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Microbiological and chemical risk assessments of the addition of selected cereal grains as non-dairy ingredients to dairy products

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ABSTRACT

Food poisoning cases involving non-dairy ingredients such as cereal grains have been reported. The addition of cereal grains to dairy products in the dairy industry has increased in recent years. This has the potential to contaminate final products with pathogenic, spoilage and toxic chemical contaminants. In this study, the microbial and chemical risks involved in the addition of cereal grains to dairy products were assessed using semi-quantitative risk assessment method.

The results showed that the most critical microbiological hazard in the selected cereal grains is *Bacillus cereus* due to its ability to form spores and persist in cereal grains. The addition of cereal grains to dairy products with high water activity/moisture content such as liquid breakfast products were found to pose the highest risk. Cyanogenic glycosides (hydrocyanic acid) were found to be the most critical chemical hazard among natural plant toxins in selected cereal grains due to their adverse health effects and abundance in most cereal grains. The addition of cereal grains to dairy products with high solid content was found to pose a very low risk.

The results have identified some knowledge gaps in conducting risk assessments and have also provided background information about the microbial and chemical risks involved in the addition of cereal grains to dairy products. The results highlight the importance of effective implementation of Hazard Analysis and Critical Control Point (HACCP), Good Manufacturing Practices (GMP) and Good Hygienic Practices (GHP) in the dairy industry.

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LIST OF ACRONYMS AND ABBREVIATIONS

ADI	Acceptable daily intake
ARfd	Acute reference dose
Aw	Water activity
BW	Body weight
CDC	Centers for Disease Control and Prevention
CoI	Cost of illness
CRA	Chemical risk assessment
DALY	Disable-adjusted life year
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
FDA	Food and Drug Administration
GAP	Good agricultural practices
GHP	Good hygiene practices
JECFA	Joint Expert Committee for Food Additives
HACCP	Hazard analysis and critical control point
HALY	Health-adjusted life year
HBGV	Health-based guidance value
ML	Maximum level
MPI	Ministry for Primary Industries
MRA	Microbiological risk assessment
MRL	Maximum residue limit
NORS	National outbreak reporting system
NOAEL	No observed adverse effect level
NOEL	No observed effect level
RA	Risk assessment
RASFF	Rapid alert system for food and feed
RMQs	Risk management questions
TDI	Tolerable daily intake
US	United States of America

CHAPTER 1. INTRODUCTION

1.1. Background

Cereal grains are often formulated into dairy products. The reasons for the addition of cereal grains to dairy products include increasing the nutritional value of the final product, novel development of new products which increases consumer interest and production of functional foods. Liquid breakfast product is an example of a convenient product that combines non-dairy and dairy ingredients. In 2017, the global liquid breakfast product market was valued at approximately USD 302.06 billion and is estimated to generate around USD 448.23 billion by 2024, at a compound annual growth rate (CAGR) of around 5.8% between 2018 and 2024 (Zion market research, 2019).

There have been several food safety issues associated with cereal grains. The Centers for Disease Control and Prevention (CDC) in 2016 reported 383 foodborne outbreaks that involved grains and beans in the United States (CDC, 2018b). In the same year, contamination of cereal milled product (flour) by *E. coli* O 121 caused 63 cases of food poisoning. There was another outbreak in New Zealand in 2008-2009 which was associated with *Salmonella* Typhimurium contamination in wheat flour leading to 67 cases of food poisoning (McCallum et al., 2013).

In regards to chemical contamination associated with cereal grains, 460 cereal and bakery products in the European Union were found to be contaminated with mycotoxins in 2000-2019 (RASFF, 2019b). These are indicative of the potential risks which can arise from cereal grains. However, no studies have been conducted to assess the risks involved in the addition of cereal grains to dairy products. Therefore, the purpose of this study was to conduct a risk assessment for the addition of cereal grains to dairy products. The outcome of this study will provide background information for the dairy industry to help manage the food safety risks associated with cereal grains when they are added to dairy products.

1.2. Research questions

The purpose of this study was to review available information to answer the following risk management questions (RMQs):

1) What are the microbial and chemical food safety risks of greatest concern in selected cereal grains in New Zealand?
2) What are the microbial and chemical food safety risks of selected cereal grains when added to dairy products in New Zealand?
3) What mitigation efforts are recommended to reduce these risks?

1.3. Aims and objectives

This study aims to identify the gaps in knowledge, assess the most critical risks for the addition of non-dairy origin ingredients (cereal grains) to dairy products and recommend the mitigation strategies to reduce any food safety risk.

The objectives of this research were as follows:

1. To conduct a microbiological and chemical risk assessments of selected cereal grains as non-dairy ingredients;
2. To develop and apply a risk ranking method to rank the most critical microbiological and chemical hazards from a global food safety perspective;
3. To assess the microbiological risks associated with cereal grains addition into three types of dairy products representing low, intermediate and high water activity;
4. To assess the risk based on estimated residue levels of chemical hazards from cereal grains added to three types of dairy products: (1) high solid content products such as milk powder, (2) intermediate solid content products such as hard cheeses and (3) low solid content products such as UHT milk/liquid breakfast product;
5. To recommend mitigation strategies to reduce microbiological and chemical risks.

CHAPTER 2. LITERATURE REVIEW

This chapter explains the potential health risks of non-dairy ingredients, especially, cereal grains and dairy products to the public health and reviews possible food safety concerns in both non-dairy ingredients and dairy products. It describes current risk assessment methods to assess the microbiological and chemical risk of selected non-dairy ingredients and the addition of non-dairy ingredients to dairy products. Finally, it explains the recommended method to best estimate the microbiological and chemical risk of cereal grains as non-dairy ingredients and their addition to dairy products.

2.1. Non-dairy ingredient: Cereal grains

Two categories are used mainly in this present study: *dairy* and *non-dairy*. Dairy includes "names, designations, symbols, pictorial or other devices which refer to or are suggestive, directly or indirectly, of milk or milk products" (CAC, 1999a). Non-dairy ingredients include "nutritive and non-nutritive sweeteners, fruits and vegetables as well as juices, purees, pulps, preparations and preserves derived from, cereals, honey, chocolate, nuts, coffee, spices and other harmless natural flavouring foods and/or flavours" (FAO/WHO, 2012).

Cereal grains are widely used as human food globally and may constitute up to 80% of the daily diet (Olsson, Börjesson, Lundstedt, & Schnürer, 2000). Cereal grains are a primary source of human dietary energy, protein and fibre requirements (Rasane, Jha, Sabikhi, Kumar, & Unnikrishnan, 2015; Wrigley, 2017b). Cereal grains commonly belong to the family of *Gramineae* or *Poaceae* and refer to crops harvested solely for dry grain (FAO, 1994b; Morrison & Wrigley, 2016). They are diverse and distinguishable in terms of morphology and composition although they are under the same taxonomic classification (Wrigley, 2017a). Cereal grains can be classified under three categories, i.e. cereals, pseudo-cereals and grains legumes (pulses). Cereals are monocotyledonous plants, while pseudo-cereals including both monocotyledonous and dicotyledonous plants. A cereal grain has an embryo and endosperm coated inside a seed. A pseudocereal grain has an embryo surrounding perisperm but does not have endosperm (R. J. Fletcher, 2016). Pulses are of *Leguminosae* family which produce edible seeds and refer to legumes harvested for dry grain only (FAO, 1994a). The three main cereal species based on the volume of global production in the world are wheat, maize, and rice (Wrigley, 2017b). The other cereal grains of economic significance include rye, barley, millet, oats, rice,

sorghum, triticale, and pseudo-cereals such as buckwheat and quinoa (Bullerman & Bianchini, 2009; Koehler & Wieser, 2013; Wrigley, 2017b).

2.1.1. Cereal grains of interest

Cereal grains can be used as ingredients to produce functional foods or value-added products (Charalampopoulos, Wang, Pandiella, & Webb, 2002). Functional food is a term used for food products which have been supplemented with natural constituents that have particular health promoting and/or physiological preventive effect (Vukasović, 2017). For instance, oat consumption may provide many health benefits including hypocholesterolaemia and anticancer characteristics (Rasane et al., 2015). Value added product is a term used for a product that enhances its value through a business strategy or the physical separation of an agricultural product in a way that results in the improvement of the product's value (USDA, 2019). For example, soy protein isolate was found to contain higher protein than soybean flour (Singh, Kumar, Sabapathy, & Bawa, 2008).

Cereal grains which have global economic significance have potential to be added to dairy products in a form of whole and milled grain products such as flour, rolled and flakes for oats, pearled barley, flour and germ for wheat. The quality of raw materials used is one aspect of concern in the food safety (FSANZ, 2006). Thus, it is vital to understand the intrinsic and extrinsic characteristics of cereal grains that may affect the food safety.

2.1.2. Intrinsic and extrinsic characteristics

Intrinsic and extrinsic factors of food can influence microbial growth and survival in food (Jay, Loessner, & Golden, 2005). The intrinsic factors refer to characteristics of the food itself such as pH, nutrient content, water activity, and antimicrobial agents. On the other hand, the extrinsic factors are coming from the environment that may impact the food and microorganisms such as the temperature of storage, relative humidity, occurrence and level of gases.

2.1.2.1. Intrinsic characteristics

Intrinsic characteristics of cereal grains are similar. They have low moisture content (8-14%), variable fat content (0.7-7%), moderate protein content (7-17%) and high carbohydrate content in the forms of starch (68-80%) (Gilbert, Lake, Cressey, & King, 2010; Martín-Belloso et al., 1997; Qin, Wang, Shan, Hou, & Ren, 2010; Zhu, 2017).

The pulses have higher protein content (22-25%) and lower carbohydrate (54%) than cereals and pseudo-cereals (Boye, Zare, & Pletch, 2010; Yasmeen et al., 2017). Cereals usually undergo a post-harvest process to remove the external layer of the grains such as rice polishing, flour milling, or barley pearling (Thielecke & Nugent, 2018). These processes result in reduced fat and protein content while increasing the starch proportion of the cereal (Gilbert et al., 2010).

Cereal grains are regarded as low moisture foods and therefore, considered as low-risk foods (Rachon, Peñaloza, & Gibbs, 2016). This means cereal grains are less likely to support bacterial growth compared with high moisture animal or vegetable based foods. However, cereal products are rich in carbohydrate and protein which may support the growth of micro-organisms (Jay et al., 2005). For instance, rice contains carbohydrate (79%), protein (6-7%), fat (1-2%), vitamins, minerals and has neutral pH suitable for microbial growth if there is sufficient moisture present (Lake, Hudson, & Cressey, 2004). Lake et al. (2004) revealed that spores of *Bacillus cereus* (*B. cereus*) can survive well in dry rice products that could raise food safety issue.

2.1.2.2. Extrinsic characteristics

The storage condition of cereal grains is essential as it may influence the growth of micro-organisms (Jay et al., 2005). The moisture content of cereals may increase through exposure to water from the environment such as high relative humidity of the storage room, water vapour condensates from equipment and improper cleaning (Gilbert et al., 2010). When sufficient water activity occurs, the growth of *Bacilli* and moulds is induced. Spore forming bacteria may produce enzymes such as amylase that helps them to make use of flour to provide energy for the bacteria to grow. In addition, mould growth that occurs may be identified by distinctive spores and mycelial growth, for example, the genus *Rhizopus* forms black spores on flour (Jay et al., 2005). Hence, it is crucial to keep the moisture content of the cereals to less than 12% to prevent microbial growth (Harris, Shebuski, Danyluk, Palumbo, & Beuchat, 2012; Hesseltine & Graves, 1966).

2.1.3. Food safety of cereal grains

There are three types of hazards which can occur in cereal grains. This includes microbiological, chemical and physical hazards. The focus of the present study is to assess the microbiological and chemical hazards; therefore, under this section, only microbiological and chemical hazards will be discussed.

2.1.3.1. Microbiological safety of cereal grains

In the United States, it is predicted that 48 million people get ill, 128,000 people are hospitalised, and 3,000 people die because of the foodborne disease annually. The surveillance for foodborne disease outbreaks in the United States in the 2016 report identified the most outbreak-related illnesses under several food groups. Grains and beans were one of the most outbreak-related groups with 383 cases from eight outbreaks. Five of these eight outbreaks were linked to cereal grains (CDC, 2018b).

The microbiological issue of cereal-based food has not been of high concern (Alldrick, 2017). This is because cereals are usually cooked (thermal treatment) to be palatable for consumption. The cooking process generally destroys microorganisms. On the other hand, there is a possibility that bacterial spores are not eliminated under typical processing conditions. Lack of adequate cooking time can elicit the activity of spore formers in the contamination of food. The risk for food poisoning by Bacillus spp. are influenced by the length of the cooking time and the storage temperature of the cooked product. The vegetative cells of *Bacillus* spp. are destroyed by frying, roasting, grilling, and pressure-cooking, while spores inactivation depends on the strain and food (MPI, 2015). For example, cooking rice in 100°C for 1.2 to 7.5 min and cooking oily food such as pumpkin pie in 120°C for 3.4 min (MPI, 2015). Alldrick (2017) highlighted that *Bacillus spp.* are the most critical bacteria in cereal products as heat shock can induce spore germination in the cooled cooked food. Food poisoning cases may be caused by *Bacillus spp.* associated with cooled cooked pasta, and rice (Raevuori, Kiutamo, Niskanen, & Salminen, 1976; Rajkovic et al., 2008). Storage of cereal-based foods after heat treatment should be done in a rapid way to change to a low temperature so as not to allow the growth of bacterial spores. It was reported that storing heat-treated cereal foods at a temperature range of 10-50°C was able to cause the spores to germinate and multiply to levels capable of causing illness (MPI, 2015).

Cereal grains can be consumed directly as a whole grain or as the milled product (flour, meal, rolled oats). The primary use of cereal grains is as processed products such as flour (Estrada-Girón, Swanson, & Barbosa-Cánovas, 2005; Gilbert et al., 2010). Flour has low water activity which means there is a low likelihood of microbial contamination (Berghofer, Hocking, Miskelly, & Jansson, 2003; Eglezos, 2010). Conversely, the consumption of flour and cereal products have been linked to many foodborne illness

outbreaks and led to product recalls (Hoffmann et al., 2015; McCallum et al., 2013; Zhang et al., 2007). Cereal-based products involved in outbreaks include dry mixes or high-moisture batter for cake or ice cream mix (King & Bedale, 2017). Table 1 shows the summary of foodborne illness outbreaks related to cereal grain products. The format for this table was adapted from (Harris & Yada, 2018).

Table 1. Summary of foodborne illness outbreaks related to cereal grain products

Product (Company/Condition)	Pathogen	Year	Country (States/Provinces)	Total cases	Recall	Reference(s)
Wheat flour						
Flour, raw (Goodman Fielder's Champion, Edmonds, Homelife and Pam's)	*Salmonella* Typhimurium phage type 42	2008-2009	New Zealand	67	Yes	(McCallum et al., 2013)
Flour (General Mills, Kansas City, MO)	*E. coli* O121, *E. coli* O26	2015-2016	USA (24 states)	63	Yes	(CDC, 2016; FDA, 2017)
Flour (Ardent Mills, Saskatoon, SK)	*E. coli* O121	2016-2017	Canada (6 provinces)	30	Yes	(PHAC, 2017)
Wheat flour products						
Cake mix, raw in ice cream (NA)	*Salmonella* Typhimurium	2005	USA (11 states)	25	Yes	(Zhang et al., 2007)
Frozen pot pies (ConAgra Foods)	*Salmonella* I 4,[5],12:i:-	2007	USA (41 states)	401	Yes	(CDC, 2008b)
Pre-packaged, raw refrigerated cookie dough (Nestle Toll House)	*E. coli* O157: H7	2009	USA (30 states)	77	Yes	(CDC, 2009; Neil et al., 2011)
Dough mix, dry (NA)	*E. coli* O157:H7	2015-2016	USA (24 states)	63	Yes	(CDC, 2016; Gieraltowski et al., 2017)
Pasta salad (Household)	*B. cereus*	2003	Belgium	5	NA	(Dierick et al., 2005; Rajkovic et al., 2008)
Pasta salad (NA)	*B. cereus*	2002-2006	Belgium	50	NA	(Rajkovic et al., 2008)
Spaghetti (Hospital)	*B. cereus*	2002-2006	Belgium	60	NA	(Rajkovic et al., 2008)
Cereal grain products						
Toasted Oats Cereal (Malt-O-Meal, Inc)	*Salmonella* Agona	1998	USA (11 states)	418	Yes	(CDC, 1998)
Rice and Wheat Puff Cereals (Malt-O-Meal, Inc)	*Salmonella* Agona	2008	USA (15 states)	28	Yes	(CDC, 2008a; Hoffmann et al., 2015)
Wheat Puff Cereals (Kellog's)	*Salmonella* Mbandaka	2018	USA	135	Yes	(CDC, 2018a)

Product (Company/Condition)	Pathogen	Year	Country (States/Provinces)	Total cases	Recall	Reference(s)
Rice products						
Fried rice	*B. cereus*	1971-1982	Japan	686	NA	(Agata, Ohta, & Yokoyama, 2002) (Rajkovic et al., 2008)
Rice (Take-away Chinese restaurant)	*B. cereus*	2002-2006	Belgium	6	NA	(Rajkovic et al., 2008)

NA: not available

2.1.3.2. Chemical safety of cereal grains

Chemical contamination of food and cereals in particular can be derived from a numbers of factors (Alldrick, 2014). These include naturally occurring toxins (e.g. plant toxins, mycotoxins), bioaccumulation (e.g. heavy metals), crop handling/agricultural practice (pesticides), acquired through primary and secondary processing equipment (e.g. cleaning and sanitising agents), formed through food processing (e.g. acrylamide), and intentionally added adulterants (e.g. melamine in wheat bran) (Hanlon, Hlywka, & Scimeca, 2015).

In cereal and bakery products in the European Union, there were 460 notifications related to mycotoxins in 2000-2019 (RASFF, 2019b), 97 notifications associated with pesticide residues 1984-2019 (RASFF, 2019d), 11 notifications regarding environmental pollutants (e.g. mineral oil, benzo(a)pyrene, toluene, and kerosene oil) in 2003-2019 (RASFF, 2019a), 33 notifications linked with natural toxins (mostly rye alkaloid, atropine and scopolamine) in 2003-2019 (RASFF, 2019c).

Natural toxins or inherent plant toxins are commonly known as natural pesticides due to their role in the defence against predators, insects, fungi, bacteria, and viruses (Essers et al., 1998; Schilter, Constable, & Perrin, 2014). Inherent plant toxins are non-nutrients secondary metabolites which have the potency to cause toxicity in humans. Examples are cyanogenic glycosides and ergot. One characteristic of some plant toxins is a strong bitter taste to prevent the plant from being eaten by the mammals (Schilter et al., 2014). For instances, the presence of cyanogenic glycosides cause bitterness in cassava and almonds (Jones, 1998). Natural toxins are also produced in reaction to environmental stress such as drought or extreme humidity (WHO, 2018b). The level of cyanogenic glycosides were found to be higher in plants that are stressed due to frost (Haschek, Rousseaux, Wallig, Bolon, & Ochoa, 2013).

Glyphosate is the most widely used herbicide. The International Agency for Research and Cancer (IARC) classified glyphosate as a “probably carcinogenic to human” (IARC, 2015). New Zealand Ministry for Primary Industries (MPI) tested pea and wheat crops in 2015-2016 and found no detected levels of food safety concern in wheat and no detected glyphosate residues in peas (MPI, 2019).

In 2016, the MPI surveyed the exposure of diets to agriculture chemicals as well as environmental contaminants to foods (New Zealand Total Diet Study). It was found that the highest amount of dietary exposure found was to be 2.9 % of respective health based guidance value, which had no significant health concern to the public. However, aluminium was found to be higher than the normal levels in some baked foods such as muffins and scones. This high level of aluminium was identified as a potential concern for the young population (MPI, 2018).

2.2. Milk and dairy products

The total global production of milk reached 770 billion litres valued at USD 328 billion in 2013 (FAO, 2016). Milk and milk products represent 14% of world agricultural trade (FAO, 2016). Skimmed milk, cheese and butter represent over more than 90% of dairy products (FAO, 2016). In 2017, New Zealand became the main exporter of caseins (30.8%), butter/dairy fats (24.4%) and powders (23.5%) (Coriolis, 2017).

The dairy industry has an essential role in public health. Milk and dairy products play a vital role in maintaining healthy human nutrition and development through life especially in childhood (FAO, 2013). Thorning et al. (2016) indicated that milk and milk products intake help to achieve nutrient recommendations and may provide benefit in protecting against the most prevalent and chronic non-communicable diseases such as diabetes and cancer.

Milk and milk products are classified as high-risk foods (Griffiths, 2010). ANZFA (2001) defined high-risk foods as foods that can favour the growth or toxin formation of pathogenic bacteria. High-risk foods typically contain high protein, high moisture and need to be stored under refrigeration. The hazards of milk and milk products are inherent to their intrinsic properties such as pH, nutrient content and moisture content.

2.2.1. Intrinsic characteristics of milk

Milk and dairy products are commonly rich in nutrients and contain high moisture content/water activity thus provide an ideal growth environment for many micro-organisms (FAO, 2013; Nero & De Carvalho, 2018). Milk consists of water, particular proteins, fats, carbohydrate/sugar, vitamins and minerals (Flint, Jamaludin, Somerton, Palmer, & Brooks, 2015). The water content of milk ranges from 82.1 to 87.8%. The

main carbohydrate in milk is lactose which is usually around 5.0%. The fat content of milk is in the range of 3.3 to 7.5% depending on the cattle breed (FAO, 2013).

Another intrinsic factor that is believed to favour the proliferation of microorganisms in milk is the pH (Nero & De Carvalho, 2018). Unfortunately, fresh milk has a pH of around 6.6 (FAO, 2013) which is in the range of optimum pH values of most organisms (Jay et al., 2005).

2.2.2. Dairy product classification

Food processing and preservation in the industry are regulating the concentration of water in foods (Early, 1998a). All microorganisms need moisture to grow as well as nutrients, either the presence or absence of air and suitable temperature. Hamad (2012) explained that nutrients taken by microbes must be dissolved in water to pass through the membranes and get into the cell. Water also has a role in chemical reactions in the cell and for the transport of nutrients and wastes in and out of the cell.

In general, foods/dairy products can be classified into three categories based on water activity, moisture content and total solids (Early, 1998a; Jay et al., 2005; Schmidt & Fontana Jr., 2008). Table 2 compares the three classifications of dairy products.

Table 2. Classification of foods/dairy products based on water activity, moisture content and total solids

Parameter	Low	Intermediate	High
Water activity (a_w)	0.00 - 0.60	0.60 - 0.85	>0.85
Moisture content	<25%	15-50%	above 50%
Total solids	high solids product (>50% total solids)	≤50% total solids	low solids product (10-20%)
Examples of dairy products and a_w	Milk, non-fat dry 0.137 -0.277	• Butter, salted 0.83 • Parmesan cheese 0.69-0.73 • Whey concentrate 0.815	• Butter, unsalted 0.96 – 0.98 • Cheddar cheese 0.95-0.98 • Milk 0.98-0.99 • Yoghurt 0.97-0.99

Water activity (a_w) is vital from a food safety perspective. As seen in Table 2, low water activity foods have a_w less than 0.60, intermediate water activity foods have a_w 0.60-0.85, and high water activity foods have a_w more than 0.85. Low water activity does not favour the growth of pathogenic micro-organisms. Intermediate water

activity inhibits pathogenic bacterial growth excluding *Staphylococcus aureus (S. aureus)*. *S. aureus* is able to grow and may produce toxin at a_w close to and lower than 0.90 (NZFSA, 2001c). Such products need to be kept at temperatures < 8°C to hinder the growth of *S. aureus*.

Moisture content influences the microbial safety of food products. Spoilage in low moisture products is normally due to moulds and yeasts which may also spoil intermediate moisture food. Intermediate moisture content products allow the possibility for aerobic spore-forming bacteria to grow such as *Bacillus licheniformis* (Early, 1998a). High moisture content permits the growth of most pathogenic bacteria (Jay et al., 2005) and may pose a food safety risk if they are not appropriately handled.

There is a relationship between water activity and moisture content (Early, 1998a). Some foods may have relatively high moisture content but low a_w. The hydrogen bond between food constituents (e.g. protein, sugars, and starches) and water may make water unavailable for microbes. In addition, water may be immobilised by sugar or other humectants. Sweetened condensed milk has a moisture content of 27% and a_w 0.83 (Early, 1998a; Fernandes, 2009). In the production of sweetened condensed milk, sucrose addition to the milk increases the osmotic pressure and decreases a_w that help to preserve the food (Bylund, 2015).

2.2.3. Dairy processing

The dairy industry must implement Good Manufacturing Practice (GMP), Good Hygiene Practice (GHP) and Hazard Analysis and Critical Control Point (HACCP) (CAC, 2004). Factors that influence the risk level of dairy products are the formulation, effective processing/handling, and prevention of product recontamination. The dairy industry are required to identify any processing step that is crucial to assuring food safety and ensuring the adequate safety process are employed, maintained and reviewed (Roberts & Greenwood, 2003). The addition of non-dairy ingredients whether before or after heat treatment can have an effect on food safety. The holding time during heat treatment (pasteurisation) is a critical control point for ingredients added before heat treatment (Fernandes, 2009). There is a possibility of post-pasteurisation contamination which may originate from the environment, e.g. equipment, manufacturing plant, personnel, or contamination of final products with raw materials (FAO, 2013).

Cereal grains as non-dairy ingredients could be added to three categories of dairy products based on moisture content. Milk powder, Parmesan cheese and liquid breakfast product are used as examples in this present study. Milk powder represents a low moisture content/high solid content product, Parmesan cheese represents an intermediate moisture content/intermediate solid content product, and liquid breakfast product represents a high moisture content/low solid content product. For example, the amount of cereal grains (in the form of whole grain oat flour) added in a 250 mL liquid breakfast product is 2.5% or 6.25 g (Sanitarium, 2019).

2.2.3.1. Milk powder

Food drying is a traditional food preservation technique (Rahman & Perera, 2007). Drying is the removal of water, inhibiting microorganisms from growing (Early, 1998a). Dried milk or milk powder does not need to be kept in refrigeration like liquid milk (Bylund, 2015). Schematic diagram of skimmed milk powder production is given in Figure 1.

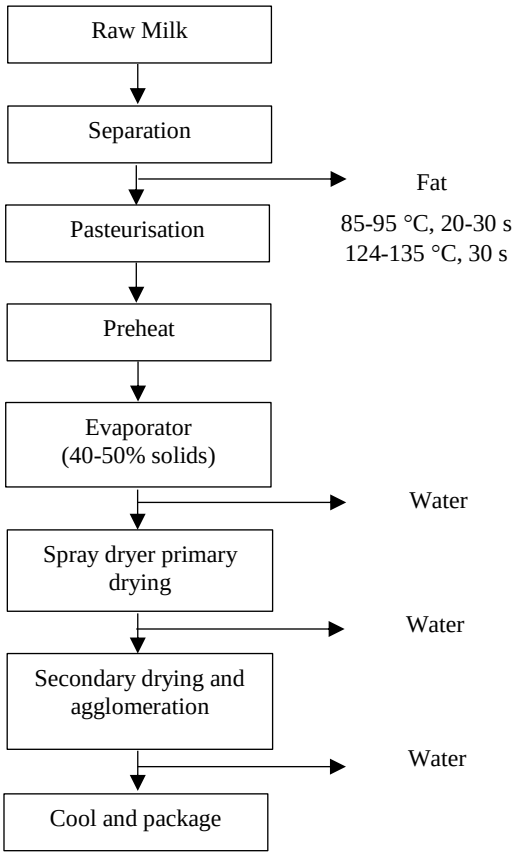

Figure 1. Schematic diagram of skimmed milk manufacturing

Adapted from "Microbiology handbook: Dairy products,"(Fernandes, 2009, p. 28). © 2009 Leatherhead Food International Ltd.

Powdered milk and dairy products include whole milk powder, skimmed milk powder, whey powder, fat-filled milk powder, demineralised whey powder, fat-filled whey powder, dry buttermilk, non-fat dry milk, casein and caseinates, and whey protein concentrate (Bylund, 2015; Early, 1998a). Non-fat dry milk and skimmed milk powder share similar characteristics in moisture content (<5% by weight) and milk fat content (<1.5%) (Bylund, 2015; CAC, 1999a). Skimmed milk powder is required to have a minimum milk protein content of 34%, but there is no minimum requirement for non-fat dry milk (CAC, 1999a).

Milk powder is useful for further food processing into a variety of food products (Augustin, Clarke, & Craven, 2003). Milk powder can be used as an ingredient in food products and a substitute for eggs in bread and pastry. Milk can be reconstituted from milk powder for the manufacture of a number of consumer products including milk chocolate production, ice cream and baby foods (Bylund, 2015; Early, 1998a).

Skim milk powder manufacturing is summarised in Figure 1. Spray dryers mix pre-heated atomised milk droplets with heated air at an inlet range temperature of 180 to 220 °C and an outlet range temperature of 50 °C (Fernandes, 2009). This results in milk powder with very low a_w (0.3 to 0.4). It is important to ensure that non-dairy ingredients are treated to reduce any food safety risk before their addition to milk powder. Blending of dried ingredients is a relatively safe approach in the manufacture of dairy products enhanced with non-dairy ingredients (Fernandes, 2009).

2.2.3.2. Cheese

Non-dairy ingredients used in cheese making must be treated to ensure that it is safe from any microbial contaminants (Tamime, 2011). Non-dairy components may introduce bacterial spores, chemical residues and physical fragments such as stones, animal and fish bones. The supplier must provide a warranty that plant-based non-dairy ingredients do not contain chemical residues of herbicide or pesticide. Some tests need to be carried out in the raw material, e.g. water content and microbiological testing to ensure the quality of the raw ingredients (Tamime, 2011).

Cheese manufacture is a complex process involving many manufacturing steps and biochemical transformations (Nassar, Lundin, Iordache, Hailu, & Kide, 2015;

Tamime, 2011). The process flow in the production of hard and semi-hard cheese is depicted in Figure 2.

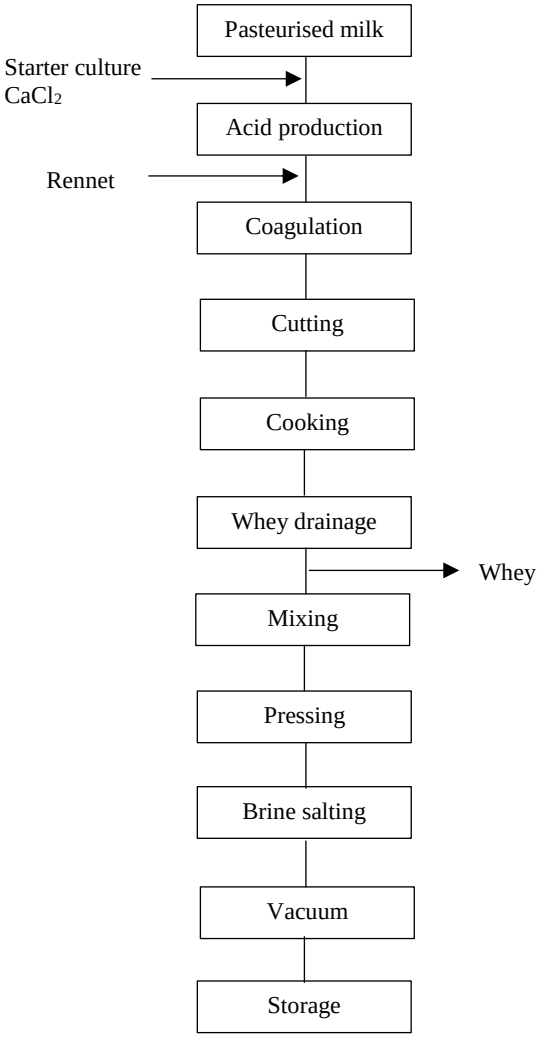

Figure 2. Process flow in production of cheese

Adapted from "Development of a potential probiotic fresh cheese using two *Lactobacillus salivarius* strains isolated from human milk," (Cárdenas et al., 2014, p. 2). CC BY 3.0.

New ingredients might affect the microflora and environmental conditions of cheese (Fernandes, 2009). The level of added non-dairy components should not have a significant effect on the consistency and texture of cheese (Tamime, 2011). This means ingredient addition should not affect the bacteriological process of cheese making. For example, ingredients containing high levels of acid or salt ingredients may interfere with the cheese making process by causing coagulation of casein and water drainage (Tamime, 2011).

The safety and stability of cheese should be closely monitored during development (Fernandes, 2009). There are two ways of non-dairy ingredients incorporation to cheese production: addition to the raw milk (prior pasteurisation) or at the mixing process (after pasteurisation) (Tamime, 2011). Post pasteurisation contamination may occur due to poor sanitation control, during cheese handling and ripening, packing and storage (Choi, Lee, Lee, Kim, & Yoon, 2016; Gould, Mungai, & Behravesh, 2014). Post pasteurisation contamination of cheese can be prevented by avoiding poor sanitation during handling, packaging and storage.

2.2.3.3. Liquid breakfast product

Trends in global food and drink have shown that consumers would like to see breakfast-to-go options in convenience stores (Mintel, 2018). Liquid breakfast product is one example of the breakfast-to-go option that combines non-dairy with a dairy ingredient. Inclusion of cereal grains into the liquid milk may increase its nutritional value in terms of protein and fibre content (Rasane et al., 2015).

There are two heat treatment methods during liquid breakfast manufacture: pasteurisation or ultra high temperature (UHT). A typical flavoured milk and UHT milk production diagram is given in Figure 3 (SSP, 2019). Non-dairy constituents such as cereal grains are added after the standardisation process of milk. Pasteurisasion and the UHT process occur after the mixing and is the critical control point.

Pasteurisation is intended to reduce the number of vegetative pathogens to an acceptable levels (Fernandes, 2009). Regular high temperature short time (HTST) pasteurisation at 72–73 °C for 15–20 s is the most commonly applied in the dairy industry (Bylund, 2015). However, spore-forming microorganisms in the spore state that are able to survive pasteurisation can cause serious problems when the product is not stored refrigerated (Fernandes, 2009).

UHT treatment applies heat with high temperature for specific time to continuously flowing milk with aseptic filling into sterile containers (Bylund, 2015; Fernandes, 2009). Normally, UHT treatment temperature ranges from 135 to 150 °C in combination with appropriate holding times (1-2 s) to obtain commercially sterile product (Bylund, 2015; Jay et al., 2005). Post-process contamination generally

happens because of failures in the integrity of aseptic filling system, i.e. packaging defects such as pinholes or faulty seals (Fernandes, 2009).

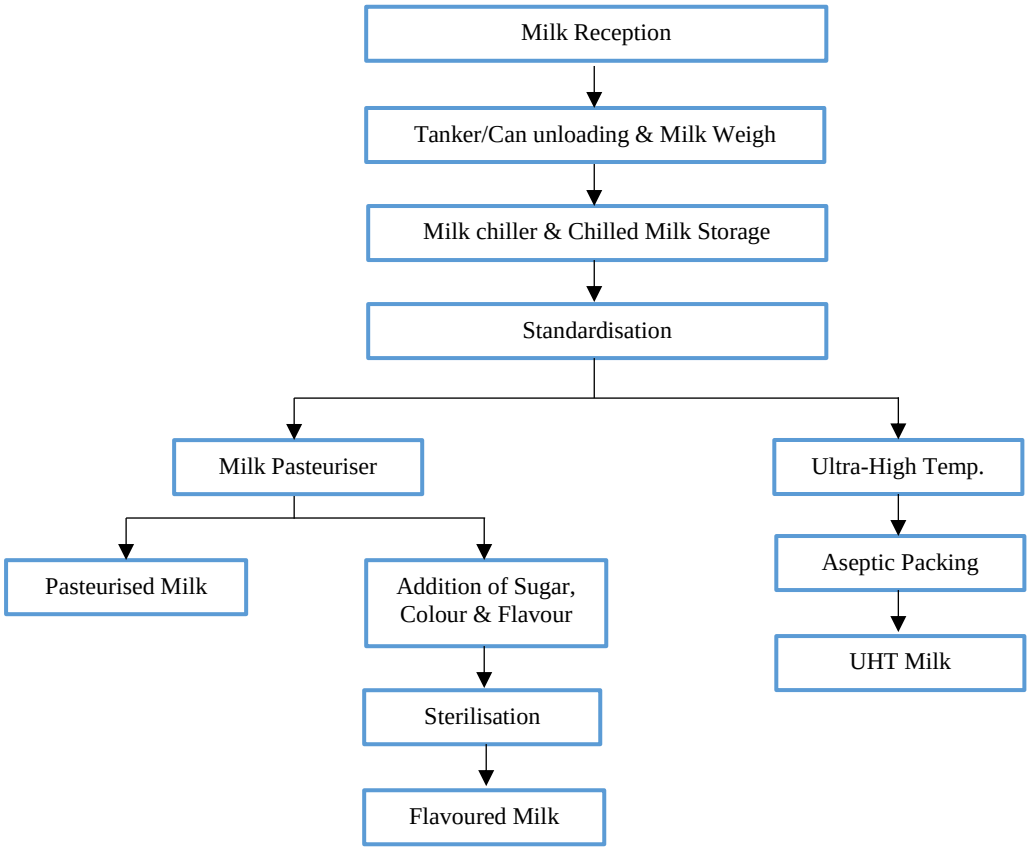

Figure 3. Processing diagram of flavoured milk and UHT milk/liquid cereal
From "Liquid milk processing plant," (SSP, 2019). Reprinted with permission.

2.2.4. Food safety of dairy products

2.2.4.1. Microbiological safety of dairy products

Raw milk is a source of food-borne pathogens that depends on the health of dairy herd, quality of raw milk, hygiene of animal, environment and personnel (FAO, 2013). Heat treatment (pasteurisation) is effective in destroying most of the micro-organisms (FSANZ, 2006). However, foodborne illness related to dairy products is still happening. Table 3 presents the microorganisms of concern in the safety of dairy products.

Table 3. **Significance of pathogens associated with milk and dairy products**

Pathogens	The implication in dairy products
Bacillus cereus	- Vegetative cells do not survive pasteurisation, but spores do - At refrigeration temperature, *B. cereus* is outgrown by gram-negative psychotrophs. But, in their absence, *B. cereus* may grow into high numbers. - A hazard in extended shelf life products
Campylobacter spp.	- Easily eliminated by pasteurisation - Its presence may be caused by post pasteurisation contamination in the environment
Cronobacter spp. *(Enterobacter sakazakii)*	- Not survive pasteurisation - Recontamination - Cannot grow in the dry substrate but can survive a long period of time - Contamination and following growth may occur during reconstitution and preparation
Escherichia coli	- Heat-sensitive and does not survive pasteurisation
Listeria monocytogenes	- Destroyed by pasteurisation - Related to post pasteurisation contamination - Can grow at 0°C
Salmonella	- Destroyed by pasteurisation - Present in the environment - Related to post pasteurisation contamination - Non-dairy ingredients can be an essential source of contamination
Staphylococcus aureus	- Destroyed by heat-treatment but toxins are heat stable - Does not grow well at refrigeration temperature
Yersinia enterolitica	- Destroyed by pasteurisation - Post pasteurisation contamination - A hazard in prolonged shelf life products

Adapted from "A risk profile of dairy products in Australia" (FSANZ, 2006, p. vi). In the public domain.

Figure 4 shows foodborne outbreaks related to dairy and causal pathogen 2007-2015 in New Zealand and the US. Foodborne outbreaks data was collected from the National Outbreak Reporting System (NORS) for the US and annual summary of outbreaks report for New Zealand.

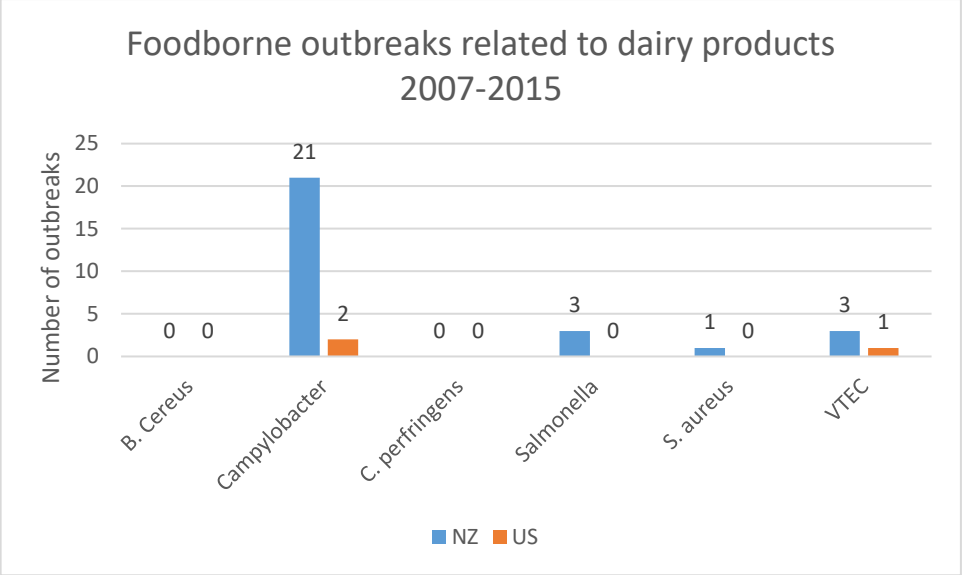

Figure 4. Foodborne outbreak related to dairy and causal pathogen 2007-2015

In New Zealand, *Salmonella* was responsible for three foodborne outbreaks related to dairy products in 2010 (ESR, 2011). Verotoxigenic *Escherichia coli* (VTEC) was responsible for three foodborne outbreaks related to dairy products during 2013-2014 (ESR, 2014, 2015). During 2007-2015, *Staphylococcus aureus* was associated with one outbreak (ESR, 2011) and no outbreaks were associated with *Bacillus cereus* and *Clostridium perfringens*. Notably, *Campylobacter* caused foodborne outbreaks linked to dairy products each year during 2007-2015 (ESR, 2016) and the high incidence of outbreaks that was linked to the consumption of raw milk (MPI, 2013). Outbreaks related to dairy products in the US showed similar results to New Zealand with fewer numbers than New Zealand (CDC, 2018b).

2.2.4.2. Chemical safety of dairy products

In Australia and New Zealand, robust regulatory and control measures in the dairy industry and the dairy supply chain result in minimal public health and safety concerns with chemical contamination of dairy products (FSANZ, 2006). Monitoring of chemical residues has revealed a high level of compliance with the regulations. Current management practices in chemical monitoring programs through the primary production chain need to be carried out continuously.

According to FAO (2013), chemical hazards that may pose a threat to dairy products are as follows:

- Agricultural and veterinary chemicals used in primary production, e.g. pesticide and insecticides, antibiotics, growth promoters;
- Environmental contaminants e.g. heavy metals, organic pollutants, dioxin, poly-biphenyl (PCBs);
- Naturally-occurring chemicals found in plants such as plant, fungal or bacterial toxins e.g. mycotoxins, cyanogenic glycosides, ergot alkaloid;
- Food processing by-products such as 3-monochloropropane-1,2-diol or 3-chloropropane-1,2-diol (3-MCPD);
- Food additives, processing aids and food contact substances that may migrate from packaging;
- Adulterants e.g. melamine.

2.3. Risk-based approach to assessing food safety

In developing value-added products, the dairy industry needs to be aware of food safety issues. As mentioned earlier, the quality of raw materials is one aspect of concern in food safety (FSANZ, 2006) but there are potentially additional issues in combining dairy and non-dairy ingredients, generating an environment that has properties that differ from the traditional intrinsic properties of dairy products and possibly revealing new food safety issues (Fuller, 2011). The dairy industry can use a risk-based approach to assess food safety issues.

2.3.1. The Codex risk analysis framework

In order to protect public health and to ensure fair practices in the international food and food product trade, Food and Agriculture Organization (FAO) and World Health Organisation (WHO) established Codex Alimentarius (FAO/WHO, 2017). Codex Alimentarius is a compilation of guidelines, standards, and codes of practice that governments may choose to implement. Since it was established in 1963, Codex Alimentarius Commission (CAC) has been developing many international standards, guidelines and codes of practices under the Joint FAO/WHO Foods Standards Programme.

The Codex risk analysis framework is a systematic approach to assess and examine public health and safety regarding risks related to food. Risk analysis is a process

comprising three different but closely related components: risk assessment, risk management and risk communication (Figure 5).

Figure 5. Risk analysis framework
Reprinted from "Risk analysis," (FSANZ, 2014a). CC BY 3.0 AU.

Risk assessment is a scientific-based evaluation which measures the probability and the impact of a hazard (ICMSF, 1998). Risk management is a process that involves considering policy options, discussion with all stakeholders, taking into account risk assessment and other aspects to protect consumer health, to promote fair trade practices, and if necessary, decide on appropriate prevention and control options to manage the risk (CAC, 2016; Forsythe, 2002). Risk communication is the exchange of information and opinions regarding the risk between stakeholders throughout the risk analysis process (CAC, 2016).

2.3.1. Risk assessment (RA)

Risk assessment (RA) is a systematic process to assess the risk related to any kind of hazard that could be biological, chemical or physical (CAC, 2016). RA aims to characterise the nature and likelihood of hazard as a consequence of hazard exposure in food. Risk characterisation includes qualitative and quantitative information with scientific uncertainty.

A hazard is a biological, chemical or physical agent that can cause an adverse reaction influencing human health. Risk is a function of the likelihood of an adverse health reaction to occur and the severity of that effect due to a hazard in food (CAC, 2016).

RA comprises of four stages (WHO, 2019). The first stage is hazard identification, which includes collecting and evaluating data concerning a hazard. Secondly, hazard characterisation which correlates the hazard (pathogen/chemical agent) and adverse reactions. Third, exposure assessment which estimates the level of the hazard. Lastly, risk characterisation which includes evaluating the risk and related information.

There are two approaches to RA, i.e. qualitative and quantitative which are illustrated in Figure 6 (Bassett, Nauta, Lindqvist, & Zwietering, 2012). Qualitative risk assessments are descriptive categories of risk, while, quantitative RA are mathematical analyses of numerical data. A quantitative RA is preferred if the quantitative information is available. A qualitative RA can be done when resources are limited or as an initial evaluation to determine whether the risk is significant and requires further analysis (Lammerding & Fazil, 2000). In circumstances when some quantitative information are available, a semi-quantitative approach that combine qualitative and quantitative inputs can be carried out. The output of semi-quantitative risk assessment is expected to be more precise that a qualitative risk assessment although less than a quantitative risk assessment (Voysey, Jewell, & Stringer, 2002).

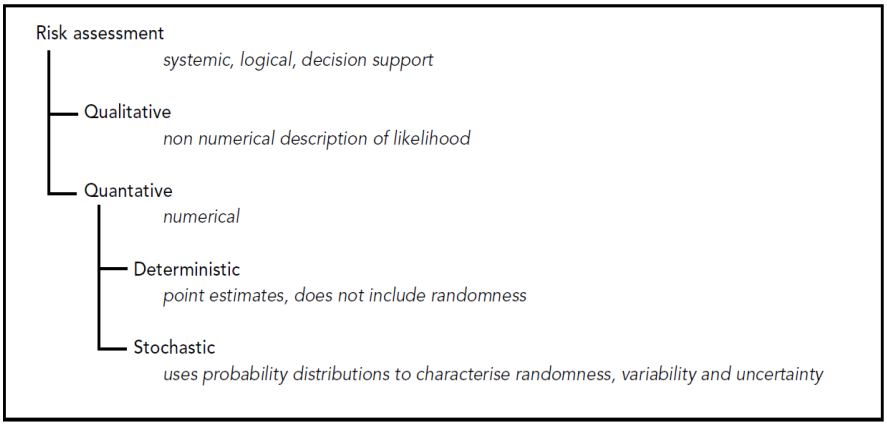

Figure 6. Risk assessment approach

Reprinted from "Tools for microbiological risk assessment," (Bassett et al., 2012, p. 7). ©2012 by ILSI Europe. In the public domain.

CAC published a guideline containing working principles for risk analysis in the framework of the Codex Alimentarius. There are several aspects that need to be considered in conducting RA:

- The scope and purpose of the specific risk assessment should be clearly stated.
- RA should be based on available scientific data. Both available quantitative and qualitative information should be taken into account with emphasis on the quantitative data.
- RA should consider pertinent production, storage and handling practices all through the food chain including traditional practices, methods of analysis, sampling and inspection and the prevalence of specific adverse health effects.
- The report should specify any constraints, uncertainties, assumptions and their impact on the risk assessment.
- RA should search out and include epidemiological surveillance data, analytical and exposure data globally including that from developing countries.
- It is worth mentioning that other factors, such as how consumers use specific food products, should be considered in risk assessments. Preparation of certain foods by certain cultures is done in the soil, which is a habitat for many microorganisms, some of which can be potential pathogens. This shows that valid risk assessment should take into consideration the specific location where the assessment is being done.
- However, sufficient data may not be available for such locations to be assessed. This makes it difficult to carry out the risk assessment. In such cases, RA needs to consider the role of expert elicitation where data may be insufficient.
- The conclusion of the risk assessment including risk estimate should be presented in a readily understandable and useful form to risk managers, risk assessors and interested parties.

RA is essential in assessing food safety (FSANZ, 2013). As a part of risk analysis, RA can measure the risk and includes the identification of hazards and factors that may influence the frequency and degree of hazard (CAC, 2016). Microbiological RA is one of the systematic tools that can assist the food industry to detect a hazard and evaluate risk to prevent food safety issues (ICMSF, 1998).

2.3.2. Microbiological risk assessment (MRA)

A microbiological risk assessment (MRA) framework offers a structured and scientific approach to assess the complex issues linked to food hygiene and foodborne diseases. The main objective of MRA is to estimate the probability of disease occurrence.

2.3.2.1. Hazard identification

Hazard identification should identify and describe the microbiological hazards that will be examined through subsequent stages of risk assessment. It should cover inputs to the supply chain such as micro-organisms or toxins from the raw materials and ingredients used in the products and possible sources of contamination during manufacturing and storage (M. Brown, 2002b). Potential microbiological hazards in food are bacteria, toxins, viruses, protozoa, and parasites. In 2011, the causal agents of foodborne infections in the United States were norovirus (58%), nontyphoidal *Salmonella* spp.(11%), *Clostridium perfringens* (10%), and *Campylobacter* spp. (9%) (Scallan et al., 2011). Among the biological hazards, bacteria are most important because they cause 90% of foodborne illness (M. Brown, 2002b). Outcomes of the hazard identification include the intended use of final product (e.g. ready-to-eat or for cooking) and way of preparation by the consumer; the probable sensitivity of consumers; control of the survival and growth of hazard by preservation during distribution and storage (e.g. cold supply chain) (M. Brown, 2002b).

2.3.2.2. Hazard characterization

Hazard characterisation focuses on comprehensive explanations of the factors affecting the disease process that could impact the dose-response relationship or the severity of disease. A description of adverse health reactions of the host, the pathogen and food matrix that influence the likelihood of the disease or other public health outcome and the data and model used to describe the dose-response relationship. Adequate information is needed to reproduce the dose-response model, including sources of data, assumptions used, goodness of fit of the distribution, uncertainty, and variability (Dennis, Miliotis, & Buchanan, 2002).

2.3.2.3. Exposure assessment

Microbiological exposure assessments are models of the level of pathogens or toxins in foodstuffs transferring through the supply chain (Lammerding & Fazil, 2000). It

explains the potential route of contamination and control measures, combined with the information regarding the pathogen's characteristics and how it is used to estimate the level or the likelihood of toxin occurrence in a portion at consumption. Assessment can use simple descriptions, point estimates or ranges of values to describe variables such as temperature, time, or pathogen concentration. Variability, uncertainty, and assumption need to be clearly explained and show in what manner the control measures regulate food hazards (M. Brown, 2002a).

2.3.2.4. Risk characterisation

Originally, RA was developed due to concern for toxic chemicals in food. Chemical RA is based on toxicology and carcinogenetic studies but it may not be applicable to micro-organisms. Micro-organisms are difficult to compare to chemical and environmental contaminants. Bacteria can multiply as conditions change as food moves through the farm to table continuum. Therefore, predictive models and other tools were developed to quantify the estimate of risk. Mathematical models are commonly used to illustrate the introduction of pathogens into food, the multiplication of microbes in food over time, the number of microbes at the point of consumption and consequent illness. A probability distribution is a mathematical demonstration of the relative likelihood of a certain value. Monte Carlo simulation of the model provides an estimate of the level of human illness and the uncertainty related to that estimate (WHO, 2019).

2.3.3. Chemical risk assessment (CRA)

Chemical risk assessment (CRA) needs sufficient toxicological information based on standardised testing protocols which have been accepted internationally. A credible risk assessment could incorporate data defined by a recognised body such as the Joint Expert Committee for Food Additives (JECFA), Joint FAO/WHO Meeting on Pesticide Residues (JMPR), Food and Drug Administration (FDA), Environmental Protection Agency (EPA) (FAO/WHO, 1995).

CRA requires abundant data (FAO/WHO, 1995). Data regarding the hazard, dose-response, and exposure information of certain substances may vary immensely in size, scope and quality. The data may be minimal and hard to acquire especially for contaminants and naturally-occurring compounds. Risk assessors need to maximise the use of the available information and deal explicitly with uncertainties.

Uncertainties in risk assessment are associated with data and the appropriate model selection. In particular, data uncertainties come from limited available data, evaluation and interpretation of data attained from toxicological and epidemiological studies.

2.3.3.1. Hazard identification

Hazard identification is effectively performed using a weight-of-evidence (WoE) approach (FAO/WHO, 1995; GOC, 2017). It is generally known as a method that involves the consideration of multiple sources of information. The WoE approach needs a fair and documented review of relevant scientific knowledge obtained from appropriate databases, peer-reviewed literature and any unpublished studies from the industry. The WoE approach helps to avoid the reliance on one source of information or lines of evidence to support the conclusion (GOC, 2017).

2.3.3.2. Hazard characterization

The chemicals in food include food additives, pesticides, veterinary drugs and contaminants. In food, they are usually present at low concentration, i.e. part per million or less. Animal toxicological studies must be carried out at high levels which may exceed several thousand parts per million. The main query in the hazard characterisation is whether or not the adverse effects detected in high-dose animal studies correlate with the low-dose human exposure (FAO/WHO, 1995).

The toxicological and human studies of chemical hazards are explained in the hazard characterisation. Toxicological studies are classified into *in vitro* and *in vivo* studies. *In vitro* studies take place in the laboratory and utilise cultured micro-organisms or cells obtained from laboratory animals or humans, while i*n vivo* studies use laboratory animals or humans (FAO/WHO, 2009a). Hazard characterisation requires data i.e. dose-response extrapolation, dose-scaling, genotoxic and non-genotoxic carcinogens, threshold approach, and non-threshold approachs (FAO/WHO, 1995).

Genotoxic and non-genotoxic carcinogens are treated differently by the food safety authorities. Non-genotoxic carcinogens may be regulated using a threshold approach, e.g. No-observed-effect level (NOEL) safety factor (FAO/WHO, 1995). Genotoxic carcinogens are regulated under the assumption that they may pose a cancer risk for humans even at very low doses (Nohmi, 2018).

2.3.3.2.1. Threshold approaches

The acceptable daily intake (ADI) is obtained from an experimental NOEL or No-observed adverse effect level (NOAEL) after applying a safety factor (Essers et al., 1998). ADI is defined in equation 1. JECFA and JMPR applied a safety factor to consider uncertainties. A safety factor of 100 comprises two factors of ten: one ten-fold factor to allow for inter-species differences and one ten-fold factor to allow for human variability (Benford & Tennant, 2012; FAO/WHO, 2009a).

$$ADI = \frac{NOAEL}{Uf} \quad (1)$$

Where:

ADI is the level intake of chemical that can be ingested daily over a lifetime without risk to health

NOAEL is No-observed adverse effect level is the highest dose at which there was not an observed toxic or adverse effect.

Uf is uncertainty (or safety) factor = 100

There are two classes of toxic effects: deterministic and stochastic. The severity of deterministic effects usually increases with increasing dose, thus, demonstrating a dose-dependent frequency distribution in the exposed population. On the contrary, the severity of stochastic effects is not dependent on the dose. Stochastic effects increase in incidence with increasing dose. One example of a stochastic effect is a genotoxic carcinogen which does not have a threshold dose but the likelihood of adverse effect increases as with increasing dose (Essers et al., 1998; WHO, 1994).

2.3.3.2.2. Non-threshold approaches

The NOEL safety factor approach is not suitable for genotoxic carcinogens (FAO/WHO, 1995). Two approaches are available: to ban the commercial use of chemical or to set a level of risk that is small to be deemed negligible.

2.3.3.2.3. Guidance or guideline value

Health-based guidance values (HBGV) were developed completely from toxicological and epidemiological data. Table 4 presents guidance and other values commonly used in chemical evaluations (WHO, 2010). ADIs have been developed for pesticides by the JMPR and for food additives by JECFA). Tolerable daily intake (TDI), provisional tolerable weekly intake (PTWI) and provisional tolerable monthly intake (PTMI) have

been developed for food contaminants by JECFA and acute reference dose (ARfD) have been developed for pesticides by JMPR.

Table 4. Guidance and other values commonly used in chemical evaluations

Type of outcome	Guidance value		Definition
Non cancer	Acceptable daily intake Tolerable daily intake Provisional tolerable weekly intake Provisional tolerable monthly intake	ADI TDI PTWI PTMI	An estimate of the amount of a substance in air, food, soil or drinking water that can be taken daily, weekly, monthly per unit body weight over a lifetime without appreciable health risk
	Acute reference dose	ARfD	Amount of a substance, normally in food or drinking-water, that can be ingested in a period of 24 h or less per unit body weight without appreciable health risk to the consumer
Cancer potentially relevant to human	Slope factor	SF	An estimate of the cancer associated with a unit dose of a chemical through ingestion or inhalation per unit body weight over a lifetime
Cancer highly relevant to humans	Benchmark dose	BMD	Amount of contaminant derived from studies in which experimental animals are given daily doses that produce a predefined cancer incidence (e.g. 5% or 10%)

Adapted from "WHO Human Health Risk Assessment Toolkit: Chemical Hazards," (WHO, 2010, p. 18). ©2010 by World Health Organization. Adapted with permission.

2.3.3.3. Exposure assessment

Information on the consumption of related foods and the level of the chemical of interest is needed to obtain an estimation of dietary intakes (FAO/WHO, 1995). Consumption data includes total diet studies, selected studies of specific foods, and duplicate portion studies.

The levels of pesticides, veterinary drugs, and additives are specified by their permitted conditions of use. Although the actual concentration of additives and pesticides present in foods are often well below the maximum levels permitted. Maximum residue limits (MRLs) is used for pesticide and veterinary drugs while maximum levels (ML) is used for additives (Benford & Tennant, 2012).

2.3.3.4. Risk characterization

Risk characterisation provides the estimation of the likelihood of adverse health effects in human populations as a consequence of exposure. Risk characterisation

considers the results from hazard identification, hazard characterisation and exposure assessment. For chemical agents that have a threshold limit, the population is characterised by comparison of the ADI with exposure. The likelihood of adverse health reactions is zero when exposure is less than the ADI. For non-threshold acting agents, the population risk is the product of exposure and potency (FAO/WHO, 1995).

RA involves the application of default assumptions to fulfil the gaps in knowledge and data. This is vital to ensure consistency in approach as well as to minimise or remove manipulations when performing a risk assessment to meet goals. Another method is to enable risk assessors to remove defaults in particular cases of chemical agents where the scientific data are available (FAO/WHO, 1995).

2.3.4. Differences between MRA and CRA

Several differences between MRA and CRA are identified in Table 5 (Langerholc, Lindqvist, & Sand, 2018). The key difference is that MRA estimates the pathogenic contamination of food at the point of consumption as well as numbers of people getting sick after consuming food, while the CRA estimates the exposure of the contaminant by food at the point of consumption and calculates whether the exposure is below or above the threshold limit, e.g. TDI or ADI (van der Fels-Klerx et al., 2018).

Table 5. Differences between MRA and CRA

	MRA	CRA
Acute or chronic hazard	Microbiological hazards that resulted in an acute sickness are identified and the association to the food chain.	Risks related to low exposure concentration of chemical hazards after a long period (chronic) are identified.
Dose-response model	Non-threshold models are commonly used including pathogen, host and epidemiologic parameters.	Threshold models are commonly used for most chemicals.
Exposure assessment	The multistep analysis is required to estimate the level of microbial contamination estimation at the point of consumption by consumers.	The multistep analysis is not required as chemical are usually stable during storage and handling. On the contrary of microorganisms that can multiply.
Purpose	Risk of illness related to estimated exposure is measured.	Estimated exposure is assessed against the recognised health-based guidance values (HBGV), i.e. ADI for food additives and TDI for food contaminants.
Variability	Variation within the human population and microbial genetic strains.	Diversities within the human population is considered.

	MRA	CRA
Uncertainty	Uncertainty along and within the food chain.	Uncertainties are when the level of chemicals is lower than the limit of detection (LOD) or limit of quantification (LOQ), route-to-route extrapolation, the dose-response curve, nature and severity of the effects, exposure duration in experimental animal studies
Exposure source	Applicable for the different scenario of exposure source including one food and one pathogen, one food and several pathogens, one pathogen and several foods or a food category, several foods and several pathogens.	Cumulative risk is measured for a particular compound found in different foods as the chemicals not only occurs in a single food.
Risk ranking method	- Cost of illness (CoI), - Health-adjusted life years (HALY) - Expert judgements	- Risk ratio - Scoring - Flow charts - Risk matrices

2.3.5. Strengths and weaknesses of RA

RA is typically employed for one identified chemical or microbiological hazard which occurs in a specific food commodity and for a predefined population, to characterise the related health risk (van der Fels-Klerx et al., 2018). Available scientific and technical information and data, variability and uncertainties are systematically organised and analysed in a RA (van der Fels-Klerx et al., 2018). A RA provides the opportunity to address uncertainties in a transparent way, e.g. via sensitivity analyses and/or modelling and simulation.

Numerous RA approaches for chemical and microbiological hazards in food apply different combinations of deterministic, probabilistic or stochastic, qualitative, semi-quantitative modelling. Various approaches are used for the exposure assessment and the hazard characterisation steps (van der Fels-Klerx et al., 2018).

When RA is used optimally, it should deliver key information concerning risk from exposure to food hazards to the policy maker, decision makers and the public. A RA is very beneficial in providing insights into gaps in knowledge and issues linked to high levels of uncertainty (van der Fels-Klerx et al., 2018).

A RA for one chemical or microbiological hazard usually requires abundant time, data and knowledge. Outcomes of individual RAs will require more resources and RAs are

often hindered by the absence of quantitative data. Ranking risks related to various hazards in food using outcomes of individual RAs will take even more resource. Lack of data, selection of models to fit to the data, and assumptions that need to be made increase the uncertainties in the outcomes. There is a need for the development of harmonised approaches and future studies on cumulative exposure assessments (van der Fels-Klerx et al., 2018).

2.3.6. Government versus industrial risk assessment

Risk assessment reports are usually published by governmental agencies or food safety authorities. There is a paucity of industrial risk assessment reports available in the literature. It is believed that government and industry have different approaches in conducting risk assessments. Risk assessments conducted by industries are not as much as the assessments conducted by government agencies. The lack of industry-based risk assessment can result in a wide variance when compared to risk assessments conducted by the government agencies. Schothorst (2002) describes the dissimilarities between governmental and industrial risk assessment in Table 6.

Table 6. Dissimilarities between governmental and industrial risk assessment

Governmental risk assessment	Industrial risk assessment
Estimate number of people that become sick as a consequence after the consumption of food containing a certain level of a specific microorganism.	• Estimate the concentration of a specific microorganism in the food to be marketed • To compare with a similar food with a good safety record (food safety benchmarking).
Investigate different scenarios with different control options to estimate the risk	Foundation of safety record are HACCP and GHP.
Estimation number of illness will be assessed for implementation and when appropriate considered for insertion in HACCP plans.	Effect of new formulations, new technologies and new equipment to the safety of the end product will be estimated as a part of the HACCP system.

Furthermore, Schothorst (2002) explained the industrial hazard analysis process in Figure 7. Industrial hazard analysis uses the same methodology as described in MRA and may utilise models such as predictive models and Monte Carlo simulations, but the end-point will be an exposure assessment. However, Roberts and Greenwood (2003) argued that risk assessment in a food production process should identify and characterise the hazards in the process, evaluate the exposure and finally, characterise the risk.

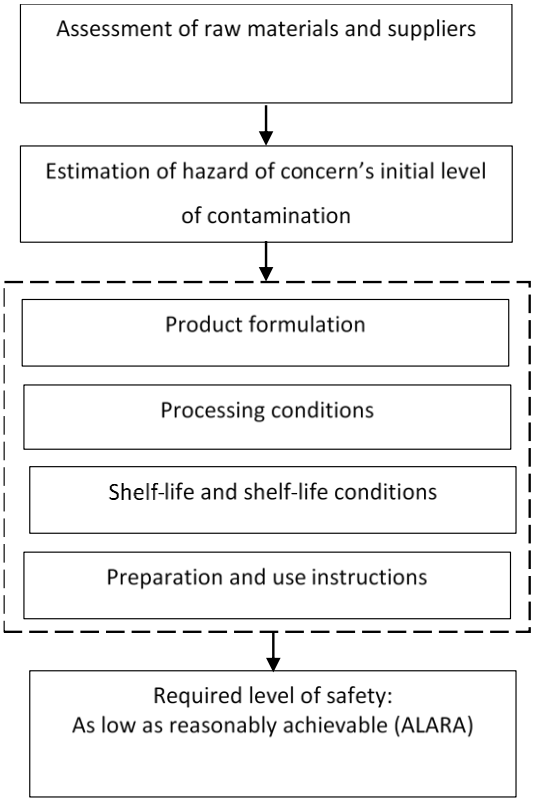

Figure 7. Industrial hazard analysis process

2.3.7. Review of published risk assessment reports

Many food safety associated agencies which include government, non-government and international bodies such as World Health Organisation (WHO) and Food and Agriculture Organisation of the United Nation (FAO) have conducted risk assessments of different micro-organisms and different foods. Joint FAO/WHO expert meetings on microbiological risk assessment (JEMRA) have produced some publications under microbiological risk assessment series (FAO, 2019). Microbial risk assessments which have been conducted include a risk assessment of *Listeria monocytogenes* in ready-to-eat foods by the FAO in 2004. The FAO also conducted another risk assessment about the prevalence of *Enterobacter sakazakii* (now *Cronobacter* sakazakii) and other micro-organisms in powdered infant formula in 2007 and a recent one in 2018 about Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterisation, and monitoring. In all the risk assessments mentioned above, FAO used a quantitative assessment approach and the data collected was based on particular pathogens in a specific food. All the reports incorporated full codex risk assessment and the results represented an ideal risk assessment report with substantial models which can be used

by other countries. The disadvantage of these quantitative risk assessments is the lengthy time frame since it involved many experts from different countries including the United States, Canada, Australia, Sweden and The Netherlands (WHO & FAO, 2004).

The United States Food and Drug Administration (FDA) has also published some risk assessment reports (FDA, 2019). These include *Vibrio* in raw oysters risk assessment in 2005, joint FDA / Health Canada quantitative assessment of the risk of listeriosis from soft-ripened cheese consumption in the United States and Canada in 2015 and *Salmonella* on tree nuts in 2017. Most risk assessments are quantitative risk assessment and a large amount of data is needed in conducting such risk assessments. The FDA usually request for scientific data, information and comments from the public, including various institutions, the food industry and consumer-advocacy groups (FDA, 2016b). Such data are provided by different sources and it is finally collated by the FDA. Compilation of such data can provide a comprehensive report which considers all the information provided by different sources.

Although most risk assessments are quantitative, a qualitative risk assessment can be carried out for reasons such as limited time to conduct a risk assessment, a paucity of available information and ease of explaining to stakeholders. The FAO/WHO (2009c) recommends starting with a qualitative risk assessment which studies the literature available and followed by a quantitative assessment if it is needed and when there is adequate information. According to (CAC, 2016), a risk profile which is also known as qualitative risk assessment is a food safety description and its purpose is to identify areas that need further work to carry out a quantitative risk assessment. In this type of risk assessment, there are no simulations required to predict outcomes (FDA, 2011).

There is limited data about risk assessments on cereal grains in the world. The New Zealand Ministry for Primary Industries conducted risk assessment of *Salmonella* (non-typhoidal) in cereal grains in 2010. A risk profile on *Salmonella* in cereal grains was carried out due to outbreaks associated with *Salmonella* after consumption of raw cake batter in New Zealand which happened in 2008-2009. The risk profile report described the aspects that are similar to risk assessment: hazard and food, evaluation of adverse health effects, exposure assessment, and evaluation of risk and control

measures. The report was therefore useful as a foundation to conduct a risk assessment of cereal grains in the present study.

The number of pathogens that are associated with cereal grains may create challenges for food safety authorities and the food industry. There is the need to rank food safety risk of pathogens in order to help the food industry to prioritise their resources. Risk ranking for food safety is the basis for risk-based priority setting and resource allocation. Thus, it is needed to fill the gap by conducting a study on pathogens associated with cereal grains.

Most microbial risk assessments have only focussed on a specific pathogen in specific foods. One question that needs to be asked, however, is whether introducing new ingredients to dairy products can create a new hazard. Therefore, it is essential for us to assess the risks involved when combining dairy and non-dairy ingredients.

Several chemical risk assessments related to cereal grains have been reported. These include a risk assessment conducted by the FDA about arsenic (heavy metals) in rice and rice products (FDA, 2016a) and carcinogenic risk of pesticide residues in food (Gold, Slone, Ames, & Manley, 2001). In regards to mycotoxin, EFSA scientific opinion on the risks for public health related to the presence of zearalenone (a mycotoxin in food) (EFSA CONTAM Panel, 2011; Gold, Slone, Ames, & Manley, 2001) and the New Zealand Ministry of Primary Industries has published some risk assessments about mycotoxins in foods which include aflatoxin, ochratoxin A and trichothecenes (Cressey & Pearson, 2014). The chemical risk assessments that have been conducted until now have focused on pesticides, mycotoxins, and heavy metals rather than naturally-occurring toxins. There is a lack of studies on risk assessment of naturally-occurring toxins which could generate food safety risk. It is therefore essential to conduct risk assessments of these naturally-occurring toxins.

2.3.8. Risk ranking of food-related hazards

Recognising major food hazards such as microbial pathogens and toxic chemicals in particular foods has been a major development in the food science for decades. Determining hazards in particular foods of greatest risk to consumers can be done through risk ranking used by government, policy makers and industry to protect public health (Morris Jr, Hoffmann, & Batz, 2011).

Risk ranking for food safety is the basis for risk-based priority setting and resource allocation. It enables governmental and food safety authorities to prioritise efforts and assign their resources efficiently to the most significant public health problem (Morris Jr et al., 2011; van Kreijl, Knaap, & Van Raaij, 2006) increasing the monitoring efficiency and decreasing cost of inspection (Reist, Jemmi, & Stärk, 2012).

A number of risk ranking methods including qualitative, semi-quantitative and quantitative methods are available to aid in prioritising food safety risks (Cope, Frewer, Renn, & Dreyer, 2010; Romero-Barrios, Hempen, Messens, Stella, & Hugas, 2013). The majority of the methods are based on the technical concept of risk (which takes into consideration all the risk ranking methods together) whereas risk is a function of the presence of hazard and severity to human health (Van Asselt, Sterrenburg, Noordam, & Van der Fels-Klerx, 2012). Risk-ranking approaches enable comparison between hazards that can pose acute or chronic health effects (Almutairi, 2016).

2.3.8.1. Comparative analysis of risk ranking methods

Different risk ranking techniques are used for assessing microbiological and chemical hazards. van der Fels-Klerx et al. (2018) identified risk ranking methods that are commonly used to assess both microbiological and chemical hazards: 1) Risk Assessment (RA), 2) Comparative risk assessment (CRA), 3) Risk ratio method, 4) Scoring method, 5) Risk matrix, 6) Flow charts (including decision trees and influence diagrams), 7) Cost of illness (CoI), 8) Health adjusted life years (HALY), 9) Multi criteria decision analysis (MCDA), 10) Stated preference methods, and 11) Expert judgment. Characteristics of different risk ranking methods are given in Table 7.

Several risk ranking techniques have been developed for microbiological hazards that depend on the purpose, time and availability of data (van Asselt, van der Spiegel, Noordam, Pikkemaat, & van der Fels-Klerx, 2013). HALY, CoI, and expert judgement are common risk-ranking methods for microbial hazards. On the contrary, risk ratio, risk matrices and scoring are mainly used for chemical hazards (van der Fels-Klerx et al., 2018).

Table 7. Characteristics of risk ranking methods

Characteristic	Ratio (exposure/ effect)	Risk assessment	Comparative risk assessment	Scoring method	Risk matrix	Flowchart	Cost of illness	Health-adjusted Life Year	Willingness to Pay	Multi Criteria Decision Analysis	Expert judgement
Quantity of resources (time, money)	Moderate	High	High	Moderate	Low	Low	Moderate	Moderate	High	High	Moderate/ Low
Output level	Semi-quantitative	Quantitative	Quantitative	Semi-quantitative	Qualitative/ semi-quantitative	Qualitative	Semi-quantitative	Semi-quantitative	(Semi-) quantitative	Semi quantitative	Qualitative
Easy to explain to stakeholders	Yes	No	No	Yes	Yes	No	Yes	No	No	Yes	Yes
Insertion of uncertainty	Possible	Possible	Possible	Possible	Not possible	Not possible	Possible	Possible	Possible	Possible	Possible
Insertion stakeholder perception	Not possible	Not possible	Not possible	Possible	Not possible	Possible	Not possible	Not possible	Possible	Possible	Possible
Insertion of economic impact	Not possible	Not possible	Not possible	Not possible	Not possible	Possible	Possible	Not possible	Possible	Possible	Possible
Insertion human incidences	Not possible	Possible	Possible	Not possible	Not possible	Possible	Possible	Possible	Possible	Possible	Possible
Insertion of weights for the risk ranking criteria	Not possible	Not possible	Not possible	Possible	Not possible	Not possible	Not possible	Not possible	Not possible	Possible	Possible
Presentation of result	Tables	Graphs/Tables	Graphs/Tables	Tables	Graphs	Decision tree	Graphs/ Tables	Graphs/ Tables	Graphs/ Tables	Graphs/ Tables	Tables

Adapted from "Critical Review of Methods for Risk Ranking of Food-Related Hazards, based on Risks for Human Health," (van der Fels-Klerx et al., 2018, p. 181). CC BY-NC-ND 4.0.

2.3.8.2. Risk matrices

The risk matrix is a method of assigning quantitative data to qualitative information to provide an easy to use scoring system. Risk matrices apply scoring of consequence and likelihood of occurrence. Risk matrices typically contain 4x4 or 5x5 matrices, where frequency of occurrence is plotted on one axis (e.g. vertical axis) and consequences are drawn on the other axis (e.g. horizontal axis). Risk matrices are commonly employed for limited quantitative data of microbiological or chemical hazards (van der Fels-Klerx et al., 2018). Risk matrices are used for ranking the risk of nanomaterials (O'Brien & Cummins, 2011; Zalk, Paik, & Swuste, 2009).

The likelihood of occurrence and the consequences are categorised into one of several categories (van der Fels-Klerx et al., 2018). Each category represents a score from one to five accordingly. Examples of categories for likelihood of occurrence are: rare (1), unlikely (2), possible (3), likely (4), and almost certain (5). Examples of categories for consequences are: insignificant (1), minor (2), moderate (3), major (4), and severe (5). Classes of risk are determined by combining the consequences and likelihood of occurrence into Low, Medium, and High. The division of these classes is subjective and may differ one to another. Table 8 is an example of a semi-quantitative risk matrix.

Table 8. Semi-quantitative risk assessment matrix example

Likelihood level	Consequence level				
	1 Very low	2 Low	3 Medium	4 High	5 Severe
1 Highly unlikely	1	2	3	4	5
2 Unlikely	2	4	6	8	10
3 Possible	3	6	9	12	15
4 Very likely	4	8	12	16	20
5 Almost certain	5	10	15	20	25

From "Risk Assessment and Management: A Guide for Integrated Urban Water Systems," (Blackmore et al., 2008, p. 16). ©2008 by eWater Cooperative Research Centre. In the public domain.

As an advantage, the risk matrix provides a visualisation of consequences and likelihood of occurrence (van der Fels-Klerx et al., 2018). It allows a clear understanding of how both elements affect the overall risk of hazard. A hazard may pose a high risk because the

likelihood is high despite low severity. On the contrary, a hazard may exhibit a high risk because it has high toxicity although low exposure.

Another advantage of risk matrices is that they offer comprehensive information to the risk manager to communicate with stakeholders (van der Fels-Klerx et al., 2018). Risk matrices provide more information than other techniques that simply show the overall risk. The disadvantage of this technique is difficulty in accurately and reproducibly allocating a score to a subjective assessment.

2.3.9. Summary and conclusion

There has been no risk assessment for the addition of cereal grains to dairy products. The present research is the first risk assessment that assesses the microbiological and chemical risks of cereal grains as non-dairy ingredients for their addition to dairy products. This risk assessment will enable us to identify critical gaps in knowledge, characterise the most important risk factors in the food chain, help to identify strategies for risk reduction, and provide guidance for determining research priorities in public health and food safety (Lammerding, 1997). A transparent and structured risk ranking technique will be needed to rank microbial and chemical hazards from non-dairy ingredients that pose the highest risk. Incorporating risk matrices into risk assessment will help to make the risk assessment result to be easily understood by stakeholders.

CHAPTER 3. METHODS

3.1. Data collection

Data required in each risk assessment step was collected using specific databases including Web of Science and Google Scholar. The general search engine Google was used to search reports, publications, and regulatory data from government institutions and agencies (e.g., EFSA, FDA, CDC, FSANZ, MPI), relevant international organizations (e.g., WHO, FAO/WHO, CAC, JECFA, IARC), and industry databases. Theses and dissertation were identified using Massey University Discover and ProQuest databases. The literature focused on articles and reports published in English.

A search strategy was applied, resulting in an initial set of search results (Appendix A. literature research procedure). The references from the initial set of search results were screened for their relevance by applying the evaluation criteria. The first screening of relevance was done by examining the title, abstracts and keywords of each reference, resulting in a list of references. The second screening of relevance was determined by reading the full text of the references obtained in the first screening.

Evaluation criteria used for screening the references were:

1) Relevant references with the purpose of the literature research included:
    - References reviewing microbiological and chemical hazards in food including cereal grains (cereals, pseudo-cereals and grain legumes), and/or dairy,
    - References describing risk analysis and risk assessment methods related to food safety and human health and/or,
    - References explaining risk prioritisation or risk ranking application of food-related hazards to human health including drinking water.
2) References originating from international peer-reviewed journals or scientific articles and reports from notable government institutions and agencies as well as recognised international bodies.
3) Reference containing methods that were possibly applicable to the present study.

3.2. Samples

Select cereal grains of interest in this present study are shown in Table 9. For the purpose of this study, cereal grains is a term used to represent three categories, i.e. cereals, pseudocereals and grains legumes (pulses). These cereal grains were selected due to their popularity and high possibility to be used in developing more appealing dairy products (Bullerman & Bianchini, 2009; Koehler & Wieser, 2013; Wrigley, 2017b).

Table 9. List of selected cereal grains to be evaluated

Category	Ingredient's name	Scientific name
Cereals	Barley	*Hordeum vulgare*
	Maize (Corn)	*Zea mays*
	Millet	*Pennisetum glaucum*
	Oats	*Avena sativa*
	Rye	*Secale cereal*
	Black glutinous rice	*Oryza sativa var glutinosa*
	Brown rice	*Oryza sativa*
	Wheat	*Triticum aestivum*
Pseudo-cereals	Buckwheat	*Fagopyrum esculentum*
Grain legumes (pulses)	Adzuki beans (red mung bean)	*Vigna angularis*
	Garden peas	*Pisum sativum*
	Hyacinth beans	*Lablab purpureus*
	Mung beans	*Vigna radiate*
	Soybeans	*Glycine max*
	Black soybeans	*Glycine max* (L) Merrit

3.3. Risk assessment methods

The microbiological risk assessment was conducted according to the Codex Committee on Food Hygiene Principles, and Guidelines for the Conduct of Microbiological Risk Assessment (CAC, 1999b). The chemical assessment was conducted according to Principles and Methods for the Risk Assessment of Chemicals in Food (FAO/WHO, 2009b). Microbiological and chemical risk assessments have the same four steps of hazard identification, hazard characterisation, exposure assessment and risk characterisation (Figure 8).

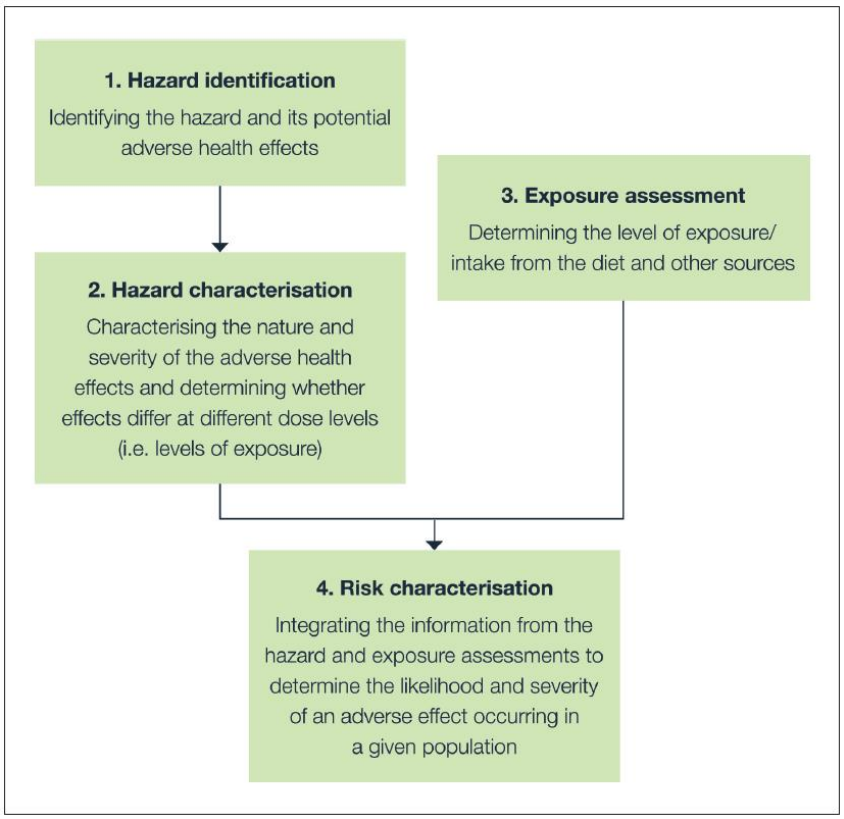

Figure 8. The steps in risk assessment and explanations
Reprinted from "Risk analysis in food regulation," (FSANZ, 2013, p. 39). In the public domain.

This present study employed a semi-quantitative approach that combine qualitative and quantitative inputs due to limited number of studies on microbiological and chemical risk assessment of cereals and grains added to dairy products. This can then lead to a quantitative risk assessment in the future (Lammerding & Fazil, 2000).

Several tasks were undertaken in each step of risk assessment as follows:

1. Hazard identification

    1) The microbiological (including microbial toxin) and chemical hazards in the cereal grains categories were identified.

    2) The potential adverse health effects in each category were identified.

    3) The origin and distribution of microbiological and chemical hazards were identified.

    4) The possible sources of microbiological and chemical contamination through processing and storage were identified.

5) The relevant data on hazards such as clinical studies, epidemiological reports and surveillance, characteristics of microbiological and chemical agents were collected.

2. Hazard characterisation

1) The nature and severity of adverse health effects caused by the microbiological and chemical hazards were characterised.

2) The dose-response information for the microbiological and chemical hazards in humans (where available) was described.

3) Effects of different dose levels were recorded.

4) Expert elicitations were used to describe hazard characterisation when the known dose-response relationship is not available.

3. Exposure assessment

1) Data on the occurrence, frequency of contamination and the levels of the microbiological and chemical hazards in the selected cereal grains were collected.

2) Data on the consumption pattern of the selected cereal grains were collected.

3) For microbiological hazards, relevant factors affecting contamination such as food handler hygiene, abusive temperature/time, intrinsic characteristics of food (pH, nutrient content, moisture content or a_w, the presence of antimicrobial constituents and competitive microflora) were identified.

4) The production to consumption pathways and possible routes of contamination were described.

5) The likelihood of microbiological and chemical hazard occurrence in the cereal grains at the point of consumption was determined, within the various levels of uncertainty. The chemical risk was assessed if the estimated exposure is below the established health-based guidance values (HBGV).

6) For chemical hazards, expert knowledge was used to set the percentage of non-dairy ingredient addition.

4. Risk characterisation

    1) Information from the hazard identification, hazard characterisation, and exposure assessment to obtain a risk estimate was integrated.

    2) In estimating the most critical microbiological and chemical risk in the selected cereals and grains, the likelihood and severity of the adverse effects which could occur for a given population was determined in the form of semi-quantitative risk assessment matrix.

    3) In estimating the risk of cereal grains addition to dairy products, a qualitative measure of likelihood based on European Food Safety Authority (EFSA) terms was used to describe prevalence (Table 10).

Table 10. Qualitative measures of likelihood

Prevalence	Descriptor
>70%	Extremely High
>50% to 70%	Very High
>20% to 50%	High
>10% to 20%	Medium
>1% to 10%	Low
0.1% to 1%	Very Low
<0.1%	Rare

From "The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011," (EFSA, 2013, p. 248). In the public domain.

    4) The uncertainties, variabilities and assumptions associated with the final estimation of the risk estimates were described.

3.3. Developing risk-ranking methods/risk assessment criteria

Risk ranking methods/risk assessment criteria were developed to rank the microbiological and chemical risks of most significant concern in selected cereal grains from a global food safety perspective. In estimating the risk of hazards, risk assessment criteria from which to measure and score need to be established and defined (Popov, Lyon, & Hollcroft, 2016). Steps to conduct risk-ranking methods/risk assessment criteria are shown in Figure 9.

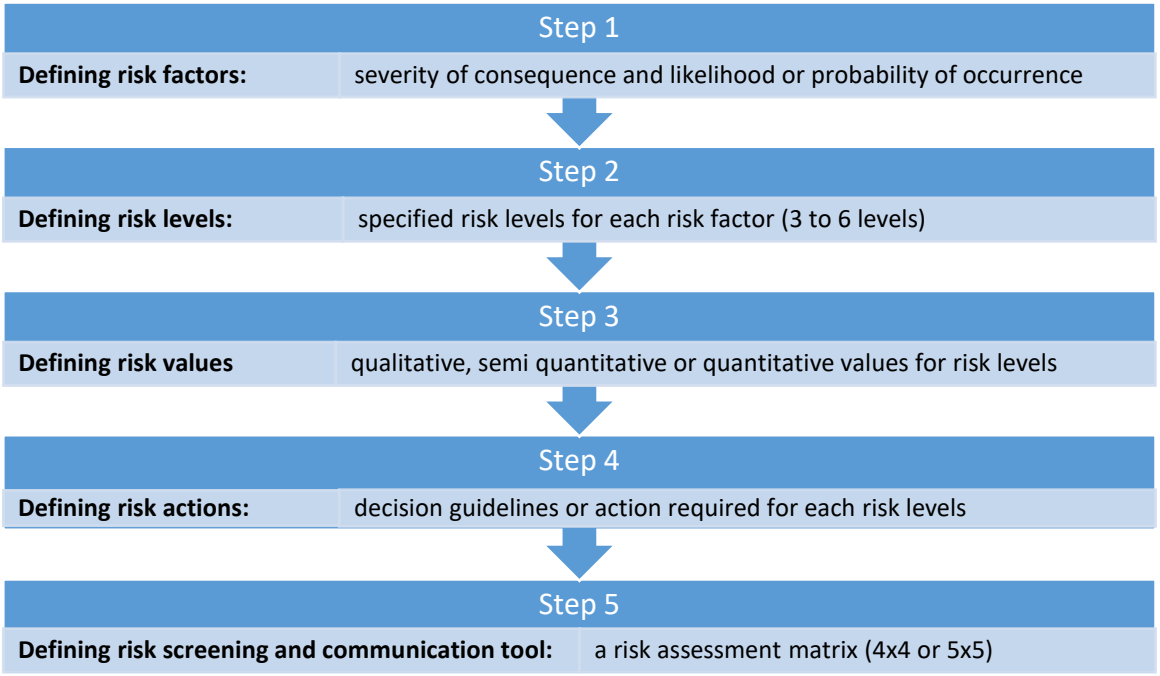

Figure 9. Steps in developing risk-ranking method/risk assessment criteria

The severity of consequence and likelihood of occurrence were chosen as the risk factors. It is fundamental for any accurate risk analysis to have procedures for determining appropriate consequences and likelihood levels. Adequate descriptions of each level of likelihood and consequence are required to get precise results and prevent vagueness in applying risk ratings (W. J. Fletcher, 2005).

3.3.1. Microbiological hazards

The consequence levels for microbiological hazards that were used in the research were insignificant (1), minor (2), moderate (3), major (4) and severe (5). Five levels of consequences and their qualitative descriptions are shown in Table 11.

Table 11. Qualitative description of consequence

Consequence level	Score	Description
Severe	5	Severe hazard for vulnerable population (category III.B based on ICMSF): Life-threatening, substantial chronic sequelae, long duration.
Major	4	Severe hazard for the general population (category III.A based on ICMSF): Life-threatening, substantial chronic sequelae, long duration.
Moderate	3	Serious hazard (category II based on ICMSF): Incapacitating but not life-threatening, sequelae infrequent, moderate duration.
Minor	2	Moderate category (category I based on ICMSF): Not usually life-threatening, no sequelae, usually short duration, symptoms are self-limiting, can be severe discomfort.
Insignificant	1	Not significant.

Adapted from (ICMSF, 2018) and (FAO/WHO, 2009c).

The risk levels identified for the likelihood of occurrence were rare (1), unlikely (2), possible (3), likely (4), and almost certain (5). The five likelihood levels and their qualitative descriptions are presented in Table 12.

Table 12. Semi-quantitative description of likelihood

Likelihood level	Score	Description	No. of outbreaks	Prevalence
Almost Certain	5	is expected to occur in most circumstances	>60	>85%
Likely	4	Will probably occur in most circumstances	41-60	50-85%
Possible	3	Might occur or would occur at some time	21-40	21-49%
Unlikely	2	Could occur at some time	11-20	1-20%
Rare	1	May occur only in exceptional circumstances	0-10	<1%

Adapted from (FAO/WHO, 2009c, p. 34) and (Popov et al., 2016).

Risk is a quantification of the probability/likelihood of an uncertain future event and severity of consequence which can be defined in the following equation:

$$\textbf{Risk (R) = Severity (S) x Likelihood (L)} \quad (2)$$

The overall risk score for each microbiological hazard was calculated by multiplying the severity and likelihood scores. Then, the result was plotted into a semi-quantitative risk assessment matrix (Table 13).

Table 13. Semi-quantitative risk assessment matrix

Likelihood	Consequences				
	Insignificant (1)	Minor (2)	Moderate (3)	Major (4)	Severe (5)
Almost certain (5)	Medium 5	High 10	High 15	Very high 20	Very high 25
Likely (4)	Medium 4	Medium 8	High 12	High 16	Very high 20
Possible (3)	Low 3	Medium 6	Medium 9	High 12	High 15
Unlikely (2)	Low 2	Medium 4	Medium 6	Medium 8	High 10
Rare (1)	Low 1	Low 2	Low 3	Medium 4	Medium 5

Adapted from (Blackmore et al., 2019) and (FAO/WHO, 2009c).

The overall risk score was categorised into risk rating (Very High, High, Medium and Low). Risk rating represents the risk management step that need to be taken to reduce the risk (Table 14).

Table 14. Qualitative risk characterisation measures

Risk score	Rating	Risk management
20-25	Very high	Intolerable under any circumstance, immediate action required
10-16	High	Unacceptable, action plan is to be given high priority
4-9	Medium	Tolerable under specific circumstances, action plan is to be taken at an appropriate time
1-3	Low	Acceptable, specific monitoring or procedure required to ensure risk level maintained

3.3.2. Chemical hazards

The severity levels for chemical hazards were low (1), medium (2), high (3) and severe (4). Four levels of the severity and their qualitative descriptions are given in Table 15.

Table 15. Qualitative measures of severity of toxicity

Criteria	Categories of the severity of toxicity			
	Severe (4)	**High (3)**	**Medium (2)**	**Low (1)**
Acute Reference Dose (ARfD) (µg/kg bw/day)	<10	10-50	50-200	≥200
Tolerable Daily Intake (TDI) (µg/kg bw/day)	<1	1-10	10-30	≥30
The severity of acute effects	High	Moderate	Low-moderate	Low
Toxicity medically treatable	Unlikely	Possible	Yes	Yes
Carcinogenic	Knowns in humans	Shown in animals	Unlikely	No
Reproductive and developmental toxicity	Knowns in humans	Shown in animals	Unlikely	No
Reversibility of toxicity	Unlikely	Possible	Probable	Probable
Chronic effects	Probable	Possible	Unlikely	No

Adapted from (Hanlon et al., 2015) and (van der Fels-Klerx et al., 2018).

The risk levels for the likelihood of occurrence were unlikely (1), possibly (2), likely (3), and almost certain (4). Four likelihood levels and their qualitative descriptions are presented in Table 16.

Table 16. Qualitative measures of likelihood

Criteria	Categories of Likelihood			
	Almost certain (4)	**Likely (3)**	**Possibly (2)**	**Unlikely (1)**
Historical data demonstrating presence of a contaminant in a commodity category or safety limits	Residues detected at MRL/ML or above in 1% of samples	Residues detected at MRL/ML or above in ≤1% of samples	Residues detected during last 10 years at concentrations below MRL/ML	No evidence for residues
Manufacturing process	Highly unlikely to remove the contaminant	Unlikely to remove the contaminant	Likely to remove the contaminant	Highly likely to remove the contaminant

Adapted from (Hanlon et al., 2015) and (van der Fels-Klerx et al., 2018).

Risk is a quantification of the probability/likelihood and severity of consequence can be defined in the following equation:

$$\textbf{Risk (R) = Severity (S) x Likelihood (L)} \quad (3)$$

Combination of severity and likelihood were mapped in the semi-quantitative risk assessment matrix (Table 17) and get a risk rating (Table 18) for prioritising potential food safety issues. Risk ratings used were low, medium, and high.

Table 17. Qualitative risk assessment matrix

Likelihood	Severity			
	Low (1)	Medium (2)	High (3)	Severe (4)
Almost certain (4)	Low 4	Medium 8	Medium 12	High 16
Likely (3)	Low 3	Medium 6	Medium 9	Medium 12
Possible (2)	Low 2	Low 4	Medium 6	Medium 8
Unlikely (1)	Low 1	Low 2	Low 3	Low 4

Adapted from "A risk-based strategy for controlling chemical contaminants as relevant hazards in food ingredients," (Hanlon et al., 2015, p. 97). ©2015 International Association for Food Protection.

Table 18. Qualitative risk characterisation rating measures

Risk score	Rating	Risk management
16	High	Immediate action required/high priority
6-12	Medium	Action plan required/medium priority
1-4	Low	Specific monitoring or procedure required/low priority

CHAPTER 4. MICROBIOLOGICAL RISK ASSESSMENT RESULTS

This chapter covers the microbiological risk assessment results of the present study. Section 4.1 presents the microbiological risk assessment of selected cereal grains resulting in several pathogens as microbial hazards. *Bacillus cereus* was the most critical microbial hazard after application of a risk ranking method (i.e. semi-quantitative risk assessment matrix). Section 4.2 describes the microbial risk assessment of oats as the selected cereal addition to three types of dairy products, i.e. milk powder, Parmesan cheese and liquid breakfast.

4.1. Microbiological risk assessment of selected cereal grains

4.1.1. Hazard identification

Cereal grains have a diverse microflora which includes bacteria (psychotropic, mesophilic, and thermophilic/thermoduric), moulds, yeast, rope-forming bacteria (*Bacillus* spp.), bacterial pathogens, Enterococci and coliforms (Bullerman & Bianchini, 2009). Most of the bacteria found in grains are under the families of *Bacillaceae*, *Lactobacillaceae*, *Micrococcaceae,* and *Pseudomonadaceae* (Laca, Mousia, Díaz, Webb, & Pandiella, 2006). Some members of these families contain pathogenic bacteria, spoilage micro-organisms and mycotoxin producing moulds.

Several researchers found cereal grains as the sources of foodborne pathogens and faecal micro-organisms such as *Bacillus cereus (B. cereus), Clostridium botulinum (C. botulinum), Clostridium perfringens (C. perfringens), Escherichia coli (E. coli), Listeria monocytogenes (L. monocytogenes), Salmonella* spp., *Shigella* spp., and *Staphylococcus aureus (S. aureus)* (Bullerman & Bianchini, 2009; Forsythe, 2002; NZFSA, 2010b). The presence of faecal micro-organisms in grains such as coliforms and enterococci are used as indicators of improper sanitary handling and processing conditions (Bullerman & Bianchini, 2009).

The common spoilage micro-organisms in cereal grains are moulds or filamentous fungi (Bullerman & Bianchini, 2009). Moulds mostly found in grains include *Alternaria, Cladosporium, Fusarium,* and *Helminthosporium* (Laca et al., 2006), but another genera can also be present such as *Aspergillus, Penicillium* and *Eurotium* (Berghofer et al., 2003). These filamentous fungi can produce mycotoxins in the field and during the

storage of cereals (Los, Ziuzina, & Bourke, 2018). Mycotoxins are regarded as a chemical hazard which will be discussed in the chemical risk assessment.

A diagrammatic representation of the microflora and potential pathogens associated with cereal grains is illustrated in Figure 10.

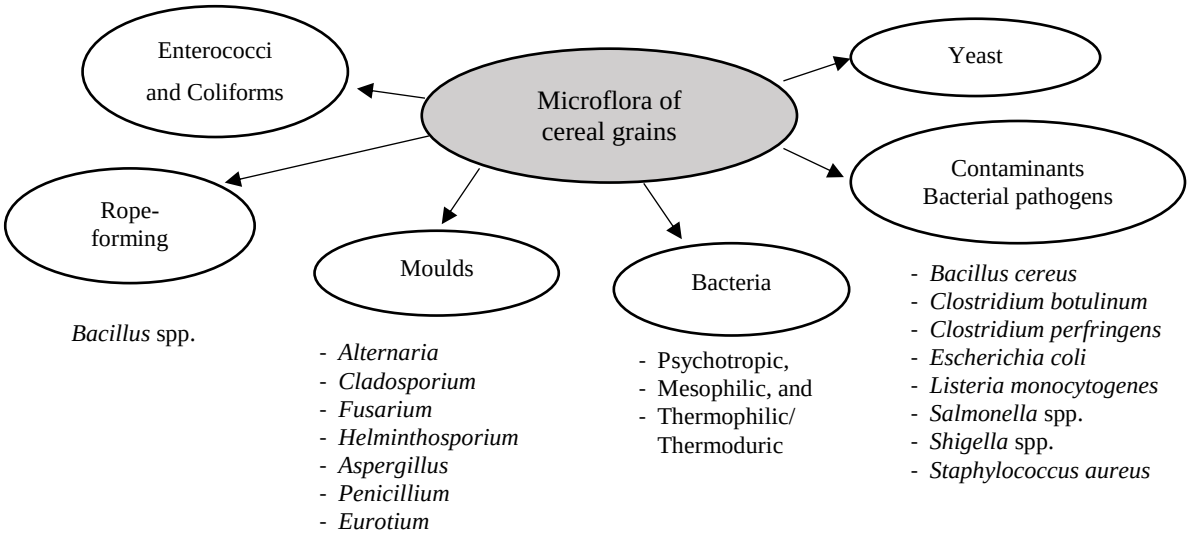

Figure 10. Microflora and potential pathogens associated with cereal grains

Summary of the microbiological hazards identified in selected cereal grains are shown in Table 19.

Table 19. Summary of microbiological hazard identification in selected cereal grains

Ingredient name	Microbiological hazards	References
Cereals		
Barley	*Bacillus cereus*.	(Daczkowska-Kozon, Bednarczyk, Biba, & Repich, 2009; Forsythe, 2002; Ok, Kim, Cho, Oh, & Chun, 2009)
Corn (Maize)	Moulds, Yeasts, *Escherichia coli*, Coliform.	(Sperber, 2007)
Millet	*Bacillus cereus*.	(Kimanya et al., 2003)
Oats	*Bacillus cereus*.	(Rosenkvist & Hansen, 1995)
	Salmonella spp.	(Sperber, 2007)
Rye	*Bacillus cereus*.	(Eglezos, 2010; Rosenkvist & Hansen, 1995)
Black glutinous rice	*Bacillus cereus*, *Cronobacter* spp. *(Enterobacter sakazakii)*.	(Forsythe, 2002; L. Lin & Beuchat, 2007)
Brown rice	*Bacillus cereus*, *Cronobacter* spp. *(formerly Enterobacter sakazakii)*.	(Forsythe, 2002; L. Lin & Beuchat, 2007)

Ingredient name	Microbiological hazards	References
Wheat	*Bacillus cereus*, Yeast, Mould. *Salmonella* Thypimurium, *Salmonella* Agona, *Salmonella* Mbandaka, *Escherichia coli* O157:H7, *Escherichia coli* O121, *Escherichia coli* O26, Coliform.	(CDC, 2016; Eglezos, 2010; FDA, 2017; NZFSA, 2010b)
Pseudocereal		
Buckwheat	Yeast, Mould, Coliforms, *Bacillus cereus*, *Staphylococcus aureus*.	(Losio et al., 2017)
Grain legumes		
Adzuki beans (red mung bean)	*Staphylococcus* spp., *Escherichia coli*, *Salmonella spp.*	(Neumayr & Krämer, 1990) (Yang et al., 2013)
Garden pea	Nonpathogenic *Escherichia coli*, *Salmonella* Typhimurium.	(Saroj et al., 2006)
Hyacinth beans	*Escherichia coli, Salmonella* spp.	(Yang et al., 2013)
Mung beans	*Salmonella* spp., *Salmonella enterica*, *Salmonella enteritidis*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, Nonpathogenic *Escherichia coli*, *Salmonella* Typhimurium.	(Ding & Fu, 2016; Saroj et al., 2006; Trząskowska, Dai, Delaquis, & Wang, 2018; Yang et al., 2013)
Soybeans	*Staphylococcus* spp., *Salmonella* spp., *Escherichia coli*.	(Adepehin, 2018; Yang et al., 2013)
Black Soybeans	*Staphylococcus* spp., *Salmonella* spp., *Escherichia coli*.	(Adepehin, 2018; Yang et al., 2013)

4.1.2. Hazard characterisation

Hazard characterisation of each micro-organism identified in the hazard identification step is described in Appendix B. A summary of the characteristics of the microbial hazards identified is presented in Table 20. This summary was aimed at understanding how the pathogens will behave in food processing environments and recommend practical risk mitigation efforts for their control.

Table 20. Characteristics of identified microbiological hazards in cereal grains

Microbiological hazards	Relevance to cereal grains	Survive heat treatment	Physiological features linked to heat resistance	Minimum a_w to grow and toxin formation	pH range to grow	Dose-response to cause illness	The severity of illness#	References
Bacillus cereus	Spores can survive in dry environments	Yes	Spores: $D_{95°C}$ 1.2–36 min; z-value 7.9–9.9°C	0.92-0.93 for growth and emetic toxin formation	4.5-9.5, optimum 6-7	Emetic: 10^5-10^8/g Diarrhoeal: 10^5-10^7/g	Moderate	(FSAI, 2016) (Schraft & Griffiths, 2006)
Clostridium botulinum	Spores can survive in dry environments	Yes*	Psychotropic spores: $D_{100°C}$, 0.1 min; z-value 7–10°C	0.97 for psychotropic and 0.94 for mesophilic	Spores can survive at pH<4.6; toxins are stable at low pH	Toxin A & B: 0.1-1.0 µg; Toxin E & F: 10 µg	Severe	(Silva & Gibbs, 2010) (MPI, 2017c)
Clostridium perfringens	Spores can survive in dry environments	Yes	Spores: $D_{95°C}$ 17.6–63 min	0.93 for growth	5-8.3, optimum 6-7	10^6/g	Severe	(Labbé & Juneja, 2013)
Cronobacter spp. (*Enterobacter sakazakii)*	Survive in a_w 0.25-0.30	No	$D_{60°C}$ 3.52 – 3.58 min	Maximum salt concentration permitting growth 9.1%	Minimum 3.89, optimum 5-9	1000 cells	Severe^	(FSAI, 2011a) (NZFSA, 2010a)
Escherichia coli O157: H7	Ability to survive in dry foods	No	$D_{63°C}$ 0.5 min; z-value 6°C	0.95 for growth	4.4- 9.0, optimum 6-7	0.3-0.4 cells/g	Severe	(NZFSA, 2001a)
Listeria monocytogenes	Ability to survive in dry foods	No	$D_{60°C}$ 1.6–16.7 min in food substrates; 70°C for 2 min	0.90-0.93 for growth	4.4-9.4, optimum 7.0	Invasive: 100-1000 cells Non-invasive:>10^5 cells/g	Severe^	(NZFSA, 2001b)

Microbiological hazards	Relevance to cereal grains	Survive heat treatment	Physiological features linked to heat resistance	Minimum a_w to grow and toxin formation	pH range to grow	Dose-response to cause illness	The severity of illness#	References
Salmonella spp.	Survives for weeks, months, or years in low-a_w foods (up to a_w 0.30)	No	$D_{60°C}$ 0.1–10 min; z-value 4–5°C; heat resistance is greatly increased in low-a_w moreover, high-fat foods	0.94 for growth	3.8 – 9.5 optimum 7-7.5	Low-attack: 4-45 cells; High attack: 10^5-10^6/g	Serious	(NZFSA, 2001c) (Blackburn & McClure, 2009)
Shigella spp.	Survive better in low moisture foods.	No	-	0.96	Minimum 4.8-5.0 in 3.8-5.2% NaCl. Maximum 9.3 in the presence of 5.2% NaCl.	10-100 cells	Serious	(NZFSA, 2001d)
Staphylococcus aureus	Can survive for months in dry foods	No**	$D_{60°C}$ 1–2.5 min in phosphate buffer; z-value 8–10°C	0.83-0.85 for growth, 0.87 for toxin formation	4.2-9.3, Optimum 7.0-7.5.	1.0 µg of toxin, but toxin is produced when population >10^5/g	Moderate	(NZFSA, 2001c) (ICMSF, 2018)

#classification based on (ICMSF, 2018) ; *neurotoxin is heat labile; **enterotoxin is heat stable; ^for vulnerable population

4.1.3. Exposure assessment

4.1.3.1. Exposure model

Cereal grain contamination may originate from different sources such as soil, water, air, dust, insects, fertiliser and animal faeces (Laca et al., 2006). Contamination can occur at pre-harvest, harvest and post-harvest process. Pre-harvest contamination usually occurs during crop growth, while transport and storage are crucial contamination points for post-harvest (F. Li, Li, Luo, & Yoshizawa, 2002). Figure 11 shows the potential sources and aspects of microbiological contamination throughout the cereal grains manufacturing chain (M. Brown, 2002a; Los, Ziuzina, & Bourke, 2018).

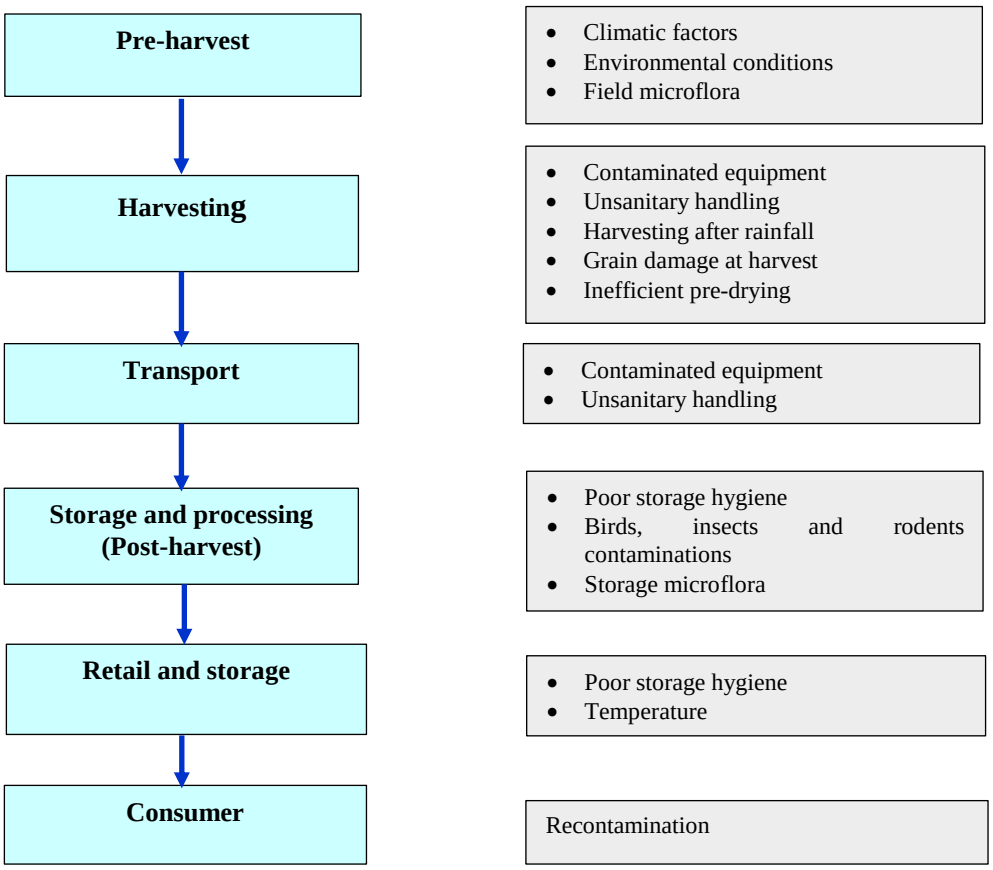

Figure 11. Cereal grains supply chain and potential sources of microbiological contamination

4.1.3.1.1. Pre-harvest

Significant contamination sources of enteric pathogens such as *Salmonella* and *E. coli* come from the faecal matter of humans and animals. Two possible routes of contamination in cereal crops are direct exposure to pathogens contained in animal faeces and direct exposure to soil or dust that has been previously exposed to the animal faeces. Fortunately, cereal crops are covered with an outer casing that may shield the grain from contact with the animal faecal matter until harvest (Gilbert et al., 2010).

4.1.3.1.2. Harvesting

During harvesting, potential sources of contamination may come from inefficient pre-drying, contaminated equipment, unsanitary handling and harvesting after rainfall (Los, Ziuzina, & Bourke, 2018). Since harvested cereal grains usually contain high moisture, drying is needed to reach a moisture content between 10% and 14% (Alldrick, 2010; Los, Ziuzina, & Bourke, 2018) equivalent to a_w <0.70. These moisture contents generate a hostile environment for mould growth. If the drying is insufficient, micro-organism growth will occur (Miskelly, Batey, & Suter, 2010).

4.1.3.1.3. Post-harvest

4.1.3.1.3.1. Transport and storage

The risk of contamination may also occur during transport and storage due: poor cleaning of the container/vehicle; inadequate rodent control that allows the birds and vermin to enter the storage room and contaminate the product; and mishandling of the grain by the workers (Gilbert et al., 2010; Miskelly et al., 2010).

4.1.3.1.3.2. Milling

Milling includes exclusion of debris and outer material, conditioning to regulate the moisture levels; exclusion of bran and/germ; and grinding into flour, grit or meal (ICMSF, 1996b). Some end products of milling which can be used in the food industry include buckwheat as wholegrain cereals, millet as hulled cereals, barley and wheat as grits, wheat as flour and germ, oats as flakes, corn and semolina as meals (Daczkowska-Kozon et al., 2009).

Milling and the environment influence the microbiological quality of cereal grains (Berghofer et al., 2003). Milling may reduce the microbiological contamination of cereal grains. Microbial contaminants are concentrated in the outer layer of grains. During the milling process from grain to flour, the outer layer of grain which may contain contaminants is detached. The inner endosperm contains fewer microorganisms. The inner endosperm then is crushed into refined flour that is relatively uncontaminated. Microbial contamination within a cereal grain is given in Figure 12.

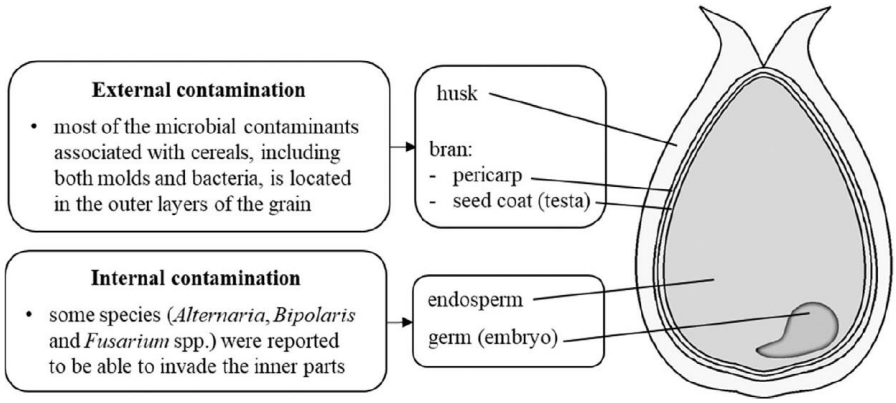

Figure 12. Microbial contamination within a cereal grain.
From "Current and future technologies for microbiological decontamination of cereal grains," (Los, Ziuzina, & Bourke, 2018, p. 1488). ©2018 by Institute of Technologist. Reprinted with permission.

Milling may also be responsible for adding to the microbiological load of the flour. Conditioning grains may increase the bacterial, yeast and mould counts (Hocking, 2003). Accumulation of residue attached to the equipment in milling plant may contribute to microbial contamination. Spore-forming bacteria such as *Bacillus* may reside in milling equipment, which can increase the microbial level in particular midstream products (Berghofer et al., 2003).

Salmonella is not commonly isolated from flour, while, *B. cereus* is more common (Berghofer et al., 2003). A survey on the microbiological status of Australian wheat and the distribution of microorganisms in flour milling fractions and end products was conducted in 1997-1999. The study found that *B. cereus* was one of the most frequently detected microorganisms throughout the survey. *Salmonella* was not detected in the incoming wheat or end product. The ability of *B. cereus* to form spores that survive in harsh environments could be an explanation.

4.1.3.1.3.3. Storage

The moisture content grains in storage is important from the food safety point of view. Usually, grains are stored at a moisture content of 12-14% (Zwer, 2017) or water activity lower than 0.60. For example, flour and maize meal have a critical moisture content of 12% or less. This is because the moisture content does not favour microbial growth including spoilage fungi.

Storage facilities need to avoid the possibility of water exposure of the grains to moisture. Some possible routes of water exposure include high humidity, condensates from equipment and improper cleaning procedures (Gilbert et al., 2010). Condensation on equipment may be caused by the heat that is generated during grinding and sifting.

4.1.3.1.3.4. General controls

Some general controls have been recommended to reduce the microbiological contamination of cereal grains (Gilbert et al., 2010), as follows:

- Chlorinated water used to condition the grains;
- Regular cleaning of equipment in the milling plant. Implement dry cleaning for the dry product section;
- Rodent, insect, and bird control programme;
- Keep the manufacturing plant dry and minimise the possibility of water condensate falling into the products.

The essential control measures for low moisture foods such as cereal grains include preventing contamination from occurring during harvest, post-harvest and processing by sound implementation of good agricultural practices (GAPs), good hygienic practices (GHPs), good manufacturing practices (GMPs), and hazard analysis and critical control point (HACCP) programs (Beuchat et al., 2013; FAO/WHO, 2014; Finn, Condell, McClure, Amézquita, & Fanning, 2013; Podolak, Enache, Stone, Black, & Elliott, 2010).

4.1.3.2. The occurrence of pathogens identified in cereal grain products

Databases from three different countries (US, New Zealand and Taiwan) were selected to get an overview of databases from both developed and developing countries. Foodborne outbreak data was collected from the National Outbreak Reporting System (NORS) for the US, and annual reports for outbreaks in New Zealand and Taiwan. The NORS is an

online platform developed by the Centres for Disease Control and Prevention (CDC) for local, state, and regional health departments in the US to report foodborne disease outbreaks (CDC, 2018c). In NORS, search criteria for foods include cereal, cereal products, grains, beans, and legume. The annual summary of outbreaks in New Zealand classifies the foodborne outbreaks by causal agent and implicated vehicle/source. Implicated vehicle/sources used include rice and grains/beans. However, foodborne outbreaks by causal agent and implicated vehicle/source data were not explained in the annual outbreak summary before 2007 and after 2015. Due to the limited information available on foodborne disease outbreaks by causal agent and implicated vehicle/source data for Central Taiwan, this report shows only outbreaks from 1991 to 2000. The occurrence of pathogens identified in cereal and grain products is presented in Table 21.

Table 21. The occurrence of pathogens identified in cereal grain products

Pathogens	Number of outbreaks			
	USA[1]	Taiwan[2]	New Zealand[3]	Total
Bacillus cereus	41	27	1	69
Clostridium botulinum	NS	NS	0	0
Clostridium perfringens	87	NS	12	99
Cronobacter spp.	0	NS	0	0
Escherichia coli enteropathogenic	NS	1	0	1
Escherichia coli O157:H7 (STEC)	4	NS	0	4
Listeria monocytogenes	0	NS	0	0
Salmonella spp.	26	0	4	30
Shigella spp.	1	NS	0	1
Staphylococcus aureus	14	25	1	40
Norovirus	NS	NS	18	18

[1] The foodborne outbreaks in USA 1998-2015 (CDC, 2018b) associated with cereal, cereal: oat, cereal: puffed wheat, cereal: puffed rice, cereal: unspecified, dry cereal, grains, grains: other, unspecified grains, beans, and legume.
[2] Central Taiwan 1991-2000 on cereal products (Chang & Chen, 2003)
[3] New Zealand 2007-2015 on grains/beans and rice category (MOE, 2015)
NS: Not stated

Viruses have also been reported to be associated with cereal grains. As seen in Table 21, the number of Norovirus outbreaks related to grains/beans and rice in New Zealand from 2007 to 2015 is the highest among all pathogens. Majority of the outbreaks happened in long-term care facilities, commercial food operators and childcare facilities (MPI, 2017a). Poor hygiene practices by food harvesters, processors and food handlers in the food facility are potential causes of Norovirus outbreaks and are therefore not caused by the cereal grains themselves. Thus, Norovirus was not classified as a concern for this risk assessment.

Different pathogens have been reported to be associated with cereals from different locations. From Table 21, it can be seen that the most common bacterial pathogen among cereal grains in New Zealand is *C. perfringens* (33.3%), followed by *Salmonella* (11.1%). *B. cereus* has also been implicated in many cereal grains related foodborne outbreaks in other parts of the world such as America and Taiwan (CDC, 2018b; Chang & Chen, 2003) and a microbiological problem in the dairy industry (Andersson, Ronner, & Granum, 1995; Montanhini, Montanhini, Pinto, & Bersot, 2013; Vasavada, Martin, Bienvenue, & Heidenreich, 2018).

The United States, central Taiwan and New Zealand show different pathogens that are related to cereal grains. Table 21 shows the number of cases in the United States, central Taiwan and New Zealand where cereal grains associated microorganisms have caused foodborne outbreaks. In the United States, the term cereal includes oat, puffed wheat, puffed rice, unspecified cereal, dry cereal, grains, other grains, unspecified grains, beans, and legume (CDC, 2018b) In New Zealand, the term cereal includes grains, beans and rice (MOE, 2015). In central Taiwan, the term includes instant cereal products and the cereal mix (Chang & Chen, 2003; Fang, Chu, & Shih, 1997). The cereal grains terms in three countries are dissimilar to a certain extent; therefore, a direct comparison of data across countries should be carried out with some caution. There is a natural bias to data collection, which is often based on funding, outbreaks, ability to culture and is not necessarily reflective of the prevalence that these pathogens might be present.

Epidemiological data for pathogens in cereal grains is needed in an exposure assessment. However, a microbiological survey of selected cereal grains is not available for New Zealand. Hence, the prevalence of pathogenic micro-organisms in selected cereal grains based on an international microbiological survey is presented in Table 22. *Salmonella* and *B. cereus* are frequently found in cereal grain products. Interestingly, there is a lack of studies on prevalence data of *C. botulinum*, *C. perfringens*, *L. monocytogenes* and *Shigella* in cereal grains.

Table 22. Prevalence (%) of pathogens in cereal grains from the global microbiological survey

Country	Year	Samples tested	Number of positive samples (%)	Route of contamination	Number of bacteria	References
Australia	1997-1998 1998-1999 wheat seasons	Wheat milling process and end products obtained from 9 flour mills, with a total of 650 samples	*B. cereus* 81% of incoming wheat 93% of wheat flour 94% of wheat bran *Salmonella* 2/412 (<0.5%) in milled samples	- Field - Milling equipment	<1 spore/g NS	(Berghofer et al., 2003)
Italy	2010-2015	1250 samples Buckwheat flour Maize flour Dry pasta: • Maize • Wheat • Maize + Rice • Rice • Buckwheat • Maize + Rice + Quinoa • Legumes	Presumptive *B. cereus* 12.5% 4.3% 0.6% 2.3% 2.4% 5.8% 4.3% 0 0	- Grain milling equipment - Flour packaging processes	Up to 4 log CFU/g	(Losio et al., 2017)
Denmark	1993-1994	116 samples of 18 raw materials for bread 350 samples of wheat grains Wheat grains rolled, bran, wholemeal, flour Rye grains, rolled, bran, wholemeal	*B. cereus* 2%	- Raw Material - Harvest	Wheat: 1.8-12.4 CFU/g Rye: 2.2-2.9 CFU/g Oat:s 9.6-29.8 CFU/g	(Rosenkvist & Hansen, 1995)
USA	1989	Wheat flour type comprises of: 1,355 soft red winter; 681 hard	*Salmonella* 40/3040 (1.32%) *E. coli* 12.8%	Field Milling	NS	(Richter, Dorneanu, Eskridge, & Rao, 1993)

Country	Year	Samples tested	Number of positive samples (%)	Route of contamination	Number of bacteria	References
		red winter; 188 spring; 816 durum				
USA	2003-2005	Milled cereal grains comprise of: 4358 wheat; 1772 corn; 714 oat; 286 whole wheat; 180 durum	*Salmonella*-positive results were only for wheat samples: 6/4358 (0.14%)	Grain handling and milling	NS	(Sperber, 2007)
Spain	2008	Raw popcorn	*Salmonella* 8-13%	NS	NS	(Anaya, Aguirrezabal, Ventura, Comellas, & Agut, 2008)
China	2019	Brown rice White rice	*Cronobacter* 42/86 (48.8%) *Cronobacter* 7/32 (21.9%)	Tillering, jointing and filling stage	NS	(Lou et al., 2019)
Northern Italy	2017	Maize flour Buckwheat flour	*Presumptive B. cereus 1/23(4.3%)* *Coagulase Positive Staphylococci 2/8(21.7%)* *Presumptive B. cereus 3/23 (12.5%)*	NS	NS	(Losio et al., 2017)
Turkey	2009	Wheat flour	*C. perfringens 14/142 (9.8%)* *B. cereus 6/142 (4.2%)* *E. coli 72/142 (50.7 %)*		$>10^2$ CFU/g $> 10^1$ CFU/g $10^2 – 10^6$ CFU/g	(Aydin, Paulsen, & Smulders, 2009)
France	2003	Thickening agent including starch	*C. botulinum 4/25 (16%)*		3-7 MPN/kg	(Carlin, Broussolle, Perelle, Litman, & Fach, 2004)

NS: Not stated

4.1.3.3. Consumption data

Cereals are essential in the human diet in many cultures, including New Zealand (Olsson et al., 2000). The most recent available data from the Food and Agriculture Organisation of the United Nations (FAO) food balance sheets for New Zealand is 2013. A summary of food balance sheets for cereal and pulses is shown in Table 23 (FAOSTAT, 2013). Wheat and products are the most frequently consumed cereal (78.5%) followed by other cereals such as oats (3.4%) and barley (0.4%) in New Zealand. Pulses (3.66 kg/capita/year) are well below the total cereal consumption (98.02 kg/capita/year).

Table 23. New Zealand food balance sheets per capita supply in 2013

Item	Food balance sheets Kg/capita/year (%)
Total cereal consumption	**98.02 (100%)**
Wheat and products	76.91 (78.5%)
Rice (Milled Equivalent)	9.16 (9.3%)
Maize And Products	4.43 (4.5%)
Oats	3.29 (3.4%)
Cereals, other	3.82 (3.9%)
Barley and products	0.4 (0.4%)
Rye and products	0 (0%)
Pulses	**3.66 (100%)**
Beans	1.64 (44.8%)
Pulses, other and products	1.25 (34.2%)
Peas	0.77 (21.0%)

Adapted from (FAOSTAT, 2013).

The available data for cereal consumption is from the 1997 National Nutrition Survey (1997 NNS) for New Zealand's adult (Table 24). This is similar to the data obtained from the FAO food balance sheets showing wheat flour consumption in New Zealand is very high compared with other cereals.

Table 24. Consumption of cereal grains in New Zealand

Cereal	Per cent consuming in 24- hours period (%)	Average daily consumption, all (g/day)	Average consumption, consumers only (g/day)	97.5th percentile consumption, consumers only (g/day)
Cereal grain fractions	98.3	127.3	129.5	370.1
Wheat flour	98.0	106.6	108.7	347.3
Rice, polished	20.4	10.2	50.0	213.8
Maize flour	23.0	3.2	14.1	68.2
Cereal brans, processed	13.6	0.9	6.7	49.9
Rye, wholemeal	23.5	2.3	9.9	27.1
Oats	22.5	5.9	26.1	99.3
Millet	2.1	0.1	6.0	27.9

'All' means the overall set of respondents, comprising people who did not report consuming cereals in the previous 24-hours. 'Consumers' means only to those who reported consumption of cereals in the previous 24-hours.

From "Risk Profile: *Salmonella* in cereal grains," (Gilbert et al., 2010, p. 15).© 2010 by Institute of Environmental Science & Research Limited. In the public domain.

4.1.3.4. Exposure evaluation

For exposure evaluation, the same approach described by Gilbert et al. (2010) was used. New Zealand data from the 1997 NNS, 2002 Children's National Nutrition Survey (2002 CNNS) and the 2008/09 Adult Nutrition Survey (2008/09 ANS) were analysed. The number of participants age 15 + years old was 4,636 from the 1997 NNS and age 5 to 14 years old was 3,275 children from the 2002 CNNS. The cereal grain consumption from the 1997 NNS was used because the information in 2008/09 is not available. Cereals may be added into a food serving as a major or minor ingredient, where major ingredient means the amount was more than 20% by weight. One or more cereals are a major ingredient in 17,528 servings from the 1997 NNS and in 14,490 servings from 2002 CNNS. With the current New Zealand population of 4,965,538 (StatsNZ, 2019) the proportions based on the latest 2013 census i.e. adults (15+ years; 79.6%) and children (<15 years; 20.4%) were used in the calculation of total number of servings. The diet of children less than 5 years old was assumed to be similar to children aged 5 to 14 years.

Annual number of servings (total population) = 4,965,538 x ((0.204 x 14,490/3,275) + (0.796 x 17,529/4636)) x 365

= 4,965,538 x (0.903+3.009) x 365

= 7.1×10^9 servings

The result shows a very high number of servings and this was predicted because cereal grains serve as a staple diet. The number of servings depicts the total number of cereal servings. Cereal grains which are consumed directly and their main processed products such as flour were assumed to have little contribution to these servings. However, this data did not allow food identification and practices such as eating raw cake batter. In 2008-2009, eating raw cake batter practice was associated with foodborne illness outbreaks in New Zealand.

4.1.3.5. Exposure summary

Bacterial pathogen contamination may occur throughout the cereal grain manufacturing chain. High consumption of cereals reflects the staple diet New Zealand. Fortunately, cereal grains are consumed mostly after cooking or heat treatment, which inactivates the pathogens. The probability of bacterial pathogen contamination in raw cereal grains New Zealand is unknown.

4.1.4. Risk characterisation

4.1.4.1. The most critical microbial risk

Risk characterisation exemplifies the integration of the hazard identification, hazard characterisation and exposure assessment to provide a risk estimate. In order to identify the most critical pathogen in cereal grains, this risk assessment used the qualitative measure of consequence (Table 11) from the hazard characterisation and qualitative measures of likelihood (Table 12) from the exposure assessment in the form of a score. The score obtained from the consequence and likelihood were multiplied to give the overall risk score (Appendix C. Table C1. Risk characterisation calculation).

The risk score was then extrapolated to a semi-quantitative risk assessment matrix (Table 25) to be more understandable. *B. cereus* scored the highest and is regarded as high representing the pathogen of most critical risk in cereal grains. Other pathogens that also high risk are *C. perfringens*, *Cronobacter* spp. *E.coli* (STEC) and *Salmonella*. Pathogens representing a medium risk are *S. aureus*, *C. botulinum*, *L. monocytogenes*, and *Shigella* spp.

Table 25. Semi-quantitative risk assessment matrix result

Likelihood	Consequences				
	Insignificant (1)	Minor (2)	Moderate (3)	Major (4)	Severe (5)
Almost certain (5)	Medium	High *C. perfringens*	High *B. cereus*	Very high	Very high
Likely (4)	Medium	Medium	High	High	Very high
Possible (3)	Low	Medium	Medium *S. aureus*	High	High
Unlikely (2)	Low	Medium	Medium	Medium *C. botulinum*	High *Cronobacter* spp. *E. coli (STEC)* *Salmonella* spp.
Rare (1)	Low	Low	Low	Medium *Shigella* spp.	Medium *L. monocytogenes*

4.1.4.2. Uncertainties, variabilities, and assumptions

There were several assumptions made at the outset this study: (1) Cereal grains are always subjected to control strategies such as heat treatment to reduce microbiological contamination before they are used; (2) Cereal grains are of good quality and harvested according to Good Agricultural Practice (GAP); (3) Cereal grains in New Zealand are manufactured under the New Zealand Crop Quality Assurance Scheme (NZCQAS) issued by The Arable Food Industry Council (AFIC).

Even though New Zealand produces most of its cereals (about 70 %)(Zydenbos, 2008), the outcome of this risk assessment might not have any significant differences between imported cereals and the ones which are grown here. This is because imported cereal grains pass through the same control processes which are applied to the locally grown cereals. In most cases, the imported cereals even go through more stringent control measures by border control agencies since they are coming from different countries. Further research to compare risk assessment between imported and locally grown cereals will be worthy of investigating.

In conducting the hazard identification, assumptions were made regarding the state/form of cereal grains as well as utilisation of the available information. Cereal grains were in the form of whole grain and milled products including buckwheat as whole grain cereals; millet as hulled cereals; barley as whole grain, pearled grain, grits, or flour; wheat as grits or flour; oats as flakes, rolled or flour; corn (maize) as flour, grits, meal; rice as flour; soybeans as flour (Baik, 2016; Daczkowska-Kozon et al., 2009; Izydorczyk & Edney, 2017). In the absence of literature on particular cereal grains, the available information on the related types of cereal grains was used. For example, black soybeans used soybean data; brown rice and black glutinous rice used white rice data.

Variability and uncertainty within the cereal grain supply chain were identified. For instance, epidemiological data for pathogens in cereal grains was mostly related to cereal grains in their post-harvest stage (Berghofer et al., 2003; Losio et al., 2017). However, this is only available for most common cereal grains such as wheat and oats. Factors recognised as having influence on the growth or survival of bacterial pathogens include different in farming practices, different seasons (winter or spring) and variation in control measures to reduce microbial contamination of cereal grains (Beuchat et al., 2013; FAO/WHO, 2014; Finn et al., 2013; Podolak et al., 2010; Richter et al., 1993).

Epidemiological data for pathogens in cereal grains for New Zealand is minimal. Therefore, the present study utilised global data from countries such as Taiwan and the US, which may not represent New Zealand. Consumption data and serving estimations used the New Zealand data, although it is not up to date. The cereal consumption data was obtained from the 1997 National Nutrition Survey (1997 NNS) for New Zealand's adult population.

The risk matrix may result in different pathogens other than *B. cereus* as a priority if there is new data available for criteria used to determine the likelihood. Other pathogens that may be a concern include *C. perfringens*, *Cronobacter* spp. *Salmonella* spp. and *E. coli (STEC)*.

4.2. Microbiological risk assessment of selected cereal addition to dairy products

4.2.1. Exposure assessment

Based on the risk assessment matrix, *B. cereus* is the highest microbial risk in cereal grains. Therefore, the scenario used in this exposure assessment is cereal grains contaminated with *B. cereus* addition to dairy products (milk powder, Parmesan cheese and liquid breakfast product). *B. cereus* is a spore-forming bacterium that generally contaminates raw milk and other dairy products such as infant formula and milk powder (Shaheen et al., 2006). *B. cereus* is also capable of attaching to dairy processing equipment (Shaheen, Svensson, Andersson, Christiansson, & Salkinoja-Salonen, 2010). The ability of *B. cereus* to attach and form biofilms on processing equipment means it can also contaminate cereal grains added to the processing lines since the characteristics of some cereal grains favour the growth of micro-organisms.

4.2.1.1. Exposure model and approach of the addition of cereals to dairy products

The present study focusses on cereal grains as raw materials which will be received in the food/dairy company. Usually, the cereal grains are treated (e.g. cleaning, heat treatment) before use. Thus, it has been assumed that any contamination has been minimised. The potential routes of contamination for microbiological hazards identified after the reception are storage, processing equipment, water, packaging, storage and distribution and people. The exposure model is shown in Figure 13 (M. Brown, 2002a).

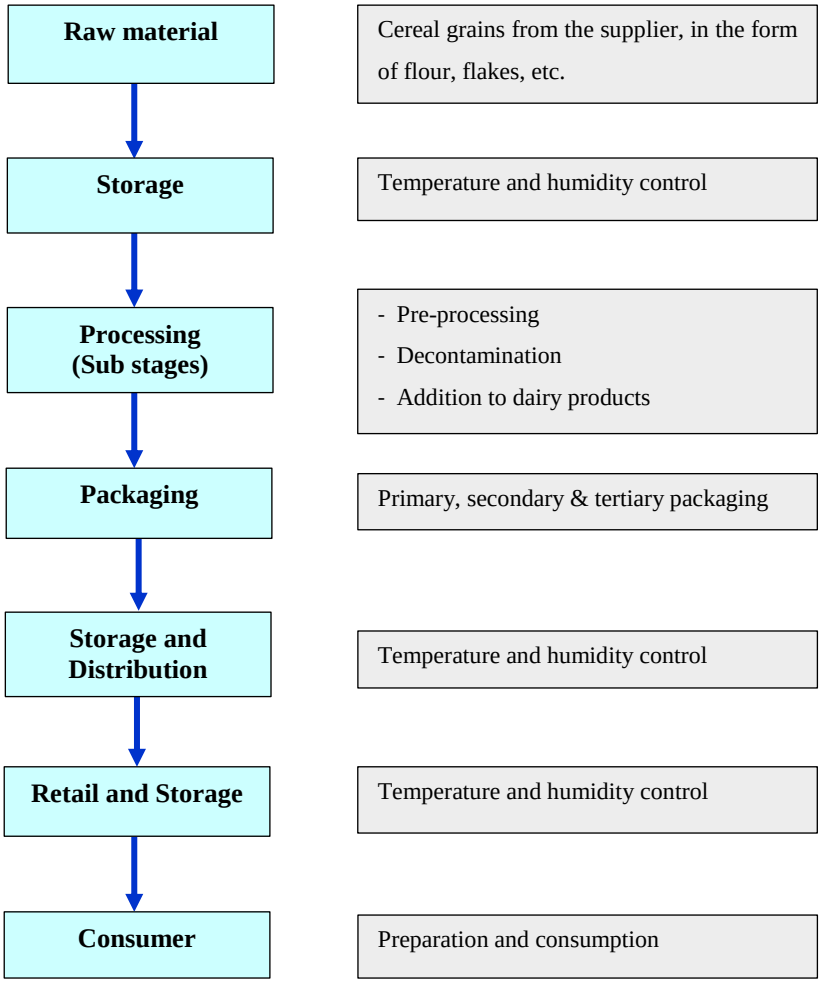

Figure 13. The exposure assessment model

4.2.1.2. The occurrence of B. cereus in dairy products

Outbreaks linked to dairy products are relatively rare (Cressey, King, & Soboleva, 2016). International foodborne illness outbreaks of *B. cereus* associated with dairy products are presented in Table 26. These outbreaks happened more than three decades ago, suggesting improvements in control measures in the dairy industry.

Table 26. Foodborne illness outbreaks of *B. cereus* associated with dairy products

Country	Year	Dairy food	People affected	Number of bacteria
Netherlands	1988	Milk, pasteurised	42 elderly people	4 x 10^5 *B. cereus*/mL in milk
Canada	1989	Milk	74 people	1.8-8 x 10^6 CFU/g in milk
Japan	1991	Ultra-high temperature milk (process failure)	201 people	NS

NS: not stated

Adapted from "Risk Profile: *Bacillus cereus* in Dairy Products," (Cressey et al., 2016, p. 93). ©2016 by Crown Copyright - Ministry for Primary Industries. In the public domain.

4.2.1.3. Consumption data

In New Zealand, domestic supply of milk products expressed as milk equivalents, excluding butter was 195 kg/capita/year (FAOSTAT, 2013). Table 27 presents the consumption of milk and cheese from 1997 NNS, 2002 CNNS and 2008/09 ANS. Milk is consumed more than cheese by New Zealand and adult consumption is higher than children. The consumption data provide information about the average level of food intake, identify the high consumption and consumption pattern of different age groups such as adult and children. Further this information can be used to estimate the risk for different age groups as well as average and high consumer.

Table 27. New Zealand consumption data on milk and cheese

Statistic	Adults (15+ Years)		Children (5-14 Years)
	2008/2009 ANS	1997 NNS	2002 CNS
Number of respondents	4721	4636	3275
MILK			
Number of servings	11342	15199	4114
Number of consumers (percentage of total respondents)	3755 (79.5%)	4067 (87.7%)	2375(72.5%)
Servings/consumer/day	3.0	3.7	1.7
Consumer mean (g/person/day)	241	272	271
Population mean (g/person/day)	192	239	197
Mean serving size (g)	79.9	72.9	157
Median serving size (g)	53.0	41.6	129
95th percentile serving size (g)	265	258	335
CHEESE (low moisture)			
Number of servings	2559	2976	1632
Number of consumers (percentage of total respondents)	1928 (40.8%)	2111 (45.5%)	1178 (36.0%)
Servings/consumer/day	1.3	1.4	1.4
Consumer mean (g/person/day)	36.7	32.3	32.5
Population mean (g/person/day)	15.0	14.7	11.7
Mean serving size (g)	27.6	22.9	23.4
Median serving size (g)	21.0	16.9	18.0
95th percentile serving size (g)	70.8	60.0	60.0

Adapted from "Risk Profile: *Bacillus cereus* in Dairy Products," (Cressey et al., 2016, p. 24). ©2016 by Crown Copyright - Ministry for Primary Industries. In the public domain.

4.2.1.4. Exposure summary

Limited studies in the literature provide the prevalence of *B. cereus* in the selected cereal grains. Table 28 shows a summary of the prevalence of *B. cereus* in cereal grains. Data are only available for maize, oats, rye, brown rice, wheat and buckwheat.

Table 28. The prevalence summary of *B. cereus* in cereal grains

Ingredient	Prevalence	Concentration	References
Barley	High (21%)	NS	(Park et al., 2009)
Corn (Maize)	Low (4.3%)	<4 log CFU/g	(Losio et al., 2017)
Millet	NA		
Oats	Low (2%)	9.6-29.8 CFU/g	(Rosenkvist & Hansen, 1995)
Rye	Low (2%)	2.2-2.9 CFU/g	(Rosenkvist & Hansen, 1995)
Black glutinous rice	High (37%)	NS	(Park et al., 2009)
Brown rice	High (37%)	NS	(Park et al., 2009)
Wheat	Low to Extremely high (2%-94%)	<1 spore/g to 12.4 CFU/g	(Berghofer et al., 2003)
Buckwheat	Medium (12.5%)	<4 log CFU/g	(Losio et al., 2017)
Adzuki beans	NA		
Garden pea	NA		
Hyacinth beans	NA		
Mung beans	NA		
Soybeans	NA		
Black Soybeans	NA		

NA: Not available; NS: Not stated.

The output of the exposure assessment of cereal grains contaminated by *B. cereus* to three different dairy products is presented in Table 29.

Table 29. The output of exposure assessment of *B. cereus* contamination in cereal grains addition to milk products*

	Exposure assessment	Remarks	References
The occurrence in raw material			
The frequency of contamination:	Low (2%)	Prevalence of *Bacillus* spores in raw material	(Rosenkvist & Hansen, 1995)
The level of contamination:	9.6-29.8 CFU/g	*Bacillus* spores surviving heat treatment at 100°C in oats (grains, rolled, wholemeal) was 9.6-29.8 CFU/g	(Rosenkvist & Hansen, 1995)
The effect of storage before processing:	Survive	- The high resistance of the spores to desiccation allows *B. cereus* to survive in most dried food products - Number of spores remains the same after 48 weeks with a water activity of 0.27-0.28	(Nicholson, Munakata, Horneck, Melosh, & Setlow, 2000) (Jaquette & Beuchat, 1998)

Processing addition to dairy products	**Exposure assessment**			**Remarks**	**References**
	a_w<0.60 Milk powder	**a_w 0.60-0.85 Parmesan cheese**	**a_w>0.85 Liquid breakfast product**		
Effect of pre-processing/decontamination of raw material					
The intended effect of all processing/ decontamination (pasteurisation 72 – 73 °C for 15 – 20 seconds, UHT 135 - 150 °C- 140°C for 1-2 seconds) on the level of *B. cereus*:	Complete inactivation (Pasteurisation)	Complete inactivation (Pasteurisation)	Complete inactivation (Pasteurisation or UHT)	Spores: $D_{95°C}$ 1.2–36 min; z-value 7.9–9.9°C	(FSAI, 2016) (Schraft & Griffiths, 2006)

Processing addition to dairy products	Exposure assessment			Remarks	References
	a_w<0.60 Milk powder	**a_w 0.60-0.85 Parmesan cheese**	**a_w>0.85 Liquid breakfast product**		
The occurrence of toxin?					
The likelihood of toxin presence if the microorganism can produce toxin and is present in the raw materials, product or process environment:	Rare (0-0.1%)	Rare (0-0.1%)	Rare (0-0.1%)	A large number of viable cells (10^5 to 10^8/g) is required to produce a toxin	(MPI, 2015)
The likelihood of contamination/ growth:	Low	Low	High	*B. cereus* requires a minimum a_w of 0.93-0.95 to grow	(FSAI, 2016; MPI, 2015)
The level of contamination:	NA	NA	NA		
Contamination after processing or decontamination					
1. Manufacturing equipment					
The frequency of recontamination of the product in the manufacturing plant after processing or decontamination, thus, the hazard is present in the final product:	High (46%)	High (46%)	High (46%)	- *B. cereus* is capable of attaching to dairy processing equipment - 46% of the milk collected just after the pasteurisation process was contaminated with gram-positive spore-forming bacteria like *Bacillus*	(Shaheen et al., 2010) (Eneroth, Christiansson, Brendehaug, & Molin, 1998)
	Very high (56%)	Very high (56%)	Very high (56%)	- *B. cereus* was found in 257/458 (56%) pasteurised milk after pasteuriser with concentration 10^3 CFU/mL to 3 x 10^5 CFU/mL	(Becker, Schaller, von Wiese, & Terplan, 1994)
	High (25.9%)	High (25.9%)	High (25.9%)	- The occurrence of *B. cereus* was decreasing from raw milk to end products. As much as 25.9% of isolated strains were taken from pasteurisation tanks. Few of *B.*	(Y. Lin, Ren, Zhao, & Guo, 2017)

Processing addition to dairy products	Exposure assessment			Remarks	References
	a_w<0.60 Milk powder	**a_w 0.60-0.85 Parmesan cheese**	**a_w>0.85 Liquid breakfast product**		
				cereus was still be detected in samples after UHT treatment	
The likely level of re-contamination after processing or decontamination:	1×10^3 - 3×10^5 CFU/mL	1×10^3 - 3×10^5 CFU/mL	1×10^3 - 3×10^5 CFU/mL	Psychotropic strains of *Bacillus* spp. are introduced into the milk as spores from pasture or as the result of inadequate cleaning of bulk tanks.	(Champagne et al., 1994)
The variability of recontamination:	NA	NA	NA		
2. Packaging line/faulty packages					
Whether or not the product put in its primary packaging before decontamination step: If the answer is no, the frequency of recontamination of the decontaminated product prior packaging?	No	No	No Very high (64%)	Gram-positive spore-forming bacteria like *Bacillus* contaminated 64% of the milk in the filled and sealed packages.	(Eneroth et al., 1998)
The level of recontamination after packaging:	NA	NA	10^4 CFU/mL		(Eneroth et al., 1998)
3. Storage and distribution					
The conditions during storage and distribution and how does this affect the level of hazard in the product after manufacture:	Medium (14.08%) >100 CFU/g	Medium (14%) < 200 CFU/g		- 14.08% of the powdered formula for infants and young children were contaminated with *B. cereus*	(Y. Li, Pei, Yang, & Li, 2014)

Processing addition to dairy products	Exposure assessment			Remarks	References
	a_w<0.60 Milk powder	**a_w 0.60-0.85 Parmesan cheese**	**a_w>0.85 Liquid breakfast product**		
		Medium (10.04%) 4×10^1 - 3.8×10^5 CFU/g in cheese	Medium (10.04%) 1×10^1 - 1.1×10^3 CFU/mL	- 14% of cheddar cheese samples although the level of contamination did not exceed 200 CFU/g - 10.04% (26/259 samples) of full-fat milk and cheese were found to be spoiled with presumptive *B. cereus*	(Champagne et al., 1994) (Yibar, Cetinkaya, Soyutemiz, & Yaman, 2017)
The effect of storage on the level of hazard at the point of sale:	NA	NA	NA		
4. **Consumer use (Abusive temperatures, unhygienic consumer behaviour)**					
The likelihood and level of recontamination/growth:	High (45.9%) 0.64×10^1 - 5.96×10^3 spores/g Very high (59%) >103 CFU/g	Very high (55%) in parmesan cheese	Medium to extremely high (13%-100%)	- 45.9% (175/381) samples of dried milk products (milk with rice, milk substitute, milk powder, milk-cereal-rice, pudding milk, flan, and mousse) - 55% in parmesan cheese - 13%-100% *B. cereus* in pasteurised milk - *B. cereus* was detected in 59% reconstitute formula which was stored for 24 hours at temperatures higher than 25°C	(Reyes, Bastias, Gutiérrez, & Rodríguez, 2007) (Zeinab, Refaat, Abd El-Shakour, Mehanna, & Hassan, 2015) (Salustiano et al., 2009; te Giffel, Beumer, Granum, & Rombouts, 1997) (Haughton, Garvey, & Rowan, 2010)

Processing addition to dairy products	Exposure assessment			Remarks	References
	a_w<0.60 Milk powder	**a_w 0.60-0.85 Parmesan cheese**	**a_w>0.85 Liquid breakfast product**		
The variability or uncertainty of this estimate:	NA	NA	NA		
Food intake by a consumer					
The likely quantity of the food consumed by a customer on a specified occasion or over a period of time?	<241 (g/person/day)	<36.7 (g/person/day) Cheese low moisture	<241 (g/person/day)	Milk (including milk powder and liquid milk) in total consumer means based on 2008/2009 ANS NZ	(Cressey et al., 2016)

*Adapted and modified from key questions and table (M. Brown, 2002a).

4.2.2. Risk characterisation

4.2.2.1. Risk in raw material/cereals

Kiln drying is heat treatment applied in oat manufacture (Zwer, 2017). Oat groats whose outer husk has been removed contain high levels of oil which is prone to lipid oxidation caused by enzymes, which may result in rancidity of the end products. Heat treatment is essential to deactivate enzymes. Kilning is conducted by putting the groats in a long vertical cylinder that comprises several columns and then steam and air are injected into the column (Gates, 2007). Steaming temperatures are usually at 95 to 105 °C for 10 to 30 min to increase the moisture content to 16 to 17% (Decker, Rose, & Stewart, 2014; Gates, 2007). The efficiency of enzyme inactivation increases as the moisture content increases (Decker et al., 2014). Then, the groats are subjected to dry heat >95 °C for more than 70 min followed by air injection for 30 min to evaporate excess moisture to a final water content of 10%-13% moisture (Decker et al., 2014; Salovaara, 1993). However, spore formers and other thermophilic bacteria can withstand such high temperatures and survive (Meer, Baker, Bodyfelt, & Griffiths, 1991).

Heat treatments in oat processing kill pathogenic bacteria, yeast and mould (Decker et al., 2014). From the microbiological perspective, high temperature processing up to 105 °C for 30-45 minutes is sufficient to eliminate some of the pathogens such as *E. coli* O157: H7, *L. monocytogenes* and *Salmonella.* D-values are the time required to kill one log of a particular bacterium at a particular temperature (Jay et al., 2005). D-values for *E. coli* O157: H7 at 63 °C is 0.5 min (NZFSA, 2001a), for *L. monocytogenes* at 60 °C is 1.6–16.7 min (NZFSA, 2001b) and for *Salmonella* at 60°C is 0.1–10 min (NZFSA, 2001c). The low water activity of cereal products like oats promotes the heat resistance of *Salmonella* (NZFSA, 2001c). Since the oat processing comprises both dry and wet heat treatment, *Salmonella* may be eliminated from oats. On the contrary, a spore of *B. cereus* is more resilient to heat (MPI, 2015), which means the spores can remain in the oats.

The probability of randomly selected cereal grains being contaminated with *B. cereus* varies depending on factors such as sample size. The prevalence of *B. cereus* in wheat flour in Australia was reported to be 93% with <1 spore/gram (Berghofer et al., 2003). The prevalence of *B. cereus* in raw material for bread (such as wheat, rye and oats) in Denmark was reported to be 2%, whereas the *Bacillus* spore numbers surviving heat treatment at 100 °C for 10 min in wheat (grains, rolled, bran, wholemeal, flour) was 1.8-

12.4 CFU/g, in rye (grains, rolled, bran, wholemeal) was 2.2-7.3 CFU/g and in oats (grains, rolled, wholemeal) was 9.6-29.8 CFU/g (Rosenkvist & Hansen, 1995). Conversely, the two studies described above indicate that considerable variability by region/country is likely. Some spores in dry infant rice cereal remain the same after 48 weeks with a water activity of 0.27-0.28 (Jaquette & Beuchat, 1998). The likelihood of toxin present in raw material is negligible because a large number of viable cells (10^5 to 10^8 /g) is required to produce a toxin (MPI, 2015).

4.2.2.2. Cereal addition to low water activity dairy product

Skim milk powder and non-fat milk powder are examples of low water activity dairy products (a_w 0.00 - 0.60) (Early, 1998b). As cereals do not go through a sterilisation process, they may contain *B. cereus* spores. Spores are persistent in dry foods such as cereals (Beuchat et al., 2013). As mentioned earlier, oats as a raw material may contain *Bacillus* spores 9.6-29.8 CFU/g, which is considered a low concentration (Rosenkvist & Hansen, 1995).

In milk powder, there are several possibilities for contamination with *B. cereus*: from manufacturing equipment, packaging line/faulty package, storage, distribution and consumer use. Contamination during storage and distribution of milk powder shows variability in the prevalence of *B. cereus* ranging from of 10.3% to 19.3% (Becker et al., 1994; Reyes et al., 2007) and similarly, the prevalence in powdered infant and young children formula was reported as 14.08% with >100 CFU/g (Y. Li et al., 2014), meaning the likelihood of contamination can be classified as medium. Table 30 summaries the prevalence of B. cereus in milk powder in several countries. As seen in Table 30, the prevalence varies from 10.3% to 100% with a maximum level of 10,000/g. These findings show that variability exists between different countries.

Table 30. Incidence of *B. cereus* in dried milk

Country	Type of product	Number of positive samples/ Number of examined samples (% Positive samples)	Concentration *B. cereus*/g
Hungary	Dried milk	27/52 (51.9%)	60-100
Poland	Dried milk	6/27 (22.2%)	100-2000
Poland	Dried milk	64/332 (19.3%)	10-1000
USA	Skim milk powder	3/8 (37.5%)	200-600
Brazil	Skim/whole milk powder	40/40 (100%)	≤1000
Belgium	Milk powder	57/60 (95%)	0.2-53
Finland	Dried milk	2/13 (15.4%)	10-100
Poland	Milk powder	12/25 (48%)	10-1000
Japan	Skim milk powder	31/302 (10.3%)	<300
USA	Skim/whole milk powder	5/8 (62.5%)	30-270
Egypt	Milk powder	7/10 (70%)	10-9500
Brazil	Whole milk powder	24/30 (80%)	>1000
India	Milk powder	4/9 (40%)	<10,000

Adapted from (Becker et al., 1994; Cressey et al., 2016).

The high prevalence suggests that the likelihood of contamination during manufacture is high, although the number is still below the dose required to cause a diarrhoea (10^5 to 10^7 total cells).

The addition of cereals contaminated with *B. cereus* to milk powder will add to the food safety risk from any *B. cereus* already present in milk powder. *B. cereus* in milk powder may spoil foods manufactured using this milk powder. *B. cereus* requires a minimum water activity of 0.93-0.95 to grow (FSAI, 2016; MPI, 2015); therefore, it is not likely to grow in low water activity dairy product such as milk powder. However, the spores can grow and potentially produce toxins when the milk powder is reconstituted and stored for a long time at a suitable growth temperature or used as a component in a product such as a dairy desert stored for some time before consumption (Jaquette & Beuchat, 1998; Rowan & Anderson, 1997).

Temperature abuse by consumers is an additional concern allowing any *B. cereus* present in reconstituted milk to grow to levels that may be a food safety hazard. The likelihood and level of contamination are high as demonstrated in the result of a study by Reyes et al. (2007). They found 45.9% (175/381) of dried milk products (milk with rice, milk powder, milk substitute, milk-cereal-rice, flan, pudding milk, and mousse) in school food services in Chile contained 0.64×10^1 to 5.96×10^3 *B. cereus* spores/g. Another study by Haughton et al. (2010) found 24 samples of 100 powdered infant formula in Ireland were positive for *B. cereus* with a mean level of 190 CFU/g and maximum level of 570 CFU/g.

B. cereus was detected in 59 out of 100 samples with more than 10^3 CFU/g from reconstituted infant formula was stored for 24 hours at temperatures higher than 25°C.

B. cereus in cereal added to milk powder is generally low during manufacture and processing. However, there is potential of contamination after production and for abuse of reconstituted product to produce a food safety hazard.

4.2.2.3. Cereal addition to intermediate water activity dairy products

Intermediate water activity dairy products (a_w 0.60 - 0.85) include Parmesan cheese and salted butter (Schmidt & Fontana Jr, 2008). *B. cereus* requires a minimum a_w 0.93-0.95 to grow (FSAI, 2016; MPI, 2015), which means it may not be able to grow in Parmesan cheese. All the ingredients, including milk and cereals if added to milk are pasteurised. However, Messelhäusser et al. (2010) revealed that *B. cereus* spores are not killed by pasteurisation. Fortunately, the processes involved in cheese manufactures such as the addition of salt and intrinsic characteristics of the end product (a_w of 0.69 – 0.73 and low pH <4.5) can suppress the growth of *B. cereus*. The likelihood of *B. cereus* spore contamination from cereals germinate in Parmesan cheese is expected to be low.

The likelihood of contamination of Parmesan cheese with *B. cereus* during storage and distribution is predicted to be low due to its intermediate water activity. However, *B. cereus* already present in the other cheese products (e.g. high moisture cheese) has the ability to germinate under refrigeration. Sadek, Fathi, and Salem (2006) found that 4 out of 9 isolates of *B. cereus* from processed cheese were able to grow at 7°C.

In Scotland, eight samples of 25 artisanal cheese (32%) made from raw milk were found to contain *B. cereus* at 10^2 to 4 x 10^4 CFU/g (Williams & Withers, 2010). However, the authors explained that *B. cereus* was not detected in cheese made with pasteurised milk. The authors were able to get 20 isolates and found that all the isolates produced enterotoxin.

Contamination of Parmesan cheese with *B. cereus* was reported by Zeinab et al. (2015). The author found *B. cereus* in 55% of Parmesan cheese. This prevalence is quite high and therefore, additional research is needed to determine how widespread this contamination is (i.e., more than one study) and whether it represents a food safety hazard (what numbers of *B. cereus* are involved).

From the studies above, it can be seen that even though there is a high potential of *B. cereus* contamination during the process of adding cereals to Parmesan cheese, the risk involved is low.

4.2.2.4. Cereal addition to high water activity dairy product

High water activity dairy products (a_w > 0.92) include milk, cream, cheddar cheese, unsalted butter and yoghurt (Schmidt & Fontana Jr, 2008). Liquid breakfast product, which is a combination of non-dairy such as cereal grains and a dairy ingredient such as milk has become common in the market. The high water activity of such products is excellent for the growth of many microorganisms (Jay et al., 2005). Many pathogenic bacteria such as *C. botulinum*, *E. coli* and including *B. cereus* are capable of growing in these food products (Jay et al., 2005), making high water activity dairy products a concern in food safety. The likelihood of *B. cereus* spore contamination to be transferred from oats to liquid milk is predicted to be high.

In a study conducted by Eneroth et al. (1998), contamination after processing occurred in the manufacturing plant. 46% of the milk collected just after the pasteuriser was spoiled by Gram-positive spore-forming bacteria like *Bacillus*. Similarly, some authors report that *B. cereus* could survive pasteurisation (Postollec et al., 2012; Rezende-Lago, Rossi Jr, Vidal-Martins, & Amaral, 2007) but unlikely to survive UHT treatment (Pacheco-Sanchez & Massaguer, 2007; Rangasamy, Iyer, & Roginski, 1993).

Contamination after processing can occur during packing. This happened due to faulty packaging by sealing off the milk boxes resulting in 64% of the milk being spoiled by Gram-positive spore-forming bacteria like *Bacillus* with 10^4 CFU/mL (Eneroth et al., 1998). Contamination of *B. cereus* during processing (in the manufacturing plant) and after processing (packaging) can be very high.

Contamination after processing during storage and distribution of pasteurised and UHT milk was reported by some authors (Table 31). As can be seen from Table 31, the prevalence of *B. cereus* in pasteurised milk is extremely high but varies from rare to medium in UHT milk. Nevertheless, contamination of UHT milk would only occur if there were a serious fault in the UHT treatment or some source of contamination after UHT treatment (Cressey et al., 2016).

Table 31. ***Incidence of B. cereus in pasteurised and UHT milk***

Country	Type of product	Number of positive samples/ Number of examined samples (% Positive samples)	Concentration *B. cereus*	References
Brazil	Pasteurised milk	9/9 (100%)	0.4-71 CFU/mL	(Salustiano et al., 2009)
China	Pasteurised full fat milk	26/54 (48%)	3-43 MPN/mL	(Zhou, Liu, He, Yuan, & Yuan, 2008)
Netherlands	Pasteurised milk	38/38 (100%)	<0.3 CFU/mL	(te Giffel et al., 1997)
Brazil	UHT milk	4/30 (13%)	NS	(Rezende-Lago et al., 2007)
Brazil	UHT milk (130-150 °C) 2-4 sec)	0/6500 (ND)		(Pacheco-Sanchez & Massaguer, 2007)

NS: Not stated, ND: Not detected

High moisture dairy foods such as yoghurt and cheese have intrinsic properties that can protect them from pathogens. A study on the growth of *B. cereus* showed that there was no *B. cereus* growth observed in yoghurt.

4.2.2.5. Risk estimate summary

A summary of risk estimate (Table 32) showed that the risk estimate for oats as a raw material is low, but, contamination of products from manufacturing equipment and packaging plus the growth of any contaminants during storage and distribution may vary along with the potential for consumer abuse influence the risk estimate.

Table 32. Summary of risk estimation of oats addition to dairy products

	Risk estimate		
	***B. cereus* in milk powder**	***B. cereus* in Parmesan cheese**	***B. cereus* in liquid breakfast product**
The occurrence in raw material (cereal):	Low (2%)	Low (2%)	Low (2%)
The likelihood of contamination/growth in the dairy product:	Low (low a_w)	Low (intermediate a_w)	High (high a_w)
Effect of decontamination process (pasteurisation or UHT):	Complete inactivation	Complete inactivation	Complete inactivation
The occurrence of toxin:	Rare (0-0.1%)	Rare (0-0.1%)	Rare (0-0.1%)
Contamination after pasteurisation or UHT process:			

	Risk estimate		
	***B. cereus* in milk powder**	***B. cereus* in Parmesan cheese**	***B. cereus* in liquid breakfast product**
- Manufacturing equipment:	High to very high (25.9%-56%)	High to very high (25.9%-56%)	High to very high (25.9%-56%)
- Packaging line/ faulty package	NA	NA	Very high (64%)
- Storage and distribution	Medium to extremely high (10.3%-100%)	Medium to high (10.04%-14%)	Medium to extremely high (13%-100%)
- Consumer use	High to very high (45.9%-59%)	Very high (55%)	Medium to extremely high (13%-100%)

NA: Not available

4.2.2.6. Uncertainties, variabilities, and assumptions

In conducting the exposure assessment, assumptions were made regarding the manufacture of dairy products. Dairy products are manufactured under Good Manufacturing Practice (GMP) and Good Hygiene Practice (GHP) which includes the implementation of HACCP programme.

There are substantial uncertainties and variabilities considered in the exposure model. Processing conditions in three types of dairy products that are different in terms of heat treatment and holding times and include the times when cereals are added to milk (i.e. whether it is before or after heat treatment, the state/form of cereal products used (grains, flour, flakes) as well as, methods of preparation and storage of products by consumers. These factors may influence the accuracy of the risk assessment.

The aim of this risk assessment was to understand the New Zealand setting, however, the data were acquired globally due to lack of local information. Moreover, the reference studies show high variability in the magnitude of prevalence (high and low) depending on the condition and situation of the country in which the studies were undertaken. Hence, the prevalence may not represent New Zealand. Some of the references regarding dairy products contamination with *B. cereus* were documented more than ten years ago which may not be relevant anymore due to improvement in dairy processing.

CHAPTER 5. CHEMICAL RISK ASSESSMENT RESULTS

This chapter covers the chemical risk assessment results of the present study. Section 5.1 shows the chemical risk assessment of selected cereal grains, resulting in several natural toxins being identified. Cyanogenic glycoside was the highest chemical hazard following a using a semi-quantitative risk assessment matrix. Section 5.2 describes the chemical risk assessment of raw defatted soy flour as the selected grain added to three types of dairy products, i.e. milk powder, Parmesan cheese and liquid breakfast product.

5.1. Chemical risk assessment of selected cereal grains

5.1.1. Hazard identification

Chemical hazards of food, and cereal in particular, can be derived from a number of sources. These include naturally-occurring toxins (e.g. plant toxins, mycotoxins), bioaccumulation (e.g. heavy metals), crop handling/agricultural practice (pesticides), toxins acquired through primary and secondary processing equipment (e.g. cleaning and sanitising agents), toxins formed through food processing (e.g. acrylamide), and intentionally added adulterants (e.g. melamine in wheat bran) (Alldrick, 2017; Hanlon et al., 2015).

Generic chemical risk issues include pesticides residue, heavy metals, allergens and mycotoxins. Mycotoxin contamination occurs due the growth of toxin producing fungi at warm temperatures (20-37 °C) and high moisture levels (18 to 30%) (Bullerman & Bianchini, 2009; Gizachew, Hsu, Szonyi, & Ting, 2019). Each generic chemical risk is described in Appendix D1.

Several authors suggest that inherent plant toxins raise more safety concerns than the synthetic chemicals due to their toxic potency and likely high exposure levels (Essers et al., 1998; Mattsson, 2007; Schilter et al., 2014). For plant toxins, the margin of safety between the actual exposure/intake adverse reactions in humans appears to be low. For example, cassava roots contain cyanogenic glycosides (Linamarin) of 240-890 mg $kg^{-1,}$ but varieties with high content may contain 1300-2000 mg kg^{-1} (EFSA, 2009). On the other hand, the level capable of causing human illness is 0.5 -3.5 mg kg^{-1} bw (Speijers, 1993). Cyanogenic plants can undergo a process called cyanogenesis, which results in the formation of free hydrogen cyanide/hydrocyanic acid (NZFSA, 2017a). In plants,

concentration of cyanogenic glycosides is measured as the level of hydrogen cyanide or hydrocyanic acid released as result of enzymatic activity (NZFSA, 2017a) .

Anti-nutrients are chemicals that may lessen the nutritional value of the plant food. Examples are phytates preventing absorption of minerals such as iron, protease inhibitors blocking protein digestion (Schilter et al., 2014). A summary of anti-nutrients and inherent plant toxins identification in cereal grains is presented in Table 33. Some of these anti-nutrients and natural plant toxins identified in the hazard identification step is described in Appendix D2.

Table 33. Summary of anti-nutrients and inherent plant toxins identification in cereal grains

Ingredient name	Anti-nutrients and inherent plant toxins			Remarks	References
	Chemical agent	Part of plant / Raw material	Concentration		
Barley	Cyanogenic glycoside (Epiheterodendrin)	Leaves	Not determined	No cyanogenic glycosides found in roots and seeds	(NZFSA, 2017a) (Jones, 1998) (Crevel & Cochrane, 2014) (Nielsen, Olsen, Pontoppidan, & Møller, 2002)
	Lectin	Not determined	Not determined	Improve blood lipid profile	(Peumans & Damme, 1998) (Sidhu, Kabir, & Huffman, 2007)
	Oxalates	Whole grains Flakes Pearl flakes	15.5 -27.3 mg 100 g^{-1} 8.2-25.3 mg 100 g^{-1} 11.6-12.0 mg 100 g^{-1}		(Siener, Hönow, Voss, Seidler, & Hesse, 2006)
	Protease inhibitors	Seed	Not determined		(I. E. Liener & Kakade, 1969)
Maize (Corn)	Oxalates	Whole grain	38.6 mg 100 g^{-1}		(Siener et al., 2006)
	Cyanogenic glycoside (Dhurin)	Not determined	Not determined		(Ganjewala, 2010)
	Protease inhibitors	Seed	Not determined		(I. E. Liener & Kakade, 1969)
	Phytates	Not stated	9.8-21.3 mg g^{-1}	Lower plasma glucose	(Greiner & Konietzny, 2006) (Sidhu et al., 2007)
Millet	Cyanogenic glycoside (Triglochinin)	Not determined	Not determined		(Agnihotri & Shrivastava, 2008) (Jones, 1998)
	Oxalates	Hulled grain	19.2-21.0 mg 100 g^{-1}		(Siener et al., 2006)

Ingredient name	Anti-nutrients and inherent plant toxins			Remarks	References
	Chemical agent	Part of plant / Raw material	Concentration		
		Flakes	3.6-7.6 mg 100 g^{-1}		
	Goitrogen	Not determined	Not determined		(Taylor, 2017)
	Phytates	Not determined	585 (180–990) mg 100 g^{-1} dry basis 354–796 mg g^{-1}		(Abdalla, El Tinay, Mohamed, & Abdalla, 1998) (Taylor, 2017)
	Tannins	Not determined	Not determined		(Taylor, 2017)
	Oxalates	Not determined	Not determined		(Taylor, 2017)
Oats	Oxalates	Whole grain Flakes Bran and germs	13.8 -16.3 mg 100 g^{-1} 6.2-22.0 mg 100 g^{-1} 11.0-32.0 mg 100 g^{-1}		(Siener et al., 2006)
	Phytates	Not determined	Oat flakes: 8.4–12.1 mg g^{-1}		(Greiner & Konietzny, 2006)
	Cyanogenic glycosides		Not determined		(Jones, 1998)
Rye	Lectins	Not determined	Not determined		(Peumans & Damme, 1998)
	Oxalates	Whole grain Flakes Wholemeal flour	32.2 mg 100 g^{-1} 12.5-44.0 mg 100 g^{-1} 22.6-27.9 mg 100 g^{-1}		(Siener et al., 2006)
	Protease inhibitors	Seed	Not determined		(I. E. Liener & Kakade, 1969)
	Cyanogenic glycosides		Not determined		(Jones, 1998)

Ingredient name	Anti-nutrients and inherent plant toxins			Remarks	References
	Chemical agent	Part of plant / Raw material	Concentration		
Black glutinous rice	Lectins	Not determined	Not determined		(Peumans & Damme, 1998)
	Protease inhibitors	Seed	Not determined		(I. E. Liener & Kakade, 1969)
Brown rice	Lectins	Not determined	Not determined		(Peumans & Damme, 1998)
	Oxalates	Long grain, unpolished Flakes	13.8 mg 100 g^{-1} 4.2-12.2 mg 100 g^{-1}		(Siener et al., 2006)
	Protease inhibitors	Seed	Not determined		(I. E. Liener & Kakade, 1969)
	Phytates	Cooked rice	1.2-3.7 mg g^{-1}		(I. Liener, 1969)
Wheat	Cyanoglycosides (Dhurin, Linamarin, Lotaustralin, Epilotaustralin)	Not determined	Not determined		(Jones, 1998)
	Lectins	Flour Germ	Not determined		(Peumans & Damme, 1998)
	Oxalates	Whole grains Flakes Wholemeal flour Flour Semolina Brans Germs	53.3 mg 100 g^{-1} 17.3-75.6 mg 100 g^{-1} 34.0-70.0 mg 100 g^{-1} dry basis 2.4-45.0 mg 100 g^{-1} 2.6-12.5 mg 100 g^{-1} 131.2-457.4 mg 100 g^{-1} 27.3-44.1 mg 100 g^{-1}		(Siener et al., 2006)
	Protease inhibitors	Germ	Not determined		(I. E. Liener & Kakade, 1969)
	Phytates		1167 (1080–1350) mg 100 g^{-1} dry basis		(Taylor, 2017)

Ingredient name	Anti-nutrients and inherent plant toxins			Remarks	References
	Chemical agent	Part of plant / Raw material	Concentration		
Buckwheat	Quercetin	Not determined	Not determined	Quercetin is intentionally added to buckwheat tea as bitterness flavour.	(David, Arulmoli, & Parasuraman, 2016)
	Protease inhibitors	Seed	Not determined		(I. E. Liener & Kakade, 1969)
	Fagopyrin	Leaves Stems Flowers Hulls Groats	0.4-0.6 mg g^{-1} dry mass 0.04-0.12 mg g^{-1} dry mass 0.64 mg g^{-1} dry mass 0.02 mg g^{-1} dry mass Not detected	Fluorescent phototoxic fagopyrins.	(Kreft, Janeš, & Kreft, 2013) (Benković & Kreft, 2015)
	Phytates	Not determined	9.2-16.2 mg g^{-1}		(Greiner & Konietzny, 2006)
Adzuki beans (red mung bean)	Not determined	Not determined	Not determined		
Garden pea	Lectins	Not determined	Not determined		(Lawley, Curtis, & Davis, 2008)
	Protease inhibitors	Seed and germs	Not determined		(I. E. Liener & Kakade, 1969)
	Cyanogenic glycosides	Not determined	2 mg 100 g^{-1}		(Chandra, 2010)
Hyacinth beans	Lectins	Not determined	Not determined		(Saha, Tuhin, Jahan, Roy, & Roy, 2014)
Mung beans	Protease inhibitors	Leaves and cotyledons Stems and roots	High Low		(I. E. Liener & Kakade, 1969)
Soybeans	Genistein	Soy milk Soy milk formula	21 mg kg^{-1} 19–23 mg kg^{-1}	Primary anticancer	(Schilter et al., 2014) (Skibola & Smith, 2000)
	Lectins	Raw soybean seed Whole soybean flour Defatted soybean flour	3,600 µg g^{-1} 3,600 µg g^{-1} 4,583 µg g^{-1}	May lower plasma glucose	(Dolan, Matulka, & Burdock, 2010)

Ingredient name	Anti-nutrients and inherent plant toxins			Remarks	References
	Chemical agent	Part of plant / Raw material	Concentration		
					(de la Barca, Vázquez-Moreno, & Robles-Burgueño, 1991) (Sidhu et al., 2007)
	Goitrogen	Not determined	Not determined		(Dolan et al., 2010)
	Phytates	Not determined	9.2-16.7 mg g^{-1}		(Greiner & Konietzny, 2006)
	Saponins	Not determined	Not determined	May lower plasma glucose	(Essers et al., 1998)
	Isoflavones	Not determined	Not determined		(Essers et al., 1998)
	Protease inhibitors	Seed	Not determined		(I. E. Liener & Kakade, 1969)
	Cyanogenic glycosides	• Protein • Shell • Whole soybean meal • Commercial raw defatted soy flour • Soy protein isolate	• 0.03-0.07 mg kg^{-1} • 1.24 mg kg^{-1} • 0.26 ± 0.09 µg g^{-1} • 0.08 ± 0.02 µg g^{-1} • 0.18 ± 0.04 µg g^{-1}		(EFSA, 2007) (Honig, Hockridge, Gould, & Rackis, 1983)
Black soybeans	Genistein	Not determined	Not determined		(Schilter et al., 2014)
	Lectins	Not determined	Not determined		(Dolan et al., 2010)

5.1.2. Hazard characterisation

Hazard characterisation of each anti-nutrient and natural plant toxin identified in the hazard identification step is shown in Table 34.

Table 34. Hazard characterisation of inherent plant toxins

Chemical agent	Health impacts	Occurrence (incidence and outbreaks)	Health-based Guidance Value (HBGV)	Legislation (maximum limit)
Cyanogenic glycoside(hydrocyanic acid) - Dhurin - Linamarin - Lotaustralin - Epilotaustralin - Epiheterodendrin - Triglochinin	- Cytotoxic and hinder the cytochrome oxidase's activity - Acute cyanide poisoning: rapid breathing, raised pulse rate, drop in blood pressure, headache, diarrhoea, vomiting, confusion, twitching, stomach pain, convulsion and death - Chronic cyanide poisoning: malnutrition, diabetes, growth retardation, neurological disorder, congenital malformations and myelopathy (Lawley et al., 2008) - Acute high dose: headache, nausea, vomiting, dyspnea, hyperpnea, convulsion, death - Moderate dose: neurological effects (konzo) (Schilter et al., 2014)	In New Zealand, two cases of cyanide poisoning associated with apricot kernels consumption were reported. In 2001, Waikato hospital reported that 30 apricot kernel (3 mg cyanide g^{-1} kernel) was the causal agent of the cyanide poisoning. In 2006, one woman was hospitalised after ingestion of a mixture of 60 ground apricot kernels and orange juice (Cressey & Thomson, 2007)	- NOAEL of 4.5 mg cyanide kg^{-1} bw/day - PMTDI of 0.023 mg cyanide kg^{-1} bw/day - 0.5 mg kg^{-1} for hydrocyanic acid (Council of Europe, 2008) - ARfD: 90 µg kg^{-1} bw - PMTDI: 20 µg kg^{-1} bw/day (JECFA, 2011a) - Acute lethal dose 0.5-3.5 mg kg^{-1} (Jones, 1998)	**Codex** The safe level of cyanide in cassava flour: 10 mg kg^{-1} (JECFA, 2011a) **Australia & New Zealand** Total hydrocyanic acid Confectionery: 25 mg kg^{-1} Stone fruit drinks: 5 mg kg^{-1} Marzipan: 50 mg kg^{-1} Alcoholic beverages content: 1 mg kg^{-1} per 1 % alcohol (FSANZ, 2016)
Fagopyrin	Cause fagopyrism in humans: burns, cold sensitivity and tingling and numbness in the hands (Benković & Kreft, 2015)		NA	No legislation
Genistein	Various hormonal effects (Schilter et al., 2014)		NA	No legislation
Isoflavones	Estrogen-like characteristic may lead to unpleasant impacts in specific population group such as postmenopausal women (Messina, 2016)		NA	No legislation

Chemical agent	Health impacts	Occurrence (incidence and outbreaks)	Health-based Guidance Value (HBGV)	Legislation (maximum limit)
Lectins	- Toxic symptoms (nausea, vomiting, bloating and diarrhoea) (Lawley et al., 2008) - Decrease nutrients absorption - Allergens and hemagglutinins (Kaushik, Singhal, & Chaturvedi, 2018)	- In 1948, partially cooked beans consumption causing the west Berlin people suffered from gastroenteritis - In 1976, an acute outbreak (diarrhoea and sickness) affected a group of schoolboys due to soaked kidney beans consumption (Lawley et al., 2008)	NA	- No legislation - FDA provides a recommendation to cook prior consuming legumes
Goitrogen	Affect the thyroid gland's function (Chandra, 2010)		NA	No legislation
Oxalates/oxalic acid	- Oxalates bind calcium, magnesium and other minerals. Oxalate complexes with calcium, causing hypocalcaemia. - Consumption of additional oxalic acid may cause stone formation in the urinary tract. - Calcium oxalate precipitates in the renal tubules and vasculature, resulting in renal failure.("Oxalic acid," 2009) (Kaushik et al., 2018)		Lethal doses: 15-30 g.("Oxalic acid," 2009)	No legislation
Phytates	Mineral deficiencies or decreased protein and starch digestibility (Dolan et al., 2010).		NA	No FDA regulations or guidelines. (Dolan et al., 2010)

Chemical agent	Health impacts	Occurrence (incidence and outbreaks)	Health-based Guidance Value (HBGV)	Legislation (maximum limit)
Protease inhibitor	Interfere with the enzyme digestion (trypsin and chymotrypsin) in the human gastrointestinal tract. (Kaushik et al., 2018)		NA	No legislation
Quercetin	Gastrointestinal effects such as nausea (Lakhanpal & Rai, 2007)		NA	No legislation
Saponins	Growth impairment and throat-irritating (Kaushik et al., 2018)		NA	No legislation
Tannins	Reduce the protein digestibility in humans and animals as well as affect dietary iron absorption. (Kaushik et al., 2018)		NA	No legislation

NA: Not available

5.1.3. Exposure assessment

Cereal grain production has an impact on chemical safety, especially in the pre-harvest and post-harvest stages. The focus of this risk assessment was mainly on cyanogenic glycosides due to the available data about cyanogenic glycosides in New Zealand as well as the limited information about other anti-nutrients

5.1.3.1. Pre-harvest

Several conditions cause variability in the levels of cyanogenic glycosides in plants. The cyanogenic glycoside level may be high in young plants that grow in cold and humid weather. It is also found to be high in plants that are severely fertilised, plants that are treated with a particular herbicide as well as stressed due to frost or drought (Haschek et al., 2013). For example, drought in a planting area in Mozambique caused the cyanide levels to be surprisingly high (Toxnet, 2018). All plant tissues contain cyanogenic glycosides with the highest concentrations found in the leaves (Pinto-Zevallos, Pareja, & Ambrogi, 2016).

5.1.3.2. Post-harvest

Post-harvest processing may influence the levels of cyanogenic glycoside in a plant. Cyanogenic glycosides need to be hydrolysed into cyanide, prussic acid or hydrocyanic acid in order to become toxic. This transformation is assisted when the plant undergoes processing such as crushing, freezing and chewing or withering. On the other hand, processing such as drying or ensiling the plants may reduce cyanogenic glycosides due to the gradual degradation and release of cyanide over time (Haschek et al., 2013).

Food processing practices are believed to reduce the hydrogen cyanide level by involving plant enzymes and leaching. Processes including drying, soaking and fermentation enable the transformation of cyanogenic glycoside into cyanide and further exposure to air and water will let the cyanide leach out of the food matrix (NZFSA, 2017a).

5.1.3.3. General control of cereal grains

Inherent plant toxins exist in different parts of plants but mostly not in the edible part of the plant, e.g. leaves or seed. It is suggested to consume only the edible parts of the cyanogenic plant. Processing methods to reduce or eliminate anti-nutrients and intrinsic plant toxins need to be used before consumption of the non-edible parts of plants. Control measures for plant toxicants are as follows:

- Most of the anti-nutrients such as lectins and cyanogenic glycosides are removed using heat (Dolan et al., 2010).
- Non-heat processing that can remove toxins includes soaking, dehulling, fermentation and germination/sprouting for heat-stable toxins, e.g. as tannins, phytates and saponins (Schoeninger, Coelho, Christ, & Sampaio, 2014).
- Advanced methods such as microwave cooking and irradiation (Kaushik et al., 2018).

The evidence presented thus far supports the idea that heat treatment is not the only technique to reduce cyanogenic glycosides in the plants. Several authors have reported different processing methods that successfully reduce the toxin content. Agbor-Egbe and Mbome (2006) found that soaking cassava root in three periods reduces total cyanogen content: 13-52% reduction after 24 hours, 73-75% after 48 hours and 90% after 72 hours. Total cyanogen content reduction of 50-64% after storage of cassava was reported by Schoeninger et al. (2014). Steaming cassava resulted in 74-80% reduction of cyanide content (Obilie, Tano-Debrah, & Amoa-Awua, 2004).

5.1.3.4. The chemical survey of cereal grains

A chemical survey of cyanogenic glycosides content in selected plant-based foods available in New Zealand market has been reported by Cressey, Saunders, and Goodman (2013). As mentioned earlier, concentration of cyanogenic glycosides is measured as the level of hydrogen cyanide or hydrocyanic acid released (NZFSA, 2017a). Selected plant-based foods assessed include cassava, bamboo shoots, pome fruit products (apple), almond and almond products, flaxseed/linseed, stone fruit products and miscellaneous products such as taro and vine leaves, spinach, passion fruit and passion fruit products. This survey found that hydrocyanic acid content in the samples shows consistency with or less than level stated in the literature. The authors identified the possibility of some foods being consumed regularly and in substantial amounts (e.g. linseed-containing bread and apple juice) which may lead to an acute reference dose (ARfD) of 0.09 mg kg^{-1} body weight being exceeded. This study raised the possibility of cyanogenic glycosides as a food safety issue even in New Zealand where cassava and bamboo shoots are small part of the diet. However, a chemical survey of cyanogenic glycosides in cereal grains of interest in this study such as rice, wheat and soybean in New Zealand is not available.

5.1.3.6. Estimate of dietary exposure

Safety assessments conducted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the International Programme on Chemical Safety (IPCS), and Food Standards Australia New Zealand (FSANZ) do not establish a safe level of exposure to cyanogenic glycosides because of insufficient toxicological and epidemiological data (NZFSA, 2017a).

A survey of cyanogenic glycosides in plant-based foods in Australia and New Zealand was conducted in 2010-2013 (FSANZ, 2014b). The plant-based foods assessed included cassava, bamboo shoots, pome fruit products (apple), almond and almond products, flaxseed/linseed, stone fruit products and miscellaneous products such as taro and vine leaves, spinach, passion fruit and passion fruit products. A dietary exposure assessment was conducted to estimate the level of chronic and acute dietary exposure to hydrocyanic acid (HCN). Estimated dietary exposure for consumers of food containing cyanogenic glycosides (measured as total HCN) is shown in Table 35.

Table 35. Estimated chronic dietary exposures for consumers of foods containing cyanogenic glycosides (measured as total HCN) for New Zealand population groups

					All respondents		Consumers only			
		(% consumers)			Mean		Mean		90th percentile	
		LB	UB		LB	UB	LB	UB	LB	UB
5-14	3,275	2,105	3,146	µg/day	48	74	74	77	154	166
		(64.3)	(96.1)	µg/kg bw/day	1	2	2	2	4	5
≥15	4,636	2,296	4,237	µg/day	58	85	118	93	206	205
		(49.5)	(91.4)	µg/kg bw/day	1	1	2	1	3	3

Note: Lower bound (LB) results are calculated by assigning concentrations below the LoD as zero; upper bound (UB) results are are calculated by assigning concentrations below the LoD as a concentration equal to the LoD.
Reprinted from "Survey of cyanogenic glycosides in plant-based foods in Australia and New Zealand 2010-13," (FSANZ, 2014b). In the public domain.

5.1.4. Risk characterisation

5.1.4.1. The most critical chemical risk

Risk characterisation considers the results from hazard identification, hazard characterisation and exposure assessment. To recognise the most critical chemicals in

cereal grains, this study used a qualitative measure of severity (Table 15) from the hazard characterisation and qualitative measures of likelihood (Table 16) from exposure assessment in the form of a score. The score acquired from the severity and likelihood were multiplied to get the overall score (Appendix E. Table E1.1. Risk characterisation calculation). The risk score was then plotted into the qualitative risk assessment matrix (Table 36) for practical interpretation. Hydrocyanic acid is regarded as medium, representing the chemical of most critical risk in cereal grains. Chemicals representing a low risk are genistein, goitrogen, lectins, oxalate, phytates, protease inhibitor, saponins and tannins.

Table 36. Qualitative risk assessment matrix

LIKELIHOOD	SEVERITY			
	Low (1)	Medium (2)	High (3)	Severe (4)
Almost Certain (4)	Low	Medium	Medium	High
Likely (3)	Low	Medium Hydrocyanic acid	Medium	Medium
Possible (2)	Low	Low	Medium	Medium
Unlikely (1)	Low Fagopyrin Genistein Goitrogen Isoflavones Lectins Oxalate Phytates Protease inhibitor Quercetin Saponins Tannins	Low	Low	Low

5.1.4.2. Uncertainties, variabilities and assumptions

There were several assumptions made at the outset this study: (1) Cereal grains are always subjected to control strategies to reduce chemical contamination before they are used; (2) Cereal grains are of good quality and harvested according to Good Agricultural Practice (GAP); (3) Cereal grains in New Zealand are manufactured under the New Zealand Crop Quality Assurance Scheme (NZCQAS) issued by The Arable Food Industry Council (AFIC); (4) The Ministry for Primary Industries (MPI) of New Zealand: (a) monitors the risk of pesticide residue under The Food Residue Surveillance Programme, (b) monitors the risk of heavy metals under The National Chemical Contaminants Programme and (c)

characterise and quantifies the risk of mycotoxins in the food supply to the New Zealand public under The New Zealand Mycotoxin Surveillance program.

Generic chemical risk issues are pesticide residues, heavy metals, allergens and mycotoxins. There are many mitigation strategies to overcome these chemical hazards, and the risk is assumed to be managed.

Studies on cyanogenic plants and its risk mitigation mostly focus on cassava, sorghum, red kidney beans and apricot. However, there is a lack of studies on other cereal grains (e.g. rice, oats, rye, wheat and millet), pseudo-cereals (e.g. buckwheat) and legumes (e.g. red mung beans, hyacinth beans and garden peas). This could be due to the plant's overall low content of hydrocyanic acid and that the edible part does not contain cyanogenic glycoside at levels that raise a safety concern.

5.2. Chemical risk assessment of selected grains addition to dairy products

5.2.1. Exposure assessment

The present study focuses on cereal grains as raw materials received by a dairy company to incorporate into dairy products. It is assumed that the cereal grains will be treated (e.g. cleaning, heat treatment) before use as a dairy ingredient. Thus, it has been assumed that any contamination has been minimised.

Based on the risk assessment matrix, cyanogenic glycoside is the highest chemical risk in cereal grains. In plants, cyanogenic glycosides are converted into hydrocyanic acid (hydrogen cyanide/prussic acid/cyanide) and can cause adverse health reactions. Therefore, the scenario used in this exposure assessment was cereal grains containing hydrocyanic acid added to three types of dairy products (high, intermediate and low solids content). The high solids content product is milk powder, the intermediate solids content product is Parmesan cheese, and low solids content product is liquid breakfast. Limited data is available in the literature for the addition of cereal grains to the three types of dairy product. Expert knowledge (Abernethy & Lindsay, 2019) was used to set the percentage of non-dairy ingredient addition in order to assess the risks of non-dairy ingredient addition.

From the literature available, no work has been done on the estimated exposure to hydrocyanic acid in the three dairy products (milk powder, Parmesan cheese and liquid breakfast product). The maximum levels of hydrocyanic acid in milk powder, Parmesan

cheese and liquid breakfast product have not yet been established. Maximum levels (ML) of hydrocyanic acid have been reported in cassava flour (JECFA, 2011a) and several foods such as confectionary, stone fruit juices, marzipan, ready-to-eat cassava and alcoholic beverages (FSANZ, 2016). It has been recommended to make the most use of the available knowledge to address the uncertainties in food research surveys (FAO/WHO, 1995). Therefore, the ML of hydrocyanic acid in cassava flour was used for milk powder since both can be classified as high solid products with extremely low moisture, while ML of hydrocyanic acid in stone fruit juices was used for liquid cereal products. Risk assessment allows expert judgement to address uncertainty. The present study used expert judgement (Abernethy & Lindsay, 2019) and previously reported data to determine the percentage of non-dairy ingredients to be added to dairy products.

The dairy industry can add any proportion of non-dairy ingredients to high solid content products such as milk powder, however if more than 50% of non-dairy ingredients on a solid basis are added, this will not comply with the requirements for milk powders under standards such as the Codex standard for milk powders and cream powder (FAO/WHO, 1999) and Standard 2.5.7 for dried milk, evaporated milk and condensed milk (FSANZ, 2017b). Therefore, the addition of non-ingredients up to 50% is used as an assumption.

For the intermediate and low solid dairy products, there is a limitation in the addition of non-dairy solids before the functionality changes. These products are already high in protein, fat, lactose, and some other ingredients such as sucrose or stabilisers. It is reasonable to expect up to 10% of the solids could be non-dairy ingredients.

Intermediate solid dairy products such as soft cheeses contain a maximum of 50% total solids, of which 10% can be non-dairy ingredients. This means that as much as 5% of cereal grains can be added to the product. On the other hand, low solid products such as UHT milk and yoghurt contain 10-20% total solids, of which 10% can be non-dairy ingredients. This means as much as 2% cereal grains can be added to the product. A summary of expert knowledge is illustrated in Figure 14.

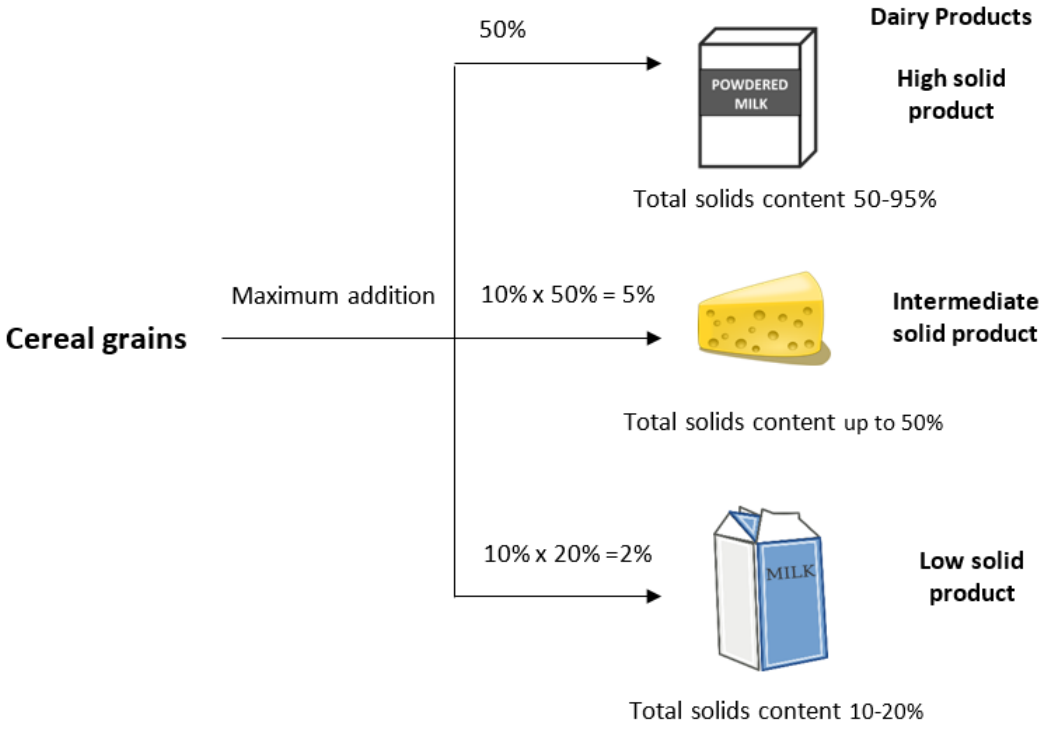

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Figure 14. Diagram of maximum addition of non-dairy ingredient to dairy products

5.2.2. Risk characterisation

5.2.2.1. Risk estimate in raw material

Soybeans are rich in essential nutrients and an exceptional source of protein in comparison with other protein foods (Singh et al., 2008). For decades, soy products have been used as a nutritional and functional food ingredient in many food categories including dairy products. One recent example is the high protein liquid breakfast cereal.

Soybeans were used as the selected grain for addition to dairy products. Defatted soy flour and grits are the most basic forms of high soy protein and the soy products used in the most significant volume in foods (Shurtleff & Aoyagi, 2013). Defatted soy flour is obtained from solvent extracted flakes and contains less than 1% oil (Berk, 1992). Soy flour is normally prepared from dehulled, usually heat-processed whole soybeans or defatted soybean flakes (Shurtleff & Aoyagi, 2013). The levels of hydrocyanic acid in soybeans are presented in Table 37.

Table 37. The levels of hydrocyanic acid in soybeans

Chemical hazard	Raw material	Concentration (μg g^{-1})
Hydrocyanic acid	• Commercial raw defatted soy flour	• 0.08 ± 0.02
	• Soy protein isolate	• 0.18 ± 0.04
	• Whole soybean meal	• 0.26 ± 0.09

Adapted from "Determination of cyanide in soybeans and soybean products," (Honig et al., 1983, p. 274).

To estimate the risk of commercial defatted soy flour containing hydrocyanic acid addition to three types of dairy products, the maximum level of hydrocyanic acid and cyanide were used as described in Table 38. The prevalence of hydrocyanic acid in defatted soy flour is very low (Table E1.2).

Table 38. Maximum level (HBGV) of natural toxicants and contaminant

Natural toxicants	Food	Maximum level (mg/kg)	References
Hydrocyanic acid, Total	Confectionary	25	(FSANZ, 2016)
	Stone fruit juices	5	
	Marzipan	50	
	Ready-to-eat cassava	10	
	Alcoholic beverages	1 mg per 1% alcohol content	
Cyanide	Cassava flour	10	(JECFA, 2011a)

5.2.2.2. Addition to high solid content dairy product

Commercial raw defatted soy flour known to contain hydrocyanic acid at 0.08 mg kg^{-1} used as raw material undergoes several processes to mitigate hazards (storage, soaking, heat treatment (steaming), and drying to help control toxin levels. Heat treatment is the best mitigation technique to reduce hydrocyanic acid content (74-80% reduction). Although, drying has been shown to provide an 88% reduction, the effectiveness of this method can vary (13-88% reduction). Nevertheless, the risk ratio between the level of hydrocyanic acid after the addition to dairy products and the maximum residue limit resulted in a very low risk. Water is added to milk powder for consumption, which further dilutes the amount of hydrocyanic acid in the product. This means less risk than very low.

As discussed earlier, the maximum addition for a non-dairy ingredient in a high solid dairy product is assumed to be 50%. The risk estimate for the addition to high solid dairy products is presented in Table 39 and the calculation shown in Table E1.3 (Appendix E).

As shown in Table 39, the risk estimate for raw defatted soy flour added to milk powder is very low.

Table 39. Risk estimate for grain addition to high solid content dairy product

Chemical hazard	Raw material (mg kg^{-1})	Method-Residue (mg kg^{-1})	Addition to dairy products (mg kg^{-1})	Maximum residue limit (mg kg^{-1})	Risk level
Hydrocyanic acid	Commercial raw defatted soy flour 0.08	Storage 0.03-0.04	0.02	10	Very low
		Soaking 0.04-0.07	0.035		Very low
		Steaming 0.02	0.01		Very low
		Drying 0.02-0.07	0.035		Very Low

5.2.2.3. Addition to intermediate solid content dairy product

The maximum addition of the selected ingredients for intermediate solid dairy products is assumed to be 5%. Risk estimate for grain addition to intermediate dairy products is presented in Table 40, and the calculation is shown in Table E1.4 (Appendix E). Initially, the hydrocyanic acid content in the commercial raw defatted soy flour is very low (0.08 mg kg^{-1}). Different mitigation methods further reduce hydrocyanic acid content, which results in a rare risk. The addition of a small amount of cereal grains (2%) does not raise a safety concern.

Table 40. Risk estimate for grain addition to intermediate solid content dairy product

Chemical hazard	Raw material (mg kg^{-1})	Residue (mg kg^{-1})	Addition to dairy products (mg kg^{-1})	Maximum residue limit (mg kg^{-1})	Risk level
Hydrocyanic acid	Commercial raw defatted soy flour 0.08	Storage 0.03-0.04	0.002	5	Rare
		Soaking 0.04-0.07	0.0035		Rare
		Steaming 0.02	0.001		Rare
		Drying 0.02 - 0.07	0.0035		Rare

5.2.2.4. Addition to low solid content dairy product

The maximum addition of the selected ingredient for low solids dairy products (e.g. UHT milk/liquid breakfast product) is assumed to be 2%. Risk estimate for addition to low solids dairy products is presented in Table 41, and the calculation is shown in Table E1.5 (Appendix E). Addition to low solids dairy product poses a rare risk.

Table 41. Risk estimate for grain addition to low solid content dairy product

Chemical hazard	Raw material (mg kg^{-1})	Residue (mg kg^{-1})	Addition to dairy products (mg kg^{-1})	Maximum residue limit (mg kg^{-1})	Risk level
Hydrocyanic acid	Commercial raw defatted soy flour 0.08	Storage 0.03-0.04	0.0008	5	Rare
		Soaking 0.04-0.07	0.0014		Rare
		Steaming 0.02	0.0004		Rare
		Drying 0.02 - 0.07	0.0014		Rare

5.2.2.5. Risk estimate summary

A summary of risk estimate is shown in Table 42. The levels of hydrocyanic acid in defatted soy flour is very low. Food processing which uses risk mitigation strategies reduces the levels of hydrocyanic acid.

Table 42. Summary of risk estimation of defatted soy flour addition to dairy products

	Risk estimate		
	HCN in milk powder	HCN in parmesan cheese	HCN in liquid breakfast
In raw material (grain):	Very low (0.8%)	Very low (0.8%)	Very low (0.8%)
After food processing (storage, soaking, steaming or drying)	Very low (0.2-0.7%)	Rare (0.02-0.07%)	Rare (0.008-0.028%)

Note: HCN: hydrocyanic acid

5.2.2.6. Uncertainties, variabilities and assumptions

Two types of uncertainty exist in risk assessment: lack of knowledge and the randomness of nature (Benford & Tennant, 2012). The first type includes lack of knowledge regarding experience in many chemicals, the specificity of human metabolism, toxicity mechanism, limited models available, difficulties in obtaining a true exposure level and chemical interactions. The second type, the randomness of nature is a simple uncertainty.

Inadequate data regarding toxicological information and exposure for most of the inherent plant toxins and anti-nutrients is a limitation in conducting this study. Health-based guidance values (e.g. ADI, TDI, and NOAEL) are vital in assessing the severity and exposure assessment required to assess the probability. The ADI for most inherent food plant toxicants is not available due to no NOAEL values being generated from animal models. The possible reason is that there is little direct economic motivation to carry out

comprehensive toxicological tests of inherent food plant toxicants. Therefore, it is not possible to do an accurate risk assessment.

Cyanogenic glycoside is mostly studied in cassava and particularly in the African continent. This is because cassava is the staple food of many countries in Africa (Nhassico, Muquingue, Cliff, Cumbana, & Bradbury, 2008). The roots and leaves of cassava contain cyanogenic glycoside that is hazardous to human health. Attempts to reduce the cyanogenic glycoside concentration have been made through drying, cooking and boiling. Hence, risk mitigation of cyanogenic glycoside of cassava is well studied, but this is not the case for other plants, including many cereal grains.

Dietary exposure estimation of anti-nutrients and natural plant toxins from selected cereal grains in New Zealand was not possible to determine. However, the estimated chronic dietary exposures acquired from several plant-based foods could provide a background to the present study.

Factors identified to have an impact on dietary exposure, include variability in toxin levels, food matrices and cooking procedures (CFS, 2007). Toxin levels in cereal grains vary as a result of their species, growth condition, as well as, geographical aspects. Food matrices and cooking techniques may vary widely and, hence, affect exposure.

Several factors need to be considered regarding the number of toxins at the point of consumption (CFS, 2007). These include bioavailability, individual susceptibility and sensitivity to a plant toxin that may result in the appearance of adverse health reactions. Hence, the quantity of intake of that may pose adverse health risk may vary among individuals.

CHAPTER 6. DISCUSSION

This chapter discusses the results of microbial risk assessment of selected cereal grains (section 6.1) and microbial risk assessment of selected cereal (oats) added to three types of dairy products, i.e. milk powder, Parmesan cheese and liquid breakfast (section 6.2). Sections 6.3 discusses the results of chemical risk assessment of selected cereal grains. Section 6.4 discusses commercial raw defatted soy flour as the selected grain added to three types of dairy products, i.e. milk powder, Parmesan cheese and liquid breakfast. Lastly, section 6.5 discusses the limitations of the present study and recommendations.

6.1. Microbiological risk assessment of selected cereal grains

The risk assessment matrix was useful for identifying the most critical risks for microbiological hazards in selected cereal grains. The most critical risk microbiological hazard in the selected cereal grains is *Bacillus cereus* (*B. cereus*). *B. cereus* was the highest for both criteria (i.e. number of outbreaks and prevalence) in assessing the likelihood of a microbial hazard. The findings are in agreement with (Alldrick, 2017; K. L. Brown, 2000). According to (Alldrick, 2017; K. L. Brown, 2000), the most significant indigenous bacteria in cereal products are *Bacillus* spp. which includes *B. cereus*. They attributed this to the ability of *Bacillus* spores to activate after cooking (thermal shocks) followed by slow cooling and storage at room temperature causing outgrowth in the cooled cooked product.

B. cereus is among the micro-organisms that persist in low moisture conditions (MPI, 2015). Spores of this bacterium survive dry conditions and antimicrobial treatments providing a food safety risk (MPI, 2015). The result from the present study shows that, although the prevalence of *B. cereus* is high (up to 94%), the microbial load is relatively low (up to 29.8 CFU/g). However, this bacterium can cause sickness due to possible temperature abuse that allows the micro-organism to grow. A good example is *B. cereus* in cooked rice (FAO/WHO, 2014; Gilbert et al., 2010).

To assess the consequences of microbial hazards, a modified version of the ICMSF classification was used (ICMSF, 2018). This led to the categories ‘insignificant’, ‘minor’, ‘moderate’, ‘major’ and ‘severe’ being used. In spite of *B. cereus* scoring the highest microbial hazard, the severity of its consequences scored below *C. botulinum*, *Cronobacter* spp., *E. coli STEC* and *L. monocytogenes*. This is because the symptoms

associated with other pathogenic bacteria such as *C. botulinum* (cause infant botulism which can result in paralysis of the respiratory muscles, legs and trunk), *Cronobacter (*causes death in infants less than 6 months old with mortality rate among neonates up to 70%), *E. coli* O157:H7 (STEC) (which can lead to Haemolytic-uremic syndrome (HUS) in children which is characterised through renal failure and its consequences) and *L. monocytogenes* (a life threatening disease which can lead to abortion in pregnant women) are more severe than *B. cereus* (which causes diarrhoea and death is rare) (ICMSF, 2018). It is important to note that, up to date, none of these pathogens mentioned above has been associated with cereal grain related foodborne outbreaks in New Zealand.

Heat treatment is the common risk mitigation for the microbiological safety of cereal grains (Gilbert et al., 2010). Although heat treatment can eliminate most micro-organisms, it may induce spore germination (Alldrick, 2017; Lake et al., 2004). To avoid the spore germination after heat treatment, alternatives to heat treatment can be used. These alternatives include cold plasma, high hydrostatic pressure, ultrasonication, use of chemicals (fermented ethanol or supercritical carbon dioxide or sodium hypochlorite dip or citric acid dip), irradiation (microwave, gamma or electron beam) and combination other of treatments that have shown their effectiveness in reducing the contamination of *B. cereus*, *Salmonella*, *E. coli* and *S. aureus* in cereal grains (FAO/WHO, 2014; Los, Ziuzina, & Bourke, 2018). Details of recommended alternatives to heat treatments are shown in Appendix F (Table F1.1). Not all countries allow the use of gamma irradiation for food products. For example, Australia and New Zealand approve irradiation using gamma rays to a limited range of commodities such as herbs and spices, herbal infusions, and some fruits (e.g. blueberry, raspberry, persimmons) and vegetables (e.g. tomato, capsicum) (FSANZ, 2017a).

A risk ranking method using a risk-based control approach is useful for prioritizing hazards in food combinations (Van Asselt et al., 2012). The risk assessment matrix is one example of a risk-based control approach (Van Asselt et al., 2012), unlike other approaches such as multi-criteria decision analysis (MCDA). Recently, the MCDA approach was used to rank low moisture foods of greatest concern based on the microbiological food safety perspective by FAO/WHO (2014). Criteria used were international trade, burden of disease, vulnerabilities due to food consumption and vulnerabilities to food production. However, the MCDA was not used in this risk

assessment because the method is not a risk-based approach and criteria used are more applicable to policy makers (including government and international agencies) (Baltussen & Niessen, 2006) whereas the use of a risk assessment matrix has wider context and may be suitable for assessing the risks in food product development for the food industry.

The risk matrix provides a visualisation of the consequences and likelihood of occurrence of a hazard. To assess the likelihood of a microbial hazard, the prevalence of the hazard in a food and the number of outbreaks were used. The number of outbreaks criteria was taken to represent the burden of illness. The data from three different countries including Taiwan, New Zealand and the United States were used depending on the available data in the literature from 1991 to 2015. One limitation is the unavailability of data from the countries used in the time period assessed. For example, for Taiwan, data from 1991-2000 was available to be used whilst data from 1998-2015 was available to be used for the United States. A high number of outbreaks of *B. cereus* food poisoning associated with cereal grains has been shown in the US and Taiwan but not in New Zealand. A possible explanation for this might be that illness caused by *B. cereus* is not a notifiable disease in New Zealand (Lake et al., 2004).

The use of outbreak data in assessing the likelihood/probability may not represent the true burden of illness. Batz et al. (2005) reveals that outbreak data may contain inherent bias. Outbreaks that are large, have short incubation period, produce serious illness and involve food premises e.g. restaurants, tend to be investigated and reported. On the other hand, sicknesses caused by pathogens that are difficult to identify or do not often cause a large outbreak are underreported, hence understated. Another way to describe the burden of illness is by using Disability Adjusted Life Years (DALY) (McKenna, Michaud, Murray, & Marks, 2005). The DALY approach requires abundant data including the quantitative estimates of incidents, disease burden and the costs for a country in a specific time frame and these data are often limited (Mangen et al., 2015). In 2011, New Zealand adopted the US model to estimate the numbers of cases of illness, hospitalisations and deaths due to foodborne agents (Cressey & Lake, 2011). However, the authors claimed that the model is under development (Cressey & Lake, 2011).

This study was unable to perform a comprehensive exposure evaluation. The exposure evaluation results did not indicate the form/state of cereal products (food identification) and practices such as eating raw cake batter. It is important to note that cereal grains are

not often consumed directly in the form of grains (e.g. wheat grains) or their main processed product (i.e. flour). Instead, cereal grains are usually consumed in the form of secondary processed products including bread, biscuits, cakes and pasta. These secondary processed products involve heat-treatment or drying that will kill many micro-organisms (Alldrick, 2017).

6.2. Microbial risk assessment of selected cereal addition to dairy products

Cereal grains (oats) contaminated by *B. cereus* incorporated into high water activity dairy products such as milk pose a high risk to the safety of dairy products (Table 32). Conversely, low and intermediate moisture dairy products pose a low risk. Although *B. cereus* is unlikely to grow in the low and intermediate moisture dairy products, its spores, if they exist in raw material, can survive throughout the manufacturing process and may be present in the final product. This result supports the hypothesis that the addition of non-dairy origin ingredients to dairy products may pose microbiological risks depending on product's characteristics such as water activity.

The addition of *B. cereus* contaminated cereal grains to dairy products contaminated with *B. cereus* can exacerbate the risk already present from *B. cereus* that may naturally be found in milk. It is crucial for the dairy industry to ensure that cereal grains from suppliers comply with microbiological criteria for such ingredients.

Microbiological quality of raw material (cereal grains and milk) used in dairy products is paramount (FSANZ, 2006). This is because bacteria and fungi are capable of producing toxins or causing invasive illness especially when they exist in high numbers in raw material. For some toxin producing micro-organisms, heat treatment will inactivate the vegetative forms of the microorganisms however many toxins are heat stable and survive heat treatment. The only acceptable solution is to control the microbiological quality of cereal grain ingredients.

From the exposure assessment, the number of bacterial spores is low in the raw material. However, the prevalence shows that *B. cereus* spores are frequently reported in the dairy processing and manufacturing plant (Becker et al., 1994; Eneroth et al., 1998; Shaheen et al., 2010). Milk after pasteurisation and UHT has been found to contain *B. cereus* spores. However, their presence in UHT product would suggest faulty operations in the processing plant (Fernandes, 2009). This indicates the importance of maintaining suitable

holding times and appropriate temperature for heat treatment in the dairy industry. Moreover, the ability of *B. cereus* to form spores as well as grow in a temperature range (30-37 °C) (MPI, 2015) make it possible for this bacterium to thrive before and after pasteurisation and in the final product until consumption. Some *B. cereus* strains can grow up to 55 °C while others can grow as low as 4-5°C (Ehling-Schulz, Fricker, & Scherer, 2004; Lake et al., 2004).

Bacterial spores can be activated by several factors such as low pH, availability of nutrients and sub lethal heat (Lake et al., 2004). *B. cereus* and its spores occur naturally in most raw foods (Jay et al., 2005), including dry foods, dried herbs, and spices (MPI, 2015). The microbial load of *B. cereus* in raw material is relatively low (<100 spores/g or mL) (Heyndrickx, 2011) and it is impractical to eliminate low numbers of spores from foods. Therefore, Lake et al. (2004) suggests preventing spore germination and growth to a high numbers that threaten food safety.

Addition of non-dairy ingredients contaminated with bacterial spore to dairy products that are nutrient dense could lead to spore germination. Pasteurisation is the main method for microbiological control and in the dairy industry with high-temperature short time (HTST) treatment at 72 °C for 15 s as the standard pasteurisation conditions (Bylund, 2015). While this will not inactivate spores it will inactivate the vegetative cells that have resulted from spore germination. The holding time during heat treatment is a critical control point for ingredients added before heat treatment (Fernandes, 2009).

There is also the possibility of contamination after pasteurisation with contamination originating from the manufacturing equipment, packaging line, storage, distribution and consumer use (Becker et al., 1994; Y. Li et al., 2014; Salustiano et al., 2009; Yibar et al., 2017). There is some variability in the prevalence of post pasteurisation contamination depending on the conditions in the manufacturing plant and the country in which the studies were undertaken. In New Zealand the prevalence of post pasteurisation contamination is expected to be less than many other countries as New Zealand has good hygienic practices in the dairy industry including HACCP, GMP and GHP implementation.

The prevalence of contamination at the consumer level reflects the importance of risk communication to educate the consumer regarding proper food safety behaviour. For example: preparation, storage and handling of reconstituted milk should properly done by diluting the milk powder in water at a minimum temperature of 70 °C, consuming milk right after each preparation and storing reconstituted milk at <5 °C. Many foods need to be completely reheated before consumption; rapid and efficient cooling of cooked foods is needed for storage (Setlow & Johnson, 1997; Turck, 2012).

Regardless of the high incidence of *B. cereus* in milk, very few *B. cereus* associated foodborne outbreaks have been reported. Currently, there is no evidence of dairy product contamination with *B. cereus* as a concern to public health in New Zealand as *B. cereus* has not been associated with any foodborne outbreak related to dairy in New Zealand from 2007-2015. This may be due to several factors such as their presence in low number (10^2/g to 10^3/g), the presence of competitive microflora in dairy products and unfavourable growth conditions which do not allow them to grow to high numbers that can reach the infective dose (10^5-10^8/g) (Champagne et al., 1994; Granum & Lund, 1997); Spanu (2016). One of the characteristics of *B. cereus* is that it is a poor competitor allowing other spoilage microorganisms to overgrow and spoil dairy products before *B. cereus* becomes a risk. Spoiled dairy products marked with sour flavour prevent people from consuming the contaminated products.

6.3. Chemical risk assessment of selected cereal grains

This study evaluated the chemical risk assessment of selected cereal grains added to dairy products. The risk assessment matrix identified cyanogenic glycosides as the highest chemical risk. It is worth mentioning that there is very limited data about the anti-nutrients and natural plant toxins found in most cereal grains which can cause risk when added to dairy products. This is because most of the studies done to identify the chemicals only report their presence but not their concentration. Therefore, it is difficult to estimate the risk when the concentrations of the chemicals as well as the concentrations required to elicit negative response are not available. For example, several authors (I. E. Liener & Kakade, 1969; Peumans & Damme, 1998; Siener et al., 2006) found the presence of cyanogenic glycoside, lectins and protease inhibitors in barley; however, none of them mentioned their concentration. There have been very few studies which have been able to identify the anti-nutrients and natural toxins in plants as well as detecting their

concentrations. For example, Greiner and Konietzny (2006) found the levels of phytates in maize to be 9.8-21.3 mg g^{-1} and (Siener et al., 2006) found the concentration of oxalates in maize to be 38.6 mg 100 g^{-1}.

From the present study, the only natural toxin which has been found in most of the selected cereal grains (barley, maize, pearl millet, oats, rye, garden pea, soybeans) is cyanogenic glycoside. Its concentration in some cereal grains has also been reported. Chandra (2010) found the cyanogenic glycoside level to be 2 mg 100 g^{-1} in garden peas. In another study, the amount of detected cyanogenic glycoside was 0.26 ± 0.09 µg g^{-1} in whole soybean meal, 0.08 ± 0.02 µg g^{-1} in raw defatted soy flour, 0.18 ± 0.04 µg g^{-1} in soy protein isolate (Honig et al., 1983).

The available data and studies which have been done on cyanogenic glycosides (which is measured by the release of hydrocyanic acid) might depend on their toxicity in comparison to other chemicals. This has led to several international agencies such as JECFA to prioritise and monitor levels in foods. It is important to note that health-based guidance values (ARfD and TDI) are only used for hydrocyanic acid (JECFA, 2011a). JECFA has not yet established health-based guidance values for anti-nutrients such as genistein, protein inhibitors, tannins, saponins, lectins, goitrogens, phytates and oxalates. Also, the TDI for most inherent food plant toxicants has not yet been established due to the absence of NOAEL values being generated from animal models (Essers et al., 1998; Schilter et al., 2014). This highlights the possible reason why many studies have been conducted on cyanogenic glycosides but not the other chemicals.

Hydrocyanic acid/cyanide is toxic to humans and may result in acute cyanide poisoning (NZFSA, 2017a). Acute cyanide poisoning symptoms include rapid respiration, dizziness, mental confusion, twitching, convulsions and in extreme case, respiratory and cardiac arrest (Dolan et al., 2010; Lawley et al., 2008). Acute cyanide poisoning has been reported to be associated with the consumption of cyanogenic plants such as cassava, apricot pits, bitter almonds and apple seeds (Davis, 1991; NZFSA, 2017a). Most of the research work that has been conducted has focused on those plants (CFS, 2007; EFSA et al., 2019; FSANZ, 2014b) which are the main established sources and not selected cereal grains.

The health effects of hydrocyanic acid (some of which have been mentioned above) outweigh that of other anti-nutrient compounds. Generally, anti-nutrient compounds have no inherent toxicity but may restrict the absorption of dietary nutrients which lead to nutrient deficiency (Cressey & Thomson, 2007). For example, oxalate can bind with minerals such as calcium to form a complex (calcium oxalate). This complex is capable of causing hypocalcaemia (low calcium in human blood serum) and hyperoxaluria (extreme urinary excretion of oxalate). Hyperoxaluria is a primary risk factor in the development of calcium oxalate stone disease (Siener et al., 2006). Anti-nutrients such as lectins could elicit symptoms such as nausea, vomiting, bloating and diarrhoea (Lawley et al., 2008).

Stress factors have been found to affect the levels of cyanogenic glycosides in plants. These include climatic conditions and addition of fertilisers. Cold and humid weather have been reported to increase the cyanogenic glycoside content in young plants. Fertiliser application to plants has been reported to increase the level of cyanogenic glycoside in plants. On the other hand, post-harvest processes such as drying and crushing have been reported to reduce the level of cyanogenic glycosides (Haschek et al., 2013).

The knowledge about the harmful effects of natural toxins found in plants such as cyanogenic glycosides has been known many years ago. Several authors have reported control measures which can be used to reduce the level of these natural toxins. Control measures which have been used to reduce the levels of cyanogenic glycoside in plants include soaking, fermentation, steaming and drying since these treatments are able to leach out cyanogenic glycosides. A study by Obilie et al. (2004) was able to reduced cyanogenic glycosides content by 74-80 % through steaming.

In New Zealand, two cases of cyanide poisoning, associated with apricot kernel consumption have been reported (Cressey & Thomson, 2007; NZFSA, 2017a). In 2001, Waikato hospital reported that 30 apricot kernels (3 mg cyanide g^{-1} kernel) were the cause of cyanide poisoning. In 2006, one woman was hospitalised after ingesting a mixture of 60 ground apricot kernels and orange juice. These incidences led to a chemical survey on cyanogenic glycosides in New Zealand plant based foods (Cressey et al., 2013). The foods which were used in the survey were cassava, pome fruit products (apple), bamboo shoots, almond and almond products, stone fruit products, flaxseed/linseed and miscellaneous products such as taro and vine leaves, spinach, passion fruit and passion fruit products.

From the survey, they found out that cyanogenic glycosides were present in most foods sampled which raises a food safety concern in New Zealand. Their study, however, did not include cereal grains such as rice, wheat and soybeans and therefore, there is no available literature about the risks of cyanogenic glycoside in cereal grains in New Zealand. Further investigation about the risk of cyanogenic glycoside in cereal grains will be helpful for the New Zealand food industry.

In this risk assessment of cereal grains, the qualitative measures of severity and likelihood were used to assess the risk of hydrocyanic acid contamination. This was the only chemical which had the highest score in the risk assessment matrix and therefore, it was regarded as the most critical chemical risk in cereal grains. Other chemicals which were included in the risk assessment matrix such as genistein, oxalate, goitrogen, phytates, lectins, saponins, protease inhibitor and tannins were all classified as low.

6.4. Chemical risk assessment of selected grain addition to dairy products

In this risk assessment, the addition of cereal grains containing hydrocyanic acid to three types of dairy products were evaluated. The three types of dairy products were selected based on their solid contents: high, intermediate and low solid contents. Milk powder was chosen as an example of a high solid product whereas Parmesan cheese was selected as intermediate solid product. Liquid breakfast was used as low solid product.

In this risk assessment, soybean was chosen as the selected cereal grain to be added to dairy products due to its popular use in the food industry as well as its high nutrient content (Shurtleff & Aoyagi, 2013). From the risk characterisation, the addition of commercial raw defatted soy flour containing hydrocyanic acid to milk powder was found to be very low. This is because the initial concentration of hydrocyanic acid in raw defatted soy flour (which is very low) coupled with control measures (soaking, heat treatment, drying) used in the industry are able to reduce the content of hydrocyanic acid (70-80% reduction).

In the risk characterisation, the addition of commercial raw defatted soy flour to both low and intermediate solid content dairy products was found to be rare. This is because the maximum amount of soy flour which is allowed to be added to low and intermediate solid content product is 2% and 5%, respectively (Abernethy & Lindsay, 2019). This represents the percentage of soy flour which can be added since total solid content in both low and

intermediate solid content dairy product should not exceed the given amount. This makes the concentration of hydrocyanic acid found in the soy flour to be added negligible. Also, control strategies employed in the manufacturing process by the industry are capable of reducing the content of hydrocyanic acid further. These factors contributed to them being classified as rare.

The forms of raw material used in the dairy industry play an important role. Flour is a milled product that undergoes several processes that might reduce the hydrocyanic acid level. Soybeans as raw material are available in the form of whole soybean meal, commercial raw defatted soy flour and soy protein isolate which have variable hydrocyanic acid content (Honig et al., 1983).

6.5. Limitations and recommendation for future work

There are some disadvantages in the use of the risk matrix tool. Risk matrices are predicted to be less accurate than other techniques which use a quantitative approach by considering concentration data and dose-response relationships or toxicological reference values (Elmontsri, 2013; van der Fels-Klerx et al., 2018). Another limitation of using a risk matrix is the subjectivity of the consequence levels. The risk matrix may be a blunt tool and often requires a number of value judgements to be made, which have the potential to bias the assessment. Nevertheless, the risk matrix can be used as a preliminary step in the prioritisation of risk (Cressey, 2019, May 31).

There is limited information about risk assessment of non-dairy ingredient addition to dairy products. Nonetheless, many products that combine non-dairy ingredients with dairy ingredients are sold worldwide. This supports the importance of conducting a risk assessment to get an overview of safety in these products. The present study was unable to provide a risk estimate of microbial and chemical hazards for New Zealand due to unavailability of local information. Hence, this risk assessment gives a general idea on the global scale of non-dairy ingredient addition to dairy products. Some of the references regarding dairy products contamination with *B. cereus* were documented more than ten years ago which may be irrelevant anymore due to improvement in dairy processing.

The present study highlights the significance of *B. cereus* contamination in cereal grains and dairy products. Complete removal of this bacterium through decontamination processes is not possible. The food industry must apply proper handling and storage of

cereal grains as well as dairy products to prevent the proliferation of *B. cereus* to levels that can cause foodborne illness. It is, therefore, recommended to carry a quantitative risk assessment as well after addressing the knowledge gaps.

The present study provides a foundation for future work. This study was able to identify knowledge gaps for future work in microbiological risk assessment. There is lack of studies on prevalence data for pathogens such as *C. botulinum*, *C. perfringens*, *L. monocytogenes* and *Shigella* spp. in the selected cereal grains in New Zealand, including those which are domestically produced or imported. In order to improve the exposure assessment, predictive modelling is needed for a real overview on the level of *B. cereus* from the farm to fork. Information regarding consumption frequency and serving sizes of skim milk powder, Parmesan cheese and liquid breakfast is also required.

The present study was able to identify information gaps for future work in chemical risk assessment. There is a need to conduct laboratory testing to determine the concentration of each anti-nutrient and natural plant toxins in selected cereal grains to perform the risk assessment accurately.

CHAPTER 7. CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusions

The most critical microbiological hazard in the selected cereal grains is *Bacillus cereus*. This bacterium is a micro-organism that persists in low moisture conditions in products such as cereal grains. Spores of this bacterium survive in both dry conditions and antimicrobial treatments providing a food safety risk. Therefore, it is recommended to prevent spore germination and prevent multiplication of bacterial cells.

The addition of cereal grains to dairy products poses a microbial risk. Oats contaminated with *Bacillus cereus* added to milk powder or Parmesan cheese were found to pose a low risk, whereas their addition to liquid cereal was found to be a high risk. There is the possibility of post-pasteurisation contamination originating from the manufacturing equipment, packaging line, storage, distribution and consumer use. This indicates the importance of this issue and the need for the dairy industry to ensure effective implementation of HACCP, GMP and GHP. A high prevalence of contamination at the consumer level indicates the importance of risk communication to educate consumers regarding appropriate food safety behaviour.

Cyanogenic glycosides (hydrocyanic acid) were found to be the most critical chemical hazard among natural plant toxins in selected cereal grains. Inherent plant toxicants raise more safety concerns than the synthetic chemicals due to their potency and high likelihood of exposure. In addition, the margin of safety between the actual exposure/intake and the level documented for adverse reactions in humans is low. However it has been identified that there is very limited information about the presence and concentration of cyanogenic glucosides in cereal grains. Thus, this risk assessment has provided some important information in helping to identify the gaps in knowledge relating to a high level of uncertainty in the risk assessment of cereal grains added to dairy products.

From this study, the addition of cereal grains pose a chemical risk to dairy products. Commercial raw defatted soy flour containing hydrocyanic acid addition to milk powder was found to pose a very low risk, while the addition to both Parmesan cheese and liquid breakfast was found to be rare.

This risk assessment highlighted the importance of decontamination of non-dairy ingredients before their addition to dairy products. Heat treatment used as a critical control point in dairy processing, in addition to the implementation of good hygienic practices, appropriate cleaning procedures in the manufacturing plant and the maintenance of the cold supply chain in storage and distribution, were all found to be very effective to in reducing both microbiological and chemical risks.

7.2. Recommendations

1. A comprehensive survey for the risk assessment for the addition of non dairy ingredients to dairy products is required. This is because there is a lack of information around the contamination of grains, particularly for New Zealand.
2. Further research is needed to evaluate cereal grains other than wheat, corn and rice (e.g. millet, black glutinous rice, brown rice and legumes such as adzuki beans, garden peas and hyacinth beans) as potential sources of bacteria pathogens such as *Salmonella* and *Clostridium perfringens*.
3. Validation of this risk assessment with laboratory testing to determine the concentration of each anti-nutrients and natural plant toxins in selected cereal grains.
4. To set up a database to streamline update work and access to collated data. Data should include any incident that occurred due to natural plant toxin ingestion as well as how the cereal grains are normally consumed or incorporated into commercial food products, especially dairy products. Stakeholders, including government, universities and research agencies, can work together to obtain useful data regarding anti-nutrients and natural plant toxins.

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APPENDICES

Appendix A. Literature research procedure

The search strategy encompasses three main steps. Combination of search strings were used, first with a wide screening for methods for risk-ranking and prioritisation in food-related issues, then narrowing down to the microbiological and chemical hazard in non-dairy ingredients including selected cereal grains

Step 1: acquired methods and tools for risk ranking/risk prioritisation

TOPIC = risk* OR hazard*

TITLE = rank OR method* OR tool* OR categor* OR matric* OR "risk matrix"

TOPIC = food* OR agri* OR agro*

Step 2: acquired microbiological hazards in non-dairy ingredients including cereals, pseudocereals, and grain legumes. Search strings used were as follows:

TOPIC = "risk assessment" OR assessment OR "food safety hazard*" OR hazard* OR "food safety" OR safety OR outbreak* OR poisoning OR illness* OR "risk mitigation" AND

TOPIC = microbio* OR bacter* OR "*Bacillus cereus*" OR "B. cereus" OR "*Clostridium botulinum*" OR "*C. botulinum*" OR "*Clostridium perfringens*" OR "*C. perfringens*" OR "*Escherichia coli*" *OR* "*E. coli*" OR "*Listeria monocytogenes*" OR "*L. monocytogenes*" OR "*Salmonella* spp." OR "*Shigella* spp." OR "*Staphylococcus aureus*" OR "*S. aureus*" AND

TOPIC = "non-dairy ingredient*" OR cereal OR "cereal grain*" OR "pseudocereal grain*" OR "grain legume*" OR barley OR "hordeum vulgare" OR maize OR corn OR "zea mays" OR millet OR "pennisetum glaucum" OR oats OR "avena sativa" OR rye OR "secale cereal" OR rice OR "oryza sativa" OR "black glutinous rice" OR "brown rice" OR wheat OR "wheat flour" OR "wheat germ" OR "triticum aestivum" OR buckwheat OR "fagopyrum esculentum" OR "adzuki bean*" OR "red mung bean" OR "vigna angularis" OR "garden pea*" OR "pisum sativum" OR "hyacinth bean*" OR "lablab purpureus" OR "mung bean*" OR "vigna radiate" OR soybean* OR "black soybean*" OR "glycine max"

Step 3: acquired chemical hazards in non-dairy ingredients including cereals and grains. Search strings used were as follows:

TOPIC = "risk assessment" OR assessment OR "food safety hazard*" OR hazard* OR "food safety" OR safety OR outbreak* OR poisoning OR illness* OR "risk mitigation" AND

TOPIC = chemical* OR "natural toxin*" OR "plant toxin" OR "inherent plant toxicant*" OR phytotoxin OR "cyanogenic glycoside*" OR lectin* OR phytates* OR "oxalic acid" OR mycotoxin* OR "pesticide*" OR "heavy metal*" OR allergen* AND

TOPIC = "non-dairy ingredient*" OR cereal OR "cereal grain*" OR "pseudocereal grain*" OR "grain legume*" OR barley OR "hordeum vulgare" OR maize OR corn OR "zea mays" OR millet OR "pennisetum glaucum" OR oats OR "avena sativa" OR rye OR "secale cereal" OR rice OR "oryza sativa" OR "black glutinous rice" OR "brown rice" OR wheat OR "wheat flour" OR "wheat germ" OR "triticum aestivum" OR buckwheat OR "fagopyrum esculentum" OR "adzuki bean*" OR "red mung bean"

OR "vigna angularis" OR "garden pea*" OR "pisum sativum" OR "hyacinth bean*" OR "lablab purpureus" OR "mung bean*" OR "vigna radiate" OR soybean* OR "black soybean*" OR "glycine max"

Step 4: acquired occurrence and prevalence of microbiological hazards in non-dairy ingredients including cereals and grains. Search strings used were as follows:

TOPIC = "prevalen*" OR "microbial survey*"OR "microbiological survey*" OR survey* AND

TOPIC = microbio* OR bacter* OR "*Bacillus cereus*" OR "B. cereus" OR "*Clostridium botulinum*" OR "*C. botulinum*" OR "*Clostridium perfringens*" OR "*C. perfringens*" OR "*Escherichia coli*" OR "*E. coli*" OR "*Listeria monocytogenes*" OR "*L. monocytogenes*" OR "*Salmonella* spp." OR "*Shigella* spp." OR "*Staphylococcus aureus*" OR "*S. aureus*" OR Cronobacter OR "*Enterobacter sakazakii*" OR "*E. sakazakii*" AND

TOPIC = "non-dairy ingredient*" OR cereal OR "cereal grain*" OR "pseudocereal grain*" OR "grain legume*" OR barley OR "hordeum vulgare" OR maize OR corn OR "zea mays" OR millet OR "pennisetum glaucum" OR oats OR "avena sativa" OR rye OR "secale cereal" OR rice OR "oryza sativa" OR "black glutinous rice" OR "brown rice" OR wheat OR "wheat flour" OR "wheat germ" OR "triticum aestivum" OR buckwheat OR "fagopyrum esculentum" OR "adzuki bean*" OR "red mung bean" OR "vigna angularis" OR "garden pea*" OR "pisum sativum" OR "hyacinth bean*" OR "lablab purpureus" OR "mung bean*" OR "vigna radiate" OR soybean* OR "black soybean*" OR "glycine max"

Step 5: acquired risk mitigation/intervention strategies for microbiological hazards in cereals and grains. Search strings used were as follows

TOPIC = "mitigation*" OR "risk mitigation" OR intervention OR heat OR "non-heat" OR microwave OR "high hydrostatic pressure" OR "cold plasma" OR "pulsed UV light" OR "irradiation" OR "organic acid*" OR chemical* AND

TOPIC = microbio* OR bacter* OR "*Bacillus cereus*" OR "B. cereus" OR "*Clostridium botulinum*" OR "*C. botulinum*" OR "*Clostridium perfringens*" OR "*C. perfringens*" OR "*Escherichia coli*" OR "*E. coli*" OR "*Listeria monocytogenes*" OR "*L. monocytogenes*" OR "*Salmonella* spp." OR "*Shigella* spp." OR "*Staphylococcus aureus*" OR "*S. aureus*" OR Cronobacter OR "*Enterobacter sakazakii*" OR "*E. sakazakii*" OR spore* OR "spore-forming" AND

TOPIC = "non-dairy ingredient*" OR cereal OR "cereal grain*" OR "pseudocereal grain*" OR "grain legume*" OR barley OR "hordeum vulgare" OR maize OR corn OR "zea mays" OR millet OR "pennisetum glaucum" OR oats OR "avena sativa" OR rye OR "secale cereal" OR rice OR "oryza sativa" OR "black glutinous rice" OR "brown rice" OR wheat OR "wheat flour" OR "wheat germ" OR "triticum aestivum" OR buckwheat OR "fagopyrum esculentum" OR "adzuki bean*" OR "red mung bean" OR "vigna angularis" OR "garden pea*" OR "pisum sativum" OR "hyacinth bean*" OR "lablab purpureus" OR "mung bean*" OR "vigna radiate" OR soybean* OR "black soybean*" OR "glycine max"

Appendix B. Hazard characterisation of microbiological risk assessment

1. *Bacillus cereus*

Bacillus cereus (*B. cereus*) is a Gram-positive, facultative aerobic, spore-forming organism which is extensively spread in nature, thus is readily isolated from soil, dust, vegetation, cereal produces, water, air and sediment (FSAI, 2016; MPI, 2015). *B. cereus* and its spores occur naturally in most raw foods (Jay et al., 2005), including dry foods, dried herbs, and spices (MPI, 2015). According to Glasset et al. (2016), food-borne outbreaks by *B. cereus* in France from 2007 to 2014 were associated with vegetables and starchy foods such as rice.

1.1. Bacterial growth

B. cereus can grow in the pH range of 4.5 to 9.5 with an optimum pH 6 to 7. It requires a minimum water activity between 0.93-0.95 in the presence of NaCl and water activity of 0.93 with glycerol. The microorganism can grow at temperatures of 4 to 55 °C and optimum 30-37 °C, while the emetic strains need a minimum temperature of 10 °C (Ehling-Schulz et al., 2004). *B. cereus* is capable of producing toxin at temperatures of 10-40 °C and with maximum toxin production at 20 to 25 °C (MPI, 2015).

1.2. Disease characteristic

B. cereus causes two types of foodborne illness, diarrhoeal or emetic syndromes (MPI, 2015). The emetic syndrome occurs because of emetic toxins (cereulide) ingestion that is formed when the vegetative cell count exceeds 10^5 CFU/g. Importantly, the toxins are highly stable (minimum two months at 4° C), heat resistance (90 min at 126 °C), pH resistant ($2 \leq pH \leq 11$) and unaffected to proteolytic enzymes (IDF, 2016). Symptoms of an emetic syndrome include vomiting, nausea, malaise and is sometimes followed by diarrhoea, appearing within six hours after consumption of food contaminated with the pre-formed toxin (Rajkovic, 2014). Emetic syndrome symptoms are similar to illness caused by *Staphylococcus aureus* (Glasset et al., 2016). Duration of sickness is 6 to 24 hours.

Diarrhoeal syndrome arises due to ingestion of bacterial cells that further create enterotoxins in the small intestine. Symptoms such as occasional nausea, abdominal pain, and watery diarrhoea generally appear within 8 to 16 hours (FSAI, 2016). The infection happens when the concentrations of *B. cereus* surpass 10^6 CFU/g in the food and adequate amounts of the enterotoxins are formed. The enterotoxins are heat-labile and sensitive to acid conditions or proteolysis (MPI, 2015).

1.3. Dose-response

Diarrhoeal syndromes are often linked to *B. cereus* counts of 10^5 to 10^8 cells or spores (Granum & Lund, 1997). Before toxins are detected in the food, a large number of viable cells (10^5 to 10^8 /g) is required. A very low emetic toxin level in the range of 0.01 to 1.28 µg/g was associated with an outbreak in Japan (Agata et al., 2002). Another measure of emetic toxin level of 8 µg/kg body weight has been proposed as the intoxication dose (Paananen et al., 2002). The diarrhoeal syndrome is often associated with meat, vegetables, milk and milk products (Pexara & Govaris, 2010). Emetic intoxication is often linked with the consumption of raw starchy foods such as rice, noodles, pasta, pastries, and potatoes (Pexara & Govaris, 2010). Cooked or fried rice is involved in 95% of emetic cases. Lesson learned from the food poisoning cases associated with cereal-based products is not to let the foods cool down slowly and not to store in the range of 10 to 50°C as this causes the spores to germinate and multiply up to level enough to cause illness (MPI, 2015).

2. *Clostridium botulinum*

Clostridium botulinum (*C. botulinum*) is a Gram-positive, anaerobic bacterium which is commonly found in soil and marine sediment. *C. botulinum* can contaminate crops cultivated in or on the soil (MPI, 2017c). It typically exists in the form of dormant spores, but, once it gets into a favourable condition, the spores propagate into active bacteria and produce toxins. Vegetative cells of *C. botulinum* and sometimes *C. butyricum* and *C. baratii* bacteria produce a toxin which is known as Botulinal neurotoxin (BoNT) (CDC, 2017). There are seven types of toxin (A through G), which are believed to be the most potent toxins known, including A, B, E and F types which cause botulism in humans.

2.1. Bacterial growth

C. botulinum can grow at temperatures of 10 °C – 48 °C, with optimum 35-40 °C. Group I which produces of toxins A, B and F grow at pH 4.6 and water activity of 0.94 in 10% NaCl. Similarly, group II which produce toxins B, E and F grow at pH of 5 and water activity of 0.97 in 5% NaCl (MPI, 2017c).

2.2. Disease characteristic

Foodborne botulism is a severe intoxication caused by ingestion of foods contain BoNT. Botulism was formerly associated with the consumption of preserved low acid and low oxygen foods such as canned foods. BoNT affects the central nervous system and can cause breathing difficulties, muscular paralysis, and even death due to respiratory failure. There are five clinical classifications of human botulism: foodborne botulism; wound botulism; adult infectious botulism; infant botulism; and other types of intoxication such as botulinum toxin injection (WHO, 1999).

Symptoms of botulism include nausea, diarrhoea, vomiting, and paralysis of the eyes, mouth, throat and eventually, muscles within 12 to 36 hours after consumption. *C. botulism* can grow and produces toxins in the intestines of babies and causes infant botulism with symptoms of constipation, fatigue, floppiness and breathing difficulties (MPI, 2017c).

Nowadays, the rate of dying from botulism is lower because of the development of antitoxins and modern medical care. It has reduced from 50/100 to <5/100 people dying dying with botulism. However, some patients still die because of infections or other problems caused by being paralysed for several weeks or months. Patients that survive from botulism still have fatigue and breathing difficulties for years and may require therapy (CDC, 2017).

2.3. Dose-response

The dose for type A and B toxins to cause death in human are estimated between 0.1 and 1.0 µg (ICMSF, 1996a) while the dose for types E and F toxins are roughly 10 µg (Bell & Kyriakides, 2000).

3. *Cronobacter* spp.

Cronobacter, previously known as *Enterobacter sakazakii* (*E. sakazakii*), is a Gram-negative, facultative anaerobic, rod-shaped, non-sporulating pathogenic bacterium which can cause foodborne sickness, mainly to infants and immunocompromised adults. This bacterium can cause meningitis, bacteraemia and necrotising enterocolitis (FDA, 2012a). *E. sakazakii* was reclassified into *Cronobacter* genus which comprises of six species: *Cronobacter sakazakii*; *C. malonaticus*; *C. turicensis*, *C. muytjensii* and *C. dublinensis*. Cronobacter have been isolated from environments such as domestic environments, manufacturing plants, foods (e.g. Powdered Infant Formula (PIF), fermented bread and cheese) (FSAI, 2011a).

3.1. Bacterial growth

Cronobacter spp. can grow at temperatures of 6 to 45 °C with an optimum temperature 37-43 °C. Generation time at 22 °C is 37-44 minutes (FSAI, 2011a).

3.2. Disease characteristic

The infection generally has a case-fatality rate ranging from 10-80%. New born infants are at risk, with infants older than 6 months hardly affected. Premature or low birth weight infants have higher case fatality rates. The highest mortality was reported in healthy term infants who suffered septicaemia. In infants, symptoms occur in a few days. The disease in adults is not common and food sources usually have not been determined (FDA, 2012a).

Symptoms are frequently severe and may include poor feeding response, jaundice, irritability, seizures, and fluctuation of body temperature, brain abscess, developmental delay and hydrocephalus. Duration of symptoms varies from 2 to 8 weeks. Death may occur within a few hours to several days after sepsis (FDA, 2012a).

3.3. Dose-response

The infectious dose of *Cronobacter* has not been determined. However, scientists estimated the dose might be similar to *E. coli* O157:H7 i.e. 10 to 100 micro-organisms (FDA, 2012a; FSAI, 2011a).

4. *Escherichia coli* O157: H7

Escherichia coli (E. *coli*) is a Gram-negative bacterium that naturally inhabit the gastrointestinal tract of humans and other warm-blooded animals. Most *E. coli* strains are not likely to cause harm, but some forms can cause severe disease. Shiga toxin-producing *E. coli* (STEC), also known as verocytotoxigenic *E. coli* (VTEC), are virulent and is responsible for the majority of human illness (NZFSA, 2017b).

4.1. Bacterial growth

E. coli can grow at temperatures of 7-8 ^{o}C to 46 °C with an optimum temperature of 37 °C. They grows at pH of 4.4 to 9.0 with optimum pH of 6 to7. *E. coli* require a minimum water activity of 0.95 and optimum growth is observed at 0.99 (NZFSA, 2017b).

4.2. Disease characteristic

STEC attacks the gut and then produces a toxin that causes infection. STEC infection is characterised by mild or severe diarrhoea and abdominal pain that occurs 3 to 9 days (with a mean of 4 days) after ingestion. Infants under four years and older people above 65 years are at risk as they can acquire a fatal condition such as acute kidney disease (NZFSA, 2001a).

This disease has severe forms, such as haemorrhagic colitis (HC), haemolytic uremic syndrome (HUS), and thrombocytopenic purpura (TTP). HC symptoms are severe stomach pain, bloody diarrhoea, vomiting. HUS took place after HC and resulted in renal dysfunction, seizures, coma and death. HUS generally affects children and occurs in approximately 10% of children infected by *E. coli* O157: H7. Fortunately, the fatality rate can be reduced to less than 10% if the appropriate care is given (NZFSA, 2001a).

TTP is a form of HUS that commonly happens in the elderly. TTP symptoms are HUS symptoms and also the loss of platelets, seizures, and stroke. Duration of illness is two to nine days. Hospitality rate is one-third of cases. Long-term effects of HUS are problems related to kidney, hypertension and neurological deficiency. The death rate in the USA is less than 5% and around 1% for New Zealand (NZFSA, 2001a).

4.3. Dose-response

The dose of 0.3 to 0.4 cells/g has been associated with outbreaks. The amount of cells needed to produce a 50% probability of disease has been predicted at 5.9 x 10^5 CFU/g (NZFSA, 2001a).

5. Listeria monocytogenes

Listeria monocytogenes (*L. monocytogenes*) is naturally found in soil and water (NZFSA, 2001b).

5.1. Bacterial growth

L. monocytogenes can grow at temperatures of 1.5 to 45 °C with an optimum at a temperature of 37 °C. It can grow at the pH range of 4.4 to 9.4 with optimum pH at 7. This pathogen requires water activity of 0.92 to grow in 11.5% NaCl solution (NZFSA, 2001b).

5.2. Disease characteristic

L. monocytogenes can cause two kinds of disease, i.e. the invasive (listeriosis) and a non-invasive (febrile gastroenteritis). The invasive usually occurs in susceptible groups, while, the non-invasive disease can occur to the general population due to ingestion of a high number of *L. monocytogenes* cells (>10^5 cells/g) (MPI, 2017b).

Listeriosis and febrile gastroenteritis have similar symptoms. Febrile gastroenteritis disease is gastroenteritis related to mild 'flu-like' symptoms (such as a headache and fever) and other symptoms of non-invasive illness including muscle pain, diarrhoea and less common for vomiting and abdominal pain with a duration of 11 hours to 7 days (MPI, 2017b). Symptoms of listeriosis include diarrhoea, vomiting, fever, headache, septicaemia, meningitis, and spontaneous abortion in pregnant women. Duration of listeriosis is one to 90 days, and hospitalisation rate is high (92%).

Listeriosis rarely occurs but is potentially life-threatening. Compared to salmonellosis and campylobacteriosis; listeriosis has a high death rate (approximately 30%) especially for the immune-weakened people such as newborn babies, pregnant women, older adults and immunocompromised people. In pregnancy, *Listeria* infection has mild symptoms, but it can cause miscarriage, premature birth or severe disease in a newborn child (MPI, 2017b).

5.3. Dose-response

Estimated dose to cause illness for invasive disease is estimated to be lower (100-1000 cells) than non-invasive disease (>10^5 cells/g) (MPI, 2017b).

6. *Salmonella* spp.

Salmonella are a Gram-negative, non-spore former, rod-shaped bacteria under the family Enterobacteriaceae. *Salmonella* are extensively distributed in nature. They inhabits the gastrointestinal tract of humans and animals such as cattle, pets and wildlife. In addition, and may be found in the sediment of pond-water. *Salmonella* may contaminate the soil, water, meat, food processing equipment, hands, and utensils (FDA, 2012b).

There are two species of non-typhoid Salmonellae, i.e. *Salmonella enterica* and *Salmonella bongori* (García & Heredia, 2009). *Salmonella enterica* has six subspecies (enterica, arizonae, salamae, houtanae, diarizonae, and indica), where the most significant subspecies is *S. enterica* subspecies *enterica* because it can cause foodborne disease (Lawley et al., 2008).

Salmonella may contaminate cereals through animal or human faecal material. Post-harvest contamination by rodents and birds may occur when the storage is insufficiently maintained. Insufficient storage means that storage facilities do not have a program to prevent rodent and bird to enter the storage room and defecate there. Cereals and their milled products have low water activity that suppresses the growth of *Salmonella*, but, it encourages the heat resistance of Salmonella. (Gilbert et al., 2010; NZFSA, 2001c).

6.1. Bacterial growth

Salmonella is a mesophilic bacterium which means that it can multiply at a temperature of 4 to 15 °C with optimum growth at 35-37 °C (García & Heredia, 2009; NZFSA, 2001c). Moreover, it can grow in the pH range of 3.6 to 9.5 with the optimum pH of 7 to 7.5. It requires water activity

of 0.94 and maximum growth with water activity above 0.99. Nevertheless, *Salmonella* can survive in dehydrated environments for months (NZFSA, 2001c).

6.2. Disease characteristic

Non-typhoid Salmonellae cause a foodborne illness known as salmonellosis. It is a gastrointestinal disease with symptoms such as diarrhoea, nausea, vomiting, abdominal cramps and fever that could last 1 to 7 days. The incubation period for salmonellosis is 6 to 48 hours, but commonly 12 to 36 hours (Lawley et al., 2008). The susceptible group consists of older people, infants, and people with the weakened immune system may develop septicaemia and reactive arthritis in the long term (NZFSA, 2001c). The hospitalisation rate is predicted at 22.1%. Mortality rate of non-typhoid *Salmonella* is estimated at 0.8% and the rate could be higher for the elderly (Lawley et al., 2008; NZFSA, 2001c)

6.3. Dose-response

The dose of non-typhoid *Salmonella* required to cause illness are varies, and many factors are involved such as individual susceptibility, type of food and serotype. Ingestion of food containing 10-100 *Salmonella* cells can cause sickness in the elderly or young. The infective dose at low attack rates is between 4 to 45 cells, while at a high attack rate is generally in the range of 10^5 to 10^6 cells (Gilbert et al., 2010; NZFSA, 2001c).

The risk of contaminated cereal grains causing human salmonellosis is considered as low. An outbreak associated with flour suggests that it is likely to impact large numbers of people although is caused by unusual consumer behaviour such as consumption of uncooked home baking materials (Gilbert et al., 2010).

7. *Shigella* spp.

Shigella spp. comprises four species: *S. dysenteriae*, *S. boydii*, *S. flexneri*, and *S. sonnei* (ECDC, 2017).

7.1. Bacterial growth

Shigella spp. can grow at temperatures of 6-7 ^oC to 45-47 ^oC. They require a water activity at 0.96 (Duckworth, 2012). This microorganism can grow at a minimum pH of 4.8-5.0 in 3.8-5.2% NaCl solution, pH of 5.5 in the presence of 300-700 mg/litre $NaNO_2$, and maximum pH of 9.3 in 5.2% NaCl solution (NZFSA, 2001d).

7.2. Disease characteristic

Shigella spp. can cause an illness called bacillary dysentery or shigellosis (FDA, 2012c). It has an incubation period of 12 hours to four days. Shigellosis is a gastrointestinal infection described as diarrhoea where faeces contain mucus and sometimes blood coupled with fatigue, fever, abdominal pain, and malaise. In three days, the illness may develop to a colonic phase that is characterised by intense cramps with repeated and painful bowel movements that continue to happen for 3 to 14 days.

Shigella may cause severe disease in infants, older people, or immunocompromised people including cancer, diabetes, HIV/AIDS, and kidney failure disease patients (CDC, 2010). No toxin is produced in foods. Septicaemia is a severe bloodstream infection that may happen to individuals with a weakened immune system (NZFSA, 2001d).

7.3. Dose-response

The dose required to cause infection is estimated at 10-100 cells (NZFSA, 2001d).

8. Staphylococcus aureus

Staphylococcus aureus (*S. aureus*) is an abundant micro-organism present on the skin and mucous membranes of humans and also most warm-blooded animals such as cows (NZFSA, 2001c). It is usually found in foods of animal origin, for example, raw milk and raw meat. *S. aureus* rarely causes food poisoning in raw food, except for milk obtained from a mastitis cow.

8.1. Bacterial growth

S. aureus can grow at temperature of 6 to 48 °C with an optimum of 37 °C. It requires pH 4.2 to grow and maximum 9.3 with optimum growth at a neutral pH (7.0 to 7.5). 0.1% acetic acid solution of (pH 5.1) inhibits *S. aureus* from growing. *S. aureus* is unaffected by drying. It may grow in the food with a water activity of 0.85 and produce enterotoxins although its optimum water activity is 0.99. It is resistant to NaCl, as it grows at a NaCl level of 7 to 10% and up to 25% (NZFSA, 2001c).

8.2. Disease characteristic

S. aureus can produce staphylococcal enterotoxins (SEs) that cause staphylococcal food poisoning (NZFSA, 2001c). The toxin is produced when the concentration of the enterotoxigenic strains exceeds 10^5 CFU/g. It is hard to remove SEs from foods once it is formed as they are resistant to heat, irradiation, and freezing. Due to its heat resistant property, SEs can survive commercial pasteurisation and even the canned food sterilisation process. To date, 16 types of SE have been recognized, they are A, B, C1, C2, C3, D, E, G, H, I, J, K, L, M, N and O. There are several factors affecting the formation of SEs, for instance, water activity, pH, temperature, redox potential, and antimicrobial constituents such as starter culture in the fermentation of milk products are able to prevent the growth of *S. aureus* and thus, SE production.

Staphylococcal food poisoning (SFP) occurs due to the ingestion of the SEs (NZFSA, 2001c). The human strains of *S. aureus* generating SE (A) and SE (D), with the majority of strains generating only SE (A) are the primary cause of SFP. Symptoms include diarrhoea, nausea, vomiting, and abdominal pains that generally appear 1 to 7 hours after ingestion. The quantity of toxin to make people sick depends on the vulnerability of the person. Epidemiological studies revealed that food poisoning could be caused by a tiny amount (1μg) of SE. Collapse may happen in severe cases, but the recovery is within two days (FSAI, 2011b).

8.3. Dose-response

Toxins are produced when the number of *S. aureus* exceed 10^5 per gram. The dose of the toxin to cause the symptoms of illness is less than 1.0 μg (NZFSA, 2001c).

Appendix C. Risk characterisation of microbiological risk assessment

Table C1. Risk characterisation calculation

Microbiological hazards	Severity of illness	Consequence score (C)	Exposure assessment $\frac{\text{Outbreaks} + \text{Prevalence}}{2}$	Likelihood score (L)	Risk Score (R=C x L)
Bacillus cereus	Moderate	3	(5+5)/2	5	15
Clostridium botulinum	Severe	5	(1+2)2	1.5	7.5
Clostridium perfringens	Moderate	3	(5+2)/2	3.5	10.5
Cronobacter spp.	Severe	5	(1+3)/2	2	10
Escherichia coli O157:H7 (STEC)	Major	4	(1+4)/2	2.5	10
Listeria monocytogenes	Severe	5	(1+1)/2	1	5
Salmonella spp.	Major	4	(3+2)/2	2.5	10
Shigella spp.	Major	4	(1+1)/2	1	4
Staphylococcus aureus	Moderate	3	(3+3)/2	3	9

Appendix D. Hazard identification of chemical risk assessment

D1. Generic chemical hazards

1. Allergens

Food allergies are adverse health reactions that involve the human immune system (FSANZ, 2013). Some foods are known to be responsible for causing food allergies. According to FSANZ (2019), milk, soy and wheat are amongst the most of allergy-causing foods. Proteins from these foods produce different allergic reactions in allergic individuals that are mediated by antibodies called immunoglobulin E (IgE).

Allergic reactions can vary from very slight (e.g. pruritus, eczema and rashes), to severe (e.g. angioedema) and sometimes fatal (e.g. shortness of breath and anaphylactic shock) depending on the dose, the individual and other factors (Crevel & Cochrane, 2013).

Adverse reactions of the human body toward foods can be classified into three groups, i.e. involved immunological, non-immunological, and microbial (FDA, 2008) that explained in Figure D1.1.

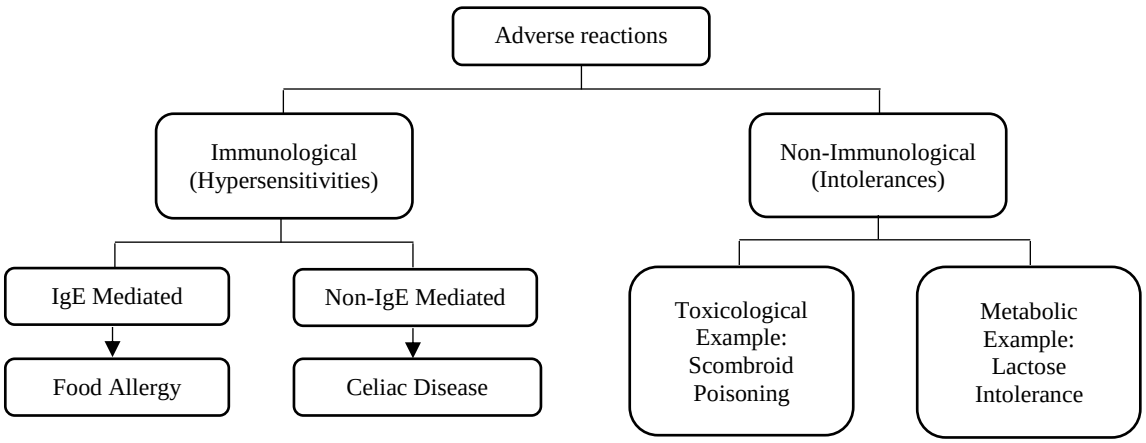

Figure D1.1. Classification of adverse reactions to foods

From "Approaches to establish thresholds for major food allergens and for gluten in food," (FDA, 2008, p. 1047).

The celiac (or coeliac) disease is an auto-immune disorder that causes a response to gluten which is a protein found in wheat, and related cereals such as rye and barley (Di Sabatino & Corazza, 2009). After gluten exposure, auto-antibodies are formed against specific endogenous proteins. This leads to atrophy of the cells lining the small intestine which lessens its ability to absorb nutrients. Celiac disease is classified as a food allergy resulting in an auto-immune disease.

FODMAPs is an acronym for "Fermentable Oligo-, Di-, Mono-saccharides And Polyols" (Gibson & Shepherd, 2005). FODMAPs can cause digestive discomfort in irritable bowel syndrome (IBD) due to microbes digesting them in the intestine. They are short-chain carbohydrates that are poorly absorbed in the small intestine but fermented by the bacteria in the small and large intestine producing gas that potentially results in bloating and flatulence (Tuck, Muir, Barrett, & Gibson, 2014). Examples of oligosaccharides include fructans (source: wheat, rye, barley) and galactans (source: pulses and beans).

Management of allergens in the food industry requires protection of allergic consumers through allergen declaration on the packaging label. Industry must ensure whether or not the allergens are present unintentionally in the food product (e.g. contamination in the processing equipment), products do not comprise allergens in amounts that can pose an unacceptable risk to allergic individuals, or the use of preventive labelling (Crevel & Cochrane, 2014). Allergen management

is widely implemented in the food industry so allergens are assumed to be managed in this risk assessment.

2. Heavy metals

Heavy metals are naturally-occurring and cannot be eliminated. Many elements are classified as heavy metals. Among heavy metals, those of most concern in food due to biotoxic effects are arsenic, lead, cadmium, and mercury. Their tendency to accumulate in the human body over time makes these elements toxic at low concentrations (Lawley et al., 2008). Importantly, heavy metal elements such as cadmium, chromium and arsenic are classified as carcinogenic (cancer producing elements) (Kulkarni, 2017).

Cereal grains are prone to heavy metal exposure especially those which are grown in the contaminated soil and sediments. Heavy metals can contaminate the soil because of the use of irrigation water from municipal waste or industrial effluents, metal-based pesticides and fertiliser (Kulkarni, 2017). There have been many studies on contamination of a wide range of heavy metals in a wide range of in cereal grains in different parts of the world (Abtahi et al., 2017; Batista, de Oliveira Souza, da Silva, & Barbosa Jr, 2010; Hensawang & Chanpiwat, 2017).

The FDA (2016a) conducted a recent risk assessment of arsenic in rice and rice products. They focused on inorganic arsenic because it is the primary toxic type of arsenic. Rice and rice products were chosen because arsenic levels tend to be greater in this food category and a typical diet in the United States. Quantitative risk assessment by the FDA suggested that even low doses of heavy metals can cause cancer such as lung and bladder cancer.

3. Mycotoxins

Cereal grains are commonly contaminated by moulds or filamentous fungi (Bullerman & Bianchini, 2009). Moulds mostly found in grains include *Alternaria*, *Cladosporium*, *Fusarium*, and *Helminthosporium* (Laca et al., 2006), but another genera can also be present such as *Aspergillus*, *Penicillium* and *Eurotium* (Berghofer et al., 2003).

The filamentous fungi may contaminate cereal grains in the field or storage (Doyle & Buchanan, 2013). *Alternaria* and *Fusarium* species are some of the field fungi that infect the grains (Mohapatra, Kumar, Kotwaliwale, & Singh, 2017), while, *Aspergillus* and *Penicillium* are examples of the storage fungi that produce mycotoxins during the storage of cereals (Los, Ziuzina, Akkermans, et al., 2018).

Mycotoxins are toxic secondary metabolites produced by filamentous microfungi (WHO, 2018a). Many mycotoxins can contaminate cereal grains including aflatoxins, deoxynivalenol, ergot alkaloids, fumonisins, ochratoxin A, and zearalenone (Bullerman & Bianchini, 2009). Among mycotoxins, ergot alkaloids, fumonisins, trichothecenes, and zearalenone are important mycotoxins in cereals. In New Zealand, mycotoxins of most concern are aflatoxins, ergot alkaloids, fumonisins, ochratoxin A, patulin, trichothecenes, zearalenone years (Cressey & Pearson, 2014). Major mycotoxins, producing fungi and vulnerable cereals are shown in Table D1.1.

Table D1.1. Major mycotoxins, produced fungi and most frequently contaminated crops

Mycotoxin	Fungi classification	Producing fungi	Vulnerable cereals
Aflatoxin	Storage fungi	*Aspergillus flavus, A. parasiticus,*	Maize, rice
Deoxynivalenol	Field fungi	*Fusarium graminearum, F. poae, F. culmorum, F. crookwellense, F. sporotrichioides, F. tricinctum, F. acuminatum*	Wheat, maize, barley, oat, rye
Fumonisin	Field fungi	*F. proliferatum, F. verticillioides*	Maize, sorghum
Ochratoxin A	Storage fungi	*Aspergillus section Nigri, A. ochraceus, Penicillium verrucosum*	Cereals
Zearalenone	Field fungi	*F. equiseti, F. graminearum F. verticillioides, F. culmorum,*	Barley, maize, rye, wheat

From "Prevalence of mycotoxins in foods and decontamination," (Patriarca & Pinto, 2017, p. 51). © 2017 Elsevier Ltd.

Mycotoxins pose a significant risk to human and animal health (Bullerman & Bianchini, 2009) by affecting specific organs. Aflatoxins target the liver and can cause liver cancer, ochratoxin A attacks the kidney while deoxynivalenol affects the gastrointestinal system and zearalenone invades the reproductive system. Other mycotoxins such as trichothecenes have many toxic effects in both humans and animals while fumonisins and Alternaria toxins are highly linked to oesophageal cancer in specific populations (Patriarca & Pinto, 2017). Hazard characterisation of major mycotoxins is presented in Table D1.2.

Environmental factors such as humidity affect fungal growth and mycotoxin production. Field fungi and storage fungi have different requirements in terms of humidity. The field fungi need high water activity (>0.9), high moisture content (18 to 30%), and high relative humidity (90 to 100%) to survive, but the storage fungi can grow in lower water activity (0.70–0.75), lower moisture content (14 to 16%), and lower relative humidity (65 to 90%) (Bullerman & Bianchini, 2009; Fleurat-Lessard, 2017). Therefore, good agricultural practice can prevent fungal growth and mycotoxin production in the pre and post-harvest stage. Post-harvest good agricultural practice is when the cereal grains are appropriately stored.

Mycotoxins have been a global concern including in New Zealand. The MPI investigates, characterises and quantifies the risk of mycotoxins in the food supply to the New Zealand public under The New Zealand Mycotoxins Surveillance program (Cressey & Pearson, 2014). To date, MPI has published nine mycotoxin reports. These include, i.e.:

- two risk profiles of mycotoxin reports in 2006 and 2014;
- three aflatoxin reports in 2008, 2010, and 2011;
- one ochratoxin A report in 2011 ;
- one aflatoxin and ochratoxin report in 2009;
- one trichothecenes report in 2014;
- one ochratoxin and trichothecenes report in 2014

Table D1.2. ***Hazard characterisation of major mycotoxins***

Chemical agent	Commodity	Health impacts	Occurrence (incidence and outbreaks)	Health-based Guidance Value (HBGV)	Legislation (maximum limit)
Aflatoxin	Maize, rice	Hepatocarcinogenic	- In 1974, a significant outbreak of hepatitis due to consumption of maize contain aflatoxin 2 and 6 mg was documented in India, causing in an estimated 106 deaths. - In 1981, a significant aflatoxin outbreak after the ingestion of maize contained 12,000 parts per billion (p.p.b.) of aflatoxin B1 was reported in Kenya, resulting in 12 died. - Since 2004, multiple aflatoxicosis outbreaks have been reported worldwide, resulting in 500 acute illness and 200 deaths (P. Kumar, Mahato, Kamle, Mohanta, & Kang, 2017)	NOEL AFM1: <2.5 µg/kg bw/day (Kuiper-Goodman, 1990) Tolerable intake is not established because it is a genotoxic carcinogen (JECFA, 2016a)	**EU** Nuts, spices, cereals, dried fruits - AFB1: 2-8 µg kg^{-1} - Total aflatoxins (B1, B1, G1, G2): 4-15 µg kg^{-1} Milk and milk products - AFM1: 0.050 µg kg^{-1} Infant foods - AFB1: 0.10 µg kg^{-1} - AFM1: 0.025 µg kg^{-1} **USA** All foods - Total aflatoxins (B1, B1, G1, G2): 20 µg kg^{-1} Milk - AFM1: 0.5 µg kg^{-1} (Lawley et al., 2008) **Australia, Canada and New Zealand** Peanuts and tree nuts - Total aflatoxins (B1, B1, G1, G2): 15 µg kg^{-1} (FSANZ, 2016)
Deoxynivalenol	Wheat, maize, barley, oat, rye	Nausea, vomiting, abdominal pain, diarrhoea		Acute NOEL: 0.25 mg kg^{-1} bw in the diet NOEL: 100 µg kg^{-1} bw /d PMTDI: 1 µg kg^{-1} bw/d	**EU** - Unprocessed cereal: 1250 -1750 µg kg^{-1} - Unprocessed durum wheat, oats, and maize: 750 µg kg^{-1}

Chemical agent	Commodity	Health impacts	Occurrence (incidence and outbreaks)	Health-based Guidance Value (HBGV)	Legislation (maximum limit)
				(JECFA, 2011b)	- Bread, biscuits, breakfast cereals, and cereal snacks: 500 µg kg^{-1} - Infant foods: 200 µg kg^{-1} **USA** Finished wheat product: 1000 µg kg^{-1} **Australia and New Zealand** No maximum regulatory limits for DON (FSANZ, 2016) (Lawley et al., 2008) (Cressey & Pearson, 2014)
Ergot (Ergotamine, ergometrine, ergosine, ergocristine, ergocryptine, ergocinine)	Rye, wheat, millet, barley, maize, oats	Gangrene, burning sensations, and hallucinations (Cressey & Pearson, 2014)	• In 1978, a serious outbreak of gangreneous ergotism occurred in Ethiopia, when 93 cases were reported and 47 deaths • In 1975, ergotism outbreak occurred in India due to infected millet consumption (Lawley et al., 2008)	(ARf) of 1 µg kg^{-1} bw and a group TDI of 0.6 µg kg^{-1} bw/day (Vettorazzi & López de Cerain, 2016)	**EU** Unprocessed cereals (placed on the market for first-stage processing) except for corn and rice: 0.5 g kg^{-1} ergot sclerotia (EC, 2015) **Australia and New Zealand** Cereal grains used in human food: 500 mg kg^{-1} (FSANZ, 2016)
Fumonisins	Maize	Oesophageal carcinoma (Cressey & Pearson, 2014)		PMTDI: 2 µg kg^{-1} body weight/day (JECFA, 2016b)	**EU** **Combination of FB1 and FB2** Unprocessed maize 4000 µg kg^{-1} Maize and maize-based food intended for direct human consumption 1000 µg kg^{-1}

Chemical agent	Commodity	Health impacts	Occurrence (incidence and outbreaks)	Health-based Guidance Value (HBGV)	Legislation (maximum limit)
					Maize-based cereals and snacks 800 µg kg^{-1} Maize-based foods for infants and young children 200 µg kg^{-1} **USA** Combination of FB1, FB2, and FB3 maize 2000-4000 µg kg^{-1} (FAO, 2004) **New Zealand** Fumonisins are currently not regulated in New Zealand (Cressey & Pearson, 2014) (FSANZ, 2016)
Ochratoxin A	Cereals	Endemic nephropathy, urothelial tumours		PTWI: 112 ng kg^{-1} bw/week (JECFA, 2007)	**EU** - Unprocessed cereals: 5.0 µg kg^{-1} - Processed cereal product intended for direct human consumption: 3.0 µg kg^{-1} - Processed cereal-based foods for infants and young children: 0.50 µg kg^{-1} (FAO, 2004; Lawley et al., 2008)
Zearalenone	Maize, barley, wheat, rye	An estrogenic effect, cervical cancer		PMTDI: 0.5 µg kg^{-1} body weight/day (500 ng kg^{-1} body weight/day) (Cressey & Pearson, 2014)	**EU** Most unprocessed cereals: 100 µg kg^{-1} Unprocessed maize: 350 µg kg^{-1} Maize intended for direct consumption and maize-based snacks and cereals: 100 µg kg^{-1}

Chemical agent	Commodity	Health impacts	Occurrence (incidence and outbreaks)	Health-based Guidance Value (HBGV)	Legislation (maximum limit)
					Bread, cereal snacks, biscuits, pastries, and breakfast cereals 50 µg kg^{-1} Food for babies and young children: 20 µg kg^{-1} (Lawley et al., 2008) (FAO, 2004)

4. Pesticides

According to WHO (2018c), pesticides are chemical mixtures for eradicating pests, including rodents, fungi, insects, and weeds. Furthermore, pesticides are broadly specified as insecticides, herbicides, fungicides, molluscicides, rodenticides, nematicides, plant growth regulators and others (Aktar, Sengupta, & Chowdhury, 2009). Approximately more than a thousand different pesticides are currently registered and used globally to safeguard food from pests (Macneale, Kiffney, & Scholz, 2010; WHO, 2018c).

Pesticide residues are found in soil, air and in surface and ground water that could contaminate the crops such as cereal grains. Lozowicka et al. (2014) investigated pesticide residues in cereal grains (wheat, barley, oats, and rye). Pesticide residues found were classified into four chemical classes as chloroorganic insecticides (IC), pyrethroid insecticides (IPYR), organophosphorus insecticides (IP) and fungicides (F). They found that chlorpyrifos methyl, diazinon, pirimiphos-methyl (IP group), malathion, dichloro-diphenyl-trichloroethane (DDTs) including metabolites, aldrine, γ-HCH (IC group), deltamethrin, cypermethrin, and tebuconazole (group F) residue in the range of 0.02 to 0.88 mg kg^{-1}. Fortunately, consumer health assessments associated with pesticide residues at the highest concentrations (0.88 mg kg^{-1}) in cereals samples shows that it does not impose serious health problems. This is because the consumer exposure to pesticides does not exceed the value of 100% ADI and ARfD.

Barley, wheat and oat that have been grown in the UK from 2005, 2006, 2007, and 2008 harvest years were studied for the pesticide residue (Baxter, Byrd, & Slaiding, 2009). The authors reported that the concentrations of all identified pesticides was under EU's Maximum Residue Limit (MRL). In New Zealand, a total diet study in 2016 suggested that all of the estimated dietary exposures for the agricultural chemicals are well within the health-based guidance value (HBGV)s (MPI, 2018). In conclusion, pesticide residues detected pose a negligible hazard to consumers, but they still need to be monitored.

D2. Natural plant toxins

According to Schilter et al. (2014), chemical constituents of plant-derived foods can be classified into two categories, i.e. intrinsic and extrinsic constituents. Intrinsic constituents are the plant's inherent components, and extrinsic constituents are the chemicals that come from natural or industry, get into the foods either by direct addition (food additives), by agricultural practices (e.g. pesticide residues), or by contamination (e.g. mycotoxins, pollutants).

Intrinsic components consist of the following chemicals:

- Macronutrients (e.g. sugars, protein, and lipids) and micronutrients (e.g. vitamins) that can be used to measure the nutritional level of plant food.
- Anti-nutrients - chemicals that may lessen the nutritional value of the plant food. Examples are phytates preventing absorption of minerals such as iron, protease inhibitors blocking protein digestion.
- Inherent plant toxins - non-nutrient secondary metabolites which have the potency to cause toxicity in humans. Examples are cyanogenic glycosides and ergot.

Table compares the definition of inherent plant toxins from Europe and the USA. The EU-AIR concerted action NETTOX is a European network compiling and evaluating human health risks associated with naturally-occurring plant toxicants (O'brien, Weir, Moody, & Liu, 2013).

Table D2.1. ***Comparison of inherent plant toxins definition from Europe and the USA***

EU-AIR concerted action NETTOX	US-FDA
"Inherent food plant toxicants are plant constituents which might give rise to adverse effects in humans when the plant or plant products are ingested."(Gry et al., 1998)	"Inherent food plant toxicants are naturally occurring poisonous or deleterious substances that are inherent natural constituents of a food which are not the result of environmental, agricultural, industrial, or other contamination." (Ely, 1989)

Inherent plant toxins are commonly known as natural pesticides due to their role in the defence against predators, insects, fungi, bacteria, and viruses. One characteristic of some inherent plant toxins is a strong bitter taste to prevent the plant from being eaten by the mammals (Schilter et al., 2014). For instance, the presence of cyanogenic glycosides causes bitterness in cassava and almonds (Jones, 1998). Natural toxins are also produced in reaction to environmental stress such as drought or extreme humidity (WHO, 2018b). The level of cyanogenic glycosides is found to be high in plants that are stressed due to frost (Haschek et al., 2013).

1. Cyanogenic glycosides

Cyanogenic glycosides or cyanoglycosides are the products of the plant's secondary metabolism. Chemically, cyanogenic glycosides are glycosides of α-hydroxinitriles and amino acid components (Vetter, 2000). They are classified as plant toxins belonging to a group recognised as cyanogen. Cyanogenic plants can undergo a process called cyanogenesis which resulted in the formation of free hydrogen cyanide (NZFSA, 2017a). Figure D2 describes the chemical structure of cyanogenic glycoside and process of cyanide release.

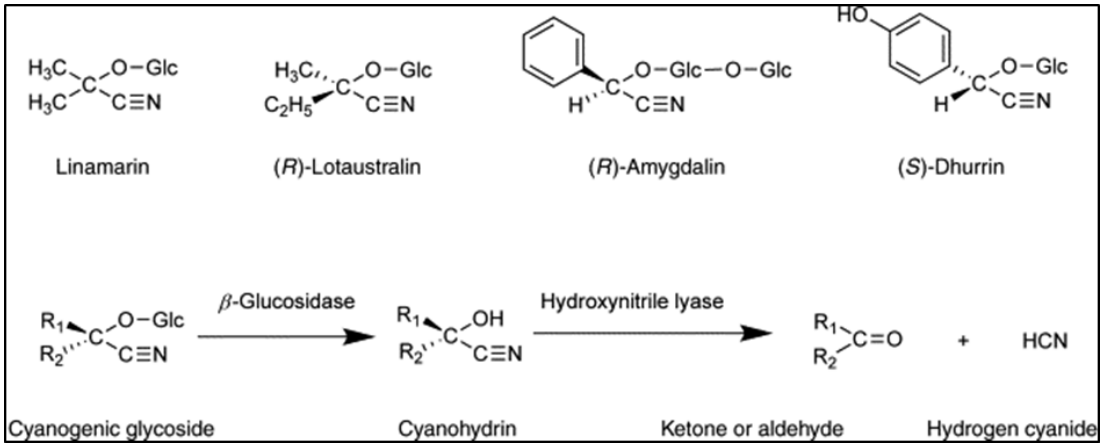

Figure D2. Cyanogenic glycoside and process of cyanide release

From "Comprehensive natural products II chemistry and biology," (Yamane et al., 2010, p. 344).

Hydrogen cyanide (also known as prussic acid, hydrocyanic acid, or cyanide) is a useful chemical defence against herbivores, insects, and predators. Thus, cyanogenic species are relatively free from pests and competitive herbivores.

Hydrogen cyanide content usually varies in cyanogenic plants, the different part of the plant and between the same parts of different individuals of the same species (Jones, 1998). For example, cyanide content is 6 mg kg^{-1} in sorghum grain, 240-890 mg kg^{-1} in cassava roots and 1040 mg kg^{-}

[1] in cassava leaves. Providentially, hydrogen cyanide is easily removed by food processing before consumption.

2. Lectins

Lectins are abundant in nature and found in many foods. Lectins are a group of proteins or glycoproteins that bind the carbohydrate. Lectins are generally known as phyto hemagglutinins (HA) because of their ability to agglutinate red blood cells which are used for blood typing (Lawley et al., 2008).

Lectins are usually found in legumes (e.g., soybeans, black beans, lima beans, lentils, and kidney beans) and grains (e.g., barley, rye, and rice) (Dolan et al., 2010). Lectin concentration in the seed is up to 2 g kg^{-1}, while in germ is up to 0.5 g kg^{-1} (Peumans & Damme, 1998). In cereals, lectins are mostly present in the seed (Peumans & Damme, 1998). Lectins isolated from uncooked red kidney beans can cause oral toxicity. Symptoms of toxicity are described as nausea, vomiting, bloating and diarrhoea (van Buul & Brouns, 2014). Therefore, beans and grains need to be cooked or fermented to decrease lectin content prior to consumption.

Appendix E. Risk characterisation of chemical risk assessment

Table E1.1. Risk characterisation calculation

Chemical hazards	Severity description	Severity score (S)	Exposure assessment	Likelihood score (L)	Risk Score (R=S x L)
Fagopyrin	Low	1	Unlikely	1	1
Genistein	Low	1	Unlikely	1	1
Goitrogen	Low	1	Unlikely	1	1
Hydrocyanic acid	Medium	2	Likely	3	6
Isoflavones	Low	1	Unlikely	1	1
Lectins	Low	1	Unlikely	1	1
Oxalates	Low	1	Unlikely	1	1
Phytates	Low	1	Unlikely	1	1
Protease inhibitors	Low	1	Unlikely	1	1
Quercetin	Low	1	Unlikely	1	1
Saponins	Low	1	Unlikely	1	1
Tannins	Low	1	Unlikely	1	1

Table E1.2. Prevalence of hydrocyanic acid in raw material

Chemical hazard	Raw material (mg kg^{-1})	Prevalence/ Likelihood	Risk
Hydrocyanic acid	Commercial raw defatted soy flour 0.08	0.08/10#x100%=0.8%	Very low
	Soy protein isolate 0.18	0.18/10x100% =1.8%	Low
	Whole soybean meal 0.26	0.26/10x100%=2.6%	Low

Note: # is the maximum level of cyanide in cassava flour=10 mg kg^{-1}

Table E1.3. Risk estimate calculation for commercial raw defatted soy flour addition to high solid content dairy product

Chemical hazard	Raw material (mg kg^{-1})	Mitigation method	Reduction	Residue (mg kg^{-1})	Addition to dairy products (mg kg^{-1})	Maximum residue limit (mg kg^{-1})	Prevalence/ Likelihood	Risk level
Hydrocyanic acid	Commercial raw defatted soy flour 0.08	Storage	50-64%	0.03-0.04	50% x 0.04 =0.02	10	$\frac{0.02 \text{ x } 100\%}{10\#} = 0.2\%$	Very low
		Soaking	13-52%	0.04-0.07	50% x 0.07 =0.035		$\frac{0.035 \text{ x } 100\%}{10} = 0.35\%$	Very low
		Heat treatment (steaming)	74-80%	0.02	50% x 0.02= 0.01		$\frac{0.01 \text{ x } 100\%}{10} = 0.1\%$	Very low
		Drying	13-88%	0.02-0.07	50% x 0.07= 0.035		$\frac{0.035 \text{ x } 100\%}{10} = 0.35\%$	Very low

Note: # is the maximum level of cyanide in cassava flour=10 mg kg^{-1}

Table E1.4. Risk estimate calculation for commercial raw defatted soy flour addition to intermediate solid content dairy product

Chemical hazard	Raw material (mg kg^{-1})	Mitigation method	Reduction	Residue (mg kg^{-1})	Addition to dairy products (mg kg^{-1})	Maximum residue limit (mg kg^{-1})	Prevalence/ Likelihood	Risk level
Hydrocyanic acid	Commercial raw defatted soy flour 0.08	Storage	50-64%	0.03-0.04	5% x 0.04= 0.002	5	$\frac{0.002 \text{ x } 100\%}{5\#} = 0.04\%$	Rare
		Soaking	13-52%	0.04-0.07	5% x 0.07 =0.0035		$\frac{0.0035 \text{ x } 100\%}{5} = 0.07\%$	Rare
		Heat treatment (steaming)	74-80%	0.02	5% x 0.02= 0.001		$\frac{0.001 \text{ x } 100\%}{5} = 0.02\%$	Rare
		Drying	13-88%	0.02-0.07	5% x 0.07=0.0035		$\frac{0.0035 \text{ x } 100\%}{5} = 0.07\%$	Rare

Note: # is the maximum level of hydrocyanic acid in stone fruit juices=5 mg kg^{-1}

Table E1.5 ***Risk estimate for addition to low solids dairy products***

Chemical hazard	Raw material (mg kg^{-1})	Mitigation method	Reduction	Residue (mg kg^{-1})	Addition to dairy products (mg kg^{-1})	Maximum residue limit (mg kg^{-1})	Prevalence/ Likelihood	Risk level
Hydrocyanic acid	Commercial raw defatted soy flour 0.08	Storage	50-64%	0.03-0.04	2% x 0.04= 0.0008	5	$\frac{0.0008 \text{ x } 100\%}{5\#} = 0.016\%$	Rare
		Soaking	13-52%	0.04-0.07	2% x 0.07 =0.0014		$\frac{0.0014 \text{ x } 100\%}{5} = 0.028\%$	Rare
		Heat treatment (steaming)	74-80%	0.02	2% x 0.02= 0.0004		$\frac{0.0004 \text{ x } 100\%}{5} = 0.008\%$	Rare
		Drying	13-88%	0.02 - 0.07	2% x 0.07 =0.0014		$\frac{0.0014 \text{ x } 100\%}{5} = 0.028\%$	Rare

Note: # is the maximum level of hydrocyanic acid in stone fruit juices=5 mg kg^{-1}

Appendix F. Risk mitigation strategies

F1. Risk mitigation strategies for microbial hazards

In order to mitigate the risk of contaminated cereal grains in addition to dairy products, the following steps are suggested:

1. Screening raw material from the supplier

The dairy company might use microbiological criteria for accepting raw material (cereal grains and milk) from the supplier. A supplier needs to conduct control procedures (Good Hygiene Practices, HACCP) that will be verified periodically by the dairy company. The frequency of sample testing by the criteria depends on the confidence of the supplier's control procedures (Scott et al., 2015).

2. Decontamination treatment

Cereal grains need to be decontaminated before its use in dairy manufacture. Some options for decontamination treatment are:

2.1. Irradiation.

Since the 1950s, irradiation has become one of microorganism's controls on flours and cereals. USFDA has approved the radiation dose of 0.5kGy for preservation and decontamination of many crops (Los, Ziuzina, & Bourke, 2018). Irradiation has advantages over heat treatment, where heat treatment may destroy nutrients. However, in Australia and New Zealand, irradiation is approved to a limited range of commodities such as herbs and spices, herbal infusions, and some fruits (e.g. blueberry, raspberry, persimmons) and vegetables (e.g. tomato, capsicum) (FSANZ, 2017a).

2.2. Heat treatment

New Zealand imports some cereal grains from overseas and requires the imported grain to be heat treated at a core temperature of 85°C and 40% relative humidity for 15 hours nonstop (Gilbert et al., 2010).

Pasteurisation is a typical heat treatment for milk in the dairy industry. Options available are low-temperature long time (LTLT) at 63°C for 30 minutes, high-temperature short time (HTST) at 72°C for 15 minutes or ultra-heat treatment (UHT) at 141°C for 2 seconds. An additional control measure is to ensure hygiene in the manufacturing environment to prevent post-pasteurisation contamination.

2.3. Alternative microbial decontamination processes are microwave treatment, pulsed UV light, cold plasma, and organic acid (Los, Ziuzina, & Bourke, 2018).

Intervention to reduce selected microbial contamination in cereals and grains is shown in Table F1.1.

Table F1.1. Intervention to reduce contamination of selected microbial hazards in cereals and grains

Food category	Mitigation	Conditions	Microbial hazards	References
Dry cereal mixes and flours	Storage conditions	Increased temperature (5-45 °C), Increased aw (0.27-0.28), decreased pH (5.6-6.7; 1-36 weeks)	*B. cereus*	(Jaquette & Beuchat, 1998)
	Irradiation	Microwave (2450 MHz; 56.7-82.2 °C,; 3.9-10 min)	*Salmonella* spp.	(Bookwalter, Bothast, Kwolek, & Gumbmann, 1980)
	Fermentation	Lactic acid bacteria (72 hr)	Generic *E. coli*	(Kimmons et al., 1999)
Wheat grains	Cold plasma	ACP (44 kV) dielectric barrier discharge for 0 (control), 5, 10, 15 and 20 min.	*E. coli* O157:H7, *Salmonella enterica* and natural microflora	(Thomas-Popo et al., 2019)
Wheat	High pressure treatment	10 min at 300 MPa and 30 °C		
Legume (Mung bean, Lucerne & Chickpea)	Combination treatment	Ultrasonication: sonicated (4–10 min; 40–50 °C) Blanching: 50–70 °C for 4–10 min Ascorbate dip: (0.25%, 5% and 1%) up to 10 min at 4 ± 1 °C. Gamma irradiation: 1–2.5 kGy	Coliform, *S. aureus*	(S. Kumar & Gautam, 2019)
Rice	Chemicals	Fermented ethanol (10-70%; 5-60 min)	*B. cereus*	(Kim, Lee, Park, & Rhee, 2013)
		Supercritical carbon dioxide (36-44 °C; 100-200 bar; 10-30 min)		(Kim et al., 2013)
		Fermented ethanol + supercritical CO_2		(Kim et al., 2013)
		Sodium hypochlorite dip (100ppm; 25-60 °C; 3-6 hr)		(Park et al., 2009)
		Citric acid dip (1%; 25-60 °C; 3-6 hr)		(Park et al., 2009)
	Irradiation	Electron beam (1.1-7.5 kGy)	*B. cereus* Generic *E. coli*	(Sarrıas, Valero, & Salmeron, 2003)
	Multiple	Gamma irradiation (0.1-0.3 kGy) + sodium hypochlorite (10-1000 ppm; 2 min) + ultrasound (18 min)	*B. cereus*	(Ha, Kim, & Ha, 2012)
		Citric acid dip + acidic and alkaline electrolyzed water (3-6 hr)		(Park et al., 2009)

Food category	Mitigation	Conditions	Microbial hazards	References
Other grains	Chemicals	Sodium hypochlorite dip (100ppm; 25-60°C; 3-6 hr) Citric acid dip (1%; 25-60°C; 3-6 hr)	*B. cereus*	(Park et al., 2009)
	Electrolyzed water	Acidic electrolyzed water (3-6 hr) Alkaline electrolyzed water (3-6 hr)	*B. cereus*	(Park et al., 2009)
	Multiple	Citric acid dip + acidic and alkaline electrolyzed water (3-6 hr)	*B. cereus*	(Park et al., 2009)

F2. Risk mitigation strategies for chemical hazards

Prevalence of adverse reactions due to food toxins is moderately low, either caused by naturally-occurring compounds or are formed through processing or handling. Regulatory agencies, e.g. US FDA, EFSA and MPI NZ, provide solutions through specifications, warning labels and prohibitions which makes low prevalence of adverse reactions. Manufacturers play a significant role in setting limits on specific substance as well as developing mitigation techniques to reduce natural plant toxins and process-induced toxins (Dolan et al., 2010).

Processing techniques have been developed to reduce antinutritional mixtures in plant foods. Most of the anti-nutrients such as lectins can be removed with heat. Hence, cooking can eradicate anti-nutrients before consumption. On the contrary, phytates, saponins and tannins are heat stable that needs different processing such as soaking, dehulling, and germination. Several processing methods used to reduce the intrinsic plant toxins are presented in Table F2.1

Table F2.1. Risk mitigation of selected plant toxicants in cereals and grains

Plant toxins	Processing method	Plant	Conditions	Reduction	References
Cyanogenic glycosides	Soaking	Cassava root	24 h 48 h 72 h	13-52% 73-75% 90%	(Agbor-Egbe & Mbome, 2006)
	Fermentation	Cassava pulp or dough	4-5 days	52-63%	(Obilie et al., 2004)
		Bitter apricot kernels	Soaking and fermentation	70%	(Tunçel, Nout, Brimer, & Göktan, 1990)
		Cocoyam	Fermentation	98.6%	(Igbabul, Amove, & Twadue, 2014)
		Sorghum leaves		84.6%	(Prasad & Dhanya, 2011)
	Storage	General	in temperature (35±2 °C), cyanogenic glycosides volatilize at temperature of 26 °C)		(Onabolu, Oluwole, Rosling, & Bokanga, 2002)

Plant toxins	Processing method	Plant	Conditions	Reduction	References
		Cassava product (gari)	in room temperature for 4 weeks	50-64%	(Onabolu, Oluwole, & Bokanga, 2002)
	Cooking	Cassava product (akyeke)	Steaming	74-80%	(Obilie et al., 2004)
			Garification: fermented and dried cassava mash simultaneously cooked and dried in a shallow wok	90-93%	(Agbor-Egbe & Mbome, 2006)
			98-102 °C for 148-180 min	97%	(Ferreira, Yotsuyanagi, & Carvalho, 1995)
	Drying	Cassava chips	40-70°C, air velocity of 2.03 m s^{-1}, 2.25 m s^{-1}, 2.45 m s^{-1} and 2.75 m s^{-1}	13-88%	(Famurewa & Emuekele, 2014)
Lectins (HA)	Milling	Garden pea		15–60%	(Coffey, Uebersax, Hosfield, & Bennink, 1993)
	Soaking, drying, milling and extruded	Garden pea		100%	(Kelkar et al., 2012)
Phytates	Cooking	Garden pea	Boiling using Mattson cooker, seed-to-water ratio 1: 4 (w/v), 18.5 min,	5.3–10.8%	(Wang, Hatcher, & Gawalko, 2008)
			Boiling, seed-to-water ratio 1:3 (w/v), 100 °C	24.4–33%	(Bishnoi, Khetarpaul, & Yadav, 1994)
			Pressure, seed-to-water ratio 1:2 (w/v), 15 psi, 10 min	41%	(Bishnoi et al., 1994)