



Natural resources and
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Improving quality and treatment of water and vegetables in fresh-cut vegetable processing

Doctoral Dissertation

Marja Lehto

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Academic dissertation

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Abstract

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Fresh-cut vegetables have been cleaned, peeled, chopped, sliced, or diced and then packaged but not heated. The fresh-cut vegetable processing industry uses large volumes of water. This water is utilized by hygiene and cleaning processes and for cooling of the products. Knowledge has been lacking about waters created and the water use in different stages of the fresh-cut vegetable processing. Obtaining information about the water use and waste water production is important for recognizing critical phases for risk management and for evaluating the need of water treatments. The aim of this study was to improve the processing of fresh-cut vegetables through collecting information on the hygienic level of waters and vegetables, decontamination methods and their efficacy, water use and waste waters which helps companies to improve their processes and self-monitoring activities. One aim of this study was to also evaluate on-farm waste water treatment systems carrying out peeling of vegetables.

Water consumption, measured in six fresh-cut processing companies in this study, was 2.0–6.5 m³/t per finished product. The water consumption varied in the same company between months and according to season, volumes of vegetables processed, and the quality of raw material. Through regular measurement of water consumption, it is possible to decrease water use in fresh-cut vegetable processing. In the present study, water consumption decreased by 15% over the course of the three-year period examined. This may decrease costs and improve sustainability of the production.

Vegetables contain 90–96% water; the remainder is composed of components such as carbohydrates, proteins and nutrients. In vegetal cells, water is present in different forms; part of this water can easily be removed and a part cannot. Depending on their size, the substances of which vegetables are composed form different kinds of solutions in combination with water. Most of the organic load and nutrients of the vegetables processed were released into water from the peeling of root vegetables, whereas the volume of the water came primarily from the rinsing and washing of vegetables. Washing is an important step in fresh-cut vegetable processing; it removes soil and debris, and reduces microbial populations residing on the vegetable surface. Washing is often the only step that can remove foreign material and tissue exudates, as well as inactivate pathogens. Water plays a dual role in the fresh-cut vegetable processing: it both reduces and transmits microorganisms to vegetables. The high quality of water used in processing is important, and can be attained through water decontamination or by using new potable water that is changed continuously during the process. The high operational cost of water use has resulted in the industry-wide common practice of the reuse or recirculation of process water.

Fresh-cut vegetables may be contaminated by pathogens in different stages and different ways after harvest. Pathogenic microorganisms can cause severe outbreaks of foodborne disease. The microbiological quality of vegetables changes during processing. The total microbial counts in peeled and cut carrots were lower than in whole washed carrots, but higher in grated than in cut carrots. The total microbial count was lower in process water than in wash water of carrots. Pathogenic *Yersinia enterocolitica* was detected in many carrot and water samples by sensitive RT-PCR, but not by the cultivation method.

The data concerning treatment of process water of fresh-cut vegetable processing is quite scarce, in particular concerning the effect of treatments on yersinia. Water decontamination methods neutral electrolyzed water (NEW), chlorine dioxide (ClO₂), organic acids and UV-C was evaluated, specially on yersinia, *E. coli* and *Candida lambica* (yeast) in this study. The effect of decontamination on different microbes in water differs with, e.g., time, concentration, decontamination method, and turbidity of water. Technically- and economically effective chlorine-alternative decontamination technologies are the goal of the fresh-cut industry. In Finland, and in many other EU countries as well, chemical treatments of vegetable process waters are restricted in food legislation, but allowed in other countries.

Published information concerning the functioning and feasibility of small on-farm waste water treatment plants are few. Waste water generated from vegetable production contains high concentrations of biochemical oxygen demand (BOD) and suspended solids (SS). One aim of this study was to evaluate on-farm waste water treatment systems carrying out peeling of vegetables. Primary treatments of waste water remove coarse solids, reduce organic matter content and adjust pH. Secondary, biological, wastewater treatment removes soluble organic matter and nutrients from water. Biological waste water treatment, such as a sequencing batch reactor or a trickling filter, are used for treating of vegetable processing waste water in small scale companies in rural areas. In the case of both systems, the requirements set in legislation were met. Tertiary treatment can be used if waste water is reused in subsequent vegetable processing or recycled for irrigation of food crops.

Fresh-cut vegetable processing companies produce high-quality fresh-cut produce with appropriate inputs and processes. Each company must establish its own specific validation protocols for evaluating their processes. The aim is to minimize the risks and produce healthy, safe, fresh and easy-to-use vegetables for consumers.

Keywords: Decontamination, carrot, fresh-cut vegetable, lettuce, microbiological quality, processing, process water, wash water, waste water treatment, water use

Tiivistelmä

Tuorekasvikset on puhdistettu, kuorittu, pilkottu (viipaloitu, silputtu tai kuutioitu) ja pakattu, mutta niitä ei kuumenneta missään prosessin vaiheessa. Tuorekasvisten prosessoinnissa käytetään paljon vettä; sitä tarvitaan raaka-aineiden, tuotteiden ja tilojen puhdistuksessa sekä hygienisoinnissa. On vain vähän tutkittua tietoa siitä, missä tuorekasvisten prosessoinnin vaiheissa ja miten paljon vettä käytetään ja miten paljon jätevesiä muodostuu. Tutkimustieto yritysten veden käytöstä ja jätevesien muodostumisesta on tärkeää, jotta voidaan tunnistaa riskien hallinnan kannalta kriittiset prosessien vaiheet ja arvioida jätevesien käsittelytarvetta. Tämän tutkimuksen tavoitteena oli kehittää tuorekasvisten prosessointia keräämällä tietoa vesien ja kasvisten hygieenisestä laadusta, vesien hygienisointimenetelmistä ja niiden tehokkuudesta, veden käytöstä sekä jätevesistä. Tavoitteena oli myös arvioida tilakohtaisia kasvisten prosessoinnin jätevesien käsittelymenetelmiä. Tämä tieto auttaa yrityksiä kehittämään prosessejaan ja tehostamaan omavalvontaansa.

Veden määrä, jota mitattiin tässä tutkimuksessa kuudessa tuorekasviksia prosessoivassa yrityksessä, vaihteli eri yrityksissä välillä 2,0–6,5 m³ lopputuotetonnia kohden. Veden käyttö vaihteli myös tietyssä yrityksessä eri kuukausina riippuen käsiteltävien kasvisten määrästä, raaka-aineen laadusta ja vuodenajasta. Yrityksissä, joissa seurattiin säännöllisesti veden käyttöä, saatiin veden kulutusta pienennettyä. Tässä tutkimuksessa veden kulutus laski yhdessä yrityksessä 15 % kolmen vuoden seurantajakson aikana. Säästämällä vettä voidaan pienentää kustannuksia ja parantaa tuorekasvisten prosessoinnin kestävyyttä.

Kasvikset sisältävät vettä 90–96 % painostaan; loppuosa koostuu muun muassa hiilihydraateista, proteiineista ja muista ravintoaineista. Suurin osa kasvisten prosessoinnissa jäteveteen päätyvästä orgaanisesta aineesta (BOD, biological oxygen demand) ja ravinteista siirtyi tutkimuksen mukaan veteen juuresten kuorintavaiheessa, kun taas pääosa veden kulutuksesta tapahtui kasvisten pesussa ja huuhtelussa. Kasvisten pesu on tärkeä vaihe tuorekasvisten prosessoinnissa; siinä kasviksista poistuu maa-ainesta ja kasvisten pintakerrosta ja se vähentää mikro-organismien määrää kasvisten pinnalla. Pesu on usein ainoa vaihe, jolla voidaan poistaa epäpuhtauksia ja muuta vierasta materiaalia kasviksista. Vedellä on kuitenkin kaksitahoinen rooli tuorekasvisten prosessoinnissa: mikro-organismien vähentämisen lisäksi vesi voi myös levittää niitä kasviksiin. Tuorekasvisten prosessoinnissa käytettävän veden laadun täytyy olla hyvää, ja laadun hallinnassa voidaan käyttää erilaisia veden puhdistusmenetelmiä tai vettä voidaan vaihtaa jatkuvasti prosessin aikana. Veden korkean käyttökustannuksen vuoksi yritykset pyrkivät kierrättämään tai käyttämään vettä uudelleen.

Kasvisten mikrobiologinen laatu muuttuu prosessoinnin aikana. Tautia aiheuttavat mikro-organismit voivat saastuttaa kasviksia prosessin eri vaiheissa ja aiheuttaa ruokamyrkytyksiä. Tässä tutkimuksessa kokonaismikrobien määrä kuorituissa ja pilkotuissa porkkanoissa oli alhaisempi kuin kokonaisissa, pestyissä porkkanoissa, mutta määrä oli

korkeampi porkkanaraasteessa kuin pilkoutuissa porkkanoissa. Kokonaismikrobien määrä oli alhaisempi porkkanoiden prosessi- kuin pesuvedessä. Patogeenista *Yersinia enterocolitica*-bakteeria löydettiin monista porkkana- ja vesinäytteistä kun käytettiin herkkää PCR-menetelmää, mutta bakteeriviljelymenetelmällä niitä ei havaittu.

Aiempia mittaustuloksia tuorekasvien prosessivesistä on melko vähän saatavissa, varsinkin yersiniaan liittyen. Tässä työssä vertailtiin veden puhdistusmenetelmiä, kuten neutraalia elektrolysoitua vettä (NEW), klooridioksidia (ClO_2), orgaanisia happoja ja ultraviolettivaloa (UV-C), ja arvioitiin menetelmien tehoa yersinia- ja *E. coli* -bakteereihin sekä *Candida lambica* -hiivaan. Puhdistuksen tehokkuuteen vaikuttaa näissä vesissä erityisesti veden sameus. Tuorekasvien prosessoinnissa tavoitteena on löytää teknisesti ja taloudellisesti tehokas veden puhdistusmenetelmä, jossa ei käytetä klooria. Suomessa ja monessa muussa EU-maassa kemiallista käsittelyä, esimerkiksi kloorin käyttöä, kasvien prosessoinnissa on rajoitettu elintarvikelainsäädännössä, mutta klooria käytetään monissa muissa maissa.

Pienen kokoluokan yrityskohtaisesta jätevedenkäsittelystä on olemassa vähän julkaistua tietoa. Kasvien prosessoinnissa muodostuva jätevesi sisältää paljon orgaanista ainetta sekä kiintoainetta. Jäteveden esikäsittelyllä voidaan muun muassa vähentää veden kiintoaineen ja orgaanisen aineen pitoisuuksia sekä säätää happamuutta. Biologista jäteveden käsittelyä, kuten panospuhdistamoja tai biosuodinta, voidaan käyttää kasvien prosessoinnissa muodostuvien jätevesien käsittelyssä viemäriverkostojen ulkopuolisilla alueilla. Tässä tutkimuksessa molemmilla menetelmillä (panospuhdistamo ja biosuodin) saavutettiin lainsäädännön vaatimukset. Jäteveden puhdistusta ja hygienisointia tarvitaan jäteveden käsittelyn jälkeen, jos jätevettä käytetään uudelleen kasvien käsittelyprosessissa tai kasteluvetenä kasvintuotannossa.

Tuorekasviksia prosessoivien yritysten tavoitteena on tuottaa korkealaatuisia tuotteita yrityksen kokoluokkaan ja resursseihin suhteutetuilla panostuksilla ja prosesseilla. Yritykset laativat oman, yrityskohtaisen omavalvontaohjeistuksensa, jolla he arvioivat prosessejaan ja koko tuotantoketjuaan. Tavoitteena on pienentää riskejä ja tuottaa terveellisiä, turvallisia ja helppokäyttöisiä kasviksia kuluttajille.

Asiasanat: Dekontaminaatio, jätevesi, mikrobiologinen laatu, porkkana, pesuvesi, prosessivesi, prosessointi, salaatti, tuorekasvis, veden käyttö

Forewords

The need for the study arose from discussions with representatives of a company that was concerned about the quality of their production and vegetable products. This happened over 15 years ago at the time when companies in Finland had begun to extend their activities from primary production to fresh-cut vegetable production including processing. There was little information on what should be measured, how to ensure that products were safe, and how the waters and waste water used in production ought to be treated so that customers and the authorities were satisfied.

In the projects belonging to this study we have cooperated with several Finnish fresh-cut vegetable companies. Several measurements have been performed and samples have been taken in the companies studied. Companies have also actively participated in planning and giving information of their production. I am grateful to all the companies studied for their co-operation, help, kindness and interest in our research, and for valuable information of the branch of activity.

I sincerely thank Professor Laura Alakukku, Docent Hanna-Riitta Kymäläinen and Senior Scientist Maarit Mäki for contributing of final form of this dissertation thesis. I gratefully acknowledge Professor Francisco Artés Hernández and Professor Hülya Ölmez for pre-examination of the text and for their valuable comments on the manuscript. I also would like to thank Senior Expert Ilkka Sipilä and Research Coordinator Risto Kuisma for their contributions for the study and so much more. I also would like to thank my colleagues and co-authors Jenni Määttä, Maarit Hellstedt and Sanna Sorvala for co-operation as well as Senior Scientist Leena Hamberg who helped me with statistical methods.

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Finally I wish to thank my family for the opportunity to think of other things.

List of original publications

This thesis is based on the following publications:

- I Lehto, M., Sipilä, I., Alakukku, L. & Kymäläinen, H-R. 2014. Water consumption and wastewaters in fresh-cut vegetable production. *Agricultural and Food Science* 23, 246–256.
- II Määttä, J., Lehto, M., Kuisma, R., Kymäläinen, H-R. & Mäki, M. 2013. Microbiological quality of fresh-cut carrots and process waters. Research note. *Journal of Food Protection* 76, 1240–1244.
- III Lehto, M., Kuisma, R., Kymäläinen, H-R. & Mäki, M. 2017. Neutral electrolysed water (NEW), chlorine dioxide, organic acid product and ultraviolet-C for inactivation of microbes in fresh-cut washing. *Journal of Food Processing and Preservation*, 42, 1, e13354. <https://doi.org/10.1111/jfpp.13354>
- IV Lehto, M., Sipilä, I., Sorvala, S., Hellstedt, M., Kymäläinen, H-R. & Sjöberg, A-M. 2009. Evaluation on-farm biological treatment processes for wastewaters from vegetable peeling. *Environmental Technology* 30, 1, 3–10.

Contributions

The following table presents the contributions of the authors to the original articles of the dissertation:

	Article I	Article II	Article III	Article IV
Initial idea	ML	ML, MM, JM	ML, MM, RK	ML, MH
Planning the experiment	ML, IS	MM, ML, JM	ML, MM, RK	ML, IS
Conducting the experiment	ML, IS	MM, JM	MM, RK	ML, IS, SS
Processing of results	ML, IS	MM, JM, ML	MM, ML, RK	ML, IS, SS
Manuscript preparation	ML, H-RK, IS, LA	ML, H-RK, RK, MM	ML, MM, H-RK, RK	ML, H-RK, A-MS

LA	Laura Alakukku, University of Helsinki
MH	Maarit Hellstedt, Natural Resources Institute Finland (Luke)
RK	Risto Kuisma, University of Helsinki
H-RK	Hanna-Riitta Kymäläinen, University of Helsinki
ML	Marja Lehto, Natural Resources Institute Finland (Luke)
MM	Maarit Mäki, Natural Resources Institute Finland (Luke)
JM	Jenni Määttä, University of Helsinki (present affiliation: Forenom)
IS	Ilkka Sipilä, Natural Resources Institute Finland (Luke)
A-MS	Anna-Maija Sjöberg, University of Helsinki (emerita)
SS	Sanna Sorvala, MTT Agrifood Research (present affiliation: Yhtyneet Medix Laboratoriot Oy)

Abbreviations

BOD	Biochemical oxygen demand
CFU	Colony forming unit
Clean water	Clean water is natural water or treated water: e.g., lake water
COD	Chemical oxygen demand
DAF	Dissolved air flotation
Decontamination	The process of cleansing an object or substance to remove contaminants such as micro-organisms
DBP	Disinfection/decontamination by-products
DM	Dry matter
Drinking water	The quality of drinking water which meets the legal requirements of drinking water
EOW	Electrolysed oxidising water
FPW	Fresh Produce Wash [®]
HRT	Hydraulic retention time
IS	Interfering substance
LOX	Lipoxygenase
MLSS	Mixed liquor suspended solids
NEW	Neutral electrolysed water
NTU	Nephelometric Turbidity Unit
Process water	Drinking water or clean water which is transferred to the food process. This water can remain in a portion of the produce, or it can be removed completely (EC 852/2004).
Q	Volumetric flow
rpm	Revolutions per minute
RT-PCR	Real-time polymerase chain reaction
SBR	Sequencing batch reactor
SS	Suspended solids
TDS	Total dissolved solids
TKN	Total Kjeldahl nitrogen
TN	Total nitrogen
TP	Total phosphorous
True solution	A homogeneous mixture of two or more substances
TS	Total solids
TSS	Total suspended solids
US	Ultrasound
UV	Ultraviolet
UV-C	Ultraviolet-C

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1. Introduction

Fresh-cut produce is defined as “any fresh fruit or vegetable or any combination thereof that has been physically altered from its original form, but remains in a fresh state” (IFPA 2005). Fresh-cut vegetables have been cleaned, cored, peeled, chopped, sliced, or diced and then packaged (Francis et al. 2012). The markets for fresh-cut vegetables vary between countries and trends in consumption seem to reflect the trends for the total production of vegetables in the different European countries (Rojas-Graü et al. 2011; Baseline et al. 2014). Consumption of fresh-cut produce in Europe has been expected to increase by 12% from 2015 to 2020 (Euromonitor 2015).

As a group, fresh-cut vegetables satisfy the consumer demand for easy-to-use, convenient and healthy food: low in fat, but high in vitamins, minerals and fibre. Such foods are also rich in components known as phytochemicals or phytonutrients: e.g., carotenoids and phenols (Cox et al. 1996; Craig & Beck 1999; Francis et al. 2012). Consumers of fresh-cut products are retail dealers or food service establishments such as schools, hospitals, catering services and restaurants, as well as households. The main advantages to consumers of fresh-cut vegetables are: the reduced preparation time, decrease in labor required for produce preparation, its characteristics as a fresh food, the uniformity and consistency of a high-quality product, the easy supply of healthy products, the reasonable price and its ease of storage, requiring little storage space and generating low quantities of waste. All these factors have led to the rapid growth of this industry in recent years (Artés & Allende 2005; Garcia & Barrett 2005; Francis et al. 2012). The fresh-cut vegetable industry is significantly different compared to that of ready-to-eat cooked foods because there is no thermal step in the food processing chain for reduction and control of microorganisms. Disadvantages of the fresh-cut products are: rapid deterioration, short shelf life of the products in the marketplace, and the potential health hazards associated with spoilage (Brecht et al. 2004). Concurrently, there has been a large number of foodborne disease outbreaks linked to fresh produce (Harris et al. 2003; Lynch et al. 2009; da Silva et al. 2013; CDC 2017).

The fresh-cut vegetable industry is very diverse, including many products, each with its own structure at the point of production. The production of fresh-cut produce requires investment in facilities as well as investment in employees and their education, technology, equipment, management systems and strict observance of food safety principles and practices in order to ensure product quality (James & Ngarmasak 2010). In addition, high-quality water is required for processing. However, fresh-cut vegetable processing involves adding value to an agricultural business (Francis et al. 2012).

The water content of vegetables and water used in processing, have a significant effect on maintenance of the quality of vegetables: microbes cannot grow without water. The fresh-cut industry is the most water-intensive sector of the food industry, and almost all food processing techniques for fresh-cut vegetables involve the use of water (Kirby 2003; Ölmez 2013). Water is used in vegetable processing for many purposes, including: cleaning, processing, cooling, rinsing and conveying of vegetables, and for

cleaning of production facilities. The availability of freshwater resources, both in quantity and quality, is important to food production and food security and safety (Ölmez 2013; Vaclavik & Christian 2014).

A lack of data has been reported on the amount of water consumed and discharged at specific steps of the processing line of the fresh-cut vegetable industry (Ölmez 2013). The processing steps of fresh-cut vegetables and the effect of these steps on vegetables and waters have seldom been reported. The safety and quality of fresh-cut vegetables must be taken into account in the entire processing line.

The primary focus of this thesis is water in fresh-cut vegetable processing: what the quality of water and water treatment is, both during and after the processing of vegetables. Figure 1 illustrates fresh-cut vegetable processing, water use in such processing, and related issues such as the framing of the content of this thesis.

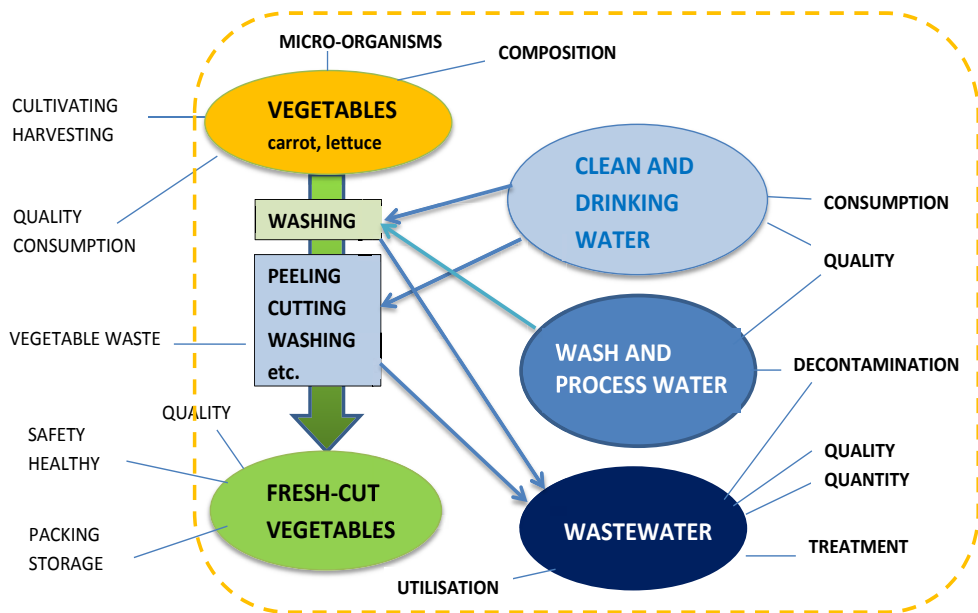


Figure 1. Fresh-cut vegetable production, waters involved in the process and related issues as the framing of this thesis. The dotted line indicates the (system) boundaries of this study. Process water is drinking water “which is transferred to the food process. This water can remain in a portion of the produce or it can be removed completely” (EC 852/2004). Wash water can be clean water: lake water, among other things.

1.1. Vegetables and water in fresh-cut vegetable processing

Vegetables consist of plant cells, which in turn contain a cell wall, chloroplasts, a vacuole and a nucleus. The cell wall has an intrinsic role to play in the quality characteristics of a vegetable (Waldron et al. 2003). Plant epidermal tissue functions as protection against infections, insects and physical damage, in order to maintain turgor pressure within the tissue by preventing water loss, and to provide for gas exchange between internal cells and the environment (Frank 2001).

Vegetable cells are cut and bruised when vegetables are peeled, cut and grated. Large areas of internal tissue are exposed, disrupting some subcellular compartmentalization. Enzymes are released from the cells and oxygen becomes accessible for reactions. Exposing of the cytoplasm provides micro-organisms with a versatile source of nutrients as compared to intact produce. Stress response reactions lead to increased respiration rates and to the synthesis of lignin (Bolin & Huxsoll 1991; Barry-Ryan et al. 2000; Damoraran 2017). Solutes of vegetables and water used in processing become mixed resulting in altered properties of both constituents.

Vegetables contain generally 90–96% water, but other various components as well. The relationships between cellular components and water determine the textural differences of vegetables. The degree and tenacity of water binding or hydration depends on a number of factors including: the nature of the nonaqueous constituent, salt composition, pH, and temperature (Damodaran 2017). In vegetal cells, water is present in the following forms (Vaclavik & Christian 2014):

- Bound water that cannot be extracted easily and which is bound to polar and ionic groups
 - It is not free to act as a solvent for salts and sugars.
 - It can be frozen only at very low temperatures (below freezing point of water).
 - It exhibits essentially no vapor pressure.
 - Its density is greater than that of free water.
- Free water that can be extracted easily from foods by squeezing, cutting or pressing
- Entrapped water that is immobilized in capillaries or cells, but if released during cutting or damage, flows freely. It has properties of free water and none of the properties of bound water.

Various substances from vegetables, such as salts, sugars, carbohydrates among other things, are either dissolved, dispersed, or suspended in water depending on their particle size and solubility (Table 1).

Table 1. Dissolved, dispersed, or suspended substances in vegetal cell water of vegetables (Vaclavik & Christian 2014).

	Dissolved	Dispersed	Suspended
Particle size	Small molecules, < 1 nm	1–100 nm	> 100 nm
Solutions	True solutions – ionic or molecular	Colloidal dispersion	Suspension with water particles settled out
Substances	Salts, sugars, water-soluble vitamins	Cellulose, pectic substances, gums, some proteins	Starch

The dry matter of vegetables consists of biomolecules (carbohydrates, proteins and lipids), minerals, vitamins, and phytonutrients. The main component (more than 90%) of the dry matter of vegetables is carbohydrates (Sanchez-Moreno et al. 2006; Butnariu & Butu 2014). Nutrient content and biochemical composition vary with vegetable products, because they come from different vegetables and different parts of the plants. Roots are rich in fibers and skeleton-type tissues with high lignin and cellulose (Butnariu & Butu 2014). These constituents are also dispersed in process and waste waters during vegetable processing.

The two major groups of micro-organisms found in vegetables are bacteria and fungi, the latter consisting of yeasts and moulds. Most microorganisms that are initially observed on whole vegetable surfaces are soil inhabitants, members of a very large and diverse community of microbes (Barth et al. 2009). The high level of water activity and the approximately neutral pH of vegetable tissue facilitate rapid microbial growth. Bacterial communities differ with respect to both the taxonomic structure and produce type of vegetable (Leff & Fierer 2013).

Carrot and lettuce as the example vegetables for this study

The carrot (*Daucus carota*) is one of the most popular root vegetables grown throughout the world (Sharma et al. 2012). Unpeeled and unwashed carrot raw material can be stored 6 to 8 months at 0–1 °C and at a relative humidity of more than 95% without loss of quality, provided that pathogens do not develop (Edelenbos 2010). The moisture content of carrots varies from 86–89%. Carrots contain a significant amount of phytonutrients, as well as carbohydrates and minerals such as Ca, K, Na, Fe and Mg. Carrots are high in dietary fiber (2.5–3.0%) and pectin (1.4%)(Bao & Chang 1994).

Lettuce is a commonly used vegetable in the EU (Freshfel 2014). There are four basic types of lettuce: crisphead or iceberg (*Lactuca sativa* var. *capitata*), butterhead (*L. sativa*, var. *Flandria*); cos or romaine (*L. sativa*, var. *longifolia*); and leaf (*L. sativa*, var. *crispa*) lettuces. Iceberg lettuce is considered one of the most popular of fresh-cut vegetables (Ragaert et al. 2004). Lettuce should be quickly cooled and maintained as close to 0 °C as possible with 98–100% relative humidity. Head types (iceberg, butterhead and

cos) are better adapted to prolonged storage than leaf lettuces, but none keep longer than 4 weeks at 0 °C (Saltveit 2004). Because lettuce is very fragile, it must be handled with care. Lettuce contains about 95% water. The structure of a leaf can be viewed as a construction in which the outer layers form a 'skin' that protects the plant from rapid breakdown (Glenn et al. 2005).

1.2. Processing of fresh-cut vegetables

The steps of fresh-cut vegetable processing are depicted in Fig. 2. Fresh-cut vegetables are altered in form by peeling, slicing, chopping, shredding, coring, or trimming, with or without washing or other treatment, prior to being packaged for use by the consumer or a retail establishment. The vegetable raw material to be processed should be of premium quality (Turatti 2011).

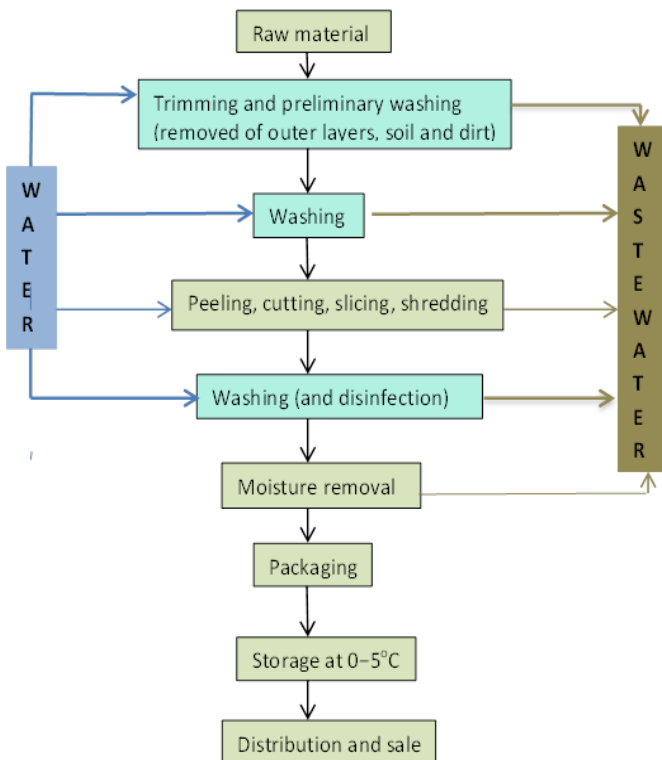


Figure 2. A general process flow diagram of fresh-cut vegetables, modified from Oliveira et al. (2015). Points where water is used and waste water is formed are marked with brown arrows.

1.2.1. Processing steps in which water is used or removed

Water is an essential part of vegetable processing; it is used in many steps of the process (Fig. 2). The quantity and quality of water involved in fresh-cut vegetable processing is depicted in section 1.3. Fresh-cut vegetable processing includes many phases and different kinds of equipment and techniques:

Preliminary washing

Roller brush washers are used for handling of round- or oval-shaped produce. A roller brush washer rotates or tumbles produce on a series of revolving brushes (Hall & Sorenson 2006). In the initial polishing of vegetables, clean or circulated water can be used. Soaking is used as a preliminary stage in the cleaning of root vegetables, which are heavily contaminated by soil. The efficiency of soaking is improved by moving the water relative to the product by means of caged propeller-stirrers built into the tank or by means of slow-moving paddles (Lo & Argim-Soyza 2005).

Washing

Washing is an important step in fresh produce processing, because it removes soil and debris and lowers the amount of microbial populations found on the surface of vegetables (Luo 2007; Palma-Salgado et al. 2014). Washing of vegetables generally reduces the microbial load by 100 to 1000-fold (Narender et al. 2018). Produce washers are designed according to the physical characteristics (size, shape, fragility, etc.) of harvested produce (Sapers 2003). There can be several stages in the washing process (Fig. 3). Fresh-cut products can be single-washed, double-washed, or triple-washed, or various wash-and-spray combinations can be implemented (Luo 2007).

According to Pao et al. (2012), two types of produce washers are used by the industry. Immersion washers wash produce by dumping, submerging, and/or floating produce in process water (Ahvenainen 2000). Non-immersion washers wash produce by spraying or rinsing produce on flat or curved wash beds or in a basket or drum (Pao et al. 2012). Depending on the product to be rinsed, the water temperature must be as cold as possible. 0 °C is the optimal water temperature for most products.

Vegetables can be cut before washing/decontamination or cut after washing/decontamination. According to Palma-Salgado et al. (2014), the reduction of *Escherichia coli* was 1.04 log₁₀ when iceberg lettuce was first cut and then washed with water and 1.33 log₁₀ when first washed and then cut. The difference was larger when decontamination (e.g., utilizing chemicals) was used during washing. The washing-before-cutting process will help the produce industry enhance the efficacy of sanitization and reduce microbial hazards.

Moisture removal

Wet fresh-cut carrots and lettuce decay considerably more rapidly compared to those that have been well dewatered (Turatti 2011). Free moisture must be removed gently after washing (Artes & Allende 2014). Centrifugation is generally used, and is the best

method for vegetables. However, alternatives are utilized such as vibration screens and air blasts. The centrifugation time and rate should be chosen carefully, so that centrifugation removes only loose water, but does not rupture vegetable cells (Ahvenainen 2000). Lettuce centrifuged at 2000 rpm resulted in increased desiccation of the product and increased storage life (Bolin & Huxsoll 1991).

Peeling of carrots

Peeling of carrots removes the epidermis and some sub-epidermal tissue. It bruises underlying tissue and leaves the new outer layer of cells damaged, causing leakage of cellular fluids which encourages microbial growth and enzymatic changes (Barry-Ryan & O'Beirne 2000). The primary peeling methods for vegetables are: lye peeling, steam peeling, and mechanical peeling. Mechanical peeling is most common in small-size vegetable processing companies; this process can be dry or wet. The types of mechanical peelers are: abrasive devices, drums, rollers, knives and milling cutters (Shirmohammadi et al. 2011; Sumonsiri & Barringer 2014). When root vegetables are peeled with a knife, the final result is a "peeled by hand" look. Using a sharp knife reduces the physical damage to cut vegetables, and less stress is observed in the cells of produce (Ahvenainen 2000). Abrasive peelers utilize abrasive surface rollers to remove the outer skin from the product. In general, knife peeling is more gently than abrasive peeling (Kleiber et al. 2005). Wet peelers contain a water spraying unit which washes vegetables and increases water use (Singh & Sukhla 1995).

1.2.2. Effect of processing on the quality of fresh-cut vegetables and wash waters

Processing of fresh-cut vegetables causes injury to plant tissue such as mechanical damage, biochemical changes, microbiological growth and physiological spoilage (Guerzoni et al. 1996; Allende et al. 2004). The composition of vegetables determines the type of spoilage (Ragaert et al. 2011; Fig. 3).

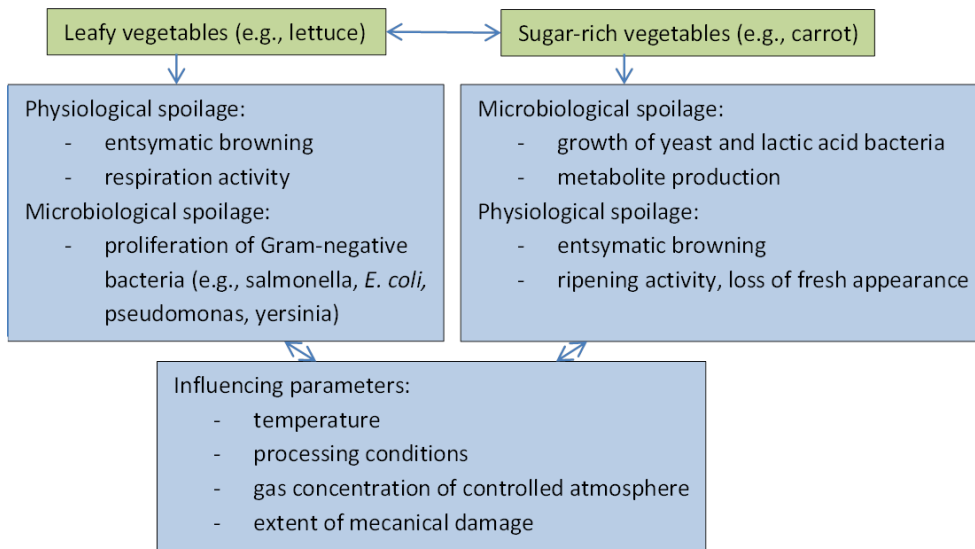


Figure 3. Dominant mechanisms of spoilage and influences on spoilage of leafy vegetables versus sugar-rich vegetables (modified from Ragaert et al. 2011).

Effect of washing on the quality of fresh-cut vegetables and wash waters

Many studies have shown that the rate of microbial reduction during washing is influenced by several factors, including the quality of washing water and the efficacy of sanitizers for microbial inactivation (Zhang & Farber 1996; Gonzalez et al. 2004; Rodgers et al. 2004; Das et al. 2016). Washing is often the only step that can remove foreign material and tissue exudates, as well as inactivate pathogens (Gil et al. 2009). In the study by Luo et al. (2018), organic load increased gradually over time as more products were washed in the same flume water. Lopez-Galvez et al. (2018) measured organic load in lettuce and shredded vegetables wash waters. The concentration of chemical oxygen demand (COD) increased from 72 to 298 mg/l during washing after three to five hours. Turbidity increased during lettuce washing from 4 to 21 NTU, and total dissolved solids (TDS) from 0.55 to 0.75 g/l. In shredded vegetable washing, COD increased from 448 to 7092 mg/l, turbidity from 1 to 287 NTU and TDS from 1.2 to 7.2 g/l.

When fresh-cut produce is fully submerged in water, either for washing or as a means of cooling, such produce is likely to have wash water infiltration into the tissues. The reason is that microorganisms, including human pathogens, have a greater affinity to adhere to cut surfaces than uncut surfaces (Seo & Frank 1999; Takeuchi & Frank 2000; Liao & Cook 2001) or in punctures or cracks in the external surface (Burnett et al. 2000).

Effects of peeling and cutting on the quality of fresh-cut vegetables and wash waters

Peeling and slicing of root vegetables cause tissue disruption, breaking of protective epidermal layers and the release of nutrients and enzymes (Adams et al. 1989). In the study by O'Beirne et al. (2014), coarse abrasion peeling of carrots disrupted the surface of the carrot tissue. The damage caused by abrasion peeling did not affect the underlying tissue. No cracks or fissures were detected at the surface or at 1000 µm below the surface. Hand peeling did not cause severe surface damage and did not appear to cause any damage to the underlying tissue (O'Beirne et al. 2014).

In the study by O'Beirne et al. (2014), there was no significant difference between different peeling methods on the number of *E. coli* O157:H7 colonising or penetrating into the peeled carrot tissue. According to Gleeson & O'Beirne (2005), *E. coli* survived better on carrots sliced with a blunt machine blade than on carrots sliced with a sharp blade. Below the surface of the carrot, bacteria did not penetrate into carrot cells, but remained in the intercellular spaces (Auty et al. 2005). Optimum cutting during processing might also increase the efficiency of washing and anti-microbial dipping treatments in reducing pathogen counts (O'Beirne et al. 2014).

Cutting and shredding of lettuce causes disruption of cells in lettuce, which induces an increase in ethylene and phenolic compounds such as formation of volatiles (Saltveit 2003; Belitz et al. 2004). The cutting direction of lettuce has been observed to have an influence on emitted volatiles and sensory perception of the lettuce. In the study by Deza-Durand & Petersen (2011), cutting the lettuce transverse to the midrib caused more severe damage to the tissue than did longitudinal cutting, based on aroma production of lipoxygenase (LOX) volatiles. Sharp rotating blades gave better results in cutting lettuce (lower respiration and lower microbial count during storage) than sharp stationary blades (O'Beirne 1995). In the case of shredded iceberg lettuce, blade sharpness has been observed to have a small effect; however, stationary blades increased respiration rate and microbiological counts, and reduced acceptability (Ahvenainen 2000).

Microbial contamination of fresh-cut vegetables

Vegetables can become contaminated at any stage of food production and preparation, from the field to the consumer. Water can play a dual role in fresh-cut vegetable processing, in both reducing and also transmitting microorganisms to vegetables. Process water can constitute a source of cross-contamination of vegetables with microorganisms (Gil et al. 2009). Cross-contamination can take place even when large quantities of water are used, or even in the presence of sanitizers (Nguyen-the & Pruner 1989; Francis et al. 1999; Lopez-Galvez 2009). The washing procedure can also create produce mechanical injury and thus promote internalization of microbiological and chemical contaminants of vegetables (Allende et al. 2004; Pao et al. 2012).

The epidermis of root vegetables, which provides a protective barrier against the development of microbes on the vegetable surface, is removed during processing (Martn-Belloso et al. 2006). The destruction of vegetable surface cells exposes the cyto-

plasm and provides micro-organisms with a richer source of nutrients as compared to intact produce (Barry-Ryan et al. 2000). Therefore, processing can increase microbial spoilage of fresh-cut produce due to the transfer of microflora from the surface to the vegetable, which acts as a complete medium for growth (Quadri et al. 2015).

The total counts of microbiological populations on fresh-cut vegetables after processing are known to range from 3.0 to 6.0 $\log_{10}$ units (Ragaert et al. 2007). Shredding and slicing steps in fresh-cut processing have resulted in increased microbial populations by 1–3 $\log_{10}$ on cut lettuce (Garg et al. 1990) and at least a 1 $\log_{10}$ increase for lettuce salads (von Jockel & Otto 1990). Lactic acid bacteria and several species of yeasts and moulds are commonly found on fresh-cut vegetables (Nguyen-the & Carlin 1994; Kakiomenou et al. 1996; Zagory 1999). As they have higher sugar content, they likely undergo microbial fermentation. Lactic acid bacteria increased in shredded or sliced carrots, achieving counts of 10^8 cfu/g (Fonseca 2006).

Fresh-cut vegetables can be contaminated with pathogens (disease-producing agents) in the course of primary production (Bartz et al. 2017). Numerous pathogens have been isolated from fresh-cut vegetables (Ragaert et al. 2011). Pathogens dislodged from contaminated vegetables can survive in wash water and spread to others (Holvoet et al. 2012). Some pathogens are capable of growing in the cold temperatures applied by the fresh-cut vegetable industry: for example, *Aeromonas* spp. and *Yersinia* spp. (Janda & Abbott 1998; Jaxsens et al. 1999).

1.2.3. Quality properties of vegetables

Quality consists in a combination of characteristics that determines the value of produce to the consumer and customer. The quality of vegetables is related to several attributes, including appearance, texture, flavor, nutritional and safety aspects (Francis et al. 2012). The quality parameters of vegetables vary with the commodity, its intended use, and the preferences of the consumer (Saltveit 2003) or other customer (Grunda 2005; Table 2). Freshness is probably the most important quality parameter for fresh vegetables (Lappalainen et al. 1998; Ragaert et al. 2004; Peneau et al. 2005; Peneau et al. 2009). Legislation in the European Union (EU) and national legislation in different countries adopted to improve food safety includes: standards regarding the characteristics of the final product, production practices in the supply chain, traceability within the supply chain and legal liability of the supply chain (Yossoff et al. 2015). Fresh-cut vegetables are perishable products and susceptible to the effects of temperature abuse, and therefore must be kept continuously at temperatures between 0 and 6 °C during processing, distribution and marketing (Hui 2015).

Table 2. Value and quality criteria of vegetable raw material and fresh vegetable product (Grunda (2005), Barrett et al. (2010), and Francis et al. (2012) (modified)).

Raw-material criteria		Product criteria		
Market value	Processing value	Product value	Sensory quality	Nutritional value
Color	Temperature	Freshness	Flavor (taste and aroma)	Vitamins
Size	Freshness	Shelf life	Odor	Minerals
Shape	Shape	Transportability	Color	Phytonutrients
Freshness	Size	Storability	Appearance	Carbohydrates, dietary fiber
Consistency	Processability	Cold chain	Textural properties	
Extraneous ingredients	Defects			
	Allergens			

1.3. Process water

Process water is “drinking water or clean water, which is transferred to the food process, can remain in a portion of the produce or it can be removed completely” (EC 852/2004). Drinking or potable water meets the legal requirements of European Council Directive 98/83/EC, and is used as process water in the fresh-cut vegetable industry in Finland. If the water is potable, then it is probably acceptable for all food contact uses (ILSI 2008). Wash water can be clean water, which is natural water: e.g., lake water, or treated water, in which there are no micro-organisms or harmful pollutants to such an extent that it could have a direct or indirect impact on the health status or the quality of the food (Kekki 2013).

1.3.1. Water use and quality

The fresh-cut vegetable processing industry uses high volumes of water in the amount of 2.4–11 m³/t of processed product (Derden et al. 2002; Ölmez 2013). High water use in the food sector is primarily caused by the hygiene and cleaning demands of processes and products, such as the need to cool the vegetable products (Ölmez 2013; Ölmez 2014; Hellman & Simola 2016).

Process water or purified waste water can be circulated and used for washing of vegetable raw material (Derden et al. 2002). Directive 98/83/EC permits processors to reuse or recycle water unless the water poses a risk to product safety (Ölmez 2013). Process water contains soluble compounds and dry matter from vegetables (sugars, proteins, organic acids, phenols and other compounds) (section 1.1) (Teng et al. 2018) as well as microorganisms. Care is needed in recycling water so as not to introduce new risks of increased microorganisms to be produced during washing. Safe water reuse in a food company can be controlled and managed by using Hazard Analysis and Critical Con-

trol Points (HACCP), a set of risk-based self-monitoring principles (Casani & Knøchel 2002; Casani et al. 2005).

The high operational cost of water use has resulted in the industry-wide common practice of reuse or recirculation of process water. In the study by Luo (2007), water quality deteriorated rapidly during produce washing as a result of the accumulation of cut produce tissue fluids, solids, and other foreign matter in the course of fresh-cut vegetable processing. Using new potable water that is changed continuously during the process could be a possible solution, but it will be very expensive for the fresh produce industry to do so (Manzocco et al. 2015).

1.3.2. Physical, chemical and biological decontamination methods for process water quality

Physical, chemical and biological water decontamination methods and their combinations are used in the fresh-cut vegetable industry (Fig. 4). In order to increase shelf life and enhance the microbial safety of vegetables, in the fresh-cut industry chlorine is commonly applied as hypochlorous acid (HOCl) and hypochlorite (OCl⁻) as a disinfectant of waters at concentrations varying between 50 and 200 ppm of free chlorine, and for a maximum exposure time of 5 min (Rico et al. 2007; Goodburn & Wallace 2013). The washing of vegetables with chlorine is common. However, in many European countries, including Finland, such decontamination is not approved and because of health and environmental factors, washing has to be done with water only (Artés et al. 2007; Rico et al. 2007; Artés et al. 2009; Tirpanalan et al. 2011). Figure 4 presents the physical, chemical and biological decontamination methods for process water and their advantages and disadvantages.

Water decontamination methods

Water decontamination methods studied in this thesis were: neutral electrolyzed water (NEW), chlorine dioxide (ClO₂), organic acids and ultraviolet-C (UV-C). Chlorine compounds are also active in EOW and ClO₂ methods. EOW was generated by the electrolysis of a sodium chloride solution. Electrodes are separated by nonselective membranes. EOW is usually generated on-site by passing a dilute salt solution (sodium chloride, NaCl, potassium chloride, KCl) through an electrolytic cell. In the conventional process, a dilute salt solution is electrolyzed with a membrane partition, resulting in the production of acidic EOW, pH 2.5–3.5, at the anode and alkaline EOW, pH 10–11.5, at the cathode (Izumi 1999; Umimoto et al. 2013). At the anode acidic EOW is obtained, production of various chlorine compounds and ions such as hypochlorous acid (HOCl), hypochlorite (OCl⁻), and chlorine gas (Cl₂) (Gil et al. 2015). An electrolysed acid with HOCl is a more effective sanitizer compared to hypochlorite (OCl⁻), and lower concentrations can be used (Buck et al. 2002; Len et al. 2002).

Biological (bacteriosins, bacteriophages, enzymes, phytochemicals) + do not cause major harms to the environment + natural preservatives (produced by lactic acid bacteria, etc.) - increased cost compared with chlorine - can affect the organoleptic properties (taste, odor etc.) of the vegetables - not very efficient individually	Physical (UV, US, filtration, etc.) + do not cause major harms to the environment + not usually produce DBP + safe - increased cost - not very efficient individually - can affect organoleptic properties
	Combination (physical-chemical, physical-physical, chemical-chemical, chemical-biological and biological-biological) + Increase effectiveness on microbes - high cost
Chemical (ClO₂, EOW, O₃, organic acids, etc.) + effective for many microbes with lower concentration than chlorine - increased cost compared with chlorine - decontamination by-products (DBP) formed - allowed maximum concentration quite low	Chlorine (widely used and widely prohibited) + low cost + wide spectrum of antimicrobial effectiveness - DBP formed - corrosive

Figure 4. Schematic overview of the advantages and disadvantages of chlorine and the alternative methods of decontamination (physical, chemical and biological, and their combination) of process waters (Meireles et al. 2016, modified)(UV = ultraviolet, US = ultrasound, EOW = electrolyzed oxidizing water, O₃ = ozone, DBP = decontamination by-product).

Chlorine dioxide (ClO₂) has increasingly been used as an alternative to sodium hypochlorite and it has been observed to have an equal or greater antimicrobial potency than chlorine. ClO₂ is a monomeric free radical and readily dissolves in water without reacting with it, unlike chlorine. ClO₂ remains stable and does not ionize in solution between pH 2 and 10 (Lopez-Galvez et al. 2010; Chen & Zhu 2011; Feliziani et al. 2016).

Organic acids such as acetic, citric, malic, tartaric and propionic acids, can act as antimicrobials, because many microbes generally cannot grow at pH values below 4.5 (Parish et al. 2003). Antimicrobial activity varies among the organic acids. For example, lactic acid and citric acid can be considered more effective than acetic acid for fresh-cut lettuce decontamination (Tirpanalan et al. 2011).

Physical decontamination technologies, such as ultraviolet-C (UV-C), have not usually produced decontamination by-products (Keyser et al. 2008; Gil et al. 2010). The UV-C portion of electromagnetic spectrum encompasses wavelengths from 200–280 nm, the absorption maximum at 273 nm. UV energy penetrates the outer cell membrane of the microbe, passes through the cell body and disrupts its DNA, preventing reproduction. The degree of inactivation of microbes by ultraviolet radiation is directly related to the

UV dose applied to the water. UV-C light processing is confirmed to be easy to use and is characterized by favorable costs of equipment, energy and maintenance (Linden et al. 1998; Lazaroova et al. 1999; Bintsis et al. 2000; Keyser et al. 2008; Ignat et al. 2015; Artes-Hernandez et al. 2017). According to Pilkington (1995), if water is highly turbid and colored, it is unsuitable for decontamination by chlorination, ozonation, or UV.

Table 3. Evaluation of decontamination treatments (Natrium hypochlorite (NaOCl), Chlorine dioxide (ClO₂), electrolysed oxidizing water (EOW), organic acids and UV-C) applied to fresh-cut vegetable process water.

Treatment	Water / COD (mg/l)	Ability to inhibit cross-contamination*	References
Natrium hypochlorite NaOCl, ≥ 5 ppm	Clean water Process water, COD = 500–1000	+++ +++	Luo et al. (2011, 2012); Tomas-Callejas et al. (2012); van Haute et al. (2013); Gomez-Lopez et al. (2014); Lopez-Galvez et al. (2010)
ClO₂ , ≥ 3 ppm	Clean water	+++	Lopez-Galvez et al. (2010); Pao et al. (2007)
EOW	Process water, COD = 3–14	+	Ongeng et al. (2006)
EOW, pH 6.5, < 1 ppm FC	Process water, COD = 500	+	Gomez-Lopez et al. (2015)
EOW + 0.5 % salt, ≥ 5 ppm FC	Process water, COD = 500	+++	Gomez-Lopez et al. (2015)
Organic acids	Lettuce wash water	+	van Haute et al. (2013)
Lactic acid, pH 2.5, 20 000 ppm	Process water, COD = 500–700	+	Lopez-Galvez et al. (2010)
UV-C, 0.1 kJ/m²	Clean water Lettuce wash water	+++ ++	Ignat et al. (2015)
UV-C, 0.4 kJ/m²	Lettuce wash water	+++	Ignat et al. (2015)

*- = non, + = low, ++ = middle, +++ = good, FC = free chlorine

Protein/peptide concentration contributes most of the chlorine demand in water decontamination, and its removal will help to ensure the safety of wash water when chlorine is used (Teng et al. 2018). According to Luo et al. (2018), surviving microbes in the wash water correlated closely with free chlorine concentration below 10 mg/l

throughout the processing of fresh-cut vegetables, irrespective of the organic load in the wash water. Table 3 presents the effect of decontamination treatments on process waters of fresh-cut vegetables.

Combined techniques

Different decontamination methods could be combined in order to increase their antimicrobial efficacy (Fig. 4). Combinations of physical-chemical, chemical-chemical, chemical-biological and biological-biological methods have been studied by Singh et al. (2002), Arevalos-Sánchez et al. (2012) and Gabriel (2015). A combination of diverse methods may allow a wider antimicrobial action than a single treatment (Goodburn & Wallace 2013). In addition to the previously-mentioned combinations, a physical-physical combination of ultrasound (US) and UV-C light may be a promising energy efficient decontamination technology for fresh-cut wash water effluents when taking into account quality and safety parameters (Petri et al. 2015; Millan-Sango et al. 2017).

Decontamination by-products

In the process water of fresh-cut vegetables there is a large quantity of organic matter in water effluents from the exudates of the cut tissues. When water decontamination is utilized, decontamination by-products (DBP) can be formed (Gil et al. 2016; Gil et al. 2019). Decontamination by-products which have been defined as carcinogenic compounds are: trihalomethanes, haloacetic acids, haloketones and chloropicrin (Nikolaou et al. 1999), as well as other toxic compounds without a proven carcinogenic potential such as chlorate (WHO 2017). The generation and accumulation of DBP can occur in wash water effluents, but also transmitted from the water to the final fresh produce. In order to reduce the formation of decontamination by-products, producers try to avoid the use of chlorine-based compounds for the decontamination of process water (Fig. 4). A rinsing step after washing decreases trihalomethane concentration below the detection limit in vegetables (Gomez-Lopez et al. 2013; Gomez-Lopez et al. 2017). According to Gil et al. (2019), activated carbon filtration treatment significantly reduced the concentration of DBPs in vegetable process water, leading to a lower concentration of chlorate in the washed produce.

1.4. Waste water from processing of fresh-cut vegetables

1.4.1. Waste water quantity and quality

Waste water generated from vegetable production contains high concentrations of biochemical oxygen demand (BOD) and suspended solids (SS) (Derden et al. 2002; Liu 2007). Common quality parameters and their concentrations of waste water are presented in Table 4. In addition, the waste waters from the carrot washing process generally contain a high concentration of nitrogen and phosphorous (Mebalds & Hamilton 2002). In general, 70 to 80% of the total organic matter in fresh-cut vegetable waste

waters is in the dissolved form, and is not easily removed from waste water by conventional mechanical means such as sedimentation (Liu 2007).

Table 4. Common quality parameters of waste water and their characteristics (Karttunen 2004; Puchlik & Struk-Sokołowska 2017).

Parameter	Characteristics	Concentration, waste water from vegetable processing (mg/l)
BOD, Biochemical oxygen demand BOD₇, BOD₅	Estimates the degree of organic content by measuring the oxygen required for the oxidation of organic matter by the aerobic metabolism of microbial communities. BOD ₇ is biochemical oxygen demand for 7 days and BOD ₅ for 5 days.	860–3200 (BOD ₅)
COD, Chemical oxygen demand	Estimates the total organic matter content of waste waters, and is an approach based on the chemical oxidation of the organic materials in the waste water. It involves either oxidation of the organic matters by permanganate or oxidation by potassium dichromate (K ₂ Cr ₂ O ₇). COD analysis using dichromate is the most common method, and it is possible to use for continuous monitoring of biological waste water treatment systems.	920–3700
Solids: total solids (TS), Total suspended solids (TSS) (non-dissolvable) and dissolved solids (DS)	SS is non-dissolvable and DS dissolved solids. Total solids is a measure of the suspended, colloidal, and dissolved solids in water.	250–420 (TSS)
Nitrogen (N) and phosphorous (P)	The sources in food and agricultural waste water can include chemical fertilizers, synthetic detergents used in cleaning food processing equipment, and metabolic compounds from proteinaceous materials.	40–60 (N) 9–16 (P)

Physicochemical processes, such as adsorption and chemical oxidation or membrane-based technologies, are capable of removing dissolved solids in relatively low concentrations at higher costs (Liu 2007). In addition to microbiological risks, the high amounts of organic matter in waste water are challenging for the efficient operation of

waste water treatment systems (Derden et al. 2002). Most vegetable industries have applied conventional waste water treatment methods such as anaerobic and aerobic biological processes (Chen 2015).

According to Hamilton et al. (2005), the high levels of organic matter from vegetable processing in waste water could potentially encourage the growth of plant pathogens. When it is used to irrigate vegetables, contaminated waste water can result in the transmission of many disease agents and cause outbreaks in countries world-wide (Kirby et al. 2003).

The conventional biological treatment of waste water requires a high biodegradable influent, where a high BOD_5 / COD ratio is usually necessary (Chen 2015). Suspended solids are a nuisance in waste waters from vegetable processing, because they can either settle on the bottom or float on the surface of the tank or the basin (Liu 2007).

1.4.2. Waste water treatment in fresh-cut vegetable processing companies

Waste water treatment systems are classified as primary (mechanical), secondary (biological) and tertiary (polishing) treatments (Isosaari et al. 2010).

Primary waste water treatment

Possibilities for primary treatment of vegetable processing waste water include: screening, flotation, flocculation, sedimentation, and (sometimes) granular sand filtration (Table 5). Coagulation and flocculation are widely used for food industry waste waters to precipitate out particulate and dissolved matter (Hafez et al. 2007; van Haute et al. 2015). They are intended to remove coarse solids and to reduce organic matter content and adjust pH prior to the secondary treatment processes (Joshi 2000; Paranychianakis et al. 2006; Liu 2007).

Sedimentation is used in biological treatments such as activated sludge and trickling filters for solid removal. Suspended solids, which have higher densities than that of water, are removed from waste water within a reasonable period of time by the action of gravity in the bottom of a settling tank or equalization basin (Karttunen 2004). The purpose of an equalization basin is to balance out process parameters such as flow rate, organic loading, the strength of waste water streams, pH, and temperature. The purification efficiency of clarifying to phosphorous, nitrogen and organic matter is 10–20%. A correctly dimensioned sedimentation basin can decrease the amount of precipitated and settleable solids by about 70% (Rontu & Santala 1995).

Table 5. Primary treatment methods of waste water in general, also used in vegetable processing.

Treatment	Substances removed	Method/equipment	Property	Reference
Screening	Relatively large solids, > 0.7 mm	Screen, e.g., static	Cheap, quick	Liu (2007)
Flotation	Fine and light suspended particulates	Air bubbles make floating particles lighter than water, rise to the surface, removed with mechanical skimmers	Particulates to aggregate	Karttunen (2004)
Sedimentation	Suspended solids	Action of gravity within a reasonable period of time	Solid removal	Karttunen (2004)
Coagulation	Colloid particles 0.1–0.01 μm	Negative charged colloidal particles are neutralised by chemicals (e.g., alum and polyaluminium chloride)	Bigger flocks	Liu (2007)
Flocculation	Colloid particles 0.1–0.01 μm	Destabilisation of colloidal particles, form aggregates with added water-soluble polymers	Bigger particles	Liu (2007)
Filtration	Flocs (or bioflocs), solids, precipitates	Sand, crushed antrachite coal, diatomaceous earth, perlite, powdered or granulated carbon	Used in every waste water treatment stage	Liu (2007)
Clarifying	Precipitated and floating matter	Particles separated from water		Rontu & Santala (1995)

Secondary (biological) waste water treatment

Biological treatment of waste water aims to remove of soluble organic and inorganic matter from water. Microorganisms, primarily bacteria, utilize organic matter and inorganic salts in waste water. Table 6 presents a characterisation of the sequencing batch reactor (SBR) and trickling filter used in treating high strength organic waste waters. Biological processes are the more effective the more easily biodegradable the organic ingredients are. Biological treatment is widely used for vegetable processing waste water, either by using anaerobic treatment (Moises et al. 2001; Moody & Raman 2001; Chen 2015), aerobic treatment or a combination of both (Austermann-Haun et al. 1999; Mulkerrins et al. 2004). Aerobic processes are only generally applicable and cost-effective when the waste water is readily biodegradable (EU 2006).

SBR is simple and cost effective, and can provide very effective treatment for the removal from waste water of BOD, TSS, ammonia, and nutrients such as nitrogen and phosphorus. SBR systems are suited for waste water treatment applications characterized by low or intermittent flow conditions, and can easily be adapted to variable pollutant concentrations (Jang et al. 2004; Mahvi 2008). A trickling filter is one type of conventional biofilm reactor (Grady et al. 1999). The filter medium is stationary: e.g., plastic covered with bacteria. The waste water is distributed over the filter, trickles down through the medium, circulates and is collected under the medium and removed. Microorganisms grow on the filter media and form biofilm. Waste water comes into contact with the biofilm and air, pollutants are diffused to the biofilm, and are converted into harmless compounds (Zhu & Rothermel 2014).

Table 6. Characterisation of sequencing batch reactor (SBR) and trickling filter.

Characteristic/criteria	SBR	Trickling filter
BOD removal (%)	89–98	80–90
TSS removal	85–97	75–85
Nitrification (%)	91–97	-
Total nitrogen removal (%)	>75	66–70
Biological P removal (%)	57–69	-
Hydraulic retention time (h)	12–40	13–14
Advantages	Single reactor vessel Operating flexibility and control	Tolerance for variations in loading
Advantages/ disadvantages	Expertise needed Maintenance needed	Flexibility and control are limited Moderate level of skill and expertise needed
Operating costs	+	+
Investment costs	++	++
Suitability	Low or intermittent flow conditions Easily adapted to variable pollutant concentrations	All kinds of biodegradable waste waters
References	EPA (1999); Mahvi (2008); Jang et al. (2004); Lam et al. (2015)	Joshi (2000); Karttunen (2004); Daud et al. (2018)

- = no information, + = low ++ = middle, +++ = high

Natural processes include land application, constructed wetlands, and various pond systems (Isosaari et al. 2010). Land application systems are typically designed to provide secondary or tertiary treatment for pretreated waste water (Crites et al. 2006). Land application systems are perceived as low-technology options that do not require compli-

cated engineering structures and continuous process control. Its designs have been classified as: slow-rate systems, soil-aquifer treatment, and overland flow. The rates of most natural processes are highly temperature-dependent. Furthermore, experience from field sites shows that the quality expected to result from the process has not always been achieved (Isosaari et al. 2010). Treatment efficiency in pond systems can be improved, for example, by use of floating elements which can be used to improve hydraulic characteristics of the treatment (Craggs 2005; Crites et al. 2006). Bubble aeration ensures aerobic conditions throughout the pond and prevents settling of suspended solids (Liu 2007).

Tertiary treatments

Tertiary treatment can be used if waste water is reused in vegetable processing or recycled for irrigation of food or landscape crops (Gerba 2008). Examples of tertiary treatment processes which reduce the number of pathogens are:

- Filtration
- Flocculation and filtration processes
- Membrane processes
- Detention in ponds or reservoirs
- Natural systems: wetlands, soil-aquifer treatment
- Chemical and physical treatments

Reduction of pathogens in waste water treatment

Waste water detention in ponds can significantly reduce the concentrations of enteric pathogens in waste water. Inactivation and removal of pathogens are controlled by temperature, sunlight, pH, adsorption to or entrapment by settleable solids and settling of the larger organisms. Indicator bacteria and pathogenic bacteria can be reduced by 90–99% or more, depending on retention times (Gerba et al. 2008).

Trickling filters are generally less effective in removing pathogens from waste water than the activated sludge treatment. Trickling filters can remove 20–80% of enteric bacteria (Feachem et al. 1983). In the study by Arimi et al. (1988), sedimentation and trickling filter in combination were found to remove 99.9% of the *Campylobacter* spp. from waste water.

In the activated sludge process, removal rate for all pathogens has been reported to range from 40 to 99%. Most of this removal is due to sedimentation and adsorption, or incorporation into the biological flocs which form during the process (Gerba 2008). In the study by Ottosen et al. (2006), a membrane bioreactor removed *E. coli*, enterococcus and coliphages more efficiently than activated sludge involving denitrification by sand filtration and upflow anaerobic sludge blanket (UASB) treatment. The membrane bioreactor process consists of a biological reactor integrated with membranes that combine clarification and filtration of an activated sludge process (Fazal et al. 2015).

Reuse of waste water from vegetable processing in washing or irrigation

Reuse of waste water from vegetable processing should contribute to a saving of water and reduce costs. Waste water can be used to irrigate a crop, or for initial washing of vegetables, without adversely affecting food quality or safety (Rajkowski et al. 1996; Hamilton et al. 2005). For many countries, irrigation of fields with waste water from the food industry remains the main reuse application (Angelakis et al. 1999; Muñoz et al. 2009). Irrigation with waste water carries with it environmental risks such as pollution of groundwater and surface water, degradation of soil quality and impacts on plant growth. Other risks include the transmission of diseases via the consumption of waste water-irrigated vegetable, and even increased greenhouse gas emissions associated with pumping large volumes of waste water to an irrigation district. The significance of such risks will plainly be dependent on the reuse scheme at hand. Ultimately, the challenge facing waste water reuse is minimisation of such risks so as to maximise the net environmental gain (Hamilton et al. 2005).

In the study by Xu et al. (2010), when reclaimed water was used for irrigation of fresh produce (e.g., leafy greens) microbiological food safety could lead to the prohibition of its use on agricultural products. In the study by Libutti et al. (2018), waste water from vegetable processing was secondarily treated by means of activated sludge treatment and sedimentation, and tertiarily treated with ultrafiltration and UV radiation. Vegetables (tomato and broccoli) were irrigated with treated waste water by the drip irrigation method. The microbiological quality of the vegetables was not affected by the irrigation water used. Dissolved air flotation (DAF) and centrifuge were able to remove solids more than 95% and these treatments followed by ultraviolet (UV) disinfection make it possible to reuse waste water for washing, rinsing and processing applications (Mundi & Zytner 2015).

The quality of irrigation water must meet requirements, because when in irrigation in contacts the edible part of the plant, it plays a major role in plant safety. According to the regulation of primary production (1368/2011), in Finland the water used on edible parts of plants at the source site for irrigation must be clean enough, which means that it fulfils the requirements concerning *E. coli* and intestinal enterococci, for example.

1.5. Summary of the literature

The main focus of the present study is water used in fresh-cut vegetable processing. The single most abundant component of fresh vegetables is water, which may account for up to 90% of the total mass of a vegetable. Part of this water cannot be extracted easily, and another part is released easily: for example, during cutting. In the course of processing, cells of vegetables are cut and bruised so that solutes are mixed with and released to the process water. Vegetable raw-material processed should be of the best quality, and processes should be suitable for the vegetables to be processed. Produce quality and safety are the most important targets which must be taken into account at every stage of the process. The characteristics of vegetables, as well as processing and

water consumption, have an influence on wash and waste water quality and quantity (section 1.1).

Fresh-cut vegetable processing includes many phases, many kinds of equipment and techniques. For example, vegetables are first polished and washed, then peeled, cut and shredded. Water is an essential part of these processes. Processing causes mechanical, biochemical, microbiological and physiological changes to the quality of fresh-cut vegetables and to their cellular structure, leading to leakage of nutrients and cellular fluids (Heard 2002; Varoquaux 2002). Fresh-cut vegetable processing operations make fresh-cut produce more susceptible to microbial attack in comparison to intact produce. However, fresh-cut vegetables can be contaminated at any stage of food production chain. Water plays a dual role both in reducing and in transmitting microorganisms to vegetables (section 1.2).

Water used in the vegetable food industry often is directly linked to the safety of vegetables. To ensure the quality of fresh-cut vegetables, enough high-quality water should be available for use. Different kinds of water-decontamination methods are utilized in order to ensure water quality. However, chemical decontamination is forbidden in many European countries, including Finland (see section 1.3).

Waste water from fresh-cut vegetable processing contains high concentrations of biochemical oxygen demand (BOD) and suspended solids (SS) such as nutrients (Derden et al. 2002; Mebalds & Hamilton 2002; Liu 2007). Waste water treatment can be divided into the following types: primary (mechanical), secondary (biological), and tertiary (reducing pathogens) treatments. Biological treatment is widely used for food-processing waste water, either by using aerobic treatment, anaerobic treatment, or a combination of both. Tertiary treatment is necessary if waste water has been recycled (section 1.4).

There is a limited amount of prior information about vegetable processing companies and their use of water, information such as: the quality of water, formation and treatment of process water and waste water, and the way of using water in order to maintain the quality and safety of vegetables. Water decontamination methods and their efficiency on microbes were discussed. Fresh-cut vegetable processing, in which any decontamination was not used, also has been little explored and is presented in this thesis. In addition, wash- and process water formation in the course of the different processing stages was monitored in the study. Previously there was little publicly accessible data about this kind of water use. In addition, prior information concerning on-site waste water treatment plants for vegetable processing is sparse.

For the vegetable processing industry, the major environmental issue to be solved is the high consumption rate of high-quality water and the generation of waste water containing high levels of organic load (Derden et al. 2002). In Finland, there is a large quantity of water available, but water treatment is expensive and water use needs to be controlled. In many countries in which water scarcity is an important national issue, water is commonly recycled in vegetable processing (Gómez-López & Gogate 2018). In Finland, waste water is not usually reused or recycled in vegetable processing.

2. Objectives of the study

The overall objective of this study was to improve food safety and sustainability of fresh-cut vegetable production. This study has a strong link with companies, and the aim was also that the results help the HACCP (Hazard Analysis and Control Points) processes in companies. In this study existing fresh-cut vegetable processing was examined and data of wash-, process-, and waste waters as well as of the microbiological quality of vegetables during processing was collected.

The detailed aims of this study were the following:

1. To determine the water use in different vegetable processing companies, as well as its quantity and the microbiological, physical and chemical quality of the wash-, process- and waste waters generated from the different steps of process lines at fresh-cut vegetable processing companies (Article I).
2. To examine the level of microbiological quality of fresh-cut vegetables and the changes in the microbiological quality of products during processing. Wash- and process waters were also analysed. The general aim was to improve the process hygiene of fresh-cut vegetables (Article II).
3. To evaluate the effectiveness of decontamination methods, including NEW, ClO₂, organic acids and UV-C, in wash waters for fresh-cut vegetables, in particular the effectiveness of these methods on *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, *Escherichia coli* and *Candida lambica* (Article III).
4. To evaluate the performance of two biological and one chemical waste water treatment system on farms carrying out the peeling of vegetables. The general aim was to obtain information concerning farm-scale waste water treatment for developing secondary trades in rural areas and agriculture (Article IV).

3. Materials and methods

The fresh-cut vegetable processing industry and the quality of the activity of fresh-cut vegetable processing in Finland was studied. We collaborated with companies in which we examined the quality of process waters, the formation of process and waste waters, and the decontamination of process waters as well as the microbiological quality of fresh-cut vegetable products. The companies were situated in southern, western and eastern Finland (Häme, South-west Finland, Satakunta and South Savo). Carrot and lettuce are among the most common vegetables in Finland (Luke 2017); these were also processed in the companies studied and were used as example vegetables in the study.

Figure 5 presents wash, process and waste waters generated by the processes of carrot, lettuce and other vegetables described and studied in this thesis (articles I–IV). In addition, the microbiological quality of carrot (II) was measured.

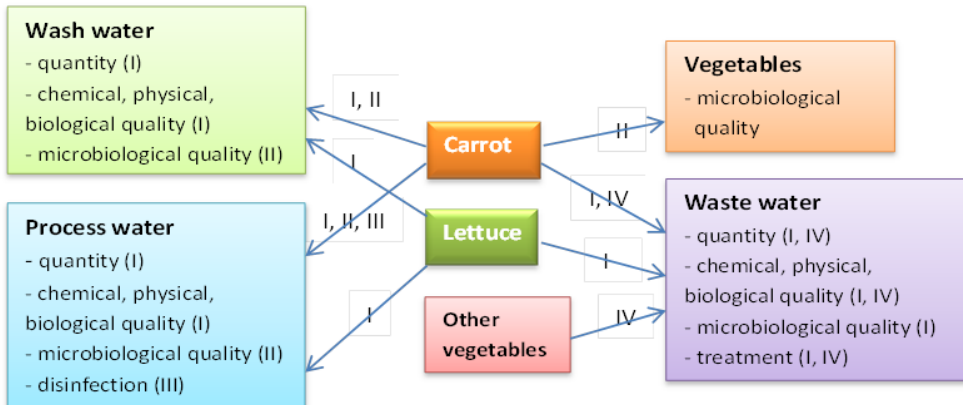


Figure 5. Waters and vegetables studied in articles I – IV.

3.1. Examination of vegetable production companies' processes, vegetables and waters

Samples were obtained from 11 vegetable processing companies. The examined vegetable processing varied in production, volumes, processes, production stages and products (Fig. 6); some purchased their raw material washed, while others treated or processed vegetables from their own farm.

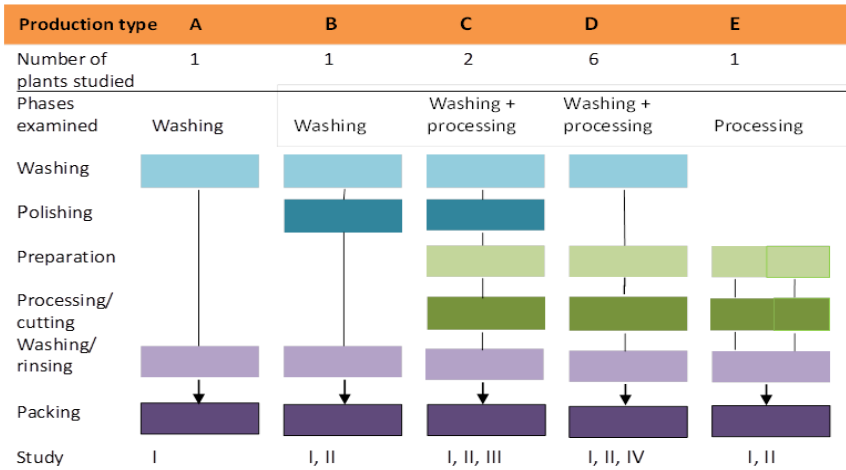


Figure 6. Types of fresh-cut vegetable production companies examined in this study. Letters indicate production types: washing of root vegetables (A, B), washing and processing of carrots (C), processing of different vegetables (D), production of vegetable salads (E).

Article I elaborated upon the topic of process waters and waste waters in different kinds of production phases and in vegetable processing companies: washing of root vegetables, washing and processing of carrots and other vegetables, and production of vegetable salads. Article II examined the microbiological quality of untreated whole and washed, peeled, peeled and cut, and peeled and grated carrot, as well as their wash-and process waters. Article III was addressed to the decontamination of process waters for carrots and other vegetables. Article IV examined waste waters from different kinds of vegetable processing. Articles I–IV focused on examining wash, process and waste waters (Table 7).

In addition, water consumption was measured in seven companies (I, IV). Article III evaluated four different decontamination methods (neutral electrolyzed water (NEW), chlorine dioxide (ClO_2), organic acid based product (FPW) and ultraviolet-C (UV-C)) with different microbes with a suspension test (>100 analyses), as well as decontamination of industrial carrot process water treated with UV-C.

Table 7. Number of vegetable processing companies, production type and samples examined in articles I–IV. – = not examined.

Article	Number of companies	Production type	Samples			
			Vegetables	Wash water	Process water	Waste water
I	4	B, C, D, E	-	X	X	X
II	6	A, B, C, 3*D	X	X	X	-
III	1	C	-	-	X	-
IV	3	3*D	-	-	-	X

3.1.1. Water consumption levels of different processing stages of vegetable processing

Water consumption at different measuring points (washing of vegetable raw material, peeling, and other operations such as cleaning of premises and machines) were measured using water meters (Model GSD, B-Meters) during a four-week period in 2009 in two fresh-cut vegetable processing companies (article I), and in 2005 and 2006 in three companies (IV). The water consumption involved in washing, processing, use of pressure washers and other uses in company D was monitored over three years (I).

3.1.2. Microbiological quality of vegetables

From 2009 to 2011, raw vegetable samples were taken from six carrot processing companies in Finland. The levels of processing hygiene of the companies were evaluated simultaneously with the vegetable and water sampling (Lehto et al. 2011). The level of microbiological quality was measured from whole, peeled, peeled and cut, and peeled and grated carrots (II). Microbial analyses from the carrot samples are described in Table 10 (section 3.2).

3.1.3. Physical, chemical and microbiological quality of wash- and process waters

Wash water samples intended for microbiological and chemical analyses were taken at the end of the working day from a washing basin or from a water pipe (I, II). The analyses of these water samples are described in Table 10 (section 3.2). Samples of wash waters of root vegetables for the purpose of microbiological and chemical analyses were taken from an outgoing washing water in 2009–2011 (I). The sub-samples were collected in the course of one working day (between 7 a.m. and 16 p.m.), once per hour, resulting in 9–10 samples per day. The sub-samples were then combined and a two-litre sample was taken to the laboratory. Samples were kept in a refrigerator before the microbiological and chemical analyses (I).

Samples of process water from carrot peeling were taken from the peeling machine of the company studied and samples of process water from lettuce batch washing were taken from the basin used for lettuce batch washing (I). The process water sample (30 liters) of study III was taken the day before the testing from a container in which whole carrots had been washed before they went to filling machines. Industrial process water was tested in a laboratory experiment (III) (Table 8 in section 3.1.4) with UV-C with and without. The UV-C treatment was applied using a TIO-UV ECO 2000 lamp. The water was continuously filtered through a 150 µm filter before treatment (III).

3.1.4. Effect of decontamination on microbiological quality of process waters

Decontamination methods are utilized in order to maintain the good hygienic quality of process water. The efficiency of decontamination methods on different microbes in carrot process water was tested with a suspension test and a test of industrial washing water (Table 8). Article III evaluated neutral NEW, ClO₂, FPW and UV-C in processing waters and their effect on *E. coli*, *Candida lambica* (yeast), *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. The methods employed were: a suspension test with and without interfering substance (IS), carrot juice (1%), and UV-C decontamination of carrot processing water from the company, with and without filter.

Suspension tests (EN1276:1998) on pure cultures of microbes were conducted with and without 1% of sterile carrot juice as an interfering substance (IS) at a low temperature between 5 °C and 10 °C (Fig. 7). *Candida lambica*, *E. coli*, *Yersinia enterocolitica*, and *Yersinia pseudotuberculosis* were used as test microbes. The suspensions were diluted to contain approximately 10⁵ cfu/ml in test solution at the beginning of the test. The cell concentration of *C. lambica* in suspensions was lower, around 3 log₁₀ cfu/ml. Suspension tests are used for indicating disinfectant efficacy (Holah 2014).

Table 8. Studied decontamination methods of water (article III).

Test and microorganisms tested	Treatment (abbreviation)	Concentrations
Suspension test <i>Y. enterocolitica</i> , <i>Y. pseudotuberculosis</i> , <i>E. coli</i> , <i>C. lambica</i>	Neutral electrolyzed water (NEW)	30, 50, 100 ppm free chlorine ^b
	Chlorine dioxide (ClO ₂)	10, 50, 100 ppm free chlorine
	Commercial wash* (FPW)	0.125, 0.25, 0.5% dilutions
	UV-C (254 nm)	30 mJ/cm ²
Test of industrial washing water Total colony count	UV-C (254 nm)	-
	UV-C (254 nm + filter 150 µm)	-

* Commercial citric acid-based produce wash (FPW).

^b The available chlorine was analysed by means of titration with 0.1 M sodium tiosulphate (Tamine 2008).

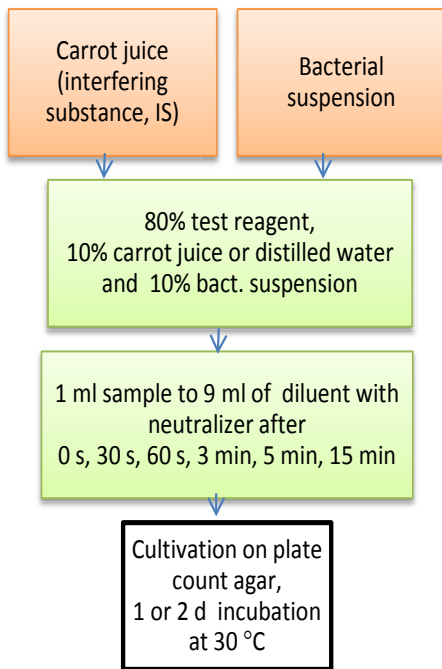


Figure 7. Procedure of the suspension test for testing decontamination of vegetable process waters (III).

3.1.5. Quality and treatment of waste waters

Three full-scale waste water treatment systems of the processing companies studied were evaluated on site. Waste waters from vegetable processing were treated in sedimentation basins and with chemical precipitation (I, IV), in a trickling filter (IV) and in a sequencing batch reactor (SBR) (IV).

Wash water of root vegetables was piped to two sedimentation basins (110 m³ and 90 m³) (I) and waste water from washing and processing was piped to the basin (8000 m³) without any mixing (I). Water remained in the basin from four to six months. Microbes were not detected in the middle or in bottom level of the basin.

In the study (I), half-litre waste water subsamples were collected in the course of one working day, once per hour. The subsamples were then mixed and a two-litre sample was taken to the laboratory. Samples were kept in a refrigerator before microbiological and chemical analysis (article I).

In the study (IV) sampling was performed three times, in spring, autumn and winter, in the years 2005 and 2006. The processes are illustrated in article IV. An ISCO 6700 automatic water sampler was used to collect a sample of 100 ml coming from the peeling process every half-hour during the working day. One combined sample per day of 500 ml was taken from the container and stored at -20 °C. Sedimentation chemicals used in the study are presented in Table 9.

Table 9. Sedimentation and neutralization chemicals used for waste water pretreatment (I, IV).

Chemical	Formula	Product	Producer/Importer	Article
Ferrous sulphate	FeSO_4	Kemira COP	Kemira, Finland	IV
Ferric sulphate	$\text{Fe}_2(\text{SO}_4)_3$	PIX-115	Algol Chemicals, Finland	I
Polyaluminium chloride	$\text{Al}_n(\text{OH})_m\text{Cl}_{3n-m}$	Eka WT91	Eka Chemicals, Finland	I, IV
Aluminium sulphate	$\text{Al}_2(\text{SO}_4)_3$	33%-solution	Tamro, Finland	I, IV
Calcium carbonate	CaCO_3	Liquid lime	Kemira, Finland	IV
Sodium hydroxide	NaOH	Lye	Tamro, Finland	IV

3.2. Microbiological methods

Microbiological analyses used in the study are summarized in Table 10. Detailed descriptions of the methods are presented in the original publications I-IV.

Table 10. Microbiological methods used in the study.

Samples	Microbe type	Method	Article
Carrot	Aerobic plate count	ISO 4833:2003: Plating on Plate Count Agar (PCA), incubated at 30 °C for 3 days.	II
	Presumptive <i>E. coli</i>	•Plating on Violet red bile agar (VRB) incubated at 44.5 °C for 24 h. •NMKL 125:2005. Plating on Tryptic Soy Agar combined with VRB.	II
	Coliform bacteria	ISO 4832:2006 (E). VRB incubated at 37 °C for 24 h.	II
	Enterobacteria	ISO 21528-2:2004 NMKL144:2005 VRB glucose agar (KVVY LAB M) incubated at 37 °C for 24 h.	II
	Yeasts and moulds	ISO 21527-1 NMKL 98:2005 (KVVY). Plating on Dichloran Rose Bengal Chloramphenicol agar (DRBC), incubated at 22 °C for 5d.	II
	<i>Y. enterocolitica</i>	RT-PCR and ISO 10273	II
	<i>Y. pseudotuberculosis</i>	RT-PCR and Bacteriological Analytical Manual Online, modified.	II
Water	Heterotrophic plate count	Plating on R2A agar (LabM Ltd), incubated at 30 °C for 3 days SFS-EN ISO 6222, 1999. HUA (Water Plate Count agar), incubated at +22.0 ± 2.0 °C for 68 ± 4 h.	I, II
	Faecal coliform bacteria	SFS 4088:2001. Filtration through a Millipore 45 µm filter and incubation on mFC agar at 44 °C for 24 h.	I, II
	Coliform bacteria and <i>E. coli</i>	Filtration through a Millipore 45 µm filter, incubation on Harlequin™ <i>E. coli</i> /coliform agar (LabM Ltd) at 37 °C for 24 h. Blue-purple colonies were counted as presumptive <i>E. coli</i> and all blue-purple and magenta colonies were counted as presumptive coliforms; Colilert® Quanti-Tray 18®-protocol (IDEXX), incubated at +36 °C ± 1 °C for 18–21 h.	I, II
	<i>Y. enterocolitica</i> <i>Y. pseudotuberculosis</i>	RT- PCR LA517H	I
	Sulphite reducing clostridia	SFS-EN26461-2, 1993	I
	Enterococci	SFS-EN 7899-2, 2002	I
	Total solids	SFS-EN 872, 2005	I, IV
	Total phosphorus	SFS-EN ISO 6878, 2004 modified, SFS-EN 1189 (IV)	I, IV
	Total nitrogen	SFS 5505, 1988 modified	I, IV
	COD _{Cr}	ISO 15705, 2002, SFS 5504 (IV)	II, IV
	BOD ₇ (ATU)	SFS-EN 1899-2, 1998 modified	I, IV
	pH	SFS 3021	IV
	NH ₄ -N	SFS 3032	IV

3.3. Improving of the function of the trickling filter (Article IV, case B)

The construction of the trickling filter examined in study IV was observed to be insufficient for the kind of waste water process in which the volume of sludge generated was high. The sludge could not be properly removed from the filter, and the volume of the trickling filter was too small to treat the biological load. Due to these factors the trickling filter had to be cleaned from sludge and solids which were accumulated in the filter.

Because of its lack of functioning, the system was improved after the publication of article IV. The software of the process control unit was finally checked and adjusted (Fig. 8). The lower part of the filter was replaced. In addition, the old, flat bottom was partly removed and a new tapered (coniform), solid bottom, made from stainless steel, was installed. The new bottom was designed at Luke, (the former MTT), and was built by Stainless Team Oy. These improvements increased the active storage capacity of the filter from 1.8 m^3 to 3.2 m^3 and the volume of the aeration unit from 5.5 m^3 to 6.9 m^3 . A 1000 W pump (Leader BVP, Italy) was installed in the bottom of the cone, which circulated water from the bottom up onto the filter material (plastic particles) of the filter. The water nozzle was also changed so that it constantly spread water on the filter.

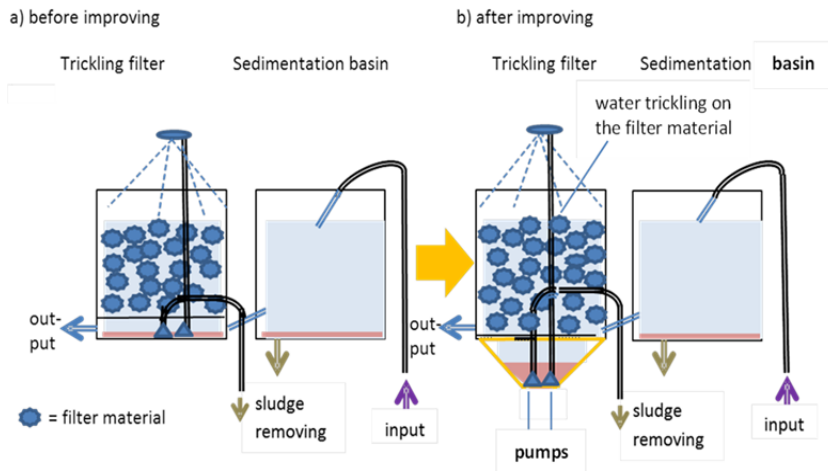


Figure 8. Trickling filter used in study IV for treatment of waste water (input) a) before (presented in article IV) and b) after improvement.

3.4. Evaluation of the data and methods of the present study

In this study, the level of quality of fresh-cut vegetable processing in Finland was studied by defining the microbiological quality of vegetables during processing, as well as water use and the quality and handling of water. The total amount of fresh water consumed in the processes was measured with water meters installed at the companies. Samples were collected from eleven companies. Water samples from each day were combined, and sub-samples from waters were collected over the course of the week. This was done because the quality and quantity of water fluctuates over the course of the day. Combination samples describe the level quality of wash-, process- and waste water well. Chemical and microbiological analyses were conducted in three replicates. The results are expressed as an average value of replicates. Ranges of chemical and microbiological analyses were calculated. In the suspension test, the sample was taken at certain intervals; it was found that the results were consistent.

The microbiological quality of fresh-cut vegetables was measured in article II. The level of microbes of the longer time is important to know and follow. The findings on the trend of microbe level in the processing companies helps in developing preventive strategies for improving the quality and safety of fresh-cut vegetables (Tango et al. 2018). The microbial quality of carrot wash- and process waters was studied in article II and biological, chemical and microbial quality as well in article I. The quality of process waters needs to be sufficiently high and it should be monitored regularly. Process water can act as a means of spreading microbial contamination in the production batch (Luo et al. 2012; Gil et al. 2016). The suspension tests (Holah et al. 1990; Gibson et al. 1995) gained important knowledge of the effect of these methods for inactivating various microbes (*E. coli*, *Y. enterocolitica*, *Y. pseudotuberculosis* and *C. lambica*).

Yersinia was analysed by means of cultivation and with RT-PCR (real-time polymerase chain reaction). RT-PCR has provided better estimates and more rapid results for the occurrence of *Yersinia* than the cultivation method (Fredrikson-Ahomaa & Korkeala 2003; Thisted Lamberz et al. 2008; Fukushima et al. 2011). In our study, when RT-PCR positive samples were cultivated *Yersinia* was not detected.

In the present study, level of functioning of waste water sedimentation basins and three waste water treatment plants was evaluated on site. In order to make the biological waste water treatment process work well, the system needs good control and care, such as knowledge of waste water treatment. Only a small amount of information on this kind of on-site vegetable waste water treatment plants has been published (Mundi & Zytner 2015; Mundi et al. 2017). More practical solutions were needed, however, laboratory- and pilot-scale studies of these kinds of waste water treatment plants have been performed (Sterritt & Lester 1982; Vanerker et al. 2013; Chen 2015; Moore 2015).

Statistical analysis

Statistical analysis was performed from microbiological assays using reasonable analytical amounts. The number of samples varied and in some cases was quite small.

The differences between the number of cfu values in the different samples of carrots and carrot processing waters were investigated using linear mixed models with function lme in library nlme in the statistical program R (R Core Team 2018). Log transformation was applied to the cfu values when the models were estimated. In the water models, the phase of a process (a factor with three categories: 1 = carrot wash water, 2 = carrot process water, and 3 = lettuce wash water) was used as an explanatory variable, and in the carrot models, the phases (a factor with four categories) were: 1 = washed, whole carrots, 2 = washed and peeled carrots, 3 = washed, peeled and cut carrots, and 4 = washed, peeled and grated carrots. The code for a factory was used as a random factor in the models to take into account the fact that samples from the same origin could be more similar than randomly collected samples.

Other biological, chemical and volume measurements were implemented to find out the overall concentration levels and in these cases no statistical comparisons were performed.

4. Results

Water consumption and quality were measured in different companies, as well as the microbiological quality of fresh-cut vegetables processed at those companies. The water decontamination of process water of carrot- and on-site waste water treatment was also tested.

4.1. Water consumption of different processing stages of vegetable processing

Different kinds of raw material, processes and processing machines are used in vegetable processing, a fact which influences water use and quality. Water consumption, measured in six fresh-cut processing companies, was 2.0–6.5 m³/t of finished product (Table 11).

Table 11. Water use in different vegetable companies (I and IV).

Processing of vegetables	Article	Company	Water used (m ³ /t of product)
Washing of root vegetables	I	B	2.0
Washing of carrots	IV	A	2.5
Processing of root vegetables	I	C	3.5
Processing of vegetables	I	D	4.4
Processing of lettuce	un-published	E	2.8
Processing of root vegetables	IV	D	6.5

The volume and organic matter content of wash and process water were measured in two companies (C, D). The total volumes of water from the washing and polishing phases (38 and 43%) and from peeling and rinsing (45 and 48%) were rather similar in both companies. Most of the organic load (BOD₇) and nutrients were released into the water from the peeling of root vegetables, whereas the volume comes primarily from rinsing and washing (Fig. 9)(I).

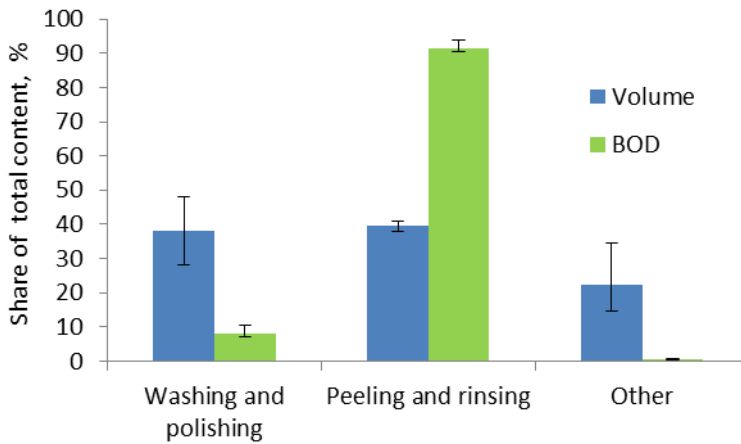


Figure 9. Comparison of carrot washing and polishing, peeling and rinsing as well as other water use during carrot processing. The mean and ranges (min-max) of the volume (%) and BOD₇ (%) of water were measured at two plants (I), $n = 2$.

Water consumption was measured over the course of three years in a company which washed and processed root vegetables (I). Water was used for washing (30%), low pressure cleaners (14%), for processing (42%) and washing of premises and machines (13%). The total water use varied among months according to the season, volumes of vegetables, and quality of raw material. When water consumption was measured over the course of three years, consumption was found to have decreased by 5% during the first two years and by 10% during the third year, compared to the situation at the beginning of the taking of measurements. Reduced consumption was achieved in the washing phase, which was made more effective by changing water feeders to more efficient ones and by monitoring water consumption with water meters (I).

4.2. Microbiological quality of vegetables

During the processing of fresh-cut vegetables the counts of microbes in the vegetables changed at different stages of the process (Fig. 10). In the article II, washed, unpeeled whole carrots in general contained the highest total microbial counts (mean 5.3 log₁₀ cfu/g) and coliform bacteria (mean 3.8 log₁₀ cfu/g). An indicative difference was found in the total microbial count which was lower in peeled carrots ($p = 0.095$) than in whole carrots. Statistically significant difference in the total microbial count was found between whole and cut carrots ($p = 0.014$): the microbial count was lower in cut than in whole carrots. An indicative difference was found in the count of coliform bacteria; microbial count was lower in peeled than in whole carrots ($p = 0.060$). There was also an indicative difference in the cfu values of molds between whole and cut carrots ($p = 0.072$); the count of molds seemed to be lower in cut carrots than in whole carrots. Other differences were not statistically significant. *Escherichia coli* was not detected in any carrot sample (II).

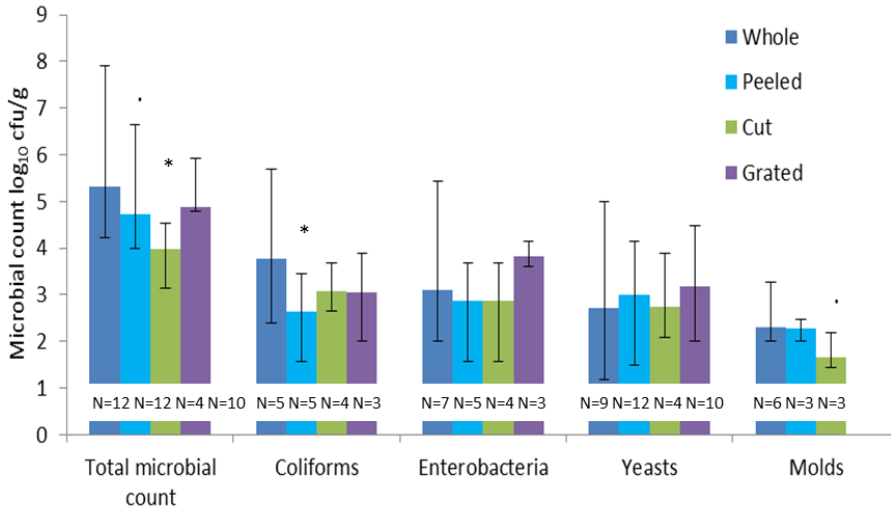


Figure 10. The means and ranges (min-max) of total microbial count, coliform bacteria, enterobacteria, yeasts and molds in whole, peeled, cut and grated carrots (II). Statistically significant differences ($p < 0.05$) between whole carrots and the processed carrots are indicated with (*), and indicative difference ($0.05 < p < 0.10$) with (.). The numbers (N) of samples analysed are marked in the figure.

4.3. Microbiological and chemical quality of wash and process waters

The microbiological quality of wash and process waters was measured in three companies in which root vegetables were washed (II). In addition, in the article II wash and process waters were collected from six carrot washing and processing companies. The results are collected in Fig. 11.

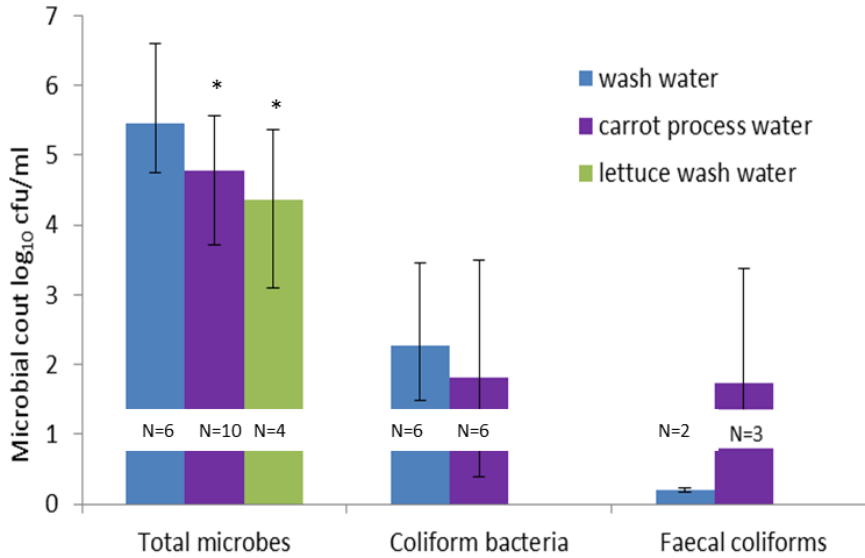


Figure 11. The means and ranges (min-max) of microbes of wash- and process waters of root vegetables (primarily carrots) and process waters of lettuce (I, II). The numbers (N) of samples analysed are marked in the figure. Significant differences ($p < 0.05$) between wash water and the other waters are indicated with (*), $p < 0.05$). Differences in faecal coliforms were not analysed statistically.

A statistically significant difference in the counts of total microbes was found between carrot wash water and carrot process water ($p = 0.012$): the microbial count was lower in process water than in wash water. In addition, a significant difference in the total microbial count was found between carrot wash water and lettuce wash water ($p = 0.031$). There was no difference in coliform bacteria counts between carrot wash-, process- and lettuce wash waters ($p > 0.10$). These statistical analyses have not been presented before. Non-pathogenic *Yersinia enterocolitica* was found in almost all wash water samples, but in only two of the ten process water samples (I) (Table 12).

Table 12. The presence of *Y. enterocolitica* in carrot and water samples analysed by cultivation (II).

Sample details	Total N of samples	N of PCR positive samples by cultivation	% positive samples
Carrot, washed	9	5	67
Carrot, washed and peeled	5	3	60
Carrot, washed, peeled and cut	4	4	100
Carrot, washed, peeled and grated	2	1	50
Wash water	5	4	80
Process water	10	2	20
Waste water	6	5	83

N = number

Some carrot and process water samples were analyzed by means of RT-PCR, which is more sensitive than cultivation. With the RT-PCR method, pathogenic *Y. enterocolitica* was observed in all washed carrot samples and in almost all peeled carrot samples. However, when the positive RT-PCR samples were cultivated, pathogenic *Y. enterocolitica* was not detected (II) (Table 13).

Table 13. Presence of pathogenic *Y. enterocolitica* in carrot and process water samples analysed by PCR (II).

Sample	Total N of samples	N of PCR positive samples	% positive samples
Carrot, washed	3	3	100
Carrot, peeled	7	5	71
Carrot, grated	3	1	33
Process water	7	2	29

The chemical quality of wash- and process waters was determined in three companies which wash vegetables, in three companies which wash and process root vegetables and in one lettuce processing company (I). Concentrations of total solids (TS), biological oxygen demand (BOD₇), chemical oxygen demand (COD_{Cr}), and nutrients P and N of the samples of washing and processing waters of root vegetables and process waters of lettuce are presented in Figures 12 and 13.

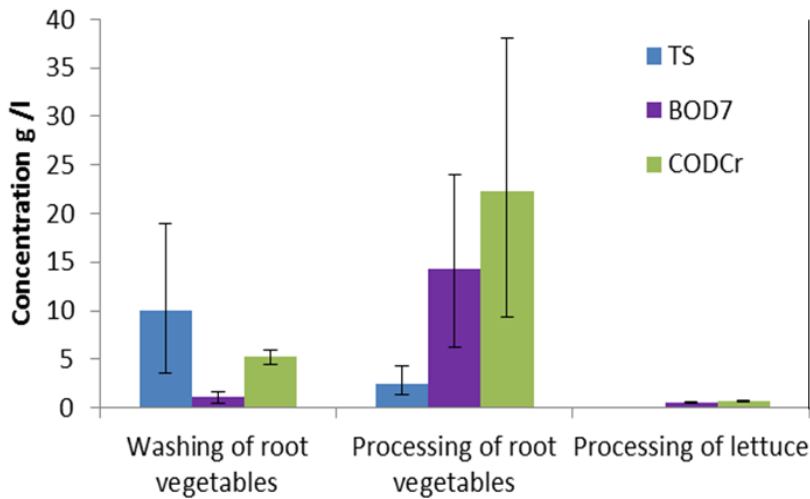


Figure 12. Mean concentrations and ranges (min-max) of TS, BOD₇ and COD_{Cr} of wash- and process waters of root vegetables (N = 6) and process water of lettuce (N = 2) (I).

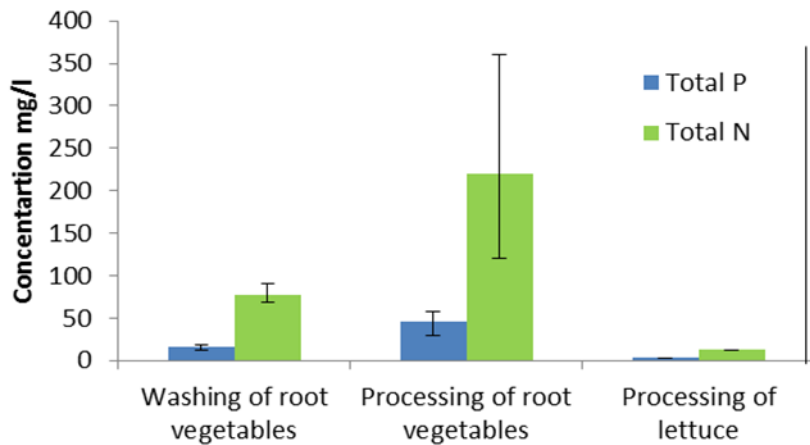


Figure 13. Means and ranges (min-max) of concentrations of nutrients P and N of wash- and process water of root vegetables (N = 6) and process water of lettuce (N = 2) (I).

4.4. Effect of decontamination on microbiological quality of process waters

Suspension test

In the suspension test, 50 ppm NEW inactivated *Y. pseudotuberculosis* in 5 minutes in the presence of interfering substance IS (III). At the lower concentration of 30 ppm, inactivation took 15 minutes. A 5 log₁₀ cfu/ml reduction of *Y. enterocolitica* was achieved by 30 ppm NEW in 5 minutes in the presence of IS. *E. coli* was more sensitive than yersinia, because it was inactivated by 30 ppm NEW in 3 minutes. A 3 log₁₀ cfu/ml reduction of *C.*

lambica took 0.5 minutes. These results were from samples in which an interfering substance IS was added (III).

ClO₂ efficiently decreased *Y. enterocolitica*, *Y. pseudotuberculosis* and *E. coli* counts in water (> 4 log₁₀ cfu/ml reduction), but at 10 ppm of ClO₂ concentration IS impaired the effect. In the presence of IS at 10 ppm ClO₂ showed no effect on *C. lambica* (Table 14).

Table 14. Log reductions of *Yersinia enterocolitica*, *Y. pseudotuberculosis*, *E. coli* and *C. lambica* colony counts in water suspension treated with NEW, ClO₂ and FPW (III). Reaction time = 30 s. *Y. enterocolitica* EELA 56 and *Y. pseudotuberculosis* EELA 549 were obtained from the culture collection of the Finnish Food Authority. *E. coli* DSM 787 and *C. lambica* VTTC-00360 were obtained from the VTT Culture Collection.

Treat- ment	Concen- tration	Microbe							
		<i>Y. entero- colitica</i> EELA 56		<i>Y. pseudotu- berculosis</i> EELA 549		<i>E. coli</i> DSM 787		<i>C. lambica</i> VTTC-00360	
		No IS	With IS	No IS	With IS	No IS	With IS	No IS	With IS
NEW^c	30 ppm	**	*	***	*	*	NE	*	*
	50 ppm	***	*	**	**	***	**	*	*
	100 ppm	***	***	***	***	***	***	*	*
ClO₂^c	10 ppm	***	*	**	NE	***	*	*	NE
	50 ppm	***	**	***	**	***	***	*	*
	100 ppm	***	***	***	***	***	***	*	*
FPW	0.125%	-	-	-	-	**	*	*	NE
	0.25%	-	-	-	-	***	*	*	*
	0.5%	-	-	-	-	***	**	*	*

^c Concentration of free chlorine, IS = interfering substance

*** = > 4 log₁₀ reduction, ** = > 3 log₁₀ reduction, * = > 2 log₁₀ reduction

NE = mild or no effect, less than 2 log₁₀ reduction, – = not measured

FPW solutions reduced the numbers of *E. coli* by 5 log₁₀ cfu/ml within 3 minutes. IS diminished the effect so that the maximum reduction was attained in less than 3 min: 4.2 log₁₀ cfu/ml reduction for 0.5% FPW solution and 2.5 log₁₀ cfu/ml reduction for 0.25 and 0.125% FPW solutions. *C. lambica* was inhibited to the limit of detection (2.8 log₁₀ cfu/ml), at all concentrations with or without IS, except for the 0.125% solution with IS, with which the maximum log reduction was achieved in 15 minutes (III).

The effect of UV-C on logarithmic reductions of *E. coli* was more than 2 log₁₀ cfu/ml in 5 minutes and 5 log₁₀ cfu/ml in 15 minutes. The influence of the interfering substance

was minor. The reduction of *C. lambica* counts in water suspension treated with UV-C was 2.0 in 5 minutes and reached the limit of detection, 2.5, in 15 minutes. When the interfering substance was used, the respective reductions were 1.5 and 1.8 log₁₀ cfu/ml (Table 15).

Table 15. Log₁₀ reductions of *Yersinia enterocolitica*, *Y. pseudotuberculosis*, *E. coli* and *C. lambica* colony counts in water suspension treated with UV-C (III). Reaction times were 0.5–15 minutes.

UV-C	Microbe							
Time	<i>Y. enterocolitica</i> EELA 56		<i>Y. pseudotuberculosis</i> EELA 549		<i>E. coli</i> DSM 787		<i>C. lambica</i> VTTC-00360	
	No IS	With IS	No IS	With IS	No IS	With IS	No IS	With IS
0.5 min	*	*	*	*	*	*	NE	NE
5 min	*	*	*	*	*	*	NE	NE
15 min	***	***	***	***	***	***	*	NE

IS = interfering substance

*** = > 4 log₁₀ reduction, ** = > 3 log₁₀ reduction, * = > 2 log₁₀ reduction

NE = mild or no effect, less than 2 log₁₀ reduction

UV-C treatment of industrial processing water (III)

The water from carrot processing was treated with UV-C with and without filtration. At the beginning of the test, the total colony count of the processing water was 7.1*10⁵ cfu/ml and pH 6.58. After 10 minutes, the colony count decreased by 2.5 log₁₀ cfu/ml using UV-C treatment and by 3.5 log₁₀ cfu/ml when the UV-C was combined with filtration (150 µm filter). After 30 minutes, the reductions were 2.5 and 5.0 log₁₀ cfu/ml, respectively (III).

4.5. Quality and treatment of waste waters

With regard to waste water treatment, sedimentation basins and their efficiency (I, IV), three on-site waste water treatment plants (IV) and tests of chemical precipitation (I) were studied. Reductions of treatments of article I are presented in Fig. 14 and treatments of article IV in Fig. 15. The trickling filter was improved and reductions after this improvement are found in Table 16.

On-site waste water treatment

Reductions of TS, BOD, COD, TP and TN were compared in two sedimentation basins. Colony counts of microbes were at the same level in the incoming water and in the egress of the basin and not detected in the middle or in the ground of the basin. The pH of the waste water was between 5 and 6 (I).

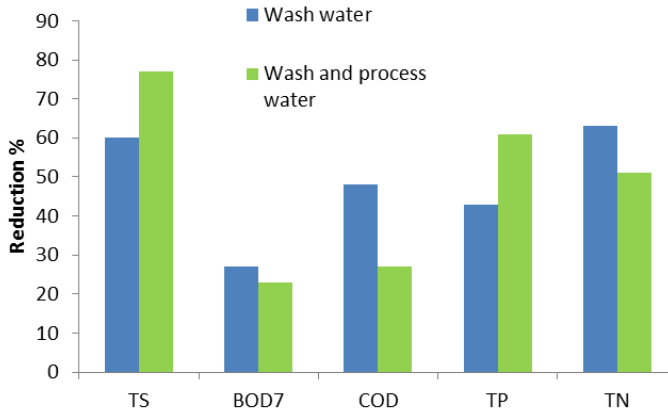


Figure 14. Comparison of the reductions of TS, BOD₇, COD, TP and TN of two waste water sedimentation basins of a company carrying out root vegetable washing, and washing and processing (II). TS = total solids, TP = total phosphorus, TN = total nitrogen.

Three waste water treatment methods (trickling filter, sequencing batch reactor and sedimentation with chemicals) were compared in the article IV. Waste water from processing of root vegetables was treated in a sequencing batch reactor (SBR); the reduction of BOD₇ was more than 99%, COD 97%, phosphorus about 95% and nitrogen 94% (IV). Samples were taken three times, in the spring, autumn and winter.

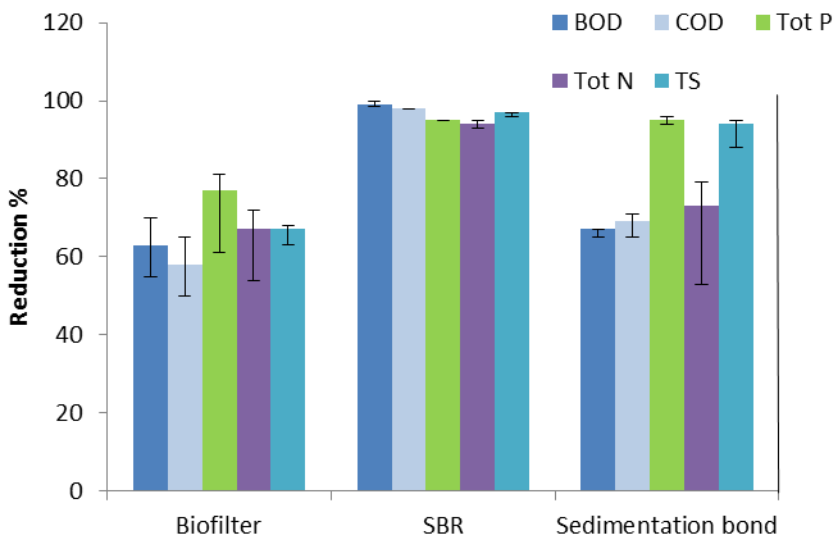


Figure 15. The means and ranges (min-max) of reductions of different-quality parameters of waste waters (N = 3) treated with different waste water treatment methods: biofilter/trickling filter, SBR (sequencing batch reactor) and sedimentation bond (IV). Treatment took place year round. The cold season did not influence the biofilter and SBR, because the trickling filter was in a warm place and the SBR was below ground. Waste water treatment processes also produce heat. The sedimentation bond was covered by ice during the winter.

The reduction of BOD₇ was only 63% for waste water treated with the trickling filter (IV). The filter was improved later (chapter 3.4); after these improvements, reductions of BOD₇ and COD were 93% and 92%, respectively (Table 16).

Table 16. Properties of untreated and treated waste waters before (IV) and after improving the trickling filter system.

Parameter	Before improvements (IV)					After improvements (previously unpublished)		
	Influent		Effluent		Reduction	Influent	Effluent	Reduction
	mean	range	mean	range	%			%
BOD₇	910	820–1000	340	250–450	63	662	44	93
COD	1445	1240–1600	610	430–800	58	1050	80	92
pH	4.7		4.5		-	5.0	7.2	-
Q	7 m ³ /d		-		-	6 m ³ /d	-	-

- = not applicable

Chemical precipitation

All tested chemicals (Table 9, section 3.1.5) improved the precipitation of organic matter and nutrients of waste waters. With the precipitation of vegetable washing water, about 80% of organic matter was precipitated (COD was reduced from 2700 to 500 mg/l) (II). In the carrot processing water, the best results were achieved using a 0.05%-solution of ferrisulphate and polyaluminium chloride and 20–25% of the organic matter was precipitated. When the waste water of vegetable washing was examined, the best dosages of aluminium sulphate and ferrisulphate were 0.1% and 0.05% solutions, respectively. In the article IV aluminium sulphate Al₂(SO₄)₃ was used in a sedimentation basin in which there was no aeration. Water remained in the basin four to six months. The basin was nearly anaerobic. The reduction of BOD₇ was 67% (IV).

5. Discussion

The objective of fresh-cut vegetable processing was to produce high-quality fresh-cut produce with reasonable inputs (Gil et al. 2009; Ölmez 2013). Water is an important part of food processing (Kirby 2003). In the present study, fresh-cut vegetable processing and waters were examined over the course of many years in 11 fresh-cut vegetable companies in Finland. In Finnish fresh-cut vegetable companies, the production of fresh cut vegetables was based on high-quality water and clean processes. Chemical water decontamination was not used; nevertheless, the quality of vegetable products was good. In Finland, water of high quality was available abundantly, but special attention must be paid to process hygiene and process water quality in order to maintain product safety.

5.1. Water consumption of different processing stages of vegetable production

Fresh-cut vegetable industry is one of the major water intensive sectors of the food production and processing (Kirby 2003; Ölmez 2013). The minimization of water use and waste water discharges are big challenges for the fresh-cut industry that will be increasingly required to implement sustainable strategies for water saving (Ölmez & Kretzschmar 2009; Gomez-López et al. 2013; Manzocco et al. 2015). In the present study, water use in the different vegetable processing companies was 2.0–6.5 m³/t of finished product (I), and these volumes primarily came from the washing stage (I). The recommended quantity of water to be used in the fresh-cut industry is 5–10 m³/t of product before peeling and cutting, and 3 m³/t after peeling and cutting (Ahvenainen 2000). According to Ölmez (2013), water consumption in the vegetable production industry was in the range of 2.4–11 m³/t of product. The present results (I) of water consumption in Finland were lower when compared with the recommendation and the result mentioned above. However, the present results were similar to the results of Mundi et al. (2017), who estimated that up to 5 m³ of waste water was generated per tonne of produce in post-harvest processing of vegetables in Canada. The measured quantity of water in the present study clearly differs from other results in the literature. This could be caused by the different habits of water use and a lack of water meters, leading to water quantities presented only as estimates in the literature.

When water consumption is measured regularly, it is possible to control water use. In the present study, water consumption decreased by 15% during the three-year period examined. According to Ölmez (2013), a systematic approach to water management can lead to about a 30–50% decrease in total water use of water in fresh-cut vegetable processing. When the costs of water rise, different means of water recycling and reuse will become a viable operation (Hancock 1999). The quality of process water in fresh-cut vegetable processing is critical for vegetable quality. It is recommended that water used during minimal processing be monitored to assess its microbial quality (EFSA 2014). Liu (2007) states that the generalized description of waters from vegetable processing

needs to be understood as an approximation for explaining a complex issue. This complexity was also observed in the present study, when water use was monitored over three years (II). For example, the water consumption varied between months and according to season, volumes of vegetables processed, and the quality of raw material used in the same company. In the autumn, when root vegetables were just harvested and in high seasons, e.g., before Christmas, water consumption was high. Daily water consumption in the washing period varied in different companies from 3.5 to 212 m³ and in processing from 22 to 105 m³ (I). Water consumption was thus shown to vary not only according to natural variation during the periods of production, but also depending on the effectiveness of the processes themselves. Water used and waste water generated should be monitored systematically, so that the quantitative and qualitative water requirements for each particular process can be identified (Kirby et al. 2003; Ölmez 2013).

5.2. Microbiological quality of fresh-cut vegetables and wash, process and waste waters

Self-monitoring of food safety focuses on the evaluation and control of foodborne hazards. The identification of spoilage organisms carried by fresh-cut vegetables is an important step in developing approaches to inhibiting and controlling these organisms (Fan & Song 2008). In this study level of microbes in wash, process and waste waters from fresh-cut vegetable processing plants was evaluated and samples were collected from companies. In the present study, *E. coli* values in vegetables were < 10 cfu/g (below detection limit), but in other studies *E. coli* has been detected in processed vegetables (Bohaychuk et al. 2000; Abadias et al. 2008; Olaimat et al. 2012; Jeddi et al. 2014). According to the present study, the total microbial counts and coliform bacteria counts in peeled and cut carrots were lower than in whole washed carrots (II). Total microbial counts can be seen to be higher in grated than in cut carrots (II). Processing of fresh-cut vegetables increases their perishability (Cantwell & Suslow 2002; Abadias et al. 2008), and each step in their processing affects the quality and microflora of fresh-cut vegetables. In this study, grated carrots contained the highest microorganism counts. In addition, Torriani & Massa (1994) found that the peeling of carrots reduced the amount of total microbes about 2 log₁₀ cfu/g, but the amount of microbes increased during cutting.

Microbes found in carrot and lettuce, come mainly from soil but also from post-harvest handling and processing (Delaquis 2005; Siponen & Niskanen 2006; Martinez-Vaz et al. 2014). The ability to remove naturally present microorganisms from fresh-cut produce by means of washing is limited (0.5–2.0 log₁₀ reduction) (Tirpanalan et al. 2011; Olaimat & Holley 2012; Goodburn & Wallace 2013; Van Haute et al. 2013). On the other hand, according to Holvoet et al. (2012), during the production of fresh-cut vegetables the washing step has been identified as a potential pathway for dispersion of microorganisms to the end product. The first washing phase of harvested carrots removes soil from the surface of carrots, which leads to microbiological load of the washing waters.

In the present study, the total microbial count was lower in process water than in wash water of carrots (I,II). However, the counts of faecal coliforms were $1.5 \log_{10}$ higher in process water than in wash water (II). As an example, at the depth of 0–15 cm, total microbial counts of $8\text{--}9 \log_{10}$ cfu/g have been observed in soil (Hoorman & Islam 2010).

In spring, when carrots have been stored for more than six months, the risk of yersinia has been observed (Finnish Food Authority 2009). In the present study, nonpathogenic *Yersinia enterocolitica* was found in several water and carrot samples by cultivation, but by more sensitive RT-PCR, also pathogenic *Y. enterocolitica* was observed in many carrot samples: all washed carrot samples and in 70% of peeled carrots (II). Nonpathogenic *Y. enterocolitica* was also observed in almost all our wash and waste water samples, but in only two of the ten process water samples (II). According to Bari et al. (2011), water is probably a significant reservoir for nonpathogenic *Y. enterocolitica*. In the present study, *Yersinia pseudotuberculosis* was not detected in the vegetable samples. If the environment of growth is favorable for nonpathogenic yersinia, possibly there is also a risk for the growth of pathogenic yersinia. The effect of decontamination methods on yersinia in vegetables has been examined only in a few studies (Escuerda et al. 1999; Velazquez et al. 2009; Delibato et al. 2018), but in the present study decontamination of yersinia was not examined on vegetables, but on waters instead. Earlier studies about decontamination on yersinia on process or waste waters were not found. In the study by Selma et al. (2006) ozone treatments (1.4 and 1.9 ppm) for 1 min decreased the *Y. enterocolitica* population in clean water by 4.6 and $6.2 \log_{10}$ cfu/ml, respectively. More generally, the effect on decontamination has typically been measured on vegetables and studies on waters are very few.

5.3. Chemical quality of wash and process waters

In the present study, the majority of the organic load (> 90%) and nutrients were transferred to water from the processing stage (peeling and polishing) and most of the total solids from washing. Concentrations of nutrients were the highest in process waters from processing of root vegetables, and the lowest in lettuce process waters (I). Concentrations of TS and nutrients in the lettuce washing and processing waters were low when compared to that of root vegetables (I). In addition, Mundi et al. (2017) found that the lower values of BOD, COD, total P and N in lettuce wash water, when compared to root vegetables, and processing produced higher levels of these values. Water from vegetable processing contains dissolved organic solids from various operations and debris from mechanical processing (Liu 2007). In the present study, most of the organic load came from the peeling phase (I).

5.4. Effect of decontamination on microbiological quality of process waters

The outbreaks associated with fresh-cut vegetables have been increased in the recent years (Jeddi et al. 2014). Washing of fresh-cut produce with sanitizing solutions has been considered the only step to achieve a reduction in spoilage micro-organisms (Alegre et al. 2013). The effect of decontamination on different microbes in water differs with time, concentration, decontamination method, and turbidity of water. The ability of chemical substances or physical decontamination methods to remove natural microorganisms from fresh-cut produce is limited. Some microbial reductions occur, but total reduction is unachievable with most methods (Tirpanalan et al. 2011; Olaimat & Holley 2012; Goodburn & Wallace 2013; van Haute et al. 2013). Organic load and turbidity of water decrease the efficiency of the methods. In the present study, interfering substances impaired the effect of chlorine dioxide (ClO_2), neutral electrolyzed water (NEW), FPV and UV-C (III). Publications of water treated with UV-C decontamination of vegetable process waters are few, but instead this method has widely been used to decontamination of vegetables (Artes et al. 2009; Turtoi 2013; Fan et al. 2017).

In the present study, the effect of UV-C on *E. coli* in water was more than 2 $\log_{10}$ cfu/ml in 5 minutes and 5 $\log_{10}$ cfu/ml in 15 minutes in the suspension test (III). Ignat et al. (2015) used UV-C light to treat lamb's lettuce wash water. Water was obtained from 5 washing cycles and about 3 $\log_{10}$ reductions in total viable count were achieved by exposure to UV-C light. Iceberg lettuce and oak leaf lettuce wash water was treated with UV-C and reduction was detected of 3.2 and 2.1 $\log_{10}$ cfu/ml of total bacteria, respectively (Wulfkuehler et al. 2013). In the study by Selma et al. (2008), a 2.4 and 3.9 $\log_{10}$ cfu/ml natural microflora reduction was observed when an UV-C light was applied for 60 min for the decontamination of onion and escarole wash water, respectively. With longer treatment times, good reductions in process waters are attained. The decontamination efficacy of UV-C light is highly related to the presence of suspended particles (Milan-Sango et al. 2017) and these systems may also require an additional capital cost of a filtration system to remove particulate matter from the water stream (Garrett et al. 2003).

In the present study the efficiency of decontamination methods on different microbes in carrot process water was tested with a suspension test in a laboratory. However, this method is not commonly used in analysing of vegetable processing waters. The test gave a good indication of time and concentration at which a certain level of decontamination was reached. Suspension tests are useful for indicating general decontaminant efficacy and for assessing parameters such as contact time and interfering matter (Reybrouck 1998).

Organic acids such as acetic, citric, malic, tartaric and propionic acids are classified as GRAS (Generally Regarded as Safe) compounds by the FDA (2018), and can be added to vegetables as preservatives (Feliziani et al. 2016). In the present study, the commercial citric acid based product (FPW, Fresh Produce Wash) was tested by suspension test

(III). An interfering substance (IS, carrot juice) diminished the effect of reducing numbers of *E. coli* so that the maximum reduction with IS was reached in less than 3 minutes and reduction was $4.2 \log_{10}$ cfu/ml for 0.5% FPW solution and $2.5 \log_{10}$ cfu/ml for 0.25% FPW solution. *C. lambica* was inhibited to the limit of detection ($2.8 \log_{10}$ cfu/ml) in concentrations 0.5 and 0.25% of FPW with or without IS (III). Virto et al. (2006) studied the inactivation of *E. coli* by organic acids, citric (0.01–0.15 mg/l) and lactic (1–60 mg/l) acids, at different temperatures (4, 20 and 40 °C) with modelling. The bactericidal effect of both acids was dependent on time and temperature of exposure and on acid concentration. Lactic acid was more effective than citric acid. According van Haute et al. (2013), weak organic acids were inefficient water disinfectants. In addition, in the present study the reductions detected were less than $3 \log_{10}$ cfu/ml, when the manufacturer's recommended FPW concentration of 0.25% was used.

Common recommendations do not exist for the decontamination of process water of vegetables. Drinking water is disinfected and chlorine is present in most drinking water at concentrations of 0.2–1.0 mg/l (WHO 2017). Decontamination is utilized to maintain the quality of the wash water of fresh cut vegetables despite limited, direct microbial benefits on the produce (Gil et al. 2009). In Finland, as well as in many other EU countries, chemical treatments of process waters are restricted by food legislation. However, UV-C, for example, is suitable for decontamination of processing water of vegetables (Kekki 2013). The implementation of UV-C decontamination technology could decrease the water use of the process as well the risk for residuals of toxic chemicals in the final product (Ignat et al. 2015). The effect of UV-C light is highly related to the presence of suspended particles in water (Millan-Sango et al. 2017)(III). Further research at the laboratory scale on the efficacy of decontaminants on washing water is recommended, in particular with experimental designs reflecting industrial conditions (Banach et al. 2015). Technically and economically effective chlorine-alternative decontamination technologies are the main goal of the fresh-cut industry (Petri et al. 2015). Regulations should be re-examined toward the global of harmonization of processing aids for water decontamination (Gil et al. 2009; Coroneo et al. 2017). It is recommended that the best quality potable water be used for the final rinse of intact vegetables prior to fresh-cut processing (Derden et al. 2002).

5.5. Quality and treatment of waste waters

The environmental issues of vegetable processing are mainly related waste water and treatment of by-products from the process (Helsky et al. 2006). The waste water from vegetable processing consists of organic substances, like cellular fluids, starch and other carbohydrates. The load of untreated waste water of a small vegetable production company is corresponding to the untreated waste water load of more than 100 persons (Helsky et al. 2006). In general, in Finland waste water from vegetable processing should be piped to a municipal waste water treatment plant. If this is not possible, waste water should be treated on-site (Finnish Government Decree 157/2017). In Finland, companies

processing fresh-cut vegetables are often located in rural areas (Isosaari et al. 2010). Many municipalities in Canada and in Finland as well impose a surcharge fee on food processors for the waste water that is discharged into the sewer system (Lam 2015).

According to the EU water framework directive (2000/60/EC) and the national regulations of the Finnish Council of State (Finnish Government Degree 157/2017), the advisory reductions resulting from domestic waste water treatment systems should be at least 80% for organic matter, 70% for total phosphorus and 30% for total nitrogen. For the food industry, there is also the recommendation of the Baltic Marine Environment Commission (Helcom 1996) for treatment plants with a waste water flow exceeding 25 m³/d. Small plants are also obliged to reach the corresponding purification levels of treated waste water, except for removal of nitrogen. In the present study (IV), only the sequence batch reactor (SBR) reached the requirements of current legislation, as well as the trickling filter after improvement (section 3.3).

A waste water treatment process should be capable to clean up polluted waste water in a sustainable way; economical, safe and accessible (Aderibigbe et al. 2017). In the present study (IV), the reduction of BOD₇ in the trickling filter was only 63%. The main problem was the pH of the waste water, which was too low for efficient biological activity and the high organic load. If the biological process is not effective enough, organic matter may be precipitated onto the plastic particles of the filter. Due to the poor performance of the trickling filter system, it was partly reconstructed after publication of article IV. The active storage capacity of the filter was increased from 1.8 m³ to 3.2 m³ and the volume of the aeration unit from 5.5 m³ to 6.9 m³. The reduction of BOD₇ was 93% after the improvement of the filter. Reductions of total solids of root vegetable wash water in sedimentation basins was 60% and that of carrot wash- and process water 77% (I). In the sedimentation pond, in which the pH of waste water was adjusted with liquid lime (CaCO₃) to pH 6–7 and aluminium sulphate (Al₂(SO₄)₃) was added to precipitation, reduction of TS was 94% (IV).

Levels of *E. coli*, coliform bacteria and enterococci were similar in wash- and process waters of root vegetables. The levels of *E. coli*, coliform bacteria and enterococci were 1.1 log₁₀, 2.5 log₁₀ and 0.4 log₁₀ cfu/100 ml higher in waters from lettuce processing than in waters from root vegetable processing, respectively (I). *Yersinia enterocolitica* was found in all waste water samples (I). A few publications are available concerning the formation and hygiene of waste water in the fresh-cut industry. Water reuse is becoming an increasingly common component of water resource planning worldwide as the costs of waste water disposal rise and drought become more common (Radcliffe 2004; Apostolodis et al. 2011). The waste water of vegetable processing should be treated before reuse because of the organic load and the microbial quality of waters. The Codex Alimentarius (2003) states that, if water is circulated for re-use, no risk to the safety and suitability of food should be caused. In Finland, waste water is rarely used for irrigation, and if it is used for that purpose, not for irrigation of vegetables. In the companies in this study, when biologically treated waste water reached the legal requirements it was piped to a ditch.

5.6. Applicability of the data to fresh-cut vegetable production

The need for this kind of research has come from Finnish companies that process vegetables. Companies need knowledge and information for developing their business. In most cases, companies have first cultivated vegetables, and then expanded their operations to vegetable processing when the demand for fresh-cut vegetables grew. From this point of view, companies needed information about process hygiene, microbial quality of vegetables, process water quality, waste waters, waste water treatment and water conservation.

The companies also understood the existing risks of fresh-cut vegetables and hoped for ways to manage them. This research has been designed to meet the needs of companies, and was accomplished in co-operation with them. Environmental authorities require that waste water and wastes are managed appropriately, and the Finnish Food Safety Authority also has its hygiene requirements. In addition, according to the requirements of the trade, the entire production chain must be in order.

Fresh-cut vegetable processing requires a lot of water, and waste waters contain large amounts of organic matter which were observed to cause a notable load on natural water systems. Companies needed information on the quality, quantities and treatment methods of waste water; the lack of waste and waste water treatment is a barrier to business. In this study, various waste water treatment solutions were introduced for the companies. In addition, for example, biological waste water treatment plants were built in several companies. With regard to vegetable processing companies and interest groups, guidance for best practices in the fresh-cut vegetable processing industry was found in the projects related to these studies collected, documented and reported in guidance for best practices evaluated by the Finnish Food Authority (2015)

There are many research studies of different techniques for the decontamination of process water (Gil et al. 2009; Banach et al. 2015, Gil et al. 2015; Gomez-Lopez et al. 2015, Gomez-Lopez et al. 2017). In Finland, as in many other European countries, the use of chemicals such as chlorine in the rinsing water of fresh-cut vegetables is forbidden (Kekki 2013). According to this thesis, fresh-cut vegetables of good quality could be produced without any water decontamination.

Each company must establish its own specific validation protocols for evaluating wash system performance, and each company is responsible for the efficacy of those systems (Gombas et al. 2017). Not all risks can be eliminated; the aim is to minimize the risks (EFSA 2011). Hygiene and health problems with pathogens should be prevented as early as possible in the production chain. The level of microbes in vegetables and process water should be monitored in the company with HACCP (Hazard Analysis and Critical Control Points). Self-monitoring and trend analysis of microbial counts in the process and products are ways of forecasting and preventing microbial contamination of products, and should be examined in the long term (Lehto et al. 2015).

Future trends in the field of fresh-cut vegetable processing should include: more gentle processing techniques with less waste and waste water, larger production com-

panies with process automation, automatic sampling, rapid tests and more safe products. In addition, different kinds of vegetable raw material suitable for processing and products should be developed.

6. Conclusions

1. The quantity and quality of water streams from the vegetable processing vary considerably with the operations of the processing and seasons. There are no two companies with similar processes and processing capacity of vegetable products, who have similar water use and generation of waste waters. There are too many variables (technical or otherwise) in the process. Most of the waste water in this study was generated from the washing and processing of vegetables, but most of the organic load (90%) came from the vegetable peeling phase. Water consumption could be decreased by regular monitoring of water consumption with water meters and by localizing the main consumption points. Information about, which part or parts of the process used the most water and which parts generated the most waste water, helps in directing the control operations of water use and quality management to the right steps of the process. Designing the processes to allow separation of solids would help waste water treatment because of lower concentrations of organic matter and nutrients. Waste water should be treated before re-use. Pretreatment of waste waters using precipitation chemicals and sedimentation in basins decreases the organic load and total solids in the waste water.
2. The quality of vegetables changes during processing. The peeling of carrots reduces the amount of microbes, but microbial counts can be increased during cutting. Wash- and process water plays a dual role: both reducing and transmitting microorganisms to vegetables. The process water samples contained less microbes than wash waters. Water quality should be monitored continuously and it should be changed often enough. In Finland, carrots are stored 6–8 months during the winter. This situation makes it possible for yersinia to grow, if the storage environment is favorable. Counts of pathogenic yersinia were very low. When carrot process water samples were analysed by RT-PCR, pathogenic *Y. enterocolitica* was detected almost in all samples, but when these positive samples were cultivated, no pathogenic yersinia was detected.
3. A suspension test was used for testing of the decontaminating effect of NEW, ClO₂, FPW and UV-C to *Y. enterocolitica*, *Y. pseudotuberculosis*, *E. coli* and *C. lambica* with and without interfering substance. The decontamination of carrot processing water was tested with UV-C with and without filtering. The inactivation times of different decontamination methods for different microbes differed. In most cases, the organic matter in water impaired the effect of the treatments, the reaction times were longer and concentrations needed to be stronger to cause inactivation. The turbidity of the water can be decreased by filtration.
4. Two biological systems and one chemical waste water treatment system were evaluated and in addition one waste water sedimentation systems. A waste water system should be proportioned correctly and good care should be taken regarding the functioning of the system to ensure that good treatment results can be attained. The biological waste water treatment methods examined were suitable for waste waters from vegetable processing, because of the high organic loads of the waste water. The sequencing batch reactor (SBR) and trickling filter were both found to be suitable for waste waters

from vegetable processing, and with both systems the requirements mandated in legislation were attained. Sedimentation and chemical precipitation were suitable as waste water pre- or post-treatment method, but their ability to remove organic loading was insufficient. Operation of waste water treatment in a cold climate condition is challenging and must be taken into account, because land applications (for example) are not applicable all year round in a cold climate. Small-scale waste water treatment should be cost-efficient.

Water is an important part of the fresh-cut vegetable industry. High-quality water is greatly needed to ensure the safety of products, but water should be also conserved and its use should be controlled to improve sustainability. Water use should be measured continuously; in addition, processing machines and techniques should be water-saving. Suitable water decontamination can be used to prevent microbial cross-contamination from water to vegetable products. Waste water from fresh-cut vegetable production has a high organic load and also contains nutrients. Waste water should be treated on-site or piped to a communal waste water treatment plant.

This study gave valuable information about waters created and the water use in different stages of the fresh-cut vegetable processing, the quality of process and waste waters and processed vegetables, as well as about the efficacy of some decontamination methods on water and on-site treatment of waste waters. The results help companies to improve their processes and self-monitoring activities. The study is also an important contribution for the scientific community of the branch.

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