RESFOOD

RESOURCE EFFICIENT AND SAFE FOOD PRODUCTION AND PROCESSING

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Part 1 Final publishable summary report

Executive Summary

The increasing demand for healthy and nutritious food requires an efficient use of the natural resources allocated to food production and processing. On average over 40 % of the water abstraction in the EU is used for agriculture and 20 to over 50 % of the water and the nutrients used disappear to surface and ground water. Also in food processing a lot of water is spilled, together with the energy in the water. Finally over 40 % of the food production is lost and wasted in the different stages of the food chain. The EU funded project RESFOOD develops technologies and tools to overcome barriers to a resource-efficient food chain, leading to a reduction of water, nutrients and energy use and the recovery of valuable materials. Besides research and demonstration of water-treatment, disinfection and reuse, much attention is paid to improve food safety through new and fast monitoring and detection methodologies.

In horticulture ICT solutions resulted in a 40 to 60 % water and nutrients saving in soil based cultivation. Using recirculation in soilless cultivation can lead to a further reduction of the water and nutrient use to over 90 %. To prevent accumulation of salts, pathogens and plant protection products the treatment of the water by filtration (f.i. electro-dialyses) and disinfection (ozone) was successfully tested in practice.

In the fresh-cut vegetable industry the treatment and reuse of washing water has been demonstrated in practice, resulting in a 30-50 % reduction of energy and water use. A new developed washing machine makes a reduction of water use of 20 – 30 % possible. This machine will be brought to the market at short term. Peroxy acetic acid can be applied as alternative disinfection chemical for chlorine, avoiding cross contamination and reduce health and environmental impact. Also in fruit juice production, water re-use showed to be possible with the use of AiRO.

Different valuable products, like carotenoids, polyphenols and terpenes could be recovered from food waste. In general the recovery of these minor products is economically only feasible in combination with a major compound. Proteins – a major product - are successfully extractable from endive at pilot scale and dried to a protein powder with 50 % proteins.

Food safety is the most important point of attention in developing new food production and processing concept. An innovative biosensing concept is developed and tested at pilot scale, decreasing the detection time of pathogens to less than an hour. Also the IS-Pro technology, first developed for medical applications was further developed and tested for food applications, together with a new filtering device.

The broad dissemination of the results via all type of media and the very successful final conference make a very important contribution to the more intensive cooperation between the water and the food sector and will speed up the market uptake of the solutions developed.

1. Project context and objectives

1.1 Background and concept

Improving resource efficiency within and across all sectors of our society is not only recognized as the way to go to reduce our societal ecological footprint and to preserve our fragile environment and its ecosystems services, but also as an opportunity to create a new European economy with strong global competitive advantage by 2020. In the food chain many natural resources (water, minerals in fertilizers, soil, biomass and energy) are used both in crop cultivation and food processing and additionally significant amounts of bio-wastes are produced. Besides huge water and energy losses, 20 -50 % of the nutrients and 30 % of all food produced in Europe is lost and wasted. A growing population, production scale up, higher need for resources and competition with other users of these resources require the sectors to become more efficient.

Resource efficiency in the food chain makes a very valuable contribution to a circular economy. Re-use and recycling of water, but also of other resources, reduces the pressure on these resources and the emissions to the environment.

The project RESFOOD addresses the essential topics in the food chain towards resource efficiency, also taking into account the most important precondition of food safety. This will lead to:

- Reduced use of resources input by recycling and re-use (Nutrients, Water, Energy)
- Maximized resource productivity (Nutrients, Energy, Biomass)
- Recovery of valuable bio-based compounds
- Increase of food safety through efficient environmental friendly disinfection strategies and new fast detection and monitoring

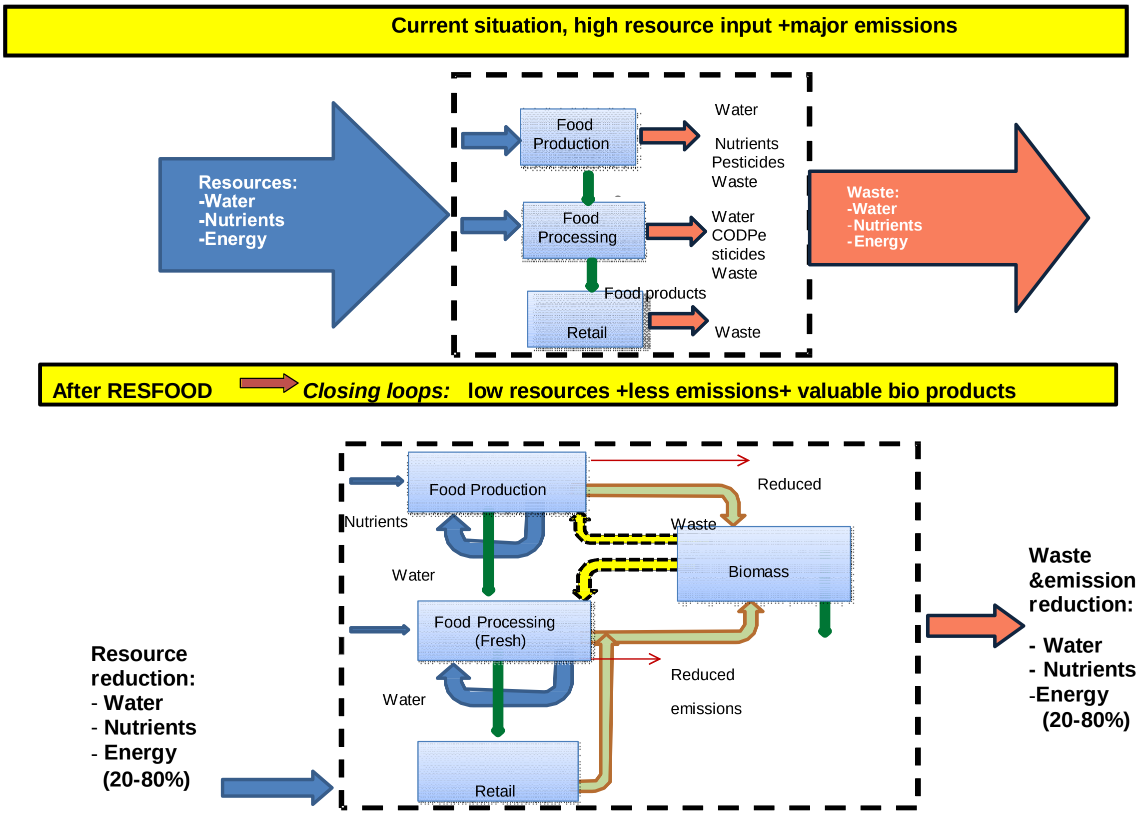

Figure 1 the RESFOOD concept

Key role of water

Within the Food Chain water plays a crucial role and also in relation to resource efficiency it is, besides an important resource, also related to most of the other resources. Therefore, the RESFOOD concept is strongly focusing at water saving, re-use and recycling of water results in an efficient use of most of the other resources. Additionally, it reduces the emissions to surface water and groundwater and reduces other environmental impact of cultivation and processing.

Focus at fruits/vegetables in horticulture, fresh cut food processing, recovery of valuable materials and advanced rapid detection methods

The food chain is very diverse in terms of both horticulture and food processing, as well as in the final products. As water is regarded as the key-factor RESFOOD focuses on a number of links of the food chain where water saving, re-use and recycling leads to big opportunities for increasing the resource efficiency and safety:

- Food Production: Focus at Horticulture: savings in soil based and in soilless cultivation
- Food Processing: Fresh Cut Vegetables and Fruit Juice: savings in warm and cold water reuse
- Re-use of valuable waste: Biomass for recovery of valuable products
- Food safety: Improved disinfection and advanced rapid detection

1.2 Overall objectives

The development and transfer of innovative technologies for resource efficient and safe food production and processing, realizing**:**

- Minimization of input and maximization of productivity of resources
- Minimization of water emissions and biomass wastes
- Re-use/recycle of the resources: water, nutrients (fertilizer), energy
- Recovery valuable, bio-based compounds from biomass wastes
- Affordable, safe and effective disinfection of pathogens of human concern
- Detection and monitoring of pathogenic contamination to secure food safety

As the development of technologies that are applicable in practice is one of the main objectives a lot of attention has been paid to pilot and demonstration. Four horticulture locations (Netherlands and Spain) and three food processing industries (Netherlands, Spain, and Turkey) are selected to test the developed technologies and methods in practice. For technologically successful solutions the aim was also to do an assessment and develop implementation plans.

Finally, based at general concept (see Figure 1) and using the results of the laboratory and pilot testing it was the objective to develop more integrated resource efficient (green) concepts taking into account the whole food chain and the related resources.

Objectives Horticulture:
Developing new technologies and methods for

- Minimize the input and maximize the productivity of resources (water, nutrients)
- Minimization of water emissions
- Recovery and re-use/recycle of the resources: water and nutrients (fertilizers)

leading to

- up to 70% reduction of intake of fresh water resources
- up to 80% reduction of losses of nutrients/fertilizer and related emissions to surface- and groundwater

In Horticulture we distinguish the conventional or optimized (stages 1 and 2) *soil based* cultivation and the *substrate* or hydroponics (soilless) cultivation (stages 3 and 4); see Figure 2 below.

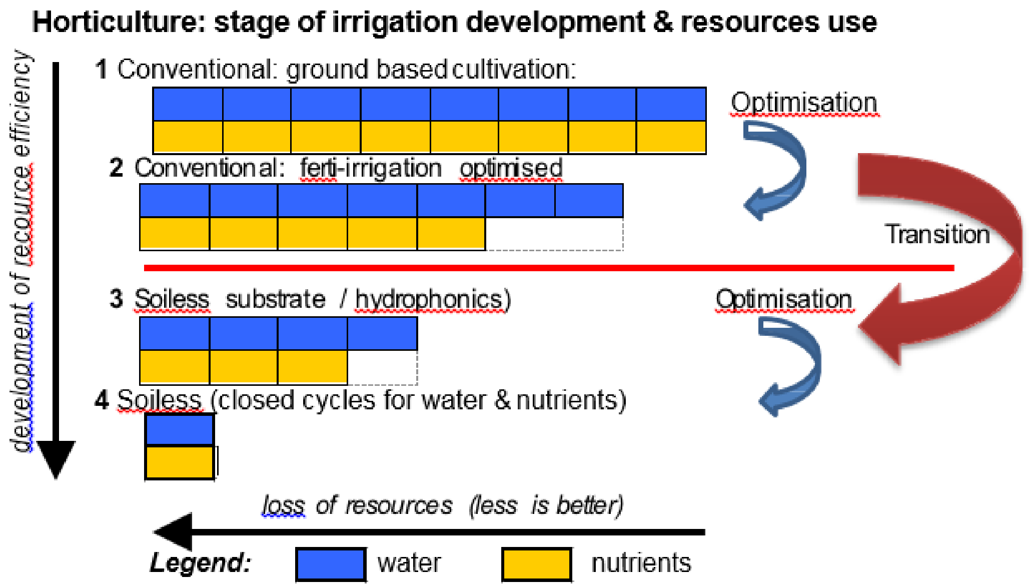

Figure 2 Horticulture stage of irrigation development & resource use

RESFOOD pays attention to both levels. For the soil based horticulture steps will be made to optimize water use and combined with this the use of nutrients (ferti-irrigation), while ultimately developing soilless solutions. In soilless cultivation crops, further innovation steps will be made by closing the water cycle, leading to increase of resource efficiency. The possibility of stage- changing depends heavily on the crop and the local situation (soiltype, climate, socio-economic situation).

Objectives Food processing (fresh cut vegetables and fruit juice)

- Development of new treatment and disinfection technologies for re-use/recycle of water to minimize the input and maximize the productivity of the main resources: water and energy
- Effective disinfection/decontamination of fresh-cut vegetables by chemical and physical treatment as alternative for chlorine-based processes
- Rapid detection and monitoring of pathogenic contamination to secure food safety
- Validation, verification and demonstration of the IGC's at pilot/demonstration scale

leading to

- 30 - 75% reduction of water use
- 25 - 60% energy savings
- Minimization of water emissions and waste
- Safe fresh-cut vegetables (3-4 log cycle reduction)
- Less chlorine by products (50% reduction up to complete removal)

The focus will be at the fresh-cut and fruit juice industry and the treatment and reuse of washing/process water at low and high temperature. This leads to a combined saving of water and energy of 25 to 75%. A holistic approach based on methods capable of removal organic and inorganic compounds and disinfecting efficiently both the process water and the product, allowing a high ratio of recycling without undermining health and safety issues.

For the treatment both existing and emerging technologies will be further developed for these applications under the specific circumstances. For disinfection different technologies (other than chlorine) will be tested for the inactivation of the most important pathogens. To optimize the sanitizing effectiveness of fresh-cut fruit and vegetables, the effectiveness of a combination of physical and chemical treatments (multi hurdle tool) will be assessed. The expected benefits are higher sanitation effectiveness and reduction of chemical emissions, like carcinogenic chlorinated compounds. This topic is a response to increasing consumer demand for products, which are free from harmful chemicals, are safer for human health and more sustainable for the environment.

Objectives By-product valorization from horticulture, food processing and retail

- Identification of biomass resources for recovery of valuable compounds
- Development of technologies for extraction of valuable products
- Development of a cascade approach for re-use and valorization of biomass with:
  - Valorization of by product/waste as food, feed, material
  - Maximum production of energy
  - Optimal loop closure of nutrients and organic carbon to agriculture

This should lead to:

- **Environmental friendly pre-treatment** methods to make extraction of useful bio-products effective:
- **Extraction technologies** for a number of bio-active compounds sesquiterpene lactones from endive, pectins from citrus apple, carotenoids and colorants from endive or citrus) based on the use of a food grade extractant (e.g. water, alcohol, CO_2):
- **Decision tool** to support the food processing industry to decide which type of valorization (product, extract, energy production,…) and which application (food, feed, material, energy) is applicable for their by-products. This will enable companies to choose
- **A methodology for a cascade approach** to achieve maximum return of the agricultural products as food, feed, material and energy with conservation of the soil fertility as a very important limiting condition. Enough nutrients and organic carbon need to be returned to the land to achieve a sustainable production.
- **A cost-benefit analysis** will be carried out in order to assess the economic feasibility of extraction and isolation of the bio-active compounds

Objectives Advanced detection of pathogens

Development of rapid detection and monitoring methodologies of pathogens in (recycled) water streams by:

- Optical biosensing
- ISpro technique (new PCR-based profiling technique)
- Molecular techniques
- Design of a prototype and testing of the detection methodologies in practice

This will lead to

- More early and accurate identification of pathogenic bacteria
- Better insight in the bacterial population
- Improved monitoring of food quality and safety

2. Main S/T results and foreground

Horticulture

2.1 Water recirculation in soil-less cultivation of strawberries and blackberries: direct recirculation vs disinfection treatments

At the research station of ADESVA four recirculation systems were studied for soil-less cultivation of strawberries:

- NR (No Recirculation
- RND (Recirculation with No Disinfection)
- RDIO (Recirculation with Disinfection by Ionized Oxygen)
- RDO (Recirculation with Disinfection by Ozone).

The three recirculation systems consumed approximately half the water of the non-recirculating system.

From analysis of the studied parameters it was concluded that there are no significant statistical differences, with a confidence level of 95%, in the vigour of the plants nor firmness or ºBrix of the fruit, between the four systems studied. In the case of early production, 1st category and total production there were no significant statistical differences between three recirculated systems. However, there were significant statistical differences compared with the non-recirculated system. The best results (highest values) were obtained with RND, RDIO and RDO. Also, there were significant statistical differences showed in 2nd category production and the average weight of fruit between NR and RDO, getting better results higher in the last one.

The concentrations of nutrients in the fertilizing solutions were lower than designed, which had a negative effect on crop yield, so that this was lower than expected in all four soilless cultivation systems.

Regarding the results of water treated with ionized oxygen, the disinfection values were not good enough. However, disinfection with ozone showed very positive overall results in the first and second analysis, providing lower bactericidal effect at the end of the season.

Comparing the electrical consumption of each of the disinfection systems along the season the ozone system consumed approximately 53% less energy while obtaining much better results in pathogen control.

In the case of the pilot on the commercial farm Agricola El Bosque (black berries):

1. There were significant statistical differences in the production of fruit both in the disinfection of water as in the applied irrigation flow indicates that both variables adversely affect fruit production.
2. There were no significant statistical differences either in the average weight of the fruit, or firmness or in brix degrees, or vigour of the plant, that indicates that neither the disinfection of drainage water neither the different kinds of irrigation flow would affect the aforementioned parameters.
3. There are statistically significant differences in the postharvest behavior of fruit produced with reference the type of employee irrigation flow indicates a marked decrease in the damage caused by fungi along the life span of fruit harvested according the contribution of irrigation water is reduced.
4. The yearly savings for Agricola El Bosque will be around € 45.000 year. For moderate growers in Huelva it will be around € 20.000/year. Besides this however the availability of enough water in the future and the environmental impact (see below) will be even more important

Environmental impact

In table 1 the **consumption of water** over the course of the whole season in the 4 systems in the trial is shown. The three recirculation systems have a very similar consumption to each other and with respect to the non-recirculation system the **water savings is approximately 40%.**

Table 1 Water Consumption in the pilot at ADESVA research station

Cultivation system	Water consumption (m^3/ha)
NR: No recirculation	2635
RND: Recirculation without disinfection	1576
RDIO: Recirculation disinfection ionized oxygen	1583
RDO: Recirculation disinfection ozone	1564

In the ADESVA experiments, it was not necessary to do “purges” (emptying part of the contents of the fertilizer tanks) in any of the three recirculation systems, as there were no recorded atypical electrical conductivity or pH values which might represent a risk to the normal functioning of the strawberry plants. Fertilization Unities consumed (N, P2O5, K2O, OCa y OMg) in ADESVA´s trial along season 2014-15, comparing recirculated systems with no recirculated systems, decreased between 38 and 43%. This is shown in the following tables 2, 3, 4, 5 and 6.

Table 2 Fertilizer units (N)

Cultivation system	Fertilizer units of Nitrogen (Kg/ha)	Differences between recirculated systems and no recirculated system (NR) (%)
NR	260	-
RND	154	41
RDIO	149	43
RDO	151	42

Table 3 Fertilizer units (P2O5)

Cultivation system	Fertilizer units of P2O5 (Kg/ha)	Differences between recirculated systems and no recirculated system (NR) (%)
NR	116	-
RND	68	41
RDIO	71	39
RDO	69	41

Table 4 Fertilizer units (K2O)

Cultivation system	Fertilizer units of K2O (Kg/ha)	Differences between recirculated systems and no recirculated system (NR) (%)
NR	272	-
RND	164	40
RDIO	161	41
RDO	163	40

Table 5 Fertilizer units (OCa)

Cultivation system	Fertilizer units of OCa (Kg/ha)	Differences between recirculated systems and no recirculated system (NR) (%)
NR	193	-
RND	117	39
RDIO	114	41
RDO	115	40

Table 6 Fertilizer units (OMg)

Cultivation system	Fertilizer units of Omg (Kg/ha)	Differences between recirculated systems and no recirculated system (NR) (%)
NR	66	-
RND	41	38
RDIO	39	41
RDO	40	39

In the commercial farm AGRICOLA EL BOSQUE, along season 2013-14 two irrigation strategies were applied, one conventional irrigation (100%) and one with a reduced watering (58%), obtaining very positive results. There were no statistical differences in yield and fruit quality between both trials. **It was achieved to reduce the water and fertilizer consumption up to 42%.** In season 2014-15 (deliverable 8.2), the reduced strategy of 58% and another more reduced strategy of 26% was applied. For the last one, yield was significantly lower in relation with the strategy of 58%. In conclusion, 26% reduced strategy is not viable.

2.2 Fertirrigation management optimisation in the cultivation of strawberries in soil.

The water consumption (m^3/ha) and fertilizing units (kg/ha) for each of the theses was monitored. Moreover, the yield and fruit quality were followed according to the needs of the crop, measuring the following parameters: precocious production, 1st category production, 2nd category production, total production, average fruit weight, plant vigour, firmness and ºBrix of the fruit.

There are only statistical differences, at a confidence level of 95%, in the **vigour** of the plants, **production of 1st category** and in **total production**, between the three irrigation tapes (2.5, 3.8 and 5.3 l/h and l.m.). For the remaining parameters, both those associated with production (early production, 2nd category production, average weight of fruit) and with the quality of the fruit (Brix degrees and firmness), the plants showed a similar agronomic behavior in the three irrigation tapes studied. With respect to the other two tapes, the tape with the least flow (2.5 l/h) had minor losses of water and fertilizers towards deeper layers, which are of no use for the plant: drainage percentages are of around 21%, 38% and 32% for the 2.5, 3.8 and 5.3 l/h l.m. tapes respectively, which represents between 55% and 49% less leaching: this implies environmental benefits due to a reduced contamination of the soil and underground and surface waters."

However, taking into account the crop profitability, it can be concluded that under conditions of present study, the tape achieving the **greatest efficiency**, with the best use of water and nutrients by the plant, was the **5.3 l/h and l.m.** tape. This tape gave a production of 4.3 kg/ha higher than the 3.8 l/h tape and 6.1 kg/ha than 2.5 l/h tape. This represents a production increase of 8.8% and 12.5 % respectively. This was achieved with a similar water consumption comparing with 3.8 l/h tape and 20% higher than 2.5 l/h tape.

Environmental impact

In Table 1 the cubic meters of water consumed per hectare are exposed to each of the irrigation tapes tested in the study (following RESFOOD methodology) compared to the average consumption of water in the cultivation of strawberries in Huelva.

Table 7 Water Consumption

Input	Resfood (m3/ha)	Huelva (m3/ha)	Difference
Water	2052 (2.5 l/ha)	5400	62%
	2471 (3.8 l/ha)		54%
	2551 (5.3 l/ha)		53%

The average consumption in the province of Huelva shown in Table 7 is provided by University of Cordoba research: "Towards Sustainability in the strawberry crop: Analysis of the real demand for irrigation and possibilities for improvements". In this study 23 commercial farms were characterized, obtaining an average of water consumption per season of strawberry crop of 7027 m3/ha, being the most frequent value 6200 m3 / ha.

It must be taking into account this study was developed for an average time of 7.5 months per season. However, pilot test carried out in "Las Acciones" was developed in 6.5 months due to the sharp decrease in the price of strawberries in the market. For this reason, the value shown in table 1 is obtained prorating the most frequent value (6200 m3/ha), for one month less of season (6.5 months), resulting 5400 m3/ha approximately.

According to the data shown in Table 1, a reduction of water consumption around 60% for the lowest flow is observed in relation to the other two tested irrigation tapes.

Table 8 Fertilizer Consumption

Water Consumption	Fertlizer Unities (kg)				
	N	P2O5	K2O	OCa	OMg
Huelva (5400 m3/ha)	250	100	270	200	80
Resfood (2551 m3/ha)	127	51	184	101	41
Diferencia (%)	49.2	49	31.8	49.5	48.7

Table 8 shows the average fertilizer units consumed in Huelva for 5400 m3/ha of water applied in the cultivation of strawberries. If it is compared the fertilizer units used in the RESFOOD project for 2551 m3/ha of water applied with irrigation tape 5.3 l/h (this is the tape which provided higher production), it is possible to reduce the macronutrient used in 30% of K2O and 50% of the rest how it can see in Table 2.

All of it entails an important reduction in loss of water and fertilizers through leaching towards deeper layers, thereby reducing pollution of groundwater and surface water.

In Table 9 it can be seen the tape 5.3 l / h had a value of water footprint similar to the other two tapes and a significantly higher production.

Table 9 Water footprint for each of the three tapes irrigation

Tape irrigation	Total m3/ha	Production (kg/ha)	Water Footprint (l/kg)
2.5	2052	42636 b	48
3.8	2471	44439 b	56
5.3	2551	48744 a	52

Values followed by the same letter are not significant different with an $\alpha = 0.05$

According to previous studies, the water footprint of growing strawberries in Huelva ranges from 140 m3/t (Aldaya et al., 2010) and 173 m3/t (Adams et al., 2009).

Regarding the fertirrigation management optimisation in the cultivation of strawberries in soil, the pilot tests performed by Adesva validated the use of the ICT methodology to achieve the highest efficiency in the use of water and fertilizer in this crop reducing the potential for contamination of groundwater and surface water. The optimum needs of water and fertilizers in the cultivation of strawberries, remaining profitable and with similar fruit quality were established, allowing a reduction around 50% of water consumption and 30 to 50% of fertilizers consumption, compared to the average consumption in the cultivation of strawberries in the region.

2.3 Treatment of drainage water of substrate growth and re-use of Water and Nutrients

Current state of the art in greenhouse horticulture production is the use of soilless growing systems using substrate materials and drain water recycling and is very efficient in terms of water footprint. This recycle however also causes the accumulation of sodium and other monovalent ions as ballast components to harmful levels since these are not being used by the crops. As a result of this water is still being drained. Because this also implies emissions of crop protecting agents, it is expected that measures such as closing the water cycle or a water treatment unit will become compulsory.

Based on desk and laboratory research it was concluded that electro-dialysis (ED) offers the best perspective for closing the water cycle in greenhouse horticulture growing systems. Water and nutrients are both valuable resources which are to be recovered as much as possible, while salts (Na+), growth inhibitors, micro-organisms and crop protection agents should be removed or degraded to prevent damage to the crops and/or emissions. Based on the results it was expected that ED treatment should capable of removing Na+ at the same rate as it is brought in via the irrigation water. This means that ED can be used to maintain a steady state concentration of Na+ in the drain water, thus preventing accumulation. The use of ED not only prevents Na-accumulation but also produces a concentrate suitable as fertilizer for third parties (e.g. agriculture).The ED process concentrates the monovalent ions to a Na+, K+,NO3- concentrate. The valuable multivalent ions are retained and recycled to the greenhouse irrigation system.

Therefore pilot testing of an ED-R electrodialysis installation using monovalent selective membranes, on-site at the Demo Nursery, under real conditions was performed. The aim was to investigate:

- The capability to maintain a steady state concentration of Na+ in the drain water
- The capability to control the rate of monovalent ion removal
- The effect of a steady Na+ concentration on crop growth
- Possible bottlenecks and solutions, which were not yet encountered at lab-scale

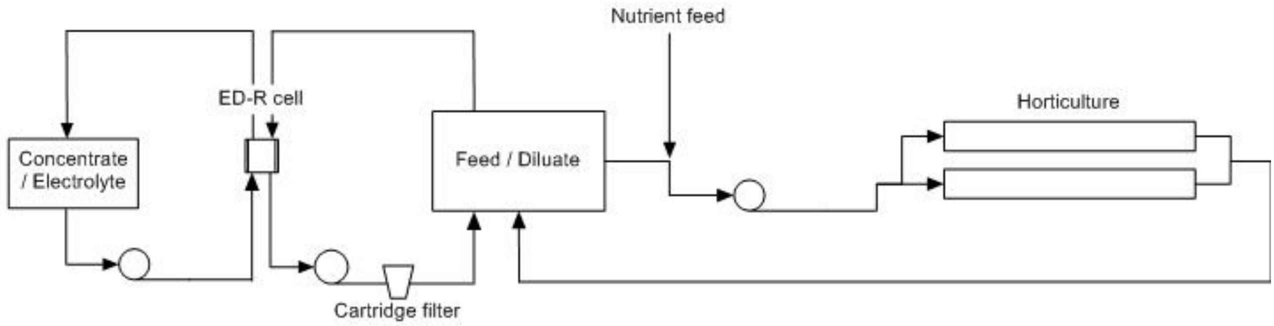

Figure 3 Flowchart ED-R pilot setup

Figure 4 Pilot setup with different growing systems and ED-R pilot

The results are that selective removal of monovalent ions from water has been demonstrated in both lab and practical setup. An average of 70% monovalent ion removal selectivity was achieved in the pilot setup under strained conditions. Operation of an ED-R system in a horticultural environment is capable of good performance if sufficient pre-treatment is available. Pre-treatment is required to prevent biological fouling and to prevent any organic material from clogging the ED-R cell. Taking into account that the pilot setup was a converted lab setup, it can be expected that a large scale set-up will be a more resilient system.

Continuous operation of an ED-R in a horticulture water supply can decrease the concentration of sodium in the circulation water. A disadvantage of continuously running the ED-R is the removal of nutrients like nitrate and potassium.

The research to the effect of the ED-R technology on crop production performance and quality was not conclusive however. There were effects of the artificial elevated sodium levels in the irrigation water on the crop production but this could not been determined as a quantitative significant effect. The levels adjusted might be too low to influence the tomatoes, but for other types of crops these are too high levels. With the experiments it is proven that with ED-R it is possible to maintain the lower levels and make further closure of the water cycle possible. Depending on the circumstances the system can be turned on or switched off.

Food processing

2.4 Exploration of the reuse options in food-industries using the Water Quality Management methodology and software

The boundary conditions for cold and hot water reuse have been determined by assessing and characterizing the water systems of the three participating food companies (VEGAMAYOR, VEZET, and ARISU). For each of these companies the possibilities for reusing water streams and technologies suitable for achieving the required water quality have been determined using the Water Quality Management methodology developed within the FP7 Aquafit4use project. The step-by-step approach of the WQM methodology is described in Table 10.

Table 10 Step-by-step approach Water Quality Management methodology

Step	Approach
Inventory of the water system Selection of relevant parameters Inventory of the flow and composition of the water streams	Questionnaire for end-users
Simulation of current system	Simulation in WESTforINDUSTRY
Formulation of re-use options	Assessing possibilities offered by using alternative water sources and/or by implementing treatment technologies
Simulation of re-use options in WESTforINDUSTRY	Simulation in WESTforINDUSTRY
Evaluation of simulations results and advised for technology research in Task 3.2 – 3.4	

The re-use options have been simulated using the WESTforINDUSTRY software tool[1] to determine the effect of implementing treatment technologies on the water composition and to determine whether this composition complied with the boundaries set for the processes in the water system. The results have been discussed with the experts of TNO and the companies involved. The main conclusions of this research are:

- Based on the use of the WESTforINDUSTRY tool disinfection + removal of BOD/COD, micro-pollutants (e.g. pesticides, TOC, polyphenols and additives) and turbidity is necessary for re-use of water from washing/cleaning and rinsing for both VEZET and VEGAMAYOR.
- Membrane filtration technologies (MF, NF, RO) are possible options for removal of the bulk COD and (partial) disinfection; fine sieves are an option for removal of TSS/turbidity, selective or additional treatment with Membrane Assisted Affinity Separations (MAAS) is possible for removal of micro-pollutants.
- For disinfection conventional treatment technologies might be applicable (UV, membrane processes) but also the more emerging technologies like PEF, Ultrasound and Cold combustion.

The work performed clearly showed the benefit of the WQM Methodology, which helps to gather all required information and determine reuse options for the water system in a structured way. The software tool allows for simulating the water system using the data gathered, determining possible bottlenecks for both the current practice and the defined reuse options and enabling the user to test

[1] See, amongst others, http://www.aquafit4use.eu/userdata/file/Final%20Conference%20Presentations/Session%204%20and%205/Optimization_of_waternetworks_in_foodindustry_052012.pdf

the effects of various new treatments. In this way, the software tool gives the end-user a very clear insight into the measures that can be taken to enable reuse and overcome any potential bottlenecks and help to decide which technologies are most suitable for tests and demonstration on-site.

2.5 Development and demonstration of innovative fouling prevention concept for membrane filtration

TNO have been working on the development of a hybrid system for adsorption and membrane filtration. As adsorption proved to be economically unfeasible (due to unexpectedly low adsorption rates), the focus was shifted to fouling prevention by the addition of particles to the membrane system. The hypothesis was that fouling prevention would enable the use of more open membranes, which could give considerably higher fluxes (m3/m2 membrane/h) at low differential pressure without a loss in production due to fouling.

The test with Low pressure particle assisted micro/ultrafiltration (LPPA-MF/UF) were started with a setup comprising of a number of flat sheets membrane holders in a submersible frame.The particles were added to the water in the tank and circulated alongside the membranes by a mixer.

The results obtained with the flat sheet membrane setup showed that the particles and mixing were successful in maintaining a higher and near constant water production per unit area of membrane. In comparison, without particles the flux was often up to 50% lower and tended to decrease to less than 20% of initial production within several hours.

Although the technology showed good results, it was not yet demonstration ready. Therefore, it was chosen to demonstrate the ceramic ultrafiltration of Logisticon at VEZET in WP8. TNO has continued the research in LPPA-UF to develop if further and make it ready for demonstration and market introduction.

It was then chosen to change from flat sheets to vertically mounted tubular membranes for the following reasons:

- The required mixing for the flat sheet setup is still quite energy intensive. Other modes of operation were likely to significantly reduce energy use per m3 of water produced
- Using the particles within a tubular membrane allows for keeping the particles in the system at all times, thus removing the risk of particles being released into in the food-processing process and making recovery/replacement much easier
- Tubular membranes are used for high demanding, strong fouling applications. A fouling prevention system is of most value in such a market, thus maximizing its market potential.

Various waste water streams, showing a strong fouling (>50% loss of flux within 1 hour) during standard crossflow, were tested with the new system: the particle-assisted system made it possible to generate a comparable membrane flux (i.e. capacity of the membrane) to that obtained via the usual cross-flow filtration without particles, with 30-50% of the energy use. See also Figure 5.

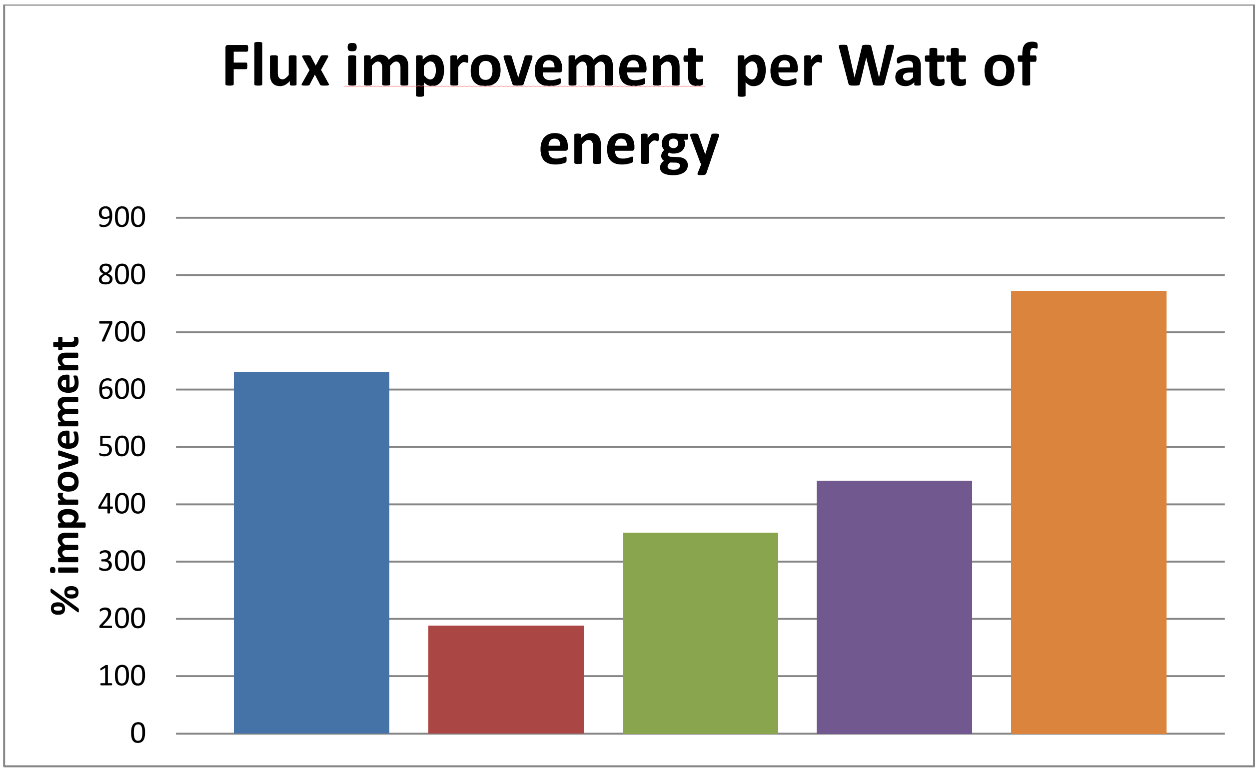

Figure 5 Flux improvement per Watt of energy obtained with particle assisted tubular UF for different fluids (patent pending, details cannot yet be shared)

Furthermore, a constant flux can be obtained without the need of backwashing to remove the fouling layer or intermediate cleaning, thus permitting a higher throughput. Washing water from the fresh-cut vegetable industry was also tested, however this was less successful and no or very little effect was measured. The fouling particles in the washing water are likely to be very small, they can easily enter the pores of the membrane but cannot be removed by the particles added to the system.

The results show that the particle-assisted system permits to reduce the formation of the fouling layer on the membrane, though it cannot stop pore fouling. This makes the system optimal to treat waste water streams that contain large fouling particles (>120 KDa); in addition, it can be an interesting alternative to the membranes currently used to treat strongly fouling waste water streams (e.g. slurries, high COD flows), since it is both more energy efficient and able to provide a constant flux without involving the usual maintenance steps like backwashing and cleaning.

These results opens up promising prospects of industrial usability: a patent application is in progress. Furthermore, TNO is in contact with industry for a follow-up-project aimed at upscaling and market uptake of this new technology.

2.6 Innovative UV methods found to be best option for hot water (70-75 °C) disinfection

Heat resistant bacteria, such as Alicyclobacillus spp (ACB), can survive well at the thermal and acidic conditions in juice manufacturing and can persist during typical pasteurization regimes applied in the sector. Once present in the water and/or on the product it can multiply easily and spoil the product. Methods to prevent intake of ACB (e.g. careful transportation and proper cleaning of product before washing/processing) are crucial. Next to prevention, mitigation of any ACB or other unwanted bacteria is crucial. As normal pasteurization does not work to kill these bacteria alternative disinfection methods are necessary.

The primary water source at Arisu containing ACB, is the condensed water. This is water (70-75 °C) that is obtained after condensation of the vapours from the evaporators used to concentrate the juice products. Various methods were tested to remove/degrade ACB in the condensed water:

- Pulsed UV
- Acidic (AEW) and neutral electrolyzed water (NEW)
- Filtration
- Active coal adsorption

A first series of tests showed that filtration and adsorption are able to remove a significant amount of COD (up to 60%) and bacteria from the condensed water. However, due to the high COD levels, the amount of active coal is large and the fluxes during membrane filtration low. As a result, both processes are economically unattractive.

Pulsed UV was tested a various settings and showed a very good performance, as shown in **Error! Reference source not found.** below. Other advantages of UV application are the lack of residual compounds and the absence of applied chemicals disinfectants and preservatives. The significant microbial reductions in very short treatment times, the limited energy cost and its great flexibility are some of the major benefits of the technique.

Table 11 Microbial counts after pulsed UV-treatment at various settings

Condensed water pH 4.44	Water depth (mm)	Shelf Distance of shelves (cm)	Counts before treatment UV	Counts after UV and 3 days of incubation (cfu/ml)	Number of Pulses						
					5	10	12	14	16	18	20
Counts after UV treatment (cfu/ml)	3 (15ml)	8	$3.0x10^6$	After UV	$4.0x10^2$	10^2	0	0	0	0	0
				After 3 days	2.0	0	0	0	0	0	0
	5 (20ml)	8	$3.0x10^6$	After UV	$1x10^3$	$1.5x10^2$	0	0	0	0	0
				After 3 days	6.0	0	0	0	0	0	0
	8 (40ml)	8	$3.0x10^6$	After UV	6~~5~~$.10^2$	10	0	0	0	0	0
				After 3 days	4.0	0	0	0	0	0	0

Application of NEW showed strongly similar results. A technical and economic evaluation showed that UV technology is the best treatment in terms of cost-effectiveness, energy and water saving for the disinfection of the hot condensed water. Transmittance is one of the most critical factors for the efficiency of UV disinfection. Frequent measurement of transmittance and implementation of precautions to standardize the condensed water composition is strongly advised.

2.7 Ultrafiltration + UV very promising concept for resource-efficient and safe reuse of cold water in fresh-cut industry

Based on the assessment performed in Task 3.1, using the Water Quality Management methodology, the following technologies were tested at VEZET (Dutch market leader fresh-cut industry) to treat washing water at 2-4 °C and reuse it in afterspraying, which is the final step of the washing process:

- Pretreatment with metal sieves (10-100 µm)
- Ceramic UF
- Low pressure particle assisted UF
- UV disinfection

The main goals were to reduce COD, remove turbidity and to achieve full disinfection. The results are shown in Table 12.

Table 12 Results obtained for pretreatment, ceramic UF, LPPA-UF and UV disinfection

	Current approach	Particleassisted UF	Ceramic UF	Ceramic UF + UV
COD removal	-	45%	30%	30%
COD removal by prefiltering (additional to UF)	-	25%	25%	25%
Turbidity removal	-	99%	99%	99%
Conductivity effect	-	0%	1%	1%
Disinfection	Log 0.5-1 (product) Ice water microbial load ≈ 0	Log 2.7 Microbial load without UV treatment ≈ $2.8*10^3$ cfu/ml With UV tr. = 1 cfu/ml	Log 2-3 Microbial load treated water ≈ $1.7*10^3 – 9.6*10^3$	Log 3.5-4 Microbial load ≈ 1
Temperature increase	-	Max 1-2°C	Max 1-2°C	Max 1-2°C

It can be seen that the combination of UF + UV is well able to fully disinfect the water and achieve a >99% removal of turbidity. Total COD removal (including pre-treatment with sieves) was found to be around 55-70%. From a microbial point of view the treated water is very suitable for reuse. Demonstration tests on-site are required to determine if the COD removal with pre-filtering + UF is sufficient for reuse.

The tests also showed that the washing water has a strong fouling tendency. Fouling prevention measures for the membranes are crucial. This can be achieved both by a high cross-flow combined with frequent backwashing of the membranes and by using particles to continuously remove the fouling layer on the membrane.

Looking at the economy, the payback period for 3 of technology concepts evaluated (particle assisted UF, ceramic UF and ceramic UF + UV) is expected to be 4-5 years, at a water price of 1.5 €/m3 (excluding cooling costs) , 1 m3/h ice water and 2.3 m3/h recirculation. Lower water prices and less recirculation will result in longer payback periods (>5 years). Lower water prices have a strong effect (>4 years added), while the effect of less recirculation is smaller (1-2 years added). Based on the results obtained in WP8 the economic evaluation will be updated.

2.8 Study of the antimicrobial effectiveness reached by combined treatments (multi hurdle approach)

Initially, research was based on the multiple hurdles concept, where a variety of physical-chemical hurdles are applied simultaneously in combined form. The principle of this concept is that the use of combined preservative factors may have greater effectiveness at reducing/eliminating microorganisms than the use of any single factor. Taking into account that: a) some bacteria are attached/internalized in sites inaccessible for these sanitizers and, therefore, limiting their effectiveness and b) the hydrophobic nature of vegetable surface that protects microorganisms from exposure to chemicals, it is clear that more work into this area was required in order to improve the disinfection step.

Thus, an innovative strategy based on hurdle technology (positive or negative pressures + sanitizers in a simultaneous application) was explored for its potential to inactivate pathogens from the surface of fresh-cut produce by enhancing the contact between antimicrobial solution and bacteria. Use of positive pressures (+1, +2 and +3 bar) and negative pressures (-400, -200, -100 and -30 mbar) could help in the disruption of such interaction by removing gas or liquid barriers than block penetration of the sanitizing agent.

In the present work, the effectiveness of chlorine and other sanitizer agents (chlorine, organic acids, hydrogen peroxide, neutral electrolyzed water, ozone, peroxyacetic acid and chlorine dioxide) was evaluated on the microorganism's reduction, both on produce and on washing water. Different sanitizer concentrations (based on literature and on manufacturer's recommendation) were tested. The two vegetable species selected were iceberg lettuce and carrots.

<u>The overall conclusions drawn with the **multi-hurdle approach** were:</u>

- The use of combined treatments with +/- pressures, to improve the contact of the chemical agent with the bacteria, does not show enough added benefit (weak synergistic effect) regarding the treatment performed at atmospheric pressure (standard conditions).
- Hence, this strategy does not seem to be a promising alternative for improving decontamination efficiency in the sanitation of fresh-cut vegetables, as expected in the theoretical approach posed. In addition, these decontamination techniques have shown an indirect detrimental effect on fresh-cut product quality by affecting plant tissue physiology and structure.

Therefore, bearing in mind that sanitizing treatment typically achieve 1-2 log units' reductions in microbial populations on fresh-cut vegetables and they are unlikely to greatly increase efficacy in decontaminating produce, the incremental improvements of work was addressed to:

- select the most adequate sanitizing strategies at atmospheric pressure for the **prevention of waterborne product cross-contamination**
- **improve the water quality during washing for water reuse**. So, the disinfection capacity and efficacy in preventing cross-contamination of product as well as their effect on physicochemical quality of wash water will be evaluated

2.9 Efficiency of different sanitizing treatments to prevent cross contamination in the process wash water

It has been recently acknowledge that the primary role of disinfectants in washing fresh-cut produce is avoiding cross-contamination (Gil et al. 2009; Lopez-Galvez et al., 2010), since the decontaminant effect of the microbial reduction caused on product is readily lost during storage (Allende et al., 2008) and once cross-contamination occurs it is not possible to eliminate the pathogen from produce (Lopez-Galvez et al., 2010, Luo et al., 2011).

So, this approach focuses the disinfection process towards the washing water rather than towards the product. The main results regarding the quality of the process water, by means of removing pathogen microorganisms and avoiding the formation of potentially hazardous DBP, as well as the impact of the treatment in the fresh cut vegetables, are detailed below:

- **Chlorine (sodium hypochlorite):**
  a. The use of sodium hypochlorite at a level that will maintain residual free chlorine levels around 2 ppm in the wash water would be a short-term alternative for the fresh-cut industry as an effective way of wash water sanitization with little disinfection by-products formation.
- **Neutral electrolyzed water:**
  a. With regards to fresh-cut vegetables, NEW75 and NEW100 reported similar sanitation efficacy than chlorinated water.

b. At doses tested (50, 75 and 100 ppm), this sanitizer completely eliminates microorganisms in suspension in process water, but due to the free chlorine concentration of this wash water solutions the THM's values are similar to chlorinated water solutions, exceeding in some cases the authorized limit set by legislation for drinking water.
c. This sanitation technique does not compromise product quality.

- **Hydrogen Peroxide:**
  a. At doses tested (1%, 2% and 5%), no further benefit was provided by using H2O2 instead of chlorine (CW) on product. The required doses of application to obtain any benefit are not appropriate and realistic for the industry, since they are too high.
  b. Regarding, the washing water, there is absence of microbial load for all doses tested. Also regarding the final quality of water it is good to take into consideration that hydrogen peroxide produces no residue as it is decomposed into water and oxygen.
  c. There is no negative impact on the overall appearance of product.
- **Ozone:**
  a. With regards to fresh-cut vegetables, ozone reported a reduced sanitation efficacy.
  b. Taking into account the aquatic toxicity test results, ozone still remains to be the most environmentally friendly alternative for wash water sanitization.
  c. Since the high organic load of the wash waters makes it impossible to maintain a residual ozone level during washing that will help to keep water free of microorganisms, other sanitizing agents that could be used in combination with ozone should be investigated. Moreover the potential use of ozone for disinfecting the waste wash water before recirculating it to the system should not be overlooked.
- **Peroxyacetic acid based sanitizers (PAA):**
  a. Two commercially available brands have been tested: TSUNAMI (from ECOLAB) and CITROCIDE (from CITROSOL).
  b. In the study of the antimicrobial effectiveness on product, PAA based sanitizer, at 375 and 500 ppm, provide more benefit than chlorine on product.
  c. Regarding, the washing water, there is absence of microbial load for all doses tested. According to the quality parameters of the effluent, a significant increase on COD values was observed when using CITROCIDE PC at doses tested (0,5, 0,6 and 0,7 %). This was not observed for washing solutions with CITROCIDE PLUS (0,025, 0,0375 and 0,05%). Besides, THMs were not generated by using this sanitation technique.
  d. This sanitation technique does not compromise product quality.
  e. This approach therefore could be used as an effective way to sanitize fresh product by food processors (tuning with WP8_ Pilot testing).
- **Lactic acid:**
  a. At doses tested (0,25% and 0,1%) no further benefit was provided by using LA instead of chlorine (CW) on product.
  b. The use of lactic acid for sanitation purposes has an impact on the processed water quality, by increasing the COD values even at the minimum dose tested.
  c. There is a sensorial impact on the product due to the acidification.
- **Chlorine dioxide (commercial brand CLODOS PURO):**
  a. In the study of the antimicrobial effectiveness on product, it has been demonstrated that chlorine dioxide, at 20 and 30 ppm, eliminates completely microorganisms in suspension in process water.
  b. It has a reduced cross-reactivity with organic matter. No DBPs formation by using this sanitation technique.
  c. This approach therefore could be used as an effective way to sanitize fresh produce by food processors (tuning with WP8_ Pilot testing).

The overall conclusions for the sanitizing agents tested are that PAA and ClO_2 treatments have comparable microbial decontamination properties as chlorine, maintaining the overall quality of washed vegetables. Additionally, their potential health impact and environmental effects regarding the by-products generated from the reaction with organic matter are scarce. In summary, these sanitation agents demonstrate their potential as an alternative sanitizing method to maintain safety on the process wash water of the fresh-cut industry, avoiding cross-contamination during processing. Hence, more realistic evaluation of their potential to replace chlorine under simulated industrial conditions was needed and successful executed.

Besides the knowledge gap about the use of these different disinfectants, especially in this sector, there are also a lot of uncertainties on European regulation and the situation is quite complex, also because there are big differences in the different countries (from obligation to use disinfection to a complete prohibition). However, the risk of cross-contamination is not removed by using large quantities of water in those countries where it is prohibited. For this reason different EU projects (Vegi-trade, Susclean, RESFOOD) paid attention to this topic to give more clearness on this subject and extensive data regarding the practical validation of the importance of cross-contamination during the washing stage.

The European Commission is planning to develop more detailed regulations governing the use of processing aids. Taking into account that legislation on processing aids is not yet harmonised at European Community level, a global approach and an adequate normative framework regulating to processing aids is needed. Therefore, the purpose of the disinfectant trials performed within the framework of the project, was to provide updated information on all those alternative disinfection strategies, with experimental designs reflecting industrial conditions, for both scenarios that are presented in the European Union.

2.10 Design and development of a pilot scale prototype for washing fresh-cut vegetables

Besides the decontamination of fresh-cut vegetables by alternative technologies, equipment used in the production of such minimally processed products must enable to tackle with the huge challenge in reducing water consumption and wastewater discharge rates while ensuring food safety of product. In this sense, the objective of **RESFOOD** project was to contribute with the design and construction of a new washing machine that incorporates several innovations that meets the environmental concerns, such as reducing water requirements by means of internal water recirculation, improving filtration of the water before reuse, increasing cleaning efficiency, improving energy consumption; and other important issues such as, improvement the mechanical movement of water and product, optimizing contact time between chemical agents and product, etc.

The design of the washer, developed by KRONEN, was based on several modifications of conventional washers to increase their effectiveness. This prototype system was a full scale prototype of an immersion washing system adapted for industrial uses that incorporates the principle of water recirculation.

Description of washing system

Globally, water flows in the opposite direction to the movement of product through the different unit operations. After trimming and cutting, the vegetables are transported to the washing machine with a conveyor belt. Basically, the washing system consists in an immersion washer followed by a two-step spraying system. A pre-washing step to remove dirt and cell exudates from the cut surfaces is optional depending on the product.

In the washing tank, the water movements bring the floating product under water. The product is washed with chemicals agents, guarantying a selected contact time of at least 45 seconds. After the wash tank, the vegetables are separated from the water with a mesh belt. Spraying systems are used on the vegetables to remove possible residues of sanitizing agents. After this spraying step, the vegetables are transported with a conveyor belt to a drying unit and later on the packaging station. Figure 6 details the washing and spraying units of the prototype machine.

a **b**

Figure 6 Washing system: a) Washing unit and b) Double spraying system

The next step after the built prototype was to make the required test to be sure that system was working as it was expected.

Innovations of prototype washing system

KRONEN designed and constructed this new prototype of the washing machine to improve the mechanical effect of water on the surface of vegetables addressing for major challenges: energy saving, water saving, food safety and cleaning potential.

The mechanical washing is important to remove soil and to reduce the microbial content on the foodstuffs and thereby to guarantee the quality of the product. In addition to obtaining a mechanical effect, the aim is to guarantee the contact time between the product and water. The best mechanical effect was achieved using simulation of the water flows in the pipes and in the wash tank (Figure 7).

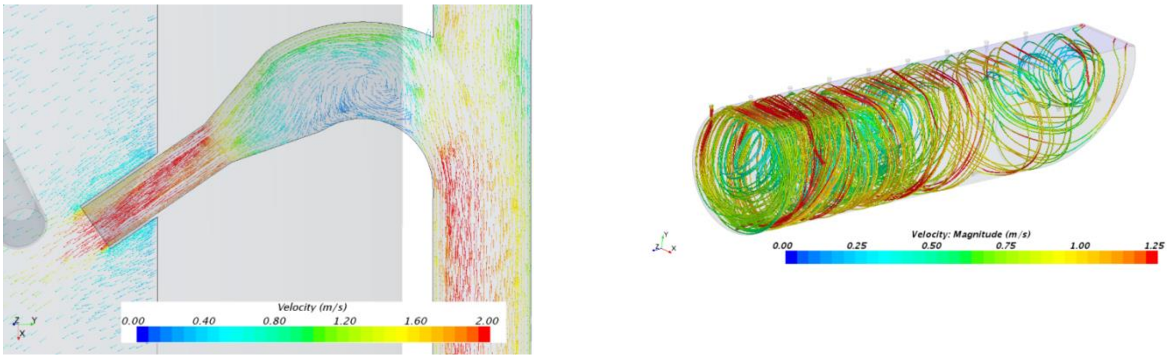

Figure 7 Water movement simulation within the nozzles and the washing unit

Water flow simulation was useful to assess water and energy consumption. Simulation also permits a better accessibility for cleaning and a reduction of the “dead-zones”, i.e. those zones where water barely moves or does not move at all. In such areas leaves may stuck for a long time thus increasing the risk of generating bio-film (aggregation of microorganisms). Avoiding cross-contamination was a priority and a guideline for the whole project and simulation has proven a successful method to attain this objective.

In addition, the prototype incorporates the principle of water recirculation providing a higher recirculation rate within the system.

2.11 Applications in food processing practice for reduction of water and energy use ensuring food safety and quality

In fresh cut vegetables processing, 3 main concepts were tested in practice with the aim of minimizing water and energy consumption, taking into account safety issues:

- Direct reuse of washing water from the after-spraying to the pre-spraying step, using water treatment technologies that allow obtaining good quality water for its reuse. The selected technologies were a disk-filter followed by an ultrafiltration (UF) and a UV treatment. A pilot system including these technologies was constructed by Logisticon.
- Addition of chemical agents in the washing water, alternative to chlorine, to decrease the need of water and to improve food safety avoiding risk contamination. The selected disinfection agents were peroxyacetic acid (PAA) and chlorine dioxide ($ClO2$)
- Use of a new washing equipment developed by Kronen addressing four major challenges: energy saving, water saving, food safety and cleaning potential.

Regarding the performance of the water treatment system, the cold water reuse is technically feasible. The pilot test performed in Vezet showed that enough flow of permeate can be produced for prolonged periods without problems. The fouling can be well controlled when operating in optimal conditions.

It's possible a 50% of reuse with a suppletion of ice water in other parts of the washing line. With this approach, a net decrease in water intake and discharge of approx. 35% can be achieved. Regarding the impact on the final product, no effect on microbial load and product quality, safety and shelf-life was found at 50% reuse. Unfortunately, from the economic point of view, the treatment concept is not economically feasible under the current conditions at Vezet. The current costs for the treatment (3-3,5 €/h) are about 1 €/h higher than the savings in water and energy (2-2,5 €/h). As capital costs of the system are the main part, higher fluxes and a bigger scale can have a positive effect and need to be further explored

The pilot test performed in Vega Mayor with CNTA showed that the use of sanitizing agents in water is a tool to prevent cross-contamination in the washing step. Peroxyacetic acid (PAA) and Chlorine dioxide (ClO2) has been proven as promising alternatives to chlorine to prevent cross-contamination showing similar antimicrobial activity compared to chlorine. The use of PAA and ClO2 did not lead to the formation of THMs. However, the use of ClO2 causes the formation of chlorates.

On the other hand, this pilot test allowed checking the performance of the new washing equipment in field conditions. During the tests some critical points were detected. Then, a new prototype was constructed. The new washing machine improves the mechanical effect of water on the surface of vegetables to remove soil and reduce their microbial content with a less energy consumption. Water and product come to the outfeed after the expected 45 seconds of contact time. In terms of water saving, this washing system incorporates the principle of water recirculation.

Recirculation of wash water in the processing of fresh cut vegetables could be a viable way to reduce the amount of effluent and reduce the amount of fresh water required. The reuse of water (from the after-spraying step to the washing step) and the use of PAA and ClO2 at doses tested did not cause detrimental effect on the sensory quality or the shelf life of fresh-cut lettuce. With this approach a reduction of, at least, 20% in the water consumption rates could be achieved.

In apple juice processing, the aim was the reuse of two streams, the regenerate from the juice clarification resins and the washing water from the final washing step. Pilot scale treatability studies were carried out for these streams using a rotating drum filter and AiRO technology, an innovative RO technology where air is used instead of chemicals in a vertical positioned membrane.

AiRO is successful in maintaining a constant transmembrane pressure for both the regeneration wastewater (400-1800 mg/l COD) and the fruit washing wastewater (1,000 -18,000 mg/l COD) during short duration operation (< 1 production day). A water recovery of about 65% is achieved. Removal of organic material from the regeneration wastewater was >80%. Average removal of COD, TOC and TSS from the fruit washing wastewater was approx. 50%, using AiRO. Then AiRO can be well used to treat both water streams and make them suitable for reuse. Reuse reduces the discharge by up to 80-90%.

Pilot scale treatability studies were carried out also for the apple washing wastewater using a rotating drum filter. The removal efficiencies for COD and turbidity were obtained up to 58-65% and about 85%, respectively. Whereas, the TSS removal attained was rather low.The pesticides were also detected in ppb level.

The economic evaluation showed that all in cases a positive business case is obtained. When 240 m3/h of regeneration water is treated with RO, a payback period of 2.9 year is obtained which is considered acceptable. For the washing water, it can be seen that both AiRO and rotating drum filter have acceptable payback period of respectively 3.7 years and approx. 0.6-1.3 years (for 2-4x lower capital costs for the drum filter compared to AiRO). If direct reuse, after drum filter treatment, is sufficient then a solution with a very acceptable payback period is obtained.

Anyway, more tests are advised to evaluate the possible effects of reuse on water quality in and after the process and possible effects on product quality and safety.

Biomass

2.12 Food waste biorefinery: cascade approach with decision model and techno-economic feasibility

Food processing by-products are often used as feed or bio-energy source. In general, these by-products still contain useful major compounds like carbohydrates, proteins and fats and minor compounds like polyphenols or carotenoids. In a food waste biorefinery, the food by-product is refined into a combination of a major compound (protein/fat/polysaccharide) and a minor compound (carotenoid/polyphenol/terpene/…) to make the process economically feasible. The RESFOOD project aimed at the extraction of sesquiterpene lactones, pectins, carotenoids and polyphenols from several food by-products like endive waste, carrot dejuiced pulp and apple pomace. Furthermore, a multiple valorisation of food by-products was aimed at, i.e., extraction of several chemicals from the same food by-product.

It is important for the food processing industry to decide which type of valorisation (food, feed, material, energy) is applicable for their by-product. Important parameters for this decision are quantity, time of production, dry matter content, uniformity, valuable compounds, need for nutrients in the agricultural area around the plant. A decision model that can be used by companies was made. Based on a limited set of parameters a quick decision can be made on the preferred valorisation of the food processing by-product. Valorisation of food processing by-products is complex due to the complex composition of the biomass matrix, making separation processes expensive. The decision model can be a guidance for food processing companies in their search for an alternative valorisation of their by-product.

The RESFOOD project showed that extraction of major compounds (pectins) and minor compounds (carotenoids, polyphenols, sesquiterpene lactones,…) is technically feasible. However, the major bottlenecks are i) the **functionality** of the extract and ii) the **economic feasibility** (e.g. polyphenol extraction from apple pomace is not economically feasible). Therefore, the bulk of the food-processing by-product should first be valorised towards a major compound (protein, fat, carbohydrate or fibre). During the refining of the first crude extract to a more pure extract, minor compounds are often separated from the crude extract. At this point during processing, the possible valorisation of the minor compound should be kept in mind. For example, the main source for the production of natural carotene is palm oil. However, palm fruits are not produced for carotene, but for their oil. By refining the red palm oil into a yellow oil, i.e. separation of carotenes from the oil, the carotene as minor compound can be valorised as high-added value chemical.

2.13 Sesquiterpene lactones from endive by-products: extraction and functionality

Pressing of endive by-products from Vega Mayor with a screw press facilitated cell disruption and a fast separation of press pulp and juice. It was found that 91% and 74% of the initial content of (dihydro)lactucopicrin, the most important sesquiterpene lactone in endive, was obtained in the total juice for Frisée (curly endive) and endive cutting waste from Vega Mayor, respectively. Therefore, the juice was used for the recovery of the sesquiterpene lactones. Clarification of the juice was done by decreasing the pH with HCl, which caused clogging of the protein particles and allowed their removal by centrifugation. It was found that 44% and 28% of the initial (dihydro)lactucopicrin content were obtained in the clarified juice for Frisée and endive cutting waste from Vega Mayor, respectively. Therefore, clarified juice was further used for the isolation of the sesquiterpene lactones. By employing column adsorption, more than 99% of (dihydro)lactucopicrin from the clarified juice could be recovered.

This process was used by TNO to prepare a sesquiterpene lactone rich extract for anti-fungal testing at BT9 to examine for antifungal properties using the Kirbey-Bauer zone of inhibition test. The hyphomyceteous fungi, *Alternariaalternata*, *Penicilliumdigitatum*, *Aspergillus niger* and *Botrytis cinerea* were used as models. Following two consecutive trials the tested material did not demonstrate antifungal properties using the Kirbey-Bauer method.

2.14 Endive biorefinery with focus on extraction of proteins on pilot scale

The increasing demand of food production, together with the limited amount of natural resources available, ask for the development of new technologies. These new tools should aim at the valorisation of already existing resources. In the RESFOOD project, research was done on the extraction of pectins, colorants, beta-carotene and other minor components like sesquiterpene lactones from vegetable waste streams like endive. It was shown that on these components alone it is not possible to build a viable business case. Broadening of the biorefinery into other components was investigated and it was concluded that the inclusion of protein extraction and the use of the dietary fibre has the potential to make it an economically viable business case.

There is an increasing need of protein in the European countries, for both human and feed consumption. Europe imports roughly 77% of its protein necessities, where soybean meal, the most important source, has the majority of the share. Valorisation of remnants from the vegetable processing industry is seen as a plausible way to satisfy the need mentioned. Endive and other green vegetables are seen as potential by-products in terms of economic return and extraction on industrial scale. The work aims to provide scientific basis to sustain the extraction of Ribulose-1,5-bisphospate carboxylase/ oxygenase, commonly called RuBisCo, out of endive remnants. Rubisco is a soluble protein present in the stroma of chloroplast of plants. Indeed, it is interesting for the human consumption for two main purposes: the good nutritional value and the functionality into the processing industry. However, there are some drawbacks that hinder the introduction of Rubisco in food. These disadvantages are related to the extraction, purification and drying steps. The phases of extraction and purification are connected to the presence of undesired compounds such as chlorophyll, polyphenols and off-flavours molecules. Moreover, the simultaneous extraction of co-products, such as fibre and polyphenols, might be the key to reach an economic way of processing.

An innovative method to extract Rubisco, on both large scale and batch scale, has been developed by TNO under the patent WO 2014/104880 A1. It briefly consist of five main phases of: 1) mechanical disruption of vegetable cells, 2) treating the plant juice to cause aggregation of chloroplast membrane, 3) removing the aggregate chloroplast membrane by precipitation and/or microfiltration, 4) subjecting the plant juice to ultrafiltration, 5) subjecting the soluble plant protein concentrate to hydrophobic column adsorption to remove undesired compounds. The introduction of hydrophobic adsorption is a very convenient method because it permits to eliminate many undesired compounds in a single step, while reducing chemicals inputs. The innovation also provides an apparatus and system for plant protein isolation.

Experimental work has been carried out in order to test the protein yield out of endive, a feedstock with relative high water content (dry solids only 2 % on a wet mass basis). Protein pellets, with up to 9% of protein, were extracted that could be excellent dried in dry protein powders with up to 50% of protein. The work has resulted in a close collaboration between industry and CRO’s which has led to the creation of a new business in the ‘Greenprotein’ group. In this group enterprises and academia involved in the whole value chain work together to produce and market the Rubisco, but also dietary fibre and other by-products. Florette will provide the raw material and the industrial facilities to install the extraction module, Provalor and TNO will supply the engineering and scientific know-how and Bionet the tailored made equipment. Ruitenberg ingredients will be in charge of the commercialization and Norland company will be instituted with the aim of managing the sales of the products.

2.15 Pectins from food processing by-products: extraction and functionality

Pectic polysaccharides are one of the components of plant cell walls. Rhamnogalacturonan I (RGI) represents a major proportion of pectic polysaccharides in food side streams. RGI pectins were isolated from various agricultural side streams like apple pomace, potato pulp, okra, carrotdejuiced pulp and endive waste. Preliminary studies were made to determine the influence of chemical (alkaline extractions) and enzymatic (use of polygalacturonase) extraction routes on RGI yield and chemical properties using apple pomace. The results illustrate that the enzymatic extraction method could promise higher RGI yields (34% vs 25%). However, mole percentage of arabinose for enzyme extracted RGI was 13% lower than alkaline extracted RGI (45% vs. 32%). The results indicate that higher arabinan side chain degradation results from enzymatic extraction. Since functionality in RGI is determined by side chain composition and length, alkaline extraction was adopted for a larger scale extraction.

In the next step, the physico-chemical properties of RGI extracted from apple pomace, potato pulp and okra cell wall materials were determined. The isolated RGI from apple pomace using chemical treatment was high in arabinan. Okra RGI are bigger molecules compared with potato and apple RGI. Shear thinning behavior of RGI solutions was observed under certain conditions. Okra RGI was much more viscous compared with apple RGI and potato RGI. It was assumed that adding RGIs into a gel system could lower the texture profiles of the gels. In low methoxy (LM) and high methoxy (HM) gel systems, weaker gels were formed as what was assumed. However, in a whey protein system, adding apple and okra RGI could significantly improve the gel strength. Also, for ALM (amidated LM) gels, apple RGI showed a similar texture with higher ALM concentration reference gels. For the gelatin gels, weaker gels were formed when RGI substitution level became higher. However, this might mainly because of the decreasing of gelatin content. Gels with apple RGI led to a better water holding capacity comparing with potato RGI and okra RGI substituted gels. This could because of the higher mobility of high branched arabinans reported in previous literatures. Potato RGI absorbed more moisture than okra RGI because of the longer galactan side chains presented in potato RGI comparing with okra RGI.. The extracts can be used as dietary fibre. Pectins with gelling properties can be extracted from a lot of agrofood by-products, but the two major by-products on industrial scale are citrus peels and apple pomace. Since pectins are complex polysaccharides, the extraction of hairy pectins with other properties than gelling properties is an important field of research, but a good technical and/or bioactive functionality is a necessary condition to claim industrial use.

2.16 Carotenoids from carrot pulp and endive by-products: extraction and functionality

Carotenoids are natural anti-oxidants and colorants present in some agro-industrial by-products. Different extraction techniques have been tested to extract as much as possible the initial content from the raw materials. For carrot, supercritical CO_2 extraction is the most efficient extraction technique since it provides around 53% of initial carotenoids content. It is a solvent free technology and provides carotenoid oleoresins of high quality. Optimal extraction conditions were 80 °C, 300 bar and 2 hours, using 10% ethanol as co-solvent. In comparison, conventional organic solvent extraction showed around 35% of recovery values. For endive, conventional solvent extraction followed by chlorophyll precipitation is the recommended technology for carotenoid extraction and purification from endive by-products. This process provides around 34% of recovery values. Supercritical CO_2 extraction is not recommended for endive due to its low recovery values (about 17%) and the need of further purification steps to eliminate chlorophylls that take away the main advantage of supercritical CO_2 extraction technology, i.e., the presence of organic solvents in the final extracts.

Biological properties of carotenoids open up a wide range of commercial applications. Recent interest in the carotenoids has been mainly for their nutraceutical properties. A large number of scientific studies have confirmed the benefits of carotenoids to health and their use for this purpose is growing rapidly. The most relevant biological functions of carotenoids in humans are linked to their antioxidant properties, which directly are related to large molecular structure. These antioxidant properties were measured. In addition, carotenoids have traditionally been used in food and animal feed for their color properties. Moreover, carotenoids are also known to improve consumer perception of quality; an example is the addition of carotenoids to animal feed to enhance color of produced food (fish, eggs, etc...).

The increasing demand for natural extracted carotenoids was the trigger to explore the techno-economic feasibility of supercritical fluid extraction to obtain natural oleoresins from dejuiced carrot pulp. Supercritical CO_2 extraction can be used technically for carotenoid extraction from carrot dejuiced pulp, but the economic assessment showed that the cost for extraction is too high to be feasible in a lot of industries.

2.17 Polyphenols from apple pomace: extraction and concentration by membrane technology at lab and pilot scale

Polyphenols are moderately water-soluble plant metabolites. They have antioxidant properties and therefore have a positive impact on human health. The world market for polyphenols is significant and was estimated to be about 200 million USD in 2009. Polyphenols can be extracted for example from apple juice and apple pomace.

After pressing of apple in anaerobic (no oxygen) conditions, the initial concentration of polyphenols remains in juice and pomace, compared to pressing in open air due to oxidation losses. Therefore, the value of juice and pomace after anaerobic pressing is higher than after aerobic pressing since polyphenols have anti-oxidant properties and hence a positive effect on human health.

Polyphenols were extracted from apple pomace with acetone/water, ethanol/water or water and the polyphenols in the extraction solvent were concentrated by membrane technology. Although acetone/water showed a better extraction efficiency compared to ethanol/water, a polyphenol extract in ethanol is more convenient than in acetone, based on input from industry. After screening of membranes and a laboratory feasibility test, the polymeric nanofiltration membrane NFX (Synder Filtration) was selected. To scale-up this process to pilot level, a 3838 spiral-wound NFX module (3.8 inch diameter x 38 inch length) with membrane surface area of 8.9 m² was used.

The pressing of the apple was performed at the Food Pilot of ILVO (Institute for Agricultural and Fisheries Research) in Belgium under the supervision of VITO with a multicut and spiral filter press. In total, 200 kg of apple (Golden Delicious) were pressed with a yield of 83% apple juice. By pressing with the spiral filter press, a fractionation of 70% of polyphenols in the apple juice and 30% in the apple pomace was shown.

The extraction of the polyphenols from the apple pomace was performed at the chemical pilot facility of Agfa-Gevaert NV in Belgium under the supervision of VITO. The extraction was performed with ethanol 56% at 80 °C during 31 minutes under continuous stirring. The total amount of polyphenols in the apple pomace is 4249 µg/g dry weight (935 µg/g wet pomace) with a yield of 84% for polyphenol extraction from apple pomace.

1) The concentration of the polyphenols from the ethanol:water filtrate was performed at the Solvent membrane pilot facility of VITO with a spiral-wound NFX membrane module. After a volume reduction by a factor of about 7, the obtained retentate was further concentrated on a bench-top rig. During the pilot test, the permeate flux decreased from 6.8 to 0.4 $L.m^{-2}.h^{-1}$. The total volume concentration factor was about 29 for pilot unit and bench-top rig unit together. The total polyphenol concentration was increased from 60 mg/L in the extract

(feed) to 1269 mg/L in the final retentate, i.e. with a factor of about 21 for pilot unit and lab unit together. Since both factors are not equal, some polyphenols were lost during pilot testing possibly due to fouling of the membranes or oxidation of polyphenols. The average retention of the major polyphenols was 97-98%, except for quinic acid (92%) and epicatechin (87%). The membrane pilot test showed good flux and concentration of polyphenols, indicating the technical feasibility of membrane technology for efficient concentration of polyphenols in an ethanol:water solvent. The use of membrane technology for concentration of compounds present at low level in an organic solvent is very important for industry. Several companies are interested in this technology. Therefore, the pilot test on polyphenol extraction and concentration with membrane technology was performed to **increase the knowledge** on this topic. Furthermore, the selected nanofiltration membrane is a common membrane for aqueous streams, but not for a stream with 56% ethanol. Although the membrane at lab and pilot scale were the same, the **module at pilot scale** (spiral wound) was different from lab scale and an important **scale-up issue**. An extra challenge of the polyphenol pilot test was also the use of an organic solvent at elevated temperature for extraction and concentration with ATEX proof (**explosion safety**) equipment.

An economic assessment of a (double) valorization of apple pomace towards polyphenols (and pectins) was also performed. Due to the relatively low amount of polyphenols in apple pomace, an organic solvent extraction to valorize apple pomace to polyphenols is not economically feasible. On industrial scale, apple pomace is already used for pectin extraction as second feedstock after citrus peels and an extra valorization to polyphenols is not recommended.

Monitoring

2.18 Label-free optical biosensing platform

In the framework of RESFOOD project, Technion team has developed a novel biosensing platform for rapid detection and identification of microbial contamination in water lines. The optical label-free biosensing platform is based on a nanostructured oxidized porous silicon ($PSiO_2$), which designed to directly capture the target bacteria cells onto its surface with no prior sample processing (e.g., pre-concentration or lysis). Exposure of these nanostructured surfaces to the target bacteria results in “direct cell capture” onto the biosensor surface, while these specific binding events induce predictable changes in the thin-film optical interference spectrum of the biosensor.

Based on the decision regarding the target bacteria was made by RESFOOD partners, the biosensing was redesigned and synthesized accordingly. Specific capture probes against the selected target bacteria (*E. coli*) were immobilized on the $PSiO_2$, using a proper surface chemistry. The biosensor was tested using laboratory *E. coli* suspensions, with excellent and robust results of 7±1% decrease in the optical signal upon introducing to 10^4 cells/mL *E. coli* bacteria and measured Limit of Detection (LoD) of 10^3 cells/mL.

Next, VEZET water samples have been characterized, analyzed and prepared for sensing experiments. The water samples were filtrated using 6 µm filter papers and the antibiotic Tetracycline (0.05 mg/mL) was added, in order to inhibit bacteria growth during storage. Biosensing experiments with VEZET water samples have shown an intensity decrease of 5±0.5% in the optical signal, upon introducing to VEZET water samples, which contained 10^4 cells/mL *E. coli* bacteria. Control experiments with VEZET water (no *E. coli*) showed minor intensity changes of 0.6±0.4%.

BT9 has developed a prototype system for bacteria detection in fresh-produce wash water (in near real-time) based on the technology developed at the Technion laboratories. The system comprises of a custom-made integrated setup that includes all biosensing system components: optics, flow cell, pump, and hardware control board. This allows for minimal handling of the studied samples for automatic analysis by non-professional operators 'in field' (outside of laboratory environment and already next to production lines) conditions. The data is collected and displayed in real time using a software, which was also specifically developed for acquiring and analyzing the biosensing results, on an external laptop computer. Using wireless technology the data can be also monitored outside of the production floor. The system performance was tested and characterized using different simulated water samples, to mimic real-life scenarios expected in the food industry. The performance was compared to that of the Technion laboratory-system. Biosensing experiments with VEZET water samples have shown an intensity decrease of 2.4±0.7% in the optical signal, upon introducing to VEZET water samples, which contain 10^4 cells/mL *E. coli* bacteria. Control experiments with VEZET water (no *E. coli*) showed minor intensity changes of 0.6±0.4%.

The two optical biosensing platforms (Technion laboratory-system and BT9 system) were installed and tested at the Vezet plant. The aim was to confirm the feasibility of detecting bacteria "in the field", i.e. in the facility with water collected directly from the industrial washers. To this purpose, washing water samples were collected from different washers' lines and then filtrated through the portable filtering device developed by Microbiome (see next section for more details regarding this device). Finally, the water was tested for *E. coli* presence through three different methods: (i) the Technion biosensors, (ii) polymerase chain reaction (PCR)-based techniques by Microbiome, and (iii) by conventional culturing on selective media performed by Vezet.

Preliminary analyses show that the target *E. coli* bacteria are selectively captured onto the biosensors surface (based on PCR results) and good optical signals can be achieved. These results demonstrate the feasibility of using the biosensors as a rapid technique for detecting indicator bacteria in complex and dynamic water samples in an industrial environment. Currently, the results are processed in order to optimize the performance of the biosensors to dynamic industrial conditions. The developed technology is attracting attention of different stakeholders in both the food and water businesses for potential implementation

2.19 IS-Pro/qPCR for detection of microbial pathogens

In the framework of the RESFOOD project, IS-Pro technology was adjusted for use in water and food assessments. IS-Pro, which stands for interspace region profiling, is based on the differences in length of the interspace region, which is present between the 16S rRNA and 23S rRNA genes that are present in all bacteria. The length of the interspace region is different among bacterial species. Thus with a PCR using universal primers annealing in the 16S rRNA and 23S rRNA genes the IS region is amplified and its length can be accurately measured using capillary gel electrophoresis. Using fluorescently-labeled primers, one can obtain a profile assembled from all IS regions present in a sample. Using sequence information from public databases, IS lengths from different bacterial species can be calculated. These known lengths are collected in a database that can be used to assign species to IS lengths found in the sample. Microbiome has developed this technology for use in human fecal samples. During the RESFOOD project, the IS-Pro assay was successfully adjusted for use in water and food samples. Water samples from the food industry were profiled using this technique and a huge variation in the water samples was found. Moreover, the bacterial species composition of these samples was found to be very different from human-derived samples, and the current database for species determination was found to be insufficient. This can be improved by filling the IS-Pro database with more food- and water related bacterial species. Further work in this issue is needed.

During the project, the bacterial composition of 18 RESFOOD samples was determined. This resulted in over 90% of sequences attributable to bacterial families or species. Currently, extensive efforts are done to link all the collected information to IS-Pro fragments lengths, in order to fill the IS-Pro database. This work will be continued in the future.

The IS-Pro methodology for the detection of bacteria species has been also evaluated in field conditions. This technique is much faster than conventional culture (5 hours versus <24 hours) and it provides a profile of all bacteria present, instead of only those that grow as in conventional culture.

Another development by Microbiome during the RESFOOD project is a water-filtering device that can be used without any external power supply. The device contains a balloon that holds the sample to be filtered (max. 0.5 liter). A 0.22 µm filter inside a holder, together with a retainer, is inserted into the balloon. The retainer serves to keep the balloon from collapsing onto the filter. The pressure is applied to the outside of the balloon using a CO_2 cartridge thus forcing the sample through the filter. The filter is aseptically housed in a holder that can transported after disassembly of the device. The device was tested with different water samples and was found to allow fast filtration time (1 minute is needed for filtering a volume of 0.5 liter of relatively clean water.
The water-filtering device was compared to a standard bench filtering device and was found to perform equally well. We found that filters can be stored for at least 3 days without loss of bacterial DNA. Moreover, field tests at Demokwekerij, Logisticon, TNO and Vezet (where water was filtered on site) yielded good results. The great advantage of the system is that it can be used on site, without any power source, and it will allow for much better representation of the actual bacterial composition of the sample than when samples are transported in liquid form, because bacteria may grow or die within liquid samples. Also shipment of filters is much cheaper than shipment of liquids.

In terms of exploitation, Microbiome is planning to first introduce an IS-Pro kit on the market for use in human disease diagnostics which will be called Molecular Culture kit. To that end, Microbiome is currently compiling the necessary documentation to obtain CE-IVD (in vitro diagnostics) certification for this kit, in compliance with the IVD Directive 98/79/EC. Market introduction is planned in Q1 2016, first only in The Netherlands, later EU-wide. For market introduction of an environmental IS-Pro kit, Microbiome will further fill the database and perform market surveys to identify the most likely customers. The water-filtering device will be introduced into the market as a separate entity (i.e. not as part of an IS-Pro kit) in Q2 2016. Microbiome aims first at customers such as dentists who need to check their water systems for *Legionella* contamination.

2.20 Analysis of real wash water samples with DHPLC techniques

In the framework of RESFOOD project, denaturing high performance liquid chromatography (DHPLC) technique was adjusted for the identification of bacteria in process water samples from the food industry. Protocols for DHPLC, which were originally developed for mutation analysis, were adapted for the separation of PCR-amplified bacterial 16S rRNA gene fragments. PCR-amplified fragments are subjected to a column containing polystyrene/polydivinylbenzene particles. The separation of the PCR products is based on the elution of partially melted DNA molecules by the reagent triethylamonium acetate (TEAA). Bacterial DNA samples with internal sequence variations and melting domains display differences in retention times.
The DHPLC system was found to distinct between species (*E. coli* vs. *Listeria*) but not between strains (*E. coli* 3110 vs. *E. coli* O157:H7). Moreover, as currently, there are no public databases; therefore, rapid verification of the results can only be performed by direct sequencing of individual collected peaks. In the best case scenario, results can be obtained in one working day and a concentration or enrichment step may be necessary, due to the detection limit of the technique, which is approximately 10^4 cells/mL (with simulated water samples).

When "real" water samples were analysed using the DHPLC technique (*E. coli* as target strain), it was not possible to detect *E. coli* at any concentration in inoculated washing water. The DHPLC was found to be not robust enough in complex media such as washing water samples, probably due to PCR inhibition, especially when there is a high bacterial load in the water samples

3. Potential impact and main dissemination activities and exploitation of results

Potential impact

The potential impact of the RESFOOD project can be appreciated under different related perspectives: The developed resource efficient solutions make a very valuable contribution to the competitiveness of the food industries, being the major labour force sector in Europe, with almost 50 million employees, 90 % of them work in SME's. From the point of the view of the societal challenge of making the European industry production more efficient, the developed solutions make it possible to attain significant savings in terms of water and energy across the different phase of food production and processing. In addition to an economic impact, this increase in efficiency has a large environmental impact, saving natural resources and the reduction of emissions to the surface and ground water and to the soil.

As highlighted in the previous sections of the present report, each work package addressing the different objectives across the food supply chain (from crop cultivation and food processing to disinfection up to treatment of food by-products and the recovery from valuable substances) was able to deliver promising results at the research level which were eventually confirmed in the different pilots.

Impact on horticulture

On average, 44% of the total water abstraction in Europe is used for agriculture. Within the RESFOOD project, ICT solutions were developed to address this issue. As example can be mentioned the average water consumption in Huelva of strawberry crop was more than the double of the water consumption in the RESFOOD pilots, what also resulted in a comparable difference in the fertilizer consumption. Currently in the EU-27 countries on average fertilizers containing 10.4 million tons of nitrogen (N), 2.4 million tons of phosphate (P2O5) and 2.7 million tons of potash (K_2O) have been applied to 135.1 million hectares of farmland each season. 20% to 50 % of those nutrients disappear in the soil and water leading to pollution of ground and surface water. Taking into account that the costs of fertilizers are up to 20% of the production costs means that ***l****eaching/loss of fertilizer represents 4%-10% of production costs*. These results have been presented to a large number of growers in an info-session in Huelva. For the research and test centres for agriculture ADESVA and Demokwekerij the project was very important to develop this knowledge and promote it to their customers and expand their role

For the partner Agricola el Bosque the savings are € 45.000 a year, for a moderate grower around Huelva it will be € 15.000 to € 20.000

The pilots in **horticulture** showed also the possibilities of water saving (40-90 %) by far going closure of the water cycle, without negative influencing the production. Besides the reduction on use of water and fertilizers and the reduced losses of to the surface and ground water the water cycle closure has also a big effect at the emissions of plant protection products to the environment.

Impact on Food processing

In the **food processing** in two fresh cut vegetable industries and one juice industry the reuse of water was demonstrated in practice saving 35 – 70 % of water and energy, without negative influencing the product quality and safety. Especially the fresh food and fruit industries are very intensive water users (over 5 million m^3/year in the EU), so these measures save several million m^3 a year. For the fresh vegetable industries, using cold water of 4 degrees, this also saves a lot of energy. Also prototype of a new washing machine was successful tested, saving 20-30 % water and energy.

Peroxy acetic acid showed to be a good alternative disinfection chemical. These sanitation agents demonstrate their potential as an alternative sanitizing method to maintain safety on the process wash water of the fresh-cut industry, avoiding cross-contamination during processing. The potential health impact and environmental effects regarding the by-products (like trihalomethanes) are positive comparing to the use of chlorine. Being able to process healthy and safe food products with less water, energy and in a safer way (also in combination with better monitoring, see below) makes the food processing industry more competitive with less environmental impact.

For the Food processing partners Vezet, Vega Mayor and Arisu the project was very important to increase their knowledge on different topics:

Vega Mayors says that they have “a better understanding of the process that allows decision making with regard to water use (volume used, recirculation rate). After RESFOOD, we have valuable information that support the increase of the reuse of the process water without having a negative impact on product safety and quality, which would result in a decrease in the volume of water used and therefore the cost associated. We are highly interested in the industrial use of peroxy-acetic acid, although the final implementation will depend on the official evaluation and approval of the chemical agent by the Spanish authorities (the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AECOSAN).

According to Vezet: ‘The tests have shown the possibilities for water reuse, but a next step has to be made to decrease costs (what should be possible) and the footprint of the installations as we have no room in the existing production rooms to install both pretreatment and UF installations at every machine. Additional the results and discussion on disinfection (not yet allowed in the Netherlands) is useful for the future.

Arisu got much more insight in their water system, but due to the short production season not every solution is economically feasible. We know much more about our water system and the cheap solutions are already implemented yet. It is a pity that the polyphenol recovery is not economically feasible.

According to Agricola el Bosque:

- Agrícola El Bosque has got the way to do its production system more environmentally sustainable.
- Agrícola El Bosque is able to increase its grow area due to saving of water got in the RESFOOD project.

Not all the results will be implemented at the short term by the above mentioned end-users. But the further implementation of water reuse will be continued and the new washing machine of Kronen will be most probably installed when new machinery has to be bought

The washer of Kronen is especially interesting for growing companies and the replacement market and is a clear improvement compared to current washers. The simulation expertise build up by Kronen is very valuable and unique. The simulation expertise allows Kronen to improve much of their current machines, without the competition being able to do the same in the coming years. This gives Kronen a clear competitive edge

For the equipment supplier Logisticon the project is very important to further develop this market. They also got insight in the specific demands of this type of food industry

Impact Monitoring and detection

Food safety is priority in a transition towards a more resource efficient food chain. During the project two pilots about new **detection and monitoring technologies** were developed and tested: A new optical biosensing system for rapid and on-site detection of bacteria in water and the IS-Pro technology. The latter came from medical applications and was adapted to food applications. The prototype system has proved robust and performed well during two rounds of pilot tests, and the results of the second demonstrations are currently being processed and analysed in order to further optimise the performance of the biosensors. Also the IS-Pro methodology showed to be applicable.

The development of these two methodologies can have much impact at food safety as they reduce the detection time from more than 1 day to less than 1 hour.

Impact biomass recovery and valorisation

A significant part of the initial agricultural food production is lost and wasted in different stages of the food supply chain. For fruit and vegetables the total loss is 46 %, so there is a large potential impact as a lot of these residues contain considerable amounts of valuable compounds. According to the EC-funded project AWARENET, approx. 222 million tonnes of food chain waste and by-products from fruit and vegetable processing industry are produced every year across the EU.

The impact of the results of RESFOOD in the area of biomass recovery and valorization differs very much depending on the application and the type of product. For a number of products the scale was too small, to make recovery economically feasable, but recovering proteins and fibers from endive is both technical and economically feasible and thus interesting for the private sector. As there is an increasing need of protein in the European countries, for both human and feed consumption, and Europe imports over 75 % of its protein necessities, the recovery of proteins can have a large influence at the competitiveness of the European Food and Feed sector and make them less dependent on import from other countries outside Europe.

An integrated scenario

In addition, RESFOOD introduced an innovative concept and approach: it brings the different sectors of the food chain together to make the process towards resource efficiency more comprehensive, taking into account the challenges of the different sectors of the medium to long term which would arise in the transition to the circular economy. In the current situation, the water, nutrient and energy consumption during food production and processing can be rather high, and so is the amount of food loss. The water cycle is mostly not closed and the discharge of water including the emissions of pesticides and nutrients is significant. On the other hand, in an integrated scenario, measures are taken concerning the reduction of water, nutrient and energy use, waste loss and valorization of food waste. The water cycle of substrate cultivation is nearly closed by using water treatment techniques to ensure the reuse of drain water. In food processing, the water and energy use is minimized by treating process water and reuse of the cold water. Retail and consumer optimization can be obtained by reducing transport, delivery on time, longer shelf life options, tax on energy consumption, etc. Validation of food waste can be introduced for the production of valuable bio-products/compounds.
Opportunities can be found for several areas: energy, water, waste, packaging, transport and consumers. The innovative technologies developed and tested within RESFOOD contribute to model this integrated scenario of green concepts. More work is nevertheless necessary: legislation and social education can help by creating more favorable conditions for the implementation of environmental-friendly solutions and the design of a sustainable agro-food chain.

The RESFOOD strategy of addressing the different topics of the food chain having the potential to favour a systemic change was remarked by Janneke Van-Veen, Policy Officer at the EC DG Research and Innovation, while attending the final conference of the RESFOOD project.

Potential impact in summary

The RESFOOD project has been successful in terms of developing new technologies and methods for resource efficient and safe food production and processing.

In the table below the potential impact of the results of RESFOOD is summarized

Table 13 Potential impact of the results of RESFOOD

Potential impact (as given in the work program)	**Related RESFOOD** **Contribution**
Reducing the pressure on primary raw materials and Preserving the environment and reducing pollution	Reduction of water use in horticulture by 40-90 % Reduction of water use in Food processing by 20 to 70 % Reduction of energy use for cooling water and heating processes in fresh food and juice processes with 25- 50 % Reduction of use of Nutrients (N, P,K, organic) with 40- 60 % Reduction of emissions of Nutrients and pesticides to ground and surface waterand to soil (20-90 %) Reduction of organic waste (biomassby 10 to 20 %), contribution to a bio-based economy
Fostering the use of secondary raw material, including urban mining, Re-use, recycling and recovering of valuable materials notably from urban waste	Re-use of Nutrients and organic waste by 10 to 20 % Recovery of valuable materials (proteins) from biomass waste from horticulture, food processing and retail
Building up more sustainable consumption and production patterns,	By thinking more in integrated concepts, taking into account all the food sectors RESFOOD will have a large impact towards a circular economy for the food chain by closing water, energy and material cycles
Increasing the role of SMEs as end users or developers of green technologies	By demonstrating in practice of small scale systems creates awareness and convinces all users but especially SME end –users of the opportunities of green technologies, what leads to increased application Involvement of SME end-users and suppliers in development of new technologies and new applications
Opening opportunities for new start-ups and markets in the medium Term	Chances for new start-ups in Biomass processing New markets for small scale water treatment systems New chances for companies involved in monitoring and detection

Joining dissemination and exploitation objectives

It is well known that eco-innovative technologies need to be made interesting to the industry in order to reach the market and consequently the society at large. For this reason it was important to involve the industry stakeholders, beside researchers and citizens' representatives (local governments, associations) and show them the maturity, applicability and benefits of the RESFOOD technologies from an economic point of view.

To reach this final objective, a robust dissemination campaign has been put in place, along with an exploitation strategy. It is necessary to raise awareness about the project and its results among stakeholders, to create a consensus among the experts community and to show the end-users practical and concrete examples on how the project results will be used, adapted and exploited by the food production and processing industry.

All the communication, dissemination and exploitation activities of the project are executed in a holistic manner under the RESFOOD Dissemination and Exploitation Plan that was prepared to describe the objectives, targets, strategies and tools at the very beginning of the project. This plan was regularly evaluated and updated according to the rising dissemination needs throughout the project and feedbacks from the RESFOOD Dissemination Committee.

As it can be inferred from the table below, an impressive number of communication actions (over 100 summing up participation to events, media briefings, press releases, meetings with stakeholders, organisations of public workshops and events, etc.) have been taken by a very dynamic consortium.

Broadly speaking, the communication strategy revolved around two main axes: scientific and technical/dissemination activities.

As concerns the scientific communication, the preparation of manuscripts for publication in different international peer-reviewed scientific journals and trade magazines has been an important asset for the research performers and an important source of knowledge about the project outcome. Research papers have been submitted to journals in the fields of horticultural science and biotechnology, bioprocess engineering as well as food science and technology. Within the overall dissemination plan, a plan for peer-reviewed journal publications has been developed in under the supervision of the DC, paying particular attention to the interest of the industrial partners in the consortium, IPR and exploitation measures, etc. At the end of the project, two manuscripts have been sent to peer-reviewed journals, one of which is already published. In addition, a chapter was also published in a scientific book. Besides this particular dissemination, a significant effort has been made to disseminate the project results in other technical-scientific forums. In total, 8 posters and 5 oral communications have been presented in several scientific workshops and congresses.

In the second period more and more results became available and therefore the focus in external communication shifted from a more general presentation of the project to more specific dissemination of RESFOOD results, with a particular attention of stressing practical and usable results and in targeting the end-users. To this end, the RESFOOD partners maintained links with the European Technology Platforms for Food and Water (Food for Life and WssTP), which represent the European industrial sectors. This exchange was intensified throughout the second period as demonstrated by the collaboration and participation on the project communication milestones (newsletter, events) and in particular in the support of the RESFOOD final conference.
The RESFOOD final event was the occasion at which the final results and information relevant for the transfer to final up-takers was disseminated. The interest generated by the event was remarkable: over 130 people registered, including sector networks, research institutions, industries and SMEs, public authorities and media services. A rich agenda of 20 speakers featured presentations of innovative research and technologies, prototypes and best practices developed by the RESFOOD partners, information on the vision and priorities of the European Commission and the sector industries, as well as project ideas to bring RESFOOD research to a further level and thus follow up on the project's outputs.

In addition to the presentations, various materials in other formats were prepared to make the attendees aware of all the different products realized by the project and fit specific dissemination purposes: a showcase of project tools by means of posters, videos and pictures gallery was set up and presented during the conference. The portable filtering unit developed by Microbiome was displayed and its practical functioning was demonstrated during the networking breaks. Following instructions by the communication partner, Kronen prepared a clip on the innovative washing machine. The conference itself, being the final milestone of the three-year project, was covered by means of an audiovisual service in order to produce material which would serve the promotion of RESFOOD even beyond its conclusion. Video interviews to WP leaders and the coordinator presenting the main results of their work were produced as well as a short video highlighting the high-level participation and the interest attracted by the RESFOOD project. In addition to that, each attendee received a booklet describing the conference as well as the main results of the different work-packages. A media kit for the journalists and media services was prepared including audio visual materials, press releases and info-sheets.

Besides, the conference hosted the launch of the 'Water and Food Innovation' dialogue for inclusive green growth in Europe", an initiative of the European Technology Platforms WssTP and Food for Life. This dialogue will form the basis for unleashing new business potentials on both sides. As highlighted by Beate Kettlitz, Food Drink Europe and Jochen Froebrich, WssTP, at the RESFOOD conference, the interaction is often fragmented and requires different and new networks within Europe. The new Working Group addresses the challenge for jobs and growth under water scarcity and droughts in Europe from a wider perspective. Opportunities within the circular economy can provide new impulses for irrigated agriculture by producing specialized products and re-using process water. The AgriFood processing can benefit from more resilience against floods and droughts and address policy barriers with a systemic approach.

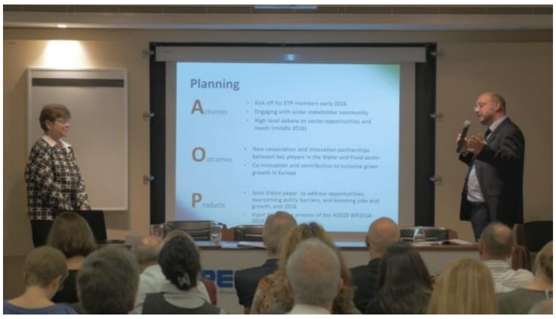

Figure 8 Beate Kettlitz, Food Drink Europe (Food for Life ETP) and Jochen Froebrich, WssTP, presenting the planning for the new Water-Food working group at the RESFOOD final conference

This dialogue suits the focus of bringing parties from the water sector and the Agri-Food processing sector together. The RESFOOD successful strategy of looking at the different topics of the food chain significantly contributed to the setup of this joint working group, which represents a tangible impact of the RESFOOD policy work on the top of the research and innovation results.

The industry and end-users were targeted also through specific mailing (the RESFOOD contact lists include over 1500 addresses of industry stakeholders) and with the participation to fairs and business event. Just to mention an example, the Kronen washing machine was for the event innovations awards by the Food Tech Jury of the international Anuga FoodTec in Cologne, on 24-27 March 2015. The Anuga FoodTec is the international supplier fair for the food and beverage industry gathering over 45,000 trade visitors from 137 countries and this selection gave the Kronen machine and the project remarkable visibility.

Technical journals were also considered as a very interesting way to expand the dissemination activities particularly targeted to the industrial sector. The target media are the professional and industrial magazines specialised in food, water, environment also including the publications for the SMEs and equipment suppliers working for the food sector. The project was already covered by the Spanish food technology magazines Alimentaria and Technifood in the first year of the project. During the second period, a major communication achievement was the publication of a full technical article in the leading bi-monthly magazine *New Food*. The article makes a comprehensive and strong picture of RESFOOD innovative solutions targeting the different topics of the food chain and their environmental benefits. The editors put RESFOOD on the cover page of the magazine, which boasts an ABC-audited circulation of nearly 14,000 across the world. This article significantly increased the impact of RESFOOD: many positive feedbacks were recorded by partners which were contacted following this article.

RESFOOD results are in line with the priorities of the upcoming programme of the European Commission. To highlight these links, during the final conferenceproject ideas addressing 6 different topics of the H2020 2016 calls related to water, agro-food, sustainable process industries themes were presented by the RESFOOD partners. These ideas have the potential to be retained for funding and thus contribute to design the sustainable food chain of the future.

All communication materials, opportunities for dissemination, participation and organisation of events, articles, news and newsletters about the project are available through the RESFOOD website (www.resfood.eu). The website was updated on a regular basis throughout the project and even beyond its official closure on 31 October 2015. In particular, the sections about news, media coverage, events calendar, link to other projects and initiatives, and video and pictures gallery were refreshed on a weekly basis in order to keep pace with the project development and link it to the context as well.

To sum up, in the course of three years, the consortium as a whole has executed the below communication actions (for more details, please refer to the Table A2: list of dissemination activities):

- organised one large final event (as described above) and four workshops/info-sessions. These information session were of special interest for the SME's and equipment suppliers: participants could get practical demonstrations of how to apply the project's technologies and implement them in their own realities and understand their benefits both in environmental and economic terms. These info-sessions served both the communication and the exploitation objectives of the project, as they help create the conditions for market uptake of RESFOOD research results. The three info-sessions were organised in three different locations: the first one in November 2013 in Germany, under the responsibility of Kronen, addressing water management and decontamination technologies; the second one in May 2014 in the Netherlands, under the responsibility of Demokwekerij, addressing re-use of water and nutrients in horticulture, in particular for soilless cultivation; and the third one in February 2015 in Spain, under the responsibility of ADESVA, addressing efficient ferti-irrigation techniques in horticulture, which was followed by a training held at the Demo Nursery under the initiative of TNO, presenting the latest advancement on water recirculation techniques for irrigation. The organising partners are a supplier active in the food processing sector, an agriculture partner active in the production sector, and a research partner developing innovative cultivation techniques, respectively. Such diversification in terms of timing, geographic and sector positioning, made each session unique in itself: it was possible to present the RESFOOD results from complementary angles, their progress and their impact all along the food chain. The format of these events – made up of a number of up-to-the point presentations describing latest technological results, followed by visits and demonstration to pilot installation - and addressing a selected and targeted audience (an average of 50 participants) composed of local professionals and business of the sector, proved effective in clearly showing the economic and practicable advantages of research and innovation actions

- participated with an oral presentation, a poster, a booth or other communication materials, to 55 events such as sector conferences, international exhibitions, business trade fairs, academic symposium, events supported by the European Commission, thus marking a potential outreach on > 50,000 professionals

- delivered three scientific publications

- published articles in 4 technical journals

- distributed news and press releases to a mailing list of over 7000 contacts including addresses from industry, research centres/universities, policy makers, media and the European

Commission from the fields of food, water and environment, plus making use of the European information services Cordis

- published and distributed three newsletters. The RESFOOD newsletters, besides delivering an update of the project research and pilots activities, hosted interviews and contributions from related projects and initiatives. The first issue featured interviews with the RESFOOD partners, highlighting the main objectives of the project along with the preliminary results achieved during the first-year laboratory tests. The newsletter also covered news about other related projects, a glossary of technical terms related with RESFOOD and a calendar of events. The second newsletter dated December 2014 and presented the results achieved by RESFOOD partners after various research and demonstration activities conducted in the first two years and also included the interview to the coordinator of two other FP7 projects (Veg-I-Trade and SUSCLEAN) active in resource efficient technologies for food production and processing. The third and last newsletter was released mid-October 2015: this issue presented the latest results of the project with a particular attention to the applicability and exploitation of RESFOOD solutions and announced the final conference in Brussels In particular, the last issue of the newsletter hosted a interview to the representatives of the Water Supply and Sanitation Platform and Food for Life European Platform, which highlighted converging objectives for the food and water industries in relation to efficiency and sustainability. Inviting external stakeholders to the RESFOOD newsletter helped enlarge impact and circulation of the project outputs.

- over 80 articles and news items about RESFOOD can be counted which appeared on European and international press services, websites, local and national press in different languages (English, plus the national languages of the partners plus Italian). See list on: http://www.resfood.eu/web/newsroom/media-review/

- maintained a presence on the web with a constantly updated dedicated website (www.resfood.eu) which totalled 10,219 unique visitors during the second period. The statistics throughout the project timeline show that number of visitors to the RESFOOD website has a rising profile: during the second half of the project, the visits counted by the website more than doubled, showing a peak in correspondence to the final event. This is mostly due to the active dissemination of the project results that were achieved on the occasion of the final conference. RESFOOD was also present on the main social media, attracting a total of around 620 followers. Among the different social media used, Twitter proves to be the most effective in managing a rapid and dynamic interaction with interested people and in multiplying the communication, by proactively linking the RESFOOD updates to the Twitter accounts of the various information services dealing with science communication and the sector networks and platforms. Among the followers, it is worth remarking public institutions like the EU Commission's DG Health & Food Safety (SANTE), cluster initiatives like Eureka for water, EU Expo2015, WssTP, information services like footprint media, Food and Drink News, Cordis - just to name a few - which boast thousands of contacts and guarantee a wide multiplying effect by favouring or re-twitting RESFOOD posts.

- produced and disseminated printed and audio-visual materials (4 videos plus various technical and scientific posters, roll-ups, leaflet, info-sheets).

Technology exploitation

During the meetings in 2nd period, exploitation and IPR management has been one of the key topics. Advice has been provided and feedback given to ensure a well thought disclosure of information during the project and optimized commercial potential of the technologies developed and demonstrated.

The exploitation manager Logisticon Water Treatment, with the support of TNO, has also made an assessment of the technologies developed within RESFOOD. For those technologies which were seen as close to market (exploitable within 1-2 years), exploitations plans were formulated, based at the canvas model.

The technologies selected were:

- Capillary UF (Logisticon)
- IS-PRO technology (Microbiome)
    - For both food and medical applications
- Water filtering device (Microbiome)
- Innovative Washer (Kronen)

The exploitations plans were written in close collaboration with Logisticon, Microbiome and Kronen. A questionnaire was used to gather the required information for exploitation. The exploitation plans discuss the following aspects:

- Short description of technology
- Unique selling points (including comparison to alternatives)
- SWOT analysis (strengths, weakness, opportunities and threats), including measures to capitalize chances and tackle issues
- Business model overview (using the business model canvas)
- Exploitation planning
- Major conclusions and recommendations

The main conclusions from the exploitation plans are:

- Fast introduction and broad dissemination of the Capillary UF technology is crucial
    - There are many other suppliers, currently only the expertise of Logisticon is (relatively) unique. IPR protection is not an option.
    - Fast introduction and broad dissemination will ensure that Logisticon is viewed as the first who introduces this technology in the food processing market ("launching company) and is best known for this technology.
- Microbiome is planning to introduce the water filtering device in both medical and food markets in 2016. It will be interesting for all companies taking water samples for microbial analysis, thus opening up a huge market. Presumably, it could become a new golden standard[2] for the industry. Microbiome is seeking collaboration with large companies, which is crucial to:
    - Obtain sufficient sales and research power to keep IS-PRO faster, easier and cheaper than DNA sequencing (large industry with rapidly decreasing product prices)

[2] Water filtering device could become a new "golden standard" for taking water samples for icrobial analysis, as the water is filtered directly and the microbial population on the filter remains unchanged during transport.

  - Obtain sufficient production capacity to be able to realize the large market potential of the water filtering device

  For the IS-Pro technology it is also important to extend the data base for food related micro-organism.

- KRONEN designed a completely new and innovative washer during the project. This work was done with help of the project partners- in particular the research centres- and also with help of simulation work. The important point was to determine the characteristics of water charged with lettuce. KRONEN can use this knowledge to improve all new washers and designs contacts and will also to bring their customers in contact with this information, thus expanding awareness on environmental and energy issues.
- The washer of Kronen is especially interesting for growing companies and the replacement market and is a clear improvement compared to current washers. Kronen is planning to add a few additional features, primarily linked to the reuse of water in the machine, after which its plans to introduce the new washer in the market mid-2016. Currently Kronen is mainly known for small machines. Although the market for big machines is small, for Kronen it is very interesting to be able to show that they can make big machines and offer one-stop solutions. Besides, the simulation expertise is also applicable to the other machines of Kronen. The market for small machines is much larger than for big machines (>2000 companies). Therefore, Kronen plans to perform similar redesigns of their other machines. The simulation expertise build up by Kronen is very valuable and unique. The simulation expertise allows Kronen to improve much of their current machines, without the competition being able to do the same in the coming years. This gives Kronen a clear competitive edge.

For the selected technologies the canvas model can be very helpful to support exploitation.
For a number of solutions that are tested in practice dissemination is the most important activity to bring the solutions to the market. This counts for instance for the application of ICT in horticulture, the use of peroxy acetic acid for disinfection and the general approach to a further closure of the water cycle in horticulture and food processing. For others, like the bio-sensing technology for fast monitoring and detection, additional research is needed to make the step from laboratory to practice.

The exploitation plans were specifically made for the equipment suppliers to support the exploitation of the products developed. For the end-users these exploitations plans gave also more insight in how to use the results of the RESFOOD project (see also above at the beginning of this chapter.