Quantitative assessment of microbial quality and safety risk: A preliminary case study of strengthening raspberry supply system in Chile

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ABSTRACT

National governments are moving to integrate risk analysis frameworks into food safety management systems at the country level. However, this process is less advanced in developing countries. In this context, the Chilean Livestock and Agriculture Service (SAG), Food Quality and Safety Agency (ACHIPIA) and the University of Nebraska-Lincoln (UNL) collaborated on a project to control generic *Escherichia coli* and Hepatitis A virus (HAV) contamination in both fresh and frozen raspberry products destined for export. The objectives of this study were to 1) identify along the raspberry supply chain the most influential factors of *E. coli* and HAV contamination in the final products; and 2) evaluate the efficacies of possible interventions to control these influential factors. To achieve these objectives, a unified quantitative model of microbial contamination in raspberries was developed to describe the impact of factors in a continuum from the farm to the destination of importation on *E. coli*/HAV contamination in fresh and frozen raspberry products. Multiple surveys were conducted to obtain country-specific data on current common practices of producing and processing raspberries in Chile for inputs into the simulation model. The model estimated mean bacterial loads of −1.64 and −5.46 logCFU/g for *E. coli* and mean viral loads of −6.45 and −6.51 logPDU/g for HAV in fresh and frozen raspberries, respectively. Sensitivity and scenario analyses indicated that reduction of *E. coli* contamination in the end products can be effectively achieved by improving the quality of water used for pesticide application, as well as by controlling the transport and storage time and temperature along raspberries supply chain. By contrast, to control HAV contamination in the end products, efforts should be focused on improving the hygiene practices of berry handlers on the farm and at the packing plant. This project provides straightforward recommendations for Chilean food safety authorities to effectively prioritize their financial and human resources to proactively prevent microbial contamination in raspberries. Moreover, this project provide a framework that can be extended to other countries to promote capability building for applying risk-based food safety management systems for public health protection.

1. Introduction

Food exports are important for the Chilean agricultural sector as well as the national economy, with total sales reaching 16 billion USD in 2016 and constituting 25% of all Chilean exports (Chilealimentos, 2017). Among food commodities, fresh fruits are the major export products. The value of these exports increased by 67.3% from 2005 to 2010, with North America accounting for the major share of export value, followed by Europe, South and Central America and Asia (Melo, Engler, Nahuehual, Cofre, & Barrena, 2014). With the increasing volume and wider range of distribution of Chilean fruit production and exports, building an effective national food safety system is essential and urgent for protecting public health not only domestically but also worldwide.

To meet the challenges of the evolving food safety environment, risk analysis has been globally endorsed as the key principle for further

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strengthening food safety systems and reducing food-borne illness. Risk analysis considers a wide range of factors in a "farm-to-fork" continuum to inform food control decision-making using a systems approach (CAC, 1999). Over the last two decades, national governments have moved quickly to integrate recommended risk analysis frameworks into national food safety management systems (FAO/WHO, 2004; Pouillot, Garin, Ravaonindrina, Diop, Ratsitorahina, Ramanantsoa, et al., 2012; USDA FSIS, 2002). In this context, the Chilean Food Quality and Safety Agency (ACHIPIA) has promoted a revamp of the Chilean National Food Quality and Safety System (SNICA). The main objective of this revamp is to shift the Chilean food safety system from a reactive to a proactive perspective to manage food risks by implementing a holistic approach to the farm-to-fork continuum and engaging all stakeholders along food supply chains. One of the key features of this paradigm shift is the integration of a risk analysis framework into SNICA, with risk assessment serving as the scientific component providing science-based information to inform the risk management process.

Challenges hindering this shift include limited coordination between organizations handling food safety issues, a fragmented regulatory system, and technical limitations in assessing potential risks, sharing information and systematically identifying and managing food safety issues. To provide a framework for addressing these challenges, a pilot study was initiated in 2015 for the purpose of effectively utilizing risk assessment tools to provide scientific evidence for food safety-related decision-making processes at a national level, based on an collaboration among ACHIPIA, the Livestock and Agriculture Service of Chile (SAG) and University of Nebraska-Lincoln (UNL).

The collaboration began with a pilot study of microbial contamination control in raspberries produced in Chile as part of the Raspberries Official Control Program (ROCP) enforced by SAG. ROCP is responsible for protecting the quality and safety of raspberry products produced in Chile, which is primarily achieved by on-site audits of participants in the ROCP. Raspberry farms exporting their products need to be registered as ROCP participants and accredited by SAG. Otherwise, their exports would be halted by Chilean customs before leaving the country. Most of the participants are small-sized raspberry farms and the accreditation consists of the completion of a small-farm oriented Good Agricultural Practices (GAP) program. This tailored the version of GAP focuses on the most common issues for small-sized farms, including water quality, hygiene measures for harvesters and animal controls on the farm (Library, 2012). The accreditation is active for one year and must be renewed annually to stay in the ROCP registry.

However, limited technical skills and capabilities have prevented SAG from adequately assessing and further improving ROCP's effectiveness. Hence, there is a need for a scientific assessment of ROCP to ensure that its resources are optimally assigned to critical steps along the production chain. To meet the need, a unified quantitative simulation model was developed to estimate the microbiological quality and safety of Chilean raspberry products and the contribution of potential contamination points to the overall microbial contamination. For a proactive prevention purpose, hepatitis A virus (HAV) is targeted as one of the microorganisms of interest, as reported outbreaks indicate that fresh produce including raspberries can be a potential source for human infection of foodborne viruses, such as HAV (Gallot, Grout, Roque-Afonso, Couturier, Carrillo-Santisteve, et al., 2011; Sarvikivi, Roivainen, Maunula, Niskanen, Korhonen, Lappalainen, et al., 2012). Generic *E. coli* was also evaluated in this study to assess the good hygienic practices along the raspberry supply chain. Microbiological testing of raspberries and water resources for the raspberry farms demonstrated the potential for fecal contamination as indicated by generic *Escherichia coli* (*E. coli*) based on communication with SAG and ACHIPIA team and supported by documented research (James, 2006). In addition, the target of generic *E. coli* was driven by the primary measure interests of importing countries. Importers of berries in Chile, such as Canada and Australia, use generic *E. coli* as one of microbiological criteria for satisfactory assessment of domestic and imported

berry products in their markets and monitor compliance with their food standards (Australian Department of Agriculture, 2019; Canadian Food Inspection Agency, 2019).

Accordingly, the collaborative pilot project was conducted with the following specific objectives: 1) identify the most influential factors along the raspberry supply chain for effective intervention implementation for *E. coli* and HAV contamination control in fresh and frozen raspberry products; and 2) evaluate the efficacies of possible interventions for controlling the influential factors. Achieving these objectives will allow food safety authorities, in this case SAG, to better allocate their human and financial resources to improve raspberry quality and safety and increase export amounts.

2. Methodology

2.1. Overview of the collaborative project

Continuous dialog between risk assessors and risk managers is essential. One way to elicit risk assessor-manager interaction is through the elaboration of a risk profile before a risk assessment project is implemented (National Research Council, 2009). Accordingly, this collaboration began with the development of a project profile consisting of problem formulation, project scope and outline, and the roles and responsibilities of the involved parties. Briefly, the team at UNL was the primary expert group conducting the quantitative simulation project and providing the main findings to address the management questions defined by SAG. SAG was also the primary data provider and collector, given its status as the agency enforcing the ROCP program and potentially utilizing the results from this project to evolve the current ROCP into a risk-based control program. ACHIPIA played a critical role as a coordinator and overseer by monitoring the progress of the project, managing the timeline and production of the required deliverables, ensuring that the overall project goals were achievable, and facilitating effective communication between UNL and SAG throughout the project's life span. Raspberry farmers and packers registered with ROCP participated in the project by providing their insights on production and processing practices with ROCP via SAG and ACHIPIA. The project was then executed by following the roadmap initially proposed in the project profile, with iterative adjustment based on agreement among the major parties (ACHIPIA, SAG and UNL) throughout the project life span.

2.2. Collection of data for input into the country-specific simulation model

During the planning stage, the data needs for simulation model development were discussed. To inform country-specific decision-making on improving food microbiological quality and safety, Chilean data were needed to capture the production and processing conditions that could extensively influence the bacterial and viral contamination in end products. The data that were necessary but not available at the project planning stage were primarily related to practices and potential risk factors along the raspberry supply chain in Chile. To fill these data gaps, three surveys were designed and launched during the raspberry harvest season in 2016 (February–March) when SAG auditing programs are usually conducted more intensively, focusing on the practices at raspberry farms, collection centers and packing plants in Chile, respectively. Surveys were distributed to 226 farms, 23 collection centers, and 36 packing plants that are reasonably representative of the variation in Chilean raspberry industry; and responses were collected and processed for all distributed surveys. These surveys provided critical information for narrowing the data gaps by providing insights on the process from a local perspective. In particular, outputs of the surveys significantly enhance the quantitative simulation model parameterization to describe how various practices influence the introduction, transmission of bacterial and viral contamination to the end products.

The data needed for model parameterization were also obtained by

searching the documented literature. The data retrieved from the literature search primarily focused on the influence of environmental conditions on microbial populations in fruits. Examples include models of microbial growth and survival under different temperatures during transportation and storage and the decay rates of microbial organisms in fruits over time. These data are related to the characteristics of microorganisms, which can be affected by the food matrix and production and processing parameters. Hence, the scope of the literature search was restricted to studies of raspberry or closely related produce under production and processing systems similar to those in Chile. Literature searches were conducted using UNL's library resources, mainly through electronic databases, including Web of Science Core Collection, Biological Abstracts, BIOSIS Citation Index, CAB Abstracts®, and MEDLINE®.

The remaining data gaps were filled through an expert consultation process coordinated by ACHIPIA. Questions from the UNL team were posted to the Food Scientists Network organized by ACHIPIA and voluntary responses were collected based on inputs from scientists in the Network. The data collected through this mechanism mainly enhanced the qualitative understanding of the potential risk factors critical for raspberry production and processing with a special consideration of conditions in Chile.

2.3. Quantitative assessment of microbial contamination in raspberry products in Chile

2.3.1. Overview of the simulation model

A unified model was established to simulate the introduction and transmission of microbial hazards of concern along the supply chain of raspberry products for export, from pre-harvest in the field through processing and transport to arrival at the destinations of importation. The final products of interest in the model were both fresh and frozen raspberries. In discussion with SAG and ACHIPIA, *E. coli* and HAV were selected as the target microbial agents. The model output was the contamination in logCFU/g of raspberries for bacterial contamination and logPDU/g for viral contamination.

A three-stage modular process with farm, collection center and packing plant modules was established as shown in Fig. 1. A general overview of the process is as follows: at the farm, raspberries are planted, irrigated, exposed to pesticide and fertilizer application, and finally harvested in summer (January–March). At the collection center, raspberries from different farms are gathered, temporarily stored and then sold to a packing plant. At the packing plant, the raspberries are inspected for quality and selected for export as fresh or frozen products (best quality), sent for processing into juice or other fruit products (lower quality), or discarded (not acceptable for consumption purposes). To estimate the behavior of *E. coli* and HAV across the three modules and to identify potential contamination sources and control measures, the data obtained from the surveys were used to populate the model from a Chilean perspective.

A one-dimension Monte Carlo simulation by Latin Hypercube Sampling with 50,000 iterations was conducted to quantify the variability and uncertainty of the model output using @Risk (version 7.5, Palisade Corporation, New York, USA).

2.3.2. Farm module

The farm module quantitatively describes how the contamination is introduced to the fruit and changes from the time of planting through harvesting to immediately before arrival at the collection center. The same farm module was used for both fresh and frozen final products. Four major factors that could potentially affect the introduction and fate of bacterial and viral agents during this stage were considered, including 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, and possible growth (mainly bacteria) or degradation (mainly virus) during transport from the farm to the collection center under varying temperatures during transport. The input variables and calculation equations for this module are provided in detail in Table 1, and the main inputs and equations are elaborated below.

One of the primary sources of microbial contamination on fruit is the water used for spraying pesticides. The water used during this step is from three sources, i.e., ground, surface or potable water, and the percentage of farming premises using each type of water was quantified (W_{type}) based on the data collected by the survey on farm practices. The contamination loads of *E. coli* and HAV in water were obtained from the literature. The contamination load on a single raspberry after pesticide application depends on the microbial concentration in water (C_w) that varies according to the water type and the volume of water attached to

FARM

| Seedlings establishment and irrigation | Pesticide application | Harvest | Transport to the collection center |
| *No factors considered* | *Water used to dilute pesticide; insufficient withholding time* | *Cross-contamination due to harvesters' hands* | *No refrigeration* |

COLLECTION CENTER

| Receipt of raspberries | Transport to the packing plant |
| *No refrigeration* | *No refrigeration; extended transport period* |

PACKING PLANT

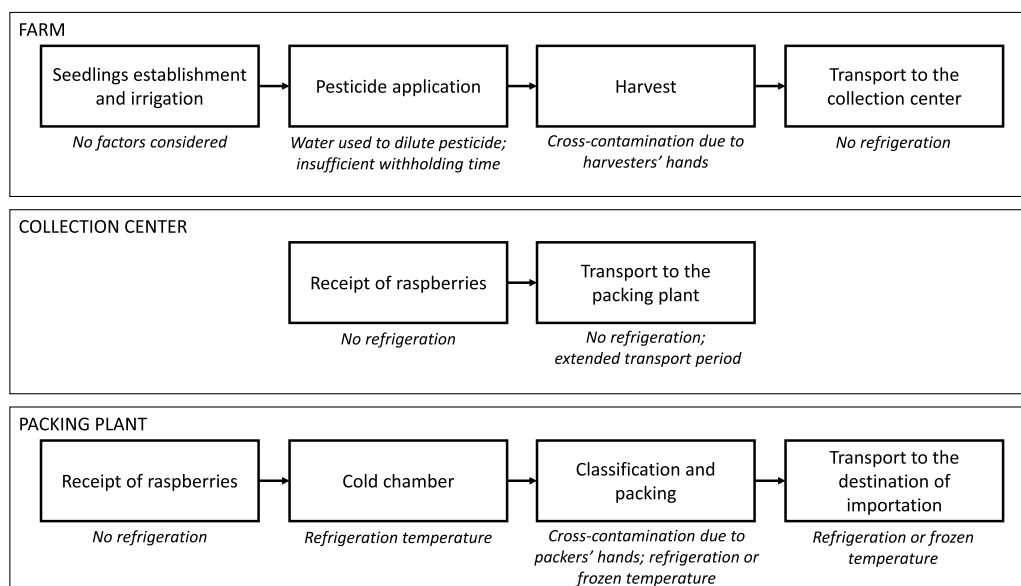| Receipt of raspberries | Cold chamber | Classification and packing | Transport to the destination of importation |
| *No refrigeration* | *Refrigeration temperature* | *Cross-contamination due to packers' hands; refrigeration or frozen temperature* | *Refrigeration or frozen temperature* |

Fig. 1. The unified flow chart of the supply chain for fresh and frozen raspberries including potential factors influencing *E. coli* or Hepatitis A virus contamination along the chain.

Table 1

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

Variable	Description	Value/Distribution/Calculation	Unit	Reference
Pre-harvest operations				
W_{type}	Type of water used for pesticide applications	Discrete		Survey
	Groundwater	71% (coded as 1)		
	Surface water	15% (coded as 2)		
	Potable water	14% (coded as 3)		
$C_{w1,bac}$	Bacterial contamination in groundwater	Uniform (0,1000)	CFU/L	WHO (2004)
$C_{w2,bac}$	Bacterial contamination in surface water	Uniform (6000,10^6)	CFU/L	WHO (2004)
$C_{w3,bac}$	Bacterial contamination in potable water	Uniform (0.01,0.1)	CFU/L	INN (2005)
$C_{w1,vir}$ [a]	Viral contamination in groundwater	Uniform (0,2)	PDU/L	WHO (2004)
$C_{w2,vir}$ [a]	Viral contamination in surface water	Uniform (0,60)	PDU/L	WHO (2004)
$C_{w3,vir}$ [a]	Viral contamination in potable water	Pert (0,0.0006,0.02) * 100	PDU/L	WHO (2004)
$C_{w,bac}$	Bacterial concentration in spray depending on the water type	$C_{w1,bac}$ if W_{type} = 1, $C_{w2,bac}$ if W_{type} = 2, $C_{w3,bac}$ if W_{type} = 3	CFU/L	
$C_{w,vir}$	Viral concentration in spray depending on the water type	$C_{w1,vir}$ if W_{type} = 1, $C_{w2,vir}$ if W_{type} = 2, $C_{w3,vir}$ if W_{type} = 3	PDU/L	
V_{surf}	Volume of spray attaching on a raspberry	BetaGeneral (2.3976,2.1805,0.0000364321,0.00021032) [b]	L/berry	Jacxsens et al. (2017)
t_{ap}	Withholding period between the last application and the harvest	Pert (0,30,120)	Days	Survey
D_{bac}	Bacterial decay rate	Triangular (0.008,0.019,0.039)	logCFU/day	Danyluk and Schaffner (2011)
D_{vir}	Viral decay rate	Normal (0.12,0.03)	logPDU/day	Stine et al. (2005)
$N_{harv,bac}$	Number of bacteria at the time of harvest	$10^{\wedge}[\log (C_{w,bac} * V_{surf}) - D_{bac} * t_{ap}]$	CFU/berry	
$N_{harv,vir}$	Number of viruses at the time of harvest	$10^{\wedge}[\log (C_{w,vir} * V_{surf}) - D_{vir} * t_{ap}]$	PDU/berry	
Harvest practices (cross-contamination)				
$P_{hand,bac}$	Bacterial prevalence on harvesters' hands	Beta (7,35)		de Aceituno et al. (2016)
$P_{fhand,vir}$	Viral prevalence on harvesters' hands	Beta (6,73)		León-Félix, Martínez-Bustillos, Báez-Sañudo, Peraza-Garay, and Chaidez (2010)
f_{prod}	Transferred proportion per touch from produce to hand	Beta (15.64,41.94)		Bouwknegt et al. (2015)
ω_{touch}	Surface area of hands that touch the produce	2.1	cm^{2} [b]	Bouwknegt et al. (2015)
ω_{hand}	Total surface area of one side of one hand	245	cm^{2} [b]	EPA (2011)
ω_{prod}	Surface area of produce	Normal (1064,167)/100	cm^{2} [b]	Bouwknegt et al. (2015)
f_{hand}	Transferred proportion per touch from hand to produce	Lognormal (-8.34,0.58)		Bouwknegt et al. (2015)
$N_{hand,bac}$	Number of bacteria on harvester's hands	$P_{hand,bac}$ * $10^{\wedge}$Uniform (1,1.9)	CFU/hand	de Quadros Rodrigues et al. (2014)
$N_{hand,vir}$	Number of viruses on harvester's hand	$P_{fhand,vir}$ * Gamma (0.14,54.6)	PDU/hand	Bouwknegt et al. (2015)
$N_{fcross,bac}$	Number of bacteria after harvesting	Eq. (2)	CFU/berry	
$N_{fcross,vir}$	Number of viruses after harvesting	Eq. (2)	PDU/berry	
Transport from farm to collection center				
$t_{trans,f}$	Transport time from a farm to its associated collection center	Pert (0.00347,0.08333,1)	Days	Survey
$T_{trans,f}$	Temperature during transport of raspberries from farm to collection center	Pert (12,28,28)	°C	Survey
$\mu_{gr,bac}$	Temperature-dependent bacterial growth rate	See Table 4		
$\mu_{red,vir}$	Temperature-dependent viral reduction rate	See Table 4		
$N_{ftrans,bac}$	Number of bacteria after transport from farm to collection center	Log ($N_{fcross,bac}$) + $\mu_{gr,bac}$ * $t_{trans,f}$	logCFU/berry	
$N_{ftrans,vir}$	Number of viruses after transport from farm to collection center	Log ($N_{fcross,vir}$) - $\mu_{red,vir}$ * $t_{trans,f}$	logPDU/berry	

[a] Viral contamination data were for all viruses, including but not limited to Hepatitis A virus. A conversion factor of 100 was used to convert the most probable number of cytopathogenic units (MPNCU) reported for potable water into PCR detectable units (PDU) (Lodder & Husman, 2005).

[b] BetaGeneral (alpha1, alpha2, min, max) defines a distribution with alpha1 and alpha2 as shape parameters, min and max defining the distribution's range.

a raspberry due to the spray (V_{surf}). A conservative assumption that water samples are always positive for *E. coli* or HAV (prevalence of 100%) was made due to the lack of prevalence data by water type. However, based on the documented data on surface water, a relatively high prevalence of *E. coli* or HAV in water for agricultural purposes can be expected (van der Poel & Rzezutka, 2017). Due to imbalanced information, data of all viruses were used to keep consistency across different types of water sources. Such a replacement may lead to an overestimation of HAV contamination in final products.

The microbial load per raspberry at the time of harvest (N_{harv}) was calculated separately for *E. coli* (CFU/berry) and HAV (PDU/berry) as a function of the contamination on a raspberry after the last pesticide application ($C_w * V_{surf}$), the withholding period between the last application and harvest (t_{ap}), and the decay rate (D) using the following

calculations proposed by Danyluk and Schaffner (2011):

$$N_{harv} = 10^{\wedge}(\log(C_w * V_{surf}) - D * t_{ap}) \qquad \text{Equation 1}$$

Different decay rates during the withholding time were incorporated in the model for *E. coli* (D_{bac} (Danyluk & Schaffner, 2011)) and HAV (D_{vir} (Stine, Song, Choi, & Gerba, 2005)). Only the last pesticide application was considered in this model because research has shown that the cumulative effect of multiple pesticide applications is negligible (Petterson, Teunis, & Ashbolt, 2001).

To assess the potential cross-contamination due to harvesting practices on the farm, the Bouwknegt model (Bouwknegt, Verhaelen, Rzetutka, Kozyra, Maunula, von Bonsdorff, et al., 2015) was adopted with adjustment. The load of bacteria or viruses per raspberry after harvest (N_{fcross}, CFU or PDU/berry) as a result of bi-directional cross-

contamination between the produce and the harvesters' hands was calculated:

$$N_{fcross} = N_{harv} - f_{prod} \frac{\omega_{touch}}{\omega_{prod}} N_{harv} + f_{hand} \frac{\omega_{touch}}{\omega_{hand}} N_{hand} \qquad \text{Equation 2}$$

where f_{prod} and f_{hand} are the proportion of microorganisms transferred from raspberries to the harvesters' hand and from the berry to hand, ω_{touch} is the area of the hand that actually touches the raspberries, ω_{prod} and ω_{hand} correspond to the total surface area of a raspberry and a harvester's hand on one side (EPA, 2011), and N_{hand} is the number of microbial particles on the hand picking raspberries.

One of the main factors affecting bacteria or virus populations is their growth or reduction due to exposure to various temperature conditions (T) over a period of time (t), which can occur at multiple stages along the raspberry supply chain. A total of four temperature-dependent models describing the impact of varying temperatures on *E. coli* or HAV contamination of raspberries over time were established (Table 4). Studies have indicated that the minimum temperature supporting the growth of *E. coli* is 4 or 5 °C (Danyluk & Schaffner, 2011). Hence, a bacterial growth model was applied at ambient temperatures above 5 °C, while separate survival models were used for refrigeration temperature (0–5 °C) and frozen temperature (below 0 °C) because different inactivation rates have been observed at these temperatures (Knudsen, Yamamoto, & Harris, 2001). Conversely, higher temperatures usually stimulate more rapid inactivation of HAV, while lower temperatures may enhance viral persistence (Butot, Putallaz, & Sánchez, 2008). Hence, a survival model was used to simulate the inactivation of HAV at environmental temperatures above 0 °C, while no changes in viral contamination were assumed otherwise.

It is noted that the environment inside fresh and frozen raspberries may not support the growth of generic *E. coli* due to its acidic property. The internal pH of raspberries ranges from 3.2 to 3.9 (Bridges & Mattice, 1939), while the minimum pH for *E. coli* growth is 4.4 (ICMSF, 1996). However, the growth of generic *E. coli* was still considered in this model due to the following reasons. It is reasonable to assume the microbial contamination is primarily on the surface of raspberries. Generic *E. coli* internalization in plants may happen but at a limited level. In a review paper summarizing the internalization of bacterial pathogens and indicators into produce and crops (Deering, Mauer, & Pruitt, 2012), one study focusing on generic *E. coli* reported infrequent detections of relative low amount of generic *E. coli* inside leaf tissues of spinach plants (Warriner, Ibrahim, Dickinson, Wright, & Waites, 2003). However, it is still unclear about the behavior of generic *E. coli* on raspberry surface due to lack of information, but it is likely that the surface environment is less acidic. A study evaluating the survival of *E. coli* O157:H7 inoculated on the surface and injected into strawberry showed that the population of *E. coli* O157 strains both on the surface and inside after a 24-h storage under room temperature was greater than the initially inoculated levels, demonstrating the possibility of growth on the surface of the fruit with a similar internal pH range of raspberries (Yu, Newman, Archbold, & Hamilton-Kemp, 2001). The growth can be explained by the higher tolerance of *E. coli* O157 to acidic conditions and may also be contributed by the possibly less acidic environment on the surface. Therefore, a conservative assumption was made that all contamination exists on the surface of raspberry that may support growth of generic *E. coli*.

Specific to transport from the farm to the collection center, the survey revealed that raspberries are usually delivered from farms to collection centers at ambient temperatures ranging from 12 to 28 °C. Hence, a growth model was incorporated in this stage to simulate the potential proliferation of *E. coli*, whereas an inactivation model was considered to simulate the reduction of HAV, as shown in Table 1. The growth rate for *E. coli* ($\mu_{gr,bac}$) and the inactivation rate for HAV ($\mu_{red,vir}$) are both temperature dependent and were calculated by Equations (3) and (4), respectively (Table 4). Specifically, at this step, the number of microbial particles per raspberry at the end of the farm module (N_{ftrans})

was calculated as a function of the microbial contamination after harvesting (N_{fcross}), transport time ($t_{trans,f}$) and transport temperature from the farm to the collection center ($T_{trans,f}$).

$$\begin{cases} \text{Log}(N_{i+1,bac}) = \text{Log}(N_{i,bac}) + \mu_{gr,bac} * t \\ \qquad \mu_{gr,bac} = (b * (T - T_0))^2 \end{cases} \qquad \text{Equation 3}$$

where N_{i+1} is the increased bacterial contamination after the occurrence of proliferation ($N_{ftrans,bac}$ in this case) during a specific period of time (t) at a specific temperature (T) based on an initial contamination ($N_{i,bac}$, $N_{fcross,bac}$ in this case) and a temperature-dependent growth rate of *E. coli* ($\mu_{gr,bac}$) as a function of T and growth model constants (b and T_0) (Table 4).

$$\begin{cases} Log(N_{i+1,vir}) = \text{Log}(N_{i,vir}) - \mu_{red,vir} * t \\ \qquad \mu_{red,vir} = 10^{-logTFL} \end{cases} \qquad \text{Equation 4}$$

where $N_{i+1,vir}$ is the reduced viral contamination after the occurrence of inactivation ($N_{ftrans,vir}$ in this case) during a specific period of time (t) at a specific temperature (T) based on an initial contamination ($N_{i,vir}$, $N_{fcross,vir}$ in this case) and a temperature-dependent reduction rate as a function of the log time required for the first log reduction (TFL) (Table 4).

Based on both published evidence (Verhaelen, Bouwknegt, Rutjes, & de Roda Husman, 2013) and personal communications with local experts in raspberry production, there was no attempt to model the transfer of microbial contamination originating from irrigation water in this study. Expert opinion indicated that the possibility of contamination originating from irrigation water is insignificant. Under high-humidity conditions, raspberries are extremely sensitive to contamination with the fungal species *Botryotinia fuckeliana*. Fruit exposed to irrigation water would spoil immediately due to this fungus and would not be harvested. Although free-roaming wild animals were investigated as a potential source of fresh produce contamination, this source was not considered in this model. Based on responses to our survey, almost all farmers responded they had never seen animals in direct contact with the fruits or animal waste in direct contact with the harvest equipment.

2.3.3. Collection center module

The collection center module covers the stages of holding raspberries at the collection center and transport from the collection center to the associated packing plant. Information on holding and transport temperatures and times were obtained from the survey focusing on the collection center. Similar to the farm module, the same model parameters were used in this module for both fresh and frozen final products. Based on our survey, raspberries are primarily held at collection centers or transported at ambient temperature, with occasional storage or transport at refrigeration temperature (Table 2). Hence, bacterial growth (Equation (3) and Table 4) or inactivation was simulated for temperatures above or below 5 °C, respectively. For temperatures between 0 and 5 °C, the bacterial survival model was incorporated with an estimated inactivation rate of 0.21 logCFU/day ($\mu_{redrfg,bac}$) (Equation (5) and Table 4). As the environmental temperature at the collection center or during transport from the collection center is also above 0 °C, inactivation of HAV is expected and was modelled in this module accordingly by following Equation (4) and Table 4.

$$\text{Log}(N_{i+1,redrfg,bac}) = \text{Log}(N_{i,redrfg,bac}) - \mu_{redrfg,bac} * t \qquad \text{Equation 5}$$

Bacterial inactivation rates were estimated from the data reported by Knudsen et al. (2001). Bacterial concentrations reported in the corresponding figures were extracted using WebPlotDigitizer version 4.2 (Rohatgi, 2019) to estimate the log reduction in *E.coli* per day for fresh ($\mu_{redrfg,bac}$) and frozen storage ($\mu_{redrfz,bac}$). Average log reduction per day was used throughout the storage period under refrigeration temperatures.

Table 2

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

Variable	Description	Value/Distribution/Calculation	Unit	Reference
Holding at the collection center				
t_{cc}	Time that raspberries stay in the collection center	Pert (0.042,0.042,0.29)	Days	Survey
T_{cc}	Temperature in the collection center	Pert (0.5,20,30)	°C	Survey
$\mu_{gr,bac}$ or $\mu_{redrfg,bac}$	Temperature-dependent bacterial growth or inactivation rate	See Table 4		
$\mu_{red,vir}$	Temperature-dependent viral inactivation rate	See Table 4		
Log ($N_{cc,bac}$)	Number of bacteria after holding period at collection center	Log ($N_{ftrans,bac}$) + $\mu_{gr,bac}$ * t_{cc} if $T_{cc} \geq 5$ or Log ($N_{ftrans,bac}$) - $\mu_{redrfg,bac}$ * t_{cc} if $0 \leq T_{cc} < 5$	logCFU/berry	
Log ($N_{cc,vir}$)	Number of viruses after holding period at collection center	$N_{ftrans,vir}$ - $\mu_{red,vir}$ * t_{cc}	logPDU/berry	
Transport from collection center to packing plant				
$T_{trans,cc}$	Temperature during transport from collection center to packing plant	Uniform (1,27)	°C	Survey
$t_{trans,cc}$	Commute time from collection center to packing plant	Pert (0.017,0.67,5)	Days	Survey
Log ($N_{cctrans,bac}$)	Number of bacteria after transport from collection center to packing plant	Log ($N_{cc,bac}$) + $\mu_{gr,bac}$ * $t_{trans,cc}$ if $T_{trans,cc} \geq 5$ or Log ($N_{cc,bac}$) - $\mu_{redrfg,bac}$ * $t_{trans,cc}$ if $0 \leq T_{trans,cc} < 5$	logCFU/berry	
Log ($N_{cctrans,vir}$)	Number of viruses after transport from collection center to packing plant	Log ($N_{cc,vir}$) - $\mu_{red,vir}$ * $t_{trans,cc}$	logPDU/berry	

2.3.4. Packing plant module

In the packing plant module, several stages are modelled, including the short wait time in the receiving area, mainly at ambient temperature; temporary storage in the cold chamber under refrigeration conditions; classification and packing of raspberries by processing handlers; and finally 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.

The changes in bacterial and viral contamination in raspberries during the first two stages and for the transport of fresh products were modelled following the same calculations used in the previous two modules (Equation (3), (4) or (5), depending on the microorganism and environmental temperature). Compared to refrigeration, frozen temperatures may trigger more rapid inactivation of *E. coli*, especially during the first day. Hence, two different reduction rates ($\mu_{redfrz,bac1}$ and $\mu_{redfrz,bac2}$) were introduced to model *E. coli* inactivation during storage and transport of frozen products, which were estimated based on data reported by Knudsen et al. (2001) extracted by WebPlotDigitizer. For a period less than one day, a $\mu_{redfrz,bac1}$ value of 1.34 log reduction/day was used, while for periods beyond 24 h, a $\mu_{redfrz,bac2}$ value of 0.05 log reduction/day was used (Equation (6)). Under frozen conditions, no change in viral contamination was assumed.

$$\begin{cases} \text{Log}(N_{i+1,redfrz,bac1}) = \text{Log}(N_{i,redfrz,bac1}) - \mu_{redfrz,bac1} * t \ if \ t \leq 1) \\ \text{Log}(N_{i+1,redfrz,bac2}) = \text{Log}(N_{i,redfrz,bac2}) - \mu_{redfrz,bac1} * 1 - \mu_{redfrz,bac2} * \\ \qquad (t-1) \ if \ t > 1) \end{cases}$$

Equation 6

Bi-directional cross-contamination can also occur between the handler's hand and fruit during processing, similar to the bi-directional cross-contamination occurring during harvest on the farm. Equation (2) was used to model the cross-contamination during processing, with adjustment by replacing the data on bacterial and viral contamination on packers' hands with data specific for packers (Table 3).

2.3.5. Sensitivity analysis

A sensitivity analysis was conducted on the baseline model to identify the most important factors influencing the model's output, in this case, the microbial concentration per gram of final raspberry products. The change in output mean was measured by varying all stochastic inputs between their extremes, which was conducted using the "Risk Sensitivity Stat Change" function in @Risk by setting the bin size of 20. The range of output mean due to varying a particular stochastic input was computed by @Risk through running 20 bins of the input and

recorded in an Excel worksheet. The results of the sensitivity analysis were visualized in tornado charts using R (R Core Team, 2016), which were generated for each combination of microorganism and product type.

2.3.6. Intervention scenarios

The efficacy of microbial control interventions that potentially can be adopted at different steps along the raspberry supply chain was evaluated through scenario analyses. Possible interventions were chosen based on the sensitivity analysis results and discussions of the feasibility of potential interventions with SAG and ACHIPIA. The goal was to provide a scientific basis for informing risk-based and reasonably feasible intervention recommendations based on the practical experiences of the stakeholders. A total of 17 scenarios were run in the model, including a baseline scenario for comparative purposes (representing current common practices as described in the three modules) and 16 intervention scenarios to predict the level of microbial safety and quality protection in raspberry products if an individual intervention technology or regulation (11 scenarios) or a combination (5 scenarios) was adopted. The list of scenarios evaluated is shown in Table 5 and included water interventions, extending the withholding time after pesticide application, reducing the time and better controlling the environmental temperature during transport, better controlling the temperature at the collection center, and more restricted hygiene requirements for workers on the farm and at the packing plant.

Water routinely used to dilute pesticide can be one of the primary sources of microbial contamination for fresh produce (James, 2006; Moncrief & Bloom,). The on-farm practice survey showed that raspberry farms in Chile mainly rely on three types of water sources: potable water, groundwater and surface water (surveys of this study), in decreasing order of microbial safety (INN, 2005; WHO, 2004). Hence, one of the water intervention actions evaluated in this study was increasing the use of potable water and/or groundwater instead of surface water as a result of improvements in the public water treatment and supply infrastructure in Chile. The changes in water sources were modelled by increasing the proportions of raspberry farms using potable and/or groundwater. To control the microbial loads in the water sources, the introduction of ultraviolet (UV) light was evaluated as another intervention action in this study, as UV lamps are easy to install, relatively inexpensive, and do not create harmful byproducts (Masse, Masse, Topp, Seguin, Ortega, Scott, et al., 2011). UV light reduces bacterial and viral contamination in water by up to 9 logs for bacteria and 4 logs for viruses (Jones, Worobo, & Smart, 2014; Nuanualsuwan, Mariam, Himathongkham, & Cliver, 2002). In the scenario of UV treatment implementation, it was assumed that microbial loads could be reduced

Table 3

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

Variable	Description	Value/Distribution/Calculation	Unit	Reference
Received at packing plant				
t_{rec}	Waiting time when receiving raspberries	Pert (0,0.0069,0.0417)	Days	Survey
T_{rec}	Average temperature in receiving space	Pert (1,25,27)	°C	Survey
Log $(N_{rec,bac})$[a]	Number of bacteria after waiting time after receipt at packing plant	Log $(N_{cctrans,bac})$ + $\mu_{gr,bac}$ * t_{rec} if $T_{rec} \geq 5$ or	LogCFU/berry	
		Log $(N_{cctrans,bac})$ - $\mu_{redrfg,bac}$ * t_{rec} if $0 \leq T_{rec} < 5$		
Log $(N_{rec,vir})$[a]	Number of viruses after waiting time after receipt at packing plant	Log $(N_{cctrans,vir})$ - $\mu_{red,vir}$ * t_{rec}	LogPDU/berry	
Storage in 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 $(N_{stg,bac})$[a]	Number of bacteria after cold storage at packing plant	Log $(N_{rec,bac})$ + $\mu_{gr,bac}$ * t_{cold} if $T_{cold} \geq 5$ or	LogCFU/berry	
		Log $(N_{rec,bac})$ - $\mu_{redrfg,bac}$ * t_{cold} if $0 \leq T_{cold} < 5$		
Log $(N_{stg,vir})$[a]	Number of viruses after cold storage at packing plant	Log $(N_{rec,vir})$ - $\mu_{red,vir}$ * t_{cold}	LogPDU/berry	
Processing practices (cross-contamination)				
$P_{hand,bac}$	Bacterial prevalence on packers' hands	Beta (7,35)		de Aceituno et al. (2016)
$P_{phand,vir}$	Viral prevalence on packers' hands	Beta (27,32)		León-Félix et al. (2010)
$N_{food,bac}$	Number of bacteria on packers' hands	$P_{hand,bac}$ * 10^Uniform (1,1.9)	CFU/hand	de Quadros Rodrigues et al. (2014)
$N_{food,vir}$	Number of viruses on packers' hands	$P_{phand,vir}$ * Gamma (0.67,1.62)	PDU/hand	Bouwknegt et al. (2015)
$N_{pcross,bac}$	Number of bacteria on raspberries after classifying and packing	Eq. (2) and Table 1	CFU/berry	
$N_{pcross,vir}$	Number of viruses on raspberries after classifying and packing	Eq. (2) and Table 1	PDU/berry	
Processing practices (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 $(N_{pack,bac})$[a]	Number of bacteria after whole processing stage	Log $(N_{pcross,bac})$ + $\mu_{gr,bac}$ * t_{pack} if $T_{pack} \geq 5$ or	LogCFU/berry	
		Log $(N_{pcross,bac})$ - $\mu_{redrfg,bac}$ * t_{pack} if $0 \leq T_{pack} < 5$		
Log $(N_{pack,vir})$[a]	Number of viruses after whole processing stage	Log $(N_{pcross,vir})$ - $\mu_{red,\,vir}$ * tpack if $T_{pack} \geq 0$ or	LogPDU/berry	
		Log $(N_{pcross,vir})$ if $T_{pack} < 0$		
Transport from packing plant to destination (fresh product only)				
$t_{trans,p,fresh}$	Time for transport to destination for fresh raspberries	Pert (0.083,0.1667,6)	Days	Survey
$T_{trans,p,fresh}$	Temperature of cooling truck during transport of fresh raspberries	Uniform (0,5)	°C	Survey
M_{berry}	Average weight of a raspberry	4	g	Iannetta et al. (2000)
$C_{ptrans,bac,fresh}$[a]	Concentration of bacteria upon arrival at destination for fresh raspberries	(Log $(N_{pack,bac})$ - $\mu_{redrfg,bac}$ * $t_{trans,p,fresh}$)/M_{berry}	LogCFU/g	
$C_{ptrans,vir,fresh}$[a]	Concentration of virus upon arrival at destination for fresh raspberries	(Log $(N_{pack,vir})$ - $\mu_{red,vir}$ * $t_{trans,p,fresh}$)/M_{berry}	LogPDU/g	
Freezing process and storage				
T_{frz}	Temperature of freezing chamber	Pert (-35,-18,-15)	°C	Survey
t_{frz}	Time in freezing chamber	Pert (0.01,3,120)	Days	Survey
Log $(N_{frz,bac})$[a]	Number of bacteria at the end of storage in freezing chamber	Log $(N_{pack,bac})$ - $\mu_{redfrz,bac}$ * t_{frz}	LogCFU/berry	
Log $(N_{frz,vir})$	Number of viruses at the end of storage in freezing chamber	Log $(N_{pack,vir})$	LogPDU/berry	
Transport from packing plant to destination (frozen product only)				
$t_{trans,p,frz}$	Time for transport to destination for frozen raspberries	Pert (0.0833,0.1667,60)	Days	Survey
$T_{trans,p,frz}$	Temperature of cooling truck during transport of frozen raspberries	Pert (-22,-18,-15)	°C	Survey
$C_{ptrans,bac,frz}$[a]	Concentration of bacteria upon arrival at destination for frozen raspberries	(Log $(N_{frz,bac})$ - $\mu_{redfrz,bac}$ * $t_{trans,p,frz}$) / M_{berry}	LogCFU/g	
$C_{ptrans,vir,frz}$	Concentration of virus upon arrival at destination for frozen raspberries	Log $(N_{frz,vir})$ / M_{berry}	LogPDU/g	

[a] For the calculation of these variables, refer to Table 4 for parameters and equations for $\mu_{gr,bac}$, $\mu_{redrfg,bac}$, $\mu_{redfrz,bac}$, and $\mu_{red,vir}$.

by 0–9 logCFU/L of water for *E. coli* and 0–4 logPDU/L for HAV. Combinations of changes in water type and reducing microbial contamination in water were also evaluated. Detailed descriptions of the intervention scenarios are provided in Table 5.

The results of the scenario analyses were expressed as 1) the mean microbial concentration in each intervention scenario for *E. coli* or HAV and fresh or frozen product; 2) the difference in output means between the intervention scenario and the baseline as log changes; and 3) the intervention efficacy as the percentage of contamination that would be reduced if the specific intervention(s) had been implemented, as calculated using Equation (7).

$$Intervention\ efficacy = \left(1 - 10^{(C_{intervention} - C_{baseline})}\right) * 100\% \qquad \text{Equation 7}$$

where $C_{intervention}$ and $C_{baseline}$ are the mean model outputs in logCFU/g or logPDU/g for fresh or frozen raspberries for *E. coli* or HAV for the intervention and baseline scenarios, respectively.

3. Results and discussion

3.1. Microbial contamination estimates under current practices

Figs. 2 and 3 show the contamination distribution of *E. coli* and HAV, respectively, in the final products estimated from the baseline

E. coli contamination in fresh and frozen raspberries
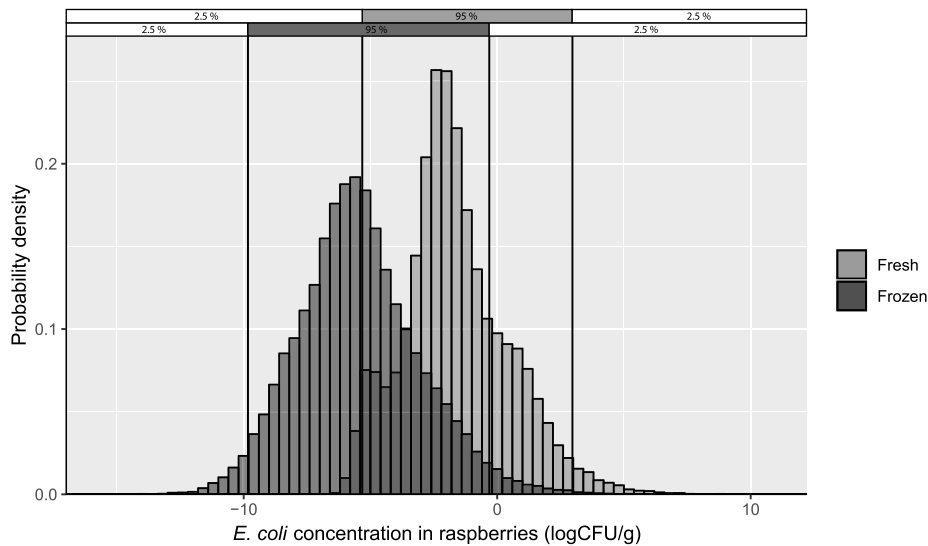

Fig. 2. The overlaid distributions of *E. coli* contamination in fresh and frozen raspberries in the baseline scenario.

model. In each figure, the contamination distributions for the fresh and frozen products are overlaid. The baseline model describes a supply chain in which only common current practices are applied, without considering the interventions of interest. Based on the responses to our survey, the baseline model is expected to provide a good representation of common practices in the raspberry industry in Chile. Under the baseline conditions, the mean contaminations of generic *E. coli* were estimated as −1.64 (95%CI: −1.65 to −1.61) and −5.46 (95%CI: −5.48 to −5.43) logCFU/g in fresh and frozen raspberries, respectively. For HAV, the estimated means were −6.45 (95%CI: −6.46 to −6.45) and −6.51 (95%CI: −6.52 TO -6.51) logPDU/g in fresh and frozen raspberries, respectively. HAV contamination was very similar between fresh and frozen products due to the negligible change in viral contamination under the frozen environment. Conversely, the log reduction of *E. coli* triggered by low temperature is the major reason for the disparity in *E. coli* contamination between fresh and frozen products.

Limited data on microbial contamination in raspberries in Chile are available for validation of the simulated results. However, the Chilean

Food Sanitary Regulation (RSA) establishes maximum detected levels of *E. coli* of 2–3 logCFU/g in fresh fruits and 1–2 logCFU/g in frozen fruits based on three-class sampling plan ($n = 5$, $c = 2$; n is the number of sample units analyzed, and c is the maximum allowable number of sample units yielding marginal results) regulated by Chilean Ministry of Health. In our simulation, the probability of *E. coli* contamination over 2 logCFU/g in fresh raspberries was estimated as 5.3%, and the probability of *E. coli* contamination over 1 logCFU/g in frozen raspberries was estimated as 1.0%. Thus, the estimated *E. coli* contamination is within the allowable range regulated by RSA. It is reasonable to assume that there is a zero-tolerance level where no HAV particles are allowed in any tested sample (e.g., 25 g of a sample unit). In this case, the contamination level would be less than 1 particle/25 g, resulting in a limit of −1.4 logPDU/g. Based on our simulation, the probability of HAV contamination higher than this limit is 0% for both fresh and frozen raspberries. A large monitoring program of foodborne viruses in berries sourced from 10 countries in Europe and North America analyzed from 2009 to 2016 showed that 0.1% berry samples were positive for HAV (2 of 2015 samples, 95% CI: 0.03–0.4%) (Li, Butot, Zuber,

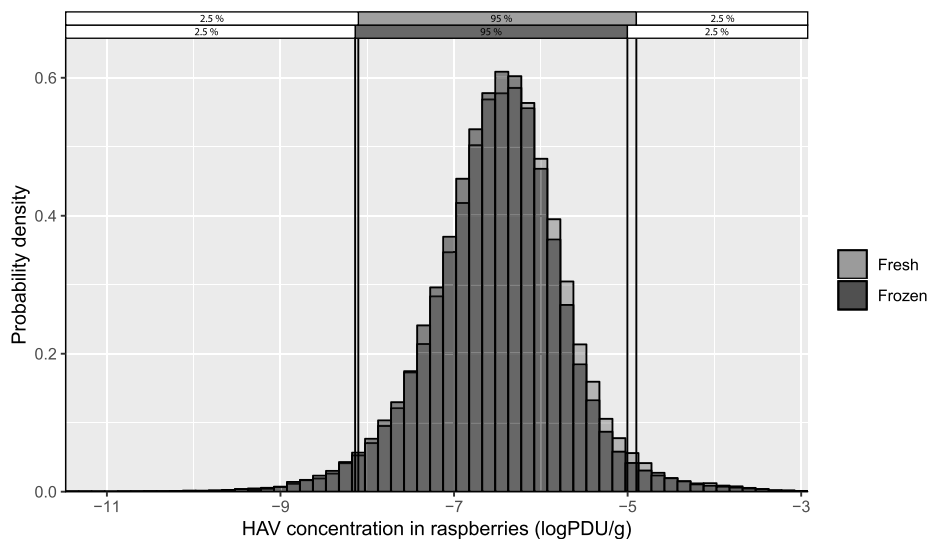## HAV contamination in fresh and frozen raspberries

Fig. 3. The overlaid distributions of Hepatitis A virus contamination in fresh and frozen raspberries in the baseline scenario.

Uyttendaele, & Profel, 2018), which indicates that our simulation results are consistent with empirical observations.

The baseline scenario may not be an accurate representation of current contamination estimates of *E. coli* and HAV contamination in raspberry products in Chile since some initial input variables, such as water contamination and hand contamination from harvesters or packers, were populated with data extracted from studies conducted in countries other than Chile. Hence, the simulation model developed in this study was primarily used for the evaluation of the relative impacts of factors along the raspberry supply chain that may influence microbial contamination in the end products and for comparisons between the potential intervention scenarios and the baseline. These analyses can provide scientific support for decisions regarding microbial contamination control measures in fresh and frozen raspberry products.

3.2. Sensitivity analysis for identifying important factors influencing microbial contamination in raspberries

The tornado charts shown in Figs. 4 and 5 indicate the factors with the most significant impact on microbial contamination in fresh and frozen products by *E. coli* and HAV, respectively. For *E. coli* contamination in both fresh and frozen raspberries, the top influential factors are the type of water used for pesticide application and the environmental conditions at various steps influencing the behavior of the bacterial population, such as the time and temperature during transport and at the collection center and the withholding period after the last pesticide application. For frozen products, the time and temperature of the freezing period and during the transport of raspberries in frozen chambers are also important influences of the final *E. coli* contamination.

According to the results, for both fresh and frozen products, the type of water used for pesticide spraying (W_{type}) is the leading factor influencing *E. coli* contamination (Fig. 4). For example, in a hypothetical situation if all raspberry farmers used surface water (the lowest-quality type), the mean concentration of *E. coli* would increase to 1.30 logCFU/g in fresh raspberries and −2.50 logCFU/g in frozen raspberries. Conversely, if potable water (the highest-quality type) was accessible and affordable for all farmers, the contamination would be reduced to −4.33 and −8.20 logCFU/g in fresh and frozen raspberries, respectively. These results emphasize the significance of water quality for raspberry famers, which is supported by several studies indicating that water is one of the main vehicles for microbial contamination in fresh produce, especially water used for pesticide application (James, 2006; Moncrief & Bloom,). Hence, scenarios with higher percentages of farmers using higher-quality water on raspberry farms are evaluated in the following analyses.

By contrast, the impact of water-quality related factors on the final HAV contamination in raspberry products ranks lower than the impact of factors related to cross-contamination due to workers' handling of raspberries at harvest and during processing (Fig. 5). Changes in the number of virus particles on harvesters' or packers' hands within the range examined may lead to a difference in contamination up to ~1.6 logs in both fresh and frozen products, whereas the water-related variables (water type and contamination) only lead to a difference of less than 0.5 logs. Given the greater variability in the model outputs attributable to hand contact and the higher contamination that can be introduced from hands compared with pesticide water (estimation based on Table 1), a decision was made to focus on evaluating the role of hygiene practices in controlling viral contamination on harvesters' and packers' hands in the scenario analyses.

Transport of raspberries among the farm, collection center and packing plant is a transitional phase but plays an important role in controlling the bacterial quality and safety of raspberry products. The time and temperature during transport of raspberries from the collection center to the packing plant are particularly critical, primarily for two reasons: great variations in both time and temperature were observed among the premises in the survey, and raspberries are mostly delivered under ambient temperature (the harvest season is in summer), which increases the potential for bacterial proliferation. Hence, reducing the transport time and regulating the temperature, such as by providing a controlled refrigeration temperature, may help maintain produce quality and reduce bacterial contamination in the end products. Conversely, inactivation of HAV can be expected under ambient temperature. Although the transport time and temperature may affect the viral population, these effects differ in direction from those for bacteria and were not identified as important for HAV compared with *E. coli*. The disparities in these factors' significance between *E. coli* and HAV are mainly due to the large differences in the bacterial growth rate and the viral inactivation rate. Based on the equations provided in Table 4, a temperature of 25 °C is associated with an increase in the bacterial growth rate of 1.9 logs per day but only a decrease of 0.01 logs per day for HAV. Hence, when exposed to the same temperature for a specific period, the change in the *E. coli* population is expected to be much larger than the change in the HAV population.

For all products and hazards, two recurring influential variables are related to the degradation of microorganisms introduced by pesticide application: the withholding period between the last pesticide application and harvest (t_{ap}) and the microbial decay rate (D_{bac} or D_{vir}). Withholding period statements can usually be found on the labels of pesticide products. The withholding period is designed to ensure that foods derived from the treated crops comply with maximum residue limits to reduce adverse health impacts. The sensitivity analysis of our simulation model also showed that the withholding time is critical for controlling microbial contamination in raspberries. The variable t_{ap} was identified as the top influential factor for HAV contamination in both fresh and frozen raspberries, resulting in ~1.5-log changes when t_{ap} was varied within its range. The changes in t_{ap} resulted in the changes in *E. coli* contamination in end products at a similar level as HAV, however, with a relatively lower rank compared to other factors that are of greater impact on *E. coli* contamination.

3.3. Scenario analyses for informing potential control measures

Table 5 lists the possible interventions examined in this study and summarizes the results. For Scenarios A-C, changing the proportions of farms using specific water types had a strong impact on the bacterial populations but not the virus populations. Increasing the proportion of farms using potable water from 14% to 90% (Scenario C) greatly reduced bacterial contamination, resulting in reductions of ~2.3 logCFU/g (almost 100% of initial contamination during rounding) in both fresh and frozen products. However, such a transition would require infrastructure development and economic investment from both the public and private sectors, which may not be achieved in a short period of time. Hence, scenarios in which the use of surface water was replaced by groundwater in combination with the transition to potable water were also examined and showed that 60–90% *E. coli* contamination decrease could be expected in the end products, representing a reduction of 0.5–1.2 logCFU/g (Scenarios A and B). Viral populations were less affected by changes in the proportion of use of different water types.

The results suggested that another intervention for improving water microbial quality evaluated in this study, UV light treatment, could significantly reduce both bacterial and viral populations. Compared with mainly using potable water (Scenario C), UV light treatment was estimated to reduce bacterial populations to similar levels and to control HAV contamination more effectively. As shown in Table 5, the log reductions in the final products achieved by this technology (Scenario D) for bacteria and viruses were as high as 2.1 logCFU/g and 0.2 logPDU/g, respectively. According to the Food Scientists Network, UV technology is currently being used in some small farms in Chile, suggesting the potential for wider recognition and application of this intervention. However, the combination of both UV lamps and increasing

Tornado Chart for *E. coli* Contamination on Fresh and Frozen Raspberries
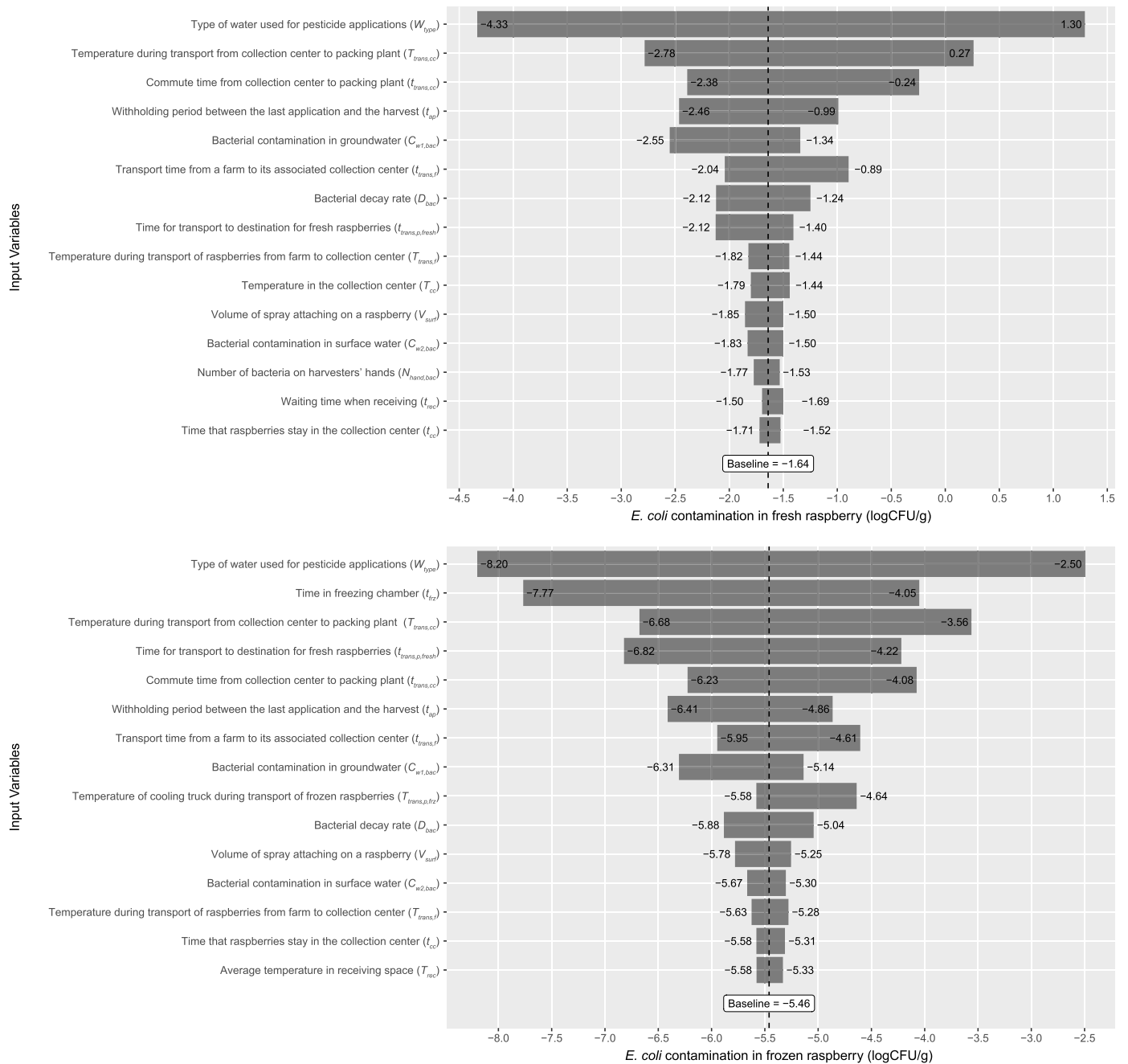

Fig. 4. Tornado plots for *E. coli* contamination in fresh and frozen raspberries.

potable water use (Scenarios A + D and B + D) did not seem to provide considerable further reduction, especially considering that the efficacy of the UV light intervention is primarily the result of reduced microbial contamination originating from groundwater.

The analyses of Scenarios E and F show that reducing the transport times from the farm to collection center ($t_{trans,f}$) and from the collection center to packing plant ($t_{trans,cc}$) may effectively control *E. coli* contamination in both fresh and frozen raspberries. In both scenarios, a transport time of 1 h was arbitrarily chosen. A combination of reduced transport time during both steps (Scenario E + F) can lead to a cumulative reduction of 1.2 logCFU/g, corresponding to a 93% reduction of the contamination level compared with current practices. From the perspective of mathematical simulation, the significant reduction in final contamination could be due to 1) the significant difference

between a transport time of 1 h and the mean values of $t_{trans,f}$ and $t_{trans,cc}$ and 2) the reduced effect of variations in transport time across different farms, collection centers and packing plant (neglected in Scenarios E and F as a deterministic value). From the perspective of practical application, the results indicate the need for more systematic design and management of the raspberry supply chain that cohesively considers production planning and inventory management among all sectors along the chain. In some scenarios, a minor increase in viruses was even estimated, which is not necessary an indication that reducing transport time will introduce viral contamination. The slight increase in mean contamination might be due to the randomness of the simulation, as variability and uncertainty were quantified and incorporated throughout the simulation process.

Compared with controlling transport time, temperature

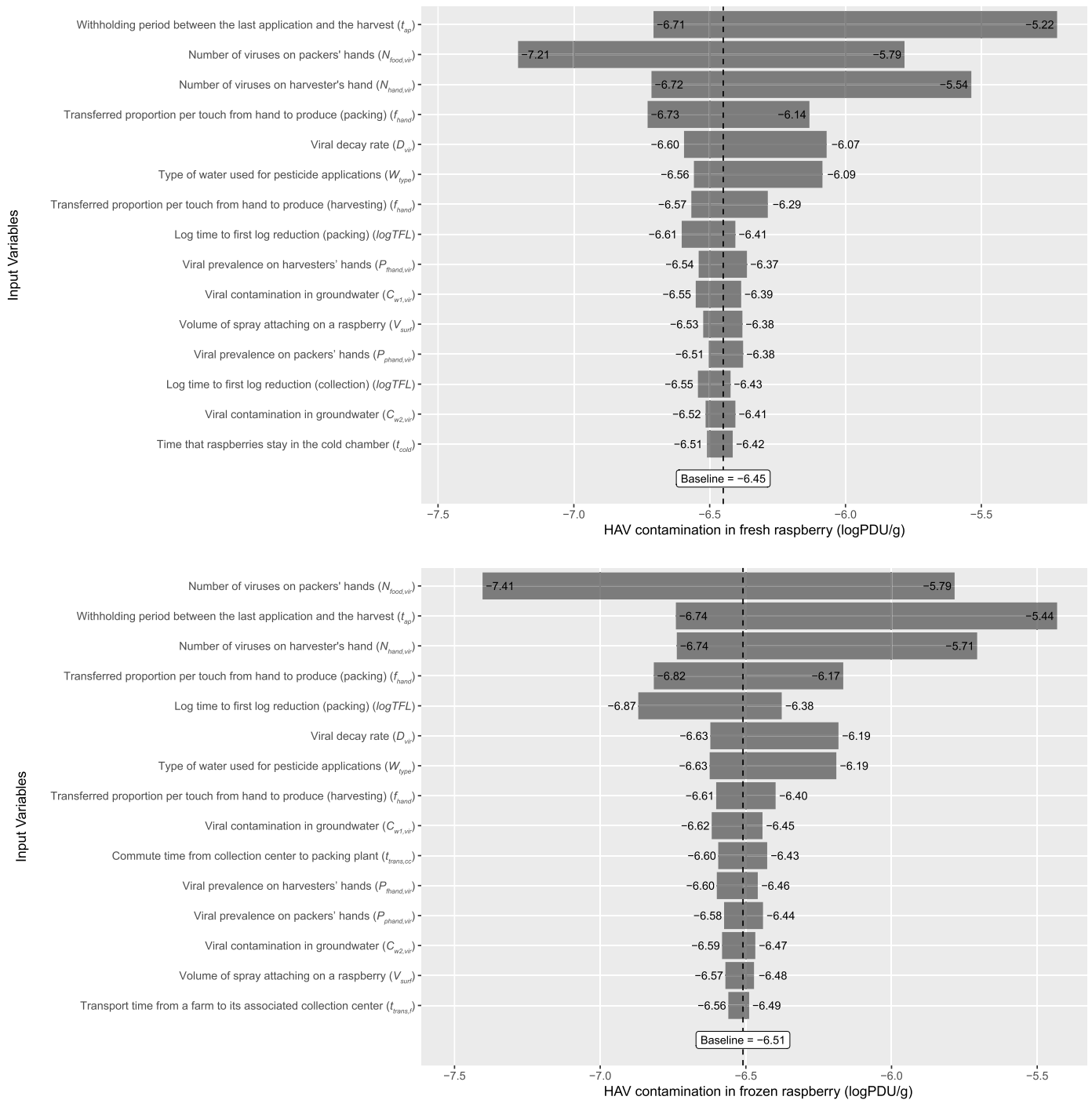## Tornado Chart for HAV Contamination on Fresh and Frozen Raspberries

Fig. 5. Tornado plots for Hepatitis A virus contamination in fresh and frozen raspberries.

management showed similar efficiency in controlling *E. coli* contamination in the final product and thus appears to be another effective intervention. According to our surveys, fresh fruit is currently received at the collection center and transported from the collection center to the packing plant under unrefrigerated conditions. In Scenario G, a refrigeration condition with a slight temperature fluctuation during transport from the collection center to packing plant was assumed to be fully implemented, resulting in a reduction of nearly 0.9 logCFU/g. However, combining the interventions of reduced transport time and temperature control (Scenario F + G) did not elicit a synergistic effect. The main reason is that bacterial growth is influenced by the interaction

between time and temperature. When the transport time is reduced, the effectiveness of the temperature intervention is discounted accordingly. Considering the apparent high monetary costs and demands on resources, a recommendation is to consider interventions to either reduce transport time through systematic supply chain management or control temperature through wide application of cold chain systems.

Although the withholding period after pesticide application before harvest appeared to be an important input in the simulations (Figs. 4 and 5), the reductions achieved for Scenarios H and I were considerably smaller than those for the previous scenarios. The practices associated with these scenarios, which are related to an increase in the

Table 4
Parameters and calculations for temperature-dependent microbial growth or survival models.

Variable	Description	Value/Distribution/Calculation	Unit
Bacterial growth model for temperature over 5°C			
$\mu_{gr,bac}$	Growth rate	$(b*(T-T_0))^2$ [a]	LogCFU/day
T	Temperature of modelled step	See Tables 1–3	°C
T_0 [a]	Temperature constant 1	2.628	°C
b [a]	Temperature constant 2	0.0616	Sqrt (log CFU/day)/°C
t	Time of modelled step	See Tables 1–3	Days
Log ($N_{i,bac}$)	Initial contamination	Output from previous step	LogCFU/berry
Log ($N_{i+1,bac}$)	Final contamination	Log ($N_{i,bac}$) + $\mu_{gr,bac}$ * t	LogCFU/berry
Bacterial survival model for temperature 0–5°C			
$\mu_{redrfg,bac}$ [b]	Reduction per day	0.21	Logs/day
t	Time of modelled step	See Tables 1–3	Days
Log ($N_{i,redrfg,bac}$)	Initial contamination	From previous step	LogCFU/berry
Log ($N_{i+1,redrfg,bac}$)	Final contamination	Log ($N_{i,redrfg,bac}$) - $\mu_{redrfg,bac}$ * t	LogCFU/berry
Bacterial survival model for temperature below 0°C			
$\mu_{redfrz,bac1}$ [b]	Reduction per day, less than or equal to 1 day at the freezing temperature	1.34	Logs/day
$\mu_{redfrz,bac2}$ [b]	Reduction per day, more than 1 day at the freezing temperature	0.05	Logs/day
t	Time of the modelled step	See Tables 1–3	Days
Log ($N_{i,redfrz,bac}$)	Initial contamination	From previous step	LogCFU/berry
Log ($N_{i+1,redfrz,bac}$)	Final contamination	Log ($N_{i,redfrz,bac}$) - $\mu_{redfrz,bac1}$ *t if $t \leq 1$ or Log ($N_{i,redfrz,bac}$) - $\mu_{redfrz,bac1}$ * 1 - $\mu_{redfrz,bac2}$ * (t-1) if $t > 1$	LogCFU/berry
Viral survival model for temperature over 0°C			
$\bar{X}_{TFL}$ [3]	Mean log time to first log reduction	$2 - T * 1.4 * 10^{-5}$	Log (day)
s_{TFL} [c]	Standard deviation of log time to first log reduction	$\sqrt{0.31 - T * 6.1 * 10^{-4} + T^2 * 1.4 * 10^{-5}}$	Log (day)
T	Temperature of modelled step	See Tables 1–3	°C
LogTFL [c]	Log time to first log reduction	Normal ($\bar{X}_{TFL}$, s_{TFL})	Log (day)
$\mu_{red,vir}$	Log reduction per day	$10^{-logTFL}$	LogPDU/day
t	Time of modelled step	See Tables 1–3	Days
Log ($N_{i,vir}$)	Initial contamination	From previous step	LogPDU/berry
Log ($N_{i+1,vir}$)	Final contamination	Log ($N_{i,vir}$) - $\mu_{red,vir}$ * t	LogPDU/berry

[a] Parameters and equations are adopted from Danyluk and Schaffner (2011).
[b] Parameters were estimated from Knudsen et al. (2001).
[c] Parameters and equations are adopted from Bertrand et al. (2012).

withholding time after pesticide applications, can be very resource-consuming and inefficient and thus do not seem to be practical interventions for controlling *E. coli* and HAV contamination in raspberry products.

Most factors examined in the scenario analysis favor the control of *E. coli* contamination. However, for reducing HAV contamination in the final products of fresh and frozen raspberries, controlling the contamination on the harvesters' ($N_{hand,vir}$) and packers' hands ($N_{food,vir}$) is more effective. The findings support the results of the sensitivity analysis in this study, which showed higher ranks of $N_{hand,vir}$ and $N_{food,vir}$, as well as the conclusion from another QMRA study that the contribution of hand contact to virus contamination is larger than that of other potential sources, including irrigation water, conveyor belts or water used for produce rinsing (Bouwknegt et al., 2015). Based on our simulation, poor hygiene of packers during the processing stage would adversely affect HAV contamination to a greater extent than poor hygiene of harvesters on the farm. This difference is primarily attributable to the storage and delivery of raspberries under either refrigerated or frozen conditions after packing. Under these conditions, the HAV population is largely preserved. Hence, the contamination introduced by packers can be maintained to the end, whereas contamination introduced by harvesters can be inactivated during transport from the farm through collection to the packing plant. Based on the results, more restrictive hygiene requirements for workers directly contacting fruit is highly recommended, with an emphasis on the processing stage.

3.4. Identification of data gaps and future steps

The lack of information pertinent to the food system being modelled is a significant limitation of this study. Data gaps can be classified into two categories: 1) non-local data and 2) non-optimized data. The first

category indicated the need to collect from Chilean source data that have not been created. For example, the lack of data on microbial contamination in different types of agricultural water resulted in significant uncertainties in the estimates of microbial contamination in raspberry products from the baseline model. The second category refers to data collected from similar but different systems than the one modelled in this study. For example, the transfer rates proposed by Stine et al. were intended for lettuce, not raspberries (Stine et al., 2005). Similarly, the microbial decay rate used in this study was reported for spinach rather than raspberries (Danyluk & Schaffner, 2011).

A summary of identified data gaps, their implementation in the model, suggestions for future studies, possible effects and relative importance are summarized qualitatively in Table 6. The most important non-local data gaps were identified as the concentration of *E. coli* and HAV in surface and groundwater in Chile ($C_{w1,bac}$, $C_{w2,bac}$, $C_{w1,vir}$, $C_{w2,vir}$) and the number of HAV on farm and plant workers ($N_{hand,vir}$, $N_{fhand,vir}$) based on the relative importance of these variables in the sensitivity analysis. The most important non-optimized data gap was viral decay rate (D_{vir}) because data were taken from a study investigating viral inactivation on the surface of lettuce rather than raspberries. Other parameters related to hand contamination ($P_{hand,bac}$, $P_{hand,vir}$) were classified as both non-local and non-optimized since these studies were conducted in different geographies with different agricultural products. Bacterial decay (D_{bac}) and growth ($\mu_{gr,bac}$, T_0, b) variables were also identified as non-optimized data gaps as the bacterial decay rate was studied on leafy greens and growth parameters did not account for specific food products, pH or moisture changes on the surface of raspberries. The direction of possible effect for irrigation water was evaluated as an overestimation of the contamination levels (conservative estimate) since used data were representing the highest observed concentrations in water sources. For the remaining variables, the direction

Table 5

List of interventions evaluated in this study and summary of scenario analysis results for bacterial and viral contamination in fresh and frozen raspberries.

Scenario	Description	Fresh Model			Frozen Model		
		Mean concentration (logCFU/g logPDU/g)	Log change (logCFU/g logPDU/g)	Intervention efficacy	Mean concentration (logCFU/g logPDU/g)	Log change (logCFU/g logPDU/g)	Intervention efficacy
Baseline	See notes for detailed description[a]	−1.64	–	–	−5.46	–	–
		−6.45	–	–	−6.51	–	–
A	**Water type:** 86% groundwater, 0% surface water, 14% potable water	−2.08	0.45	64%	−5.91	0.45	65%
		−6.51	0.06	13%	−6.57	0.05	12%
B	**Water type:** 42% groundwater, 8% surface water, 50% potable water	−2.79	1.16	93%	−6.63	1.17	93%
		−6.51	0.05	12%	−6.56	0.05	11%
C	**Water type:** 5% groundwater, 5% surface water, 90% potable water	−3.93	2.30	99%	−7.80	2.33	100%[e]
		−6.55	0.09	19%	−6.60	0.08	18%
D	**Water treatment:** UV disinfection of water	−3.78	2.14	99%	−7.65	2.19	99%
		−6.64	0.18	35%	−6.67	0.16	31%
A + D	**Water type:** 86% groundwater, 0% surface water, 14% potable water **Intervention:** UV disinfection of water	−4.00	2.37	100%[e]	−7.87	2.41	100%[e]
		−6.66	0.21	38%	−6.69	0.18	34%
B + D	**Water type:** 42% groundwater, 8% surface water, 50% potable water **Intervention:** UV disinfection of water	−4.05	2.42	100%[e]	−7.92	2.46	100%[e]
		−6.65	0.20	37%	−6.69	0.18	34%
E	**Transport time from farm to collection center:** 1 h	−1.98	0.34	55%	−5.81	0.35	55%
		−6.45	0	no change	−6.51	0	no change
F	**Transport time from collection center to packing plant:** 1 h	−2.47	0.83	85%	−6.30	0.84	86%
		−6.44	(0.01)[b]	(3%)[c]	−6.51	0	no change
E + F	**Transport time from farm to collection center:** 1 h **Transport time from collection center to packing plant:** 1 h	−2.81	1.17	93%	−6.64	1.18	93%
		−6.44	(0.01)[b]	(3%)[c]	−6.51	0	no change
G	**Temperature during transport from collection center to packing plant:** 4–8 °C (fully implemented refrigeration)	−2.51	0.87	87%	−6.33	0.87	87%
		−6.45	0	no change	−6.47	(0.04)[b]	(11%)[c]
F + G	**Transport time from collection center to packing plant:** 1 h **Temperature during transport from collection center to packing plant:** 4–8 °C (fully implemented refrigeration)	−2.49	0.86	86%	−6.33	0.87	86%
		−6.45	(0.01)[b]	(2%)[c]	−6.47	(0.05)[b]	(12%)[c]
H[d]	**Withholding period after pesticide application:** 25% increase from baseline	−1.81	0.18	33%	−5.64	0.18	35%
		−6.53	0.07	15%	−6.58	0.06	13%
I[d]	**Withholding period after pesticide application:** 50% increase from baseline	−2.00	0.36	57%	−5.82	0.36	56%
		−6.57	0.12	24%	−6.62	0.10	21%
J[d]	**Number on harvester's hand:** Reduced to 25% of baseline	−1.68	0.05	10%	−5.52	0.06	12%
		−6.57	0.11	23%	−6.61	0.10	20%
K[d]	**Number on packer's hand:** Reduced to 25% of baseline	−1.64	0	no change	−5.46	0	no change
		−6.76	0.30	50%	−6.84	0.33	53%
J + K[d]	**Number on harvester's hand:** Reduced to 25% of baseline **Prevalence on packer's hand:** Reduced to 25% of baseline	−1.70	0.06	13%	−5.53	0.07	14%
		−6.91	0.46	65%	−6.99	0.47	66%

[a] In the baseline simulation, variables were estimated as follows representing the practices at the time of this analysis: **Water type:** 71% groundwater, 15% surface water, 14% potable water; **Transport time from farm to collection center:** Pert (0.0035,0.083,1) days; **Transport time from collection center to packing plant:** Pert (0.017,0.67,5) days; **Temperature at collection center:** Pert (0.5,20,30) °C; **Withholding period after pesticide application:** Pert (0,30,120) days; **Number on harvester's hand:** See Table 1 for the distribution and calculation for *E. coli* and HAV contamination in the baseline model; **Number on packer's hand:** See Table 3 for the distribution and calculation for *E. coli* and HAV contamination in the baseline model.

[b] A number in parentheses indicates a negative value. Hence, a log reduction in parentheses indicates a log increase associated with the implementation of a particular intervention compared to the baseline.

[c] A number in parentheses indicates a negative value. Hence, an intervention efficacy in parentheses indicates that the intervention examined is not effective in controlling contamination and does not necessarily indicate that the intervention is associated with an increase in contamination. The slight increase could be due to the randomness of the simulation process.

[d] A percentage change (x %) refers to the change in microbial contamination in CFU or PDU/g in an intervention scenario relative to the baseline.

[e] 100% due to rounding up.

of the effect could not be assessed due to uncertainty. It is crucial to fill the data gaps for important variables for future quantitative risk assessments at national levels. Therefore, it is suggested to increase microbiological surveillance efforts in water sources for agricultural and industrial use at national levels and direct more research towards specific food matrices, instead of model or broth systems to reduce the uncertainty factor in the new risk assessment. In addition, model validation was conducted by comparing the estimates of *E. coli* and HAV contamination in end products with microbiological criteria for *E. coli*

in fresh produce in Chile and survey data of HAV in fresh produce in European and North American countries, respectively. To better evaluate the model's capability of producing accurate and reliable risk estimates, sufficient data on bacterial and viral contamination in fresh and frozen raspberries produced in Chile will be helpful.

Lack of information is a common challenge for the applications of quantitative risk assessment (Vose, 2008). In this situation, a good understanding of the impact of assumptions made to fill data gaps provides valuable information for identifying major risk drivers and

Table 6
Summary of identified data gaps with their implementation in the model, possible effects on risk estimates, relative importance and suggestions for improvement.

Type	Data gap	Data used	Suggestions	Possible effect	Importance[a]
Non-local	Concentrations of *E. coli* and HAV in surface water and groundwater in Chile.	High detectable concentrations of *E. coli* and viruses reported by WHO (2004). Sampling locations and measurement sensitivity were questioned.	Pathogen concentrations in water sources should be surveyed at the national level.	Data used in the model represent the high values, final contamination may be overestimated.	I
Non-optimized	Bacterial growth kinetic parameters	Growth is only considered to take place on the surface of the fruit, with pH of the surface environment uncertain. Data used for *E. coli* growth model development were derived from experimental study using nutrient broth rather than specific food.	Internalization of *E. coli* is not a concern due to limited possibility and low pH. But surface growth kinetics should be studied extensively using specific food matrix of interest, raspberries in this case.	A scenario analysis with and without bacterial growth being modelled shows that difference in significance ranking of input variables and results interpretation for risk management suggestions is not significant.	IV[b]
Non-optimized	Bacterial decay rate	Decay rate of *E. coli* on leafy greens (Danyluk & Schaffner, 2011)	Survival of *E. coli* on raspberries should be studied in laboratory conditions.	Direction of effect is not clear because of insufficient information about difference between raspberry and leafy greens. Final contamination may be over or underestimated.	II
Non-optimized	Viral Decay rate	Decay rate of HAV on lettuce (Stine et al., 2005)	Inactivation of HAV on raspberries should be studied in laboratory conditions.	Direction of effect is not clear because of insufficient information about difference between raspberry and lettuce. Final contamination may be over or underestimated.	I
Non-local and non-optimized	Bacterial concentration sourced from farm and plant workers	Concentration on workers' hands in organic lettuce farms in Brazil (de Quadros Rodrigues et al., 2014)	Field studies should be conducted in Chilean raspberry farms and collection centers for hand contamination.	Direction of effect is not clear because of insufficient information about difference between raspberry and lettuce and hygiene practices in Chile and Mexico.	III
Non-local	Viral concentration sourced from farm and plant workers	Estimated for a risk assessment study on raspberries in Europe (Bouwknegt et al., 2015)	Field studies should be conducted in Chilean raspberry farms and collection centers for hand contamination.	Direction of effect is not clear because of insufficient information about difference between practices in Chile and Europe.	I
Non-local and non-optimized	Bacterial prevalence sourced from farm and plant workers	Prevalence on workers' hands in jalapeno pepper farms in Mexico (de Aceituno et al., 2016)	Field studies should be conducted in Chilean raspberry farms and collection centers for hand contamination.	Direction of effect is not clear because of insufficient information about difference between raspberry and pepper and hygiene practices in Chile and Mexico.	IV
Non-local and non-optimized	Viral prevalence sourced from farm and plant workers	Prevalence on workers' hands in bell pepper farms in Mexico (León-Félix et al., 2010)	Field studies should be conducted in Chilean raspberry farms and collection centers for hand contamination.	Direction of effect is not clear because of insufficient information about difference between raspberry and pepper and hygiene practices in Chile and Mexico.	II
Non-local	Bacterial and viral contamination in fresh and frozen raspberries	Maximum allowable detection levels of *E. coli* by the Chilean Food Sanitary Regulation (Chilean Ministry of Health) and data from a large monitoring program of foodborne viruses in berries from countries in Europe and North America (Li et al., 2018)	Large monitoring program of *E. coli* and HAV in fresh and frozen raspberries produced in Chile	No effect on model estimates, but less confidence on model validation	I[c]

[a] Importance was based on tornado chart. If any of the related input distributions appear among the top 5 variables in any of the tornado charts, ranked I; if between 6 and 10, ranked II; if lower than 10, ranked III and if did not appear in the tornado charts, ranked as IV.

[b] The *E. coli* growth kinetic parameters didn't appear in the tornado chart as significant model input variables, because those parameters are deterministic and were therefore not evaluated in the sensitivity analyses. However, the importance of those parameters is still ranked low due to its limited impact on risk management strategies based on findings from the risk assessment.

[c] Importance score for this variable is not based on sensitivity analyses, as this is not one of model inputs. However, it is still suggested being ranked high due to its great significant impact on model validation.

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developing strategies for managing risks. For example, an assumption made in this study is that the microbial contamination is primarily on the surface of raspberry and the fruit surface is able to support the growth of generic *E. coli* at appropriate temperature ranges due to the lack of knowledge of generic *E. coli* behavior on raspberry surface. Based on this assumption, the growth model was incorporated at various stages along a passage from farm to packing plant whenever raspberries may be exposed to environment with a temperature higher than 5 °C (Table 4). Such a conservative assumption leads to an overestimation of generic *E. coli* contamination in both fresh and frozen raspberry products, indicating the real contamination level can be even lower than the model estimates. The impact of this assumption was also evaluated by comparing implications from sensitivity analyses with and without growth model being considered. Sensitivity analyses based on the current model with *E. coli* growth considered indicated the significant role of water used for mixing pesticide spray in contributing to the overall generic *E. coli* contamination in raspberry products in Chile, highlighting the importance of improving microbial quality of water. The model was rerun by excluding *E. coli* growth, results of which showed water-related variables are still of the greatest importance to the contamination in final products (data not shown). The similar variable importance ranking reemphasizes the importance of improving data collection for water-related variables. In addition, it indicates the conservative assumption regarding *E. coli* growth seems unlikely to change the suggestions on improving water quality as effective strategies to enhance microbiological quality of raspberries in Chile.

Improvement of food safety management system aided by a risk-based approach is well recognized as a highly interactive process (Vose, 2008; World Health Organization & Food Agriculture Organization, 2006). Applications of risk assessment related to safety of fresh produce have been increasingly observed (De Keuckelaere, Jacxsens, Amoah, Medema, McClure, Jaykus, et al., 2015). A common drawback shared among most of the microbial risk assessments is the lack of local data. In this perspective, significant progress was made in this study. Due to the cohesive planning between Chilean government and the UNL team, many data gaps pertinent to Chile-specific practices among raspberry industry were identified at the project planning stage and successfully filled using survey approach. However, as one of the first few works employing quantitative risk assessment framework, particularly the first application in raspberry in Chile, the reliability of microbial risk estimates needs to be further improved by refining parameterization of important model variables identified. The current work provides a preliminary but relatively comprehensive and flexible framework for estimating generic *E. coli* and HAV contamination in both fresh and frozen raspberries by considering the impact of environmental and operational conditions. Using a relatively comprehensive model describing the raspberry supply chain provides an opportunity to systematically identify data gaps and prioritize the future data collection activities. With improved knowledge of the behavior of specific microorganisms on food matrix, the current model can be easily updated to produce more reliable microbial risk estimates. In the situations when no immediate solutions are available such as data generation based on newly designed and conducted studies, systematic review could be considered as a good practice for refining the most important variable identified (EFSA., 2010)

4. Conclusion

This collaborative project is among the first few of its kind in the realm of food safety in Chile. Results of this study can be implemented by SAG to improve current ROCP practices and increase the quality and safety of raspberry products in Chile. The key findings of this study are as follows. First, higher priority can be assigned to controlling the bacterial contamination of fresh raspberry products, since less baseline bacterial contamination was predicted in frozen products. Second, to control *E. coli* contamination in the end products, improving the quality

of the water used for pesticide application and controlling the time and temperature to which raspberries are exposed before arriving at the packing plant may be most effective. Third, interventions to improve the hygiene practices of harvesters on the farm and packers at the packing plant may be most effective for reducing HAV contamination. Note that the conclusions rely on the used modelling approach and its assumptions and limitations and may not necessarily be applicable to other situations. This experience of Chilean food safety agencies represents a pilot project for enhancing the national food safety system by incorporating a risk analysis framework, which could potentially be leveraged by similar developing economies to narrow the gap between developing and developed countries in integrating science into complex food safety decision-making processes.

CRediT authorship contribution statement

Juan E. Ortúzar: Formal analysis, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Onay B. Dogan:** Formal analysis, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Gustavo Sotomayor:** Conceptualization, Data curation, Project administration, Resources, Writing - review & editing. **Constanza Jiménez:** Writing - original draft. **Jennifer Clarke:** Conceptualization, Supervision, Writing - review & editing. **Rolando A. Flores:** Conceptualization, Supervision, Writing - review & editing. **George M. Gray:** Supervision, Writing - review & editing. **John H. Rupnow:** Supervision, Writing - review & editing. **Bing Wang:** Conceptualization, Data curation, Methodology, Project administration, Software, Writing - original draft, Writing - review & editing.

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