PROJECT FINAL REPORT

Grant Agreement number: 212544

Project acronym: NAFISPACK

Project title: NATURAL ANTIMICROBIALS FOR INNOVATIVE AND SAFE PACKAGING

Funding Scheme: Collaborative project

Period covered: from 1st. November 2008 to 31st December 2011

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[1] Usually the contact person of the coordinator as specified in Art. 8.1. of the grant agreement

[2] The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index_en.htm; logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.

1. Final publishable summary report (about 40 pages)

1.1. Executive summary (max 1 page)

NAFISPACK is the acronym of the project entitled Natural Antimicrobials For Innovative and Safe Packaging.

This project has been supported by the Seventh Framework Programme (FP7) within the Cooperation Work Programme:

Theme 2 – Food, Agriculture and Fisheries and Biotechnology
KBBE-2007-2-4-04: Innovative and safe packaging
Call: FP7-KBBE-2007-1
Grant Agreement No: 212544

NAFISPACK duration has been 3 years and 2 months, starting in November 2008 and ending in December 2011. The total budget of the project has been 3,967,268 € with a total EC contribution of 2,971,360 €.

NAFISPACK has been constituted by seventeen European beneficiaries coming from countries such as Spain, Italy, Sweden, Norway, Finland, Germany and Denmark. The consortium has been represented research organisations and industries. In concrete, 9 research organisations involving professionals in the areas of packaging, food and safety, and 8 industries involving 5 small and medium size enterprises and 3 large companies have taken part of the project:

1. Instituto Tecnológico del Embalaje, Transporte y Logística (ITENE)
2. Università degli Studi di Milano (UMIL)
3. Innventia AB
4. Consejo Superior de Investigaciones Científicas (CSIC)
5. Institutet för Livsmedel och Bioteknik AB (SIK)
6. Nofima AS
7. Technical Research Centre of Finland (VTT)
8. Universidad de Zaragoza (UNIZAR)
9. Verein zur Förderung des Technologietransfers and der Hochschule Bremerhaven e.V. (TTZ)
10. Danisco A/S (Large company)
11. Metalvuoto S.P.A. (SME)
12. Aragonesa de Tintas y Barnices S.A. (SME)
13. Envaflex S.A. (SME)
14. Nordisches Lachskontor GmbH (SME)
15. Verdifresh S.L. (Large company)
16. Tommen Gram Folier (SME)
17. Nutreco Servicios S.A. (Large company)

1.2. Summary description of project context and objectives (max 4 pages)

The industry needs effective technological solutions to preserve the quality and safety of the food products along the whole distribution chain. In this sense, NAFISPACK project aimed at providing advanced packaging solutions able to satisfy these industrial needs as well as satisfying the increasing demands of the consumer for fresher, minimally processed, more convenient, and safer foods.

As Figure 1 shows, NAFISPACK focused in all the packaging aspects within the supply chain:

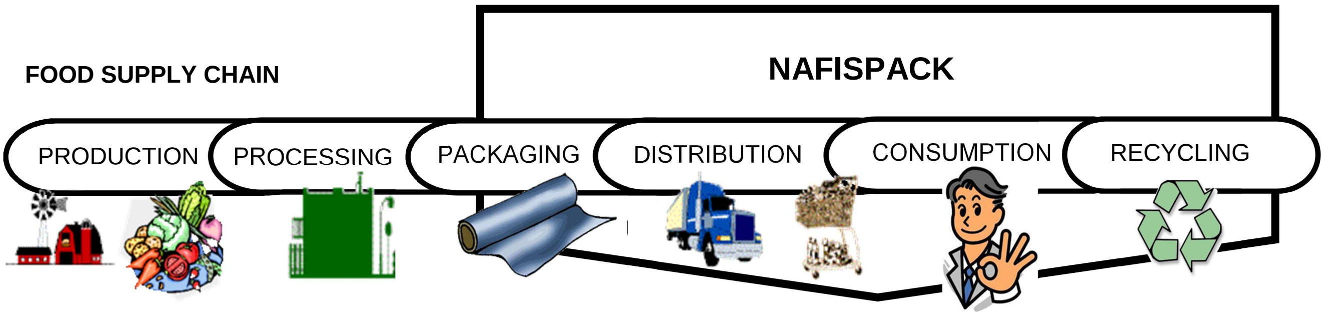


Figure 1. NAFISPACK in the supply chain.

NAFISPACK project looked at the development of innovative packaging solutions (active and intelligent) for some selected target foodstuffs: fresh chicken, minimally processed vegetables (MPVs) and fresh fish (salmon).

Food quality is related to the preservation of the fresh food products, being the action of microorganisms one of the main food spoilage mechanisms. This is the case of the target food products studied in NAFISPACK whose quality and safety remains acceptable just for some few days. NAFISPACK tried to increase the shelf life of these products by developing novel '**active packaging technologies**' based on natural antimicrobials. The use of natural antimicrobials is considered safer and better tolerable than the use of synthetic preservatives, because concern about the latter is steadily rising, due to a limited documentation on safety and tolerance. However, despite some natural preservatives have been already tested as food additives and ingredients, their use in the development of new packaging applications pose new parameters of evaluation and problems (stability, effectiveness, availability, costs, law compliance, etc.) that needed to be studied in addition to a severe risk assessment of them.

NAFISPACK was also focused on the detection or monitoring of the food quality and safety along the distribution chain by means of the development of '**intelligent packaging technologies**'. The development of new visual/measurable indicators sensible to substances indicating food quality and safety, produced during spoilage of the food, will offer more reliable information than the traditional "best before" or the "use by". In this sense, efforts were focused on developing systems able to monitor parameters related with the freshness of the most perishable target food product studied into the project; fresh salmon.

NAFISPACK offered a combined packaging solution based on active components releasing natural antimicrobials and based on intelligent functions monitoring the quality and safety state of the product, which it is a really innovative and safe solution to achieve a proper preservation of fresh products, prolong their short shelf life and assure their freshness along the supply chain.

Also, regarding scientific impacts of the project, it should be highlighted that after several years of European research focused on active and intelligent packaging materials, NAFISPACK was one of the first projects in which the active and intelligent materials developed were evaluated in terms of **chemical, toxicological and microbiological risks**. In this sense, new methodologies were developed that take into account exposure to chemical substances arising from the new materials, the toxicological exposure assessment for key potential hazards and validation of predictive models for behaviour of relevant hazards.

Furthermore, consumer demands and requirements (cost, safety, easy-to-use, recyclable, reuse, etc.) were taken into account in the development of the new packaging solutions and were evaluated as a way of unravel possibilities to improve the cost effectiveness and the consumer acceptance of the developed products. Also issues regarding **sustainability** of the developed products were evaluated through life cycle assessment approaches.

As Figure 2 shows, NAFISPACK merged all these key factors coming from the different actors within the supply chain (food and packaging industries, consumers and authorities). NAFISPACK is the result of the collaboration of R&D entities and industrial partners at European level that really contributes to joining efforts and releasing knowledge and technologies for the benefit of the European Community.

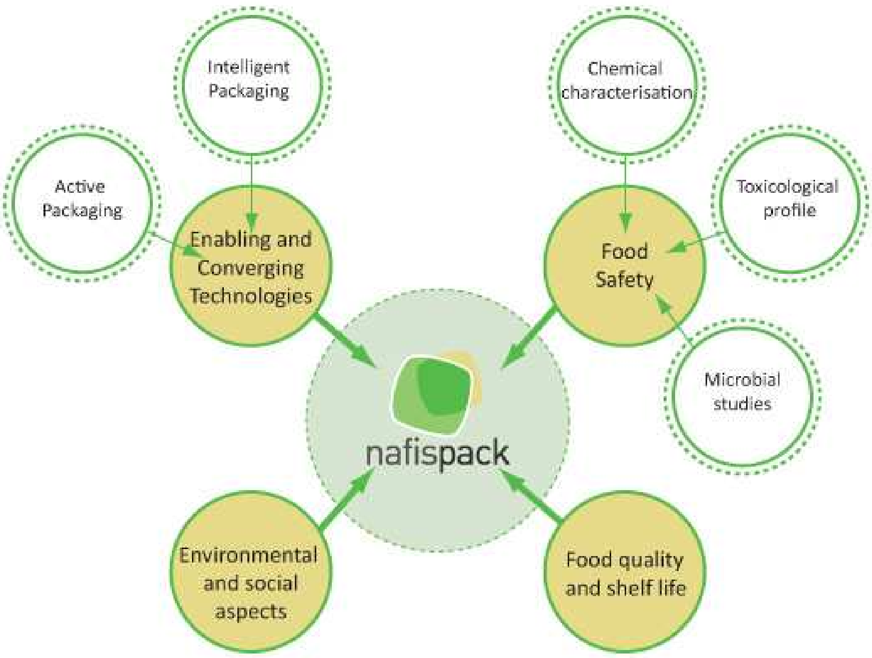


Figure 2. NAFISPACK concept.

NAFISPACK has two main objectives:

1. By one hand, the **development of active packaging systems** with (natural) antimicrobial properties to reduce the spoilage of the target foods and increase their shelf life **and** the **development of intelligent packaging solutions** to monitor the food quality and safety along the food chain. The target foods, fresh chicken, minimally processed vegetables (MPVs) and fresh fish (salmon) were selected because of their highly perishable nature and because their consumption is increasing within the European Union.
2. By the other hand, when developing novel packaging concepts that have never been used and are produced by new processes, there is the need to ensure the safety and benefits of such food packaging solutions. This issue was related to the second main objective of NAFISPACK, **the development of a safety assessment methodology** that includes the chemical characterisation and toxicological profile of intentionally and non-intentionally added substances present in these new packaging solutions which may migrate to the foodstuffs. Microbiological risk assessment was also included. Scientific results coming from this issue could be a source of information for the European reference body related to food safety, the EFSA, in order to evaluate active and intelligent packaging materials as the new Commission Regulation (EC) 450/2009 dealing with active and intelligent materials and articles intended to come into contact with food, stated in May 2009.

By the combination of both main objectives, the new packaging systems will satisfy European food safety policies, functional and marketing requirements, and will accomplish environmental, cost and consumer demands.

In order to fulfil these two global objectives, NAFISPACK has other more specific objectives related with each of the work packages (WP) included in the work plan:

1. Evaluation of suitability/feasibility of Natural Antimicrobials for food packaging applications (WP1). Identification of some natural antimicrobials agents (NAs) having the highest possibilities of being incorporated in packaging materials and being effective against the most common and dangerous target microorganisms which may be present in the target foods preliminarily chosen (fresh fish, chicken and minimally processed vegetables).
2. Development of effective antimicrobial food materials for package design (WP2). Development of polymeric materials which include the NAs using different processing strategies such as coating, extrusion and material functionalization. Characterisation of their mode of action and efficiency for the target food will be studied. Tailoring controlled release of active agents by use of novel technologies such as encapsulation and nanotechnologies.
3. Design and up scaling in pilot plants the materials previously developed to obtain a real food packaging systems (WP3). Additional advantages such as easy-to-open characteristics will be also aimed. This work will be performed together with industrial beneficiaries.
4. Provide risk assessments and new risk assessment models for new active and intelligent packaging solutions developed (microbiology, migration and toxicology) (WP4).

5. Evaluation of the effectiveness of the developed packaging solutions for providing a longer shelf-life by maintaining the chemical, physical and sensorial quality of the foods on a higher level than existing packages do (WP5).
6. Development of intelligent food packaging solutions (WP6). Tailored indicator materials that react to the presence of the quality indicating metabolites, hence providing rapid, low cost, package integrated ways to determine the quality and safety of the target food in consumer packages through the logistics chain from producer to consumer will be developed.
7. Obtain tools to assure recyclability of the new packaging solutions developed, and assess the risk versus the benefit for these materials to be used as recycled materials (WP7).

1.3. Description of the main S&T results/foregrounds (max 25 pages)

The delivery of safe food from the producer to the consumer requires meticulous monitoring at every stage in the supply chain, a concept now called “from farm to fork”. The “from farm to fork” philosophy underlines the fact that the quality and safety of food are mayor priorities for the industry and authorities. European research is focused on making food as safe and clean as possible. In this trend, packaging plays a crucial role since its most important functions are preservation and protection of food.

In the following pages are summarized the main S&T results obtained in the different Work Packages of NAFISPACK.

WP1

The overall aim of this WP was to assess preliminarily the suitability/feasibility of natural antimicrobial agents (NA) to be included in packaging materials. Thus, a preliminary goal of the project because it is clearly evident that the real and final selection derived from the tests carried out in the subsequent WPs, dealing with the final materials, the target foods selected and the storage conditions suggested also by the industrial partners.

Even if it cannot be considered a totally new idea “Antimicrobial packaging” is still innovative items and a tremendous effort has been made over the last decade to develop and test films with antimicrobial properties to improve food safety and extend shelf life. A review, appeared just before the NAFISPACK project started (Joerger RJ. 2007. Antimicrobial films for food applications: an analysis of quantitative results. Packaging Technol. Sci. 20:231–273), catalogued and analyzed the outcome of these research efforts. The bacteriocin nisin was the antimicrobial most commonly incorporated into films, followed by foodgrade acids and salts, chitosan, plant extracts, and the enzymes lysozyme and lactoperoxidase. One or two log reduction in microorganisms counts was always recorded and in many cases much stronger effects have been shown.

However, NAFISPACK Project was addressed specifically towards ***Natural Antimicrobials***, in order to lead to ***Safe and Novel Packaging*** and, therefore, accurate definitions of three key words of the project was firstly established, as a first achievement of the Project activities, through a deepened and detailed research into the scientific literature.

Antimicrobial: Generally, the term antimicrobial property includes antibiotics, antifungals, antiprotozoals and antivirals effects. In this project the attention will be focused on the antimicrobial effects against bacteria and/or fungi (yeasts and moulds) that are relevant only to the selected target foods. The antimicrobial effects are intended as the ability of killing microorganisms (lytic effect) and/or reversibly or irreversibly preventing their growth (bacteriostatic or fungostatic effect).

Natural: refers to pure substances or preparations, obtained from plants, microrganisms, or animals, either in the raw state or after processing (including drying, torrefaction, and traditional food-preparation processes), by appropriate physical (including distillation and solvent extraction), enzymatic, or microbiological processes (fermentation).

Innovative: with reference to NAFISPACK project, the term is referred to novel natural antimicrobials, never used before (therefore we exclude those already present on the market as, for example, ethanol emitters, mustard essence...), as well as on novel applications in packaging materials of already well known NAs.

The selection and the evaluation of the active substances has been addressed in the three specific NAs categories *(Peptide/Protein based substances, Volatile substances, Water and lipid soluble agents. Available as single molecule or as preparation/ mixture,…)*, accordingly to the target foods to be dealt with in the project, which were *Fresh Fish, Minimally Processed Vegetables* (MPV), and *Fresh Chicken*; actually, these 3 types of food have common and important features, as they show good market share, they are very perishable, and packaging is more important than processing for their shelf lives.

The results achieved at the end of the project by Workpackage 1
WP1 worked experimentally for about 2/3 of the entire project time, because its main goals were preparatory to the other WPs activities. The preliminary selection of natural antimicrobials was focused on **more than 50** natural substances known and available on the market, for which useful information about activity, form of availability, general characteristics, and costs were collected.

This work of documentation retrieval, led to a first, consistent result which is an accessible Database was all these information were recorded.

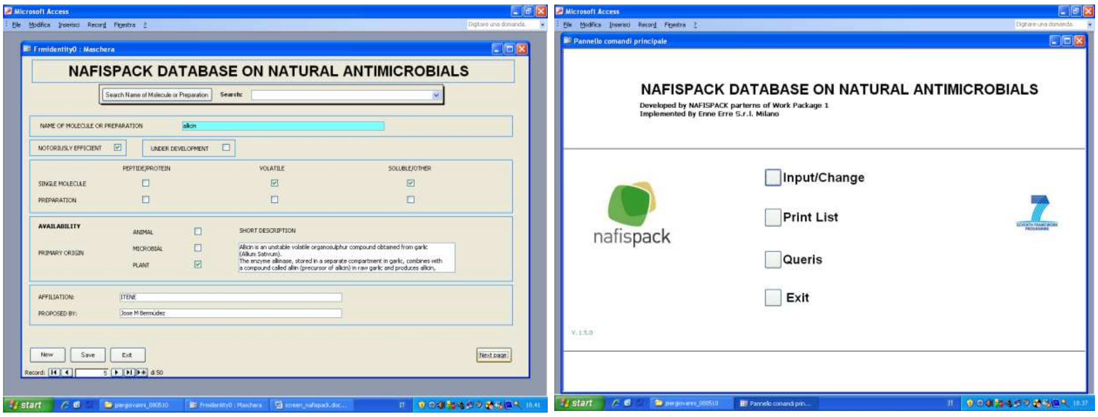


Figure 1 – Two pages of the Database developed. One feature of the Database is the implementation of some queries for the information retrieval that make the archive really useful for whoever interested in natural antimicrobials.

The some 50 NAs preliminarily selected were reduced to a shorter list in the subsequent steps of the project, according to reasons of effectiveness and availability:
About soluble NAs three substances were selected:
- Green tea; possible target foods: fresh fish, fresh chicken
- Aloe; possible target foods: fresh fish, fresh chicken
- LAE; possible target foods: fresh fish, fresh chicken

About protein/peptide NAs,the following three species were selected
- Lysozyme+Lactoferrine; possible target foods: fresh fish, fresh chicken
- Nisin A; possible target foods: fresh fish, fresh chicken

About volatile NAs: the final selection focused on three pure substances and two essential oils (E.O.):

- Citral (to be studied together with chitosan films); possible target foods: MPV, fresh fish
- Carvacrol; possible target foods: MPV, fresh fish
- Cinnamaldheyde; possible target foods: MPV, fresh fish, fresh chicken
- Oregano E.O. ; possible target foods: MPV, fresh fish, fresh chicken
- Cinnamon E.O. ; possible target foods: MPV, fresh fish, fresh chicken

After testing the antimicrobial activities (i.e. their minimal inhibitory concentration [MIC], minimum lethal concentration [MLC], and their kinetic growth) of these 10 selected NAs onto 21 yeasts and moulds and 25 Gram+ and Gram– bacteria, a final list of 6 natural antimicrobials, having high probability of becoming part of innovative and safe antimicrobial packaging materials were selected, as the next table show, representing a further consistent result of the project.

Final list of natural antimicrobials

Class of antimicrobial	Natural antimicrobial
WATER SOLUBLE	Ethyl Lauroyl Arginate (LAE)
PROTEIN/PEPTIDE	Lysozyme + Lactoferrine
VOLATILES	Citral
	Carvacrol
	Oregano essential oil
	Cinnamon essential oil

These natural antimicrobials, selected on the basis of availability and microbiological factors only, were also tested for their thermal and chemical stability with specific reference to the processes intended to be used for their implementation in packaging materials. This part of the project, actually, didn't change the original selection, because all the NAs overcame the tests performed, but permitted to achieve original and interesting technical results.

For instance, as far as thermal stability is concerned, the pure active substances tested, Cinnamaldehyde and Carvacrol, behaved differently in comparison with their original essential oils. In general they show, in fact, less thermal resistance, having the other EO constituents a protective role onto the antimicrobial molecules.

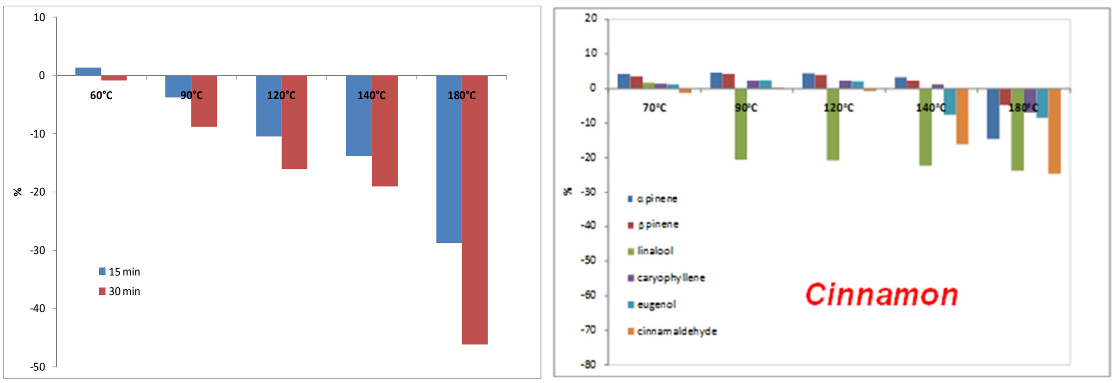


Figure 2 – Cinnamaldehyde reduction after heat treatment in the EO (left) and alone as pure substance (right).

Moreover, pure cinnamaldehyde undergoes to a time-temperature driven transformation to benzaldehyde, as also indicated by Friedman et al. (J.Agric.Food Chem, 2000, 48, 5702-5709). This is shown in Figure 3 in which the benzaldehyde appearance is indicated together with the reaction scheme of its formation. It is worth to underline that also benzadehyde has an antimicrobial effect that should be taken into account.

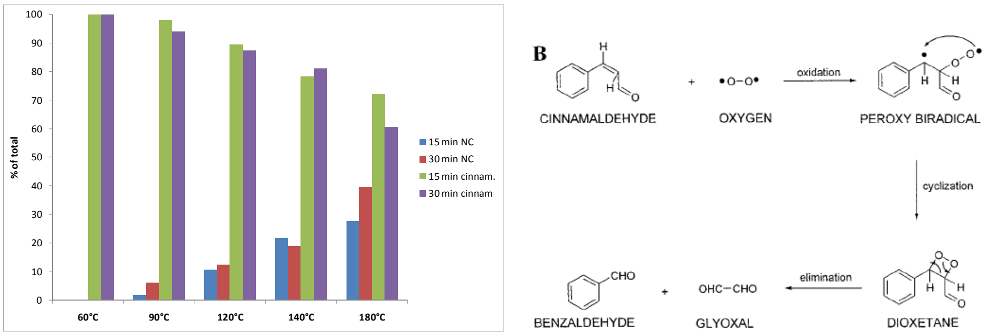


Figure 3 – Benzaldehyde appearance and cinnamaldheyde degradation after heat treatments (left) and the reaction scheme of its formation (right).

The chemical test conducted to establish the resistance of the natural antimicrobials to the different pH values also led to interesting and useful results showing different results, according to the difference NAs tested. For instance, with regard to carvacrol in oregano EO and cinnamaldehyde in cinnamon EO, it was evident that the lowest values of pH determine a larger degradation of the active principles. On the contrary, the analysis of citral with different pH ranges showed the degradation of citral in alkaline pH, and the change in ration of the two isomers, as Figure 4 shows.

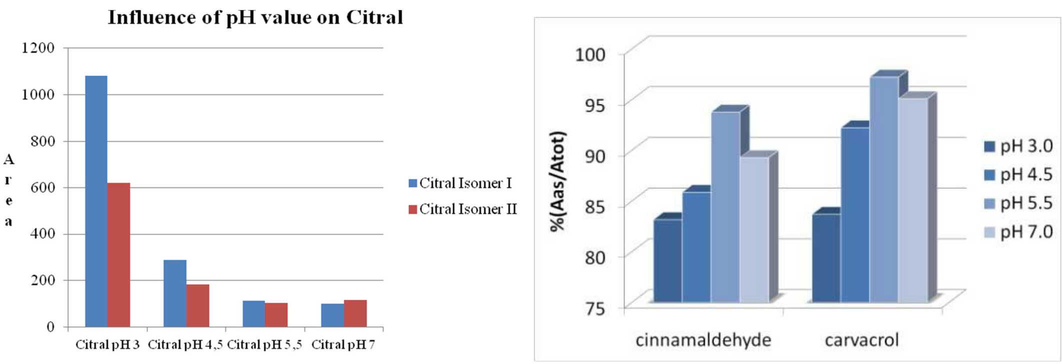


Figure 4 – Citral degradation after a treatment at high pH values (left) and Cinnamaldehyde and Carvacrol degradation after a low pH value treatment (right).

WP2

Active food contact materials are intended to extend the shelf life or to maintain or improve the condition of packaged food. They are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food. Polymers are appropriate materials for the development of active structures thanks to their mass transport characteristics – permeation, sorption and migration. The active components can be incorporated into the package walls by diverse procedures which include as solutes in polymer solutions or dispersions for coatings, as constituents which are melt-blended during plastic extrusion or by functionalization of package surface. From there, the active agent can be released into the food or headspace to make their beneficial action, can remove food or headspace components which are taken into the polymer matrix or act by food contact.

One of the main activities of the Nafispack project has been to develop polymeric active materials which contain the selected natural antimicrobial agents. Obviously, the developed materials should have got adequate antimicrobial activity to improve the protection of the food products and should provide the functional properties required for the stabilization of the diverse target products in terms of barrier characteristics, sealability, optical or thermal properties, among others, by themselves or in a suitable combination of them with conventional materials.

Three different strategies were planned to produce active materials, namely active polymeric coatings, active extruded polymer films and surface functionalization of polymeric films. Three types of antimicrobial agents were selected, volatile organic compounds from plant essential oils, lauroyl arginine ether (LAE) as a novel water soluble antimicrobial and polypeptide agents. A long list of polymeric materials was initially selected as potential matrices for NAFISPACK developments including conventional oil-derived polymers and biopolymers. First trials were indicative of several agent/polymer/process incompatible combinations as indicated in Figure 5.

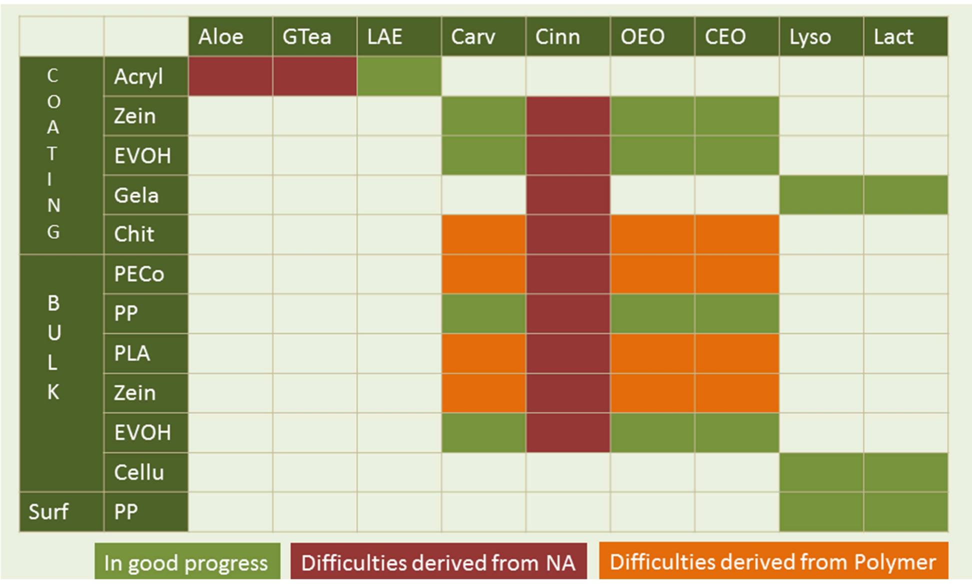


Figure 5 - Suitability of agent/polymer/process combinations based on processability/degradation/interactions issues.

Nevertheless, a suitable number of combinations were feasible and were attempted. Volatile antimicrobials which mode of action is their release from the packaging materials were incorporated on diverse polymers by both extrusion and coating processes. Peptidic agents were not valid for extrusion processes because of their thermal degradation but active materials by coating and by surface immobilization were developed. Water soluble agents could be added to acrylic coatings without difficulties. Therefore, there were a sufficient number of combinations as to propose suitable solutions for each specific target food products.

Throughout the project, the efforts were being refocused to those materials which were more promising from a technological point of view. Among the materials which were stopped but that presented attractive results, it should be mentioned the advances in surface immobilization of peptides onto plasma treated polypropylene films. This line of research presented very interesting scientific information such as the effect of atmosphere composition during plasma treatment, the effect of crosslinkers on peptide immobilization, the activity of immobilized peptides, etc. but the difficulties for up-scaling were insurmountable.

Also, very interesting results were obtained during the development of PP and PLA based extruded materials containing volatile antimicrobials. The composition of the matrix played a relevant role in the performance of the materials. Although both polymers presented an excellent capacity to incorporate the organic compounds, PLA materials showed a release kinetics so slow that resulted in ineffective materials. On the contrary, PP films presented a controlled release of the agents whose extend was fully characterized, and a subsequent measurable antimicrobial capacity, although not sufficient as to be selected for materials optimization and scale-up trials.

Very interesting were the results obtained in the development of active papers based on the incorporation of peptides. The direct addition of the peptides on the fibre paste was not sufficient to produce a suitable material and therefore the addition of diverse hydrocolloids to improve peptide retention and release into food was assessed. The final material containing a cellulose derivative produced excellent results although the difficulties to conform a packaging system for the target products avoided its final implementation.

Based on the antimicrobial activity of the films measured through in vitro tests and trials with real foods, on effects on sensory attributes of foods exposed to the films and on the feasibility of the developed materials to be adapted to the packaging needs of the target food products, seven materials were finally selected, characterized and optimized. LAE incorporated in an acrylic coating on a PP film, four antimicrobial volatile agents incorporated in an EVOH coating, two on PP and another two on PET, a coextruded PP/EVOH/PP structure with carvacrol incorporated in the central layer and a mixture of peptides in a gelatine coating on PA/PE films.

LAE, a novel antimicrobial was incorporated on the formulation of a stable, up-scalable and homogeneous coating. The development of this formulation needed to solve difficulties of agent solubility, sedimentation, coating adhesion failure, by proper selection of several additives like cosolvents, thixotropants, surfactants, etc. The final formulation presented excellent properties and good adherence to PET. The antimicrobial characteristics of the coated films were tested with very promising properties. Since the LAE is a non-volatile agent, the antimicrobial coating needs direct food contact to develop its activity; therefore, it is not applicable to fresh produce. The LAE containing coating has been characterized to prove the stability of LAE and the kinetics of release into aqueous food. The chemical and thermal stability of the antimicrobial agent has been tested to guaranty the stability of the final active material. Experimental conditions up to 120ºC for 1 hour were studied in the UNIZAR group, confirming the high stability of LAE as a pure compounds and as part of a coating applied onto a polymeric surface.

The release behaviour of the active polymer prepared with LAE as active agent was also studied, and needed the development of an improved analytical method that has a sensitivity of 9 ppb and can monitor the release kinetics. With this method, LAE was found to be released from the coating into aqueous food products by direct contact, that the release is diffusion controlled and that the coated polymer acts as a reservoir of the antimicrobial, able to supply the antimicrobial as required and when it is necessary. This LAE containing Acrylic coated PET films were tested as separators for chicken breast fillets.

Many reports have described the antimicrobial activity of diverse peptides included in protein or polysaccharide films obtained by casting although the main application of such active films is food coating and in many cases, these films loss their integrity when exposed to aqueous foods, being thus unsuitable for the application in packaging design. This line of research pursued the development of coatings based on gelatine which include antimicrobial peptides, lactoferrin and lysozyme.

A water-based coating solutions of gelatine with the antimicrobials was developed to coat plastic films using gravure printing equipment. Coated films have shown excellent optical characteristics (transparency and gloss) as well as good adhesion properties thanks to an in-line corona treatment, especially for PET and OPA. PE and PP films needed the use of a chemical primer to improve their performance.

The incorporation of protein antimicrobials was achieved successfully and trials carried out to test antimicrobial activity of the obtained films highlighted that the developed antimicrobial coatings have shown efficacy against the target antimicrobials (*Escherichia coli* and *Listeria innocua*, both lysozyme alone or combined 1/1 with lactoferrin). Tests also showed that the materials were active by direct contact and by diffusion from the coating into the agar media. PA/PE films were coated with gelatine containing lysozyme or lysozyme/lactoferrin and tested in contact with real food, salmon. All materials presented antimicrobial activity against the common microorganisms present in this product and therefore this development was selected for up-scaling.

The antimicrobial activity of several plant essential oils has been well documented on books and reviews and has been described as potential agents for the development of active packaging systems, although their inclusion in polymeric matrices by impregnation (immersion or wetting of films surface) was inefficient, their release from the matrix uncontrolled or their effect on the sensorial properties of the product due to the powerful aromatic properties of the agents unacceptable. Two research lines using extrusion and coating processing successfully incorporated these agents in the same polymeric material, ethylene-vinyl alcohol copolymer (EVOH).

By coating technologies, polymeric coatings including essential oils (or pure components) were developed which were stable in dry conditions (to ease the storability of the active films with minimum aging) but very active in humid conditions, that is, the activity is triggered by the presence of the food product. Another characteristic of the coatings is that they can be applied onto conventional films commonly use in the packaging of Nafispack target food products. EVOH presented similar properties: soluble in hydroalcoholic mixtures (fast drying) and insoluble in water (reduced interactions with aqueous food products), barrier characteristics highly dependent on humidity, and suitability for direct food contact. EVOH coatings with volatile antimicrobials: carvacrol, citral, oregano and cinnamon essential oils were obtained. Coated films were highly transparent, uncoloured, and presented a slight odour to the NA. Losses due to evaporation during film drying were ca. 20%. The coated films stored in dry conditions did not loss any relevant amount of the active agents. However, when exposed to humidity conditions, films presented a fast release of the agent which concentration was practically depleted in 24 hours. To reduce the development time, a mathematical model has been built to understand, describe and predict the release of volatile antimicrobials from hydrophilic coatings as a function of temperature and humidity. This model requires the input of mass transport parameters, mainly, equilibrium constants (solubility, S, or partition, K, coefficients) and kinetics constants (diffusion coefficients, D) which were fully characterized. Tests on antimicrobial properties of films containing carvacrol, citral and oregano and cinnamon essential oils on *Listeria innocua, Salmonella enterica and E. coli*. showed very interesting results in *in vitro* tests. To confirm the efficiency of the developed films test with real product was performed and the results were good but revealed a very relevant food matrix effect, losing part of their efficiency and the need of a more controlled release of the agents. In this line, the use of nanoclays to delay the release kinetics of volatile additives was considered appropriate. The effect of the incorporation of the bentonite in the EVOH film and in the EVOH coating applied to PP was studied and showed that the presence of the clay increases the retention of the agent during coating drying, increases the extent of the release and slows the diffusion in the matrix in a relevant way.

Based on the microbiological and sensory results obtained in tests with real products, two EVOH coated PP films were selected for the manufacture of salad bags, concretely those containing oregano essential oil and citral. Like this, two EVOH coated PET films containing citral and cinnamon essential oil were selected for the manufacture of tray lids for chicken products.

Volatile antimicrobials were also used in the development of active materials by extrusion and the manufacture of active PP/EVOH/PP containing carvacrol was fully developed. Carvacrol was successfully added into EVOH by extrusion. Losses by evaporation were reduced when multilayer PP/EVOH/PP was prepared thanks to the retention provided by the olefinic films. Figure 6 shows the thickness profile of the active multilayer structure. In dry conditions, the agent was greatly retained in the structure, while the presence of humidity (the food) fully developed the antimicrobial activity. This material not only presented attractive active characteristics but also high barrier properties to oxygen and water, fulfilling the requirements of salmon packaging. The incorporation of carvacrol in the EVOH layer did not produce any relevant modification of the permeation values. The slight deterioration of oxygen barrier observed in the active film can be a consequence of a plasticization caused by the agent, although the difference between materials is within the experimental error. With respect to the water vapour transmission, the inclusion of carvacrol appears to improve the barrier of the structure which might be caused by a decrease in polarity of the polymeric matrix. Nevertheless, both results are adequate for salmon packaging. The behaviour of the coextruded material as well as the effect of diverse variables on its activity has been studied theoretically, with the previous characterization of all mass transport characteristics.

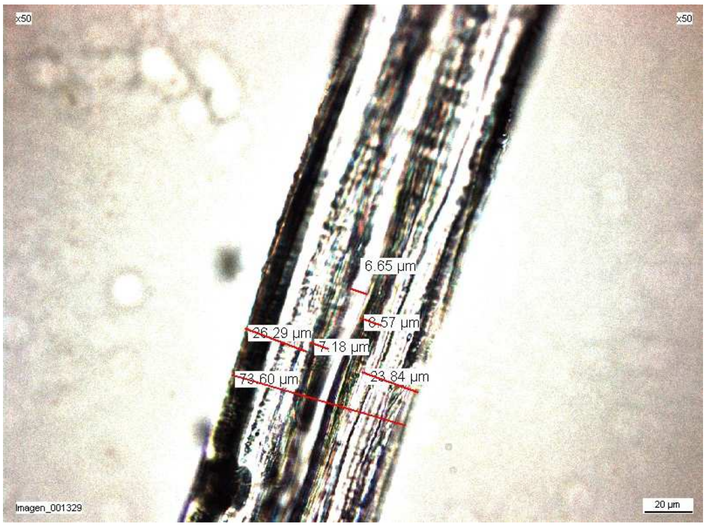


Figure 6 - Image showing the diverse layer thickness of the PP/tie/EVOH-active/tie/PP structure.

WP3

NAFISPACK also dealt with the up-scaling processes and the manufacturing performance of the new packaging materials. The objective was to make feasible the implementation of the new antimicrobial materials developed at laboratory scale to the industrial level. In this sense, the up-scaling processes introduce new variables and parameters in the production of these materials that could challenge the properties observed at laboratory scale.

A specific work package addressed the objective of designing and up-scaling the most promising packaging materials and processes needed for implement the new solutions at industrial level and aimed at evaluating if the selected antimicrobial materials were suitable for commercial production.

The up-scaled packaging materials have to be suitable to fulfil:

- Requirements imposed by the food products (barrier, optical…).
- Requirements imposed by the industrial processing machines of the packaging manufacturers.
- Requirements imposed by the industrial filling and sealing machines of the food producers.

The up-scaling processes introduced new variables and parameters in the production of the new materials that were different to the ones experienced at laboratory scale. So, this WP needed of the expertise and skills of the industrial partners since it was of great importance in defining requisites and target properties of the new materials to make possible their use in the actual equipments which cannot be very easily changed or ”re-designed”.

At laboratory scale, the development of the new packaging materials was carried out in small scale processing equipments (sometimes manually) which were perfect for the screening of the different material compositions since they are very versatile, easy to clean and to change. Laboratory processing machineries were perfect for making

”difficult” materials to good plastics with almost no material cost limits because of the small quantities needed. Results obtained at this stage were very valuable for developing very new and unknown materials and very valuable for the fundamental understanding of the behaviour of the new materials.

At industrial level, the large scale processing equipments that are obviously perfect for production introduce limitations in R&D activities. For instance, large equipments are very expensive to clean and have a low versatility since the performance is limited. In addition, working with them in R&D activities is difficult since machine time is valuable and can generate losses in production and money. However, the use of up-scaled machineries is very valuable for getting a new material on the market and as a ”proof” of their processing performance.

In NAFISPACK, the up-scaled materials were tried in conventional large scale processes like melting extrusion or coating processes. Processing windows were optimized in order to obtain antimicrobial materials with the ”appropriate” functions after manufacturing such as antimicrobial activity, adequate for overcoming filling, forming and sealing processes, economically viable and fulfil aspects such as legislation, stability, compatibility with food products, sustainability, etc.

Different antimicrobial packaging materials were experienced in this WP in function of the target food product:

Table 1. List of up-scaled materials for each target food product.

Food Product	Materials
Salmon	lysozyme + lactoferrin in gelatin coating on PA/PE carvacrol in coextruded multilayer material PP/EVOH/PP
Chicken	citral in EVOH coating on PET cinnamon essential oil in EVOH coating on PET LAE in acrylic coatings on PET
Minimally processed vegetables	citral in EVOH coating on PP oregano essential oil in EVOH coating on PP

Both monolayer and multilayer materials were planned to be manufactured. However, monolayer prototypes were found not to be appropriate to use as antimicrobial packages. It was found, that active monolayer packages were not suitable due to high losses of antimicrobial substances to the outerside of the packaging. The idea on using monolayer material was initially, that lower cost packages could be produced. However, the migration of the active antimicrobial substances was found to have a too high migration to be used in monolayer films. The “active function”, i.e. the migration of antimicrobial agents has no function on the outside of the packages and would thus be a gainless loss. Thus, a monolayer packages would lose antimicrobial agents to the outer side if not protected by an outer layer. Thus, all the up-scaled materials were based on multilayer structures as table 1 shows.

Several industrial partners were involved in the development of the up-scaled materials. Artibal sited in Spain, which is a producer of varnishes for packaging systems developed and produced acrylic coatings with LAE. Envaflex also sited in Spain, which is a printing and coating converter for packaging, performed coatings with EVOH on several substrates incorporating volatile antimicrobials. Metalvouto sited in Italy, which is a producer of barrier films for packaging, developed gelatine coatings incorporating lysozyme and lactoferrin.

In the process of up-scaling, the consortium experienced also trials in pilot scale equipments. These are machines placed somewhere in a middle stage between laboratory and industrial scales. These tests were perfect to use as an intermediate stage, specially, when the lab is too small to reflect up-scales, when the material is yet too uncertain to be run in a full scale industrial machine or when there is no possibility to run in industrial machines (e.g. because of continuous production regimes). Partners involved in the production of pilot scale materials were ITENE which developed co-extruded PP/EVOH/PP with carvacrol and VTT which produced coated gelatin materials containing lysozyme and lactoferrin. The following figure shows some pictures of the pilot lines used.

Figure 7. Pictures of the pilot plant equipments used in the project (coextruder line and coating one).

After developing the up-scaled materials, their performance in filling, forming and sealing activities were tested using the industrial packing lines available in the facilities of the food producers as Figure 8 shows.

Figure 8. Pictures of the packing test in the food producer facilities.

In the case of chicken, the up-scaled materials were used in the conventional production line of the producer (Nutreco sited in Lominchar, Toledo, Spain). The target food product was breast chicken meat which was packed in PET trays with outer dimensions of 252 x 182 x 30 mm and 1375 mL of capacity. The up-scaled active materials were used as lid for the trays. In this sense, the active films were successfully sealed to the trays and modified atmosphere (MAP) containing 20% oxygen, 70% carbon dioxide and 10% nitrogen was successfully applied.

In the case of salmon, the up-scaled materials were used in the conventional production line of the producer (Lachskontor sited in Bremerhaven, Germany). Fresh salmon fillets, with a weight of approximately 200 g each, were packed. The fillets were placed individually in the active pouches made with the up-scaled materials and sealed under vacuum.

Finally, in the case of the minimally processed vegetables, the up-scaled materials were used in the conventional production line of the producer (Verdifresh sited in Valencia, Spain). In this sense, a mixed salad (160-165 g cut iceberg lettuce, 52-58 g shredded carrots and 30-35 g shredded red cabbage) chosen as target food product was packed using a vertical form / fill / seal (VFFS) machine. The 3D packages for the salad were three sides sealed bags made with the active films. Modified atmosphere (MAP) containing 10-13% oxygen, 3.5-5% carbon dioxide and nitrogen was successfully applied.

As summary, activities within this WP have demonstrated the adequate performance of the new materials in the production line of both the packaging manufacturers and food producers. It has been a WP in which new findings and challenges were found, faced and solved thanks to the great exchange of knowledge and skills between the R&D partners and the industrial ones.

The new materials also overcome the distribution and transport from the food producer facilities to the partners involved in the characterisation of some of the food quality parameters. For instance, chicken was sent from Spain to Norway while salmon was sent from Germany to Sweden. In both cases, the 3D packages maintained their integrity and thus the integrity of the packed food products.

From the experience got into the project we could conclude that the new materials could be successfully implemented by industrial companies (both manufacturers and food producers).

WP4

NAFISPACK had a key objective on the evaluation of the food safety of the new developments. The risk assessment approach was based on characterizing both the exposure scenario and the hazard properties. In this sense, the WP dealing with the evaluation of the risks associated to the new developments focused on three pillars; the study of the chemical exposure due to migration phenomena, the toxicological characterization of the natural antimicrobials including studies on the bioavailability and cellular toxicity and the evaluation of the microbiological risks.

Packaging materials are not inert. This means that the packaging materials have distributed into their bulk mass several chemical substances that can be released from the packaging materials and be transferred to the outer media including the food product itself or its surrounding media. These chemical substances can be added during the manufacturing of the packaging materials including the production, transforming, converting and/or printing activities. Substances such as adhesives, plasticizers, UV filters, varnishes, or inks could be found in the food products.

In the case of the packaging materials developed in this project, the migration of the antimicrobial substances is something expected since the active packaging materials are designed in purpose to release chemical substances although in a gradual and controlled way. In addition, the inclusion of new substances in the manufacturing process of the packaging materials adds uncertainties regarding the possible generation of new chemical substances coming from degradation and other chemical reactions (commonly known as non-intentionally added substances or NIAS) that could be of concern in terms of food safety. In this sense, the required limit of detection for NIAS is as low as 10 parts per billion (micrograms per kilogram of food).

The groups of the University of Zaragoza and ITENE worked in the development of analytical methods and tools for the evaluation of the migration of both intentionally added and non-intentionally added substances coming from the different packaging materials developed.

In this sense, global and specific migration tests were carried out using the adequate food simulants (accordingly to the target food products) and experimental conditions (temperature and contact time assuring the worst foreseeable contact conditions). These testes were done in accordance with Directives 85/572/EEC and 82/711/CEE and the recent Regulation 10/2011. The following table summarizes the experimental migration conditions:

Table 2. Experimental conditions used in the migration tests of the final antimicrobial packages by type of food product

	Contact conditions		**Migration conditions**			
Food	**T (ºC)**	**t (days)**	**Simulant**	**T (ºC)**	**t (days)**	**Comments**
Salmon	0-2	4-7	Ethanol 10%	20	10	New simulant in Regulation 10/2011
			Isooctane	20	1	Surrogates of the fatty food simulant (rect. olive oil)
			Ethanol 95%	20	10	
Chicken	0-4	11-13	Ethanol 10%	20	10	Simulant in Regulation 10/2011
			Isooctane	20	1	Surrogates of the fatty food simulant (rect. olive oil)
			Ethanol 95%	20	10	
Salad	2-8	7-9	Ethanol 10%	20	10	New simulant in Regulation 10/2011

As main results coming from these tests;

- Overall or global migration was below the migration limits set by legislation (i.e. 10 mg/dm^2).
- Volatile antimicrobial substances like those coming from the packaging materials containing essential oils were found in at least one of the food simulants tested for each target food product. The packaging material based on PP/EVOH/PP structure containing carvacrol showed the highest migration values. All the active volatile substances included into the new packaging materials were initially included in the list of flavouring additives for food products with no applicable restrictions regarding content. However; nowadays this list is being evaluated by EFSA, so

some legal restriction values regarding maximum contents in food or food simulants might come into effect.

- In the case of non-volatile antimicrobials, specific migration tests were carried out for several active films containing LAE in their formulations. Only one simulant (water) was evaluated to represent the worst case scenario, as LAE is soluble in water. The migration values were 9 mg/Kg, well below the reference values so far given. So, a positive evaluation must be given to the studied films concerning their commercialization. In the case of materials containing lactoferin and lysozyme, according to the results obtained, no migration of lysozyme and lactoferrin is expected from the active materials developed in this study.

In the analysis of volatile NIAS a specific methodology was developed to determine volatiles at the very low detection levels required. In this sense, the methodology followed was based on the headspace solid phase microextraction (HS-SPME) of the volatiles directly from the new packaging materials. A great number of volatile substances were detected; chromatograms showed in some cases more than 100 peaks. This did not mean that all the peaks corresponded to NIAS. Just 4 NIAS of concern were identified at 5-10 ppb and only in the packaging materials incorporating citral. However, the chemical nature of these substances could not be satisfactorily determined and thus their possible origin.

In the case of non-volatile NIAS, migration into "standard" simulants was followed by UPLC_MS (QTOF) analysis to determine the identity and concentration of possible NIAS. Several chromatographic peaks were observed. The origin of most of them could be determined and linked to impurities coming from the antimicrobials and other substances used in the processing of the materials. Again in the case of materials containing citral, a couple of substances were identified as likely new substances generated by degradation of citral.

From a toxicological point of view, the ingestion of the foods packed with the materials containing lysozyme and lactoferrin, citral, LAE and cinnamon essential oil should not entail any associated risk, since, as the bioaccessibility studies conducted in the present project have shown, these compounds are not bioaccessible after digestion of the packed samples since little or no migration to the food was observed.

As for the foods packed with oregano essential oil, their major component carvacrol was detected both in the salad and in the salmon after packing and in the bioaccessible fractions of both foods at levels of 117 – 240 μg. The risk associated to these values could not be evaluated since no recommendations concerning the maximum acceptable intake of carvacrol have been made by any official organisation. Anyway, carvacrol had the lowest cytotoxicity of all the antimicrobial compounds studied in the project.

In relation to this, the order of cytotoxicity of the other antimicrobials was as follows: LAE (IC_{50} = 25 mg/L) > citral (IC_{50} = 80–90 mg/L) ≈ cinnamon essential oil (100 mg/L > IC_{50} > 50 mg/L) > oregano essential oil ($IC_{50} \geq$ 200 mg/L). Lactoferrin and lysozyme were not toxic for neither hepatocytes nor intestinal cell at the concentrations (up to 1000mg/L) studied. Figure 9 shows the cytotoxicity of oregano essential oil on hepatic and Caco-2 cells.

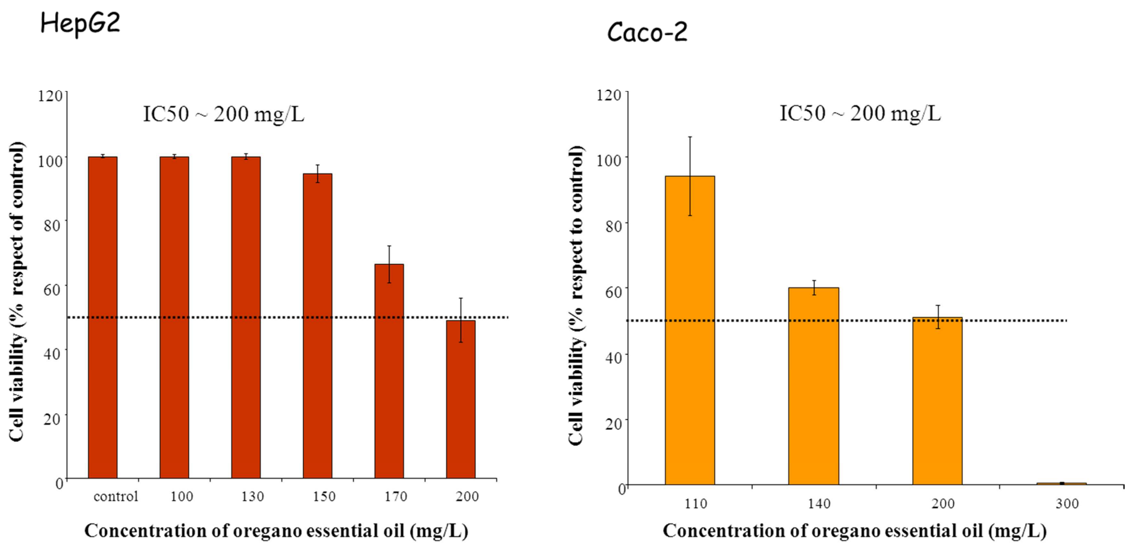


Figure 9. Cytotoxicity of oregano essential oil

LAE was the most cytotoxic compound although the bioaccessibility studies showed that LAE was not present in the fraction available for absorption.

From a microbiological point of view, the developed packaging solutions had low risk of selecting unbeneficial or resistant bacteria. In this sense, growth experiments were performed with *E.coli* and *Listeria innocua* treated at various temperatures and at various concentrations of antimicrobials such as citral, carvacrol, lactoferrin and lysozyme.

Results showed that no antimicrobial effect was found neither for lactoferrin nor lysozyme. However, both carvacrol and citral showed an in vitro antimicrobial effect on both microorganisms. Based on data from the *in vitro* bacterial growth in presence of antimicrobials, risk assessment methodology with risk characterization (such as Monte Carlo simulations), exposure assessment and effect of intervention studies were established. These tools can be used to assess risk reducing effects of antimicrobial packaging on human health. In addition, it could be seen that low temperatures in combination with antimicrobials addition to refrigerated ready-to-eat products would control *E.coli O157:H7* and *L. monocytogenes* surrogates growth (< 100cfu) during shelf life. This could be used as an intervention strategy.

As conclusion, the risks in using the antimicrobial packaging prototypes developed in the NAFISPACK project seem reasonably low. However, before going into commercial application the prototypes should be further tested in relation to current and new legislation.

WP5

The overall objective of this work package was to determine if the newly-developed packaging concepts introduced in other parts of the project were successful in prolonging the shelf-life of three selected products – mixed salad, fresh chicken fillets and fresh salmon fillets - by maintaining their quality from a chemical, physical, microbiological and sensorial viewpoint.

In order to fulfill this ambition it was essential to first select appropriate quality parameters of the chosen foods to be followed during storage for evaluating the quality of the packaged products.

Thereafter, the efficiency of a number of potential antimicrobial packaging materials was investigated by carrying out storage tests, with the objective to identify the most suitable materials for each individual foodstuff.

These materials were then sent for production of up-scaled packaging prototypes. Finally, the performance of the packaging prototypes were evaluated by carrying out storage tests with the three food products. The results from the studies of each of the three foods are dealt with separately under their heading below.

Salad

In the screening test it was observed that there was a reduction in microbial activity in several samples, especially at the beginning of the storage period. There were also indications that the sensory perception of some samples improved by storage in the new materials. A final selection of antimicrobial materials to be used for production of packaging prototypes was made based on the results from the screening tests. The chosen packaging materials were:

- 10% citral in EVOH coating on PP
- 7.5% oregano essential oil in EVOH coating on PP

The results from the final evaluation indicated that the new antimicrobial packages provided an antimicrobial effect in the mixed salad during the first day of storage. However, this effect could not be observed on or after day 4 of the storage period.

The amounts of the active antimicrobial volatiles, i.e. carvacrol and citral, present in the headspace of the packages decreased during storage. A probable explanation to the loss of antimicrobial activity is that the headspace concentration of the active substances dropped below a certain critical level.

Thus, in order to provide a prolonged antimicrobial effect, it is essential to maintain the concentration of the active substances in the packaging headspace throughout the storage. This could be obtained by a controlled release of the volatiles over time. A possible drawback of this approach could be sensory problems since it was observed that consumers preferred the smell and general acceptability of the reference sample after 1 day of storage, due to off-odours caused by the active components emanating from the packages at the time of opening the bags.

Chicken

In the screening studies there were no results that clearly pointed to a reduction of microbial growth in the samples. However, there were indications that the materials may have contributed with delaying the development of off-odours in the chicken. Based on these observations, a final selection of antimicrobial materials to be used for production of packaging prototypes was made. The chosen packaging materials were:

- 7.5% citral in EVOH coating on PP
- 7.5% cinnamon essential oil in EVOH coating on PP

In the final evaluation it was observed that these materials - especially the one containing citral - had an impact on the quality of the chicken in the early phase of the storage time. There was a delay in bacterial growth in the samples kept in the new materials compared to those stored in the conventional packaging. However, the antimicrobial material did not increase the shelf life of the chicken breast filets as the bacterial content were not significantly different between the samples at the end of the storage period. This implied that the volatile antimicrobials were predominantly released in the first days of the storage time and that the antimicrobial effect thereafter diminished.

The sensory evaluations did not reveal any effect of the antimicrobial materials in any of the evaluated attributes.

Samples kept in the new materials had significantly lower content of hexanal – a volatile substance that can be used as a marker for the oxidation processes in the product – after 14 days of storage.

In conclusion, it was difficult to establish a clear positive effect on the food quality from the antimicrobial materials. The results indicated that exposure to citral may provide a delay in bacterial growth, resulting in higher quality of the chicken in the first part of the storage time, but it did not generate an increased shelf life of the chicken fillets under the current conditions.

Salmon

Three separate screening experiments were carried out and the results from these were somewhat contradictory. At first there were indications that a few of the materials exhibited an antimicrobial effect. However, this was not as clear when the study was repeated. That was the reason that some of the materials were investigated in a third and then there were again some observations that suggested that there was some inhibition of the microbial growth. Based on the collected results from all three studies, a final selection of antimicrobial materials to be used for production of packaging prototypes was made. The chosen packaging materials were:

- 3% lysozyme + 3% lactoferrin in gelatin coating on PA/PE
- 4.8% carvacrol in extruded PP/EVOH/PP

In the final evaluation it was concluded that the main effect of the new packaging materials were of a chemical nature. It was evident that the new materials inhibited the production of unwanted volatile substances during the latter part of the storage period. However, this could not be directly correlated to a reduced microbial activity since no such was apparent. Also, the lower levels of degradation products did not have any effect on the sensory attributes. It could have been expected that the larger concentration of volatile metabolites in the reference sample would result in a lower score for this sample, especially regarding smell, but this could not be observed.

As general remarks, the results from the final evaluations with real food products were not conclusive. No material had a positive effect on all the investigated quality parameters that lasted for the entire storage period. However, some improvements regarding chemical and sensory as well as microbiological quality could be observed along the line. Similar results were observed in the different trials carried out during the project. The results suggested that the materials have potential to maintain food quality during storage, thereby prolonging shelf life, but they still need further development.

WP6

Another objective in Nafispack was to create a package integrated system indicating that the quality of a selected packaged food product has been maintained throughout the distribution chain. Thus, a novel colorimetric food quality indicator for monitoring fish freshness was produced in Work package 6.

The changes taking place during food spoilage can be attributable to microbiological growth and metabolism causing pH-changes, formation of toxic compounds, off-odours, and/or formation of gas and slime, or to oxidation of lipids and pigments resulting in undesirable flavours, formation of compounds with adverse biological reactions or discoloration. The formation of the different metabolites depends on the nature of the packaged food product, spoilage flora, the type of packaging and storage conditions. It was decided that the freshness indicator in Nafispack project targets for packed salmon (*Salmo salar*), taking into account, different kinds of packaging solutions.

When fish spoils a number of volatile substances are released into the headspace of the packed fish. On the basis of the study in Nafispack chemical indicators of salmon spoilage were in particular acetaldehyde, ethanol, 3- hydroxy-2-butanone (acetoin) and isopentyl alcohol. Some sulphurous or nitrous substances were expected to be appropriate for detecting chemical spoilage. However, they could not be detected in the used experimental setup. The indicator was designed to detect aldehydes and ketones that were identified as spoilage products for the target food product, salmon.

In order to find visible indication reactions for aldehydes and ketones, especially acetaldehyde and acetoin literature searches were carried out. The potential indicator reactions were judged with respect to several criteria: visible colour change, safety of reagents, simplicity of procedure e.g. one-step reaction, reaction conditions, feasibility in indicator structures, possibility to incorporate in printing ink carrier. The most promising reaction principle for the indicator was the detection of aldehydes and ketones using hydroxylamine hydrochloride. Release of hydrochloride gives colour change of a pH dye. The chemical detection reaction is not used in food quality indicator patents so far. It was found that the basic reagents are harmful substances and therefore a lot of effort was directed to finding alternative ingredients. Finally reagents that would be perfect for a food packaging indicator were found. The main ingredients are the reagent compound and a pH-dye, and the composition can be tuned as for the sensitivity.

Intelligent packaging systems are generally attached as labels, incorporated into a food packaging material, or printed onto a food packaging material. For a low cost indicator, printing has been identified as the preferable application technique. The reagents for the reaction were incorporated into a printable system containing solvent, binder and additives. It was seen that a number of additives are favorable for the indicator function in respect to e.g. colour stabilization and clear and rapid detection. White filter papers as carrier material helped detection of the colour change. In the first phase the indicator was designed in sticker form to be easily attached on the inner side of the package.

The indicators prepared were attached in modified atmosphere packages of salmon, which were stored in chilled conditions. The indicator responded to fish spoilage through visible colour change from yellow to orange-red (first version) and from colourless to red (second version) depending on choice of pH-dye.

starting point	***6 days***

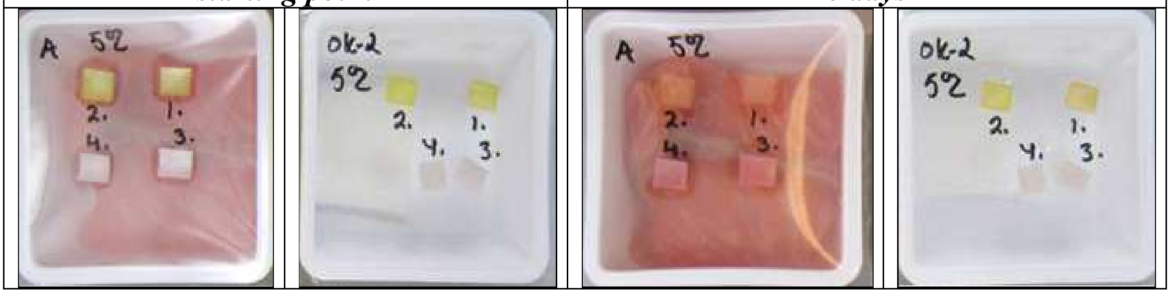


Figure 10 - Two indicator versions with two pH dyes in preliminary fish storage test +5°C.

The indicator colour changes expressed as ΔE values are illustrated in figure 2 as a function of time in a chilled temperature trial.

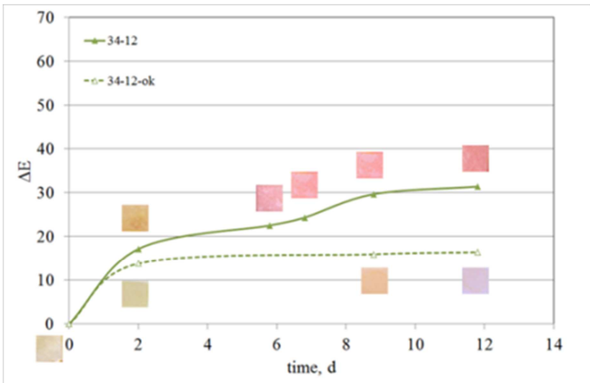


Figure 11. Example of indicator colour change in fish packages compared to emty packages at chilled temperature.

The performance of two developed indicator versions was studied in an extensive storage test with relevant fish quality analyses. The fish used in the validation study was Atlantic salmon (*Salmo salar* L).

Figure 12 - Fish packed in Nofima in trays with two types of indicators.

The packaging was performed within 36 hours after slaughtering. The salmon filets were cut into pieces of 430g and packed in white 1200 ml trays consisted of high density polyethylene, resulting in gas/product ratio of 2:1. The indicators were placed on the wall inside the trays. The trays were sealed with a top web consisting of polyethylene terephthalate multilayer barrier film by using a tray sealing machine. A pre-mixture of 60% CO_2 and 40% N_2 was used as the modified atmosphere.

The packaging was carried out at Nofima in Norway. Half of the samples were then transported refrigerated to SIK (Gothenburg, Sweden).

The samples were stored in dark at 4˚C until subjected to analyses. The following analyses were carried out:

- Gas composition
- Photography
- Colour measurement with Minolta
- Microbiological analyses
- Chemical analysis of volatile components.

The sensory evaluation aimed at identifying the sensory quality characteristics (appearance, odour, texture by hand) of fresh salmon packed in modified atmosphere during a period of two weeks. The aim of the chemical analysis was to analyse the presence of selected volatile substances in headspace of fresh salmon packages during a storage period of 15 days. The analysis was carried out at SIK.

The fresh salmon fillet samples in HDPE trays were stored in the dark at 4°C for 15 days. Initially, there were no detectable amounts of acetoin present in the headspace of the packages. This substance appeared between day 7 and day 12. During the latter part of the storage period the acetoin concentrations decreased, possibly due to further degradation. Acetaldehyde was observed in amounts close to the odour threshold of this substance already on day 1. A sharp increase of the acetaldehyde levels was observed during the last few days of storage.

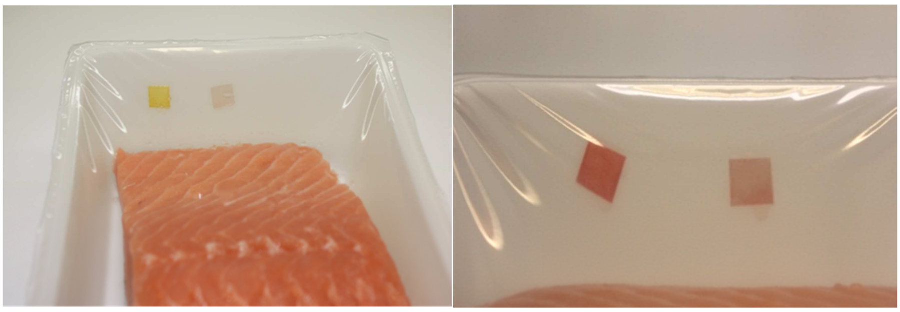


Figure 13. Salmon sample with indicator at point of packaging and when expired

Microbiological analyses were performed in Nofima on the salmon filets at the time of packaging and at six times during the period of 16 days.

The total viable counts in salmon filets increased from 3.40 log cfu/g at the time of packaging to > 8 log cfu/g at the end of storage. Relatively high number (7 log cfu/g) was reached after 11-12 days of storage. The total viable counts performed on Iron agar and Long&Hammer agar (and count of Photobacterium) were approximately the same regardless of the method (agar) used. The growth of Lactic acid bacteria (LAB) followed the same pattern as Total viable count (TVC), but somewhat lower content during storage, 2.30±0.0 at time of packaging to 7.99 ± 0.06 at end of storage. The results indicate that LAB constituted the major part of the total bacterial count in the salmon filets. The growth of hydrogen sulfide producing bacteria and yeast were, as expected, slower compared to TVC and LAB. The content of H_2S producing bacteria increased from 2.30±0.0 log cfu/g at the time of packaging and reached a relatively high number (> 5 log cfu/g) after appr. 14 days of storage, followed by a steadily increase to 16 days of storage. Yeasts were found to increase during storage from about 2.60 log cfu/g, to 4.36± 0.56 after 16 days of storage.

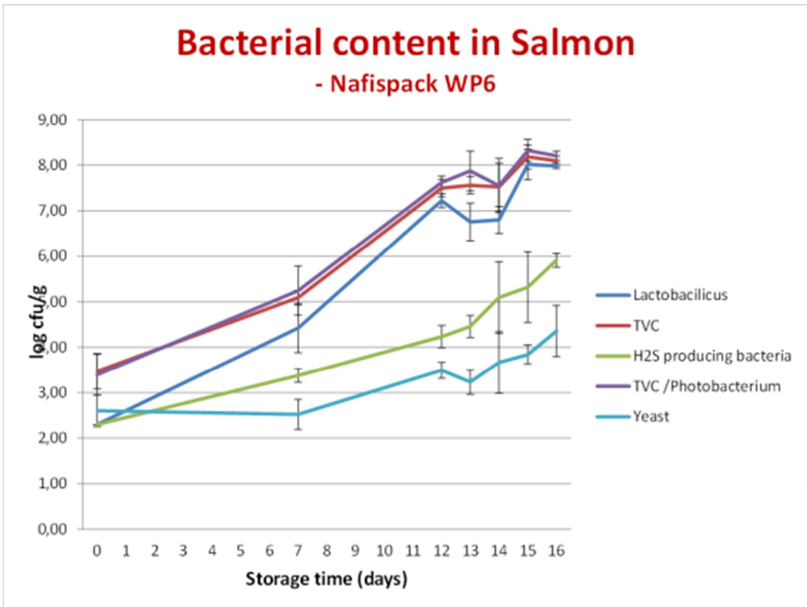


Figure 14 - Bacterial content in Salmon

In Figure 6 the measured ΔE values of two versions of indicators in fish packages and reference packages are presented as function of time. The colour measurements turned out to give results that were not as clear as expected. The reason might be that the method as adapted to the samples was not sensitive and repeatable enough. The colour change was visible in the photographs but was not distinctly observable in the Minolta measurements.

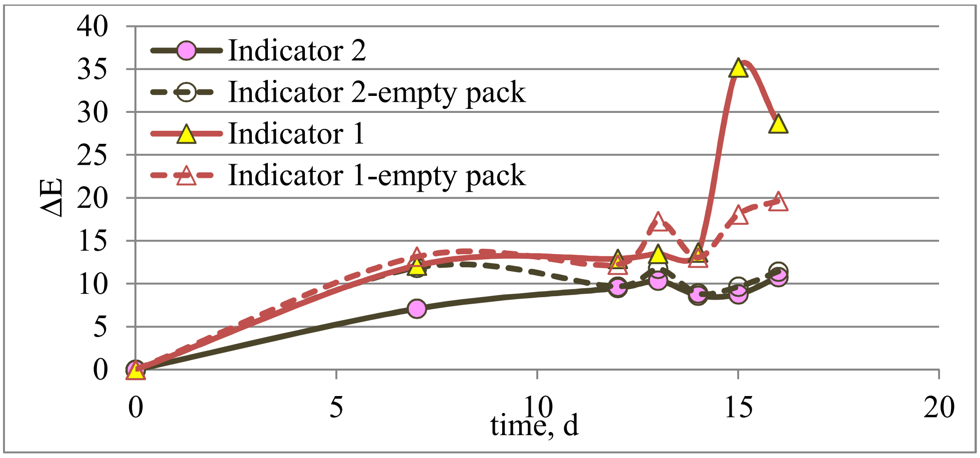


Figure 15 - ΔE values of two indicator types in fish packages and reference packages presented as function of time.

The performance of the two indicators in the storage test was analysed using principal-component analysis PCA. In order to visualize the obtained results PCA was carried out on colour change and microbiological test results. The results were then analysed observing the sensory and chemical test results. In figure 16 are included information on colour changes visible by the human eye. The points marked by a pink oval represent indicator samples that have presented a colour change while those marked by a blue oval are unchanged.

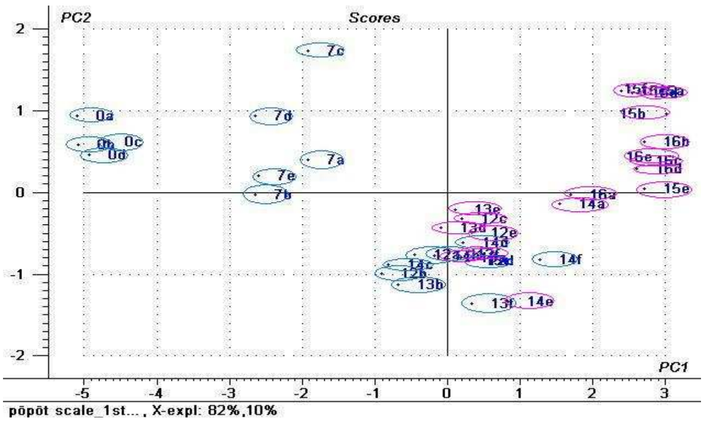


Figure 16 - Principal component score plot showing the observations in the fish packages with microbiological methods and colour measurements (visual and instrumental). Expired (pink) and non-expired (blue) indicators are marked

According to Figure 16 the expired samples are located to the right side and non-expired at the left side of a certain limit line. Only a small number of samples are incorrectly located. Samples on the limit line are of the age of 12 to 14 days.

The sensory study on the odour, texture and appearance shows that the quality of the salmon in packages was good on Day 7 but deteriorated on Day 12. Likewise the results of the chemical compounds acetaldehyde and acetoin start to increase after Day 7. In fact the validation test failed to give exact information of the critical days during the period of Day 7 to Day 12.

Performance testing simulating supply chains were carried out by Itene in order to check if the colour change of the indicators is well related with the freshness degree of packaged fresh salmon. Two different supply chains were simulated and two different temperature profiles were tested. One supply chain had a constant temperature. This temperature was set at 4º C, the other one showed breaks in the temperature. This experiment showed an irregular behaviour for the reason that the experiment set-up seems to have boosted the build-up of moisture on the indicators inside the trays which may have affected the sensitivity of the indicating compound and may have affected the reaction. Indicators inside the package changed it colour during the trial but no relation with the freshness degree of packaged salmon could be found in this exercise.

The conclusion is that the spoilage as measured by the microbiological, sensory and chemical methods was detected by the indicators, but possibly only when the spoilage had proceeded to a rather late phase. The validation suggests that the indicator formulation should be adjusted to a slightly more sensitive mode and the moisture resistance should be improved. The indicator can after some modification be applied in consumer package systems indicating that the quality of the selected packaged food product has been maintained throughout the distribution chain.

The selected chemical reaction is known and used for qualitative analysis of aldehydes and ketones but has not been used in food quality indicators so far. A reason for this is the toxicity of the conventional reagents. In this project the use of the particular safe ingredients and the processes of transferring the reaction from liquid phase into a solid phase spot detecting minor amounts of analyte is new and enables the use of indicator in food packaging application. A process to protect the innovative aspects has been initiated by VTT.

The work has been carried out in cooperation of Nofima, SIK, ITENE and VTT together with Artibal, Lachskontor and Nutreco.

WP7

WP7 aimed at the sustainability evaluation of the new packaging developments considering a life cycle thinking approach. A special consideration was made to the end of life and waste management of the new packaging development. According to this objective, a life cycle study considering environmental (Life Cycle Assessment), economic (Life Cycle Costing) and social aspects was carried out. The consumer requirements (cost, safety, easyness-to-use, recyclable, reuse, etc.) were identified and considered in new packaging developments.

Life cycle analysis (LCA) takes a systems approach to evaluating the potential environmental impact of a particular product, process, or activity from “cradle to grave.” By taking a “snapshot” of the entire life cycle of a product from extraction and processing of raw materials through final disposal. LCA is used to assess systematically the impact of each component process. Ideally, a LCA include four separate but interrelated components: goal and scope definition, an inventory analysis, an impact analysis that address both ecological and human health impacts, and the interpretation of results aimed at an improvement analysis to reduce or mitigate the environmental impact throughout the whole life cycle of the product. Life Cycle Costing (LCC) uses a parallel approach than the LCA but considering economic data.

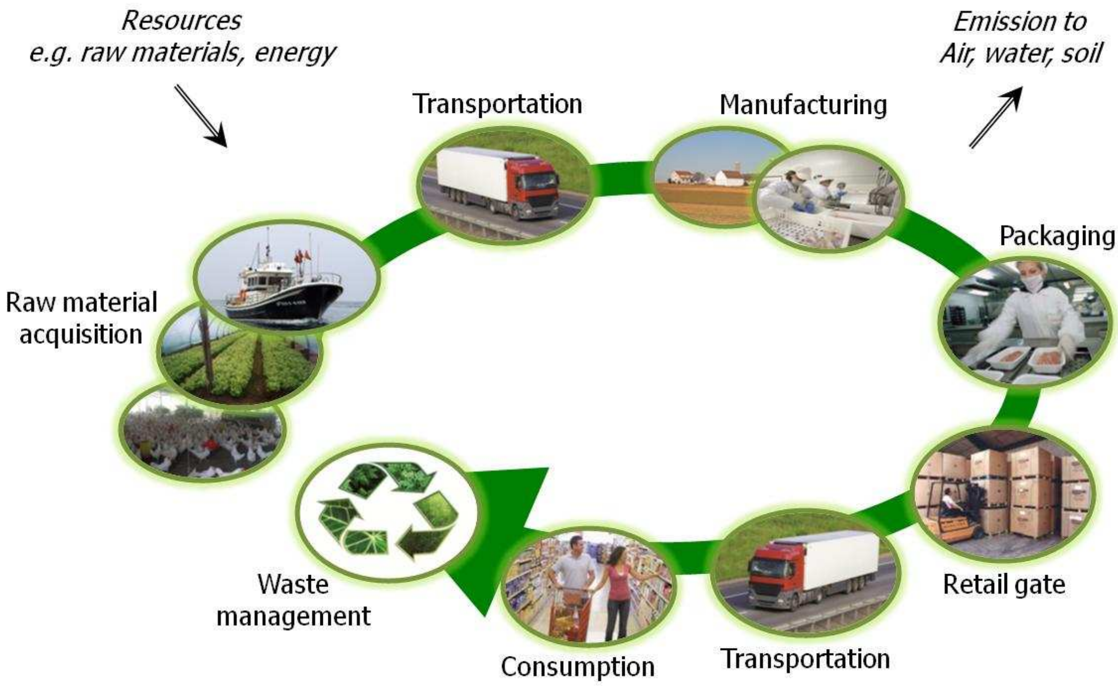


Figure 17 - Life Cycle Approach

In order to see the advantage of the new NAFISPACK packaging resulting from increased shelf-life, it was necessary to focus on food wastes and more specifically on the reduction of the food waste amount using novel packaging material in comparison to conventional packaging material (Wikström & Williams, 2010). Considering the current food loss for chicken breast, mixed salad and salmon filets, it was possible to build up realistic scenario for food waste reduction. Although the food waste minimization appeared low in some cases, it is not negligible from view of the industry: Food specialists estimate that a 2% decrease of food loss would be a significant achievement. Increased shelf life also represents more energy consumption for the storage. This aspect was integrated in the model. The breakeven point of how many more packaging are needed to have the same energy consumption was investigated.

The study of the sustainability of the NAFISPACK packaging developments in terms of LCA can be summarized as follows:

- For chicken breast, the new packaging is not bringing any improvement in term of environmental impacts studied and a break-even point between the current packaging and the improvements in term of energy demand cannot be reached.
- For salmon filet, the packaging coated with gelatin-lysozyme-lactoferrine present a decrease of environmental impact in the case of a total decrease of food loss (no food loss): break-even point between the energy demand of the current packaging and of this NAFISPACK packaging is reached if the food loss can be decreased by 52%. The new co-extruded packaging is increasing the environmental impacts studied and a break-even between the current packaging and the improvements in term of energy demand cannot be reached.

- For mixed salad, the new packaging is increasing the environmental impacts studied and a break-even point between the current packaging and the improvements in term of energy demand cannot be reached.

As in the Life Cycle Assessment, the main target of the Life Cycle Costing study was to assess and compare costs associated to the life cycle of the selected functional units, which include packaging and food products production, packing, transports, retailer and end-of-life:

- NAFISPACK packaging contributed to increase the costs along the life cycle of the food products due to high cost of the innovative materials. However the effect of the economies of scale in a near future could lead to a decrease in the cost of innovative antimicrobial packaging materials. The increment is partially compensated by the increasing in shelf life of products and consequent reduction in waste production at retailer.
- Food production represents the main contributor to the LCC of Nafispack packaging systems. Primary packaging is the LCC constituent with the least impact beyond logistics, except for mixed salad in which low product cost, lead to an outstanding contribution of packaging.

In order to achieve a better knowledge of the NAFISPACK packaging from an economic point of view, the possible break-even points in which the waste reduction at retailer level would make the total new packaging cost cheaper compared with the current packaging was determined.

- Break-even point can be reached in salmon fillets by educating consumers and adopting sustainable habits in the day-to-day routine.
- Break-even point in chicken breasts is impossible to reach since the maximum waste reduction is very small in comparison with the current waste production at retailer level.
- Break-even point in mixed salads is impossible to reach. Further research is needed to be able to substitute materials, technologies or take advantage of economies of scale.

The end-of-life available techniques and processes for the new Nafispack packaging material were investigated. Although no industrial recycling of multilayer packaging exists currently, new potential and future technologies to recycle multilayer NAFISPACK packaging materials were evaluated.

The consumer acceptance of the innovative NAFISPACK packaging was compared to the current packaging. To ascertain survey of the acceptance of European consumers (in the North and South of the EU), the consumer acceptance study was performed in Germany and in Spain.

The innovative NAFISPACK salad packaging with citral and oregano essential oil as well as the current salad packaging were evaluated as satisfying to good by the German and Spanish consumers. Nevertheless one attribute was critical:

- The smell inside the packaging had been seen as critical by the packaging developers/producers due to the use of essential oils that are indeed efficient antimicrobials but possess strong odour. It is therefore very interesting to see that the smell inside the salad packaging with citral and oregano was evaluated better as the smell inside the current packaging. In particular the German consumers considered the citral smell inside the packaging with citral as enjoyable fresh smell. The Spanish consumers preferred the smell inside the salad packaging with oregano, most probably due to a wide spread use of oregano in the Spanish cuisine.

The innovative NAFISPACK chicken packaging with citral and cinnamon as well as the current chicken packaging were evaluated as good packaging by the German and Spanish consumers. Nevertheless one attribute was critical:

- The smell inside had been seen as critical by the packaging developers/producers due to the use of essential oils that are indeed efficient antimicrobials but possess strong odour. The German as well as the Spanish consumers evaluated the smell inside the chicken packaging with citral similarly to the smell inside the current chicken packaging. The smell inside the packaging with cinnamon was considered by the consumers in Germany and Spain as bad/not satisfying.

The innovative NAFISPACK salmon packaging with lysozyme-lactoferrine as well as the current salmon packaging were evaluated as satisfying to good by the German and Spanish consumers. The innovative NAFISPACK co-extruded salmon packaging with carvacrol was evaluated as bad/not satisfying especially for the attributes smell outside, opening and smell inside. The consumers disliked the very intensive and aggressive smell of the carvacrol.

German and Spanish consumers would rather accept intelligent packaging than active packaging. The consumers are afraid of possible effect of active packaging on the human body, on health and on the taste of food. The consumers perceive active packaging as not natural and wish more information about this type of packaging (information about active and intelligent packaging is very important to 95% of the German consumers and to 63% of the Spanish consumers). Nevertheless the German and Spanish consumers agree that the food quality will be improved by the use of innovative packaging.

Approximately 30% of the consumers are willing to pay more money for food packed in an active packaging and approximately 70% of the consumers would be ready spend more money for food packed in an intelligent packaging.

As conclusion, the European consumers are ready to accept active and intelligent packaging such as the Nafispack innovative and safe packaging with natural antimicrobials. Nevertheless the food industry has to consider that the active compounds used possess only little flavor and that the flavor type pairs to the food product packed within the packaging and the cultural background of the country the food product is sold in. Oregano for example is more accepted in Spain than in Germany, most probably due to extensive use of oregano in Mediterranean countries as opposition to Germany.

1.4. Potential impact and main dissemination activities and exploitation of results (max 10 pages)

Potential impact

NAFISPACK has delivered scientific and technological knowledge in several areas related with the packaging systems aiming at extending the quality and safety of the food products. The results and products obtained along the project could have a potential impact at industrial, commercial, environmental and economic levels.

One of the main objectives of the project was the development of active packaging solutions based on the incorporation of natural antimicrobials into/onto packaging materials. Today, there is a clear trend towards natural food protection solutions since aspects such as clean labelling, healthy or organic food concepts are demanded increasingly by the consumers. Natural solutions account for 25% of the global food protectant market which accounts for 1 billion euros. A clear reflect of the growing demands is the annual growth rate of natural solutions (6-8%) which is around twice that of the food protection sector in total.

In addition, consumers are also concerned about the role of packaging. Consumers want to be assured that the packaging is fulfilling its function of protecting the quality, freshness and safety of the foods. The development of advanced packaging materials with new properties and characteristics aiming at promoting the active interaction between food and packages generates a new concept of food protection.

NAFISPACK is in alignment with consumer demands by giving technological solutions able to release natural preservatives from the packaging to the foods in a controlled way. This means that the active packaging works in a coordinated way in function of the packed food and the conditions around the packed good. This solution optimizes the amount of preservatives in the food product assuring at the same time a high level of preservation and protection. This benefit of reduction of food additives in direct contact with the food products is something that many consumers would consider as a step in the right direction.

Consumer demands are the reason of developing new packaging concepts. But, in addition to consumers, the results of the project will impact directly in the European packaging and food sectors. The innovative packaging solutions developed in NAFISPACK opens new commercial and business possibilities that can guarantee the leadership of the European industries in both sectors in the global market.

Packaging manufacturers and natural antimicrobial suppliers are the key actors involved in the production of the new packaging concepts. The project offers know-how since it improves the knowledge on antimicrobial additives from natural origin and also improves the knowledge on antimicrobial systems and materials for food applications. Even more, NAFISPACK did not only focused on the processes to develop the new materials and its implementation at industrial level but also in demonstrating the active features and effectiveness, the safety, the environmental impact, the waste management, the costs and the consumer acceptance of the new materials. All of these issues are of great importance in decisions related with launching a new solution into the market.

Downstream, the food producers will apply the new solutions with their food products. NAFISPACK has focused in three target food products; fresh chicken meat, salmon and mixed cut salad. The effectiveness of the packaging materials in maintaining the quality and safety of the food products for a longer shelf life will impact on their commercial possibilities.

Packaging materials that have the ability to maintain food quality during storage – be it from a chemical, sensory or microbiological perspective – can have a great impact on both economical and also environmental issues. A shelf life extension can result in reduced wastage and that a larger proportion of all the food that is produced is also consumed at some stage. In recent years it has been reported in many investigations throughout the world that food waste is an enormous problem that has both economical and environmental ramifications. In relation to this, the sustainability of the developed products was evaluated through inventory analysis, impact analysis that address both ecological and human health impacts, as well as social, cultural and economic impacts and improvement analysis to reduce or mitigate the environmental impact throughout the whole life cycle of a product, process, or activity. Consumer requirements (cost, safety, easy-to-use, recyclable, reuse, etc) were identified and considered in the new packaging developments. This unraveled possibility to improve the cost effectiveness and the consumer acceptance of the developed products. Moreover it provided an overall approach on what is going to happen once the packaging is in the market.

The new packaging materials have demonstrated to be compatible with the actual packaging technologies used with the target food products such as modified and controlled atmosphere or vacuum treatments. The combination of preservation technologies improves the packaged product. Moreover, the active packaging technologies allow optimizing other food production practices. The antimicrobial effectiveness of the new packaging materials could reduce the need of microbial inactivation processes applied by the food producers. For instance, in the production of mixed cut salad, the initial microbial load of the raw product is controlled by washing treatments with chlorine substances. Reduction of these treatments impacts on lower production costs and decrease the labour risks.

Consumers also demand for intelligent devices that communicate the real quality of the packed good. In some market researches (e.g. earlier EU-project SustainPack) were identified as winning technologies for retailers, freshness indicators, chip radio frequency identification (RFID), electronic article surveillance (EAS), time temperature indicators and printed electronics. Rapid growth in freshness indicators was expected due to changing consumer lifestyles, growth in modified atmosphere/controlled atmosphere technologies and consumer preference for foods with indicators. NAFISPACK evaluated the consumer perception of the active and intelligent technologies which showed the real interest for these systems.

The Nafispack project has started the process of developing a new freshness indicator for a highly perishable food product, fresh fish. The data obtained from the indicator will be presented as an easy-to-understand colour change. Having real-time knowledge about the edibility and quality of the food will have following impacts:

- fish industry: possibility to monitor the quality of the products throughout the distribution chain
- consumers: possibility to reduce health related risks and illnesses originated from consumption of expired or not properly stored food
- authorities: new tools for traceability of food
- packaging material and label industry: new business opportunities
- retailers: new possibilities for product differentiation

A final impact of NAFISPACK results is related with the identification of approaches to evaluate the risk versus the benefits introduced by the new packaging materials with active properties. For instance, new chemical methodologies aiming at the identification of non-intentionally added substances (NIAS) probably arising from the manufacturing processes have been developed for both volatiles and non-volatiles substances. Toxicological tests have been carried out with bioaccesible fractions obtained from the target food products exposed to the new packaging materials. Moreover, the impact on microbiological risk due to the development of microbial resistance and increase of virulence of the microorganisms exposed to the natural antimicrobials has been also assessed. These results and methodologies are available for food safety authorities like EFSA.

As summary of the potential impacts of the NAFISPACK project, from an industrial point of view, the implementation of the new materials would be possible and real. There were not differences of performance of the new materials with respect the actual materials, so there is no need of adapting the packaging producing and packing lines. From a commercial and economical point of view, providing new packaging solutions able to extend, in a mild but effective way, the commercial life of the most perishable products such as those selected in the NAFISPACK project (minimally processed vegetables, fresh fish and fresh chicken) can lead to many, useful consequences. It means to provide better quality to the consumers, during the same storage time, to increase profits of the producers companies, to minimize products loss and short runs along the supply chain, to expand distribution commercial range, to smooth inventory costs and, in general, to increase the sustainability of packaged foods that last for a longer time and can be distributed in a more convenient way. Finally, in some circumstances, a shelf life extension, provided by the natural antimicrobials, tested and selected in NAFISPACK, can contribute also to solve food security problems, for some specific food products and in specific world areas.

NAFISPACK team has carried out several dissemination activities in order to assure a wide diffusion of results at scientific, industrial and social levels, making in all the cases a special acknowledgement to the funding received from the European Union through the Seventh Framework Programme. The main dissemination activities carried out are presented below.

- *NAFISPACK website*

The Nafispack website (www.nafispack.com) was developed in December 2008 and it is made of static and dynamic areas. Static areas go through the project objectives, key areas of research, project structure, partners and contact details. Dynamic areas are a window of news and events related to the food packaging sector. Also can be considered as dynamic the download area, where anything related to the project can be uploaded in several formats.

When creating the Nafispack website, dynamic areas were considered very important to increase the number of visits and well position it in the main web searchers. In this way the project website became an attractive space to visit to those who followed the project and wanted to hear fresh news about research, fairs and conferences of the food sector.

- *Brochure and posters*

The Nafispack brochure described the project in broad brushstrokes, specially the “why” of Nafispack and an introduction to the Natural active and intelligent packaging to be developed. As the website, it included the project main objectives, structure, short work packages description and partners contact list. It has been written be to accessible not only to a technical level but also to a wide type of audience. Print and PDF formats were available for all partners.

The Nafispack poster gathered the two main project objectives and highlights the philosophy of the project "from farm to fork". Partners were represented by their logos and target foods salmon, chicken and minimally processed vegetables were also visible in it. The website was included as a claim to the viewer to look for further information as this poster has been conceived to be exhibited in fairs, conferences, projects meetings, etc.

- *Newsletters*

Three newsletters were released periodically (annually) along the project. Nafispack newsletter I and II were printed and PDF brochures of 10/12 pages including the main advances of the project in each term and more information of interest in the framework of the active packaging. Dissemination of these documents has been carried out through mail and e-mail to its target with a special focus on companies potentially interested in these innovative technologies. Newsletters are available on PDF format in www.nafispack.com.

Nafispack Newsletter III, the final one, has been developed as an audio-visual document of the closing conference of the project. It uses an innovative tool that interacts with the viewer, it is not only a video recording of the conferences but also a synchronized video with speaker speeches and PowerPoint slides. Visitors can easily move through the slides to their points of interest, download the full presentations in PDF and select the language (English or Spanish) to see the conferences. All information is uploaded in a website together with an interview to the coordinator, logotypes and a few lines about the main aims of the project.

To disseminate the Nafispack Newsletter III it has been developed an E-mail blast in Spanish and English and linked them to the Newletter III homepage (Conference.nafispack.com). E-mail blast has been sent to the attendees and to ITENE contacts and associates. Also, Nafispack partners have been asked to do it with the purpose of reaching as much public from as many countries in Europe as possible. This tool makes possible to attend the conference up to one year after its celebration.

It is an attractive way to present the project results with no limit of samples, just a link that can be forwarded and shared as many times as wished by the receiver. To be able to calculate the success of the Newsletter people visiting it need to introduce some basic data (name, company name and e-mail) to access to the presentations.

- *High level Strategic Conferences*

Two Strategic conferences were held at the mid-stage and final stage of the project.

I Strategic Conference

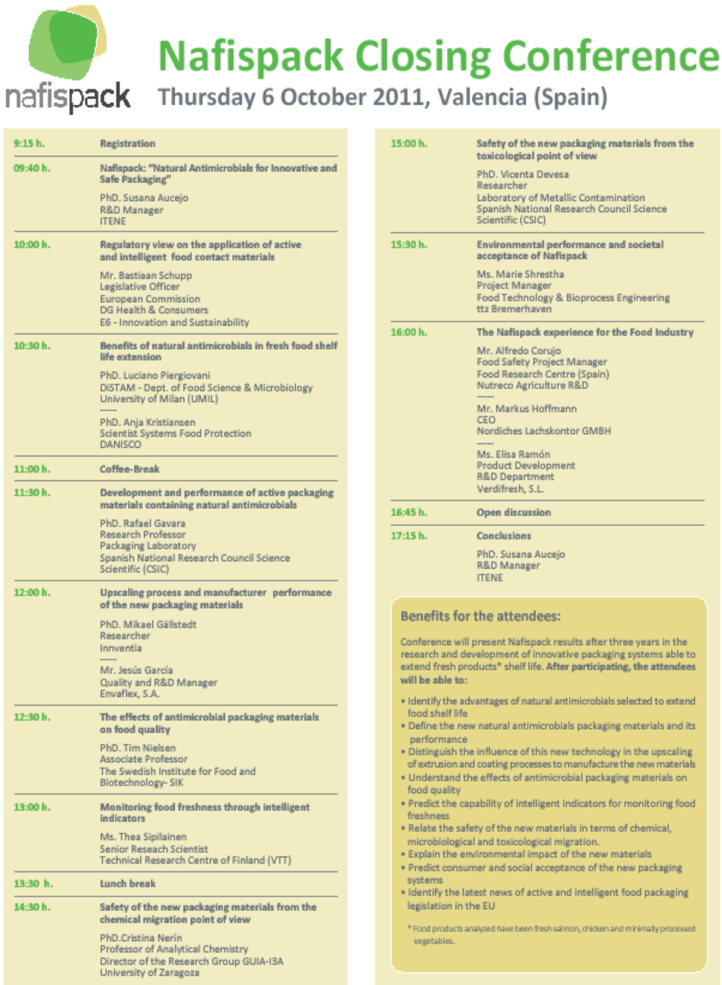
The First Strategic Conference was held in Brussels. Location selection (Leopold hotel Brussels) was conditioned by its connexions to the airport and conference room capability. Brussels was chosen as excellent meeting point in the European context.

The initial date of the Conference was 22 April 2010 but had to be cancelled due to flight disruptions caused by the volcano. The new date defined was 20 May 2010 and cancellation arrangements and new dissemination effort was done since 19 April. All partners were involved in this task and programme only suffered small changes.

Nafispack results presentation forum was completed with representatives of Nestlé, Cryovac, Keller and Heckman and University of Athens who were invited as speakers to show their point of view on active and intelligent packaging from the outside of the project consortium. This picture shows Conference room during presentations. Some registrations were lost after the date change and a final number of registrations was **54**.

II Strategic Conference

The "Nafispack Closing Conference" was held on October 6 in Valencia. ITENE hosted the event as the project coordinator and received **135 registrations**.
WP8 worked on programme definition, partners coordination, dissemination plan, conference room arrangements, dissemination materials design and distribution (brochures, banners, posters, etc.), attendees registration, etc.
After discussion it was decided by a majority of the partners that no speakers out of the project consortium were included in the programme as event was conceived as an exclusive forum about Nafispack results. Only Mr. Bastiaan Schupp, Legislative Officer of the EC was invited to speak about the "Regulatory view on the application of active an intelligent packaging food contact materials". One day programme was planned with an overview of the project, main achievements in



nafispack

Nafispack Closing Conference

Thursday 6 October 2011, Valencia (Spain)

9:15 h.	**Registration**
09:40 h.	**Nafispack: "Natural Antimicrobials for Innovative and Safe Packaging"** PhD. Susana Aucejo R&D Manager ITENE
10:00 h.	**Regulatory view on the application of active and intelligent food contact materials** Mr. Bastiaan Schupp Legislative Officer European Commission DG Health & Consumers E6 - Innovation and Sustainability
10:30 h.	**Benefits of natural antimicrobials in fresh food shelf life extension** PhD. Luciano Piergiovani DiSTAM - Dept. of Food Science & Microbiology University of Milan (UMIL) ----- PhD. Anja Kristiansen Scientist Systems Food Protection DANISCO
11:00 h.	**Coffee-Break**
11:30 h.	**Development and performance of active packaging materials containing natural antimicrobials** PhD. Rafael Gavara Research Professor Packaging Laboratory Spanish National Research Council Science Scientific (CSIC)
12:00 h.	**Upscaling process and manufacturer performance of the new packaging materials** PhD. Mikael Gällstedt Researcher Innventia ----- Mr. Jesús García Quality and R&D Manager Envaflex, S.A.
12:30 h.	**The effects of antimicrobial packaging materials on food quality** PhD. Tim Nielsen Associate Professor The Swedish Institute for Food and Biotechnology- SIK
13:00 h.	**Monitoring food freshness through intelligent indicators** Ms. Thea Sipilainen Senior Reseach Scientist Technical Research Centre of Finland (VTT)
13:30 h.	**Lunch break**
14:30 h.	**Safety of the new packaging materials from the chemical migration point of view** PhD.Cristina Nerín Professor of Analytical Chemistry Director of the Research Group GUIA-I3A University of Zaragoza
15:00 h.	**Safety of the new packaging materials from the toxicological point of view** PhD. Vicenta Devesa Researcher Laboratory of Metallic Contamination Spanish National Research Council Science Scientific (CSIC)
15:30 h.	**Environmental performance and societal acceptance of Nafispack** Ms. Marie Shrestha Project Manager Food Technology & Bioprocess Engineering ttz Bremerhaven
16:00 h.	**The Nafispack experience for the Food Industry** Mr. Alfredo Corujo Food Safety Project Manager Food Research Centre (Spain) Nutreco Agriculture R&D ----- Mr. Markus Hoffmann CEO Nordiches Lachskontor GMBH ----- Ms. Elisa Ramón Product Development R&D Department Verdifresh, S.L.
16:45 h.	**Open discussion**
17:15 h.	**Conclusions** PhD. Susana Aucejo R&D Manager ITENE

Benefits for the attendees:

Conference will present Nafispack results after three years in the research and development of innovative packaging systems able to extend fresh products* shelf life. **After participating, the attendees will be able to:**

- Identify the advantages of natural antimicrobials selected to extend food shelf life
- Define the new natural antimicrobials packaging materials and its performance
- Distinguish the influence of this new technology in the upscaling of extrusion and coating processes to manufacture the new materials
- Understand the effects of antimicrobial packaging materials on food quality
- Predict the capability of intelligent indicators for monitoring food freshness
- Relate the safety of the new materials in terms of chemical, microbiological and toxicological migration.
- Explain the environmental impact of the new materials
- Predict consumer and social acceptance of the new packaging systems
- Identify the latest news of active and intelligent food packaging legislation in the EU

* Food products analyzed have been fresh salmon, chicken and minimally processed vegetables.

research, legislation in this field and the Nafispack experience from the food industry partners point of view.

It was considered interesting to guarantee the event success to include simultaneous translation English-Spanish-English in the event.

Samples of salad bags, meet trays and film were exposed during the conference for attendees to look at them.

Both Strategic Conferences were extensively disseminated through several channels:

- **Website (Nafispack, ITENE…)**
- **E-mailings**
- **Brochure printed and PDF**
- **Personal invitations**
- **Mailing to European context**
- **Advertisements:** Different advertisements were designed and included **INFOPACK E+E/ R&D applied to Packaging and Logistics** magazine. This publication has a print run of 3.500 numbers and a periodicity of 11 numbers per year, controlled by OJD (Spanish Diffusion supporting Office).
- **Banner:** Also a banner was updated in the homepage as a claim for ITENE website visitors. All partners were invited to place the banner in their websites or send their company banner sizes for us to adapt it.

- **Dissemination in Social Networks:** Facebook, Linkedin and Twitter tools were used to announce the conference and inform about the programme and further details.
- **Posters** for exposition in the conferences were developed.

- *Other dissemination activities*

Dissemination in fairs and conferences:

The project partners have presented Nafispack in conferences, seminars and fairs in the European context. Some examples,

Fairs

1. 21-23 September 2009 Rotterdam, "Freshtec Rotterdam"
2. 3-6 Nov 2009, Valencia (Spain), "Idinova"
3. 18-19 Nov 2009, Madrid (Spain), "Easyfairs Empack"
4. 11-15 mayo 2009, Barcelona (Spain), "Hispack 2009"
5. 14-15 April 2010, Barcelona (Spain), "Easyfairs Packaging Innovations"
6. 24-25 Nov 2010, Madrid (Spain), "Easyfairs EMPACK"
7. 20-30 March 2011 Lisboa (Portugal), "Horexpo & Alimentaria Lisboa"
8. 12-18 May 2011 Düsseldorf (Germany), "Interpack"
9. 14-18 Nov. 2011 Barcelona (Spain), "Equiplast"
10. 26-27 Oct. 2011 Madrid (Spain), "Easyfairs EMPACK"

Conferences

11. 28 May 2009, Dajeon (South Korea)
12. 29 October 2008, Hamburg (Germany)
13. 28 Nov 2012, ILSI Symposium Berlin (Germany)
14. 2010 Paris (France) MATBIM (Material/Bioproduct Interactions)
15. 2010 UK Campdem Bri
16. 2010 Zaragoza, Spain 4th Shelf Life International Meeting
17. 2010 Chicago, USA, Congress IFT (Institute of Food Technologist)
18. 16 April 2010 Congress: Los aditivos como herramienta tecnológica para garantizar la calidad y seguridad de los alimentos. Universidad de Burgos
19. 9 November 2011, Talca, Chile Congress: 2º Seminario Internacional de Alimentos Procesados Hortofrutícolas. Centro de Estudio en Alimentos Procesados
20. Torremolinos, Málaga, (Spain) 2011 Congress: Biomicroworld
21. Dijon (Francia) 2011 International Workshop on Food Safety
22. Valladolid (Spain) December 2010 International Conference on Antimicrobial Research
23. December 2011 Valencia (Spain) VI Congreso Nacional de Ciencia y Tecnología de los Alimentos.
24. December 2012 International Conference of Agricultural Engineering. Valencia (España)
25. Zaragoza (Spain) 2010 SLIM (Shelf life International Meeting)
26. Tianjin (China) 2010 IAPRI World Conference on Packaging
27. Barcelona (Spain) 2010 JAI (Jornadas de Análisis Instrumental)
28. San Luis Obispo (CA, USA) 2012 IAPRI World Conference on Packaging
29. May 2011 La Rioja (Spain) Food Safety Seminar
30. Berlin (Germany) 2012 Congress ILSI
31. October 2010 Zaragoza (Spain) Food Safety Seminar
32. November 2011 Madrid (Spain) Escuela Superior de Sanidad

33. November 2011 Barilla Company (Italy)
34. Oslo, Norway, March 31 2011 Norwegian Packaging Association (DNE)
35. 18 November 2010 Oslo, Norway, Norwegian Packaging Association (DNE)
36. 9 May 2010 Packaging School, (the Norwegian Packaging Association (DNE), Oslo, Norway)
37. 22 April 2010 (Nofima Mat, Ås, Norway) FoodPack 2010 Packaging Seminar
38. 24-25 February 2010 Zeist, Netherlands Safe Consortium, TNO Seminar
39. 20-21 January 2010 Stjørdal, Norway *The Seafood days (*Sjømatdagene)

Thesis (PhD)

AUTHOR: R. Becerril.
TITLE: Estudio de las alteraciones producidas por un envase activo antimicrobiano en bacterias mediante técnicas microbiológicas y analíticas.
PLACE: Department of Analytical Chemistry. University of Zaragoza.
DIRECTOR: C. Nerin

Book chapters:
Silva, A; Belda-Galbis, CM; Zanini, SF; Rodrigo D; Martínez, A (2012) Sublethal damage of Listeria monocytogenes by non-thermal treatments and its implications on food safety. In: Listeria Infections: Epidemiology, Pathogenesis and Treatment. New York; Nova Science Publishers. In press.

Nafispack in the media:

Nafispack has attracted the attention of the media since the project kick off. Technical magazines, journals, TV news, radio programmes have published articles and interviews related to the project during this term.

Scientific papers:

Around 15-16 scientific peer review papers will be released from the activities carried out into the project. Some of these papers have been already published, others are in process of review and others are being prepared. As example the following already published papers can be mentioned:

References	**Comments**
"Antimicrobial Activity Of Lysozyme And Lactoferrin Incorporated In Cellulose-Based Food Packaging" Barbioli, A., Bonomi, F., Capretti, G., Lametti, S., Manzoni M., Piergiovanni, L., Rollini, M. Food Control (2012), doi:10.1016/j.foodcont.2012.01.046	Accepted Manuscript
"Mathematical model to describe the release of an antimicrobial agent from an active package constituted by carvacrol in a hydrophilic EVOH coating on a PP film" José Pasqual Cerisuelo, Virginia Muriel-Galet, José M. Bermúdez, Susana Aucejo, Ramón Catalá, Rafael Gavara, Pilar Hernández-Muñoz. Journal of Food Engineering, 110, pp. 26-37 (2012)	Date of publication:2012
"Modification induced by the addition of a nanoclay on the functional and active properties of an EVOH film containing carvacrol for food	Submited in December 2011

packaging" José Pasqual Cerisuelo, Jose Alonso, Susana Aucejo, Rafael Gavara, Pilar Hernández-Muñoz Journal of Food Engineering	
"Describing and modelling the release of an antimicrobial agent from an active PP/EVOH/PP bag for salmon" José Pasqual Cerisuelo, Virginia Muriel-Galet, Jose Alonso, Susana Aucejo, , Rafael Gavara, Pilar Hernández-Muñoz Journal of Food Engineering	Submited in January 2012
"Development of antimicrobial films for microbiological control of packaged Salad" Virginia Muriel-Galet, Josep P. Cerisuelo, Gracia López-Carballo, Jose M Bermudez, Rafael Gavara, Pilar Hernández-Muñoz International Journal of Food Microbiology	Submited in December 2011
"Evaluation of antimicrobial EVOH coated PP films to improve the stability of packaged salad, Packaging Science and Technology" Virginia Muriel-Galet, J.P. Cerisuelo, Gracia López-Carballo, Susana Aucejo, Rafael Gavara and Pilar Hernández-Muñoz Packaging Science and Technology	Submited in February 2012
"Antimicrobial activity of green tea decaffeinated extract, green tea antioxidant extract and lauramide argine ethyl ester" R. Becerril, R. Gómez-Lus, C. Nerín Italian Journal of Food Science. 174-177, 2011	Date of publication:2010
"Antifungal activity of the essential oil of cinnamon (Cinnamomum zeylanicum), oregano (Origanum vulgare) and lauramide argine ethyl ester" Manso S, Nerin C, Gómez-Lus R Ital J Food Sci; 151-156, 2011	Date of publication:2010
R. Becerril, R. Gómez-Lus, C. Nerín "Comparision of antimicrobial activity of lauroyl arginate ethyl ester (LAE) with oregano and cinnamon essential oil" R. Becerril, R. Gómez-Lus, C. Nerín Foodborne Pathogens and disease	Under revision
"Analytical procedure for the determination of Ethyl Lauroyl Arginate (LAE) to assess the kinetics and specific migration from a new antimicrobial active food packaging" Davinson Pezo, Beatriz Navascués, Jesús Salafranca, Cristina Nerín Analytica Chimica Acta	Under revision
"LC-Q-TOF-MS analysis of non volatile migrants from new active packaging materials" M. Aznar, A. Rodriguez, P. Alfaro, C. Nerin Analytical and Bioanalytical Chemistry	Under revision
"Analysis of migration samples from a new antimicrobial food packaging material base on lauric arginate (LAE). Bioaccesibility studies in chicken samples" M. Aznar, C. Nerin, J. Gomez, V. Devesa I Perez Food Chemistry	In Writing
"LC-MS (QTOF) analysis of migration samples from a new antimicrobial food packaging material base on lysozyme and lactoferrin proteins to salmon samples"	In Writing

P. Alfaro, M. Aznar, C. Domeño , C. Nerin Journal of Agricultural and Food Chemistry	
“Release of natural substances from active packaging materials containing oregano and cinnamon essential oils” M. Lara, J.M. Bermúdez, S. Aucejo, R. Gavara, Pilar Hernández	In Writing
“Performance of active packaging materials containing natural antimicrobials” M. Lara, J.M. Bermúdez, S. Aucejo, R. Gavara, Pilar Hernández	In Writing
“Evaluation of the formation of non-intentionally added substances (NIAS) in active packaging materials containing natural antimicrobials” M. Lara, J.M. Bermúdez, S. Aucejo, R. Gavara, Pilar Hernández	In Writing

Exploitation of results

NAFISPACK had as objective the development of active and intelligent packaging solutions for extending the shelf life and assuring the safety of fresh food products. Several materials have been developed along the project including a great number of combinations coming up from the different antimicrobials, packaging materials and processing techniques.

The number of potential materials was reduced along the project in function of its technical performance, especially regarding antimicrobial effectiveness.

Results obtained along the project indicated that the packaging materials were highly active in *in vitro* tests although it was reduced in *in vivo* tests with real food products. In this sense, chemical, microbiological and sensorial parameters were evaluated as measurement of the quality and safety of the target food products. The final storage test with real food products, it was not found any material that had a positive effect on all the quality parameters that could last for the whole storage time, although some punctual improvements were found.

These results suggest that materials could work but they still need further development. So, the exploitation of results goes in the direction of improving the materials. The background of scientific and technological know-how developed into the project will be of great importance and useful in such further actions.

1.5. Address of the project public website and relevant contact details

NAFISPACK- Natural Antimicrobials For Innovative and Safe packaging

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