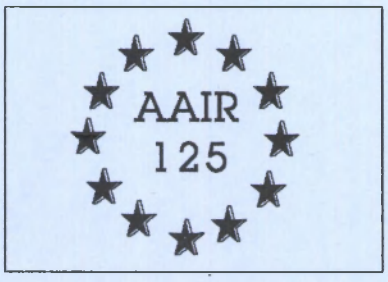

IMPROVEMENT OF THE SAFETY AND QUALITY OF REFRIGERATED READY-TO-EAT FOODS USING NOVEL MILD PRESERVATION TECHNIQUES

EC-AAIR PROJECT CONTRACT N° AIR1-CT92-0125

FINAL SYNTHESIS REPORT

JANUARY 1993 - DECEMBER 1996

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IMPROVEMENT OF THE SAFETY AND QUALITY OF REFRIGERATED READY-TO-EAT FOODS USING NOVEL MILD PRESERVATION TECHNIQUES

(AIR1-CT92-0125; 1993-1996)

Partnership

Agrotechnol. Res. Inst., Wageningen, NL (coordinator); Wageningen Agricult. Univ., Wageningen, NL; Inst. Natl. Rech. Agronomique, Montfavet, FR; Coop. Int. Rech. Agronomique, Syst.Agro-alimentaires Ruraux, Montpellier, FR; Inst. Technol. Agricult. Products, Athens, GR; Inst. Food Res.,Norwich, GB; Dept. Life Sciences, Univ. Limerick, EI; Les Crudettes, Fruidor S.A., Cavaillon, FR; Nature's Best Ltd., Duleek, EI

Objectives

With the increasing popularity of ready-to-eat, fresh and processed foods which are preserved only by relatively mild techniques, a new habitat for microbial growth may have emerged. In order to control the growth of food poisoning and spoilage microorganisms in these habitats, while keeping loss of product quality to a minimum, sound information on important factors affecting the survival and growth of such microorganisms under the mild preservation conditions is required. This project has set out to improved the safety and quality of vegetable based ready-to-eat foods, *i.e.* fresh or minimally processed preparations, and refrigerated, processed foods of extended durability (REPFEDs). These perishable, convenience foods rely heavily on refrigerated storage to asure safety and quality. In practice, proper refrigeration is not always asured and additional, mild preservation barriers were thus optimised or designed in this project for better control of quality and safety deterioration. The barriers concerned were biopreservation, modified atmosphere packaging (MAP) or coating (MAC), and coatings containing food-grade antimicrobial agents (active MAC).

Achievement highlights

The project has shown that Minimally processing/Mild preservation is not a simple concept and that, in particular, MAP is not an "off-the-shelf" technology. To obtain a quality product, the concept needs to be carefully tailored to the specific physiological requirements of the product under the exact conditions prevailing (quality of processing applied, refrigeration, selection of MAP system, logistics, expected shelf-life, etc.). All results have been extensively disseminated towards both industry and scientific audiences.

For the first time in this project, an integrated approach in disciplines and technologies to improve both the safety and the quality of minimally processed foods. On the food safety side, important new insight has been gained into the growth and survival of pathogens such as *Listeria monocytogenes* and *Clostridium botulinum* on perishable food. It was observed that a range of different food components (osmoprotectants, polypeptides) cold-tolerant pathogens and that such effects could be very aspecific. The mechanisms underlying this effect were, for the first time, studied in much detail. The balans-interaction between pathogens and epiphytes on foods, as observed for *Listeria monocytogenes* on leafy salads has pinpointed the importance of the normal microflora in food safety and has identified consequences for Good Manufacturing Practices and disinfection treatments. When disinfection is used, therefore, re-infection needs to be excluded. Proteolytic epiphytes help *L. monocytogenes* and non-prot *Cl. botulinum* to obtain essential nutrients.

Whereas it was commonly thought that microorganisms in general are sensitive to carbon dioxide, it was shown in the current project that CO_2 levels under 50% do not significantly reduce growth of psychrotrophic pathogens (i.e. *L. monocytogenes, Aeromonas hydrophila, Yersinia enterocolitica*). Low O_2, however, reduces spoilage but this may give the pathogens a competitive advantage due to which they will proliferate in.

Despite the general conception that traces of oxygen would keep *C. botulinum* from growing, it was found here that 1-2% O_2 (in the head-space atmosphere) allowed for pathogen growth. Only an atmosphere of 100% CO_2 was shown to control this cold-tolerant pathogens. Under normal MAP conditions, thus there is a potential for any psychrotrophic pathogen to grow an pose a genuine health hazard in MAP systems. As a consequence additional precautions required for optimal safety. Such precautions could be the use of bacteriocin-producing lactic acid bacteria microorganisms (LABs) or their bacteriocins. Much effort was invested in finding LABs that actively produced suitable bacteriocins at chill temperatures under MAP conditions. No LAB with this trait was found. However, a bacteriocin producing *Enterococcus* was found and thoroughly characterized. Ecologically adapted bacteriocin producers (*Pediococcus parvulus, Enterococcus mundtii*) controlled growth of *L. monocytogenes* on sterile food, but not on fresh produce. Pure bacteriocins (mundticin, nisin) controlled pathogen growth better, i.e. at low pH.

Biocoatings, totally natural and edible, were deveoped for physical protection and to carry bacteriocins and other natural antimicrobials. This area is rapidly developing now and the participants in this project have moved much to the forefront of this development. The results can easily picked up by inductries and taken further into their practice. The participating companies have experienced that improvements can be developed and implemented in a rather short time. All necessary information has been disseminated, or is still avaialble from the partners.

Based on the results from the project, food producing industry can and has been given targeted advise. Especially the SME- and end-user-level of results already disseminated via the RETUER workshops of 1996 (A FLAIR-FLOW EUROPE initiative) proved to be very successful.

A new custom-made GC-system was tested and compared to commercially available non-GC systems of the quantification of low levels of oxygen. Inaccuracies in the different systems were identified and quantified. The methods for accurate measurement were developed and described. The methods described are useful for routine true oxygen measurement by industry or regulators, also those working outside the vegetable/fruit area. For example, precisely controlled low levels of oxygen can be vital in packaging of cured and some fresh meats.

The effects of a wide range of raw material processing and storage variables were evaluated in model products. Significant effects on quality were observed for all parameters. These findings are immediately applicable to industry. The effects of processing packaging and storage conditions on losses of ascorbic acid/conversion to dehydroascorbic acid were quantified. Guidelines to minimise the loss of this vitamin are applicable in industry. Packaging materials were selected for a range of commercial products that considerably improve product-package compatibility compared with current commercial practice.

Noticably, conditions can prevail that encourage growth of pathogens, the cold-tolerant *Listeria monocytogenes* especially in prepared salads, the non-proteolytic *Cl. botulinum* in REPFEDs/sous-vide type of preparations. By current standards, the presence of these pathogens may not be completely avoided in (raw) vegetables.

Many of the aspects of non-proteolytic *Cl. botulinum* were demonstrated here for the first time. The ability of sub-lethally damaged spores to grow in suboptimal conditions (NaCl, pH, organic acid, gaseous atmosphere) at refrigeration temperatures were characterised and the ability of many cooked vegetables to support growth of non-proteolytic *Cl. botulinum* was demonstrated for the first time. Particularly interesting was the discovery that vegetable juice can aid the recovery of heat damaged spores.

Edible films and coatings have been designed and knowledge gained in the process should enable research and practice to fine-tune this technology for near-future application in minimally processed fruits and vegetables. Active edible coatings were found to be a promissing concep, allowing (bio)preservation, MA-generation and physical protection to be combined in one process. The research performed on this topic has set the course to further develop "minimal packaging" or "invisible packaging" concepts.

Mathematical modelling has, in cases, reinforced earlier empiric observations and was found to be very suitable to pin-point conditions or circumstances where research efforts needed to be focused at. In all, mathematical modelling should be instrumental in achieving a total systems approach for the minimally processing concept.

Innovation

The project has, for the first time, taken an integrated approach in disciplines and technologies to improve both the safety and the quality of minimally processed foods. On the food safety side, important new insight has been gained into the growth and survival of pathogens on such foods. It was found that a range of different food components supported the growth of certain cold-tolerant pathogens and that such eefects could be very aspecific. The mechanisms underlying this effect were, for the first time, studied in much detail. The balans-interaction between pathogens and epiphytes on foods, as observed for *Listeria monocytogenes* on leafy salads has pinpointed the importance of the normal microflora in food safety and has identified consequences for Good Manufacturing Practices and disinfection treatments. Whereas it was commonly thought that microorganisms in general are sensitive to carbon dioxide, it was shown here that the cold-tolerant pathogens are not sensitive at all and really may pose a health hazard in MAP systems, unless additional precautions are taken. Such precautions could be the use of bacteriocin-producing lactic acid bacteria microorganisms (LABs) or their bacteriocins. Much effort was invested in finding LABs that actively produced suitable bacteriocins at chill temperatures under MAP conditions. No LAB with this trait was found. However, a bacteriocin producing *Enterococcus* was found and thoroughly characterized. It was found that application of the culture was less effective than application of the pure bacteriocin. Biocoatings, totally natural and edible, were deveoped for physical protection and to carry bacteriocins and other natural antimicrobials. This area is rapidly developing now and the participants in this project have moved much to the forefront of this development. The results can easily picked up by inductries and taken further into their practice. The participating companies have experienced that improvements can be developed and implemented in a rather short time. All necessary information has been disseminated, or is still avaialble from the partners.

Based on the results from the project, food producing industry has been given targeted advise, e.g. via VALUE and FLAIR-FLOW Europe workshops. In many cases, the responses from SME and larger companie were very positive and appreciative. Especially the SME- and end-user-level of results disseminated in the RETUER workshops of 1996 (A FLAIR-FLOW EUROPE initiative) proved to be very successful

Achievements per task

Task 1. Physiology of pathogens

The protective effect of proline, betaine and L-carnitine on *in vitro* growth of *Listeria monocytogenes* Scott A at high osmolarity was studied by WAU.DFS. Betaine and L-carnitine are present in foods originating from plants and animals, respectively. L-carnitine could function equally well as an osmoprotectant as betaine, whereas L-proline was less effective. Although the osmoprotective capacity of betaine is well-known among many prokaryotic organisms, L-carnitine has not been recognized as an osmolyte up to now-.When L-carnitine and betaine were supplied together, betaine was preferentially accumulated, specially at high osmolarity. Both the betaine and the L-carnitine transporter are not significantly activated by osmotic stress, whereas the final levels of accumulation were dependent on the external osmolarity. In addition, a fairly new feature of osmoprotectants was established at WAU.DFS: cold tolerance in *L. monocytogenes* can be invoked by betaine and L-carnitine and, moreover, both compounds were shown to stimulate the growth of *L. monocytogenes* under anaerobic conditions. These observations are relevant for the growth and survival of the pathogen at refrigeration temperature and in low oxygen environments like modified atmosphere packaging.

From literature it is known that *L. monocytogenes* requires certain amino acids for growth. To obtain information on nitrogen metabolism in *L. monocytogenes*, the property of the pathogen to utilize dipeptides as a source of essential amino acids was studied at WAU.DFS. It was shown that the essential amino acids leucine and methionine supported growth of the pathogen not only when supplied as the free amino acid but also in the form of dipeptides (*i.e.* leucine or methionine combined with proline or alanine).The nutritional value of peptides for the growth of *L. monocytogenes* was further investigated by studying the utilization of oligopeptides as a source of essential amino acids for growth of the pathogen. Using growth experiments, we showed that the size limit for peptide utilization in *L. monocytogenes* is probably 8 amino acids residues. It appeared that several oligopeptides are toxic to *L. monocytogenes*, which may be an interesting observation with regard to a potential application of such peptides as antimicrobial agents.

INRA.AV collaborated with IFRN to investigate the growth potential of non-proteolytic *Cl. botulinum* on vegetables. Canned vegetables have been the cause of several cases of botulisms with proteolytic, non-psychrotrophic *Cl. botulinum*. However, cooked chilled vegetables foods are normally stored below 10°C and should not permitted growth of proteolytic botulinum. Spores of non proteolytic, psychrotrophic botulinum should survive the mild heat treatment of these foods and their growht was investigated in a wide range of vegetables at temperatures from 5 to 30°C. Cooked chilled foods are not sterile and contained numerous spore formers that may influence botulinum. The influence of *Bacillus* spp. isolated from vegetables on growth of non-proteolytic *Cl. botulinum* was investigated. Growth of non-proteolytic *Cl. botulinum* was observed in all cooked vegetables tested with pH higher than 5.0. Large variation in lag times were recorded among vegetables and temperatures whereas growth rate were similar. The presence of *Bacillus* spp. permitted growth and toxin production by non-proteolytique botulinum in media incubated in aerobic conditions.

Combinations of mild preservative factors can be used to maintain product safety whilst maximising product quality. Although the effect of individual factors such as incubation temperature, sodium chloride concentration, pH and the addition of organic acids on growth of non-proteolytic *Cl. botulinum* have been studied in isolation, information on the effect of combinations is sparse. In particular, the potential for growth of *Cl. botulinum* and toxin production in REPFEDs will depend upon the ability of the spores to survive thermal processing and subsequently germinate, outgrow and produce toxin in the prevailing conditions. Growth from heated or unheated spores may differ. Experiments at IFRN were therefore set-up to quantified the combined inhibitory effect of heat treatment with subsequent incubation in culture media at different incubation temperatures or with different NaCl concentrations, pH values or organic acid additions.

Studying combinations of heat treatment and preservative factors, IFRN found that additions of 3% NaCl (equiv.) or pH values ³6.0 were not effective in preventing growth from 10^6 non-proteolytic *Cl. botulinum* spores at 10°C, even after heating at 90°C for 15 min, if lysozyme is present in the recovery medium. Addition of 4.1% NaCl to the recovery medium, or a pH value of 5.3 prevented growth from both heated and unheated spores of non-proteolytic *Cl. botulinum* 17B when incubation was at 10°C. NaCl and pH interacted to reduce the probability of growth and/or extend the period before growth was observed. Acetic or lactic acid at 0.01M were not effective in preventing growth from non-proteolytic *Cl. botulinum* spores at 10°C, even after the spores had been heated at 80°C for 10 minutes. Potassium sorbate in broth at 10°C prevented spores leading to turbidity in all test conditions but did not prevent toxin production. Citric acid at 0.01M increased time to turbidity from unheated spores by 4 weeks and prevented turbidity occurring within 14 weeks from spores heated at 80°C for 10 minutes.

Contrast to general believe, IFRN found that not only proteolytic *Cl. botulinum* may cause botulism associated with vegetable. Many cooked vegetables were tested and a significant number was found to support growth and toxin production by non-proteolytic *Cl. botulinum* when they were incubated at 30°C and their pH value was above 5.0. Growth and toxin production also occurred at 5 to 10°C. To preliminarily investigate the practical relevance of this observation, IFRN studied toxin production in a seven commercially available, artificially inoculated vegetable based REPFEDs. Four short shelf-life REPFEDS were purchased from a retail outlet, inoculated and incubated anaerobically for 8 weeks. Although the pH and A_w were within the limits of growth for non-proteolytic *Cl. botulinum*, toxin was detected in only one of the products. This suggests that manufacturers are incorporating a considerable safety margin into their products. In three other commercial preparations, the effect of heat treatment on growth was tested. These packages contained vacuum packaged vegetable products currently given a heat treatment of 20 min at 80°C and with assigned shelf-lives of 10 days were tested. They were obtained direct from the manufacturer, inoculated with 10^6 spores of *Cl. botulinum* per pack, left unheated or heated for 20 minutes at 75°C or 85°C and incubated at 10°C for up to 20 days. Toxin was not detected in any of the samples tested. This again suggests that the safety margins in these types of vegetable products is much greater than would be predicted from studies in rich, meat based media. However, until the interaction of food matrix components and growth is better understood lower processing conditions cannot be generally recommended and such results can not be extrapolated to other products.

Studies at IFRN and elsewhere have previously shown that lysozyme can increase the measured heat resistance of spores of non-proteolytic *Cl. botulinum*. In this project, IFRN showed for the first time that adding some raw vegetable juices to culture media also can increase the recovery of heat damaged spores. Heating these juices at 75°C for 10 min failed to destroy this effect. This implies the survival of non-proteolytic *Cl. botulinum* spores in pasteurised vegetable products would be greater than predicted from studies where spores were recovered in the absence of lytic enzymes. To examine the survival of spores both heated and subsequently incubated on vegetables, heat treatments were performed on vials containing sterile anaerobic vegetable juice or culture media inoculated with between $7x10^6$ and 0.1 spore ml^{-1}. Maximum recovery occurred when spores were heated in phosphate buffer and subsequently recovered on unheated PYGS+L medium. Vegetable juices reduced the probability of growth from unheated spores compared to culture media. However, for spores heated at 80°C for 10 min and subsequently recovered in the heating medium, the number of spores able to lead to growth in bean or broccoli juice was greater than that in PYGS although less than in PYGS+L. Further study is required to determine if this effect results from a protective effect of the vegetable juice during heat treatment or improved recovery of heat damaged spores.

Task 2. Pathogen-epiphyte interaction

WAU.DFS studied the interaction of *L. monocytogenes* and *Bacillus cereus* or *Pseudomonas fragi* in milk and in a chemically defined medium with casein present as the only nitrogen source. Optimal growth of *L. monocytogenes* in milk was dependent on the growth of the other micro-organisms. Growth of *L.*

monocytogenes in medium with casein but without the essential amino acids was enhanced when this medium had been pre-incubated with either *B. cereus* or *Ps. fragi*. Collectively, *L. monocytogenes* benefits from the proteolytic activity of other micro-organisms in foods.

INRA.AV investigating the fate of foodborne pathogens in minimally processed foods in the presence of non-pathogens (epiphytes). The fate *Listeria monocytogenes* on minimally processed green salads was studied in most detail. The effect of the following factors were tested: storage conditions (temperature, carbon dioxide), type of inoculum (strain of *L. monocytogenes*, initial numbers of *L. monocytogenes*), effect of the leaf substrate and of the saprophytic microflora.

The growth of *Listeria monocytogenes* versus spoilage development was described for temperatures ranging from 3 to 20°C. It was shown at INRA.AV that on unspoiled products *L. monocytogenes* would hardly grow of more than 2 log units whatever the storage temperature, but that spoilage of the salad leaves would permit a rapid multiplication. Low storage temperatures reduced growth of *L. monocytogenes* more than that of the spoilage microflora and are therefore a factor improving safey. On the contrary, carbon dioxide concentrations of 10-20% reduced spoilage development and growth of the spoilage microflora and higher concentrations slightly increased growth of *L. monocytogenes*.

Most information on the fate of foodborne pathogens in foods are obtained from inoculation with high numbers of microorganisms, several log units higher than those found in foods naturally contaminated. On minimally processed green endive, INRA.AV studies showed that high inoculum concentrations over-estimated the maximum growth of *L. monocytogenes*. The epiphytic microflora of green endive leaves had a barrier effect against *L. monocytogenes*. A practical implication of this result was that any reduction of this microflora (by a disinfection for instance) would tend to increase the growth potential of *L. monocytogenes*. However, disinfection would also have a positive effect in reducing the numbers of *L. monocytogenes* in case of a contamination of raw material before processing. This barrier effect could be ascribed to enterobacteria (presumably by a competition for essential nutrients), but not to pseudomonads.

A need for diversification of the range of minimally processed product led manufacturers to propose mixed salad with raw and cooked vegetables, but without dressing (i.e. not acidified). INRA investigated with « Fruidor Les Crudettes » the consequences of incorporating cooked ingredients on the microbiology and safety of minimally processed fresh vegetables. A salad containing raw shredded endive and cooked sweet corn was choosen for this study. The presence of sweet corn dramatically increased the growth of *Listeria monocytogenes* and permitted development of the lactic acid bacteria *Leuconostoc mesenteroides*. Selective acidification of sweet corn with citric acid was successfully tested and the minimum concentration to reduce listeria growth to that observed in raw green salad alone was determined. Citric acid did not damaged green salad leaves. Another approach was studied: coating sweet corn with edible formulations to somehow isolate this ingredient from raw green salad (the potential source a contamination). Zein produced an edible coating with good mechanical and sensory properties, but it only slightly reduced growth of *Listeria monocytogenes* and did not improve the effect of sorbic acid.

The use of disinfectant to reduce microbial load of minimally processed fresh salads is permitted in some UE members. Results obtained by INRA.AV show that after disinfection the surviving microorganisms have an increase growth rate and rapidly reach the same level than on non-disinfected products. In addition, in the case of contamination with *L. monocytogenes* during processing, disinfection of salad leaves would reduce the antagonism from epiphytic bacteria and might increase growth of the foodborne pathogen. Therefore, the major interest of disinfectant may be more to prevent build up of contaminations in washing water during processing rather than to reduce microbioal load of raw salad leaves (Carlin et al 1996).

An evaluation of the growth of *L. monocytogenes* in minimally processed green salads based on the complete picture regarding impact of storage conditions, practical shelf-life and processing steps involved was made by INRA.AV. It was estimated that with an initial contamination lower than 1 *L. monocytogenes* per gramme, numbers of *L. monocytogenes* should not exceed the tolerance limit of 100 per gramme over

the practical shelf-life (i.e. before noticeable spoilage occur). This should be true whatever the storage temperature (because spoilage development accelerates with temperature increasing) but, in general, lowering storage temperature increase safety because *L. monocytogenes* growth is more reduced by refrigeration than spoilage (Carlin et al. 1995).

At IFRN, studies have been conducted into the behaviour of non-proteolytic *Cl. botulinum* in relation to psychrotrophic *Bacillus* species (isolated by C. Nguyen-the, INRA) and gas atmosphere. Spores of non-proteolytic *Cl. botulinum* and *Bacillus* sp. were inoculated into aerobic and anaerobic PYGS broth and incubated below 10°C. Aerobic PYGS did not support growth of *Cl. botulinum* when inoculated in isolation. However, toxin was produced when *Cl. botulinum* was co-inoculated into broth with *Bacillus*, showing that *Bacillus* could induced growth of non-proteolytic *Cl. botulinum*. This results illustrates that the initial presence of oxygen in sealed food packs may not be a guarantee of preventing growth and toxin production by non-proteolytic *Cl. botulinum*.

Task 3. Gas/microbe interactions

This tasks intended to investigate the effect of the composition of the gas atmosphere (mixtures of O_2, CO_2 and N_2, and under MAP and moderate VP conditions) on the growth of relevant spoilage organisms (epiphytes) occurring in vegetable food. ATO.DLO studied the effect of the gas atmosphere composition (O_2, CO_2, N_2) on the growth characteristics of relevant pathogens and epiphytic organisms both *in vitro* and *in situ*. A novel solid-surface model system was specifically developed for the *in vitro* tests. The dynamic flow-through equipment was employed for the *in situ* tests (on model foods). The effect of different combinations and concentrations of O_2, CO_2 and N_2 at different incubation temperatures on the growth of selected pathogens (*L. monocytogenes*, *Yersinia enterocolitica*, *Aeromonas hydrophila* and *Bacillus cereus*) and spoilage micro-organisms (freshly isolated enterobacteria, lactic acid bacteria and pseudomonads) was investigated *in vitro* by ATO.DLO. From these studies it was concluded that growth of none of the pathogens studied is inhibited significantly at low O_2 (0 - 5%) and high CO_2 (20 - 50%), except for *B. cereus* for which growth is about halved at 50% CO_2. This is in contrast to general belief that CO_2 has very pronounced antimicrobial properties. At levels of O_2 and CO_2 that are in general favourable for storage of fresh produce (1-5 % O_2, 5-10% CO_2), there is certainly no beneficial effect of CO_2. In the *in situ* studies at ATO.DLO, it was established that the specific conditions of MAP (reduced oxygen, increased carbon dioxide) can lead to marked changes in the epiphytic micro-flora, especially with chicory endive. Thus, whereas there may be no direct antimicrobial effect of CO_2, there is an influence on the composition of the micro-flora and thus on the competition that pathogens may experience in this ecosystem. It was evident from studies at ATO.DLO that, occasionally, conditions in MAP systems arrise that favour the outgrowth of such psychrotrophic pathogens as *L. monocytogenes* (Figure 2). Unfortunately, these conditions could not be clearly specified since there was no straight forward relationship with either the gas phase composition, the initial level or composition of the epiphytic micro-flora nor with the produce.

NARF studied the effect of the MA conditions on of growth of *Salmonella enteritidis* and *L. monocytogenes* on different vegetables (i.e. lettuce, tomatoes, carrots). *Salmonella enteritidis* was found to decrease in number but survived over shelf-life on all produce during refrigerated (4°C) storage in air and MA (4.9% CO_2/ 2.1% O_2/ 93% N_2 ; 5% CO_2/ 5.2% O_2/ 89.8% N_2). The rate of decrease was greatest in carrots. Partly this maybe due to the low initial pH of carrots, but it may as well indicate the inability of *S. enteritidis* to compete successfully with the lactic acid bacteria present, which produce a range of antimicrobials (lactic acid, acetic acid, bacteriocins). Concerning *L. monocytogenes*, a strong decrease was found under all experimental conditions, but again most pronounced with carrots. It has been shown before that carrots contain antilisterial phenolics. NARF studies indicate that modification of the atmosphere can not be considered as the only factor involved in the inhibition of pathogens since changes in type of vegetable, initial pH, competition of other flora also affect their growth. MAP does not present an effective

hurdle against growth of *S. enteritidis* and *L. monocytogens* when compared to growth in aerobic conditions. Additional hurdles (e.g. biopreservation, active coating) need to be conciously built in the system.

The effect of gaseous atmosphere on growth from unheated and heated spores of non-proteolytic *Cl. botulinum* was assessed at IFRN. The gasses tested were: 100% carbon dioxide; 100% nitrogen; 100% argon; 100% helium; 100% nitrous oxide; 10% hydrogen 90% nitrogen; 5% carbon dioxide 10% hydrogen 85% nitrogen; 50% carbon dioxide 50% nitrogen; 2% oxygen 98% nitrogen; 21% oxygen 1% carbon dioxide 78% nitrogen; 50% carbon dioxide 50% nitrogen; 80% carbon dioxide 20% nitrogen; 50% carbon dioxide 50% argon. With the exception of atmospheres containing oxygen, none of the gaseous atmosphere tested significantly reduced the number of unheated or heated spores leading to turbidity during prolonged storage. None of the gasses reduced the number of spores resulting in growth within 6 weeks at 10°C by a factor of 10^6 even in combination with a 10 minute heat treatment at 80°C. High levels of carbon dioxide and argon marginally increased the time for spores of non-proteolytic *Cl. botulinum* to reach turbidity but did not reduce the number of spores leading to turbidity within four weeks in medium at constant pH. However, when the headspace above culture media was flushed with carbon dioxide without maintaining the initial pH, the time to turbidity was greatly extended and the number of spores capable of growth was decreased. No growth was observed in medium containing 21% oxygen. Growth occurred in broth prepared under 2% oxygen but the number of spores required to initiate growth was increased.

Task 4. Biopreservation

The aim of this task was to study the expression of natural antimicrobial systems using modified atmosphere conditions which for instance select for lactic acid bacteria. Investigate suppression of epiphytes organisms and certain pathogens.

Since psychrotrophic pathogenic bacteria can be a specific health hazard in mildly preserved (MAP with refrigeration) fresh and minimally processed produce, ATO.DLO studied the potential to use biopreservation by employing Lactic acid bacteria (LABs) as a means to provide a natural safety hurdle for their proliferation under suitable conditions (see Task 3). At the onset of the project, over 900 LABs were isolated at ATO.DLO from fresh and minimally processed produce and screened for the production of suitable bacteriocins (activity towards a.o. *L. monocytogenes*). Three isolates were found to have the required characteristics: one strain of *Enterococcus mundtii* and two strains of *Pediococcus parvulus*. Both types produced a bacteriocin that effectively controlled growth of *L. monocytogenes* in *in vitro* studies. Both pediococci, however, only produced significant amounts of bacteriocin at temperatures over 15°C, and where not really suited for any application at lower temperature. The bacteriocin produced by both starins was fully identified and characterised and appeared to be identical to pediocin PA1, formarly only known to be produced by *Pediococcus acidilactici* (publication accepted). *E. mundtii* produced significant amounts of a bacteriocin even at 4 to 10°C. Thus, although it is not a LAB and does not have a GRAS status, the organism is a very suitable model to test the possibility that biopreservation can be the required safety hurdle towards certain psychrotrophic pathogens. The bacteriocin produced was tentatively called mundticin. It was characterised and found to be a class II bacteriocin (publication pending). Its mode of action is under study. ATO.DLO found that the application of the mundticin producer as a protective culture was very successfull on laboratory, vegetable media composed of sterilised vegetable extracts. However, on fresh, non-sterile produce, no activity was found. Most possibly, either the production of mundticin on produce at low temperature is not sufficent or the mundticin is inactivated after production (enzymatic inactivation, adsorpion to produce). Since the application of partially purified bacteriocin was found to significantly delay the growth of *L. monocytogenes*, the inactivation may not be the most prominent problem. The use of mundticin as a purified compound is not very attractive for producers of ready-to-eat vegetables. The costs to apply for a full approval are not in line with the added value. Nevertheless, use of purified bacteriocin samples with the same activity spectrum of mundticin may be a

good option for a biopreservation system.

WAU.DFS assessed the minimal inhibitory concentration (MIC) of nisin in a laboratory medium and in a food system (fat-free milk) at different temperatures (7, 21 and 37°C) using several gram-positive micro-organsims. All bacteria tested were sensitive to nisin and the MIC values were only slightly affected by temperature. The MIC values in the laboratory medium were comparable with those obtained in milk, which gives good perspectives for the application of nisin. The use of the bacteriocin nisin on minimally processed was studied further at WAU.DFS in the absence and presence of a yoghurt based salad. The number of micro-organisms increased during storage at 30°C and 7°C, whereas the addition of dressing resulted in a decrease of the bacterial population at both temperatures. Since the pH of the dressing is low (*i.e.* 3.8), this population consist of rather acid-resistance microbes, like lactic acid bacteria, yeast and moulds. The number of microorganisms capable of growth on lettuce was significantly reduced as compared to the control in the presence of nisin-containing yoghurt dressing. Inoculated *L. monocytogenes* was not able to grow on lettuce leaves containing dressing both at 7°C and 30°C, most likely due to the low pH of the dressing. In lettuce samples containing dressing with a pH of 5.4, outgrowth of *L. monocytogenes* appeared to be possible and in the presence of nisin growth was significantly reduced compared to the control situation. Comparable results were obtained with pediocin. Since the ability to develop resistance to bacteriocins is an obstacle for their application, nisin-resistant mutants of *L. monocytogenes* were isolated and characterized by WAU.DFS. The isolated mutant of *L. monocytogenes* Scott A is approximately 12 times more resistant to nisin than the parent strain. Since the primary target of nisin in sensitive cells has been shown to be the cytoplasmic membrane, the fatty acid composition of both strains was determined at 7°C and 30°C. The effect of temperature on fatty acid composition was far more pronounced compared to the difference in fatty acid composition between the two strains at one pariticular temperature. Therefore, it is likely that other factors contribute to the resistance mechanism. Results from monolayer studies with lipid extracts from both the nisin-resistant and the wild-type strain showed that nisin interacts much more efficiently with the lipids of the latter strain.

NARF isolated and identified the indigenous lactic acid bacteria dominating on different vegetables and investigated bacteriocin production. One hundered fourty five colonies were isolated from broccoli (25) and carrots (120) stored under different gaseous conditions (air; 5% CO2/5.2% O2/ 89.8% N2; 5% CO2/95% N2) at 4° or 10°C. *Lactobacillus plantarum* (2), *Leuconostoc mesenteroides* subs. *mesenteroides* (122), *Lactobacillus casei* subs. *casei* (1) and *Weissella minor* (20) were found. Most isolates (125) were screened for bacteriocin production, but no inhibition zone was observed whith *S. enteritidis* and *Staphylococcus aureus* nor with a suitable references strain. i.e. *Lactococcus cremoris* CNRZ-117. Further studies at NARF involved the effect of lactic acid bacteria on the growth of *S. enteritidis* on sterile carrots. *L. mesenteroides* was found to affect the growth of the pathogen under aerobic conditions only when it was present in very low numbers. Under MAP conditions, there was neither a significant reduction or increase *S. enteritidis* inoculated on carrots. With *L. mesenteroides* subs *mesenteroides*, which is the dominant LAB on carrots, a rapid spoilage of the product was evident. The organism grew rapidly and produced high amounts of organic acids, that lowered the pH of the carrots and destroyed their fresh organoleptic characteristics. For biopreservation of vegetables, this organism thus is not useful, unless packaging materials are used that allow for a high equilibrium oxygen tension. This, however, may only be suitable for a very limited number of highly respiring produce (broccoli, mung bean sprouts) but not for carrots.

Culture supernatants from bacteria (isolated from vegetables by ATO.DLO were tested by IFRN for their ability to inhibit proteolytic and non-proteolytic *Cl. botulinum*. Non-proteolytic *Cl. botulinum* vegetative cells and spores were susceptible to many of the supernatants tested. Encouraging the growth of bacteriocin producing bacteria on vegetables may help prevent growth by this food poisoning organism.

Task 5. Gas/product interactions

This tasks aimed at the assessment of optimal (equilibrium) gas-mixtures for the extension of the shelf life of selected vegetable products using the computerised, flow-through gas incubator system study large numbers of gas atmosphere compositions on a single batch of products concomitantly

Using automated flow-through equipment, ATO.DLO assessed the respiratory behaviour of chicory endive and mungo bean sprouts under different combinations and concentrations of O_2 and CO_2 and at two different temperatures. The data obtained were used to built appropriate mathematical models that describe the impact of O_2, of CO_2 and of the interaction between O_2 and CO_2 on the respiration of the two model products. Independent runs were performed to validate the models established.For a range of produce, equilibrium gas atmosphere compositions were determined by ATO.DLO and were fed to task 6. Independent from this, these data have been dissiminated to SMEs on a number of occasions in 1996 (especially the RETUER sequence of meetings). The organoleptical quality of mung bean sprouts and chicory endive has been assessed under a small range of different gas compostions at ATO.DLO. The most favourable conditions (at 8°C) were found to be the following conditiond *ad equilibrium* (judged by sensory aspects, respiration rate, firmness, spoilage); mungo bean sprouts (2% O_2; 20% CO_2); whole chicory endive (2% O_2; 5% CO_2); cut chicory endive (1-3% O_2; 6% CO_2). Too low oxygen tentions (about 0.5%) led to off odour development with chicory endive.

NARF invastigated the microbial biochemical and organoleptic response of shredded carrots, brocoli, lettuce and tomatos under different gaseous atmospheres (Air, vacuum pack, 100% CO_2 ; 100% N_2; 20:80 CO_2:O_2; 5:5.2:89.8 CO_2:O_2:N_2; 5:95 CO_2:N_2; 4.9:2.1:93 CO_2:O_2:N_2) at 4, 10 and 20°C. At the end of shelf-life (6 days) LABs were the dominant organisms at 10°C regardless of the packaging system. The water phase of carrots contains numerous non-volatile substances such as sugars (sucrose, glucose, fructose), organic acids (malic acid) and amino acids which could serve as energy sources to the epiphytic microflora. In the present study a pronounced drop in pH was observed during storage. The rate of decrease was higher in samples stored at 10°C than at 4°C. The pH decrease could be attributed to the production of various acids such as lactic, acetic, malic, succinic, pyruvic. Indeed a progressive increase of these acids was confirmed by HPLC analyses. In general, the production of acetic acid from lactic acid bacteria is affected by oxygen level and energy source limitation. Acetic acid production could be a beneficial trait, since it is a stronger antimicrobial than lactic acid and may efficiently reduce spoilage. NARF showed that *Pseudomonas fluorescens*, a bacterium suspected to participate in carrot spoilage was markedly inhibited. However, acetic acid accumulation will affect the vegetable odour and/or induce softening. Such organoleptic changes in texture, odour and colour were also recorded. In samples stored under 5% CO_2/95% N_2 the changes were delayed by 4 to 7 days at 4°C, as compared to storage under air or 4.9:2.1:93 CO_2:O_2:N_2. The various organic acids identified in the study (acetic, lactic, citric, tartaric, and succinic) all increased progressively during storage so they may be used as chemical spoilage indicators. This allows for a fast detection of microbial spoilage, but then they first have to be better correlated to sensory changes. In some experiments, the production of ammonia was found as well. This compound increased in concentrations as well and since it is a rather typical metabolite of pseudomonads, it may be used as a more specific spoilage indicator. Interestingly, NARF could show that the changes in the organic acid profile of vegetables during storage may be due mainly, when not only, to the metabolic activity of lactic acid bacteria. At least with sterilized carrots, the organic acids profile only changes when *L. mesenteroides* was inoculated onto the samples. No changes were observed with uninoculated samples or with samples inoculated with *S. enteritidis* in the various storage conditions used in this study. This observation could be used to confirm that LABs are the main specific spoilage organisms (SSOs) in the case of (processed) carrots packaged under aerobic/VP/MAP conditions.

ULMK.DCLS studied accurate oxygen measurement, which is important to ensure that MA packages are not anoxic and, more generally, for reliable product-package compatibility data. Anoxic packs could result in off-flavour development due to anaerobic respiration and, in extreme circumstances, to growth and toxin production by *Clostridium botulinum*. Most oxygen measurement by GC fails to separate argon from oxygen. As argon is present as 0.9% of the atmosphere, these methods give an overestimation of

about 1%. The significance of this is that an anoxic package could appear to contain 1% oxygen. In this work, a custom made GC column capable of separating argon from oxygen was located and applied to evaluate 'true' oxygen levels in commercial packages. The work showed that apparently safe commercial packages were anoxic, and that for unflushed packages an allowance of 0.9% should be made for interference by argon. In the case of flushed packages, argon levels depended on storage time and package permeability. In general, flushed packages contained about 0.1% argon and this level rose during storage to 0.9%. The rate at which it increased depended on package permeability. The ability of non-GC procedures to accurately measure oxygen levels was also investigated. These were found to be accurate at low oxygen levels (more accurate than conventional GC measurements) but somewhat less accurate and consistent at oxygen levels >10%.

Also at ULMK.DCLS, the effects of raw material variables and of alternative methods of peeling, slicing, washing and storage were investigated using MA packaged carrot discs and MA packaged shredded lettuce as models. Deterioration was monitored using sensory evaluation scores, respiration rates and microbial growth. Changes in texture, pH, cell permeability, enzymatic activity, weight loss, exudate and lignin production were also determined. Microscopic observations were made of the changes in the ultrastructure of cells from the processed tissue. Both cultivar and physiological age affected quality and storage life. Cultivar effects were probably related to intrinsic perishability properties and require further study. The poorer quality of products prepared using physiologically older raw materials was due to higher microbial loads, higher levels of exudate and higher cell permeability. Commercial abrasion peeling with a coarse carborundum plate caused considerable cellular damage, enhanced inoculation of product surface with spoilage organisms, induced stress response reactions and resulted in high rates of dehydration. Use of a fine carbonindum plate considerably reduced these effects and resulted in higher sensory scores for appearance and aroma. Slicing of carrots by machine caused greater stress reactions, physical damage and higher follow-on microbial growth rates than slicing with a razor blade. A sharp machine blade significantly reduced physiological and physical damage compared with a blunt machine blade. Similar results were found with shredded lettuce, and slicing with a blunt machine blade resulted in lowest levels of retention of ascorbic acid. Addition of chlorine (100ppm) to the washing water reduced microbial loads and improved acceptability scores. These beneficial effects were larger in shredded lettuce than for carrot discs. Overall, however, these effects were small and were lost during storage at 8°C. Of all the variables examined, storage temperature had the biggest effect on acceptability scores. A reduction in temperature from 8°C to 3°C increased storage life of carrot discs by more than five days.

Task 6. Product/film compatibility (MAP)

The objkective here was to identify suitable combinations of produce, initial gas-mixture and packaging films (or coatings) that together provide favourable equilibrium gas-mixtures *in situ* (results from Task 5). Because the optimal packaging system for a certain product depends on a multitude of external (temperature, humidity, gas composition, etc. versus time) and intrinsic factors (physiological age, processing damage, etc.), match the packaging with the product is a matter of careful match-making. Based on knowledge of the physical characteristics of packaging materials and the physiological pecularities of different types of the produce (mungo bean sprouts or chicory endive) obtained in task 5, suitable packaging systems were selected and tested by ATO-DLO. It was found that mungo bean sprouts needed packaging materials that allowed for high passage of oxygen, but selectively retainment of CO_2. For chicory endive, packaging materials needed a more equal permeability for both gases. Microperforated films (P+) should be most appropriate for mungo bean sprouts, whereas OPP foils should be suitable for chicory endive. Various microperforated (P-Plus films, Sidlaw Packaging, Avignon, France) and non-perforated films were tested at INRA.AV for the packaging of bean sprouts. Too impermeable films caused a fermentation of bean sprouts but films creating atmosphere with 10 to 20% CO_2 improved quality. This was in agreement with results obtained by ATO using controlled atmosphere cabinets. Bean

sprouts carried a very high numbers of Gram negative bacteria and of lactic acid bacteria. This microflora probably had a significant role in gas exchanges and therefore increase spoilage by hastening anoxia and fermentation.

ULMK.DCLS collaborated with NBEST to solve product-package compatibility problems in commercial packages. When a range of P Plus films were evaluated, atmosphere modifications close to optimum could be identified. For example, in the case of carrot discs, use of OPP film resulted in 'true oxygen' values of zero/close to zero. Of the P Plus films evaluated, PA 120 and PA 200 were clearly too permeable and PA 60 was closest to optimum. In the case of "Salad Bowl Mix" PA 60 was closest to optimum. In the case of "Dry Coleslaw Mix" PA 90 and PA 160 both gave good results depending on the time of the season. NBEST optimized the packaging system for a number of commercial products. The following systems were found to perform satisfactorily in practice. Mixed Lettuce: Gas flushed Mixed Lettuce packed in Standard OPP held the oxygen at the target level of <3% and performed better than flushed or unflushed PA60 packs. OPP also had a slightly lower bacterial count at the end of shelf-life. Carrot Discs: Based on sensory analysis, PA120 and Pa60 films are suitable.
Coleslaw Mix: Both PA120 and Standard OPP were suitable, although the product may go "sour" in Standard OPP at certain times of the year due to seasonal variations of the ingredients involved. Wrapping is stretch film, the current practice, gives an even better quality produce. Beansprouts: Beansprouts gave the best quality at the end of shelf-life (6-7 days) with Standard OPP film and without flushing.

Task 7. Biodegradable films and coatings

This tasks aimed at the development films and coatings from natural polymers which are food-grade and have the appropriate physico-chemical characteristics to function as MA-barriers. Due to their relatively low water vapour barrier properties, most hydrocolloide-based films can only be used as protective layers to limit moisture exchange for short-term applications. CIRAD.SAR therefore studied the design and physico-chemical properties of hydrocolloid-based films with very high water vapour permeability which should be better suitable for application with fresh foods such as fruits and vegetables in modified atmosphere packages. It was found that inclusion of lipid compounds into the film formulation drastically reduced water vapour permeability. Water is not very soluble or mobile in lipid-based films because of the low polarity and dense, well-structured molecular matrixes that can be formed by these compounds. Nevertheless, wax or lipid based materials are commercially applied to many fruits and vegetables to reduce dehydration and improve consumer appeal. For hydrophilic films at low a_w, water vapour permeability is generally relatively low. It was found that increasing the a_w leads to an increase in film moisture content (non-linear sorption isotherms), in film plasticization due to water absorption and, consequently, a decrease in water vapour barrier properties.

Investigating the control of gas exchange in edible films, CIRAR.SAR found market differences for identical films between water vapour on the one hand and gas (CO_2 and O_2) permeability on the other. Gas diffusion is known to be crucial for gas permeability, whereas both sorption and diffusion are essential for moisture transfer. Materials with suitable O_2 barrier properties are required to protect oxidizable foods (to reduce rancidity, enzymatic browning and vitamin loss), but for fruits and vegetables the O_2 and especially CO_2 permeabilities are most essential. The O_2 and CO_2 permeabilities of numerous biopolymer-based and synthetic films were evaluated. It was found that, generally, hydrocolloid-based films have impressive gas barrier properties, especially against O_2, but only when they are not moist. For instance, O_2 permeability of wheat gluten film was 800 times lower than low density polyethylene and twice lower than polyamide 6, a well know high O_2 barrier polymer. When moisture was present, the macromolecule chains apparently become more mobile which leads to a substantial increase in O_2 and CO_2 permeability. Lipids, which are very often used to delay water transfer, also have significant effect on O_2 barrier properties.

In collaboration with INRA.AV, CIRAD.SAR conducted experiments on real food that proved that the selective gluten-based films lead to the creation of very original atmospheres when used to wrap fresh

vegetables. Evolution of atmosphere composition around fresh mushrooms placed in a glass jar covered with a conventionnal microperforated film, a commercial selective polyether amide film (Pantek marketed as "Pebax" by Atochem), or a gluten film were evaluated and compared in detail. With the gluten film, the MA equilibrium gas composition was 2-3% CO_2 and 2-3% O_2, which is favourable to the mushroom overall quality.

ULMK.DCLS investigated the effects to (bio-)coating technology on the quality of carrot discs that had been subjected to coarse abrasion peeling. Normally, appearance scores decline rapidly for these products due to dehydration and stress response reactions such as lignin synthesis. Use of the commercial coating 'Nature Seal' or a laboratory prepared pectin-calcium chloride coating advised by CIRAD.sar considerably improved product appearance. Other coatings based on gluten (Opta Glaze) or oil coatings were not successful. ULMK.DCLS also evaluated commercial samples obtained from NBEST. These samples were packaged using optimal packaging films (task 6). Best quality was obtained with carrot discs in PA 60, with "Dry Coleslaw Mix" in PA 120 and with "Salad Bowl Mix" packaged in OPP and flushed with nitrogen.

Task 8. Active edible coatings (MAC)

In this task the use of edible coatings in which functional components (antimicrobials) are integrated ("active coatings") is evaluated. By their specific "retention" or "(slow-)release" properties for additives, edible coatings loaded with these compounds can modify and control the surface conditions of the coated food products very locally and targetted. Using sorbic acid as a model antimicrobial, CIRAD.SAR investigated wheat gluten and lipid based films. Diffusion of sorbic acid was found to be influenced by various parameters: film characteristics (type, manufacturing procedure), food characteristics (pH, a_w), storage conditions (temperature, time) and solute characteristics (hydrophilic properties, molar mass). The mechanism of sorbic acid migration onto model foods such as aqueous gels (1,5% agar) was studied. Predicted diffusion of sorbic acid was in close agreement with experimental diffusion.Pectin films, gluten films and gluten/monoglyceride derivative composite films containing sorbic acid, were found to delay the development of *Penicillium notatum* on a model food (0.97 a_w and 30°C) for more than 1, 2, or 4 days, respectively, as compared to controls where sorbic acid was directly deposited on the food surface.

Some of the films developed by CIRAD.SAR to which sorbic acid or natural antimicrobials (the bacteriocins munditicin and nisin) were added, were tested at ATO.DLO on minimally processed foods (mung bean sprouts, chicory endive). Their efficacy was compared to gluten and alginate coatings that are commercially available and to antimicrobial application via dipping. In a limited set of experiments, it was found that the speed at which the (natural) antimicrobials are released from the all the coating materials used was too slow to be optimally effective. In addition, with regard to the bacteriocins, the stability of the antimicrobial agents on fresh produce surfaces may not be good enough under the experimental conditions used. In fact, simple dipping was the most effective treatment, although this has the obvious disadvantage in practice that the product surface is wetted and a high dose of the preservative is applied. It is concluded that it takes careful control of both the active coating technology and the application environment to obtain sufficient activity *in situ*. To enable this level of control, more knowledge needs to be obtained on the interplay of the most crucial variables in the total, integrated system: coating, antimicrobial, produce, packaging, refrigeration.

Further active coatings studies were perfomed at INRA.AV and involved the coating of cooked sweet corn which is used by FRUID [9] in ready-to-eat dishes together with minimally processed vegetables and for which it was observed earlier that the sweet corn increases spoilage (task 2). The physical properties of different edible coating formulations were studied first in collaboration with CIRAD.SAR. Coatings based on zein (10%) and zein (10%)+Myvacet (1%), both prepared in ethanol and applied in two layers on the grains were found to give good physical properties. However, the residual ethanol was thought to have pronounced antimicrobial effects and studies with sorbic acid containing films should await until a

procedure has been designed that avoids the presence of ethanol in the coating. The required dose of sorbic acid to completely inhibit growth of *L. monocytogenes* on the surface of coated sweet corn at 10°C was found to be about 0.1%, using aqueous solutions of the compound. This dose is in the range of concentrations authorized for food use.

Task 9 and 10. Safety and quality evaluation

The objective in this task for the final year was to to study the *in situ* growth/survival of pathogens (task 9) and to perform organoleptic/sensory evaluations of product quality (task 10) regarding the improved or newly developed (whatever is appropriate) mild preservation treatments

In these task, all participants contributed their individual expertise. The safety evaluation was performed by ATO.DLO, WAU.DFS, INRA.AV, NARF and IFRN. relevant findings have been reported already with the various tasks.

As for the quality of fresh or processed vegetable-based food products subjected to improved or novel preservation techniques under practical conditions, the practical evaluation at partners 8 (FRUID) and 9 (NBEST) was adequately covered by partners 3 (INRA) and 7 (ULMK.DCLS), respectively. The findings are reported with the specific technologies discussed above.

The finding at NARF, that organis acids and ammonia profiles in stored produce can be used as chemical indicators of microbial spoilage may be open the possibility to establish fast reading systems of spoilage for use in food processing companies.

Task 11. Modelling

Aim was to integrate data on quality, safety and preservation techniques from various tasks into a predictive model (to the extend possible). Data collected by ATO.DLO, INRA.AV and ULMK.DCLS were processed by ATO.DLO through a suitable mathematical modelling software (Dmodel; author József Baranyi). This allowed for an objective evaluation of the impact of MAP conditions on the growth of *Listeria monocytogenes* on model media, raw chicory endive and mungo bean sprouts (ATO.DLO data). In addition, the influence of temperature and mixing cooked vegetables with raw vegetables on the growth rate of this pathogens were valuated (INRA.AV data). Finally, the impact of handling on the growth rate of spoilage micro-organisms was calculated using a sepearte set of data (ULMK.DCLS).

Data on the growth of non-proteolytic *Cl. botulinum* in cooked mushroom, potato and cauliflower, under different environmental conditions relevant to sous-vide products, have been collected by IFRN. These data were were fitted using the Baranyi equation. This enabled calculation of the maximum specific growth rate and the lag time. The possibility of using advanced mathematical techniques to interpolate other data sets on this project was examined.

Data obtained by NARF on the interaction between packaging/storage conditions and the rate of growth of spoilage micro-organisms (*L. mesenteroides*) and pathogens (*S. enteritidis* and *L. monocytogenes*) have been processed using the modified Gompertz and the logistic equation as well as the D-model software. For the various experimental data, the models were used to generate the maximum specific growth of the different microorganisms.