



OCEAN WISE

WP7.3.1: Living Labs of Innovation for EPS Alternatives in the Seafood Sector

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Executive Summary

Work Package 7.3, “Living Labs of Eco-innovation” set out to test some of the EPS/XPS alternative products, prototypes or materials identified within the Oceanwise project. This action was led by BIM – Ireland’s Seafood Development Agency (Ireland) and CETMAR Centro Tecnológico del Mar (Spain). As both organisations work with the seafood sector, the tests focused on alternatives to EPS fish boxes. Based on the outputs of other Oceanwise Work Packages, four alternatives were chosen for the living lab trial, three of which are comprised of foamed bioplastic (similar in weight and appearance to EPS), while the fourth comprised a coated cardboard. Timed trials were conducted to assess the quality and shelf life of different fish species packed in ice within the different boxes based on real life scenarios in both Ireland and Spain. Further laboratory tests were conducted to assess the strength of the alternative boxes relative to EPS. An additional small living lab trial was conducted, focusing on how to establish an EPS fishbox recycling management plan within Irish government laboratories.

All of the alternatives guaranteed fish quality over the time required to use the boxes e.g. 3 days for wholefish in the Spanish market and up to 13 days for Irish salmon fillets based on summer shelf life. The laboratory testing of the physical strength of the boxes returned similar results to EPS for all of the foamed alternatives. However, these are not the only factors to be considered by a seafood processing business prior to the adoption of alternatives. Issues such as cost, availability of industrial composting infrastructure at end of life and establishment of good management practices to avoid contamination of EPS recycling streams must also be considered. The Irish Government laboratory trial reinforces the fact that good management planning for EPS recycling is also a viable option.



1.0 Introduction

The environmental problems generated by the extensive use of EPS packaging in many sectors, including the seafood sector, have led to the development of packaging comprised of alternative materials. Compared with electrical items and other household goods, seafood is not well suited to many of the alternative packaging options.

Work Package 7.3, “Living Labs of Eco-innovation” set out to test some of the EPS/XPS alternative products, prototypes or materials identified within the Oceanwise project, in particular WP5 and WP7.1, to prove technical and commercial viability in comparison with current uses of EPS. This action was led by BIM – Ireland’s Seafood Development Agency (Ireland) and CETMAR Centro Tecnológico del Mar (Spain). As both organisations work with the seafood sector, the tests focused on alternatives to EPS fish boxes. The seafood sector uses a wide variety of packaging solutions (EPS, PET trays, waxed cartons etc) for the storage and transportation of fish to meet the specific requirements and needs related to food safety and seafood quality. EPS fish boxes are used at different points in the cold chain and are most widely utilised at business to business level, for example: for direct sale of wholefish at markets; between primary processing and secondary processing (especially when being exported); from the secondary processing to the foodservice market; and from secondary processing to independent retailers (fishmongers).

2.0 The Living Labs

Four alternatives were chosen for the living lab trial, three of which are comprised of foamed bioplastic (similar in weight and appearance to EPS), while the fourth comprised a coated cardboard. They have been developed under the following brands: STOROPACK <https://www.storopack.es>; BEWISYNBRA <https://bewisynbra.com>; GREEN PLANET by KANEKA <https://www.kaneka.co.jp/en>; and SUMBOX <https://sumbox.com>. All of them, as promoted on their websites, are identified as an alternative to EPS. They vary in their stage of market development and commercial availability. They represent potential real choices for use in the seafood supply chain.

The top priority for users of fish boxes is that EPS alternatives guarantee seafood quality and safety for the period of storage and transport within the supply chain. The handling and durability features are also important for users and hence the physical performance of the boxes was also tested. The aspects described in the previous paragraph represent the two basic objectives of the alternatives materials Living Labs described in this report



In its programme of work BIM tackled both objectives at a wider level, while CETMAR focused more strongly on the assessment of the quality of fish using a variety of species and wider range of quality and organoleptic tests. Both organisations contracted external companies and research groups with great expertise to conduct laboratory tests. Regarding CETMAR, the chemical and sensory analysis were carried out by the Chemistry of Fisheries Products Group from the IIM-CISC in Vigo and the microbiology analysis were carried out by the Food Products Technology Lab (Department of Analytical Chemistry, Nutrition and Food Science) from the University of Santiago de Compostela. Regarding BIM, all the tests linked to the evolution of the quality of fish were conducted at accredited laboratories at MOWI Ireland <https://mowi.com/ie/>.

Contrary to the initial plan to transport boxes storing chilled fish between Ireland and Spain, the living lab trials were carried out in situ. Physical transport of fish within the alternative fishboxes was logistically very difficult due to uncertainties linked with the Covid 19 pandemic. Instead the alternatives were subjected to laboratory / quality assured material strength testing to assess performance against traditional EPS fish boxes. The tests concerning the final condition of the packaging after use were made by the Centre for Industrial Service and Design (CISD) the Technical University of the Shannon, Ireland.

Finally, while WP7.3 was initially focused entirely on alternative materials, it became apparent during the Oceanwise project, that in certain parts of the supply chain, EPS isn't managed as effectively as it could be and needs resources to manage it as a dedicated waste stream to improve its end of life management. In response to this the scope of the tests was also extended to incorporate the field assessment of improved waste management through alternative systems for the management of EPS. An additional small living lab trial was conducted, focusing on how to establish an EPS fishbox recycling management plan within government laboratories at Ireland's Marine Institute.



2.1 Alternatives chosen for the Living Lab Trials:

WP7.3 was always planned as an action to be carried out at the back end of the Oceanwise project as it was reliant on project findings to determine what alternatives to test within the living Labs. Oceanwise research, workshop participation by various relevant stakeholders, and findings together with the outputs from the Oceans Calling Award together helped to influence the choice of alternatives chosen for the Living Labs. A detailed description of the four alternatives is provided below:

Green Planet by Kaneka

Comprised of PHBH (a plant based biopolymer). The resin from which the foam is blown is approved for food contact but approval has not yet been sought for the foam. It is home compostable, industrially compostable and biodegradable in the marine environment.

It is not yet in commercial production.



Synbra BioFoam (BEWI)

Comprised of 100% PLA (polylactic acid). It is plant derived, mainly corn starch. It is industrially compostable and can also be recycled, however the recycling infrastructure is not yet widely available. Approved for food contact.





SeaClick (by Storopack using EcoVio)

Comprised of PLA (85%) and BASF (BASF Polymer EcoFlex which includes PBAT). PLA as above. PBAT (Polybutyrate Adipate Terephthalate) is degradable but non-renewable, petrochemical derived material. It is mixed with PLA to increase the speed of biodegradation. In contrast to 100% starch based bioplastics, EcoVio is more resistant to mechanical stress and moisture. Approved for food contact.

It is in commercial production. The SeaClick box can be made in different sizes and is designed as a fish box. The click on lid removed the need for strapping and it is designed for safe stacking.



SumBox

Comprised of specially coated card, the SumBox degrades as paper. Approved for food contact. Comes flat as a two piece box or one piece box with integrated lid.





3.0 Assessment on the quality of fish

BIM and CETMAR initially agreed to follow a common methodology and analysis for the study of the quality of fish. However, upon sourcing fish and contracting laboratories to conduct the tests, there was some divergence. CETMAR adhered to a more wide-ranging methodology considering chemical, microbiological and organoleptic analysis. BIM focused more on microbiological and temperature analysis for shelf-life testing. All tests were carried out at accredited laboratories using approved methodologies and are typical for the species of fish included in the tests.

CETMAR tested four fish species that had various market presentations (gutted, round, filleted etc): pouting (*Trisopterus luscus*); sardine (*Sardina pilchardus*); megrim (*Lepidorhombus whiffiagonis*) and horse mackerel (*Trachurus trachurus*). BIM tested just one species: filleted salmon (*Salmo salar*). The tests reflected real life market presentation, quality and food safety testing linked to the fish species, chilled fish type (e.g. oily, whitefish), and box handling for storage and transport within the respective regions. Hence there are some differences. CETMAR tested whole fish while BIM tested 100g salmon filets. CETMAR punched holes in the boxes to allow ice flake melt water to escape, while BIM did not. The differing approach was aligned to standard practices for the types and presentation of fish tested.

It must be noted that there was variation in box size and volume for the alternatives tested. This is due to limited availability of prototypes and product range. This factor did limit the interpretation of trial results.

3.1 Tests developed by CETMAR

3.1.1 Description, approach and scope (species)

The marketing of chilled fresh fish species is a very popular activity in the Spanish market, especially in Galicia and in other European countries, including those in the Atlantic Area.

The EPS boxes are used, apart from placing the raw material, to add flake ice. The catch gets to port from coastal fisheries and from the Great Sole fishing area. Once downloaded and sold in the fish markets they are sent mainly to the wholesalers who place them in EPS packaging to distribute to big supply platforms and central city markets. They arrive in just a few hours to be sold to the public.

This chilled fresh fish represents the highest volume and value of seafood traded in Europe. As CETMAR is located in Vigo, the largest European fish market, it was agreed to develop the tests with some fish species that are usually placed in the market using EPS boxes. The following four



species were chosen for the study: pouting (*Trisopterus luscus*), sardine (*Sardina pilchardus*), megrim (*Lepidorhombus whiffiagonis*) and horse mackerel (*Trachurus trachurus*). Pouting and Megrim are demersal /whitefish species, while sardines and horse mackerel are oily /pelagic species. To note, small round pelagic species will always have a short shelf life compared to demersal species, especially those that are gutted and longer again if filleted.

The study for each species was carried out according to a pre-planned methodology summarised below with more details contained in Appendix V. The supply of samples came from a wholesaler called “Martínez Campos SL” located in the fish market at the port of Vigo. Once the date was fixed, the company prepared the samples following the guidelines established beforehand (similar size specimen, from the same batch and homogeneously fresh). After that, first thing in the morning, the company gave the samples to IIM-CSIC who had to send some of them to USC-Lugo by express Courier for them to do the microbiology tests.

3.1.2 Analysis of results

All the results obtained in the study of the four species, both chemical, and organoleptic and microbiologic are collected in Annexes II and III and they show the output of the work carried out by the two previously mentioned research groups hired by CETMAR.

3.1.3 Discussion / Conclusion

It must be remarked that the time for the fresh fish commercialization in EPS or alternative material boxes just takes 2-3 days. Interpretation of the following conclusions should bear this fact in mind. Further information about this issue is available in section 6.1. Living Lab Conclusions.

Sensory conclusions

Along a 9-day chilled storage:

The four alternative boxes tested led to a higher retention of sensory quality (evaluation of skin, eyes, external odour, and gills) in pouting and sardine experiments when compared to the Polyspan control batch. This effect was found more important in the case of employing *Symbra*, *Storopack*, and *Kaneka* packaging.

For the megrim experiment, variability of starting fish quality provided insignificant differences, although a sensory quality retention could be concluded for *Storopack*, and *Kaneka* boxes. Concerning the horse mackerel experiment, a higher sensory quality retention was detected for



Symbra, *Storopack*, and *Kaneka* packaging, while Control and *Sumbox* batches were not found acceptable at the end of the experiment.

Along a 3-day chilled storage:

If a short chilled time (i.e., 3 days) is taken into account, sensory quality loss was found insignificant in all alternative boxes as well as in Polyspan condition. Thus, fish was sensory acceptable in all cases, scores being slightly better for *Symbra*, *Storopack* and *Kaneka* packaging when considering the pouting and sardine experiments.

Chemical conclusions

Concerning chemical quality assessment, comparison to control Polyspan condition showed an inhibitory effect on lipid oxidation development (i.e., thiobarbituric acid and fluorescence indices) by the use of all alternative boxes. This effect was found more pronounced in the case of *Symbra*, *Storopack*, and *Kaneka* packaging during the storage experiments developed on pouting and sardine.

If a 3-day storage is considered, a low development of lipid damage (oxidation and hydrolysis) was found in all batches. Remarkably, slightly lower values for TBARS and fluorescent compounds were obtained in alternative packaging than in traditional Polyspan condition if considering the experiments carried out on pouting, sardine and horse mackerel.

Microbiological conclusions

Microbiological analyses carried out after three days of refrigerated storage indicated good microbial quality in all four fish species investigated in all five storage systems considered. On day three, aerobes were always below 4 log CFU/g, a level far below the 6 log CFU/g legal limit. With respect to Enterobacteriaceae, these were in all cases below the legal limit of 3 log CFU/g in all species and storage systems tested after three days of refrigerated storage.

When storage was extended to six days, microbiological analyses still indicated acceptable microbial quality of all four fish species in all five storage systems according to the aerobes levels, which were always below 5 log CFU/g, still below the 6 log CFU/g legal limit. Nevertheless, only *Storopack* and *Kaneka* systems kept Enterobacteriaceae levels below the legal limit of 3 log CFU/g in all species tested after six days of refrigerated storage.

No significant ($p>0,05$) difference was determined among the five storage systems for any of the microbial parameters tested, during the first six days of refrigerated storage, although a slight



slowdown of microbial growth was observed in fish specimens corresponding to *Storopack* and *Kaneka* systems.

3.2 Tests developed by BIM

3.2.1 Description, approach and scope (species)

BIM worked with MOWI Ireland who culture and process Atlantic salmon in Ireland. The salmon aquaculture sector in Ireland is important in terms of volume, value, employment and social sustainability of rural coastal communities. In 2020 production of farmed finfish was 14000 tonnes with a value of €129million. Most salmon farmed in Ireland is exported and is highly sought due to its premium quality and exclusive production to the EU Organic Certification Standard. It is mostly exported to the EU, with lesser volumes going to North America and the Near and Far East. In 2021, by value, salmon is both Ireland's top exported species at €109M and imported fish species at a value of €57M (<https://bim.ie/wp-content/uploads/2022/04/BIM-Seafood-Business-2021.pdf>). Imported salmon can be market ready (filleted and portioned) or undergo secondary processing (filleting and /or portioning) in Ireland. It is frequently transported in EPS boxes at business to business level.

Summer shelf-life trials were conducted on gutted and filleted chilled 100g salmon portions over a 16 day period, packed in the four alternative boxes and using a standard EPS fish box as a control. All salmon was one day post-harvest at the time of packing.

All boxes were lined with a plastic liner and packed with 20 x 100g salmon portions. Approximately 1.5 kg of ice in a plastic bag was placed on top of the portions before lid placement. Tidbit temperature loggers were placed in each box. Each box was strapped with nylon straps and stored under chill conditions for the duration of the trial.

3.2.2 Analysis of results

All Tidbit temperature loggers were removed from the boxes at the end of the trial and data downloaded for analysis. Microbiological testing was also conducted at post-harvest days 1, 8 & 12-16 inclusive. An upper limit of 10ma viable bacteria per gram of fish is assumed as the limit of shelflife. A limit of 100 Enterobacteriaceae per gram of fish is also applied. *Listeria monocytogenes* was also tested as the pathogen of concern for seafood.

Overall, the alternatives performed well alongside the EPS control box.

Temperature Analysis



In relation to temperature the Synbra biofoam box performed best. However, some melt water leaked through the box and at the end of the trial the box was heavier than the others indicating that it also absorbed water. Sumbox and Seaclick were the poorest performers and correspondingly no ice remained at the end of the trial. For Sumbox the ice was fully melted by day 8 of the trial, the box was soggy, but did not leak. This resulted in the portions being submerged in melt water for the remainder of the trial. The Seaclick box was the smallest of the trial boxes and after the portions were placed in the box there was limited room remaining and less ice could be added. The result of this was that by day 13 of the trial all the ice had melted. In relation to ice retention and temperature, the performance of the Kaneka box was most similar to the EPS control with sufficient ice remaining until day 13, a temperature spike on day 15 but some ice still remaining at the end of the trial.

Microbiological Analysis

In relation to the microbiological testing, no listeria monocytogenes was detected from any of the samples and all were well within the limits of e-coli throughout the trial period. The most elevated levels were in the Sumbox and Seaclick boxes corresponding with the elevated temperatures. Total viable bacterial counts@30C, (i.e. the shelf life limit) was exceeded for samples in three of the boxes: Seaclick on day 15 post-harvest, and Kaneka and Sumbox on day 16, the final day of the trial. In this instance the performance of the Synbra box was broadly the same as the EPS control with total counts well within the limit. The Kaneka box had the largest internal volume of all of the boxes used in the trial. Given that the same volume of ice and fish was added to each box, the larger air pocket may have contributed to the reduced bacteriology performance for the Kaneka box.

3.2.3 Discussion BIM

Further testing would be required to draw a full conclusion on the performance of the boxes prior to largescale uptake. The initial comparisons derived from the summer shelf-life study approach do indicate adequate performance from a microbiological and temperature control perspective. Discussions with seafood processors however, indicated that they would reserve judgement on the basis of these tests alone and that other factors would also require consideration. These are mainly, but not limited to cost and also include treatment options at end of life destination, transport performance (materials strength testing section 4.0), and availability on the market place. Concerns were raised about potential contamination of EPS recycling streams with Biofoams as they look quite similar, and thereby creating a bigger problem. On this basis SeaClick was considered better as its brown colouring means that it is easy to distinguish from the EPS



boxes. Once compacted it can also be difficult to tell if a recycling stream is contaminated. Therefore, strong SOP's for handling would be required where mixed materials are being used. Given the higher costs and limited availability of the biofoams, seafood processors speculate that at this time, uptake of alternatives may only be viable for certain product lines. e.g. top range products destined for markets with strong composting infrastructure.

4.0 Assessment of the final condition of the boxes used in the tests

4.1 Context

It was envisaged at the project outset that the living labs would include actual transport trials, and CETMAR and BIM did plan to transport fish packed in the alternative boxes, between Ireland and Spain. However, with the onset of the Covid 19 pandemic, the logistics associated with such trials made them more difficult and less reliable. As decision was taken to conduct the fish quality trials in situ using typical fish species from Spain and Ireland (as described in section 3 above). However, to enhance overall findings, an assessment of the physical performance of the boxes was still desirable. Hence, the alternatives together with an EPS control, were subjected to laboratory-based strength testing. These tests were conducted at the Centre for Industrial Service and Design (CISD) based within the Technical University of the Shannon, Ireland.

4.2 Description Approach and Scope

Samples of the four alternatives and the EPS control were each subjected to the following: Drop testing; Impact Strength testing; Compressive testing; and artificial weathering.

- 1) Drop testing: This involved dropping each box from a height 10 times, on each of the six faces, three edges and the most fragile corner. Tests conformed with ISO 2206:1987.
- 2) Impact strength testing: All of the samples were subjected to impact testing. This involved a 1kg impactor delivered from a 600mm test height. Tests confirmed with EN ISO 13245:2010...
- 3) Compression testing:
 - a) Compression testing – full box
The whole boxes were subjected to compression testing using a Zwick/Roell Tensometer. The tensometer was fitted with a 9.9kN load cell and compression jig with a compression speed of 50mm/min.



b) Compression testing – box materials

Material from each box was subjected to compression testing using a Lloyd LRX Tensometer. The tensometer was fitted with a 2.5kN load cell and as with the whole box test, operated at a compression speed of 50mm/min.

- 4) Density testing was then using a ROLBATCH RBDT-01
- 5) Weathering: the specimens were subjected to artificial weathering using the ATLAS Weatherometer for 2500 hours exposure which is approximately equivalent to 5 years weathering exposure. Following artificial weathering the impact tests and compression tests were repeated.

4.3 Analysis of Results

1. Drop testing: All of the samples either completely broke or suffered a “hanging break”.

EPS Control



Storopack



Synbra



Kaneka

Sumbox



2. Impact strength testing: None of the samples showed any evidence of a surface fracture or penetration into the sample and hence all specimens passed the test. The tests were repeated following artificial weathering and ageing and again all passed.
3. A. Compression testing – full box
The Sumbox withstood the highest stress while the Kaneka box withstood the lowest amount.
B. Compression testing – box materials
Interestingly material from the Sumbox withstood the least amount of compression while material from the Kaneka box withstood the highest amount. Both pre and post weathering EPS was in the middle of the performance range for compression loading (maximum load measured in Newtons) and stress at maximum load.
4. Density Testing: The density of the boxes in grams per cubic cm was measured pre and post artificial weathering. None of the boxes experienced a marked change in density measurement. The EPS box was the least dense and the cardboard Sumbox was the densest material. Of the expanded foam boxes the range of density varied by just 0.04g/cm³ from 0.024g/cm³ for EPS to 0.064g/cm³ for the Kaneka box. This compared with a density of 0.375 for the Sumbox.
5. Weathering
Material density was measured pre and post artificial weathering. It did not vary to any great extent across the samples.

4.4 Discussion

All of the boxes performed at a similar level and were comparable with the EPS control in the physical material strength tests. While Sumbox is a completely different product, the others being foamed bioplastics displayed behaviour closely similar to the EPS control. In general handling it was observed that the storopack box seemed to be more friable than the others. However, its design which incorporated a click on lid means that strapping is not necessary. This



helps to mitigate against such breakage. Overall, for normal handling all of the boxes demonstrate adequate performance at a level similar to that of EPS.

As the boxes are generally intended for single use in fish storage and transport, the weathering tests were not so relevant. However, it is interesting that physical performance was not greatly affected post artificial weathering. It is unlikely that boxes would be used after a period of weathering. However, there may be a scenario where bulk purchase, with the use of boxes over an extended time-period, is more economically viable. This could be a realistic scenario for a small seafood processor. It is therefore good to know that the physical properties are not likely to deteriorate during long term storage should that be required.

Taking the post artificial weathering results from another angle (with 2500 hrs in laboratory equivalent to 5 years), it could be considered that like EPS these materials would persist in the environment should they become litter. Note: the artificial weathering was for air and not saltwater, so it cannot be concluded that they would persist in the same way should they become marine litter.

5.0 Assessment of alternative management of EPS fish boxes in Ireland's Public Sector.

5.1 Context

Project research as part of Oceanwise confirmed that EPS fish boxes are often disposed as municipal waste. This is true of damaged and broken boxes used at fish processing facilities and was also the case at the national testing laboratory within Ireland's Marine Institute. An additional trial was conducted on alternative management of EPS. As EPS is made up of 98 percent air and 2 per cent plastic it has a high volume/low weight ratio. Waste compaction options are increasingly available and uptake of such services is good across the private sector from supermarkets and stores to fish processors. However, this is not replicated within the public sector. Ireland's Marine Institute (MI) provide the national reference laboratory for fish and shellfish testing. Shellfish and fish samples are sent to the MI by primary producers all around Ireland for a wide range of food safety, environmental and research testing. Upwards of 3000 boxes are received annually. The Marine Institute engaged with the Oceanwise project at stakeholder meetings and was approached by BIM to test alternative management of this EPS waste stream. Prior to the trials, all boxes received were sent to landfill. Due to fish contact,



odours, fouling and oil contamination were assumed to be a barrier to the recycling of EPS, together with varying sizes and condition of the boxes received.

5.2 Description of the Trial and Results

A mobile compacting and recycling trial was conducted over a three month period. Waste Matters Ltd, an Irish waste recycling company, were contracted to bring their mobile compactor to the site. Waste Matters Ltd already provide such a service to many commercial seafood processors. The service efficiently shreds, heats, compacts and reforms the polystyrene material into a compact polystyrene briquette which is then taken away for resale and re-use.

For the first uplift, carried out 24th Sept 2019 (2 pallets), there was an insufficient quantity of boxes to justify onsite compacting. In this instance the boxes were placed on pallets, were shrink-wrapped and transported by lorry for compacting elsewhere. Further collections were arranged so that a sufficient quantity was available to enable onsite compaction. In total, six collections were carried out between September 2019 and February 2020 as part of this trial.

The service was able to effectively handle the variety of end of life EPS boxes accumulated by the Marine Institute and prove that EPS recycling is a viable option for this waste stream.

Image 1: Pallets of Polystyrene awaiting collection in the Marine Institute



Image 2: Collection day. The compacted Polystyrene blocks



5.3 Discussion

Following the trial period and with the establishment of a Standard Operating Procedure (SOP) for box handling in house, this service has been adopted as an ongoing waste management measure by the organisation, effectively diverting 3000 plus EPS boxes from landfill annually.

There were a number of requirements that needed to be established to facilitate the recycling of the EPS boxes by the Marine Institute, but none of these were too onerous:

- Boxes are required to be washed out.
- Storage space required to store boxes for 1-month period in the Marine Institute.
- Stored on pallets and shrink wrapped if collection by lorry only.
- Will only compact on site if enough boxes for compacting.
- A 32 amp socket (3 phase power socket) is required on site if the compacting is taken place on-site (Marine Institute got this installed).

The Marine Institute have cited a number of advantages to the alternative management which justified the establishment of EPS recycling as a standard operating procedure:

- Would be the lead as a governmental agency in recycling EPS in Ireland
- An alternative waste management plan for the Marine Institute
- As part of public sector – reducing waste and recycling
- Majority of polystyrene in the MI could be recycled
- Less waste going to landfill
- Reduced landfill costs



6.0 Discussion

6.1 Living Lab Conclusions

At the outset of the Oceanwise project, Work Package 7.3 – Living Labs of Innovation, was designed to identify commercially viable alternatives to the existing model of EPS usage for the seafood sector. This deliverable primarily focused on trials carried out to test the performance of fish boxes comprised of alternative materials on the basis that these materials would have a lower environmental impact if they did end up as marine litter. The boxes were identified through the research undertaken in other areas of the Oceanwise Project and more specifically, WP5, WP7.1 and WP7.2. As the alternatives are not plastic, the initial assumption is that the alternative packages are more environmentally friendly than EPS. However as with all life cycle and waste management considerations, the conclusions/outcomes are not so straightforward.

The following sections draw together the combined conclusions from the Living Labs trials and present some wider considerations and observations to help provide some clarity about the viability of alternative materials and alternative management of EPS.

FISH QUALITY AND FOOD SAFETY

The trials were conducted to assess the performance of the boxes against an EPS for temperature control, and fish quality and safety based upon microbiological, biochemical and organoleptic variables. For seafood transport and storage, times to market are variable depending on seafood species, the market and type and presentation of seafood (oily/demersal wholefish/filleted). The short commercialization process, i.e. time from landing until reaching the consumer, of three days in Spain was not long enough to detect any deterioration in fresh fish quality across all samples. The Spanish tests were extended to 9 days to account for this and changes in quality were observed from day 5/6 onwards. The Irish case study identified summer shelf life limits from day 14 onwards. E-coli levels were within safe limits for the duration of the 16 day test period in Ireland. In the Spanish e-coli tests, the alternatives performed slightly better and in the Irish tests the performance against EPS was more mixed. Total viable bacterial counts used as a measure of shelf-life did not display rapid increases until day 13 and 14 in the Irish trials with limits exceeded from day 14 onwards by three of the four samples. According to the sensory and chemical definitions, the conditions of alternative packaging have obtained similar or even, better



quality values compared with traditional EPS packaging. All of the boxes used in the living lab trials performed well alongside the traditional EPS fish box with respect to temperature control.

In summary, all of the alternative packaging tested would guarantee the commercialisation of the raw material preserving the quality and the food safety of the different species under study. The tests on the evolution of the fish quality showed that all of the alternatives are suitable for the regular commercialisation logistics for wholefish in the Spanish market, which takes from two to four days and also for up to thirteen days for the fresh salmon fillets from Ireland.

However, before a business takes a decision to adopt an alternative fish box, it would be necessary for them to undertake targeted testing using the appropriate box size, incorporating drainage if required and considering the following factors: fish species; type (wholefish / fillet); distance to market destination; shelf life requirements; and other customer specifications.

PHYSICAL PERFORMANCE OF BOXES

The laboratory testing of the physical strength of the boxes returned similar results to EPS for all of the foamed alternatives. A problem with EPS during handling is that it can break if dropped. The sheared edges are more likely to shed foam balls from the EPS which can end up as litter / marine litter. Based on the test results, it is likely that similar issues would arise if using foamed bioplastic alternatives. In such instances, the marine litter risks may arise from the handling of the broken boxes used at fish packing /processing plants. Management appropriate to the material type needs to be in place to minimize risks.

At a non-scientific level, general observation during the trials concluded that some of the boxes were more friable than others. The Storopack box broke when strapped closed. However, it is designed to click closed and therefore removed the need for strapping (another plastic that also requires waste management).

The Sumbox comprised of lined cardboard, was completely different from the other foamed boxes and therefore was difficult to directly compare.

ALTERNATIVE MANAGEMENT OF EPS

Throughout the evolution of the Oceanwise project, in learning together, attending workshops and researching EPS and XPS as a source of marine litter, it became apparent that management of end of life EPS could be further optimised to reduce the risk of it becoming marine litter. Many users are unaware of how widely recyclable EPS is and presume that contaminated products such as fish boxes are ineligible for such transformation. The trials with Ireland's Marine Institute were



a success on this basis and shows that affordable and practical alternative end of life options do exist to divert EPS from landfill and reduce the risk of it becoming marine litter.

LOGISTICS

The choice of fish box within the seafood supply chain / by seafood primary producers, processors and retailers is strongly influenced by the logistics. This was apparent in the divergence of the living lab trials carried out in Spain and in Ireland and linked to fish species, level of processing, distance to market, time to market and customer specifications. The commercialization process for fresh seafood can vary greatly, and this in turn influences the use of ice, the need to add drainage to boxes, how boxes are sealed etc.

Often, the logistics cycle of the fresh fish commercialization is very short. From the download in the port, the sale in the fish market, packing in the fish box until the arrival to the sales spot and to the final consumer, takes just three days for wholefish in the Spanish market. This time is not enough to affect the ice. In this time period the ice flake keeps unaltered if the transport conditions have been correct and consequently, there is not any ice water to cause trouble. However, the ice might have partially melt, which makes drilling the bottom of boxes a regular practice to ease drainage. In this way, the boxes are sold with the bottom perforated or unperforated and it is the user who chooses.

Ice and Ice melt water can affect the quality of both the fish and the box in which it is contained. Fresh fish sold in fishboxes is widely packed with the addition of ice flakes. It means that although the boxes keep good isothermal conditions and are transported in refrigerated trucks, the ice may partially melt after a few days as evidenced in the trials. The ice water can accumulate in the boxes, come into contact with fish and damage the quality. The fish box material is also a key consideration. If cardboard gets wet the box will deteriorate and lose function. In the trials, the Synbra biofoam appeared to absorb some meltwater. Specifying drainage is one solution but only practical for short supply chains. For longer shelf life products and larger transport distances it may be necessary to use sealed boxes. This is also the case for air freight, home delivery and indeed individual customer specifications. Gel ice-packs or similar products can also be used to maintain temperatures.

The box dimensions and volume should be tailored to the seafood being transported. This will allow packing volume and ice to be optimised. Box size was not consistent across the living lab trials due to availability of the prototypes which are all at variable stages of commercial availability. This did impact the results as discussed previously.



As per fish quality and safety, it would be necessary for businesses considering the adoption of alternative fish boxes, carefully consider logistics requirements and test performance on this basis.

6.2 Wider Conclusions

Considering the Living Lab trials together with the wider Oceanwise findings, it is clear that the decision to adopt an alternative material to EPS is informed by multiple factors and there is no simple switch available. The Living Lab trials focused on seafood as it was assumed at the outset of the project that fish boxes were a major source of EPS as marine litter. This is not the case in reality, as project findings have revealed. However, due to the proximity to the marine environment of certain operators in the supply chain, more careful management is required to minimise risks. In addition, being a food product, fish has more stringent requirements than non-food products and following seafood contact, end of life options are more limited, due to oil, blood and odour contamination. Therefore, it was of interest in the project to examine alternatives for a more difficult scenario. Project findings could then be used to inform packaging companies, EPS users and waste managers about the options, challenges advantages and disadvantages of alternative materials and management.

- While the alternatives tested spanned from prototypes to commercially available products, the project also looked at alternative processes for management of EPS through trials with Ireland's Marine Institute for compacting and recycling EPS fish boxes. This trial was successful and the process has been adopted by the organisation, hence diverting EPS from landfill and contributing to its circularity. However, it is still a case of using plastic, and while EPS is 98% air, the boxes generally comprise virgin plastic and at end of life the fish boxes are recycled into other products, so the process is not completely circular.
- The fishboxes made from alternative materials do have potential. However, there needs to be appropriate infrastructure in place for end of life management e.g. industrial composting, logistics for collection and providing scale for the composting and this needs to be taken into consideration as part of the overall systems management for their successful adoption. Cost, overall performance compared with EPS, critical mass to justify the most appropriate life cycle management of the products and potential for contamination of EPS recycling streams are additional factors of concern for the seafood sector in adoption of alternatives. However, this certainly does not, and should not rule out their uptake in future.



- The fact remains that at present, EPS is cheap, readily available to a wide range of specifications, has excellent insulation and shock absorbing qualities, and is approved for food contact and has a waste management system available to scale (e.g. the WasteMatters solution in Ireland. Similar solutions are also available in other partner countries.)
- EPS wastes are in high demand for recycling into PS/XPS gravel to be used in the building and automobile sectors. The adoption of alternative materials that look and behave like EPS presents a risk for contamination of EPS recycling streams and could cause serious trouble in the industrial process and in the quality of the final product. In other words, they are incompatible, so wastes from both types of materials cannot be valorized in the same process. Strict handling protocols therefore need to be adopted where mixed materials are used to ensure segregation. The fact that the Storopack box is a different colour is an example of a solution to minimise the risk of contamination.
- Among the four alternatives studied only SUMBOX –made of cardboard with an inner plastic layer, causes objectively less environmental damage as it can be recycled as paper / cardboard.
- The other three alternatives comprising of expanded foams, look like EPS and are made of compostable bioplastics. Therefore, they are recyclable only where suitable composting infrastructure is available. If they reach the sea, they become marine litter, similar to EPS. Only Kaneka has been tested for degradability in the marine environment and it meets the standard. A biocompostable plastic is biodegradable in composting conditions but not in an aquatic environment.
- Additionally, the only way to recycle wastes from boxes made of compostable material would be the private companies working in this activity. Up to now they are not using these wastes because they do not need it and because they would alter their routinely process in technical terms. Added to this, there is limited agricultural demand for the compost that would be obtained.
- Taking all aspects into consideration, (except for SUMBOX), regardless of the preservation of fish quality and safety, the use of alternative materials by the seafood sector, do not at this time provide a practical widescale solution to address the problem of EPS fish boxes as marine litter. There are a number of challenges to be addressed to justify largescale uptake. In saying that however, there are opportunities, where the logistics permit, to adopt the use of alternative fish boxes for certain product ranges. In the meantime, emphasis should continue to be placed on optimizing EPS waste



management across the entire seafood sector supply chain to minimise the risks of it becoming marine litter. Work should also continue on the evolution of the alternative boxes within a wider system change that would further support their uptake, use and end of life treatment.



References

Bibliographical references

- Antonacopoulos, N.,
VerbesserteApparatuszurquantitativenDestillationwasserdampf­flüchtigerStoffe. *Z. Lebensm. Unters. Forsch.* **1960**, *13*, 113-160.
- Aubourg, S. P.; Pérez-Martín, R.; Medina, I.; Gallardo, J. M. Fluorescence formation during albacore (*Thunnus alalunga*) thermal processing. *Z. Lebensm. Unters. Forsch.* **1992**, *195*, 332-335.
- Ben-Gigirey, B.; VieitesBaptista de Sousa, J.; Villa, T.; Barros-Velázquez, J. Changes in biogenic amines and microbiological analysis in albacore (*Thunnus alalunga*) muscle during frozen storage. *J. Food Prot.* **1998**, *61*, 608-615.
- Ben-Gigirey, B.; VieitesBaptista de Sousa, J.; Villa, T.; Barros-Velázquez, J. Histamine and cadaverine production by bacteria isolated from fresh and frozen albacore (*Thunnus alalunga*). *J. Food Prot.* **1999**, *62*, 933-939.
- Bligh, E.; Dyer, W. A rapid method of total extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911-917.
- Chapman, R.; McKay, J. The estimation of peroxides in fats and oils by the ferric thiocyanate method. *J. Am. Oil Chem. Soc.* **1949**, *26*, 360-363.
- European Council Regulation, European Community (EC), No 2406/96, 26 November **1996**. *Off. J. Eur. Comm.*, L-334/2:23.12 (1996).
- Lowry, R.; Tinsley, I. Rapid colorimetric determination of free fatty acids. *J. Am. Oil Chem. Soc.* **1976**, *53*, 470-472.

Appendices

- Appendix I Summer Shelf-life Study BIM
- Appendix II Tables - chemical and organoleptic results CETMAR
- Appendix III Tables – microbiological results CETMAR
- Appendix IV Tables - Weathering & mechanical testing of boxes report BIM
- Appendix V Work Plan and analytical methods



Appendix I Summer Shelf-life Study BIM



Atlantic salmon portion shelf-life study packed in various box types.

The purpose of the trial was to assess the shelf-life extent of chilled 100g salmon portions over a 16-day period, packed in a variety of insulated boxes. The box types examined in this trial were:

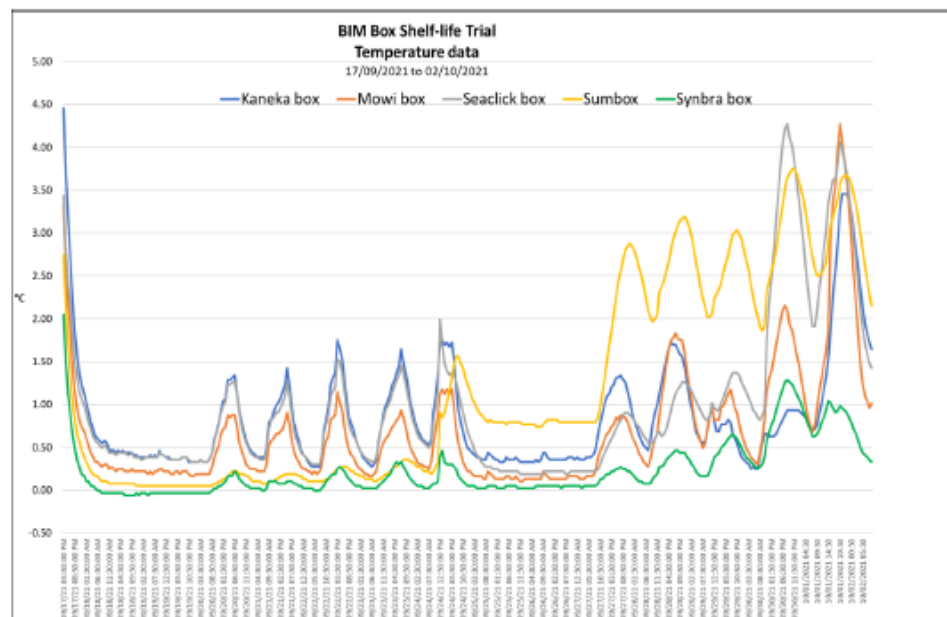
- Mowi expanded polystyrene box
- Synbra box – supplied by BIM
- SumBox – supplied by BIM
- Seadlick box – supplied by BIM
- Kaneka box – Supplied by BIM

Trial Conditions:

All boxes were lined with a plastic liner and packed with 20 x 100g salmon portions. Approximately 1.5kg of ice in a plastic bag was placed on top of the portions and before lid placement. Tidbit temperature loggers were placed in each box. Each box was strapped with nylon straps and stored under chill conditions for the duration of the trial.

Temperature recordings:

At the end of the trial all Tidbit temperature loggers were removed, and the data downloaded for analysis. Data files are available on request. The results of the temperature recordings from each box are illustrated below.





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Box Condition Observations:

Trial Day	Mowi EPS box	Synbra box	SumBox	Seaclick box	Kaneka box
1				Box fragile when strapping.	
8	Sufficient ice	Sufficient ice	Ice melted; portions covered in melt water. Box soggy but not leaking	Reduced ice. Melt water in box	Sufficient ice
12	Sufficient ice	Sufficient ice	Ice melted; portions covered in melt water. Box soggy but not leaking	Reduced ice. Melt water in box	Sufficient ice
13	Sufficient ice	Sufficient ice	Ice melted; portions covered in melt water. Box soggy but not leaking	Little ice. Melt water in ice bag	Sufficient ice
14	Reduced ice	Sufficient ice	Ice melted; portions covered in melt water. Box soggy but not leaking	No ice. Melt water in box	Reduced ice
15	Reduced ice	Some ice remaining, melt water leaked through box	Ice melted; portions covered in melt water. Box soggy but not leaking	No ice. Melt water in box	Reduced ice
16	Reduced ice	Some ice remaining, melt water leaked through box	Ice melted; portions covered in melt water. Box soggy but not leaking	No ice. Melt water in box	Reduced ice

Microbiological results:

Microbiological test results are summarised for each box type in the following tables. An upper limit 10,000,000 total viable bacteria per gram of fish is assumed as the limit of shelf life. A limit of 100 Enterobacteriaceae per gram of fish is also applied. Since *Listeria monocytogenes* is the pathogen of concern for seafood, this was included in the assay for information.

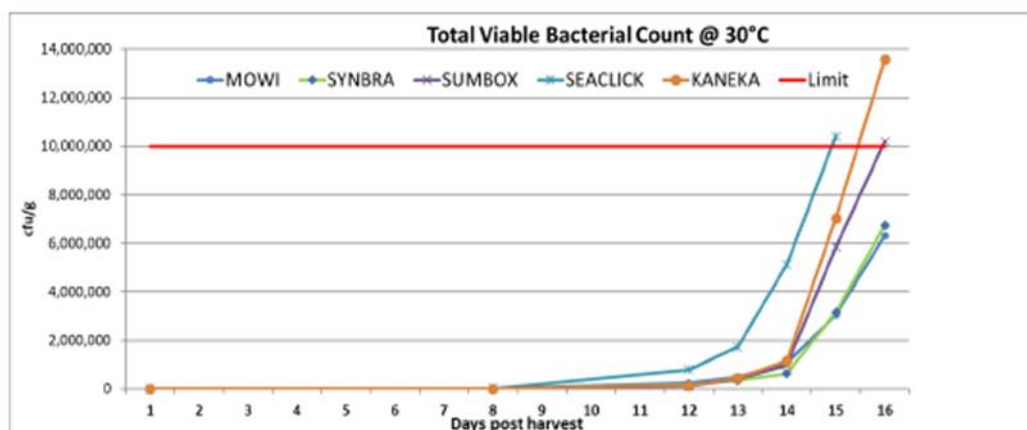
Total Viable Count @30°C			MOWI EPS box	SYNBRA box	SUMBOX	SEAClick box	KANEKA box
Day post-harvest	Sample day	Sample date	cfu/g*	cfu/g	cfu/g	cfu/g	cfu/g
1	0	17/09/2021	190	190	190	190	190
8	7	24/09/2021	3,413	437	6,332	11,226	2,087



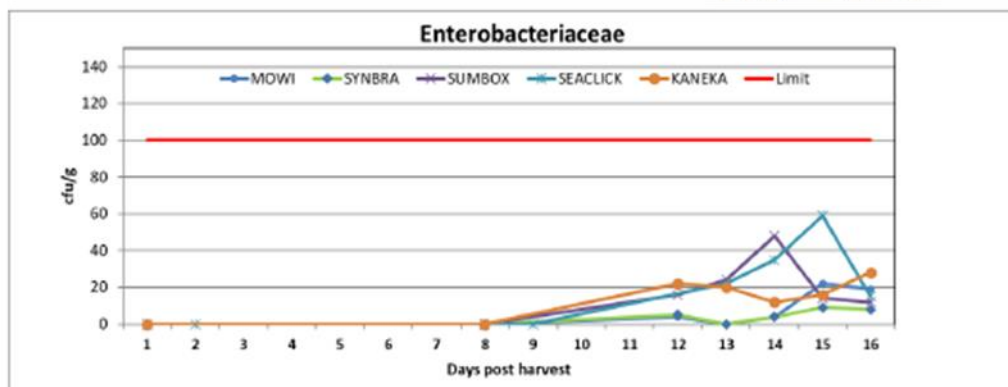
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12	11	28/09/2021	235,696	172,159	107,215	797,422	129,199
13	12	29/09/2021	479,152	343,618	421,180	1,719,327	437,578
14	13	30/09/2021	1,045,980	630,429	976,308	5,125,749	1,153,096
15	14	01/10/2021	3,045,257	3,158,988	5,840,169	10,416,694	7,046,137
16	15	02/10/2021	6,310,454	6,748,808	10,200,410	TNTC	13,590,592

*Cfu = Colony forming units



Enterobacteriaceae			MOWI EPS box	SYNBRA box	SUMBOX	SEAClick box	KANEKA box
Days post-harvest	Sample day	Sample date	cfu/g	cfu/g	cfu/g	cfu/g	cfu/g
1	0	17/09/2021	<4	<4	<4	<4	<4
8	7	24/09/2021	<4	<4	<4	<4	<4
12	11	28/09/2021	4	5	16	22	22
13	12	29/09/2021	<4	<4	24	35	20
14	13	30/09/2021	4	4	48	59	12
15	14	01/10/2021	22	9	14	15	16
16	15	02/10/2021	19	8	12	23	28


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<i>Listeria monocytogenes</i> (presence/absence)			MOWI EPS box	SYNBRA box	SUMBOX	SEACLICK box	KANEKA box
Days post- harvest	Sample day	Sample date	per 25 grams	per 25 grams	per 25 grams	per 25 grams	per 25 grams
1	0	17/09/2021	Not detected	Not detected	Not detected	Not detected	Not detected
8	7	24/09/2021	Not detected	Not detected	Not detected	Not detected	Not detected
12	11	28/09/2021	Not detected	Not detected	Not detected	Not detected	Not detected
13	12	29/09/2021	Not detected	Not detected	Not detected	Not detected	Not detected
14	13	30/09/2021	Not detected	Not detected	Not detected	Not detected	Not detected
15	14	01/10/2021	Not detected	Not detected	Not detected	Not detected	Not detected
16	15	02/10/2021	Not detected	Not detected	Not detected	Not detected	Not detected

Nigel Teape
Laboratory Quality Manager



This is an electronically signed document

* Indicates a test which is not included in the INAB accreditation schedule for this laboratory.

Indicates indicated information supplied by the customer.

Note 1: Any comments or observations made or requested are outside the scope of the accreditation.

Note 2: The results above relate only to the sample item tested.

Note 3: The report shall not be reproduced in full without the prior agreement of the Mowi Ireland Laboratory Quality Manager.

Note 4: The sample tested was in satisfactory condition unless otherwise stated in comments section.

Note 5: The information supplied by the customer is outside the control of the laboratory and can affect the validity of the results.



Appendix II Tables - chemical and organoleptic results CETMAR



TABLE 1: Sensory Scale employed for evaluating freshness of chilled fish species

Descriptor	Highest quality (E)	Good quality (A)	Fair quality (B)	Inacceptable (C)
Skin	Transparent mucus; very intense pigmentation	Milky mucus; insignificant pigmentation losses	Slightly greyish mucus; pigmentation without shine	Widely opaque mucus; important pigmentation losses
Eyes	Convex; transparent cornea; bright and black pupil	Convex and slightly sunken; slightly opalescent cornea; black and cloudy pupil	Flat; opalescent cornea; opaque pupil	Concave and milky cornea; internal organs blurred
External odour	Sharply seaweed and shellfish	Weakly seaweedy and shellfish	Slightly sour and putrid	Sharply sour and putrid
Gills	Brightly red; without odour; lamina perfectly separated	Rose coloured; without odour; lamina adhered in groups	Slightly pale; fishy odour; lamina adhered in groups	Grey-yellowish colour; intense ammonia odour; lamina totally adhered

Pouting (*Trisopterus luscus*)



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TABLE 2: Sensory analysis* of chilled pouting stored under different packaging conditions**

Descriptor	Packaging	Chilling time (days)			
		0	3	6	9
Skin	1	E	A+	A-	B-
	2				B+
	3				
	4				
	5				
Eyes	1	E	A-	B-	C
	2		A+	B+	B-
	3			B-	
	4			B+	
	5				C
External odour	1	E	B+	B-	C
	2		A+	B+	B+
	3			A-	B-
	4			B+	
	5				C
Gills	1	E	A-	B+	B+
	2		A+	A-	
	3		A-		
	4		A+	B+	
	5			A-	

*Quality categories: E (highest quality, excellent), A (good), B (fair) y C (unacceptable). For A and B categories, plus (+) and minus (-) symbols are included to indicate better and lower quality inside such quality category.

** Packaging conditions: 1 (Polyspan; control), 2 (Symbra), 3 (Storopack), 4 (Kaneca), and 5 (Sunbox).

TABLE 3: Determination of peroxide value (PV) and conjugated dienes (CD) and trienes (CT) in chilled pouting stored under different packaging conditions

Quality index	Packaging	Chilling storage time (days)			
		0	3	6	9
CD (absorbance units)	Polyspan	0.46 (0.04)	0.56 (0.02)	0.55 (0.02)	0.70 (0.07)
	Symbra		0.57 (0.01)	0.57 (0.01)	0.56 (0.06)
	Storopack		0.59 (0.00)	0.68 (0.05)	0.68 (0.02)
	Kaneca		0.53 (0.02)	0.55 (0.05)	0.62 (0.01)
	Sunbox		0.68 (0.07)	0.65 (0.04)	0.84 (0.15)
PV (meq. active oxygen/kg lipids)	Polyspan	1.29 (0.17)	2.18 (0.24)	2.00 (0.19)	2.17 (0.11)
	Symbra		1.72 (0.36)	1.75 (0.09)	1.50 (0.12)
	Storopack		1.64 (0.18)	1.83 (0.22)	1.96 (0.20)
	Kaneca		2.32 (0.41)	1.67 (0.07)	1.86 (0.15)
	Sunbox		2.04 (0.26)	2.24 (0.23)	1.91 (0.13)
CT (absorbance units)	Polyspan	0.053 (0.010)	0.068 (0.007)	0.076 (0.006)	0.116 (0.048)
	Symbra		0.062 (0.004)	0.073 (0.006)	0.073 (0.002)
	Storopack		0.069 (0.001)	0.089 (0.007)	0.097 (0.018)
	Kaneca		0.066 (0.006)	0.069 (0.004)	0.077 (0.003)
	Sunbox		0.075 (0.011)	0.085 (0.007)	0.153 (0.052)

Sardine (*Sardina pilchardus*)



TABLE 4: Sensory analysis* of chilled sardine stored under different packaging conditions**

Descriptor	Packaging	Chilling time (days)			
		0	3	6	9
Skin	1	E	A-	B+	C
	2		A+		B-
	3			B+	
	4			A-	B-
	5				
Eyes	1	E	A-	B+	C
	2		A+	A-	B-
	3				B+
	4		A-	B-	C
	5				
External odour	1	E	B+	B-	C
	2		A+	B+	B-
	3			B-	
	4		A-	B+	C
	5			B-	
Gills	1	E	A+	A-	B-
	2				
	3				
	4				
	5				

* Quality categories: E (highest quality, excellent), A (good), B (fair) y C (unacceptable). For A and B categories, plus (+) and minus (-) symbols are included to indicate better and lower quality inside such quality category.

** Packaging conditions: 1 (Polyspan; control), 2 (Symbra), 3 (Storopack), 4 (Kaneca), and 5 (Sunbox).

TABLE 5: Determination of primary oxidation compounds (conjugated dienes and peroxide value) in chilled sardine stored under different packaging conditions

Quality index	Packaging	Chilling storage time (days)			
		0	3	6	9
Conjugated diene content (absorbance units)	Polyspan	0.60 (0.05)	0.63 (0.05)	0.64 (0.06)	0.84 (0.02)
	Symbra		0.65 (0.01)	0.79 (0.16)	0.75 (0.25)
	Storopack		0.63 (0.04)	0.77 (0.05)	0.64 (0.01)
	Kaneca		0.65 (0.05)	0.69 (0.05)	0.74 (0.04)
	Sunbox		0.61 (0.06)	0.68 (0.04)	0.67 (0.09)
Peroxide value (meq. active oxygen/kg lipids)	Polyspan	4.17 (3.03)	10.84 (1.23)	17.96 (1.02)	79.45 (0.37)
	Symbra		14.32 (1.49)	28.12 (1.68)	26.97 (1.32)
	Storopack		14.38 (1.72)	30.43 (1.19)	21.61 (1.60)
	Kaneca		13.84 (0.48)	18.80 (1.63)	37.23 (1.20)
	Sunbox		9.67 (1.42)	29.79 (1.86)	28.43 (1.38)



TABLE 6: Determination of secondary oxidation compounds (conjugated trienes and thiobarbituric acid index) in chilled sardine stored under different packaging conditions

Quality index	Packaging	Chilling storage time (days)			
		0	3	6	9
Conjugated triene content (absorbance units)	<i>Polyspan</i>	0.044 (0.001)	0.042 (0.010)	0.045 (0.006)	0.085 (0.019)
	<i>Symbra</i>		0.048 (0.002)	0.066 (0.028)	0.071 (0.031)
	<i>Storopack</i>		0.044 (0.005)	0.060 (0.008)	0.058 (0.002)
	<i>Kaneca</i>		0.042 (0.006)	0.046 (0.017)	0.062 (0.006)
	<i>Sunbox</i>		0.037 (0.006)	0.055 (0.001)	0.047 (0.015)
Thiobarbituric acid index (mg malondialdehyde/kg muscle)	<i>Polyspan</i>	0.42 (0.03)	0.88 (0.20)	1.42 (0.87)	2.80 (0.38)
	<i>Symbra</i>		0.97 (0.10)	1.28 (0.20)	1.03 (0.26)
	<i>Storopack</i>		0.74 (0.24)	1.10 (0.02)	1.15 (0.31)
	<i>Kaneca</i>		0.67 (0.14)	1.78 (0.28)	1.71 (0.47)
	<i>Sunbox</i>		0.82 (0.00)	2.25 (0.53)	0.81 (0.25)

TABLE 7: Determination of free fatty acid content in chilled sardine stored under different packaging conditions

Quality index	Packaging	Chilling storage time (days)			
		0	3	6	9
Free fatty acid content (g/100 g lipids)	<i>Polyspan</i>	0.08 (0.02)	0.13 (0.03)	0.26 (0.08)	0.13 (0.07)
	<i>Symbra</i>		0.10 (0.03)	0.11 (0.04)	0.25 (0.09)
	<i>Storopack</i>		0.08 (0.01)	0.09 (0.01)	0.32 (0.13)
	<i>Kaneca</i>		0.12 (0.02)	0.08 (0.05)	0.25 (0.27)
	<i>Sunbox</i>		0.29 (0.10)	0.31 (0.09)	0.25 (0.10)



Megrin (*Lepidorhombus whiffiagonis*)

TABLE 8: Sensory analysis* of chilled megrin stored under different packaging conditions**

Descriptor	Packaging	Chilling time (days)			
		0	3	6	9
Skin	1	A+	A-	B+	B+
	2				B-
	3		A+	A-	B+
	4		A-	B+	B-
	5		A+		B+
Eyes	1	A+	A-	B+	C
	2			B-	
	3			B+	
	4			A+	
	5			B-	
External odour	1	A+	A+	B-	C
	2		B+	C	
	3		A-	B+	
	4				
	5				
Gills	1	A+	A+	B+	B-
	2			A-	
	3				B+
	4				
	5			C	

* Quality categories: E (highest quality, excellent), A (good), B (fair) y C (unacceptable). For A and B categories, plus (+) and minus (-) symbols are included to indicate better and lower quality inside such quality category.

** Packaging conditions: 1 (Polyspan; control), 2 (Symbra), 3 (Storopack), 4 (Kaneca), and 5 (Sunbox).



TABLE 9: Determination of primary oxidation compounds (conjugated dienes and peroxide value) in chilled megrim stored under different packaging conditions

Quality index	Packaging	Chilling storage time (days)			
		0	3	6	9
Conjugated diene content (absorbance units)	<i>Polyspan</i>	0.54 (0.04)	0.59 (0.02)	0.60 (0.04)	0.65 (0.18)
	<i>Symbra</i>		0.54 (0.01)	0.52 (0.00)	0.56 (0.06)
	<i>Storopack</i>		0.53 (0.02)	0.64 (0.03)	0.59 (0.00)
	<i>Kaneca</i>		0.44 (0.02)	0.55 (0.03)	0.63 (0.02)
	<i>Sunbox</i>		0.60 (0.01)	0.61 (0.02)	0.62 (0.08)
Peroxide value (meq. active oxygen/kg lipids)	<i>Polyspan</i>	1.71 (0.35)	1.73 (0.74)	1.41 (0.58)	1.62 (0.04)
	<i>Symbra</i>		1.52 (0.08)	2.60 (0.22)	1.91 (0.09)
	<i>Storopack</i>		1.50 (0.02)	2.25 (0.39)	1.86 (0.26)
	<i>Kaneca</i>		1.90 (0.16)	1.86 (0.32)	2.20 (0.16)
	<i>Sunbox</i>		0.97 (0.02)	1.52 (0.05)	1.56 (0.02)

TABLE 10: Determination of secondary oxidation compounds (conjugated trienes and thiobarbituric acid index) in chilled megrim stored under different packaging conditions

Quality index	Packaging	Chilling storage time (days)			
		0	3	6	9
Conjugated triene content (absorbance units)	<i>Polyspan</i>	0.050 (0.007)	0.042 (0.003)	0.047 (0.010)	0.067 (0.004)
	<i>Symbra</i>		0.042 (0.005)	0.078 (0.000)	0.079 (0.006)
	<i>Storopack</i>		0.050 (0.002)	0.088 (0.002)	0.082 (0.003)
	<i>Kaneca</i>		0.053 (0.006)	0.066 (0.009)	0.095 (0.004)
	<i>Sunbox</i>		0.035 (0.002)	0.073 (0.006)	0.060 (0.013)
Thiobarbituric acid index (mg malondialdehyde/kg muscle)	<i>Polyspan</i>	0.17 (0.05)	0.62 (0.04)	0.45 (0.01)	0.30 (0.02)
	<i>Symbra</i>		0.43 (0.03)	0.25 (0.01)	0.33 (0.01)
	<i>Storopack</i>		0.42 (0.10)	0.19 (0.01)	0.26 (0.04)
	<i>Kaneca</i>		0.28 (0.09)	0.28 (0.00)	0.20 (0.00)
	<i>Sunbox</i>		0.46 (0.06)	0.46 (0.17)	0.26 (0.05)



TABLE 11: Determination of trimethylamine content in chilled megrim stored under different packaging conditions

Quality index	Packaging	Chilling storage time (days)			
		0	3	6	9
Trimethylamine content (TMA; mg TMA-N/100 g muscle)	<i>Polyspan</i>	0.53 (0.24)	6.46 (1.86)	8.88 (0.65)	27.48 (4.70)
	<i>Symbra</i>		6.33 (2.51)	20.54 (2.93)	39.81 (0.61)
	<i>Storopack</i>		5.01 (1.47)	22.41 (3.44)	40.18 (3.96)
	<i>Kaneca</i>		10.08 (1.13)	13.64 (1.45)	51.95 (2.58)
	<i>Sunbox</i>		5.75 (0.82)	25.68 (1.45)	45.09 (9.89)



Horse Mackerel (*Trachurus trachurus*)



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TABLE 12: Sensory analysis* of chilled horse mackerel stored under different packaging conditions**

Descriptor	Packaging	Chilling time (days)			
		0	3	6	9
Skin	1	E	A-	A-	B-
	2		A+	A+	B+
	3			A-	
	4			A+	B-
	5			A+	
Eyes	1	E	A-	B+	B+
	2		A+	A-	B-
	3				
	4		A-	B+	C
	5				
External odour	1	E	A-	B-	C
	2			B+	B-
	3				
	4				
	5			B-	C
Gills	1	E	A+	A-	B-
	2		A-		
	3		A+	B+	
	4			A-	
	5			B+	

* Quality categories: E (highest quality, excellent), A (good), B (fair) y C (unacceptable). For A and B categories, plus (+) and minus (-) symbols are included to indicate better and lower quality inside such quality category.

** Packaging conditions: 1 (Polyspan; control), 2 (Symbra), 3 (Storopack), 4 (Kaneca), and 5 (Sunbox).



TABLE 13: Determination of primary oxidation compounds (conjugated dienes and peroxide value) in chilled horse mackerel stored under different packaging conditions

Quality index	Packaging	Chilling storage time (days)			
		0	3	6	9
Conjugated diene content (absorbance units)	<i>Polyspan</i>	0.95 (0.01)	0.99 (0.08)	0.94 (0.21)	1.11 (0.07)
	<i>Symbra</i>		0.95 (0.10)	0.85 (0.06)	0.88 (0.05)
	<i>Storopack</i>		0.87 (0.02)	0.89 (0.03)	0.91 (0.17)
	<i>Kaneca</i>		0.78 (0.09)	0.81 (0.08)	1.13 (0.03)
	<i>Sunbox</i>		0.83 (0.16)	1.04 (0.16)	0.95 (0.05)
Peroxide value (meq. active oxygen/kg lipids)	<i>Polyspan</i>	0.78 (0.03)	3.86 (0.52)	11.17 (3.41)	21.25 (4.88)
	<i>Symbra</i>		2.64 (0.43)	5.50 a (1.26)	13.11 (3.83)
	<i>Storopack</i>		4.44 (0.11)	8.54 (0.38)	24.78 (0.94)
	<i>Kaneca</i>		2.25 (0.12)	8.26 (1.01)	17.48 (0.18)
	<i>Sunbox</i>		5.55 (0.37)	15.30 (2.34)	11.50 (2.68)

TABLE 14: Determination of secondary oxidation compounds (conjugated trienes and thiobarbituric acid index) in chilled horse mackerel stored under different packaging conditions

Quality index	Packaging	Chilling storage time (days)			
		0	3	6	9
Conjugated triene content (absorbance units)	<i>Polyspan</i>	0.049 (0.006)	0.052 (0.001)	0.062 (0.022)	0.100 (0.033)
	<i>Symbra</i>		0.050 (0.005)	0.047 (0.003)	0.081 (0.009)
	<i>Storopack</i>		0.041 (0.003)	0.051 (0.007)	0.089 (0.027)
	<i>Kaneca</i>		0.035 (0.003)	0.046 (0.003)	0.108 (0.012)
	<i>Sunbox</i>		0.035 (0.005)	0.083 (0.007)	0.078 (0.007)
Thiobarbituric acid index (mg malondialdehyde/kg muscle)	<i>Polyspan</i>	0.10 (0.05)	0.22 (0.01)	0.71 (0.22)	1.49 (0.40)
	<i>Symbra</i>		0.12 (0.02)	0.57 (0.02)	1.17 (0.09)
	<i>Storopack</i>		0.28 (0.03)	0.64 (0.18)	1.50 (0.22)
	<i>Kaneca</i>		0.09 (0.02)	0.72 (0.24)	1.75 (0.04)
	<i>Sunbox</i>		0.23 (0.07)	1.02 (0.29)	1.17 (0.21)



TABLE 15: Determination of several chemical quality indices in chilled megrim stored under different packaging conditions

Quality index	Packaging	Chilling storage time (days)			
		0	3	6	9
Fluorescence ratio	<i>Polyspan</i>	0.86 (0.10)	1.12 (0.02)	1.00 (0.17)	1.48 (0.52)
	<i>Symbra</i>		0.80 (0.20)	0.78 (0.25)	1.50 (0.20)
	<i>Storopack</i>		0.88 (0.16)	0.89 (0.10)	1.76 (0.12)
	<i>Kaneca</i>		0.69 (0.03)	0.80 (0.03)	1.13 (0.38)
	<i>Sumbox</i>		0.90 (0.11)	1.13 (0.11)	1.33 (0.02)
Trimethylamine content (TMA; mg TMA-N/100 g muscle)	<i>Polyspan</i>	0.04 (0.00)	0.11 (0.01)	0.25 (0.04)	0.76 (0.06)
	<i>Symbra</i>		0.08 (0.01)	0.18 (0.01)	0.98 (0.06)
	<i>Storopack</i>		0.11 (0.01)	0.23 (0.03)	1.09 (0.08)
	<i>Kaneca</i>		0.09 (0.01)	0.22 (0.02)	0.80 (0.37)
	<i>Sumbox</i>		0.12 (0.01)	0.30 (0.06)	1.03 (0.23)

Report carried out in Vigo (Spain) on April 28th 2022

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MARTINEZ
SANTIAGO PEDRO
- DNI 36037669G

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Appendix III Tables – microbiological results CETMAR



Table 1. Microbial evolution in pouting muscle during refrigerated storage. Results are expressed as log CFU/g mean values and standard deviations between parentheses

	Pack type	Day 0	Day 3	Day 6	Day 9
Enterobacteriaceae	<i>Polyspan</i>	1,23 (0,40)	2,09 (0,16)	2,49 (0,20)	3,10 (0,95)
	<i>Symbra</i>	1,23 (0,40)	1,66 (0,10)	1,93 (0,41)	1,97 (0,35)
	<i>Storopack</i>	1,23 (0,40)	1,97 (0,20)	2,26 (0,45)	3,18 (0,25)
	<i>Kaneca</i>	1,23 (0,40)	1,43 (0,38)	2,39 (0,75)	2,23 (0,82)
	<i>Sunbox</i>	1,23 (0,40)	2,24 (0,32)	3,09 (0,36)	2,93 (0,65)
Aerobic Mesophiles	<i>Polyspan</i>	3,90 (0,35)	3,94 (0,42)	4,36 (0,32)	5,52 (0,07)
	<i>Symbra</i>	3,90 (0,35)	4,05 (0,20)	4,17 (0,26)	5,26 (0,22)
	<i>Storopack</i>	3,90 (0,35)	3,98 (0,18)	4,02 (0,32)	5,28 (0,18)
	<i>Kaneca</i>	3,90 (0,35)	3,84 (0,06)	4,41 (0,44)	5,29 (0,31)
	<i>Sunbox</i>	3,90 (0,35)	3,88 (0,83)	4,30 (0,30)	5,26 (0,14)
Psychrotrophes	<i>Polyspan</i>	3,86 (0,30)	6,86 (0,23)	7,78 (0,27)	8,54 (0,36)
	<i>Symbra</i>	3,86 (0,30)	6,10 (0,45)	7,19 (0,42)	8,21 (0,23)
	<i>Storopack</i>	3,86 (0,30)	6,28 (0,38)	6,96 (0,37)	8,08 (0,12)
	<i>Kaneca</i>	3,86 (0,30)	5,77 (0,54)	6,86 (1,49)	8,35 (0,14)
	<i>Sunbox</i>	3,86 (0,30)	5,86 (0,55)	6,79 (1,12)	8,34 (0,29)



Table 2. Microbial evolution in sardine muscle during refrigerated storage. Results are expressed as log CFU/g mean values and standard deviations between parentheses

	Pack type	Day 0	Day 3	Day 6	Day 9
Enterobacteriaceae	<i>Polyspan</i>	1,1 (0,28)	1,17 (0,15)	1,23 (0,24)	1,93 (0,85)
	<i>Symbra</i>	1,1 (0,28)	1,0 (0,00)	1,3 (0,30)	2,07 (0,50)
	<i>Storopack</i>	1,1 (0,28)	1,1 (0,17)	1,42 (0,39)	2,05 (0,23)
	<i>Kaneca</i>	1,1 (0,28)	1,0 (0,00)	1,3 (0,30)	1,63 (0,85)
	<i>Sunbox</i>	1,1 (0,28)	1,0 (0,00)	1,56 (0,23)	2,22 (0,46)
Aerobic Mesophiles	<i>Polyspan</i>	2,89 (0,11)	3,34 (0,16)	3,77 (0,18)	4,39 (0,13)
	<i>Symbra</i>	2,89 (0,11)	2,86 (0,56)	3,69 (0,25)	3,78 (0,42)
	<i>Storopack</i>	2,89 (0,11)	2,65 (0,39)	3,45 (0,21)	3,72 (0,22)
	<i>Kaneca</i>	2,89 (0,11)	2,98 (0,15)	3,40 (0,18)	3,84 (0,54)
	<i>Sunbox</i>	2,89 (0,11)	3,14 (0,16)	3,44 (0,08)	4,19 (0,39)
Psychrotrophes	<i>Polyspan</i>	3,86 (0,30)	5,55 (0,65)	5,89 (0,13)	7,81 ((0,16)
	<i>Symbra</i>	3,86 (0,30)	4,37 (0,20)	5,35 (0,48)	7,28 (0,09)
	<i>Storopack</i>	3,86 (0,30)	3,60 (0,23)	5,88 (0,29)	7,18 (0,36)
	<i>Kaneca</i>	3,86 (0,30)	3,93 (0,30)	5,86 (0,06)	7,23 (0,32)
	<i>Sunbox</i>	3,86 (0,30)	4,35 (0,11)	5,92 (0,13)	7,22 (0,21)

Table 3. Microbial evolution in megrim muscle during refrigerated storage. Results are expressed as log CFU/g mean values and standard deviations between parentheses

	Pack type	Day 0	Day 3	Day 6	Day 9
Enterobacteriaceae	<i>Polyspan</i>	1,1 (0,17)	1,64 (0,73)	1,83 (0,42)	3,77 (0,28)
	<i>Symbra</i>	1,1 (0,17)	1,59 (0,55)	1,59 (0,78)	3,50 (0,36)
	<i>Storopack</i>	1,1 (0,17)	1,36 (0,10)	2,31 (0,40)	3,37 (0,20)
	<i>Kaneca</i>	1,1 (0,17)	1,50 (0,17)	1,74 (0,09)	3,24 (0,24)
	<i>Sunbox</i>	1,1 (0,17)	1,65 (0,37)	3,09 (0,36)	2,69 (0,21)
Aerobic mesophiles	<i>Polyspan</i>	3,63 (0,06)	4,05 (0,58)	4,75 (0,64)	5,42 (0,10)
	<i>Symbra</i>	3,63 (0,06)	3,89 (0,41)	4,42 (0,39)	4,79 (0,44)
	<i>Storopack</i>	3,63 (0,06)	3,74 (0,31)	4,66 (0,31)	5,21 (0,32)
	<i>Kaneca</i>	3,63 (0,06)	3,97 (0,50)	4,98 (1,09)	5,12 (0,32)
	<i>Sunbox</i>	3,63 (0,06)	3,74 (0,61)	4,23 (0,40)	4,58 (0,28)
Psychrotrophes	<i>Polyspan</i>	5,64 (0,25)	6,39 (0,47)	7,09 (0,69)	7,81 (0,23)
	<i>Symbra</i>	5,64 (0,25)	6,34 (0,28)	6,88 (0,72)	7,67 (0,17)
	<i>Storopack</i>	5,64 (0,25)	6,57 (0,06)	7,07 (0,49)	7,22 (0,35)
	<i>Kaneca</i>	5,64 (0,25)	5,98 (0,30)	7,18 (0,46)	7,78 (0,64)
	<i>Sunbox</i>	5,64 (0,25)	6,05 (0,31)	6,72 (0,61)	6,65 (0,22)



Table 4. Microbial evolution in horse mackerel muscle during refrigerated storage. Results are expressed as log CFU/g mean values and standard deviations between parentheses

	Pack type	Day 0	Day 3	Day 6	Day 9
Enterobacteriaceae	<i>Polyspan</i>	1 (0,00)	1,23 (0,40)	3,03 (0,41)	4,28 (0,33)
	<i>Symbra</i>	1 (0,00)	1,3 (0,30)	3,36 (0,39)	4,59 (0,20)
	<i>Storopack</i>	1 (0,00)	1,36 (0,62)	2,94 (0,30)	3,93 (0,08)
	<i>Kaneca</i>	1 (0,00)	1,49 (0,20)	2,77 (0,43)	4,25 (0,29)
	<i>Sunbox</i>	1 (0,00)	1,1 (0,17)	2,90 (0,53)	4,03 (0,24)
Aerobic mesophiles	<i>Polyspan</i>	2,82 (0,24)	3,53 (0,35)	5,83 (0,31)	7,02 (0,25)
	<i>Symbra</i>	2,82 (0,24)	2,85 (0,26)	5,58 (0,36)	7,08 (0,22)
	<i>Storopack</i>	2,82 (0,24)	3,47 (0,24)	5,30 (0,38)	6,55 (0,19)
	<i>Kaneca</i>	2,82 (0,24)	3,14 (0,35)	5,13 (0,29)	6,51 (0,29)
	<i>Sunbox</i>	2,82 (0,24)	2,71 (0,85)	5,11 (0,24)	6,53 (0,29)
Psychrotrophes	<i>Polyspan</i>	3,53 (0,41)	4,14 (0,20)	6,26 (0,44)	7,41 (0,36)
	<i>Symbra</i>	3,53 (0,41)	3,65 (0,66)	6,15 (0,54)	7,75 (0,14)
	<i>Storopack</i>	3,53 (0,41)	4,13 (0,23)	6,00 (0,32)	7,02 (0,33)
	<i>Kaneca</i>	3,53 (0,41)	3,07 (0,52)	5,78 (0,42)	7,13 (0,20)
	<i>Sunbox</i>	3,53 (0,41)	3,32 (0,72)	5,34 (0,16)	7,20 (0,42)

Santiago de Compostela, April 27th 2022

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Appendix IV Tables -Weathering & mechanical testing of boxes report BIM

Five (5) samples of box product were submitted by Bord Iascaigh Mhara (BIM) to the Contract Analytical Services (CAS) of the CIRD in AIT for specimen Drop testing; Impact strength testing; Compressive Testing and Weathering tested.

Table 1.0 – Sample Description

Sample ID	Description
21017A	5 X Kingspan Foam
21017B	5 X Storopack (Brown)
21017C	5 X Synbra Biofoam
21017D	5 X Kaneka Biofoam
21017E	5 X Sumbox (Flat pack)

Five samples of box product were submitted by Bord Iascaigh Mhara (BIM) to the Contract Analytical Services (CAS) of the CIRD in AIT for specimen Drop testing; Impact strength testing; Compressive Testing and Weathering tested.

The testing was conducted using a 1kg weight at a drop height of 600mm.

Table 4.1 Summary of Results:

Sample ID	TEST – 6J
21017A 0 Hrs	PASS
21017A 2500 Hrs	PASS
21017B 0 Hrs	PASS
21017B 2500 Hrs	PASS
21017C 0 Hrs	PASS
21017C 2500 Hrs	PASS
21017D 0 Hrs	PASS
21017D 2500 Hrs	PASS
21017E 0 Hrs	PASS
21017E 2500 Hrs	PASS



Appendix V Work Plan and analytical methods

Methodology for each fish species

- Initial sample (day 0)
- Sample preserved in five packaging conditions –traditional EPS and four alternative packaging
- Number of samplings: 4 (days 3, 6, 9 and 12)
- Analysis in triplicate -three independent groups or replication.
- Protocol of action:
 - o Sample collection
 - o Removal of initial sample for analysis
 - o Ice preservation of the other samples in boxes with drainage
 - o Placing of fish and ice in cold storage at 4 °C
 - o Partial replacing of ice when needed
 - o Sample collection on the given days, according to the species and their alteration rate

Details on the types of analysis carried out and methods used are detailed below:

- Microbiological analysis: This includes measurement of aerobic mesophiles, enterobacteriaceae, and psychotrophs. The analyses will be performed on fish muscle, dissected under aseptic conditions. Extracts are prepared by homogenizing 25 g of muscle in 250 mL of 0.1% peptone water (Oxoid), according to the methodology developed by Ben-Gigirey et al. (1998 and 1999).
- Chemical analysis: Analysis of formation of volatile amine and lipid degradation. Within the first group, total volatile amines (Antonacopoulos, 1960) and trimethylamine (Tozawa et al., 1971) will be measured. Lipid degradation will be measured through formation of free fatty acids (Lowry and Tinsley, 1976), peroxides (Chapman and McKay, 1949), thiobarbituric acid-reactive substances (Vyncke, 1970) and formation of fluorescent compounds (Aubourg et al., 1992).
- Sensory analysis: Evaluation according to the methodology proposed by COUNCIL REGULATION (EC) No 2406/96 of 26 November 1996 laying down common marketing standards for certain fishery products. Descriptors such as gills, eyes, smell, colour and overall appearance will be emphasized.