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RISK-BASED EVALUATION OF TREATMENTS FOR WATER USED AT A PRE-HARVEST STAGE TO MITIGATE MICROBIAL CONTAMINATION OF FRESH

RASPBERRY IN CHILE

By

Constanza Paz Avello Lefno

A THESIS

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Under the Supervision of Professor Bing Wang

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RISK-BASED EVALUATION OF TREATMENTS FOR WATER USED AT A PRE-HARVEST STAGE TO MITIGATE MICROBIAL CONTAMINATION OF FRESH RASPBERRY IN CHILE

Constanza Paz Avello Lefno, M.S. University of Nebraska, 2021

Advisor: Bing Wang

The global agricultural sectors are facing challenges of providing food for a rapidly growing population while still meeting appropriate food quality and safety standards. The great climatic and soil diversity of Chile, a South American country, has positioned the country among the top ten agricultural exporters in the world.

Considering aspects such as historical outbreaks, contamination potential, exposure, and frequency and severity of disease, the Food and Agriculture Organization (FAO) and the World and Health Organization (WHO) consider berries a highly prioritized produce in terms of microbiological hazards. Considering the particularities and importance of raspberry production in Chile, the work presented in this thesis primarily focuses on the control of microbial contamination for enhanced quality and safety of raspberry products in Chile destined for export.

Water is one of the most significant sources of microbial contamination influencing the quality and safety of fresh produce. Based on a previous collaboration work between Chilean authorities and the University of Nebraska-Lincoln, the water used for the dilution of pesticides was identified as the most significant source for Chilean raspberries contamination with *E. coli*.

The long-term goals of this thesis project are to provide evidence- and risk-based scientific information to the Chilean food authorities to further enhance the quality of raspberry products, and to develop a framework of applying risk-based approaches for food policy development to revamp the national food safety management system in Chile.

Two studies were conducted to achieve the goals. In the *first study*, a systematic review was conducted to characterize potential water treatments suitable for the implementation on raspberry farms in Chile based on both their efficacy of reducing *E. coli* contamination in water and in-field feasibility. The *second study* employed a quantitative simulation model to evaluate the impact of water quality on *E. coli* contamination on fresh raspberries at the arrival of importers' border.

Compiling findings of the two studies, suggestions on water treatment suitable for raspberry farms were provided. Independent, science-based assessment was conducted and highlighted the most relevant aspects that will help the Chilean food safety authorities with tools to suggest solutions to raspberry producers. To my Grandparents Aquiles and Nora, the most beautiful human beings who have ever walked the Earth.

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Since I was little, my beloved Grandpa instilled in me great values, one of many, that of keeping me a curious person and never stop learning. Dear Grandpa, I am eternally grateful for the imprint you left on me, I will see you somewhere, and I will hug you forever.

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I hope I was able to contribute through this research to the work carried out by the Chilean Agency for Food Safety and Quality (ACHIPIA). You trusted me to collaborate on this project and I can't wait for the results to come to fruition.

Organization of the Chapters

This thesis is composed of four chapters that are interrelated and follow a logical order according to the two studies performed.

The first chapter aims to give an overall context on the Chilean Food Safety System and the production of raspberries, describing the most relevant concepts that aid the understanding of the following three chapters. The second chapter (*first study*) focuses on the identification of water treatments based on their efficacy against *E. coli* in freshwater sources and their feasibility analysis for a pre-harvest implementation. The third chapter (*second study*) evaluates through a quantitative model, the acceptance rate of fresh raspberries at the port of entry of importing countries, considering the efficacy against *E. coli* in the water sources used by small-raspberry farmers in Chile for the application of pesticides. Lastly, chapter four provides the overall conclusions from this research.

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CHAPTER 1. INTRODUCTION

I. Background

The water used for growing and processing fresh fruits and vegetables could contain a variety of pathogens and thus enter the food chain. At a pre-harvest stage, the irrigation water or any foliar contact water (such as water used for dilution of pesticides) in direct contact with the edible portions of the growing or mature produce has long been identified as one of the most probable sources of pathogens contamination of concern to human health (Malakar, Snow, & Ray, 2019; Suslow, 2010).

The food industry is of immense importance to the Chilean economy, and local authorities have made efforts to implement food safety risk analysis to strengthen national food safety management systems. In an effort to implement food safety risk assessment methodologies, Chilean food safety authorities and the University of Nebraska-Lincoln conducted a collaborative study, which identified the water used for the dilution of pesticides as the most likely point of entry of *Escherichia coli* contamination in the raspberry supply chain in Chile (Ortúzar et al., 2020).

According to the Chilean Ministry of Agriculture, the vast majority of raspberry producers in Chile have access to surface water, followed by groundwater, and to lesser extent access to drinking water quality to use in the growth of orchards (INIA, 2016). This means that the risk of pathogens and indicator bacteria entering the raspberry production chain through the use of these lower quality sources of water is relevant. Preventing pre-harvest contamination of fresh produce, especially when is consumed uncooked, is a priority for the Chilean government, academia, and industry stakeholders due to the major public health and economic burden of related outbreaks. Moreover, Chile's economy is driven by exports, concentrated primarily in its agricultural sectors (USDA, 2019), and raspberry producers must comply with the microbial parameters of the destination market. Generic *E. coli* is a fecal contamination indicator and is evaluated both by the main importers of Chilean raspberries as well as at the local level (Agency, 2019; Australia, 2020; Chile, 2015). Since the entry point for *E. coli* contamination has already been identified, it becomes necessary to evaluate appropriate treatment of water used at the pre-harvest stage that will lead to a reduction in the contamination of raspberry products, protecting human health, and helping strengthen the export sales in Chile.

The overall goal of Chapter I is to describe the main characteristics of the Chilean raspberry production, the relationship, and functions performed by the Chilean food safety authorities in the food supply chain, besides presenting a context of the relevance of the water used at the pre-harvest stage, and finally, corroborate the use of the systematic review and quantitative microbial risk assessment as valuable tools for decision making on food safety and quality control.

II. Chilean Institution in Charge of Food Safety and Quality The institution in charge of the safety and quality of food in Chile responds to a management model made up of multiple agencies, with different scopes of action and responsibilities related to food safety (*Figure 1*). The Chilean ministries as part of the institution are the Ministry of Agriculture (MINAGRI), Ministry of Health (MINSAL), Ministry of Economy, Development, and Tourism (MINECON), and the Ministry of Foreign Affairs (MINREL) (ACHIPIA, 2018).

Each of the ministries has public services associated to fulfill determined functions. In a very simplified description of these entities, MINSAL through the Ministerial Regional Secretaries (SEREMIs) applies routine control and surveillance procedures focused particularly on domestic consumption and production, also applying controls to imports. On the other hand, MINAGRI through the Service of Agriculture and Livestock (SAG) ensures the suitability for human consumption of primary agricultural products destined for export. The National Fisheries and Aquaculture Service (SERNAPESCA) functions similarly as SAG but primarily focusing on the compliance of target market requirements in fish and fishery products. Lastly, the Directorate of International Economic Relations (DIRECON) collaborates in the development of the country's exports, intervenes in negotiations, and promotes international treaties and agreements of economic nature. Besides, what is related to the regulations on the Technical Barriers to Trade (TBT) and Sanitary and Phytosanitary (SPS) measures is evaluated (ACHIPIA, 2018).

In 2005, the Chilean Food Safety and Quality Agency (ACHIPIA) was established to serve as an interrelating body between the entities with responsibilities associated with food safety, the Ministries, and their Services, and to strengthen the Chilean institutional framework (ACHIPIA, 2018). The Agency coordinates and conducts the Chilean National Food Quality and Safety System (SNICA), integrated by a set of policies, programs, norms, and actions carried out by various public institutions with competence in matters of food safety and quality and the private actors that participate in the food chain (ACHIPIA, 2016).

Risk Analysis is a key discipline for strengthening food safety systems (WHO/FAO, 2006). According to FAO/WHO, Risk Analysis is a structured and systematic process by which the possible harmful effects on health as a consequence of a hazard present in a food, or property of it, are examined and options to mitigate that risk are established (WHO/FAO, 2006). This science-based process has gained vast acceptance as the preferred way to assess hazards along the food chain and risks to human health and includes three major components that have also been implemented in Chile: risk management, risk assessment, and risk communication (ACHIPIA, 2016; WHO/FAO, 2006). The Chilean entities who play a key role within the Risk Analysis framework are shown in Figure 2.

ACHIPIA is the agency directly responsible for the risk assessment stage, which corresponds to the scientific analysis of known or potential adverse effects on human health resulting from exposure to foodborne hazards (ACHIPIA, 2016). Food safety officials working for national governments generally play the role of risk managers. Risk managers (SAG, SERNAPESCA, MINSAL) are responsible for choosing and implementing appropriate food safety control measures that protect the health of consumers and promote fair trade practices, considering the results from the risk assessment (WHO/FAO, 2006). The risk communication stage, within the risk analysis process, should be a cross-cutting process that will involve different SNICA actors, whose roles and functions will be established according to their levels of competence and contributions (ACHIPIA, 2016).

III. Raspberry Production in Chile

The food industry represents 25 percent of the Chilean economy and is forecast to grow to more than 35 percent by 2030. This country is among the top ten agricultural worldwide exporters and fresh fruit is one of the main exports (USDA, 2019). Berry fruits are popular for a variety of reasons, including flavor, nutrition, convenience, and their high levels of antioxidants and anti-cancer compounds (Matthews, 2014; Yang & Kortesniemi, 2015). For fresh and processed raspberries, the global demand has increased considerably during the last ten years due to their nutritional properties and health benefits (SAG, 2014). In the United States, for instance, the consumption of fresh raspberry has increased fourfold over the past six years (Matthews, 2014).

Chile is a major producer and exporting country of raspberries. The cultivation area of raspberries in Chile reaches 12,000 hectares, concentrated in the Central-South region of the country (Region of El Maule and Bio Bio) (Figure *3*) being the *heritage* variety cultivated in 80% of the national surface (SAG, 2014). According to the International Raspberry Organization, Chile is part of the 14 countries involved in 93% of the world's raspberry production (IRO, 2020) and the second world exporter of frozen raspberries, the main export form for this fruit, shipping 27,165 tons for a value of 75 million dollars in 2017 (ODEPA, 2018) The main importers of Chilean raspberries are the United States (26%), Canada (16%), and Australia (14%) (ODEPA, 2018).

The national production of raspberry in Chile is characterized by the small volumes of individual production, exploitation conditions with low technological and mechanization levels, and commercialization carried away mostly by intermediaries (SAG). The production is usually managed by small and medium producers with orchards of an average area of 0.5-0.75 hectares (ODEPA, 2018; Servicio Agrícola y Ganadero).

The Chilean raspberry supply chain can be described into three main stages: farm, collection center, and packing plant (Figure 4). At the farm, raspberries are cultivated, irrigated, treated with pesticides and fertilizer, and harvested (January-March). Harvested raspberries are then transported to the collection center, where the fruit originating from different farms is gathered, temporarily stored, and sold to the packing plant. At the packing facilities, raspberries are exposed to refrigeration temperatures, graded for quality and either exported fresh or frozen (better quality), processed into juice or other fruit products (lower quality), or discarded when not acceptable for consumption (Ortúzar et al., 2020).

IV. Risk Assessment of Chilean Raspberries: A Collaboration Project

The Chilean National Food Quality and Safety System (SNICA), is shifting from a reactive to a proactive/preventive outlook by implementing a comprehensive approach in a farm-to-fork continuum and involve all stakeholders along the food supply chain to mitigate food risks (Ortúzar et al., 2020). One of the main focuses is to implement risk assessment as an essential component of the food safety risk analysis framework (Ortúzar et al., 2020).

SAG is the official Chilean State body responsible for supporting the development of Chile's agriculture, forestry, and livestock industries by protecting and enhancing plant and animal health (SAG, 2021). When exporting animal or vegetable products, the SAG participates in its sanitary certification, which is internationally recognized for following norms and standards that regulate international trade (SAG, 2021).

The production of raspberry in Chile is centered in exportation, however, the limited technical proficiency and human resources have prevented SAG from properly evaluate and further improve the raspberry farms for exports (Ortúzar et al., 2020).

To integrate the proactive/preventive approach to mitigate food risks and to ensure that resources to provide sanitary certification are committed to crucial steps along the supply chain optimally, ACHIPIA, together with SAG and the University of Nebraska-Lincoln, engaged in a collaborative project to assess the risk on the production of raspberries destined to export.

The factors evaluated as possible contribution points to overall microbial contamination on raspberries are shown in Table *1*.

Results of the study indicated one of the top risk factors that can significantly influence microbial contamination, particularly *E. coli* level in end raspberry products, is the type of water used at a pre-harvest stage for pesticide application (Ortúzar et al., 2020).

In Chile, as a way to guarantee the fitness for human consumption of raspberry exports, the Resolution No. 3410 was enacted in November 2002 by the SAG. This resolution establishes the inscription in the list of participants of the chain of export raspberries: orchards, marketers, processing plants, collection centers, and exporters. Also, the resolution determines minimum Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) requirements for each of the participants and implements the Raspberry Official Control Program (ROCP) by auditing participants on their compliance with the established requirements (SAG, 2011). These requirements are based on the most common problems for small-scale farms, such as water quality, hygiene measures for workers, and farm animal control.

V. Water Used at Pre-Harvest Stage and Impact on Food Safety and Quality Microbial contamination in fresh produce may occur at numerous venues across the farmto-fork path (Uyttendaele et al., 2015). Pre-harvest sources of produce contamination include the soil, the interaction of workers with the produce along the supply chain, and the water used for irrigation, and the application of pesticides and fungicides (Balali, Yar, Afua Dela, & Adjei-Kusi, 2020; Uyttendaele et al., 2015). During harvesting, contamination can occur through contact with equipment, transport containers, knives and tools, and human hands and gloves, while post-harvest contamination can take place during transport, storage, and processing (Carstens, Salazar, & Darkoh, 2019).

A. Irrigation water

Water is an essential component in the production of fruits and vegetables as it is used in significant amounts in pre- and post-harvest operations. Irrigation water, the water applied through an irrigation system during the growing season, field preparation, preirrigation, weed control, harvesting, and leaching salts from the root zone (Dieter et al., 2018), is a recognized reservoir for foodborne pathogens, and its quality is an indicator of produce safety and quality (Carstens et al., 2019). Such pathogens include both human-specific as *Shigella* spp., norovirus, hepatitis A virus, *Cyclospora cayetanensis*, and zoonotic pathogens such as verocytotoxin-producing *E. coli*, *Salmonella* spp., *Yersinia enterocolitica*, and *Cryptosporidium* (Uyttendaele et al., 2015).

Access to safe and high-quality water for agricultural use is of high priority and is becoming continuously more difficult for many countries, resulting in the production of contaminated fresh produce with pathogenic microorganisms, causing an increased risk of human disease (Newman, 2004; Uyttendaele et al., 2015).

The contribution of irrigation water to the contamination of fruits and vegetables leading to subsequent outbreaks of foodborne diseases is increasingly evidenced (A. Allende & Monaghan, 2015). In the U.S., a recent investigation from the Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA) have traced E. coli O157:H7, the microorganism responsible for foodborne illness outbreaks, to canal water in the growing region (CDC, 2018) and the agricultural water reservoir on the farms (CDC, 2019). Both outbreaks involved the consumption of romaine lettuce, and one of them caused more than 90 hospitalizations and 5 deaths (CDC, 2018). Similarly, irrigation water was the source of different large outbreaks associated with the consumption of alfalfa sprouts (CDC, 2016), peppers (CDC, 2008), and tomatoes (Greene et al., 2008) contaminated with Salmonella strains. Likewise, it was the most likely source of two other large outbreaks associated with the consumption of fresh salad and iceberg lettuce in Sweden (Edelstein et al., 2014; Söderström et al., 2008). The iceberg lettuce contaminated with E. coli O157:H7 caused a total of 135 cases including 11 cases of the hemolytic uremic syndrome (Söderström et al., 2008).

The probability of produce contamination is higher when irrigation water has direct contact with the crops, therefore, indirect irrigation systems as furrow, drip, subsurface, or flood represent safer options compared with overhead spray or surface irrigation (Gil, Tudela, Luna, & Allende, 2013; Steele & Odumeru, 2004). Indirect irrigation precludes the direct contact of water with the produce, however, the water used for the delivery of pesticides or fungicides in the form of spray must make direct contact with the growing or mature portion of the crops to be effective. Thus, the quality of the water used in agrochemical applications has a direct impact on the final quality and safety of the food product.

B. Water used for agrochemical application

The water used for pesticides or agrochemicals dilution, also known as foliar contact water (Suslow, 2010), is rarely monitored and can pose a risk to human illness in the same way as irrigation water (Pachepsky, Shelton, McLain, Patel, & Mandrell, 2011; Stine, Song, Choi, & Gerba, 2011). This risk is even higher given that fungicides and insecticides are often sprayed to the edible parts of the crops just before harvest (Herwaldt, Ackers, & Group, 1997). In berries, for instance, fungicides are generally applied just before harvest to enhance quality and prolong shelf life due to their high susceptibility to fungal spoilage (Goulart, Hammer, Evensen, Janisiewicz, & Takeda, 1992). Besides, studies have suggested that pesticides may support the growth of pathogens as *Salmonella* when introduced with a source of water and may increase the risk of foliar contact application further of the water source alone (Lopez-Velasco, Tomas-Callejas, Diribsa, Wei, & Suslow, 2013), although the inactivation of *E. coli* can also happen (Pham, Min, & Gu, 2004).

The spraying of pesticides or fungicides prepared using contaminated water has been determined as the likely source of some foodborne outbreaks. One of the largest outbreaks reported in the U.S. and Canada caused more than 1,400 people infected with *Cyclospora cayetanensis* linked to the consumption of Guatemalan raspberries (Herwaldt et al., 1997). The same coccidian parasite caused 34 cases associated with the consumption of contaminated fresh green leafy herbs imported from Southern Europe in

Germany (Döller et al., 2002) and 17 cases through the consumption of basil imported from the U.S in Canada (Hoang et al., 2005).

C. The current situation in Chilean raspberry farms

Local experts in raspberry production have pointed that irrigation water appears to be an insignificant source of microbial contamination, as fruit exposed to high-humidity conditions created by irrigation would easily spoil due to the fungal species *Botryotinia fuckeliana* and will not be harvested (Ortúzar et al., 2020). However, the type of water used for the pesticide application was indicated as one of the top risk factors that can significantly influence microbial contamination level in end raspberry products in Chilean farms (Ortúzar et al., 2020).

The water sources used for primary production are highly variable and often characterized by distinct microbial quality. Various water sources have been used for agriculture operations worldwide, including rivers, lakes, rainwater, desalinated seawater, aquifers, and groundwater (Uyttendaele et al., 2015). Treated wastewater is also increasingly used (Carstens et al., 2019). Surface water drawn from lakes, streams, or rivers is generally considered to be of questionable hygienic quality. Although groundwater from wells is normally of better microbial quality, it can still get contaminated with fecal pathogens particularly in areas close to extensive livestock production and manure application sites (Leifert, Ball, Volakakis, & Cooper, 2008).

As irrigation water source, the majority of the Chilean raspberry producers have access to surface water (67%), the rest of them (22%) to groundwater water and 7% of producers have access to both types of sources (INIA, 2016), which means most of the farmers have access to a lower water quality source. Microbiological quality parameters of irrigation

water in Chile have been scarcely studied as the monitoring is in the hands of the private sector, but the presence of fecal coliforms in agricultural water (including water used for pesticide application) in Chilean raspberry farms has been demonstrated (Palacios, 2019). Regarding water quality, the microbial requirement for the dilution/application of phytosanitary products and fertilizers in raspberries is based on the levels of generic *E. coli*.

VI. Control of Generic E. coli in Raspberries

A. Generic E. coli

E. coli is a gram-negative, facultative anaerobic rod-shaped bacteria within the fecal coliform group type, and is an indicator of fecal contamination (Rock & Rivera, 2014; Zealand, 2018). Its presence in food indicates recent contamination, either directly or indirectly by feces or fecal contaminated materials (Zealand, 2018), providing evidence of poor hygiene or insufficient processing or post-process of foods.

There is no consensus on the best fecal indicator (Rusiñol et al., 2020), but generic *E. coli* is still considered appropriate as it is found in the intestines of warm-blooded animals and is not naturally present in the environment; have similar survival rates as pathogens outside the host; is less likely or slower to proliferate in the environment (Rochelle-Newall, Nguyen, Le, Sengtaheuanghoung, & Ribolzi, 2015); and the detection methods are inexpensive (Rusiñol et al., 2020).

Microbiological water quality standards are also based on indicator organisms that, although not pathogenic, are expected to correlate with the presence of other pathogens allowing the probability that potential pathogens are present in water to be predicted (Pachepsky et al., 2011; Rock & Rivera, 2014). *E. coli* was reported as a suitable index organism for *Salmonella enterica* and shiga toxin-producing *E. coli* (Ceuppens et al., 2015), but it is not a particularly good indicator of enteric viruses and protozoa (WHO, 2017). *E. coli* concentrations in water is a strong indication of recent sewage or animal waste contamination which is a known reservoir of pathogenic microorganisms, therefore is used as a hygiene indicator when assessing water quality for agricultural practices (Banach & van der Fels-Klerx, 2020; Rock & Rivera, 2014; Rodrigues, da Silva, & Dunn, 2020).

B. E. coli in Chilean raspberry and agricultural water

The Chilean Food Sanitary Regulation (RSA), regulated by the MINSAL establishes the sanitary conditions to which the production, import, elaboration, packaging, distribution, and sale of food for human use must adhere, to protect the health and nutrition of the population and guarantee the supply of healthy and safe food (Chile, 2015). Based on a three-class sampling plan (number of sample units analyzed: n=5, and the maximum allowable number of sample units yielding marginal results: c=2); the RSA establishes maximum detected levels of 2-3 log CFU/g in fresh fruits and 1-2 log CFU/g in frozen fruits of generic *E. coli* (Chile, 2015).

Relevant importers of Chilean berries (OEC, 2020) such as Canada and Australia, use generic *E. coli* as one of the microbiological criteria for the satisfactory assessment of imported berry products in their markets (Agency, 2019; Australia, 2020). In the U.S., a principal importer of Chilean raspberries, per the Food Safety Modernization Act (FSMA) and particularly on the Final Rule of Produce Safety (FDA, 2015), criteria were established for microbial quality of agricultural water directly applied to growing produce based on the level of generic *E. coli* (A Allende et al., 2018; ODEPA, 2018). To ensure an acceptable quality, the ROCP regulates that raspberry growers wishing to export under the registration of the ROCP with accreditation by SAG must guarantee the water used for the application of phytosanitary products and fertilizers to comply mandatorily with a critical limit of non-detection of *E. coli* in 100 mL (with a detection limit of 2 MPN), or a proof that water is of drinking quality (SAG, 2011).

C. Water treatments for agricultural practices in Chile

The MINAGRI through the National Irrigation Commission (CNR) elaborated a manual in 2007 where it suggests technologies to mitigate contamination in irrigation waters. Much of the technologies presented were designed and are used to make water drinkable or treat liquid industrial waste, therefore their use for agricultural purposes poses new challenges in their adaptation to more variable pollution conditions (CNR, 2007). The technologies with the greatest commercial diffusion for the control of pollutants regulated by the Chilean Norm for irrigation water (NCh. 1333 of.78) are ultraviolet light (UV light), filtration packed (bag and cartridge), microfiltration (membrane technology), ozone, and oxidants electro generation (CNR, 2007).

These technologies were selected to meet the requirements based on the Chilean standard for irrigation water (NCh1333), which is not based on generic *E. coli*, but on fecal coliforms (1,000 MPN fecal coliforms/100mL). Furthermore, while the same technologies might represent satisfactory efficacy against *E. coli*, Chilean raspberry farms under the ROCP needs to comply with the destination market *E. coli* benchmarks. Hence, there's a growing need for the adoption of science and risk-based preventive measures to reduce the contamination of produce (FDA, 2019). Currently, the treatment

recommendation for raspberry farms includes the addition of chlorine to the water, but no further treatments are proposed. Additionally, whereas water-disinfection technologies as ozone, UV (Banach & van der Fels-Klerx, 2020), and other chemical treatments are available in the market, there is a significant deficiency in their evaluation to be implemented on-farm.

VII. Systematic Review as A Tool to Enhance Risk-based Decision Making

A systematic review (SR) is an analysis of existing evidence related to a defined research question that employs pre-specified, structured methods to classify and critically appraise relevant research, as well as collect, document, and evaluate data from the studies included in the review (Cumpston et al., 2019; EFSA, 2010). Different from conventional narrative reviews, SRs adopt an explicit procedure that seeks the reduction of bias and increase transparency, resulting in more accurate outcomes from which decisions can be drawn (Cumpston et al., 2019).

SR methods have been widely applied in clinical research with a human health-care focus, and are now used in different investigation fields such as education, environmental management, international development, plant and animal health, including food production, food safety, and security (Aiassa et al., 2015; Wood, O'Connor, Sargeant, & Glanville, 2018).

Relevant international food safety entities such as the European Food Safety Authority (EFSA) and the Food and Agriculture Organization of the United Nations (FAO), have commissioned and funded recent SRs to substantiate their work (Wood et al., 2018), demonstrating the evidence-based approach of the methodology to support policy

decision making, particularly of interest in the context of risk assessment. One of the principal aims of risk assessment is to synthesize the most comprehensive, relevant and qualified set of information to risk managers, so sound science-based decisions can be made concerning a potentially hazardous situation (Aiassa et al., 2015; WHO/FAO, 2006). SR methods ensure that the risk assessment process is based on relevant and robust data, and the output of SR could be used with increased reliability as input into risk assessment models (EFSA, 2010).

VIII. Summary

Member countries and key partners from the Organization for Economic Co-operation and Development (OECD) represent about 80% of world trade and investment (OECD, 2021). Chile is a developing country and a member of the OECD but faces similar disadvantages to assure the safety of the produce as the other Latin American countries.

The global agricultural sector is faced with the challenge of increasing productivity to meet the growing demand for food, while at the same time both demands of the national market and the international trade agreements that Chile has signed comply. According to the Chilean Ministry of Agriculture, particularly the berry sector represents great relevance related to export items. National berry exports totaled 800 million dollars in 2017, positioning Chile as the fifth supplier of this item worldwide (ODEPA, 2018).

Implementing a proactive approach in the management of microbiological hazards is a priority for Chilean food safety authorities, which are seeking to reaffirm the capacities of the country in terms of the production of high-quality food commodities either for export or local consumption.

The sources of water to which raspberry farmers have access to use for the dilution of pesticides is of great microbiological variability and was determined as the most likely source of contamination by *E. coli*, an indicator of fecal contamination evaluated by the countries of commercial interest for Chile (Ortúzar et al., 2020).

Systematic Review methods in the food safety arena are increasingly influencing policy advocacy both nationally and internationally. Applying SRs methods to find mitigation options for *E. coli* in water enables a fully comprehensive search, analyzing the included studies objectively and impartially, using the best scientific knowledge available to support the decision-process made at the level of the risk managers in Chile, particularly the Agricultural and Livestock Service, SAG.

Long-term goal and specific objectives

The long-term goal of this project is to provide an evidence-based and risk-based framework integrating systems, proactive approaches to assist in revamping the national food safety management systems and effectively control microbial hazards in food produced in Chile. Such a paradigm will strengthen the capacities of the country in terms of the production of high-quality food commodities both for exportation and local consumption. Specifically, two studies were conducted to achieve specific objectives and elaborated on in separate chapters.

Objective 1 (Chapter 2): Characterize various water treatment technologies in terms of their *E. coli* removal efficacy and feasibility of implementation on the small-size raspberry farms in Chile using a systematic review approach to critically review currently existing evidence in the literature.

Objective 2 (Chapter 3): Determine expected performance criteria for water treatments

using a quantitative microbial risk assessment approach for the selection of appropriate

technologies to lower border refusals of raspberry exports.

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Figure 1.Organization of the Chilean institution in charge of food safety



Figure 2. Risk analysis paradigm in the Chilean food safety system



Figure 3. Distribution of raspberry production in Chile (SAG, 2014).



Figure 4. Chilean raspberry supply chain
Module	Factor
Farm	Contamination introduced from water through pesticide application
	Degradation during the withholding time between the last application of pesticide spray and harvest time
	Bi-directional transfer between the harvesters' hands and the fruit during harvest
	Possible bacterial growth during transport from the farm to the
	collection center under varying temperatures during transport.
Collection Center	Time that raspberries stay in the collection center
	Temperature in the collection center
	Temperature during transport from collection center to the packing plant
	Commute time from collection center to the packing plant
Packing Plant	Storage time at ambient temperature in the receiving area
	Temporary storage in the cold chamber under refrigeration conditions
	Classification and packing of raspberries by processing handlers
	Transport from packing plant to the final export destination under refrigeration conditions for fresh products and freezing
	Storage and transport to the final destination in frozen chambers for frozen products

Table 1. Most influential factors of *E. coli* contamination in raspberry

(Ortúzar et al., 2020).

CHAPTER 2: PRIORITIZATION OF WATER TREATMENTS TO MITIGATE E. COLI IN WATER FOR SMALL RASPBERRY FARMERS IN CHILE: A SYSTEMATIC REVIEW APPROACH

I. Abstract

Water has long been identified as one of the most significant sources of microbial contamination in produce influencing human health. Previous results of a quantitative microbial risk assessment model indicated that the water used for the pesticide application is the main entry point of generic E. coli in raspberries produced in small farms in Chile. The purpose of this chapter is to identify water treatments that can effectively mitigate E. coli in water and are feasible to be implemented at small-scale raspberry farms. To compare the efficacy of various treatments in controlling E. coli in water, a rapid systematic review (RR) of studies in English and Spanish was conducted by searching electronic databases including Web of Science Core Collection (1900-2019), Scopus (1959-2019), Medline (1950-2019) and CABI (1910-2019). The search focused on established water treatment technologies applied in freshwater sources (groundwater and surface water) excluding those interventions at the proof-of-concept stage. A review of reviews was conducted to collect evidence for the feasibility analysis covering technological, managerial, and sustainability criteria, considering Chile-specific situations. A total of 42 publications were considered for data extraction (RR) which included chemical disinfectants, ozone, UV light, and filtration. The efficacy rates reported were variable, but UV light, a combined technology in tandem (ozone and chlorine) achieved the highest log removal (> 7 log), while riverbank and bio sand filtration did not exceed 4 log reduction. The review of reviews identified the treatments most applied at a pre-harvest stage: chemical disinfectants (chlorine-based compounds,

peroxyacetic acid, hydrogen peroxide), ozone, UV light, and membrane filtration. Albeit significant data gap in the current literature on disinfection methods applied in agricultural water at a pre-harvest stage was identified, our study critically reviewed and analyzed data currently available in the literature, results of which can assist the Chilean food safety authorities with a science-based decision on water treatment method adoption and implementation.

II. Introduction

Based on statistics reported by the Chilean Ministry of Agriculture in 2018, a total of 5,130 orchards registered raspberries as their primary products, and approximately 57% of them are accounted in the Peasant Family Farming (PFF) program focusing on small farms of less than 0.5 hectares (ODEPA, 2018). To be eligible for export, small raspberry producers need to enroll in the Chilean Raspberries Official Control Program (ROCP), a small-farm-oriented program enforced by the Chilean Ministry of Agriculture. Under the program, compliance with Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) is required to address issues including hygiene measures for harvesters, animal controls on the farm, traceability guarantees, and water quality analysis (SAG, 2002).

Water used for pesticide application, resulting in intimate contact to edible portions of the fruit, has been long recognized as one plausible source of microbial contamination on fresh produce, which may negatively affect the end products' safety and quality (TV Suslow, 2010; Verhaelen, Bouwknegt, Rutjes, & de Roda Husman, 2013).

Ideally, fresh produce production ought to use potable water, but the use of surface water sources does happen (TV Suslow, 2010). In Chile, water used by small raspberry farmers

covers a wide range of sources with various microbial qualities. Take irrigation water for example, the majority (67%) of the Chilean raspberry producers have access to surface water, while 22% of them have access to groundwater and 7% have access to both sources (INIA, 2016). Based on a recent survey conducted by the research team, the majority of raspberry farms use groundwater for pesticide application (71%), followed by surface water (15%) and potable water (14%) (Ortúzar et al., 2020).

E. coli is ubiquitous in freshwater bodies for agricultural purposes, and high prevalence has been particularly observed and documented for fresh water (GWPP, 2017). Generic E. coli is an indicator for the good hygienic practices along the raspberry supply chain and is of primary interest for importing countries (C. F. I. Agency, 2019; A Allende et al., 2018; Australia, 2020; James, 2006). Relevant importers of Chilean berries (OEC, 2020) such as Canada and Australia, use generic E. coli as one of the microbiological criteria for satisfactory assessment of domestic and imported berry products in their markets and monitor the compliance of importing produce with their food standards (C. F. I. Agency, 2019; Australia, 2020). As one of the primary importers of Chilean raspberries, the U.S. established criteria for microbial quality of agricultural water directly applied to growing produce based on the level of generic E. coli (A Allende et al., 2018; ODEPA, 2018). In particular, a previous study using a risk-based approach to determining critical control points of microbial contamination in both fresh and frozen raspberries concluded that water used for pesticide application is a highly influential source for generic E. coli in the end products, indicating the significance of controlling generic E. coli in agricultural water to enhance the microbial quality of fresh produce for successful international trade (Ortúzar et al., 2020).

Limited technical skills and capabilities have prevented SAG from effectively take further actions for enhancing the microbial quality of agricultural water and more specifically, controlling generic E. coli contamination in water on the raspberry farm (Ortúzar et al., 2020). Since the majority of raspberry producers in Chile have access to less-qualified water sources, appropriate treatments suitable for the small-sized farms are critically important. There has been numerous studies to investigate a large number of different methods of water treatment, including filtration, oxidation-reduction, chlorination, ozonation, UV light, electronic beam processing, heat treatment, hydrodynamic cavitation, electrolyzed oxidizing (EO) water, and electrochemical disinfection (Dandie et al., 2019; Newman, 2004a). However, there is not a critical analysis of the possible water treatments to date to comprehensively compare their efficiency in generic *E. coli* reduction or to coherently take into consideration of technological, managerial, and sustainability-related factors such as maintenance, costs, safety, and biological effects on end products (Dandie et al., 2019; Pachepsky, Shelton, McLain, Patel, & Mandrell, 2011) to support the decision making of water treatment adoption.

Therefore, the objective of this study is to apply the systematic review approach to (1) evaluate the efficacy of water treatments in reducing *E. coli* contamination in water, and (2) to assess the feasibility of the use of water treatments in small-sized raspberry farms in Chile by integrating other factors with technological, managerial, and sustainability consideration. Findings from the study will provide the food safety authorities and raspberry farmers with scientific evidence to support their decision-making on the prioritization of water quality management measures. With the identification of effective

and feasible water treatments that can effectively mitigate *E. coli* and be carried out by the Peasant Family Farmers, the negative impact of water quality on raspberry products can be minimized to support farmers to positively comply with international standards to facilitate exports.

III. Materials and Methods

Rapid systematic review for comparing the efficacy of water treatments in controlling E. coli in water To quantify the efficacy of various treatments in controlling E. coli contamination in water used for agriculture, a rapid systematic review (RR) was conducted to provide a comprehensive assessment of relevant evidence due to limited timescale and human resources. In this review, recognized techniques in conventional systematic review were used for retrieving, screening, appraising, and synthesizing evidence (Tricco, Langlois, Straus, & Organization, 2017). Major deviations in the rapid approach are: the search was targeted in the most relevant bibliographic databases, and only one reviewer conducted the relevance screening and data extraction. Additional efforts were made to strengthen the screening process, including verification of a sample of articles by a second reviewer; and convening an expert panel to address questions from the primary reviewer to minimize the risk of inappropriate exclusion of relevant studies due to the single screening process. The expert panel included an environmental engineer focusing on generic water treatment and microbial contamination in the environment, a water for food processing specialist expertized in water treatment technologies in the agri-food area, a produce safety specialist with extensive experience and knowledge in fresh produce safety regulations and commonly used water treatment practices, and a food safety risk assessor emphasizing on microbial hazards.

A. Research question and eligibility criteria

The review was designed to address the question "What is the efficacy of possible treatments to control generic *E. coli* contamination in fresh water intended for agriculture?" Eligibility criteria were developed following the PICO (Population, Intervention, Comparison, and Outcomes) framework covering the following components pertinent to the review question:

Population (P): freshwater, including both groundwater and surface water, as these are the primary water sources accessible by raspberry farmers in Chile.

Intervention (I): all possible water treatment documented in the literature, including traditional, well-developed water treatment technologies such as coagulation, flocculation, slow bed sand filtration, membrane filtration, ultraviolet (UV) irradiation, ozone (O₃), peroxyacetic acid, chlorine dioxide, and emerging water treatment technologies under development such as hydrodynamic cavitation, electrolyzed oxidation water, electrochemical treatment, and advanced oxidation processes (AOP).

Comparison (C): untreated samples in control groups, or samples collected before treatment being implemented.

Outcomes (O): changes in the contamination level of generic *E. coli* in water, usually reported as logarithmic reduction or percentage of elimination.

The study design was not used as one component to control eligibility, as most articles published in this field are controlled studies or quasi-experimental studies with inoculated contamination or naturally occurring contaminants. Ideally, controlled studies with naturally occurring contamination are preferred, but studies with all types of design as aforementioned were initially considered to maximize the capture of relevant data.

B. Search strategy and data source

The search strategy integrated terms related to three main concepts: 4 population terms (i.e., water, freshwater, surface water, and groundwater), 4 intervention terms (i.e., treatment, disinfection, sterilization, and purification), and 3 outcome terms (i.e., *Escherichia coli, E. coli,* and coliforms). Key terms for each concept were combined using the Boolean operator "OR", and the concepts were combined using Boolean operator "AND". The search syntax was verified by ensuring a full capture of a list of 30 relevant articles that were obtained before the systematic search based on a hand search and recommendations from the expert committee.

The last search was conducted in October 2019, in four electronic bibliographic databases, including Web of Science Core Collection (via Web of Science, 1900 to date of search), Scopus (via the University of Nebraska-Lincoln Scopus interface, 1959 to date of search), MEDLINE (via PubMed[®], 1964 to date of search), and CAB Abstracts and Global Health (via Web of Science, 1910 to present) with no restrictions placed on the search beyond the inception dates of databases. Similarly, no restrictions were placed on language in the initial search, although publications in English and Spanish were selected during the screening process. In addition to the retrievals from these electronic bibliographical databases, the search was supplemented by reviewing the reference lists of relevant review articles to find additional pertinent publications. Search results from multiple databases were uploaded to EndNoteX9 (Clarivate Analytics, Philadelphia, PA).

Duplicated citations identified by Endnote deduplication function and hand search were removed.

C. Relevance screening

Screening of relevant citations was managed using EndNote. Two levels of relevance screening were conducted, i.e., title and abstract-based preliminary screening and full text-based advanced screening. The preliminary screening was conducted to rapidly exclude articles irrelevant to our research question. Prior to the independent screening process at this stage, the reviewer was trained on a pre-test set of 50 randomly selected citations by an expert panel member. Although generic *E. coli* was the microorganism of interest, description of coliform without *E. coli* in the title and/or abstract didn't preclude those articles, as coliform is another commonly applied indicator organism for water microbial quality, indicating a possibility of reporting *E. coli* relevant data in full texts. Articles were excluded if their focuses were sea/marine water treatment or water quality description.

The advanced screening was conducted to further confirm articles' relevance based on full texts. In addition to those in English, articles reported in Spanish were selected, due to the possibility of Chile-specific data or data originating from other Latin American countries with similar agriculture practices to Chile reported in Spanish journals. At this stage, additional exclusion criteria were applied. Articles were excluded for the following reasons: if treatments applied in deionized water, tap water, sterile water, distillate water, aqueous solution, and wastewater were reported, when the water quality is significantly different from the source water used by the Chilean farmers; or if no quantitative measure of changes in generic *E. coli* due to applied water treatments were reported. Relevant articles were categorized based on the types of water treatment technologies.

D. Data extraction and synthesis of results

Data extraction was conducted on all articles that passed the criteria and extracted data were stored in Microsoft Excel (Version 2016) as follows.

General information: author, publication year, location of the study conducted such as continent and country.

Characteristics of water applied for microbial treatment and collection process: type of freshwater (surface water/groundwater), source of water (river, lake, well, borehole), sample size, pH, temperature, turbidity (measured in NTU, Nephelometric Turbidity Units), dissolved organic carbon (DOC), total hardness (CaCO₃), total dissolved solids (TDS), and electrical conductivity.

Detailed characteristics of the water treatment: pretreatment, type of water treatment, application time, time after application.

Microorganism of interest: bacterial specie, pathogenicity (yes/no).

Efficacy measurement: detection/enumeration method, concentration without or before treatment, concentration with or after treatment, contamination change as a measure of efficacy (primarily measured as log reduction or percentage in concentration reduction).

Compliance to regulatory requirements after treatment: irrigation water quality guidelines, drinking water quality guidelines.

The initial intention of this review was to conduct a meta-analysis to quantify and compare the efficacy of various water treatment options. However, due to the lack of necessary statistical descriptors (e.g., mean, standard deviation, 95% confidence interval, or sample size), no quantitative synthesis was performed. Results were narratively presented, aided by summary tables and graphs for visualization.

<u>Review or reviews for characterizing factors affecting feasibilities of water treatment</u> <u>application</u>

Although efficacy is the major factor influencing the adoption of water treatment technologies, other criteria determining the application feasibility play equally important roles. Hence, additional evidence was collected to enable the assessment of the treatments' feasibility for Chilean small-sized raspberry farms via another rapid systematic review referred to as "the review of reviews". In this rapid search, the identification of review papers of water disinfection was focused by searching key terms "water disinfection" and "review" in Scopus and Web of Science Core Collection databases. Relevant reviews were selected by following a similar 2-phase procedure as aforementioned.

Based on a selection tool previously published to decide on a water disinfection technology in pre- and post-harvest practices of produces (Haute, Sampers, Jacxsens, & Uyttendaele, 2015), information relevant to three main criteria was extracted and evaluated from the review papers: (i) technological, (ii) managerial, and (iii) sustainability criteria (Figure 5). Further efforts were made to maximize the capture of relevant information for these criteria by a backward snowballing search (from the reference lists of selected review papers). Evidence critically assessed in this review will supplement the efficacy criteria targeted in the other review to support a multi-criteria decision-making to help the food safety policy makers and producers to scientifically, objectively evaluate the adoption of water treatment technologies for small raspberry production in Chile.

It was suggested by the expert panel that data describing water treatments that have been well established and long applied should be prioritized, as the team was aimed to provide robust recommendations to the Chilean government for a higher chance of successful implementation. The disinfection methods currently used in the field are typically classified into two categories: a) chemical (chlorine, bromine, hydrogen peroxide (H₂O₂), peracetic acid (PAA) or ozone (O_3) and b) physical treatment (filtration, UV, and ultrasound) (Raudales, Parke, Guy, & Fisher, 2014; Sigge et al., 2016). Treatment technologies such as hydrodynamic cavitation, electrolyzed oxidizing (EO) water, electrochemical treatment (Dandie et al., 2019), as well as some advanced oxidation based processes (AOP), have shown great promise for controlling waterborne microbial issues in experimental settings but uncertain for implementation in scale-up scenarios, hence were excluded in the present study for both efficacy and feasibility evaluation. Figure 6 presents the connection between the efficacy- and feasibility-focused reviews as aforementioned and lay out the primary water treatment technologies analyzed in each review which are further elaborated in Results and Discussion.

IV. Results and Discussion

Efficacy of water treatments for controlling E. coli in water

A. Study characteristics

In total, 19,244 articles were identified through the database searching. After deduplication, 11,762 publications were screened by title and abstract, followed by full-text screening, resulting in 42 articles included for data extraction and the following critical analysis. A flowchart of the rapid systemic review focusing on water treatment efficacy is shown in Figure 7.

A considerable number of studies were excluded due to their emphasis on new technologies and new materials that are still at the proof-of-concept stage. For example, nanomaterials are frequently investigated in research studies, showing great potentials. However, it is still in its infant stage and far from being widely applied in water treatment practices. Studies focused on anti-biofouling materials, cavitation treatment, ultrasound, photocatalytic reactions, and solar disinfection, are examples of technologies also excluded from our analysis. Publications assessing combined technologies were excluded when one of the technologies evaluated was out of our scope of the relevance screening.

Among the 42 articles selected for data extraction, studies were classified into the following categories based on the mode of action of water treatments, including chemical disinfectants, ozone, UV light, various filtration technologies (i.e., membrane filtration, slow sand filtration (SSF), biosand filtration (BSF), riverbank filtration, and some others), and multiple treatments implemented in tandem (referred as "combined" treatments). A summary of the distributions of the water treatments covered in this review is summarized in

Table 2. Some studies reported efficacies of multiple treatments across different categories that were tested individually or in tandem, hence these studies were counted into more than one category in the table. A complete description of the study

characteristics of the 42 articles included for the Rapid Review is shown in Table *3*. Below is a brief description of individual treatment technologies included in the review.

Chemical disinfectants

<u>Chlorine:</u> Chlorine is a strong oxidant commonly used in water treatment for oxidation and disinfection. As a primary disinfectant, chlorine is applied to disinfect and control microbial activity in the distribution system (EPA, 2020a).

<u>Calcium hypochlorite:</u> Is the solid presentation of chlorine (Ca(OCl)₂). All forms of chlorine, when applied to water, form hypochlorous acid (HOCl) (EPA, 2020a)

<u>Chlorine dioxide (ClO₂)</u>: Chlorine dioxide (+ IV oxidation state) is a powerful oxidant and disinfectant chlorine compound (EPA, 2020a). The main advantage is that yields lower levels of organic disinfection by-products compared to chlorine (Decol et al., 2019).

<u>Monochloramine:</u> Chloramines are a family of oxidants formed by the reaction of chlorine and ammonia (EPA, 2020a). Monochloramine is a preferred specie, as it is a more powerful oxidant and is less likely to cause taste and odor problems in drinking distribution systems than the other species (EPA, 2020a). Although weaker than chlorine and chlorine dioxide, monochloramine oxidizes precursors of disinfection byproducts (DBPs), inactivates microorganisms, and controls biofilm (EPA, 2020a).

<u>Ferrate Fe(VI)</u>: Because of its high oxidizing strength and non-toxicity of the ferrate decomposition product, ferrate (Fe(VI), the +6 oxidation state of iron) has gained growing popularity as a green, multi-purpose water treatment chemical, acting as an

oxidant, coagulant, disinfectant, or a combination thereof (Cho, Lee, Choi, Chung, & Yoon, 2006).

<u>Sodium dichloro-s-triazine-trione (active ingredient)/ Sodium dichloroisocyanurate</u> (<u>NaDCC</u>): It is the disinfectant base of some coagulant/disinfection product (CDP). It is a chlorinated sanitizer thought to be comparatively advantageous over calcium hypochlorite where water can have high or variable chlorine demands (Legare-Julien, Lemay, Vallee-Godbout, Bouchard, & Dorea, 2018).

Ozone

Ozone (O₃) is one of the strongest disinfectants and oxidants available in drinking water treatment. Is generated on-site by an ozone generator that uses either dried air or liquid oxygen (EPA, 2020a).

UV light

UV light inactivates pathogens by disrupting their DNA, making them non-viable and non-infectious. UV disinfection is a physical process that does not require the addition of any chemicals. This technology is known for its germicidal power in inactivating microorganisms (i.e. bacteria, viruses, algae, etc.) including chlorine-resistant pathogens, such as *Cryptosporidium* (EPA, 2020a).

Filtration

<u>Membrane filtration</u>: Membrane filtration processes commonly used in water treatment include microfiltration (MF) and ultrafiltration (UF). Membrane pore size typically ranges between 0.1 to 0.5 μ m for MF units and from about 0.01 to 0.1 μ m in UF. Both

types of membranes are principally used for particulate and microbiological contaminant removal. (EPA, 2020a).

<u>Slow Sand Filtration:</u> Slow sand filtration can be used to remove particulate and microbial constituents. In the process, water is treated by percolation through a bed of sand (EPA, 2020a).

<u>Biosand filtration</u>: A biosand filter (BSF) is an adaptation of the traditional slow sand filter for intermittent use, and is a popular household water treatment technology (Ngai, Coff, Baker, & Lentz, 2014). The study included in the RR corresponded to an adaptation of a full-scale BSF (Napotnik, Baker, & Jellison, 2017).

<u>Riverbank filtration</u>: Is an effective natural filtration process that can be effective for a variety of pollutants, pathogens, and organic DBPs and is typically described in the literature as surface water that percolates through the banks or bed of a river to an extraction well by induced filtration through pumping (Partinoudi & Collins, 2007).

Miscellaneous (Nano-adsorbents; Carbon nanotubes; Chitosan-bentonite composites; Silver nanoparticles; Activated carbon filters): Representatives from this category were still included in the RR since their classification as treatment was rather ambiguous. Some of them can be categorized under nanotechnology applications, and although is a rapidly developing science (Hassouna, ElBably, Mohammed, & Nasser, 2017), they are not in reality established technologies. The efficacy against *E. coli* reported by these studies did not reach a value greater than three log, which precludes them to be eligible for our later analysis in Chapter 3.

It was initially attempted to review articles focusing on the treatments of water used for agricultural purposes only. However, the intended use was not always explicitly

introduced in the primary studies. Among the 43 articles, only 3 described a treatment intended to be used in irrigation water: two for chlorine dioxide treatment (Lopez-Galvez, Gil, Meireles, Truchado, & Allende, 2018; Reitz, Roncarati, Shock, Kreeft, & Klauzer, 2015) and one for ozone (Martínez-Sánchez & Aguayo, 2019), while the other article is agriculture-related but irrelevant to fresh produce production, which studied on-farm water disinfection using a UV lamps system for milking equipment wash on dairy farms (Masse et al., 2011).

B. Water treatment efficacy against E. coli

The disinfection against *E. coli* showed great variability between the different categories of treatment and within the same category, as shown in Figure 8. The full table of the water treatment's efficacy against *E. coli* with the detailed characteristics of the treatment can be found in Annex I.

In general, treatments achieved better efficacies at higher doses and contact time of the disinfectant (including chemical disinfectants, ozone, and UV). For instance, when a dose of 1.4 mg/L of ferrate (VI) was used, a 3 log reduction (LR) was achieved in 5 min, whereas the same LR could be achieved in 1 min at a higher dose (6.25 mg/L) (Cho et al., 2006).

Chemical disinfectants varied from non-inactivation (cupric chloride at a dose of 0.4-0.8 mg/L \times 60 min) (Straub, Gerba, Zhou, Price, & Yahya, 1995) to 6 LR (2.5 mg/L mono chloramine + 0.4 mg/L cupric chloride \times 10 min) (Straub et al., 1995). The combined use of mono chloramine and cupric chloride showed a synergetic effect. Depending on the dose usage, these chemicals can also be used for oxidation of organic compounds which

is advantageous to generate less disinfection by-products and allow higher inactivation rate of pathogenic microorganisms (de Souza & Daniel, 2011).

Ozone as a single disinfectant and when combined with hydrogen peroxide, revealed the same log reduction (6 LR), demonstrating a weak microbicidal activity of hydrogen peroxide in water (Sommer et al., 2004). The reported efficacy of ozone alone varied from 3.5 to 6 LR depending on the different exposure times and doses examined in studies.

A pulsed ultraviolet (PUV) light system achieved the greatest efficacy (9 LR) occurring at a UV dose of $4.32 \,\mu$ J/cm² under the reported testing conditions. However, increased exposure dose didn't seem to further strengthen E. coli inactivation. (Garvey, Hayes, Clifford, & Rowan, 2015), where greater reductions in viability were observed with increased UV dose. Great between-study heterogeneity in E. coli activation was observed, with the observed minimum as 1.46 LR (Liu & Zhang, 2006), and the maximum as 9 LR (Garvey et al., 2015), but most likely ranging from 3-6 LR. Similarly, high variation was observed for the filtration technology. For this category, adsorption materials, such as kaolin clay loaded with silver nanoparticles or carbon nanotubes (Hassouna et al., 2017) and activated carbon filters (Shaheed, Wan Mohtar, & El-Shafie, 2017; Silupu et al., 2017), did not effectively exert an inactivation of more than 1 LR. On the contrary, membrane filtration systems showed higher effectivity. With a pore size of 0.2 μ m and a filter medium of polypropylene, microfiltration membrane achieved a 6 LR (Coccagna et al., 2001), while ultrafiltration membranes with a smaller pore size $(0.002 \,\mu\text{m})$ and a filter medium of hollow fiber polyvinylidene fluoride (PVDF) presented an efficacy higher than 7 LR (Huang, Jacangelo, & Schwab, 2011). Slow Sand

Filtration (SSF) results were variable, but higher inactivation rates (6 LR) were reported when the system was enhanced with materials such as acid-soluble seston extract (Weber-Shirk, 2002) and natural bauxite (Urfer, 2017). Riverbank filtration (Partinoudi & Collins, 2007) and granular activated carbon (GAC) filters (Hijnen, Suylen, Bahlman, Brouwer-Hanzens, & Medema, 2010) seemed less promising, with an efficacy varied from 0.4 to 1.74 LR.

Finally, for the category of combined technologies, a combination of water treatments in more than one category achieved greater disinfection efficacy for *E. coli*. Ozone followed by chlorine (ozone 2mg/L + chlorine 5mg/L) resulted in a 7.76 LR, one of the highest disinfection values of the review. Results from this study suggest that permutation of the used dose can be applied without interfering in the final inactivation, therefore higher doses of ozone can be used as a primary disinfectant with a respective reduction on the dose of chlorine, which could possibly minimize the presence of toxic disinfection by-products (de Souza & Daniel, 2011). When UV light was combined with hydrogen peroxide or peroxydisulfate (PDS) an additional log reduction of *E. coli* was achieved (4 LR) compared with UV irradiation alone (Sun, Tyree, & Huang, 2016). A joint effect of filtration with GAC followed by chlorine dioxide reached a little more than 2 LR at doses of 2 mg/L of chlorine dioxide (Lin, Hou, Wang, & Chen, 2017).

C. The effect of water quality on the efficacy

Based on the findings of this review, water treatment can be significantly influenced by the quality of source water. The most common design factors considered on the quality of treated water were pH, temperature, and turbidity (measured in NTU). Other water quality parameters considered to a less extent include Dissolved Organic Carbon (DOC); Hardness (CaCo₃ concentration); Total Organic Carbon (TOC); Total Dissolved Solids (TDS); Electrical conductivity; Chemical Oxygen Demand (COD); UV Transmittance (UVT); Dissolved Oxygen (DO); Biochemical Oxygen Demand (BOD); and Total Suspended Solids (TSS).

Influencing quality factors vary by water treatments. A significant change on E. coli inactivation rate was observed with Fe (VI) with decreasing pH from 8.2 (1.7 LR) to 5.6 (4.5 LR) (Cho et al., 2006). Higher organic content in water (8mg/L Total Organic Carbon) had a negative effect on the efficacy (2.5 LR) compared with the lower organic content water (4mg/L TOC, 5.1 LR) when a coagulant/disinfection product based on Sodium dichloroisocyanurate was tested (Lewis Ivey & Miller, 2013). Results from this review suggest that UV is not significantly affected by high levels of turbidity. Turbidity influenced the efficacy of UV when it was over 4 NTU, however higher UV intensity minimized the negative effect on the inactivation of E. coli (Liu & Zhang, 2006). The disinfection capacity of a low-pressure UVC lamp was not significantly impacted when tested with water containing turbidity levels from 0 to 18 NTU, and the disinfection of E. *coli* in all scenarios remained above 5 LR (Younis, Mahoney, & Palomo, 2018). The study suggests that this system would be suitable to be operated with waters that contain higher turbidities, such as surface water or sandy groundwater wells. At relatively high turbidity (28.7 NTU), UV was highly efficient to disinfect water at low doses and very high pathogen concentration in raw water (505 CFU/100 mL) (Masse et al., 2011). Similarly, to achieve the same level of deactivation of *E. coli* at different turbidity levels, exposure time needs to be adjusted (6 seconds at a 0.25 NTU to 8 seconds at 20 NTU)

(Prakash et al., 2017). The effect of turbidity on the efficacy of disinfection was not evaluated in the ozone category of publications.

It is worth noting that among the primary studies with a major focus on the evaluation of water treatment efficacy, none of them discussed the implementation feasibility of the treatments, in particular to our interest, the treatment of water with direct contact of the edible portion of produces suitable for small-scale farms. Besides, although this efficacy-oriented review shed a light on the significant roles of water quality, pH, temperature, and turbidity as the most critical quality parameters were discussed in more detail in the next section "Other factors considered for water treatment adoption" under the technological criteria.

Other factors affecting feasibilities of water treatment application

A. Characteristics of selected reviews

To critically review the evidence for the support of evaluating the feasibility of the treatments to be implemented at small raspberry farms in Chile, a "review of reviews" was conducted. A total of 241 publications from Scopus and 169 from Web of Science Core collection were initially retrieved. After a relevance screening, 20 publications were included for the consideration of technological, managerial, and sustainability criteria. The included reviews were mostly published in the last decade, between 2010-2020. Like the efficacy-oriented review, included articles mostly focused on drinking water and wastewater municipal treatments, rather than water intended for agriculture practices (Al-Juboori, Aravinthan, & Yusaf, 2010; Decol et al., 2019; Luukkonen & Pehkonen, 2017; Wei, Zhang, Hu, Feng, & Wu, 2017). Out of the 20 reviews, six centered around treatments on irrigation water (Jones, Worobo, & Smart, 2014; López - Gálvez, Gil,

Meireles, Truchado, & Allende, 2018; Majsztrik et al., 2017; Raudales, 2014; Raudales, Fisher, & Hall, 2017; Raudales et al., 2014). Although many of these reviews emphasized plant pathogens disinfection (Raudales, 2014; Raudales et al., 2014), they were still included due to their coverage of information relevant to the criteria of our interest. In this feasibility-oriented review, the following technologies were discussed, including chlorine, chlorine dioxide, ozone, peracetic acid (PAA), hydrogen peroxide (H₂O₂), membrane filtration, and UV light, as these technologies were most prevalent in the scientific and gray literature, allowing for a more accurate evaluation for their suitability at small raspberry farms (Dandie et al., 2019; Haute et al., 2015). The coverage of water treatment technologies between the efficiency- and feasibility-oriented reviews considerably overlap with each other, with exceptions due to the disparity in the evidence available of these two aspects.

B. Technological criteria

The technological criteria are related to the physicochemical and microbial parameters of the water source that will subsequently determine the requirement of the disinfection method to achieve the desired water quality (Haute et al., 2015). The effectiveness of the treatment depends on parameters such as total dissolved solids (TDS), turbidity (expressed as nephelometric turbidity units NTU), pH, total suspended solids (TSS), chemical oxygen demand (Jones et al., 2014), and temperature (Dery, Brassill, & Rock, 2019). The water source quality will also determine if a pre-treatment is needed to ensure an adequate disinfection performance for the subsequent process (I. E. P. Agency, 2011). Turbidity, pH and temperature are the physicochemical parameters discussed as they can be more routinely and directly monitored by the farmers and enforcement agencies. <u>Turbidity</u>. Generally, turbidity has a negative effect on all water treatments considered in this study. Turbidity increases with organic matter concentration, which provides substrate to protect pathogens and microorganisms from the action of ozone, UV, and chlorine (Dery et al., 2019). High levels of turbidity demand increased concentrations of chemicals to obtain the desired level of disinfection, or inclusion of a pre-treatment step such as filtration (Dery et al., 2019). Additionally, when persisting in water, organic carbon is a precursor of chemical disinfection by-products (DBPs) (I. E. P. Agency, 2011).

For the case of UV, the relation between its efficacy and turbidity is not consistent, but typically as turbidity increases, UV transmittance (UVT) and bactericidal efficacy decrease (Qian, 2011). UVT levels in water needs to be addressed when dimensioning or sizing a UV disinfection system, whereas the power requirement needed to achieve a determined UV dose is approximately doubled for every five percent reduction in the UVT (I. E. P. Agency, 2011). Although highly turbid waters might not be a good candidate for UV without previous filtration, it has been observed a 99.9% of inactivation or greater for generic *E. coli* in surface water sources with an average NTU of 19.6 (Jones et al., 2014).

The efficiency of membrane filtration depends on the load of solids and the formation of fouling during the treatment (EPA, 2020a). Systems combined with low-pressure membrane filtration followed by high pressures can reduce this problem (M. C. Collivignarelli, Abba, Benigna, Sorlini, & Torretta, 2018).

No risk-based guideline value for turbidity has been proposed, however, median turbidity ideally should be below 0.1 NTU for effective disinfection (WHO, programme, Zdrowia,

Organization, & Staff, 2004), although a less restrict turbidity level of < 2 NTUs has also been commented as adequate to facilitate treatment of microorganisms (Zheng, Dunets, & Cayanan, 2014b).

<u>pH</u>. Chlorination is more effective in water with lower pH and is not recommended for a pH above 7.5 due to a low level of hypochlorous acid (HOCl) formation (preferred form for disinfection) (Dery, Brassill, & Rock, 2020; Jones et al., 2014). Keeping the pH between 6 and 7.5 is ideal, as it can help avoid the formation of chlorine gas that can lead to workers' health issues and further equipment corrosion (Dery et al., 2020; T. V. Suslow, 2001). Pathogen inactivation with chlorine dioxide is much less influenced by pH in the 6.0 to 8.5 range than chlorine (EPA, 2020a). The activity of H_2O_2 does not differ significantly from pH within the 2.0 to 10.0 range, although other authors have suggested that its function under acidic conditions is higher (Galeano, Guerrero-Flórez, Sánchez, Gil, & Vicente, 2019). Similarly, membrane filtration processes (microfiltration and ultrafiltration) typically can tolerate a pH range from 2 to 13 (EPA, 2020a).

The water pH has a significant impact on ozonation, and ozone activity generally decreases as pH increases (EPA, 2020a; Majsztrik et al., 2017), which is mainly related to the availability of ozone in water. Lower pH (<7.0) slows down ozone decomposition resulting in higher concentrations of molecular ozone, while at pH >8 ozone decomposition increases significantly (EPA, 2020a).

UV disinfection efficacy is not significantly influenced by pH, however, it can still impact the scaling of UV lamp sleeves (Basaran, Quintero-Ramos, Moake, Churey, & Worobo, 2004; EPA, 2020a; Quintero-Ramos, Churey, Hartman, Barnard, & Worobo, 2004). <u>Temperature</u>. As temperature rises, most chemical disinfectants are more efficient for microbial inactivation, requiring a reduced dose (I. E. P. Agency, 2011; Dery et al., 2019). Moreover, this parameter also affects pH in irrigation water, as pH decrease at higher temperature (Dery et al., 2019).

Chlorine disinfection is most effective at temperatures between 18°C and 37°C, where for every 10°C increase in temperature, sodium hypochlorite will degrade 3.5 times faster (Dery et al., 2020; Manufacturing, 2019; WHO, 2013). Water temperature has a significant impact on water density and viscosity, which impacts microfiltration (MF) and ultrafiltration (UF) membrane flux (EPA, 2020a). As the viscosity and density increase, the transmembrane pressure required to pass the water through the membrane also increases (EPA, 2020a). On the other hand, ozone disinfecting and oxidative properties are relatively independent of temperature; however, as temperatures increase, the solubility of ozone in water decreases (EPA, 2020a). The major challenge with higher temperatures is the ability to transfer an adequate ozone dosage to the water. This can be accomplished by increasing the ozone concentration in the feed system and/or by providing adequate design for ozone transfer (EPA, 2020a). Likewise, the overall effectiveness of UV disinfection is not influenced by temperature (EPA, 2020a).

<u>Other consideration</u>. Due to our main focus on water used for pesticide application, the potential interaction between pesticides with active compounds of water disinfectants needs to be taken into consideration. Examples of pesticides used by small raspberry farmers in Chile are benomyl (fungicide); mancozeb (fungicide); bifenthrin (insecticide); azinphos-methyl (insecticide); cuprous oxide (fungicide) belonging to benzimidazole, dithiocarbamate, pyrethroid, and organophosphate families (INIA, 2017). Evidence has

shown that chemical treatment for water disinfection, such as chlorine, chlorine dioxide, hydrogen peroxide, ozone, and UV, may remove pesticide in water supplies, which was demonstrated in studies objectively investigating the application of water treatments to remove pesticide pollution (Chamberlain et al., 2012). Chlorination was shown to be an effective option for the removal of organophosphorus pesticides (Acero, Benitez, Real, & González, 2008). A dose of 2.5 mg/L was enough to oxidize chlorpyrifos and diazinon almost completely in surface water (Acero et al., 2008). Post-harvest treatments for the reduction of pesticides in produce have also been conducted. Mancozeb was removed by chlorine (up to 99%), chlorine dioxide (up to 87%) ozone (up to 97%), and hydrogen peroxyacetic acid from fresh apples (Hwang, Cash, & Zabik, 2001). The concentrations studied by Hwang et al., 2001, are as low as 500 ppm of chlorine and 5-10 ppm of chlorine dioxide for apples coated with mancozeb from 1-10 ppm, suggesting that residual concentrations of disinfectants used for the treatment of water used a pre-harvest stage for small farmers should be carefully monitored to ensure the effectiveness of the pesticides is maintained.

C. Managerial

<u>Cost</u>. Though the effectiveness against *E. coli* is the main criteria for the selection of technology, the cost is the most influential factor in the decision-making when the degree of disinfection effectiveness is satisfied (Haute et al., 2015). However, It is unlikely to establish a unified ranking in terms of costs of various water treatment technologies, as the cost-effectiveness relation is multifactorial (Haute et al., 2015). Matching the type and scale of technology for each specific grower situation is critical (Raudales et al., 2017). According to the literature reviewed, water treatment technologies have been

scarcely tested in water for pesticide spray especially at small-scale farms, making it difficult for the objective of this study to prioritize them based on their costs. Some relevant publications that delivered capital and operation costs for water treatments in agricultural use are discussed below.

One of the determining factors for cost-efficiency is the volume of water consumption. According to the Chilean Institute for Agricultural Development (INDAP, 2017), the required volume used per hectare of pesticide application can vary from 10 to 120 L per 1,000 m³ of vegetation depending on the foliar volume of the raspberry bushes. For instance, for a foliage volume of 3,400 m³ per hectare and a medium foliar density (70 L per 1,000 m³) the volume of application is 238 L/ha. It has also been reported by Verhaelen et al. (2013) that depending on the crop, pesticides are diluted in different amounts of water and sprayed onto the fields in volumes ranging from 200 L to 1000 L per hectare.

The majority of raspberry production in Chile are as peasant family agriculture, where the farms have an area of no more than one hectare (ODEPA, 2018). Moreover, different from the year-round application of water for irrigation, pesticide sprays are carried out in several specific productive stages of raspberry, such as sprouting, flowering, fruit set and at the beginning of winter recess. Hence, water consumption needed for pesticide spray on raspberry farms in Chile is anticipated much less demanding compared with most agriculture water use scenarios.

Considering the relatively low water consumption, some disinfection technologies, particularly physical treatments, are generally less cost-efficient for Chilean raspberry

farmers, as these technologies require substantial investments in infrastructure that hinders their adoption at small scale operation. Due to substantial costs for installation and maintenance, such as pumping, downstream processing, and rapid filter clogging, contaminant remediation using membrane filters is considered prohibitively costly (Majsztrik et al., 2017). Membrane filters, slow sand filtration, and constructed wetlands are considered more capital-intensive compared with injectable chemicals such as chlorine, and therefore are more likely to be applicable for large quantities of water where economies of scale lower the cost of capital per volume treated (Raudales et al., 2014). Among various physical treatments, UV seems a promising option, as after the relatively expensive installation, its operational cost to sustain the apparatus is fairly low as limited maintenance is required, rendering it more suitable for small-scale water disinfection facilities (Sigge et al., 2016). Additionally, the price of light-emitting diode (LED) has decreased significantly due to technical advances (Hinds, O'Donnell, Akhter, & Tiwari, 2019). A study determined UV light against ozone and ultrafiltration, as the most feasible disinfection technology in terms of microbial and cost efficacies to treat surface water for agricultural use (Banach, Hoffmans, Appelman, van Bokhorst-van de Veen, & van Asselt).

Compared with physical treatments, most chemical treatments are more promising costefficacy-wise in terms of both capital investment and operational costs. Hypochlorite (usually in the form of liquid sodium hypochlorite) is a very popular water disinfectant in the produce industry because of its ease of use and relatively low cost (T. V. Suslow, 2001). A preliminary analysis of the cost of water treatment in U.S. greenhouse operations found a broad range of treatment costs from USD 0.02 per 1,000L for calcium hypochlorite, sodium hypochlorite, or chlorine gas chlorination up to USD 5.15 per 1,000L for chlorine dioxide treatment (Raudales, 2014). Albeit the lack of data for quantitative comparison, PAA, another popular chemical sanitizer, was reported with minimum investment costs like hypochlorite but higher purchase cost due to limited production capacity (Dandie et al., 2019).

In contrast, ozonation is one of the costliest chemical solutions for water treatment due to high costs for installation and operation (electricity consumption, a key component of operation costs) for small systems (M. C. Collivignarelli et al., 2018; Luukkonen & Pehkonen, 2017; Zheng, Dunets, & Cayanan, 2014a). The capital investment for ozone generator and its setup depends on the water treatment system. As an example, the investment for municipal water system from the highest to the lowest is UV, ozone, PAA, and chlorine dioxide (C. Collivignarelli, Bertanza, & Pedrazzani, 2000) while when evaluating the investment for an irrigation water system, ozone was four times more expensive than UV (USD 16,949/1,000L- year versus USD 4,356/1,000L-year) (Banach et al.). However, it seems the high costs do not exclude ozonation application, as it has been applied to low flow systems on high-value crops including precision drip delivery for berry production (TV Suslow, 2010). Indeed, ozonation was selected as a feasible post-harvest water treatment (for processing water) to be applied on-farm with reasonable costs and allowing an operation and maintenance with no excessive dedication compared to the original situation in a Chilean vegetable farm (investment USD 6,200 and operation costs of USD 220 approximately annually) (PUC, 2020).

<u>Complexity of operation</u>. The most popular and widely used method for water disinfection is chlorination. Chlorine exists in three forms: hypochlorite (sodium

hypochlorite or calcium hypochlorite) and chlorine dioxide, and chlorine gas (Ivey & Miller, 2013). Sodium hypochlorite (liquid bleach), is a relatively easy and cost-effective method that does not require extensive technical knowledge to use and is capable to cope with supply systems of different sizes (I. EPA, 2011). Chlorination with sodium hypochlorite consists of a pump and a storage tank (Brief, 1999). Calcium hypochlorite is available as a powder, tablet, or granules (Lewis, 2010), and its storage is easier than sodium hypochlorite without requiring bulk tanks (Newman, 2004b). On the other hand, chlorine dioxide is unstable and has minimum shelf-life, which should be produced close to the application site and mixing with water reasonably immediately afterward (Masotti, 2011). It can be produced by using sodium chlorine combined with hydrochloric acid or chlorine gas (Al-Juboori et al., 2010).

Peracetic acid (PAA) is relatively stable when stored under appropriate conditions, has a long shelf life, and is easy to handle (Sigge et al., 2016; Tchobanoglus, Burton, & Stensel, 2003). The storage and dosing systems are similar to sodium hypochlorite (M. C. Collivignarelli et al., 2018), however, limited research is available for pre-harvest applications (Dandie et al., 2019). For hydrogen peroxide, it is possible to store onsite, but it is subject to degradation over time and is a hazardous substance that needs secondary containment for storage facilities (I. E. P. Agency, 2011)

Ozone is unstable and therefore must be generated in situ (M. C. Collivignarelli et al., 2018). The ozone production device requires electricity to form ozone (Majsztrik et al., 2017). The equipment includes air preparation (ozone generator, contactor, destruction unit), instrumentation, and controls. Operation and maintenance are relatively complex (M. C. Collivignarelli et al., 2018).

UV-LEDS cause no disposal problem (mercury-free), leave a small footprint (flexible architecture), are mechanically robust, and possess an instant on-off functionality (high-frequency response), low voltage, low power requirements, and long lifetimes (reduced frequency of replacement) (Würtele et al., 2011). On the other hand, UV mercury lamps installation is bulky and large, are packaged by glass which is fragile (Li et al., 2019).

Membrane filtration requires high expertise for its operation (Sigge et al., 2016). Maintenance to clean fouling clogging demands backwashing and membrane replacement on regular basis (Dandie et al., 2019). In addition, membrane failure can be catastrophic and hard to detect (Dandie et al., 2019).

Monitoring. The operation of the selected disinfection technology should allow for easy verification. Operational monitoring parameters usually evaluated in drinking water systems are turbidity, pH, chemical dosage, flow rate, head loss, disinfectant concentration x contract time (Ct), disinfectant residual, and disinfection by-products (DBPs) (WHO et al., 2004). The temperature should also be monitored in water as it directly affects the performance of the treatment (Haute et al., 2015). Because chemical reactions often increase at higher temperatures, chlorine treatments, for example, are less effective at low temperatures (WHO et al., 2004). Besides, temperature affects other water quality parameters such as pH, whereas the temperature of the irrigation water increases, pH decreases (Dery et al., 2019). The association between water physicochemical parameters and the disinfection performance of the treatment was also discussed in the technological criteria section.

Advantageously, commercial kits for on-site measurement of active ingredients are available for chlorine, chlorine dioxide, activated peroxygens, and ozone (Raudales et al., 2014). On the other hand, membrane filtration requires high expertise to run and maintain, and fouling or clogging might require backwashing and more frequent membrane replacement (Dandie et al., 2019; Haute et al., 2015).

Safety. Hypochlorite solutions are highly unstable since degradation takes place on heat and light exposure. Peroxides are highly unstable and corrosive, and exposure to PAA causes irritation and possibly permanent damage to skin (cutaneous emphysema), eyes, and the respiratory system (Cristofari-Marquand, Kacel, Milhe, Magnan, & Lehucher-Michel, 2007). Therefore, safety measures should be in place during the storage of these treatment chemicals and good ventilation should be maintained to prevent harmful health effects (Sigge et al., 2016). On the contrary, calcium hypochlorite is much safer to handle compared to both chlorine gas and sodium hypochlorite (Newman, 2004b). Exposure to UV light can bring operators with some safety problems including eye damage; skin burns from hot lamps or equipment; exposure to mercury from a broken lamp; and electrical shock (USEPA, 2020). Also, UV mercury lamps can potentially cause mercury leaks in the external environment, releasing harmful vapors into the air (Li et al., 2019). Ozone is highly corrosive and toxic, hence instrumentation should be provided for ozone systems to protect both personnel and the equipment (USEPA, 2020).

D. Sustainability criteria

<u>Corrosive materials</u>. Corrosion is the partial dissolution of the materials that integrate the water treatment and supply systems, tanks, pipes, valves, and pumps, leading to structural failure with the decayed of chemical and microbial water quality (WHO et al., 2004). Chemical disinfectants applied in water can interact with metal-based distribution systems causing the corrosion of pipe materials and the formation of deposits (Water &

Organization, 2006; Zuluaga-Gomeza, Bonaverib, Zuluagab, Álvarez-Peñaa, & Ramírez-Ortiza, 2020).

The literature reviewed in this study is normally focused on the potential effect of the disinfectants over irrigation delivery systems as pipelines and pumps (Childress, Le-Clech, Daugherty, Chen, & Leslie, 2005; Dery et al., 2020; Sigge et al., 2016; Zuluaga-Gomeza et al., 2020). Chlorine gas (derived from chlorine) (Newman, 2004a; T. V. Suslow, 2001); ozone (Trevor Suslow, 1997); sodium hydroxide (derived from sodium hypochlorite when dissociated in water); calcium hypochlorite (Newman, 2004a); chlorine dioxide; PAA and hydrogen peroxide (Haute et al., 2015) all have been described as corrosive agents in irrigation water distribution systems. Nonetheless, this type of system might not be relevant when treating the water used for pesticide application, especially at small farms. Common spraying equipment used by raspberry farmers in Chile are hydraulic and pneumatic backpack sprayers (INDAP, 2017). Backpack sprayers entirely made of corrosion-resistant materials are available in the Chilean market since 1960, including especial backpack sprayers for highly corrosive liquids, in addition to equipment maintenance products such as corrosion inhibiting oils (SOLO-CHILE, 2019). The chemicals reviewed will probably be applied to wells or storage units such as plastic containers, therefore is expected that by following manufacturer recommendations, holding to Good Agricultural Practices (GAP), and opting for the use of anti-corrosive materials, the effectivity and the water treatment structure should not be compromised.

Regardless, special attention is required when combining technologies to treat water, for instance, chlorine oxidative agents might attack the membrane of reverse osmosis membrane (Al-Juboori et al., 2010).

Availability for rural areas in Chile. According to the National Irrigation Commission (CNR) of the Chilean Ministry of Agriculture, there is a reliable supply chain for water treatment technologies in the national market. By the year 2007, there were 27 water treatment supplier companies, managing technologies such as ultrafiltration, microfiltration, activated coal, UV, ozone, greensand, KDF (Kinetic Degradation Fluxion), activated alumina, cartridge filter, filter bags, electro dialysis, and ion exchange (CNR, 2007). UV, ozone, cartridge filtration, filter bags, and microfiltration were indicated as some of the technologies with higher potential for their use in irrigation water to remove fecal coliforms (CNR, 2007). UV, ozone, and microfiltration (filtration with Bags) were validated on-farm in irrigation water since they presented a complexity that allows them to be easily integrated into the usual property management of farmers in the area and the costs were within acceptable margins (CNR, 2007).

Currently recommended technologies by the Chilean Institute of Agricultural Research (INIA) to treat low-quality water for irrigation purposes, are stabilization lagoons (biological or biotechnological), chlorination, UV, and ozone (INIA, 2014).

Agricultural producers are looking for alternatives to chlorine to avoid introducing any chemical risk in water (A Allende et al., 2018). The UV radiation water treatment technique is a practice that has been widely tested and used in the country (INIA, 2014). At the farm level, it is easy to use for the operators and the disinfection equipment requires less space than the other methods (INIA, 2014). Crops of carrots and lettuce in

the metropolitan region have been irrigated with water treated with a UV-C lamp showing satisfactory results when coupled with a *desander* and water accumulator (INIA, 2014).

As reported by the Observatory for Agricultural, Agri-food and Forestry Innovation in Chile (OPIA), in the last years, a couple of projects for the disinfection of agricultural water have been developed such as photocatalysis using solar light for the disinfection of irrigation water (OPIA, 2004, 2008). Most of these technologies have been developed to purify water, treat liquid industrial waste or desalinate water resources for potable water, so their use in agricultural water poses the challenge of working under different scenarios both in concentration and type of pollutants.

Disinfection by-products. The use of chemical disinfectants in water treatment systems typically results in the generation of disinfection by-products (DBPs) (WHO et al., 2004). DBPs are organic and inorganic compounds formed by the reaction of chemical disinfectants with byproduct precursors and natural organic matter (I. E. P. Agency, 2011; Research Group on Quality et al., 2019) during the disinfection of drinking water or water for food production (EFSA, 2015). The presence of significant concentrations of DBPs in fresh produce has triggered an intensive debate on current disinfection practices and how DBPs may enter the food supply chain, becoming a potential risk for consumers health (Research Group on Quality et al., 2019).

A relevant example is that when using chlorine, chlorine dioxide, or hypochlorite for the disinfection of water for food production, chlorate is formed as a by-product (EFSA, 2015).

Although treated process water (post-harvest) has been indicated as the main source of chlorates in fruits and vegetables (Research Group on Quality et al., 2019), there is no clear information about the risk posed by the presence of DBPs in agricultural water at a pre-harvest stage.

Chlorination of water in the presence of natural organic substances leads to the formation of halogenated, highly toxic, and hazardous DBPs (Galeano et al., 2019). Trihalometahnes (THMs), halo acetic acids (HAACs), chlorophenols, chloral hydrate, and haloacetonitriles (HANs) are all examples of chlorination DBPs (Al-Juboori et al., 2010; M. C. Collivignarelli et al., 2018). Chlorine dioxide is a potential alternative to chlorine for disinfection of agricultural water (Decol et al., 2019), as it generates fewer types of DBPs in smaller quantities compared to chlorine and chloramines (Al-Juboori et al., 2010; EPA, 2020a). During disinfection with chlorine dioxide, chlorite and chlorate are the major reaction by-products, potentially toxic (M. C. Collivignarelli et al., 2018). Ozone does not entail the formation of chlorinated by-products as THMs (M. C. Collivignarelli et al., 2018) but can form mutagenic and carcinogenic agents such as bromide (Al-Juboori et al., 2010). Regarding the use of PAA, one of the main benefits over free chlorine and ozone is the less probability to originate DBPs (Kitis, 2004). Moreover, when applied in surface water PAA form a significantly low concentration of formaldehyde compared to the guideline value in drinking water (Nurizzo, Antonelli, Profaizer, & Romele, 2005).

Chilean fresh raspberries can potentially be contaminated with DBPs through the treated water used for the dilution of pesticides, as after being harvested no further process step is applied along the supply chain (Ortúzar et al., 2020). Furthermore, as the quality of
water sources used by farmers is variable, interaction with high levels of organic matter could lead to the formation of potential carcinogens above the guideline values established by the WHO.

The risk to human health of DBPs is considerably low compared to the risks associated with insufficient disinfection, therefore disinfection should not be compromised in attempting to control such chemicals (WHO et al., 2004). Essential strategies adopted for reducing the concentrations of DBPs in drinking water (WHO et al., 2004), and that might be applicable for agricultural water are the removal of precursor compounds as the natural organic matter before the application, employ disinfectants with a lower likelihood to produce byproducts in surface water, and prefer non-chemical disinfection that does not cause the formation of by-product as UV irradiation products (M. C. Collivignarelli et al., 2018) or membrane processes.

V. Conclusion

There is limited research focusing on the microbial content of agricultural water (A. Allende & Monaghan, 2015), and the situation is not different for Chile, where the literature discussing the microbial load in agricultural water is much scarcer. According to the WHO, high detectable concentrations have been described in the literature depending on the water source. The presence of *E. coli* goes from lower to higher concentration: groundwater (0-1,000); wilderness rivers and streams (6,000-30,000); impacted rivers and streams (30,000-1,00,000) and lakes and reservoirs (10,000-1,00,000) (WHO et al., 2004). According to a governmental report and survey conducted by this team, the majority of the Chilean raspberry producers have access to surface water and groundwater water as their main sources of water for irrigation and pesticide

application (INIA, 2016; Ortúzar et al., 2020), which means most of the farmers have access to a lower water quality (Leifert, Ball, Volakakis, & Cooper, 2008).

The Chilean food safety authorities have issued guidelines on water quality for agriculture use, which, however, are not enforced mandatorily. The guidelines suggest that farmers wishing to export their fresh produce products should use water with quality equivalent to drinking water if the intended use involves direct contact with the produce (FDF, 2013). The U.S., principal importer of Chilean raspberries, as per the Food Safety Modernization Act (FSMA) and particularly on the Final Rule of Produce Safety, establishes criteria for microbial quality of agricultural water directly applied to growing produce based on the level of generic E. coli (A Allende et al., 2018; ODEPA, 2018). The water used for the application of pesticides falls under the classification of agricultural water according to FSMA, therefore, a numerical criteria based on the geometric mean of \leq 126 CFU/100 mL of *E. coli* and a statistical threshold of \leq 410 CFU of E. coli in 100 mL of water is required (FDA, 2015). Otherwise, the European Commission has established a target value of E. coli of 100 CFU/100 mL in agricultural water that has direct contact with the edible portion of the crop of fruits and vegetables intended to be eaten uncooked (EC, 2017). In a new protocol developed by the U.S Environmental Protection Agency (EPA) and the Food and Drug Administration Agency (FDA) to support registration of new treatments products or amendments to current products labels for use in agricultural water, an acceptance criterion was set as a minimum of 3 log reduction of suggested testing microorganism (EPA, 2020b). The apparent disparity between the low quality of accessible source water and high

expectation on water applied on raspberry farm highlights an urgent need for water treatments with high efficacy according to the source water quality.

It is challenging to make a definitive ranking of the possible water treatments evaluated, due to the scarcity of available evidence and the impossibility to seek one-fit-all technologies. However, the critical review allows for a totality analysis of individual treatments based on various factors that influence the feasibility of in-field implementation. In general, physical treatments generally require higher managerial demands, but more sustainable in a long run. On the contrary, chemical treatments are effective, require less front-loaded investment and infrastructure, but may last in a shorter life span. A qualitative evaluation of different feasibility-related traits is elaborated in Table *4* for the treatments with more promising potentials to be applied on the small-size raspberry farms .

(Dandie et al., 2019; Haute et al., 2015).

The search strategy followed by the RR excluded those water treatments that were not focused on microorganisms of public health concern, and even more specifically, those who did not evaluate efficacy against generic *E. coli*, that is why it is presumed there are some types of incongruity between the treatments found systematically (Table 2) compared to the results from the "review of reviews" approach (Table 4). After this last approach was conducted, it was observed that water treatments have been widely implemented on-farm at a pre-harvest stage, but the technologies or interventions are focused on the elimination and/or prevention of plant pathogens, algae growth, or materials preventing the fouling within the irrigation systems delivery, rather than measure the effectivity against microorganisms from public health concern as it is *E. coli*.

Besides, the vast majority of the interventions identified in our study corresponded to water treatments that have been developed to be implemented in large treatment plants of drinking-water or waste-water systems, being the analysis for our study to some extent challenging.

Several technologies that can undoubtedly exhibit great potential have been left out from the scope of this study (such as advanced oxidation process (AOP), electrochemical treatments, electrolyzed oxidizing water, solar disinfection (SODIS) to mention a few), nevertheless, it is expected that relevant data that supports the process of decision-making could potentially be published soon. Take the United States for instance, wherefrom a regulatory perspective it was published on July 2020 a protocol intended to help companies develop data on the effectiveness of their products in inactivating pathogens in pre-harvest agricultural water (FDA, 2020).

Given that the RR is focused on collecting scientific publications, additional relevant sources, such as commercial water treatment distributors that might have been able to carry out validations under conditions from the interest of this study, are attractive options to evaluate in the future, specifically taking into account a cost-benefit analysis for Chilean farmers.

One of the substantial contributions of the RR is that the efficacy values against *E. coli* were systematically extracted from studies in which treatments were evaluated in freshwater sources, where the performance of each technology is expected to be

influenced by water quality parameters such as turbidity, pH, or temperature. These values are assumed to be closer to the real operating conditions in the small farms, avoiding the overestimation of the effectiveness and therefore providing more reliable results.

This study displays relevant options to be considered by the Chilean food safety authorities, and those aspects that to our understanding are relevant to considered were critically analyzed. We conclude that there is no single option for treating the microbial contamination of the water used for the pesticide dilution, but each particular condition on-farm must be evaluated in detail to consider factors such as the particular water quality of each property to count on the technology that best fits.

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Figures

1) Technological criteria	2) Managerial criteria	3) Sustainability criteria
 Quality of source water Optimal water quality Process parameters 	 Costs Complexity of the technology Monitoring Safety of operators Legal considerations 	 Materials resistant to corrosion Environmental considerations

Figure 5. Main criteria of feasibility evaluated for water treatment implemented in raspberry farms in Chile. Adopted from the selection tool developed by Haute *et al.* (2015)



Figure 6. A comprehensive approach to retrieve relevant information from the scientific literature: Comparison and connection of the two reviews focusing on efficacy (rapid systematic review) and feasibility of application (review of reviews) for water treatments



Figure 7. Flowchart of the rapid systematic review focusing on treatment efficacy



Figure 8. Log reduction of E. coli by water treatments applied to freshwater sources

Tables

Category	Sub-category	Number of articles	Treatment
Chemical Disinfectants		9	Calcium hypochlorite; Chlorine; Chlorine dioxide; Monochloramine; Ferrate Fe (VI); Sodium dichloro-s- triazine-trione; Ferric sulfate + Sodium dichloroisocyanurate (NaDCC)
Ozone		5	
UV light		8	
Filtration	Membrane filtration	2	Microfiltration
		3	Ultrafiltration
	Slow sand filtration	5	
	Biosand filtration	2	
	Riverbank filtration	2	
	Miscellaneous	5	Nano-adsorbents; Carbon nanotubes; Chitosan-bentonite composites; Silver nanoparticles; Activated carbon filters
Combined treatments		4	Granulated Activated Carbon (GAC) + Chlorine dioxide; Ozone + Chlorine; UV light + Hydrogen peroxide (H ₂ O ₂); UV + Peroxydisulfate (PDS); Ozone + H ₂ O ₂

Table 2. Distribution of selected articles across different categories of water treatments

Reference	Country (Region)	Water source	Water treatment	Intended use	Experiment al setup	Spiked/ Natural	Enumeration/ Detection	Outcome		
							Method	measure		
Chemical disinfectant										
Cho, Lee, Choi, Chung, and Yoon (2006)	Korea (Asia, AS)	Surface water	Fe (VI)	Not specified	Laboratory	Spiked	Spread plating	Log reduction (LR)		
de Souza and Daniel (2011)	Brazil (South America, SA)	Ground water	Chlorine	Not specified	Laboratory	Spiked	Membrane filtration (cellulose nitrate filter method 9222 Chromocult agar.	LR		
El- Maghraoui, Zerouale, Ijjaali, and Benbrahim (2013)	Morocco (Africa, AF)	Surface water	Fe(VI) Na ₂ FeO ₄	Not specified	Laboratory	Spiked	Optical density 660 nm/ spread plating	Survival percentage		
Ferreira, Luz, and Buss (2016)	Brazil (SA)	Ground water	Calcium hypochlorite	Not specified	Laboratory	Natural	IDEXX Colilert [®] and Quanti-Tray 2000®	Prevalence after treatment		
Kfir, Bateman, and Coubrough (1995)	South Africa (AF)	Surface water	Sodium dichloro-s- triazine-trione	Drinking water	Laboratory	Spiked	Membrane filtration (APHA, AWWA, WPCF)	LR		
Legare- Julien, Lemay, Vallee- Godbout, Bouchard, and Dorea (2018)	Canada (North America, NA)	Surface water	Ferric sulfate + Sodium dichloroisocya nurate (NaDCC)	Drinking water	Laboratory	Natural	IDEXX Colilert [®] and Quanti-tray 2000 [®]	LR		
Lopez- Galvez, Gil, Meireles, Truchado, and Allende (2018)	Spain (Europe, EU)	Surface water	Chlorine dioxide	Irrigation water	Pilot-scale	Natural	Spread plating (Chromocult coliform agar) PCR (viable <i>E. coli</i>)	LR		
Reitz, Roncarati, Shock, Kreeft, and Klauzer (2015)	United States (NA)	Surface water	Chlorine dioxide	Irrigation water	Pilot-scale	Natural	IDEXX Colilert ® and Quanity-Tray 2000®	LR		

Table 3. Summary of characteristics of studies focusing on water treatments controlling *E. coli* in freshwater sources

Straub, Gerba, Zhou, Price, and Yahya (1995)	United States (NA)	Ground water	Monochlorami n + cupric chloride Cupric chloride Monochlorami ne	Drinking water	Laboratory	Spiked	Plate count (method not specified)	LR
Ozone								
de Souza and Daniel (2011)	Brazil (SA)	Ground water	Ozone	Not specified	Laboratory	Spiked	APHA 9222	LR
Izdebski, Dors, and Mizeraczyk (2011)	Poland (EU)	Surface water	Ozone	Not specified	Laboratory	Natural	Non specified	LR
Martínez- Sánchez and Aguayo (2019)	Spain (EU)	Surface /ground water	Ozone	Irrigation water	Laboratory	Natural	According to order SCO/778/200 9 on alternatives methods	LR
Sommer et al. (2004)	Austria (EU)	Ground water	Ozone	Not specified	Pilot-scale	Spiked	Membrane filtration and violet red bile agar.	LR
Zuma, Lin, and Jonnalagad da (2009)	South Africa (AF)	Surface water	Ozone	Not specified	Laboratory	Spiked	Spread Plate Technique	LR
UV light								
Garvey, Hayes, Clifford, and Rowan (2015)	Ireland (EU)	Mimics freshwa ter	UV light	Not specified	Laboratory	Spiked	Not specified	LR
Liu and Zhang (2006)	China (AS)	Mimics freshwa ter	UV light	Not specified	Laboratory	Spiked	Plate count Fuchsine sodium sulfite broth	LR
Masse et al. (2011)	Canada (NA)	Surface water	UV light	On-farm water disinfection system	Pilot-scale	Natural	mFC Basal Medium (Difco)	LR
Prakash et al. (2017)	India (AS)	Mimics freshwa ter	UV light	Not specified	Laboratory	Spiked	Plate count	LR
P. Z. Sun, Tyree, and	United States (NA)	Surface water	UV light	Not specified	Laboratory	Spiked	-	LR

Huang (2016)									
W. J. Sun and Liu (2009)	China (AS)	Surface water	UV light	Drinking water	Pilot-scale (Continuou s flow system)	Spiked	APHA 9222	LR	
Younis, Mahoney, and Palomo (2018)	United States (NA)	Mimics freshwa ter	UV light	Not specified	Laboratory	Spiked	Membrane filtration according to Standard Method 9132	LR	
Younis, Mahoney, and Yao	United States (NA)	Ground water	UV light	Drinking water	Pilot-scale	Spiked	IDEXX Colilert Quanty-Trays	LR	
(2019)		Surface water					Membrane filtration technique (Standard Method American Water Works Association, AWWA)		
Filter systems									
Hassouna, ElBably, Mohammed , and Nasser (2017)	Egypt (AF)	Ground water and Surface water	Nano- adsorbents	Not specified	Laboratory	Natural	Plate count	Percentage removal	
Lukhele, Mamba, Momba, and Krause (2010)	South Africa (AF)	Ground water and Surface water	Carbon nanotubes- nanoparticles	Not specified	Laboratory	Spiked	Membrane filtration technique- chromocult media (Biolab)	LR	
Mpenyana- Monyatsi, Mthombeni , Onyango, and Momba (2012)	South Africa (AF)	Ground water	Silver nanoparticles filter system	Drinking water	Laboratory	Spiked and Natural	Standard Methods APHA American Public Health Association	LR	
Shaheed, Wan Mohtar, and El- Shafie (2017)	Malaysia (AS)	Surface water	Adsorption- Filtration System	Drinking water	Laboratory	Natural	AOAC Official Method 991.14	Percentage removal	
Silupu et al. (2017)	Peru (SA)	Surface water	Activated carbon filters	Drinking water	Laboratory	Spiked	Luria broth- McFarland Tube Method- Mueller- Hinton Broth	Percentage removal	
Membrane filtration: microfiltration and ultrafiltration									

Coccagna et al. (2001)	Italy (EU)	Surface water	Microfiltration	Not specified	Pilot-scale	Spiked	Membrane filtration technique (Standards Methods 1995)	LR
Ujang, Au, and Nagaoka (2002)	Malaysia (AS)	Surface water	Microfiltration	Drinking water	Laboratory	Natural	Standard Plate method Standard Methods (1995)	Percentage removal
Galvañ et al. (2014)	Spain (EU)	Surface water	Ultrafiltration	Not specified	Pilot-scale	Natural	Most Probable Number	Percentage removal
Huang, Jacangelo, and Schwab (2011)	United States (NA)	Surface water	Ultrafiltration	Drinking water	Laboratory	Spiked	Colilert Quanti-tray system (IDEXX Laboratories)	LR
Praneeth, Kalyani, Ravikumar, Tardio, and Sridhar (2013)	India (AS)	Surface water	Ultrafiltration	Not specified	Laboratory	Natural	Coliform test	LR
Slow Sand Fi	iltration							
Hijnen, Schijven, Bonne, Visser, and Medema (2004)	Netherlands (EU)	Surface water	Slow Sand Filtration	Not specified	Full-scale Pilot-scale Laboratory	Natural and Spiked	Not specified	LR
Ochieng, Otieno, Ogada, Shitote, and Menzwa (2004)	South Africa (AF)	Raw water	Multistage Filtration (MSF): Slow Sand Filtration (SSF)+Pretrea tment system- horizontal flow roughing filter (HRF)	Drinking water	Pilot-scale	Natural	Not specified	Percentage removal
Rao, Malini, Lydia, and Lee (2013)	India (AS)	Ground water	Bentonite Amended Slow Sand Filter	Not specified	Laboratory	Natural	Multiple tube method MPN technique	LR
Urfer (2017)	Switzerland (EU)	Surface water	Multistage Filtration (MSF) Biosand Filter enhanced with bauxite	Not specified	Pilot-scale	Natural	Standard plate-count method according to Standard Methods (APHA AWWA WEF ,2014)	Percentage removal

Weber- Shirk (2002)	United States (NA)	Surface water	Slow Sand Filter enhanced with acid-soluble seston extract	Not specified	Laboratory	Spiked	Standard Method (APHA, AWWA, WPCF, 1998).	Percentage removal
Biosand Filtra	ntion							
Hyde and Lackey (2013)	United States (NA)	Surface water	Biological Sand Filters (BSFs)	Not specified	Laboratory	Spiked	Membrane filtration (Standard Methods 9222D)	Percentage removal
Napotnik, Baker, and Jellison (2017)	United States (NA)	Mimics freshwa ter	Biosand Filters	Drinking water	Pilot-scale	Spiked	Membrane filtration (Standard Method 9222)	LR
Riverbank filt	ration							
Cady et al. (2013)	India (AS)	Surface water	Riverbank Filtration	Not specified	Pilot-scale	Natural	IDDEXX Colilert MPN	LR
Partinoudi and Collins (2007)	United States (NA)	Ground water and Surface water	Riverbank Filtration	Not specified	Pilot-scale	Natural	Method 9223 (Standard Methods, 2005)	LR
Granular Acti	ivated Carbon							
Hijnen, Suylen, Bahlman, Brouwer- Hanzens, and Medema (2010)	Netherlands(EU)	Surface water	Adsorption Filtration	Not specified	Pilot-scale	Spiked	Sodium Lauryl- sulphate Agar incubation confirmed for indol formation	LR
Combined								
de Souza and Daniel (2011)	Brazil (SA)	Ground water	Ozone Chlorine Ozone + Chlorine	Not specified	Laboratory	Spiked	APHA 9222	LR
Lin, Hou, Wang, and Chen (2017)	China (AS)	Surface water	Granulated Activated Carbon (GAC) + Chlorine dioxide	Not specified	Pilot-scale	Spiked	Method 1204 (USEPA)	Inactivation efficiency
Sommer et al. (2004)	Austria (EU)	Ground water	Ozone + Hydrogen peroxide	Not specified	Pilot-scale	Spiked	Membrane filtration technique	LR
P. Z. Sun et al. (2016)	United States (NA)	Surface water	UV light + H ₂ O ₂	Not specified	Laboratory	Spiked	Not specified (CFU/mL)	LR
			UV + Peroxydisulfat e					
			(PDS)					

LR: Log reduction or also reported as log inactivation level, calculated as log_{10} (N/N₀) where N is the remaining count of *E. coli* after the treatment was applied, and N₀ is the initial count of *E. coli* before the treatment.

Survival percentage: Percentage of destroyed bacteria after treatment, measured with optical density. The concentration of disinfectant to achieve 0% survival (or 100% of inactivation) is reported.

Prevalence after treatment: Percentage of water samples being positive after the treatment was applied.

Percentage removal: Percentage of E. coli cells removed from the water.

Membrane retention: Percentage of *E. coli* cells retained on the microfiltration membrane, which can be interpreted as the percentage removal.

Inactivation efficiency: Calculated using the following equation: $(N_0 - N_t / N_0) \times 100\%$ where N_0 and N_t represent the initial number of *E. coli* and those at the sampling point during the process, respectively.

Table 4. Qualitative analysis of relevant criteria for selection of water treatments to be applied on small-raspberry farms in Chile



(Dandie et al., 2019; Haute, Sampers, Jacxsens, & Uyttendaele, 2015; Masotti, 2011)

Less suitable Medium suitability High suitability

CHAPTER 3: A RISK-BASED APPROACH TO SETTING MICROBIOLOGICAL SPECIFICATION OF *E. COLI* CONTAMINATION IN WATER AND FACILITATING WATER TREATMENT SELECTION FOR SMALL-SIZED RASPBERRY FARMS IN CHILE

I. Abstract

Water used on the pre-harvest stage is an important source affecting the contamination of E. coli in raspberries, an important economic crop produced in Chile for international exports. As a microbial indicator of product quality, E. coli in end products at the border of importing countries determines the border rejection. Various water treatments were critically reviewed in the previous chapter focusing on their disinfection efficacy of E. coli contamination control in water and the feasibility of in-field application with the consideration of Chilean specific conditions. However, a risk-based recommendation on microbial specification of *E. coli* in water and performance criteria of water treatments is not available. To fill the gap, a simulation model was established to quantitatively describe the dynamics of *E. coli* along the fresh raspberry supply chain in a farm-toborder continuum, where factors influencing the contamination changes were integrated. Using the model, the impact of water quality on *E. coli* in fresh raspberries, and subsequently the acceptance rate at the border of importing countries was quantified, based on which performance criteria of water treatment was informed to ensure a target acceptance rate can be met. Usage of surface water can be associated with the lowest acceptance rate of raspberries (75.41%) followed by groundwater (97.62%) and potable water (99.88%), given a compliance standard of $2 \log_{10} \text{CFU/g}$ in the major importing countries of raspberries. Results showed a positive association with a 0.96-log increase of *E. coli* in raspberry for every 1-log increase in the water. Based on the findings, $a \ge 3 \log \frac{1}{2}$

reduction was recommended for groundwater sources, while more effective technologies should be considered for surface water to reach an efficacy of up to 6 log reduction. Some of the treatments evaluated in the study that represents great efficacy, as well as great potential to be implemented to an on-farm level, are UV light, filtration methods, chemical disinfectants, and a combination of them. The present study provides a risk-oriented framework for the selection of effective water treatments based on their efficacy against *E. coli* and the target expectation on the product quality. Our findings can support the small producer's compliance with target markets as part of the ROCP (Raspberry Official Control Program) program to assist the Chilean food safety authorities with science-based recommendations for risk-management strategies.

II. Introduction

Water used at the pre-harvest stage directly contacting the editable parts has been widely recognized as a significant source of bacterial contamination in produces affecting product safety and quality. Implementation of effective and feasible water treatments has been suggested as solutions to ensure food safety and reduce the risks associated with consumers (Sigge et al., 2016). From the perspective of public health impact, large outbreaks of foodborne illnesses can jeopardize consumer confidence in the produce, and subsequently the sales of similar products (Sigge et al., 2016). From an international trade point of view, microbial quality is one of the key determinants, such as the contamination of generic *E. coli* indicating the level of hygienic compliance, for border rejections, which are a highly relevant indicator for the food safety authorities in charge of the inspection.

Raspberry is an important economic crop that provides considerable supports for the livelihood of small-size farms in the central region of Chile, and most raspberries produced in this region are destined for exports. The main market for Chilean raspberries is the United States, followed by Canada and Australia (Figure *9*)(ODEPA, 2018; OEC, 2020).

The U.S. Food and Drug Administration (FDA) is in charge of quality compliance of American food products and inspects imported foods at the border or the port of entry for indications of adulteration or misbranding (Bovay, 2016). The Region of Latin America and the Caribbean (LAC) including Chile, Costa Rica, Panama, and Ecuador have a relatively low rate of rejection in comparison with other regions exporting to the U.S. (Fonseca & Njie, 2009; Henson & Olale, 2010). Although with a low rejection rate overall, it has been reported that fruit and fruit products constitute 10.5% of total rejections of exports in this region (Bovay, 2016). According to FAO and WHO, berries are considered a highly prioritized produce in terms of concerns of microbiological hazards, by considering historical outbreaks, potential for contamination, exposure levels, and potential of control, frequency and severity of disease and trade, and economic impacts (WHO/FAO, 2008) The most common reason for a shipment of fruit and fruit products to be refused was sanitary violations or "filth".

Despite limited records about import refusals of Chilean raspberries, data have shown that the occurrence of border rejection not only has an immediate effect on the economic loss but can also influence the future management actions of the importing country against the exporting country that did not comply with the target market standards. In the U.S. for instance, it was shown that the refusal increase by 62% if there was a refusal of a related product from the same country in the preceding year (Jouanjean, Maur, & Shepherd, 2015). This is relevant for developing countries like Chile, where the food industry represents a great part of its economy, and border rejections are presumed to affect the reputation and trustability of the importing countries in Chilean sanitary standards. Specifically, contamination of *E. coli* is an important microbial criterion for the refusal determination of raspberry products. Compliance standards of *E. coli* relevant to raspberries according to the major importing countries, as well as Chile, are listed by countries in Table 5 and a benchmark of $\leq 2 \log_{10} \text{ CFU/g}$ is widely used.

To control the water-originating *E. coli* contamination in food products, a proactive, riskbased approach to water quality management is highly recommended (WHO, 2016). Table *6* lists the satisfactory quality of water used on farm regulated by the U.S. FDA and the Raspberries Official Control Program (ROCP) in Chile, respectively, but the link between these standards and the probability of exported products being accepted or refused at the border of importing countries is uncertain. In addition, numerous water treatments have been studied over the years, but a risk-based framework supporting the selection of effective technologies is not available.

Hence, the present study was aimed to use a risk-based simulation model to 1) make a linkage between the *E. coli* in water and the contamination in raspberry products; 2) quantify the impact of water quality on the acceptance rate of raspberry products at the border of importing countries; and 3) determine performance criteria for water treatments enabling the achievement of target acceptance rate to facilitate the decision making on water treatment selection. The present study will provide science-based recommendations

to help ensure the small Chilean farmers to satisfactorily comply with export standards and decrease the import refusals. (WHO, 2016)

III. Materials and Methods

The quantitative microbial risk assessment model in the present study was adopted with modification from a previously published study from the same team (Ortúzar et al., 2020). The country-specific model was developed to specifically describe the practices of raspberry production in Chile in a continuum from farm, through collection center, to processing facilities. Country specificity was guaranteed by estimating model input parameters using data collected via a series of surveys of local producers and processors to reflect the most common operating conditions throughout the supply chain in Chile. In the first stage of the collaborative project, the established model was used for the identification of critical control points along the chain that may considerably influence the contamination of *E. coli* in raspberry end products, and water used for pesticide spray on-farm was identified as the major contamination entry point. Hence, in the present study as the second stage of the collaborative project, the model was modified to quantify the impact of *E. coli* contamination in water for pesticide application on the contamination in end products, and to inform water treatment or treatments in combination to enhance the microbial quality of raspberry exports and ensure high acceptance rates at the port of importing countries.

Description of the quantitative simulation model

The model followed a modular process risk model methodology (Nauta, 2001). quantitatively describing the introduction of *E. coli* contamination from various sources and the dynamics of *E. coli* contamination on the fruit under different environmental conditions as moving towards the end of the raspberry supply chain. The final model output is defined as the concentration of *E. coli* in fresh raspberries at the port of importing countries (log_{10} CFU/g). Fresh raspberries are the major food matrix of interest in this study, as they are commonly contaminated with *E. coli* at a significantly higher level compared with frozen products (Ortúzar et al., 2020). Hence, management strategies applied to fresh raspberries will likely warrant an acceptable quality of frozen products.

The chain model connected three modules in a consecutive order of the farm module, collection center module, and processing module, as shown in Figure 10. Estimated contamination from the previous module as the modular output serves as the input of the next module. In the farm module, two contamination sources were considered before harvest, i.e., water for pesticide application and harvesters' hands. Water for irrigation was not considered, as fruits exposed to a relatively large amount of water such as for irrigation purposes are highly sensitive to fungal infection and are unlikely to be harvested. The contamination at the time of harvest was determined by the contamination transferred during pesticide application estimated based on the type of water used for pesticide mixing (W_{type}) , E. coli contamination by water type $(C_{w,bac})$, and volume of water attached on a raspberry (V_{surf}), and the inactivation during the following withholding time between the last application and harvest depending on the length of the withholding period (t_{ap}) and the decay rate of E. coli inactivation (D_{bac}) . During harvest, harvesters' hands are assumed a source of E. coli through cross-contamination, which was considered dual-directional, meaning decrease and increase in contamination on a

berry were simultaneously considered due to the transfer to and from harvesters' hands at the time of fruit-hand touching. After harvest, raspberries are transported from farm to an assigned collection center, hence, potential growth or inactivation of *E. coli* depending on transport time ($t_{trans,f}$), temperature ($T_{trans,f}$), and relevant kinetic parameters were incorporated in this stage. A list of input variables with estimated parameters, data sources, and calculations are listed in Table 7 and kinetic parameters are provided in Table *10*.

In the collection center module, E. coli load changed subject to the holding time (t_{cc}) and temperature at the collection center (T_{cc}) , and time $(t_{trans,cc})$ and temperature at the transport $(T_{trans,cc})$ to the packing plant, which is elaborated in Table 8. When raspberries are received at the processing plants, they are usually held for a transit period (t_{rec}) under ambient temperature (T_{rec}) , then stored in an extended period (t_{cold}) in the cold chambers under refrigeration temperature (T_{cold}) before entering processing chains. At processing, fruits are visually inspected for quality classification and manually packed according to assessed quality. Similar to the harvest process, packers' hands can potentially become a source of *E. coli* at processing through the occurrence of cross-contamination. Besides, *E. coli* on raspberries may proliferate or inactivate depending on the processing time (t_{pack}) and temperature (T_{pack}) . Afterward, packed raspberries are transported to importing countries ($t_{trans,p,fresh}$ and $T_{trans,p,fresh}$), and the acceptance is determined based on the border inspection via E. coli tests at the port. As the final model output, E. coli contamination at the port of importing countries ($C_{ptrans,bac,fresh}$) was estimated, which reflected the cumulative effects passed along from all the upstream steps. Details of the processing module are provided in Table 9.

As described, *E. coli* may proliferate or inactivate along the supply chain, and the increase or decrease in contamination was quantified using growth or survival models listed in Table *10*. In summary, *E. coli* growth was simulated when holding/transport/storage temperature was over 5°C; separate survival models were applied for the temperature ranging from 0 ~ 5°C and below 0°C, as different inactivation rates were observed (Dawn M Knudsen, Sheryl A Yamamoto, & Linda J Harris, 2001).

Measurement of the impact of water quality on raspberry contamination In the baseline model, *E. coli* contamination in fresh raspberries was estimated without applications of any water treatments, by setting LR_{wt} , log reduction due to water treatment as 0. *E. coli* levels in end fresh raspberry products were estimated for different types of water used for pesticide spray, representing the situation in reality that small raspberry farmers may use potable water, groundwater and fresh water given their accessibility.

To quantify the association between water microbial quality and *E. coli* in raspberries, the concentration of *E. coli* in water ($C_{w,bac,afwl}$) was set as 0 (transferred to 10⁻⁹⁹ for computation purpose), 10⁰, 10¹, 10², 10³, 10⁴, 10⁵, 10⁶ CFU/L, and the corresponding contamination in end products were estimated. These values were chosen to capture possible ranges as described by probability distributions of bacterial contamination in various water types. Through this practice, the microbial specification on *E. coli* in water can be determined to ensure the acceptable raspberry quality (2 log₁₀ CFU/g) was not exceeded. The determination of microbial specifications associated with different level of acceptance was achieved by linear interpolation of the curves representing acceptance curves of 99.7%, 99% and 90% in Figure *10*. A level of 99.7% was chosen as the ultimate target acceptance rate of raspberry exports, as this is the level of acceptance that

can be expected when FDA's microbial quality criteria for agriculture water used during growing activities with direct contact with produce are met, i.e., the geometric mean of *E. coli* in water samples of 126 CFU/100mL or less and under a threshold value of 410 CFU/100mL (FDA, 2015).

The expected reduction in *E. coli* contamination was determined for groundwater and surface water, respectively, by setting the log reduction due to water treatment (LR_{wt}) value as 1, 2, 3, 4, 5, and 6 log₁₀ CFU. By doing this, the expected performance criteria of water treatment can be determined by ensuring the ultimate target acceptance rate of raspberry experts as aforementioned is met. The estimation of expected performance criteria in combination with the consideration of effectiveness of available technologies, varying microbial quality of source water and the target food quality management objective.

Modelling and analysis methods

A one-dimension Monte Carlo simulation model by Latin Hypercube Sampling with 50,000 iterations was run to quantify the variability and uncertainty around the model output using @Risk (version 8.0, Palisade Corporation, New York, USA). When multiple simulations were needed for scenario analysis, a fixed seed was chosen to remove the between-simulation difference attributable to randomness, so the observed difference would be solely explained by the changes between scenarios. The correlation of determination (\mathbb{R}^2) of two quantities was determined using functions in StatTools (Palisade Corporation, New York, USA).

IV. Results and Discussion

Baseline model estimates

In the baseline model, the contamination level of *E. coli* in fresh raspberry products at the arrival of importing countries were estimated under the current practices of production and processing in Chile, assuming no water treatment implementation on source water before the use for pesticide application. On average, E. coli can be detected at a level of - $1.63 \log_{10} \text{CFU/g}$ (90% CI: -1.64 ~ -1.61), but a wide range was observed due to the integration of uncertainty and variability of input variables (10th percentile: -4.30; 90th percentile: $1.21 \log_{10} \text{ CFU/g}$). When the usage of different water sources were modeled, the mean contamination in raspberries changed to -4.33, -1.71, and $1.29 \log_{10} \text{CFU/g}$, if potable water, groundwater, and surface water were used, respectively. The probability distributions associated with different water usage scenarios were overlaid in Figure 10. At a national level, all three types of water can be used on raspberry farms, and this resulted in a multimodal distribution with three distinct local peaks (filled with grey), and positions of the peaks aligned well with the three distributions representing potable water (filled with turquoise), groundwater (filled with green), and surface water (filled with brown). In addition, the center peak aligning with the groundwater distribution constitutes the greatest probability mass, which can be explained by the fact that the majority of raspberry farms in Chile (71%, Table 7) use groundwater for pesticide application. These results qualitatively indicate a strong association between E. coli contamination in water and that in the end products.

Impact of water quality on *E. coli* contamination in raspberries

To further quantify the association, various levels of *E. coli* levels in water and corresponding expected means of contamination in raspberries were plotted in Figure *11*. Based on simulated results, for every log increase in the contamination in water, a 0.96 log increase can be expected in fresh raspberries (95% CI: $0.93 \sim 1.00 \log$), with an R² of 0.998.

Acceptance rate, the probability of exported goods being approved into the market of importing countries, is an important measure for setting the performance goal, and a contamination level of *E. coli* equal to or less than $2 \log_{10} \text{ CFU/g}$ is considered acceptable for fresh raspberries. When the water quality meets the standard of FDA of the U.S., one of the major importing countries, it was estimated that the acceptance rate can reach 99.7%, indicative of only a 0.3% of chance when exports would fail to pass the microbial expectations. The probability distribution of E. coli in water was determined as a lognormal distribution (RiskLognorm2 (2.10037,3.05551), truncated at a maximum of 410, in CFU/100mL) based on FDA's requirement via a trial-and-error approach. In the present study, this was selected as the optimal acceptance level for the following analysis. Based on Figure 11 different levels of acceptance require different minimum requirements of *E. coli* contamination in the water used on farm. To reach a 99.7% acceptance rate, the mean contamination in water needs to be controlled to a level below 3.67 CFU/L. Lower expected acceptance rates can be achieved by less restrictive control. For example, 99% and 90% acceptant rates corresponded to the maximum allowable mean concentrations in water as 1.64 and 4.63 \log_{10} CFU/L. As shown in Figure 12 as the water contamination increases and beyond $2 \log_{10} CFU/L$, the acceptance rate can be

exponentially decreased. It is worth noting that it was simulated that the acceptance bar could not cross the 99.9% acceptance curve, even the water contamination level was set to 10^{-99} , an input value representing no *E. coli* in water. In the model, water is one possible contamination source, and the contamination was also considered to be introduced through the cross-contamination from workers' hands that could occur both during harvest and processing. Albeit a minimum relative contribution, control measures should be implemented to eliminate the occurrence of cross-contamination and/or lower the transferred load during a cross-contamination event to further increase the raspberries acceptance rate.

Risk-based recommendation of water treatment technologies The criteria of expected performance efficacy were estimated as the log reductions required to ensure the target acceptance rate can be met for groundwater and surface water, respectively. As shown in Table *11* using potable water will guarantee a promising acceptance rate of 99.88%. In the condition without any water treatments, groundwater can be associated with an acceptance rate of 97.62%, but 24.59% of exports is likely to be rejected when raw surface water is used. To reach the target acceptance rate (99.74%), water treatment (single or combined) that can exert a 3-log reduction needs to be implemented, while a net efficacy of 6 logs is desired for the farms with access to a surface water source before the water is ready to be used for pesticide application. In conjunction with the findings in Chapter 2, treatments with desired efficacy are listed

below for the surface water and groundwater scenarios, respectively. It should be noted that the results from the efficacy-oriented systematic review heavily depend on the quality of the water considered in the study design, e.g., initial *E. coli* concentration and process parameters (such as the dose used, contact time, pore size for membrane filtration, source of UV light). Hence, the reported efficacy in selected studies does not necessarily imply the maximum capacity of the studied technology. However, these results can still be used as guidance for water treatment selection.

Surface water source

Based on the risk-based evaluation in this Chapter, surface water sources require a highly effective decontamination treatment with the efficacy round 6 log reduction. The treatments reported with the target efficacies are:

- Chemical disinfectants: monochloramine + cupric chloride (Straub, Gerba, Zhou,
 Price, & Yahya, 1995); and ozone (Sommer et al., 2004).
- UV light (Garvey, Hayes, Clifford, & Rowan, 2015) two low-pressure UVClamps; two 30 W low-pressure UV lamps (Younis, Mahoney, & Palomo, 2018).
- Enhanced Slow Sand Filtration (Weber-Shirk, 2002).
- Membrane filtration: Microfiltration (Coccagna et al., 2001); Ultrafiltration (Huang, Jacangelo, & Schwab, 2011).
- Combined treatments: Ozone + chlorine (de Souza & Daniel, 2011) and ozone + hydrogen peroxide Sommer et al., 2004).

The initial concentration of generic *E. coli* in the surface water source in our model was simulated up to 10^6 CFU/L Table *10*. Based on the systematic review, treatments that were observed to have high efficacy were usually tested in studies designed with high initial concentrations of *E. coli* (can be as high as 10^9 CFU/L, and the treatments cover chemical disinfectants (including ozone), UV light, and microfiltration (Garvey et al.,

2015; Sommer et al., 2004; Straub et al., 1995), suggesting the suitability of these treatments to be implemented in water sources with a high concentration of *E. coli*.

Groundwater source

A 3-log reduction is expected for groundwater. According to the literature reviewed, and as it is specified in Annex I, *the* majority of the treatments can achieve equal or greater efficacy than three log reduction, except for:

- Some chemical disinfectants: Sodium dichloro –s-triazine-trione (2.5%; 1.4% available chlorine (Kfir, Bateman, & Coubrough, 1995); coagulant/disinfection products (CDPs) sodium dichloroisocyanurate at high organic content water (Legare-Julien, Lemay, Vallee-Godbout, Bouchard, & Dorea, 2018); Chlorine dioxide ClO₂ solution AGRI DIS® (Lopez-Galvez, Gil, Meireles, Truchado, & Allende, 2018); and cupric chloride (Straub et al., 1995).
- A UV light system (Liu & Zhang, 2006) only achieved a 1.46 log reduction, but it is important to highlight that the system achieved a higher efficacy (3.14 log reduction) when the turbidity in water was improved (from 12 NTU to 4 NTU).
- A high level of turbidity (57.45 NTU) also prevented a higher efficacy in an ultrafiltration membrane system (Galvañ et al., 2014), affirming the importance of the monitoring of turbidity levels in water when the treatment is applied.
- The category of miscelanous filtration (or adsorptions) options as the use of kaolin clay loaded with silver nanoparticles or carbon nanotubes do not represent a good option in terms of *E. coli* efficacy (Hassouna, ElBably, Mohammed, & Nasser, 2017).
Riverbank filtration is a natural process and not a treatment as such, and can also be dismissed as an option for groundwater treatment as the maximum log removal achieved was 1.74.

The interventions above mentioned can be preliminarily dismissed from being implemented at farms where groundwater is used for the application of pesticides. The same interventions can be automatically dismissed for their use in surface water, as the required log removal for this source would not be achieved.

The water treatments identified to be suitable for surface water will be sufficient for groundwater. Additionally, the following treatments can also be considered for groundwater, which can achieve a \geq 3-log removal but insufficient to surface water.

- Ferrates (Fe (IV) (Cho, Lee, Choi, Chung, & Yoon, 2006; El-Maghraoui,
 Zerouale, Ijjaali, & Benbrahim, 2013); chlorine (de Souza & Daniel, 2011);
 coagulant/disinfection products (CDPs) sodium dichloroisocyanurate based
 (Legare-Julien et al., 2018), ozone (de Souza & Daniel, 2011; Sommer et al., 2004; Zuma, Lin, & Jonnalagadda, 2009).
- UV light (40W Low-Pressure Mercury Lamp) (Liu & Zhang, 2006), Mercury-free plasma lamp (Prakash et al., 2017); 40 W low-pressure UV lamp (P. Z. Sun, Tyree, & Huang, 2016); low-pressure UV lamps (Trojan Technologies, Canada) (Three lamps) UVT 90% (W. J. Sun & Liu, 2009); and two low-pressure UVC-lamps (Younis et al., 2018; Younis, Mahoney, & Yao, 2019).
- Full-scale bio sand filters (Napotnik, Baker, & Jellison, 2017).
- Combined technologies (UV light/H₂O₂; UV light/Peroxydisulfate (P. Z. Sun et al., 2016).

The initial concentration of generic *E. coli* in groundwater source in our model was specified up to 1,000 CFU/L (WHO, 2011). Generally, the initial concentration of *E. coli* in the water sources in which treatments were evaluated for this section (3 to < 6 log reduction), stayed within a close range, indicating a good fit for the initial concentration of the water used by the majority of farmers for the application of pesticides.

V. Conclusion

The scope of this study is focused on establishing risk-based water management options that allow raspberry farmers to comply with international standards based on generic *E. coli* concentration on fresh raspberries. Chile, like most Latin American countries, has limited technologies for reducing microbial contamination at a pre-harvest stage, especially in small-scale farms. Various obstacles, including the lack of investment in the agri-industry, have been identified as a major barrier to economic development (Da Silva, 2009). To continue maintaining economic growth within the food industry, strengthen the national food safety system, actions need to be taken towards the consideration of science- and risk-based information to guide the investment of technologies at the primary production level.

The present study is an example demonstrating the application risk assessment framework to informing water treatment options to strengthen international trade of agriculture goods in Chile. Results from this study wish to provide critical and extensive options for Chilean Food safety authorities that support the production of raspberries in Chile.

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Figures

Total: \$109M					
United	Canada	France 5.33%	1.48%		Japan 2.63%
States		Netherlands 3.42%		 	
		Australia	a	New	
38.7%	20.1%	12.2%	6	2.78%	

Figure 9. Main importing countries of Chilean raspberries (OEC, 2020)



Figure 10. Simulated *E. coli* contamination in fresh raspberries at the port of importing countries with different types of water used for pesticide application



Figure 11. Changes in *E. coli* concentration in fresh raspberries at the port of importing countries as the contamination in water used for pesticide application increases. The contamination limits of *E. coli* in water are estimated at points where the lines representing different acceptance rates intersect with the horizontal line of acceptable *E. coli* contamination in raspberries.



Figure 12. Impact of *E. coli* contamination in water used for pesticide application and the acceptance rate. Acceptance rate refers to the probability of exported goods being approved into the market of destined countries. In this case, the maximum allowable contamination of *E. coli* in fresh raspberries is 2 log₁₀ CFU/g. Acceptance rates are labeled given specific contamination levels of *E. coli* in water.

Country	Commodity	Satisfactory Assessment	Reference
Canada	Frozen prepackaged cut fruits and berries	$\leq 10^2 \text{ CFU/g or}$ MPN/g	(Agency, 2019)
Australia	Berries: ready-to-eat that will not undergo further processing	¹ n=5, c=2, m=10, M=100 CFU/g	(Australia, 2020)
Chile	Fresh fruit	2-3 log CFU/g	(MINSAL)
Chile	Frozen fruit	1-2 log CFU/g	(MINSAL)

Table 5. E. coli benchmark of interest in raspberries

n = the minimum number of sample units that must be examined from a lot of food;

c = the maximum allowable number of defective sample units i.e. that have counts between 'm' and 'M';

m = the acceptable microbiological level in a sample unit;

M = the level which when exceeded (i.e. the level is greater than M) in one or more samples would cause the lot to be rejected.

Guideline/Regulation	Country	Water	Satisfactory Assessment	Reference
Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption; Final Rule	United States	Agricultural water used during growing activities for covered produce using a direct water application method	A geometric mean (GM) of 126 CFU per 100 mL of water (GM is a measure of the central tendency of the water quality distribution) A statistical threshold value (STV) of the agricultural	(FDA, 2015)

Table 6. E. coli benchmark of interest in agricultural water

			water samples of 410 or less CFU per 100 mL of water	
Raspberries Official Control Program (ROCP)	Chile	Water for sanitary use and the application of phytosanitary products and fertilizers	Critical limit: No detectable <i>E. coli</i> in 100 mL and no detectable fecal coliforms in 100 mL Detection limit: 2 MPN	(SAG, 2011)

Table 7. List of variables, values, distributions, and calculations used in the farm module for fresh raspberries

Variable	Description	Value/Distribution/Calcul ation ¹	Unit	Reference
Pre-harvest	t operations			
W_{type}	Type of water used	Discrete		Survey
	for pesticide			
	applications			
	Groundwater	71% (coded as 1)		
	Surface water	15% (coded as 2)		
	Potable water	14% (coded as 3)		
$C_{w1,bac}$	Bacterial	Uniform(0,1000)	CFU/L	WHO (2004)
	contamination in			
	groundwater			
$C_{w2,bac}$	Bacterial	Uniform(6000,10 ⁶)	CFU/L	WHO (2004)
	contamination in			
	surface water			
$C_{w3,bac}$	Bacterial	Uniform(0.01,0.1)	CFU/L	INN (2005)
	contamination in			
	potable water			
$C_{w,bac}$	Bacterial	$C_{wl,bac}$ if $W_{type} = 1$,	CFU/L	
	concentration in	$C_{w2,bac}$ if $W_{type} = 2$,		
	spray depending on	$C_{w3,bac}$ if $W_{type} = 3$		
	the water type			
LR_{wt}	Log reduction in <i>E</i> .	0	Log ₁₀ CFU	
	coli due to water			
	treatment(s)			

$C_{w,bac,afwt}$	Resulting bacterial contamination in	$C_{w,bac}$ / (10 ^A LR_{wt})	CFU/L	
	water after water			
Vsurf	Volume of spray attaching on a	BetaGeneral(2.3976,2.1805, 0.0000364321,0.00021032)	L/berry	Jacxsens et al. (2017)
t _{ap}	Withholding period between the last application and	Pert(0,30,120)	Days	Survey
D _{bac}	the harvest Bacterial decay rate	Triangular(0.008,0.019,0.03 9)	Log ₁₀ CFU/day	Danyluk and Schaffner (2011)
N _{harv,bac}	Number of bacteria at the time of harvest	$\frac{10^{[log(C_{w,bac,afwt} * V_{surf}) - D_{bac} * t_{ap}]}{D_{bac} * t_{ap}]}$	CFU/berry	
Harvest pro	ictices (cross-contami	ination)		
Phand,bac	Bacterial prevalence on	Beta(7,35)		de Aceituno et al. (2016)
f_{prod}	Transferred proportion per touch from	Beta(15.64,41.94)		Bouwknegt et al. (2015)
ω_{touch}	produce to hand Surface area of hands that touch	2.1	cm ²	Bouwknegt et al. (2015)
ω_{hand}	Total surface area of one side of one hand	245	cm ²	EPA (2011)
ω_{prod}	Surface area of produce	Normal(1064,167)/100	cm ²	Bouwknegt et al. (2015)
fhand	Transferred proportion per touch from hand to produce	Lognormal(-8.34,0.58)		Bouwknegt et al. (2015)
Nhand,bac	Number of bacteria on harvester's hands	$10^{Uniform(1,1.9)} * P_{hand,bac}$	CFU/hand	de Quadros Rodrigues et al. (2014)
$N_{fcross,bac}$	Number of bacteria after harvesting	$N_{harv,bac} - f_{prod} \frac{\omega_{touch}}{\omega_{prod}} N_{harv,bac} + f_{hand} \frac{\omega_{touch}}{\omega_{hand}} N_{hand,bac}$	CFU/berry	x - /
Tuanantf	Constant formers to a all a sting	a anatan		

Transport from farm to collection center

<i>t</i> _{trans,f}	Transport time	Pert(0.00347,0.08333,1)	Days	Survey
	from a farm to its			
	associated			
	collection center			
T _{trans,f}	Temperature	Pert(12,28,28)	°C	Survey
-	during transport of			
	raspberries from			
	farm to collection			
	center			
$\mu_{gr,bac}$	Temperature-	See Table 10		
	dependent bacterial			
	growth rate			
N _{ftrans,bac}	Number of bacteria	$Log(N_{fcross,bac}) + \mu_{gr,bac} *$	Log ₁₀	
	after transport from	t _{trans,f}	CFU/berry	
	farm to collection			
	center			

Table 8. List of variables, values, distributions, and calculations used in the collection center module for fresh raspberries

Variable	Description	Value/Distribution/Calculation	Unit	Reference
Holding at th	he collection center			
t_{cc}	Time that raspberries	Pert(0.042,0.042,0.29)	Days	Survey
	stay in the collection			
-	center		.	a
T_{cc}	Temperature in the collection center	Pert(0.5,20,30)	°C	Survey
$\mu_{gr,bac}$	Temperature-	See Table 10		
or	dependent bacterial			
μ redrfg,bac	growth or inactivation rate			
$Log(N_{cc,bac})$	Number of bacteria	$Log(N_{ftrans,bac}) + \mu_{gr,bac} * t_{cc} \text{ if } t_{cc} \ge 5$	Log ₁₀ CFU/berr	
	at collection center	$Log(N_{firans,bac}) - \mu_{redrfg,bac} * t_{cc} \text{ if } 0 \le t_{cc}$ <5	y	
Transport fr	om collection center to t	the packing plant		
T _{trans,cc}	Temperature during transport from collection center to	Uniform(1,27)	°C	Survey
	packing plant			
t _{trans,cc}	Commute time from collection center to packing plant	Pert(0.017,0.67,5)	Days	Survey
Log(N _{cctrans}	Number of bacteria	$Log(N_{cc,bac}) + \mu_{gr,bac} * t_{trans,cc}$ if $t_{cc} \ge 5$	Log ₁₀	
,bac)	after transport from	or	CFU/berr y	

collection center to	$Log(N_{cc,bac})$ - $\mu_{redrfg,bac} * t_{trans,cc}$ if $0 \le t_{cc}$
packing plant	< 5

Variable	Description	Value/Distribution/Calculation	Unit	Reference
Received at t	the packing plant			
t _{rec}	Waiting time	Pert(0,0.0069,0.0417)	Days	Survey
	when receiving			
	raspberries			
T_{rec}	Average	Pert(1,25,27)	°C	Survey
	temperature in			
	receiving space		_	
$Log(N_{rec,bac})$	Number of	$Log(N_{cctrans,bac}) + \mu_{gr,bac} * t_{rec} \text{ if } t_{cc} \geq$	Log ₁₀	
)1	bacteria after	5	CFU/berry	
	waiting time	or		
	after receipt at	$Log(N_{cctrans,bac}) - \mu_{redrfg,bac} * t_{rec}$ if 0		
G (1	the packing plant	$\leq t_{cc} < 5$		
Storage in th	Time that	\mathbf{D}_{-1}	D	C
<i>t_{cold}</i>	11me that	Pert(0.083,0.104,0.5)	Days	Survey
	in the cold			
	ahambar			
T	Target	$P_{ort}(0,0,8)$	°C	Survey
I cold	temperature in	1 en(0,0,8)	C	Survey
	the cold chamber			
Log(Neta has	Number of	$L_{OO}(N_{rea}, h_{ac}) + \mu_{ar}h_{ac} * t_{outd}$ if $t_{ac} > 5$	$L_{0}\sigma_{10}$	
$)^1$	bacteria after	$\frac{1}{2} Or$	CFU/berry	
/	cold storage at	$Log(N_{rec,bac}) - \mu_{redrfg,bac} * t_{cold} if 0 <$	ereing	
	packing plant	$t_{cc} < 5$		
Processing p	practices (cross-con	tamination)		
Phand,bac	Bacterial	Beta(7,35)		de
	prevalence on			Aceituno
	packers' hands			et al.
	-			(2016)
$N_{food,bac}$	Number of	$P_{hand,bac} * 10^{Uniform(1,1.9)}$	CFU/hand	de
	bacteria on			Quadros
	packers' hands			Rodrigues
				et al.
				(2014)
$N_{pcross,bac}$	Number of	$N_{rec,bac} - f_{prod} \frac{\omega_{touch}}{\omega} N_{rec,bac} +$	CFU/berry	
	bacteria on	$f = \omega_{touch N}$ referring to		
	raspberries after	Jhand $\frac{\omega_{hand}}{\omega_{hand}}$ Infood, bac, felefilling to		
	classifying and	Table 7 for variables not defined in		
_	packing	this table		
Processing p	ractices (growth or	inactivation)	-	~
t_{pack}	Processing time	Pert(0.017,0.125,0.125)	Days	Survey

T_{pack}	Temperature inside processing area	Pert(-1,8,13)	°C	Survey
$\log(N_{pack,ba})^{1}$	Number of bacteria after whole processing stage	$Log(N_{pcross,bac}) + \mu_{gr,bac} * t_{pack} \text{ if } t_{cc}$ ≥ 5 or $Log(N_{pcross,bac}) - \mu_{redrfg,bac} * t_{pack} \text{ if } 0$ $\leq t_{cc} < 5$	Log ₁₀ CFU/berry	
Transport fr	om packing plant to	destination (fresh product only)		
t _{trans,p,fresh}	Time for	Pert(0.083,0.1667,6)	Days	Survey
$T_{trans,p,fresh}$	transport to destination for fresh raspberries Temperature of the cooling truck during transport of fresh raspberries	Uniform(0,5)	°C	Survey
Mberry	Average weight of a raspberry	4	g	Iannetta et al. (2000)
$C_{ptrans,bac,fres} {h^1 \over h^1}$	Concentration of bacteria upon arrival at destination for fresh raspberries	$(Log(N_{pack,bac}) - \mu_{redrfg,bac} * t_{trans,p,fresh}) / M_{berry}$	Log ₁₀ CFU/berry	`` <i>`</i>

¹BetaGeneral(alpha1, alpha2, min, max) and Beta (alpha1, alpha2) define distributions with alpha1 and alpha2 as shape parameters, min, and max defining the distribution's range.

Lognormal(mean, SD) and Normal(mean, SD) define distributions with mean and standard deviation as position and spreading parameters based on the data on the original scale.

Pert(min, most likely, max) and Triangular(min, most likely, max) define distributions determined by parameters of the minimum, maximum, and most likely values

Uniform(min, max) defines a distribution determined by parameters of the minimum and maximum values.

Variable	Description	Value/Distribution/Calculation	Unit	Reference
Received at t	the packing plant			
t _{rec}	Waiting time when receiving	Pert(0,0.0069,0.0417)	Days	Survey
T_{rec}	Average temperature in	Pert(1,25,27)	°C	Survey
$Log(N_{rec,bac})^1$	Number of bacteria after waiting time after receipt at the packing plant	$Log(N_{cctrans,bac}) + \mu_{gr,bac} * t_{rec} \text{ if } t_{cc} \ge 5$ or $Log(N_{cctrans,bac}) - \mu_{redrfg,bac} * t_{rec} \text{ if } 0$ $\leq t_{cc} < 5$	Log ₁₀ CFU/berry	
Storage in th	ne cold chamber			
t _{cold}	Time that raspberries stay in the cold chamber	Pert(0.083,0.104,0.5)	Days	Survey
T_{cold}	Target temperature in the cold chamber	Pert(0,0,8)	°C	Survey
$\log(N_{stg,bac})^1$	Number of bacteria after cold storage at packing plant	$Log(N_{rec,bac}) + \mu_{gr,bac} * t_{cold} \text{ if } t_{cc} \ge 5$ or $Log(N_{rec,bac}) - \mu_{redrfg,bac} * t_{cold} \text{ if } 0 \le t_{cc} < 5$	Log ₁₀ CFU/berry	
Processing p	Bootorial	Pote(7.25)		do
F hand,bac	prevalence on packers' hands	Beta(7,55)		Aceituno et al. (2016)
Nfood,bac	Number of bacteria on packers' hands	Phand,bac * 10^Uniform(1,1.9)	CFU/hand	de Quadros Rodrigues et al. (2014)
Npcross,bac	Number of bacteria on raspberries after classifying and packing	$N_{rec,bac} - f_{prod} \frac{\omega_{touch}}{\omega_{prod}} N_{rec,bac} + f_{hand} \frac{\omega_{touch}}{\omega_{hand}} N_{food,bac}$, referring to Table 7 for variables not defined in this table	CFU/berry	(2017)
rocessing p	Droposing time	macuvation) Dest(0.017.0.125.0.125)	Dava	Cumuon
<i>t</i> _{pack}	Processing time	Pert(0.017,0.125,0.125)	Days	Survey

Table 9. List of variables, values, distributions and calculations used in the packing plant module for fresh raspberries

T_{pack}	Temperature inside processing area	Pert(-1,8,13)	°C	Survey
$\log(N_{pack,ba})^{1}$	Number of bacteria after whole processing stage	$Log(N_{pcross,bac}) + \mu_{gr,bac} * t_{pack} \text{ if } t_{cc}$ ≥ 5 or $Log(N_{pcross,bac}) - \mu_{redrfg,bac} * t_{pack} \text{ if } 0$ $\leq t_{cc} < 5$	Log ₁₀ CFU/berry	
Transport fr	om packing plant to	destination (fresh product only)		
t _{trans,p,fresh}	Time for	Pert(0.083,0.1667,6)	Days	Survey
$T_{trans,p,fresh}$	transport to destination for fresh raspberries Temperature of the cooling truck during transport of fresh raspberries	Uniform(0,5)	°C	Survey
Mberry	Average weight of a raspberry	4	g	Iannetta et al. (2000)
$C_{ptrans,bac,fres} {h^1}$	Concentration of bacteria upon arrival at destination for fresh raspberries	$(Log(N_{pack,bac}) - \mu_{redrfg,bac} * t_{trans,p,fresh}) / M_{berry}$	Log ₁₀ CFU/berry	

¹For the calculation of these variables refer to Table *10* for parameters and equations for $\mu_{gr,bac}$, $\mu_{redrfg,bac}$, $\mu_{redfrz,bac}$, and $\mu_{red,vir}$.

Table 10. Parameters and calculations for temperature-dependent microbial growth or survival models.

Variable	Description	Value/Distribution/Calculation	Unit
Bacterial grow	wth model for temperature or	ver 5°C	
$\mu_{gr,bac}$	Growth rate	$(b^*(T-T_0))^2$	Log_{10}
			CFU/day
Т	Temperature of modelled	See Table 7, Table 8 Table 9	°C
	step		
To^1	Temperature constant 1	2.628	°C
b^1	Temperature constant 2	0.0616	Sqrt(log ₁₀
			CFU/day)/°C
t	Time of modelled step	See Table 7, Table 8, Table 9	Days
$Log(N_{i,bac})$	Initial contamination	Output from previous step	Log ₁₀
			CFU/berry

$Log(N_{i+1,bac})$	Final contamination	$Log(N_{i,bac}) + \mu_{gr,bac} * t$	Log ₁₀
			CFU/berry
Bacterial surv	ival model for temperature 0	~ 5°C	
$\mu_{\it redrfg,bac}^2$	Reduction per day	0.21	Logs/day
t	Time of modelled step	See Table 7, Table 8, Table 9	Days
$Log(N_{i,redrfg,ba})$	Initial contamination	From previous step	Log_{10}
c)			CFU/berry
$Log(N_{i+1, redrfg})$	Final contamination	$Log(N_{i,redrfg,bac})$ - $\mu_{redrfg,bac} * t$	Log ₁₀
<i>,bac</i>)			CFU/berry
Bacterial surv	ival model for temperature b	elow 0°C	
$\mu_{redfrz,bac1}^2$	Reduction per day, less	1.34	Logs/day
	than or equal to 1 day at		
	the freezing temperature		
μ redfrz,bac2 ²	Reduction per day, more	0.05	Logs/day
	than 1 day at the freezing		
	temperature		
t	Time of the modelled step	See Table 7, Table 8, Table 9	Days
Log(Ni, redfrz, ba	Initial contamination	From previous step	Log ₁₀
c)			CFU/berry
$Log(N_{i+1, redfrz})$	Final contamination	$Log(N_{i,redfrz,bac}) - \mu_{redfrz,bac1} * t$ if t	Log_{10}
<i>,bac</i>)		≤ 1	CFU/berry
		or	
		$Log(N_{i,redfrz,bac})$ - $\mu_{redfrz,bacl} * 1$ -	
		$\mu_{redfrz,bac2} * (t-1)$ if $t > 1$	

¹Parameters and equations are adopted from Danyluk and Schaffner (2011). ²Parameters were estimated from D. M. Knudsen, S. A. Yamamoto, and L. J. Harris (2001). Table 11. Association between the water treatment performance (log reduction in *E. coli* contamination in log_{10} CFU) given different types of water used for pesticide application and the probability of exported raspberries with a quality equal to or higher than microbial specification set by importing countries (100 CFU/g fresh raspberries)

	Probability of exported raspberries with a quality equal to or								
Water treatment	higher than microbial specification set by importing countries,								
performance in		100 CFU/g fresh raspberries							
raducing E cali (log.c				Water with					
CEU	Groundwator	Surface	Potable	quality					
CFU)	Gioundwater	water	water	meeting FDA					
				standard ¹					
0 (no treatment)	97.62%	75.41%	99.88%	99.74%					
1	99.05%	88.59%	-	-					
2	99.65%	94.63%	-	-					
3	99.84%	97.68%	-	-					
4	_2	98.98%	-	-					
5	-	99.61%	-	-					
6	-	99.85%	-	-					

¹FDA's microbial quality criteria for agriculture water used during growing activities with direct contact with produce require 1) geometric mean of *E. coli* in water samples of 126 CFU/100mL or less, and 2) under a threshold value of 410 CFU/100mL (FDA, 2015). The acceptance rate achieved by using water meeting the standard is considered the ultimate target.

²No further log reduction is needed, as the ultimate target of acceptance rate is achieved at a lower level of water treatment performance.

Annex I

Summary of water treatment efficacy against *E. coli* on freshwater sources

Reference	<i>E. coli</i> strain	Design Factors (Water quality parameters)	Disinfectant	Dose (mg/L)	Residual concentration (mg/L)	Contact time	Efficacy	
Cho, Lee, Choi, Chung, and	<i>E. coli</i> ATCC 8739	pH: 5.6-8.2 Temperature: 25 °C	Fe(VI)	1.4	-	5 min	3 LR	
Yoon (2006)		Dissolved Organic Carbon (DOC): 2.81 mg/L						
		Initial <i>E. coli</i> Concentration: 3 x 10 ⁵ CFU/mL						
(Cho et al.,	E. coli	pH: 5.6-8.2	Fe(VI)	6.25	-	1 min	3 LR	
2006)	ATCC 8739	Temperature: 25 °C						
		DOC: 2.81 mg/L						
	E. coli ATCC	Initial <i>E. coli</i> Concentration: 3 x 10 ⁵ CFU/mL						
(de Souza &	E. coli	pH:7.2-7.8	Chlorine	5	-	20 min	3.5 LR	
Daniel, 2011)	ATCC 11229	Ca CO ₃ : 80-108mg/L						
		Turbidity (NTU): 0.23- 0.69						
		Initial <i>E. coli</i> Concentration: 10 ⁸ CFU/100mL						
(El-	E. coli	pH: 8	Na ₂ FeO ₄	5	-	1,440	Complete	
Maghraoui, Zerouale,		Temperature: 37°C				min (1 day)	inactivation of 0.171	
Ijjaali, & Benbrahim, 2013)		Initial <i>E. coli</i> concentration: 0.171 optical density					optical density of <i>E. coli</i>	
(El-	E. coli	pH: 8	Na ₂ FeO ₄	30	-	1,440	3 LR	
Maghraoui et al., 2013)		Temperature: 37°C				min (1 day)	(>99.9%)	
		Initial <i>E. coli</i> concentration: 0.171 optical density						

(Ferreira, Luz, & Buss, 2016)	E. coli	Initial <i>E. coli</i> concentration: 1011.2 MPN/mL	Calcium hypochlorite	170	-	2,880 min (2 days)	95% wat samples negative
(Ferreira et al., 2016)	E. coli	Initial concentration of <i>E. coli</i> : Max 456.9 MPN/mL	Calcium hypochlorite	170	-	15 days	80% wat samples negative
(Ferreira et al., 2016)	E. coli	Initial <i>E. coli</i> concentration: Max 791.5 MPN/mL	Calcium hypochlorite	170	-	30 days	65% wat samples negative
(Kfir, Bateman, & Coubrough, 1995)	E. coli	-	Sodium dichloro –s- triazine- trione (2.5%; 1.4% available chlorine)	-	-	10 min	2 LR
(Legare- Julien, Lemay, Vallee- Godbout, Bouchard, & Dorea, 2018)	E. coli	pH: 7.1 \pm 0.1 Turbidity (NTU):4.8 \pm 0.7 TOC: 8 (mg/L) Temperature: 20°C Initial <i>E. coli</i> concentration: 2.6x10 ² (0.9 to 7.9) x 10 ² MPN/100mL	Coagulant/di sinfection products (CDPs) Sodium dichloroisoc yanurate based	AQS tablet/10 L	0.2mg/L (after 24h)	30 min	2.5 LR
(Legare- Julien et al., 2018)	E. coli	pH: 6.7 ± 0.2 Turbidity (NTU): 3.9 ± 3.2 TOC: 4 (mg/L) Initial <i>E. coli</i> concentration: $2.7x10^{2}(1.9 \text{ to } 4.0) \times 10^{2}$ MPN/100mL	Coagulant/di sinfection products (CDPs) Sodium dichloroisoc yanurate based	AQS tablet/10 L	0.2mg/L (after 24h)	30 min	5.1 LR
(Lopez- Galvez, Gil, Meireles, Truchado, & Allende, 2018)	Culturabl e <i>E. coli</i>	Initial <i>E. coli</i> concentration: <1-100 CFU/100mL	Chlorine dioxine ClO ₂ solution AGRI DIS®	<1	<1	-	0.2-0.3 L
(Lopez- Galvez et al., 2018)	Viable <i>E.</i> <i>coli</i>	Initial <i>E. coli</i> concentration: <1-100 CFU/100mL	Chlorine dioxine ClO ₂ solution AGRI DIS®	<1	<1	-	No reduction
(Reitz, Roncarati, Shock, Kreeft, &	E. coli	Initial <i>E. coli</i> concentration: 26-< 2,420 MPN/100mL	Chlorine dioxide	1	-	30 min - 2 hr	No E. co detected

Klauzer, 2015)						after treatm
(Reitz et al., 2015)	E. coli	Initial <i>E. coli</i> concentration: 33-548 MPN/100mL	Chlorine dioxide	3 -	30 min - 2 hr	No E. detecto after treatm
(Straub, Gerba, Zhou, Price, & Yahya, 1995)	E. coli	 pH: 6.8 CaCO₃ Calcium hardness: 96 mg/L CaCO₃ Total hardness: 120 mg/L Turbidity (NTU): 0.08 Total Dissolved solids (TDS): 210 mg/L Electrical conductivity 0.43 mS/cm Initial <i>E. coli</i> concentration: 10⁶ CFU/ml 	Monochlora mine + cupric chloride	Monochl - oramine: 2.5 Cupric chloride: 0.4	10 min	6 LR
(Straub et al., 1995)	E. coli	 pH: 6.8 CaCO₃ Calcium hardness: 96 mg/L CaCO₃ Total hardness: 120 mg/L Turbidity (NTU): 0.08 TDS: 210 mg/L Electrical conductivity 0.43 mS/cm Initial <i>E. coli</i> concentration: 10⁶ CFU/ml 	Monochlora mine + cupric chloride	Monochl - oramine: 2.5 Cupric chloride: 0.4	10 min	6 LR
(Straub et al., 1995)	E. coli	pH: 6.8 CaCO ₃ Calcium hardness: 96 mg/L CaCO ₃ Total hardness: 120 mg/L Turbidity (NTU): 0.08 TDS: 210 mg/L Electrical conductivity 0.43 mS/cm	Monochlora mine + cupric chloride	Monochl - oramine: 2.5 Cupric chloride: 0.8	10 min	6 LR

		Initial <i>E. coli</i> concentration: 10 ⁶ CFU/ml					
(Straub et	E. coli	pH: 6.8	Monochlora	Monochl	-	20 min	6 LR
al., 1995)		CaCO ₃ Calcium hardness: 96 mg/L	mine + cupric chloride	oramine: 2.5			
		CaCO ₃ Total hardness: 120 mg/L		Cupric chloride:	hloride:		
		Turbidity (NTU): 0.08		0.8	0.8		
		TDS: 210 mg/L					
		Electrical conductivity 0.43 mS/cm					
		Initial <i>E. coli</i> concentration: 10 ⁶ CFU/ml					
(Straub et	E. coli	pH: 6.8	Monochlora	5	-	60 min	6 LR
al., 1995)		CaCO3 Calcium hardness: 96 mg/L	mine				
		CaCO ₃ Total hardness: 120 mg/L					
		Turbidity (NTU): 0.08					
		TDS: 210 mg/L					
		Electrical conductivity 0.43 mS/cm					
		Initial <i>E. coli</i> concentration: 10 ⁶ CFU/ml					
(Straub et	E. coli	pH: 6.8	Cupric	0.4	-	60 min	No
al., 1995)		CaCO3 Calcium hardness: 96 mg/L	chloride				reduction
		CaCO ₃ Total hardness: 120 mg/L					
		Turbidity (NTU): 0.08					
		TDS: 210 mg/L					
		Electrical conductivity 0.43 mS/cm					
		Initial <i>E. coli</i> concentration: 10 ⁶ CFU/ml					
(Straub et al., 1995)	E. coli	pH: 6.8	Cupric chloride	0.8	-	60 min	No reduction

		CaCO ₃ Calcium hardness: 96 mg/L					
		CaCO ₃ Total hardness: 120 mg/L					
		Turbidity (NTU): 0.08					
		TDS: 210 mg/L					
		Electrical conductivity 0.43 mS/cm					
		Initial <i>E. coli</i> concentration: 10 ⁶ CFU/ml					
Ozone							
Reference	<i>E. coli</i> strain	Design Factors (water quality parameters)	Disinfectant	Dose (mg/L)	Residual concentration (mg/L)	Contact time	Efficacy
(de Souza &	E. coli	pH:7.2-7.6	Ozone	5	-	20 min	3.5 LR
Daniel, 2011)	ATCC 11229	Ca Co3: 80-108mg/L					
		Turbidity (NTU): 0.23- 0.69					
		Initial <i>E. coli</i> Concentration: 10 ⁸ CFU/100mL					
(Izdebski,	E. coli	pH: 7.4	Ozone	20	-	-	280 to 1
Dors, & Mizeraczyk,		T°: 18°C					CFU/mL
2011)		Electrical conductivity: 376 µS					
		Initial <i>E. coli</i> concentration: 280 CFU/mL					
(Martínez- Sánchez & Aguayo, 2019)	E. coli	Initial E. coli concentration (1.03 ± 0.03 CFU/100mL)	Ozone	0.354	-	-	Log CFU/100m L (1.03 ± 0.03 to <1 log CFU/100m L)
(Sommer et al., 2004)	<i>E. coli</i> ATCC 11229	Initial <i>E. coli</i> concentration: 10 ⁶ organisms/mL	Ozone	2.5	0.4	4 min	5 LR
(Sommer et al., 2004)	E. coli ATCC 11229	Initial <i>E. coli</i> concentration: 10 ⁶ organisms/mL	Ozone	3.1	0.1	10 min	6 LR

(Zuma, Li & Jonnalaga a, 2009) (Zuma et al., 2009)	n, <i>E. d</i> dd <i>E. d</i>	coli pH: Tem Initia conc 10 ⁸ C coli pH: Tem Initia conc 10 ⁸ C	4.93-9.16 perature:8°C, 25' al <i>E. coli</i> entration: 2FU/mL 4.93-9.16 perature:8°C, 25' al <i>E. coli</i> entration: 2FU/mL	Oz₁ °C Oz₁	one	0.906	-	6 : 4 :	min	4 LR 5 LR	
UV light	UV light										
Referen ce	E. coli strain	Design Factors (water quality parameters)	UV light source	Lamp dischar ge energy	Waveleng th (nm)	UV fluence (Dose) (mJ/cm ²)	UV intensit y	Distan ce from lamp	Flow rate	Efficacy	
(Garvey , Hayes, Clifford, & Rowan, 2015)	E. coli	Organic matter 10 ppm HA Initial <i>E.</i> <i>coli</i> concentratio n: 6 log CFU/mL	Pulsed Ultra Violet (PUV)	16.2 J	-	4.32 μJ/cm ²		8 cm	-	9 LR	
(Liu & Zhang, 2006)	<i>E.</i> <i>coli</i> 1337 3	Turbidity (NTU): 0.5 Initial <i>E.</i> <i>coli</i> concentration n: 10 ⁷ cells/mL	40-W Low Pressure Mercury Lamp	-	253.7	5 mJ/cm ²	0.1 mW/c m ²	-	-	3.14 LR (0.5NTU)	
(Liu & Zhang, 2006)	<i>E.</i> <i>coli</i> 1337 3	Turbidity (NTU): 4 Initial <i>E.</i> <i>coli</i> concentration n: 10 ⁷ cells/mL	40-W Low Pressure Mercury Lamp	-	253.7	5 mJ/cm ²	0.1 mW/c m ²	-	-	3.15 LR (4 NTU)	
(Liu & Zhang, 2006)	E. coli 1337 3	Turbidity (NTU): 12 Initial <i>E.</i> <i>coli</i> concentratio	40-W Low Pressure Mercury Lamp	-	253.7	5 mJ/cm ²	0.1 mW/c m ²	-	-	1.46 LR (12NTU)	

		n: 10 ⁷ cells/mL								
(Masse et al., 2011)	E. coli	Hardness (mg Ca Co ₃ /L): 157 Turbidity (NTU): 28.7 UV Transmittan ce: 55.5%	Low- Pressure Mercury Lamp Trojan UV Max TM Pro Series Model 20 (two lamps)	-	254	138 mJ/cm ²	-	-	-	505 CFU/100 mL to total disinfecti on
		Total Solids (TS): 229 mg/L								
(Masse E. et al., coli 2011)	E. coli	Hardness (mg Ca Co ₃ /L): 157	Low- Pressure Mercury	-	254	136 mJ/cm ²	-	-	-	505 CFU/100 mL to
		Turbidity (NTU): 28.7	Upstream ™ (NC15- 50) (Two lamps)							disinfecti on
		UV Transmittan ce: 55.5%								
		TS: 229 mg/L								
(Prakas h et al., 2017)	<i>E.</i> <i>coli</i> ATC C 1559 7	Turbidity (NTU): 0.25 <i>E. coli</i> Initial concentratio n: 6.2 x 10 ⁴ CFU/mL	Mercury- free plasma (MFP-UV lamp) 6 seconds contact time	-	172	-	-			4.79 LR
(Prakas h et al., 2017)	<i>E.</i> <i>coli</i> ATC C 1559 7	Turbidity (NTU): 5 Initial <i>E.</i> <i>coli</i> concentratio n: 6.2 x 10 ⁴ CFU/mL	Mercury- free plasma (MFP-UV lamp) 6 seconds contact time	-	172	-	-			4.79 LR
(Prakas h et al., 2017)	<i>E.</i> <i>coli</i> ATC C 1559 7	Turbidity (NTU): 10 Initial <i>E.</i> <i>coli</i> concentratio n: 6.2 x 10 ⁴ CFU/mL	Mercury- free plasma (MFP-UV lamp) 8 seconds contact time	-	172	-	-			4.79 LR

(Prakas h et al., 2017)	E. coli ATC C 1559 7	Turbidity (NTU): 20 Initial <i>E.</i> <i>coli</i> concentratio n: 6.2 x 10 ⁴ CFU/mL	Mercury- free plasma (MFP-UV lamp) 8 seconds contact time	-	172	-	-			4.79 LR
(P. Z. Sun, Tyree, & Huang, 2016)	<i>E.</i> <i>coli</i> ATC C 1559 7	Initial <i>E.</i> <i>coli</i> concentratio n: 4 x 10 ⁶ CFU/mL	4W low- pressure UV lamp	-	254	10.6 mJ/cm ²	-	0.44 cm	-	4 LR
(W. J. Sun & Liu, 2009)	<i>E.</i> <i>coli</i> ATC C 1.337 3	pH: 6.4-6.8 Turbidity (NTU): 0.2- 0.3 UV Transmittan ce: 92-96% TOC: 0.8- 1.4 mg/L Temperature : 22-29°C COD: 1-1.2 mg/L	Low- pressure UV lamps (Trojan Technologi es, Canada) (Three lamps). UVT 80%	-	-	60 mJ/cm ²	-	-	25,00 0 L/h	3 LR
(W. J. Sun & Liu, 2009)	<i>E.</i> <i>coli</i> ATC C 1.337 3	pH: 6.4-6.8 Turbidity (NTU): 0.2- 0.3 UV Transmittan ce: 92-96% TOC: 0.8- 1.4 mg/L Temperature : 22-29°C COD: 1-1.2 mg/L	Low- pressure UV lamps (Trojan Technologi es, Canada) (Three lamps) UVT 90%	-	-	60 mJ/cm ²	-	-	25,00 0 L/h	4 LR
(Younis, Mahone y, & Palomo, 2018)	E. coli ATC C	Turbidity (NTU)/ Initial <i>E.</i> coli concentratio n	Low- pressure UVC- lamps (two lamps)	-	254	215.6 mJ/cm 2	-	-	576 L/h	5.5 ± 0.3 LR

	1559 7	$0.16 \pm 0.03/$ 7.17 ± 0.12 log Temperature : 20 ± 1.4 °C pH of 7 ± 0.16								
		UVT of 95%								
(Younis et al., 2018)	<i>E.</i> <i>coli</i> ATC C 1559 7	Turbidity (NTU)/ Initial <i>E.</i> <i>coli</i> concentratio n $3.53 \pm 0.11/$ 7.02 ± 0.16 log Temperature $: 20 \pm 1.4 \circ C$ pH of 7 ± 0.16	Low- pressure UVC- lamps (two lamps)	-	254	215.6 mJ/cm 2	-	-	576 L/h	5.1 ± 1.0 LR
		UVT of 95%								
(Younis et al., 2018)	<i>E.</i> <i>coli</i> ATC C 1559 7	Turbidity (NTU)/ Initial <i>E.</i> <i>coli</i> concentratio n $6.62 \pm 0.21/$ 7.15 ± 0.12 log Temperature	Low- pressure UVC- lamps (two lamps)	-	254	215.6 mJ/cm 2	-	-	576 L/h	5.6 ± 1.0 LR
		: $20 \pm 1.4 \circ C$ pH of 7 ±								
		UVT of 95%								
(Younis et al., 2018)	<i>E.</i> <i>coli</i> ATC C 1559 7	Turbidity (NTU)/ Initial <i>E.</i> <i>coli</i> concentratio n	Low- pressure UVC- lamps (two lamps)	-	254	215.6 mJ/cm 2	-	-	576 L/h	6.8 ± 0.9 LR

		$\begin{array}{c} 13.30 \pm \\ 0.53/\ 6.91 \pm \\ 0.42 \end{array}$ Temperature : 20 ± 1.4 °C pH of 7 ± 0.16 UVT of 95%								
(Younis et al., 2018)	<i>E.</i> <i>coli</i> ATC C 1559 7	Turbidity (NTU)/ Initial <i>E.</i> <i>coli</i> concentratio n $17.83 \pm$ $0.32/6.93 \pm$ 0.06 Temperature : 20 ± 1.4 °C pH of 7 ± 0.16 UVT of 95%	Low- pressure UVC- lamps (two lamps)	-	254	215.6 mJ/cm 2	-	-	576 L/h	5.1 ± 0.2 LR
(Younis, Mahone y, & Yao, 2019)	<i>E.</i> <i>coli</i> ATC C 1559 7	Turbidity (NTU)/ Initial <i>E.</i> <i>coli</i> concentratio n $0.16 \pm 0.03/$ 7.17 ± 0.12 log pH: 7 ± 0.16 Temperature : 20 ± 1.4°C	30 W Low- pressure UV lamps (two lamps)	-	254	215 mJ/cm 2	-	-	564 L/h	5.5 ± 0.3 LR
(Younis et al., 2019)	<i>E.</i> <i>coli</i> ATC C 1559 7	Turbidity (NTU)/ Initial <i>E.</i> <i>coli</i> concentratio n $3.53 \pm 0.11/$ 7.02 ± 0.16 log pH: 7 ± 0.16	30 W Low- pressure UV lamps (two lamps)	-	254	215 mJ/cm 2	-	-	564 L/h	5.1 ± 1.0 LR

		Temperature : $20 \pm 1.4^{\circ}C$					
(Younis et al., 2019)	<i>E.</i> <i>coli</i> ATC C 1559 7	Turbidity (NTU)/ Initial <i>E.</i> <i>coli</i> concentratio n	30 W Low pressure UV lamps (two lamps)	254	215 mJ/cm 2	 564 L/h	5.6 ± 1.0 LR
		6.62 ± 0.21/ 7.15 ± 0.12log					
		pH: 7 ± 0.16					
		Temperature : $20 \pm 1.4^{\circ}C$					
(Younis et al., 2019)	<i>E.</i> <i>coli</i> ATC C 1559 7	Turbidity (NTU)/ Initial <i>E.</i> <i>coli</i> concentratio n	30 W Low pressure UV lamps (two lamps)	254	215 mJ/cm 2	 564 L/h	6.8 ± 0.9 LR
		$\begin{array}{l} 13.30 \pm \\ 0.53/\ 6.91 \pm \\ 0.42 log \end{array}$					
		pH: 7 ± 0.16					
		Temperature : $20 \pm 1.4^{\circ}C$					
(Younis et al., 2019)	<i>E.</i> <i>coli</i> ATC C 1559 7	Turbidity (NTU)/ Initial <i>E.</i> <i>coli</i> concentratio n	30 W Low- pressure UV lamps (two lamps)	254	215 mJ/cm 2	 564 L/h	5.1 ± 0.2
		17.83 ± 0.32/ 6.93 ± 0.06log					
		pH: 7 ± 0.16					
		Temperature : $20 \pm 1.4^{\circ}C$					

Filter systems						
Reference	E. coli strain	Design Factors (water quality parameters)	Adsorption/Filter material	Contact/Retention time	Flow rate L/h	Efficacy
(Hassouna, ElBably,	O128:k67, O157:k-,	-	Kaolin clay loaded with silver nanoparticles	2h	-	<1 LR

Mohammed, &	O111:k58		(AgNPs) (0.05-			
Nasser, 2017)	and O55:k59		0.1mg/L)			
(Hassouna et al., 2017)	O128:k67, O157:k-, O111:k58 and O55:k59	-	Kaolin clay loaded with carbon nanotubes (CNTs) (0.1 mg/L)	2h	-	<1 LR
(Lukhele, Mamba, Momba, & Krause, 2010)	Pathogenic <i>E. coli</i> ATCC 25925	Temperature: 12.5- 14.3°C Conductivity: 16.4- 79.1 mS/m pH: 7.13-8.23 Turbidity: 1.1-7.4	-Multi-walled carbon nanotubes polymer cyclodextrin (MWNT- CD, 0.3g) and -Silver impregnated carbon nanotube co- cyclodextrin polymers (Ag-MWNT/CD 0.3g) packed in solid phase extraction cartridges (SPE)	-	0.3	3 LR
(Mpenyana- Monyatsi, Mthombeni, Onyango, & Momba, 2012)	E. coli	pH: 7.22±0.14 Turbidity:1.59±0.11	2 cm diameter 20 cm length Polyvinyl chloride (PVC) column Packed with 10 cm Ag/cation resin nanoparticle filter	20 min	0.12	3 LR
(Shaheed, Wan Mohtar, & El- Shafie, 2017)	E. coli	Initial <i>E. coli</i> concentration: 23-119 CFU/ml pH: 6.26-7.31 Dissolved oxygen (DO): 4.84-8.64 mg/L Biochemical Oxygen Demand (BOD ₅):1.79-5.3 mg/L COD:10-110mg/L Total Suspended Solids TSS: 2.16- 42.45mg/L	Activated Carbon and Sand Filtration (CACSF)	87 min retention time	2.5	< 2 LR
(Silupu et al., 2017)	<i>E. coli</i> ATCC 25922	pH: 6-6.5	Adsorption of Activated Carbon Filters Pore Size 0.5-3 µm (1,000mg/L)	120 min	-	< 1 LR

Membrane Filtration: Microfiltration and Ultrafiltration									
Referen	Е.	Design	Filtration	Filter	Pore Size	Filter	Flow	Efficacy	
ce	coli	Factors		medium		depth	rate		
	strain	(water				(m)	(L/h)		

		quality parameters)						
(Coccag	Е.	pH: 8.3	Microfiltration	Polypropyle	0.2 µm	1	3,500	6
na et al., 2001)	coli	Turbidity: 2.4		ne				
		Temperature : 7.7 °C						
		1.8 x 10 ⁶ - 2 x 10 ⁶ CFU/100mL						
(Ujang,	E.	-	Microfiltration	Polyolefins	Polyolefins 0.2	-	-	100% removal
Nagaoka , 2002)				Immersed Membrane Filtration (IMF) - Powdered Activated Carbon (PAC)	PAC 1-2.5 μm			Telilova
(Galvañ et al	E. coli	Turbidity: 57.45	Ultrafiltration	Hollow fiber Zeeweed®	0.04 µm	-	15,000	2
2014)		2.4-5 log MPN/100m L		500D				
(Huang, Jacangel o, & Schwab,	E. coli CN13 (ATC	pH: 8-8.5 Conductivity : 370-404 uS/cm	Ultrafiltration	Hollow fiber polyvinylide ne fluoride (PVDF)	0.002 µm	-	1,020	>7
2011)	70060 9)	Turbidity: 0.31-1.41						
		TOC: 0.76- 1.11 mg/L						
		Initial <i>E. coli</i> concentratio n 9.21×10^2 MPN/100m L						
(Praneet	<i>E</i> .	Turbidity:	Ultrafiltration	Hollow fiber	PAN 0.01 μm	-	-	5 LR (PAN)
n, Kalyani, Raviku	coli	140 FAU (Formazin Attenuation		polyacryloni trile (PAN) and				4 LR (PES)
mar,		Unit)			PES 0.05 µm			
Tardio, & Sridhar, 2013)		Suspended Solids: 88mg/L		fone (PES)				

pH: 6.8

initial
concentratio
n
1.1×10 ³ (MPN/100 mL)

Slow Sand	Slow Sand Filtration									
Referen ce	E. coli strain	Design Factors (water quality parameters)	Filtration	Surface area (m ²)	Bed porosi ty	Diameter sand (mm)	Bed/f ilter depth (m)	Filtrati on rate m/h	Efficacy	
(Hijnen, Schijven , Bonne, Visser, & Medema , 2004)	E. coli	DOC: 1.5- 2.1 mgC/L Turbidity: 0.1-0.7 FTU (Formazin Turbidity Units) pH: 8 Temperature :9.4-11.7 °C	Slow Sand Filtration	2.56	0.27	0.3	1.5	0.3	2-3 LR	
(Ochien g, Otieno, Ogada, Shitote, & Menzwa , 2004)	E. coli	Turbidity: 30.65-123.8 Suspended Solids (SS): 30.1-116 mg/L Initial <i>E. coli</i> concentratio n 32-110 CFU/100mL	Multistage Filtration (MSF): Slow Sand Filtration (SSF)+Pretreat ment system- horizontal flow roughing filter (HRF)	-	-	HRF: 15;10;5 SSF: 0.25mm	-	HRF: 0.75 SSF: 0.2- 0.29	2 LR	
(Rao, Malini, Lydia, & Lee, 2013)	E. coli	pH: 7.65 Electrical Conductivity : 2.17 mS/cm Total Dissolved Solids: 1,409 mg/L	Bentonite Amended Slow Sand Filter (BASSF) (90%s sand- 10% bentonite)	-	-	Coarse Fraction: 4.75 mm- 2mm Medium fraction sand: 2- 0.425 mm	-	0.1-0.3 Collecti on 500- 700ml/ 5-7h	<i>E. coli</i> initial value 160MPN/10 0ml- Final value 0-50 MPN/100ml	

		Initial <i>E. coli</i> concentratio n 160 MPN/100m L				Fine fraction sand: 0.425m m- 0.075m m Bentonit e Clay: <0.002m m			
(Urfer, 2017)	E. coli	Turbidity: 0.3-39 FTU pH: 6.25- 7.89 Conductivity : 354-530 μS/cm Initial <i>E. coli</i> concentratio n: 0-1,300 CFU/100mL	Multistage filtration: Up- flow Roughing Filters (URF)+Slow Sand Filtration enhanced with natural bauxite	-	-	SSF: -Bauxite: 0.2-0.5 mm	SSF: 80cm Bauxi te	URF: 0.45 SSF: 0.15	100 % removal
(Weber- Shirk, 2002)	E. coli	-	Slow Sand Filter enhanced acid- soluble seston extract	-	-	-	18cm	0.1	6 LR
Biosand F	litration								
	munuton								
Referen ce	E. coli strain	Design Factors (water quality parameters)	Filtration	Filter medium	Grain/Sa	and size	Filter depth (m)	Flow rate (L/h)	Efficacy

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						d		
						with		
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						sand:		
						0.355		
						m		
(Napotni	Е.	TOC:	CAWST v10	Full-scale	Gravel	Grave	Max 24	3.34-3.66
k,	coli	12.5 ± 6.8	(Centre for	bio sand	Coarse sand	l: 3-		LR
Baker, &	AIC C	5.8-24.2	Affordable Water and	filters		Scm		
Jellison,	11775	mg/L	Sanitation	2,5 gal	Fine sand	Coars		
2017)		pH: 7.5±0.4	Technology	bucket BSF.		e sand:		
		7 1 0 0	charge volume			3-		
		/.1-8.8	pore volume			5cm		
		Hardness:	equal)			Fine		
		339±77.3				sand:		
		IIIg/L				10:54		
		247-492				cm		
		Turbidity: 5-						
		50						
		Initial E. coli						
		concentratio						
		n:						
		$2.8 \ge 10^3$						
		CFU/100mL						
D:	F '1							

Kiverbank Fluranon								
Referen ce	E. coli strain	Design Factors (water quality parameters	Distance of RBF well (m)	Depth of extraction well (m)	Travel time (days)	Efficacy		
(Cady et al., 2013)	E. coli	-	26	-	Min 45.2	1 LR		
(Partino udi & Collins, 2007)	E. coli	DOC: 0.46mg/L 10 ±4 - 28 ±	12.2-55	12.2-19.8	1-5	1-1.74 LR		
		6 CFU/100 mL						
Granular Activated Carbon								
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Referen ce	E. coli strain	Design Factors (water quality parameters)	Filtration	Filtration Column	Grain size (mm)	Conta ct time	Filtrati on rate	Efficacy
(Hijnen, Suylen, Bahlma n, Brouwer - Hanzens , & Medema , 2010)	E. coli	Turbidity: 0.09 ± 0.02 pH= 8.1 Conductivity : 44.8±0.5 mS/m Temperature : 14.5±0.8°C Initial <i>E. coli</i> concentratio n 1.1 x 10 ⁶ ± 3.1 x 10 ⁵ and 6.0 x 10 ⁵ ± 2.6 x 10 ⁴ CFU/L	Granular Activated Carbon (GAC) filters	Diameter 0.15 m; height 1.35 m; 1m of fresh GAC (Chemviron F400) or loaded GAC (40.000 bed volumes of filtering)	0.8-1.1	12 min	5m/h	0.4-1.1 LR

Combined						
Reference	<i>E. coli</i> strain	Design Factor (water quality parameters)	Combination	Dose (mg/L)	Contact time (min)	Efficacy
(de Souza & Daniel, 2011)	E. coli ATCC 11229	pH:7.2-7.8	Ozone + Chlorine	Ozone (2mg/L) + Chloring (5mg/L)	20	7.76 LR
		Ca Co3: 80- 108mg/L		Chlorine (Jing/L)		
		Turbidity (NTU): 0.23-0.69				
		Initial <i>E. coli</i> Concentration: 10 ⁸ CFU/100mL				
(Lin, Hou, Wang, & Chen, 2017)	E. coli	рН: 7-7.5	Granulated Activated Carbon (GAC) Filter + Chlorine dioxide	Chlorine dioxide (0.5mg/min/L)	-	<1 LR
	ATCC 10798	Turbidity: 8-15				
		DOC: 2.85-3.61 mg/L				
		COD _{Mn} : 2.01-2.27 mg/L				

(Lin et al., 2017)	<i>E. coli</i> ATCC 10798	pH: 7-7.5 Turbidity: 8-15 DOC: 2.85-3.61 mg/L COD _{Mn} : 2.01-2.27 mg/L	Granulated Activated Carbon (GAC) Filter + Chlorine dioxide	Chlorine dioxide 2.0 mg/min/L	-	2-3 LR
		Initial <i>E. coli</i> concentration : 10 ⁸ CFU/mL				
(Sommer et al., 2004)	E. coli ATCC 11229	pH: 7.6 Temperature: 11°C Initial <i>E. coli</i> concentration: 10 ⁶ organisms/mL	Ozone + Hydrogen Peroxide	Ozone (2.5 mg/L) Hydrogen Peroxide (1.5mg/L)	-	6 LR
(P. Z. Sun et al., 2016)	E. coli ATCC 15597	Initial <i>E. coli</i> concentration: 4 x 10 ⁶ CFU/mL	UV light/H2O2	UV (8.6 mJ/cm ²) + 0.3 mM H ₂ O ₂	-	4 LR
(P. Z. Sun et al., 2016)	E. coli ATCC 15597	Initial <i>E. coli</i> concentration:4 x 10 ⁶ CFU/mL	UV light/Peroxydisulfate	UV (8.8 mJ/cm ²) + 0.3 mM Peroxydisulfate	-	4 LR

CHAPTER 4: GENERAL CONCLUSIONS

The chapters developed in this thesis cover two studies that provide the Chilean authorities in charge of food safety and quality with science-based recommendations for the adoption of water treatments at the pre-harvest stage to mitigate the presence of generic *E. coli* in fresh raspberries: i) a systematic review for the identification of water treatments and the quantification of their efficacy against *E. coli* (rapid systematic review) and feasibility evaluation for the implementation on-farm (*review of reviews*), and ii) a risk-based analysis of the expected performance of the treatments to ensure the target acceptance rate to be met by relevant importing countries of Chilean fresh raspberries.

Chapter 1 presents the context where this research is initiated and points out the economic importance of the raspberry industry for Chile, the main characteristics of the production, the relevant sources of microbial contamination within the supply chain (specifically generic *E. coli*), and the proposed methodology to provide a solution.

Chapter 2 presents the results of the rapid systematic review, where a pre-established protocol was followed to identify, select, and critically appraise relevant publications to retrieve the efficacy of treatments in reducing generic *E. coli* in the water sources commonly used by the small farmers (groundwater and surface water). Additionally, since the decision-making process regarding water treatment(s) would not only consider the efficacy criteria, a second analysis was carried out, to evaluate the most important aspects such as technological, managerial, and sustainability criteria regarding the feasibility of in-field application. After analyzing more than 11,000 publications, it is concluded that the water treatments used at a pre-harvest stage, or on-farm, have not been

extensively studied for the efficacy of *E. coli*, and there is no critical analysis for the feasibility of these treatments to be implemented at a small-scale. The water treatments identified by the reviews include chemical disinfectants (chlorine-based compounds and ferrates), ozone, UV light, and filtration systems (such as riverbank filtration, biosand filters, slow sand filtration, and membrane filtration). The "review of reviews" identified those treatments generally implemented at a pre-harvest stage (usually on greenhouse operations, for irrigation systems, or for preventing plant pathogens or algae growth): chemical disinfectants (chlorine-based compounds, peracetic acid, hydrogen peroxide), ozone, UV light, and membrane filtration.

Finally, Chapter 3 provides an evaluation of the acceptance rate of fresh raspberry in relevant markets for Chile. Taking into account the *E. coli* efficacy values (log_{10} reduction of the water treatments, different scenario analyses were simulated in a quantitative model to achieve a target concentration in water of a geometric mean ≤ 126 CFU/100mL or a statistical threshold value of ≤ 410 CFU/100 mL as stated under the U.S. regulation (main destination of Chilean raspberry production). When the water used for the application of pesticides achieved this concentration, an acceptance rate at the port of entry of 99.7% was estimated.

When small-raspberry farmers use groundwater sources, $a \ge 3$ log reduction is expected to achieve the target concentration of 10^2 CFU/g in fresh raspberries and a 99.7% of acceptance rate at the port of entry of importing countries, while $a \ge 6$ log reduction is expected when surface water sources are used for the same purpose.

The results of the two studies conducted in this thesis are summarized in Table 12

List of selected water treatment scenarios RR: Efficacy against <i>E. coli</i> (log reduction required by source of water)		gainst <i>E. coli</i> required by water)	Review of reviews: Feasibility		
	Groundwater (≥3)	Surface water (≥6)	Advantages	Disadvantages	
Chemical					
Ferrates only	\checkmark	Х	-	-	
Chlorine only	\checkmark	Х	-	-	
Coagulant/disinfection	\checkmark	Х	-	-	
products (CDPs) sodium					
dichloroisocyanurate based					
Monochloramine + cupric	\checkmark	\checkmark	-	-	
chloride			I :	T	
Chlorine dioxide	-	-	and turbidity than	shelf-life	
			DBPs in smaller	to calcium	
			quantity compared to	hypochlorite, sodium	
			chlorine and	hypochlorite, or	
			chloramines	chlorine gas	
			Commercial kits for on-	Corrosive	
			site measurement	DDD	
Sodium nypochiorite	-	-	Low cost and	DBPS	
			Cope with supply	Conosive	
			systems of different		
			sizes.		
Calcium hypochlorite	-	-	Low cost and	DBPs	
			complexity	Corrosive	
			Calcium hypochlorite is		
			compared to both		
			chlorine gas and sodium		
			hypochlorite		
			Storage is easier than		
			sodium hypochlorite		
Hydrogen peroxide (H ₂ O ₂)	-	-	Low capital and	Highly influenced by	
			operational cost	turbidity	
			technology	that needs secondary	
			Effective in a broad pH	containment for	
			range	storage facilities	
			Low levels of DBPs	Corrosive	
				Low DBPs	
Peracetic acid (PAA)	-	-	Low capital and	Corrosive	
			moderate operational	production canacity)	
			Lower influence of	Low DBPs	
			turbidity than other		
			chemicals		
			Low complexity, long		
			shelf life and is easy to		
			handle		
Ozone	/	/	LOW IEVEIS OF DBPS	High capital and	
OZUIL	V	\checkmark		operational cost	
				Complex	
				Highly influenced by	
				turbidity levels	

Table 12. Characterization of the efficacy against *E. coli* and feasibility (advantages and disadvantages) of selected water treatments to be implemented in small raspberry farms in Chile, considering the risk-based evaluationⁱ

				Must be generated <i>in</i> situ
				DBPs
				Corrosive
UV light				
UV light: Two low-pressure UVC lamps	\checkmark	\checkmark	Low to medium influenced by turbidity Negligible effect of pH and temperature Low operational cost, low maintenance Normally no DBPs produced	High capital cost Safety issues UV mercury lamps potential leaking into the environment
Filtration				
Enhanced Slow Sand	\checkmark	\checkmark	-	-
Filtration				
Full-scale bio sand filters	\checkmark	X	-	-
Membrane filtration				
Microfiltration	\checkmark	\checkmark	Effective in a broad pH	Costly
Ultrafiltration	\checkmark	\checkmark	range No DBPs (not applicable)	Biofouling High complexity, high expertise to monitor Membrane failure can be hard to detect and catastrophic
Combined				
UV light/H ₂ O ₂	\checkmark	Х	-	-
UV light/Peroxydisulfate	\checkmark	Х	-	-
Ozone + Chlorine	\checkmark	\checkmark	-	-
$Ozone + H_2O_2$	\checkmark	\checkmark	-	-

ⁱ The water treatments from the RR do not represent necessarily an absolute match with the treatments from the *Review* of review approach, therefore for chlorine dioxide (which achieved efficacy values < 3 log reduction) and for the treatments from the *review of reviews* (sodium hypochlorite; calcium hypochlorite; hydrogen peroxide; and peracetic acid) the efficacy against *E. coli* was not described. In the same way, some treatments from the RR (ferrates; chlorine; coagulant/disinfection products (CDPs) sodium dichloroisocyanurate based; monochloramine + cupric chloride; slow sand filtration; bio sand filters; and combined technologies, did not match the results of the *review of review*, therefore, their feasibility was not described.