

An innovative listeriaphage-based product will be developed in order to control *Listeria monocytogenes* growth and to improve the safety of seafood products. The use of *Listeria*-infecting phages (listeriaphages) as a food safety strategy for biocontrol purposes is desirable as they are natural specific enemies of *L. monocytogenes*, are harmless to humans, animals and plants, and do not affect the existing commensal microflora or alter attractive food properties.

Apart from thermal technologies, which are very effective, there are few options for eliminating *L. monocytogenes* from foods. However, thermal treatment may cause undesirable changes in raw, lightly preserved and ready to eat seafood products. Therefore, the implementation of strict and effective control programs is essential for the prevention of *L. monocytogenes* contamination in raw and RTE seafood products. The preventive measures should include specific cleaning and disinfection programs, Good Manufacturing Practices (GMP), training of plant employees, raw material controls, processing environmental monitoring and testing and refrigeration of finished products from production to consumption. However, even under the best possible conditions, it is very difficult to reach raw seafood or processing environments completely free from *L. monocytogenes*.

Due to the reasons described before, there is a big interest of the seafood sector in the development of innovative solutions for *Listeria* control in seafood products. In this context, the use of listeriaphages appears as a promising approach to prevent *L. monocytogenes* presence and growth in raw and RTE products.

Currently, two commercial listeriaphage products are available for use in the food industry: Listex containing listeriaphage P100 (Micreos Food Safety, The Netherlands) and ListShield, a cocktail of six listeriaphages (Intralytix, USA). Both commercial listeriaphage products have US FDA GRAS (generally recognized as safe) status, and are approved as food processing aid in non-EU countries like USA, Canada, Brazil, New Zealand or Israel, i.e. export countries of processed European fish products. Yet, so far no specific European regulation exists concerning phage application in food production in Europe. EFSA has recently recognised that no safety concerns are associated with the utilization of the commercial listeriaphage P100 for raw fish decontamination. Nonetheless, EFSA also underlined that more data is needed to draw definitive conclusions on the efficacy of phages in reducing *L. monocytogenes* contamination levels in RTE products, particularly of critical parameters like phages activity during fish storage, contamination levels expected in fish, and potential development of resistance.

The innovative listeriaphage-based product will be a natural solution to reduce the risk of *L. monocytogenes* in seafood products. Its commercial application to raw and RTE seafoods should be safe and easy for operators and the cost of the final product to the consumers should not vary significantly.

5.7.2. Marine biotoxins decontamination

The decontamination process will develop a canned or pasteurized product, toxins free, with a high quality from a sanitary point of view. The obtained products consist of bivalve molluscs, mostly mussels and clams. The heat treatment starts with cleaning in fresh water, and the cooking and cooling steps followed by thermal treatment (sterilization or pasteurization in autoclave).

Nowadays, there are two official and very specific procedures to reduce toxins in shellfish:

The decontamination process by *heat treatment* is only applicable to the bivalve mollusc *Acanthocardia tuberculatum* with the objective of reducing the PSP toxin to a level lower than 800 micrograms/Kg (COMMISSION DECISION of 18 January 1996 establishing the conditions for the harvesting and processing of certain bivalve molluscs coming from areas where the paralytic shellfish poison level exceeds the limit laid down by Council Directive 91/492/EEC). This procedure is industrially applied in Spain, with high effectiveness, obtaining an income for the sector, producers and processors, around 2.5 mills € only for this bivalve produced in the South of Spain.

Other decontamination process involves the *ablation of hepatopancreas* in Pectinidae or scallops (*Pecten maximus and P jacobaeus*) contaminated with ASP. The industry has to be authorized by the Competent Authority for this procedure, for instance in Galicia (NW, Spain), there are only two establishments approved for this specific procedure (COMMISSION DECISION of 15 March 2002 establishing special health checks for the harvesting and processing of certain bivalve molluscs with a level of amnesic shellfish poison (ASP) exceeding the limit laid down by Council Directive 91/492/EEC). Only one industry can eviscerate 3000 Kg of scallops per day during the campaign of this mollusc. In particular, a total of 71,500 Kg scallops were produced in Galicia in 2018 reaching a value of 382,000 euros.

Many efforts were performed to develop a detoxification method, but none of them achieved the expected results. So, since there are not any alternatives to decrease or eliminate toxins in other shellfish, it results in an innovation opportunity to try to develop a solution for this economic and healthy burden. For instance, 20 $ millions annual economic losses on human health was estimated in USA due to HABs. The effects on fisheries and, in a minor degree tourism or recreational impacts, have to be added to this figure, reaching many hundreds of $ million monthly or annually.

When a toxic episode occurs, producers cannot harvest molluscs, then, depuration centers, manufacturers, stakeholders, even tourism and restaurants have not raw material to work with. Many industries have to stop their activity, close and fire the workers until the HAB disappears and molluscs comply the sanitary conditions again. Some harvesting areas are completely closed due to the presence of toxic phytoplankton and contaminated molluscs during more than 7 months per year. So it is an unmaintainable situation. In this context, industries are very interested in finding a solution that allows them to continue and increase their activities.

The characteristics of these new solutions have to be easily applied at an industrial scale. They should be approved by the Competent Authority after proving that the procedure can reduce or eliminate toxins and the product complies with all the sanitary requirements. This is the major problem we can find, since to be approved in a new legislation, many tests have to be performed and validated with international criteria and in different laboratories. This exercise takes too long and needs the involvement of European experts and Authorities.

In addition to get a final product that does not contain PSPs after the application of the heat treatment, this method should be sustainable from an environmental point of view with minimum water and energy consumption. This is the reason why we try to modify the published protocol in order to get the expected and best results, and reducing hydric and energy use. So, once harmonized, the procedure should be similar to the one used in a traditional way at the canning industry.

5.8. Reduction of energy and water in seafood processing

Three different technologies are being applied in order to reduce the consumption of energy and water during the processing of seafood. A fish soup has been selected as model to study Thermal Solar Energy, Radio Frequency and High Pressure Processing for sterilsation/pasteurisation.

Conventional processing consists of heating the soup in tubular heat exchanger using the energy provided by a steam generated in a steam boiler. The heat from the steam is transferred to the soup.

One of the approaches to save energy is to replace fossil energy with renewal energy. In SEAFOOD^TOMORROW, the feasibility of replacing the steam boiler with thermal solar energy to generate heat will be studied.

Another approach to save energy is to use Radio frequency (RF) to heat the soup, which consists in transferring heat in the form of electromagnetic waves instead of transferring heat through the wall of a heat exchanger. Some of the advantages of this technology are higher rate of heating inside the product that reduces the processing time and energy expenses, ultrafast heat treatment of fluids that minimises the loss of nutrient and organoleptic properties, lower fouling deposition, higher heating efficiency and lower maintenance cost

One of the main problems of tubular heat exchangers is fouling. Fouling phenomena decreases heat transfer and increases energy consumption, and the heating system must be cleaned more frequently, increasing other associated costs such as manpower and the need for chemical products for cleaning. One advantage of RF is the reduction of fouling, decreasing water and energy use during cleaning.

<u>High Pressure Processing</u> is a cold pasteurization technique which consists of subjecting food, previously sealed in flexible and water-resistant packaging, to a high level of hydrostatic pressure (pressure transmitted by water) up to 600 MPa for a few seconds to a few minutes. HPP treatment emerges as a potential nonthermal, or more correctly, adiabatic food processing technique that preserves food without excessively degrading its quality.

The primary aim of treating foods with HPP in most cases is to reduce or eliminate the relevant foodborne microorganisms that may be present. The molecular composition of the food substrate, however, can significantly affect the extent to which HPP kills the inhabitant microorganisms. It is important to consider both the effects of the food substrate on slowing the microbial inactivation kinetics and the effect of the HPP on the properties of the foodstuff, when optimizing processing conditions for specific foods.

HPP is similar to thermal processing in that there is a threshold value (specific to each microorganism) below which no inactivation occurs. Above the threshold, the lethal effect of the process tends to increase as the pressure and/or temperature increases.

The FDA has officially approved HPP as a non-thermal pasteurization technology that can replace traditional pasteurization in the food industry. Clearly defined regulations and specifications will facilitate the development of the application market to improve product quality and consumer trust. The widespread application of HPP technology has boosted the development and market demand for HPP equipment. HPP has been widely used in the production of packaged vegetables, fruits, meat, seafood, and dairy products. By the end of 2015, more than 300 units of HPP equipment were operating globally .

Compared with traditional thermal processing technology, HPP is performed at room temperature, reducing energy consumption associated with heating and subsequent cooling. In addition, the food is in packaged form and does not directly contact the processing devices, preventing the secondary contamination of food after pasteurization. Additionally, the pressure transfer medium can be recycled after processing. Hence, water consumption can be considered null or negligible. With the advantages of low energy consumption and low contamination risk, HPP technology is an environmentally friendly processing technology.

By comparing specific energy inputs of a thermal and a combined thermal and HPP sterilization process, it was estimated that specific energy input required for the sterilization of cans can be reduced from 300 to 270 kJ/kg when a high pressure is applied. In the case of high pressure processing, a compression energy recovery rate of 50% can be estimated when a two vessel system or a pressure storage system is used. Making use of energy recovery, a specific energy input of 242 kJ/kg will be required for a sterilization, corresponding to a reduction of 20% .

However, energy consumption has to be measured for each food processing technique and in industrial conditions in order to get accurate data.

The increased consumers interest in high quality foods with fresh-like sensory and additive free attributes led to the development of alternatives to conventionally heat food. Processors in the food industry must use processing technologies capable of reducing additives and the microbial food safety risk while maintaining natural flavors and food quality, which has increased the development of emerging processing technologies such as RF and HPP. Also, the increasingly public concern about environment and climate change encourage the use of eco-friendly techniques in the food industry.

4 main characteristics have been identified to meet the end users' requirements:

- Cost. In particular the return on investment that should cover the extra cost of these new technologies in a reasonable time (2-3 years).
- Maintenance. Maintenance costs should be as low as possible and keep the system downtimes as low as possible (lower than conventional technologies).
- Safety. Equal or better microbiological parameters than conventional technologies.
- Quality. The quality of the product produced with the new technologies should have at least the same quality as the product produced with conventional technologies. In this sense an increase of the shelf life would be desirable.

5.9. Digital traceability system

In SEAFOOD^TOMORROW the development of a digital traceability tool to determine the attributes of seafood products at each stage (quality, safety, nutritional level, etc.) is proposed. Such system must track and segregate the seafood from the point of sale back to a catch area or farm, and must be based in the main voluntary standards, such as GS1 guides and ISO rules. The traceability tool must also integrate QR-codes for instant information to the consumer.

The food industry has already traceability systems working as this is a regulatory requirement, but most of these systems are internal, that is, each company manages the traceability in its part of the chain, without connection with the rest of the companies that make up the supply chain. Therefore, a system that trace the information and the product along the whole value chain is desirable and will help increase the transparency.

Several companies (4 in Spain and 3 in France) have been interviewed with the objective to participate in the demonstration phase. As it was said before in all cases there were traceability systems working and it seems that they are not much in favour of changing. For instance, one company in Spain cannot change the system because it is a requirement from its client, a big retailer.

The system to be implemented should improve systems already put in place in terms of easiness to use, price and above all make easier the management not only the management of the product but also the company in general terms.

6. Conclusions

After the analysis of the end users' requirements it seems that the industry and consumers might accept the innovative solutions, but some characteristics that should be achieved are highlighted and are common to most technologies.

- Regulatory issues.
  In the case of biosensors, the results obtained from the responses highlighted the concern for regulatory aspects. It is clear the commitment of the seafood industry to comply with regulatory requirements and they do their best to guarantee the safety and authenticity of their products. Therefore, seafood products are controlled and usually these analyses are performed by external laboratories. The different agents of the seafood value chain seem not to have the facilities and resources to make their own analyses. However, they will be glad to have analytical tools that could be implemented in their processes. These new tools should offer similar characteristics to the existing methods. That means that they should be reliable and therefore accredited and considered as reference methods.
  This aspect is also important for the other technologies. There are regulations that the new solutions should also obey. Indeed, in some cases the development of new technologies is a consequence of these regulations.
- Improvement of existing technologies.
  As far as better products (higher quality and safety characteristics) are obtained there will be a place for all technologies. For example, potential end users appreciate the benefits of rapid detection kits, above all the possibility of performing the analyses themselves which would decrease the response time and speed up the management of seafood products.
- Cost.
  This is crucial. It is important to maintain the cost of the product as lower as possible and demonstrate the reproducibility and robustness of the new tools.

All these facts should be considered during the design of the new solutions. When developing a new kit different alternatives/technologies/procedures can be applied. Although they might not be the best, they should be selected according to the requirements established by the end users. In addition, developers should be in close contact with end users and explain adequately the benefits to them. The latter is critical when consumers are approached. In this sense an effort should be made to communicate adequately the benefits of the new technologies.

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8. Annex 1

Questionnaire in English

Rapid detection kits

1. Introduction

SEAFOOD[TOMORROW] is an innovation action funded by the European Union (ref. 773400; www.seafoodtomorrow.eu), and aims to validate and optimize commercial solutions for improving the socioeconomic and environmental sustainability of the seafood production and processing industry, while contributing to product quality and safety.
To achieve these objectives and fine-tune the results to the needs of the end-users, it is of crucial importance to have stakeholders' support and input. For this reason, we would be most grateful if you could devote a few minutes to complete this web-based questionnaire, which explores the demand in the seafood sector for **rapid detection methods**. Your comments and feedback will help shape the progress and outcomes of the project, in this case the development of **rapid detection kits[1] for the detection of marine toxins[2], chemical contaminants[3] and authenticity[4] of seafood species**, ensuring they are fit-for-purpose.

Your answers will be kept confidential.
Thank you very much for your cooperation.
For more information about SEAFOOD[TOMORROW], visit www.seafoodtomorrow.eu or contact Alejandro Barranco (abarranco@azti.es)

[1] **Rapid detection kits:** Commercially packaged collection of components that is intended to simplify the analytical function. It should have everything required to perform its analytical function and provide results in an easy and rapid manner.
[2] **Marine Toxins:** They are molecules of very different structure and mechanism of action, produced naturally by marine microorganisms. They can be hazardous for humans and marine organisms, directly or after being transferred through the food webs. In Europe, lipophilic marine toxins, group that includes the Diarrheic Shellfish Poisoning (DSP) toxins, Paralytic Shellfish Poisoning (PSP) toxins and Amnesic Shellfish Poisoning (ASP) toxins are regulated. Additionally, emerging marine toxins, such as tetrodotoxin, which is not regulated at the moment, may also be present in shellfish and involve risk situations.
[3] **Chemical contaminants**: chemicals that might be present in the environment naturally or as a result of man-made activity, that make food unfit for consumption or use. They can be present in foods through many potential sources, such as food production, distribution, packaging or consumption.
[4] **Authenticity:** The authenticity assurance of seafood species involves the use of methodologies that allow the unequivocal verification that the traded species are accurately identified in the accompanying label without any possibility of mislabelling that might involve substitution of valuable species with cheaper ones.

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Rapid detection kits

2. CONTACT INFORMATION

In this page you are kindly invited to provide some contact information that that will help the analysis of the results and to achieve the goals of this survey. Fields marked with * are of mandatory response and some replies are optional as they refer to personal information. By giving this personal information to us, you agree to be included in our stakeholder database. This personal data will be stored on a secure database and will never be used for any other purpose than the dissemination of information, news and events from the SEAFOOD^TOMORROW project. We will not share your data with any other organization. The responsible for this database is Dr. Siân Astley, from EuroFIR, and in case you have any concern with regard to this database contact her by email at sa@eurofir.org.

1. Name of the company (optional)

____

* 2. Country

____

* 3. Select the type of company where yours fits

- ◯ SME (<250 employees)
- ◯ Large (>250 employees)
- ◯ Cooperative
- ◯ Other (please, specify)

* 4. Activity

- ◯ Seafood supplier
- ◯ Seafood processor
- ◯ Retailer
- ◯ Other (please, specify)

* 5. Indicate the main seafood species your company works with

6. Personal details (optional)

Name and surname

e-mail

Position

Rapid detection kits

3. CONTROL OF SEAFOOD INTEGRITY (Chemical contaminant, Marine Toxins and Authenticity)

* 1. How important are these topics for your company?

	Not important	Somewhat important	Important	Very important
Seafood safety	◯	◯	◯	◯
Seafood species authenticity	◯	◯	◯	◯
Marine Toxins	◯	◯	◯	◯
Chemical contaminants	◯	◯	◯	◯
Species sustainability	◯	◯	◯	◯
Species diversity	◯	◯	◯	◯

* 2. Please specify the main toxins, contaminants or authenticity issues or authenticity issues of interest for your company. If there are none, please say so

* 3. Does your company perform routine controls of seafood authenticity, marine toxins and/or chemical contaminants?

◯ Yes ◯ No

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4. CONTROL OF SEAFOOD INTEGRITY (Chemical contaminant, Marine Toxins and Authenticity)

* 1. How often are these analyses performed?

	Daily	Weekly	Monthly	Quaterly	Only in case of suspicions	N/A
Authenticity	○	○	○	○	○	○
Marine toxins	○	○	○	○	○	○
Chemical contaminants	○	○	○	○	○	○

* 2. Where are these analysis performed?

☐ At the company facilities

☐ External laboratories

3. If the analysis are performed at the company facilities, please, specify the techniques used

	Authenticity	Marine toxins	Chemical contaminants
Chromatographic techniques	☐	☐	☐
ELISA	☐	☐	☐
PCR	☐	☐	☐
Other detection kits	☐	☐	☐

Others (please specify)

4. If samples are sent to external laboratories, please specify the techniques used (if known):

Authenticity

Marine toxins

Chemical contaminants

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* 5. Overall, how satisfied are you with the methods used and the results obtained?

- Not at all satisfied
- Somewhat satisfied
- Satisfied
- Very satisfied
- Extremely satisfied

* 6. Please, indicate the reasons for the previous answer

* 7. Annual expenditure in safety and authenticity control

	<1000€	1000-5000€	5000-10000€	10000-50000€	50000-100000€	>100000€	N/A
Authenticity							
Marine toxins							
Chemical contaminants							

* 8. Cost per measurement

	<30€	30-60€	60-90€	90-120€	120-150€	>150€	N/A
Authenticity							
Marine toxins							
Chemical contaminants							

* 9. Time of analysis (in house analysis)

	<1 hour	1-6h	6-12h	12-24h	1-3 days	>3 days	N/A
Authenticity							
Marine toxins							
Chemical contaminants							

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* 10. Response time (external laboratory)

	<12 hour	12-24h	1-3 days	4-6 days	7-10 days	>10 days	N/A
Authenticity	◯	◯	◯	◯	◯	◯	◯
Marine toxins	◯	◯	◯	◯	◯	◯	◯
Chemical contaminants	◯	◯	◯	◯	◯	◯	◯

* 11. Desired response time

	<12 hour	12-24h	1-3 days	4-6 days	7-10 days	>10 days	N/A
Authenticity	◯	◯	◯	◯	◯	◯	◯
Marine toxins	◯	◯	◯	◯	◯	◯	◯
Chemical contaminants	◯	◯	◯	◯	◯	◯	◯

* 12. Based on your experience, how important are the following aspects of the methods?

	Not important	Somewhat important	Important	Very important
It is an accredited method	◯	◯	◯	◯
It is a reference method	◯	◯	◯	◯
Response time	◯	◯	◯	◯
Cost	◯	◯	◯	◯

Other (please specify)

* 13. How likely are you to change the methodology?

Definitively will not	Probably will not	Probably will	Definitively will
◯	◯	◯	◯

Please indicate the reasons for your previous answer

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Rapid detection kits

5. ASSESSMENT OF RAPID DETECTION KITS

* 1. Indicate your degree of interest in **rapid detection kits** for the detection of marine toxins, chemical contaminants and/or authenticity:

No interest	Some interest	Moderate interest	High interest
○	○	○	○

In case you are not interested in rapid kits, please specify the reasons

* 2. In case you are interested in rapid detection kits, what is your motivation?

	Not important	Somewhat important	Important	Very important
Increase the number of analysis	○	○	○	○
Gurantee food safety	○	○	○	○
Enhance the response time	○	○	○	○
Guarantee food species authenticity	○	○	○	○
Implement corrective measures	○	○	○	○
Decrease the costs	○	○	○	○

Other (please specify)

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* 3. In a rapid detection kit, how important are the following attributes?

	Not important	Somewhat important	Important	Very important
Sensitivity	◯	◯	◯	◯
Specificity	◯	◯	◯	◯
Response time	◯	◯	◯	◯
Reproducibility and consistency of results	◯	◯	◯	◯
Robustness	◯	◯	◯	◯
Quantitative results	◯	◯	◯	◯
Design: small, portable...	◯	◯	◯	◯
No equipment needed (completely manual)	◯	◯	◯	◯
Easy to use	◯	◯	◯	◯
Price/cost per analysis	◯	◯	◯	◯
Stability and expiry date	◯	◯	◯	◯
After sales services	◯	◯	◯	◯
Availability: easy to purchase (distributors, internet...)	◯	◯	◯	◯
Number of analysis per kit	◯	◯	◯	◯

Other attribute you would like to highlight

* 4. How much would you be willing to pay for a rapid kit (10 analysis)?

* 5. How often would you use a rapid kit?

- ◯ Every day
- ◯ A few times a week
- ◯ About once a week
- ◯ A few times a month
- ◯ Once a month
- ◯ Less than once a month

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* 6. How many analysis would you perform annually?

◯ <25 ◯ 25-50 ◯ 50-100 ◯ 100-500 ◯ >500

* 7. Please choose the minimum expiry date you would accept

◯ 1-3 months ◯ 3-6 months ◯ 6-12 months ◯ >12 months

Thank you very much for your collaboration!

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