NSW GOVERNMENT Food Authority

Plant products not regulated under the NSW Plant Products Food Safety Scheme

Background information

NSW/FA/FI191/1404

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EXECUTIVE SUMMARY

The Plant Product Food Safety Scheme was introduced in 2005, and applies to the following high risk plant product industries:

- fresh cut fruit and vegetables
- unpasteurised juices
- seed sprouts
- vegetables in oil

A report prepared by Food Science Australia (CSIRO) in 2000, on the food safety risks of plant products, was used as a basis to include high risk foods in the Plant Products Food Safety Scheme.

Since that report was published, the food industry has experienced many changes as other plant-based product groups have increased their presence in the market, such as soy products, fermented vegetables, vegetable-based dips and sauces, mixed salads and fresh cut herbs. The Authority identified a need to update its plant product knowledge and understanding of the associated food safety risks. Thus, this desktop review of plant products that are not currently regulated in the Food Safety Scheme was undertaken.

The categories and product examples are:

- Soy products: tofu
- Fermented vegetables: kimchi
- Vegetable based dips and sauces: baba ghanoush
- Prepared salads: coleslaw
- Fresh cut vegetables (not already regulated): fresh herbs, and
- Edible seaweeds.

This review has found that no specific hazards would justify any of the above products being classed as high risk, and therefore would not be included in the Food Safety Scheme.

To support this finding, a survey of the microbiological quality and/or chemical properties of the product categories listed above (except kimchi and seaweed) was carried out (See *Survey of plant products not within the NSW Plant Products Food Safety Scheme*).

Overall, food safety issues with these products are rare and sporadic. Requirements set out in the Food Standards Code coupled with inspection of businesses are likely to provide adequate food safety control. Based on this assessment, further regulation is not currently warranted.

INTRODUCTION

In 2005, the Plant Products Food Safety Scheme was introduced into legislation. The Scheme required businesses (with the exception of retail businesses) handling certain plant products to be licensed and operate under a food safety program. The products covered by the Scheme include fresh-cut fruits and vegetables, vegetables (or fruits) in oil, unpasteurised juices and seed sprouts. These products were included in the scheme based on a report by Food Science Australia (2000) which identified them as high risk based on the microbiological hazards associated with these products (Table 1).

Table 1. Microbiological hazards associated with plant products

Plant product	High risk ranking	Medium risk ranking
Fresh cut vegetables – may be consumed raw	Pathogenic *E. coli* *Salmonella* spp. *L. monocytogenes*	
Fresh cut vegetables – chilled, modified atmosphere packaging (MAP) or extended shelf life	*L. monocytogenes* *C. botulinum*	
Fresh cut fruit	Pathogenic *E. coli* *Salmonella* spp. *L. monocytogenes*	*Cryptosporidium parvum* Enteric viruses
Fruit juice / drink (unpasteurised)	Pathogenic *E. coli* *Salmonella* spp.	
Vegetables in oil	*C. botulinum*	
Seed sprouts	Pathogenic *E. coli* *Salmonella* spp.	*B. cereus* *L. monocytogenes*

Adapted from *Scoping study on the risk of plant products* (FSA, 2000)

Since then the possibility of a regulatory gap was raised by experienced Food Safety Officers during a workshop as a number of plant-based products appear to have similar risks to products covered by the Scheme (Table 2). Background information about those products is assembled in this document. The information will be used to decide if there has been sufficient change since the Food Science Australia report (2000) to warrant further work on the risks associated with these products. A survey of some of those products was undertaken to supplement the information from the scientific literature.

Table 2. Plant products not within scope of the Plant Products Food Safety Scheme

Group	Example of products	Risk rating according to the scoping study (FSA, 2000)
Soy products	Tofu & fermented soy products (eg tempeh) Soy milk & milk products (eg soy yoghurt & soy cheese)	Medium risk for *B. cereus* Low risk for *Salmonella* spp. and *Y. enterocolitica*
Fermented vegetables	Kimchi	
Vegetable based dips & sauces	Sesame-based dips (eg tahini, hommus & baba ghanoush) Salsa-style dips Pesto-style dips (eg pesto, tapenades)	For tahini & hommus: Medium risk for *Salmonella* spp. and *B. cereus* For guacamole & olive tapenade: Medium risk for pathogenic *E. coli*, acid tolerant *Salmonella* spp., psychrotrophic *B. cereus* and *Cl. botulinum*
Salads (exclude fresh cut vegetables)	eg potato salad, rice salad, coleslaw, and other mixed salads	Salad with mayonnaise based dressing: Medium risk for pathogenic *E. coli* and *Salmonella* Low risk for *L. monocytogenes*
Fresh cut vegetables excluded in the FSS	Fresh herbs Snow pea sprouts	Medium for Pathogenic *E. coli*, *Salmonella* spp., *Shigella* spp. Low for *B. cereus*, *L. monocytogenes*, Enteric parasites and viruses
Edible seaweeds		

SOY PRODUCTS

Soybeans (also known as soya beans) are oilseed legumes originating from East Asia and have been used in Asian cooking for thousands of years. The consumption of soybeans and soybean based foods has increased greatly in Australia in the last few years. These products are commonly used as a substitute for meat, dairy and wheat based foods. Soybeans are available in a wide variety of colours and sizes and manufactured into a wide array of foods.

1 Tofu

Tofu or bean curd is a food made by coagulating soy milk, and then pressing the resulting curd into soft white blocks. Tofu has very little flavour and odour on its own and can be used in many dishes.

Processing steps

The production of tofu is a relatively simple process (**Error! Reference source not found.**). Soybeans are soaked in water until they easily fall apart. Time required for soaking ranges from 6 to 10 hours (various home style recipes) to overnight (Han et al., 2001a). The soybeans are then drained, rinsed and pureed. The puree is cooked until it expands to around four times its original volume. After cooking, the puree is filtered to separate the soybean milk from the okara (fibre). The okara is further pressed to remove all soymilk. Okara is dry and crumbly after pressing.

The soymilk is boiled, a coagulant is added and the mix is reheated. Three types of coagulants are commonly used: salt coagulants such as calcium sulphate (gypsum), magnesium or calcium chloride; acid coagulants such as Glucono delta-lactone (GDL); and enzymic coagulants such as papain.

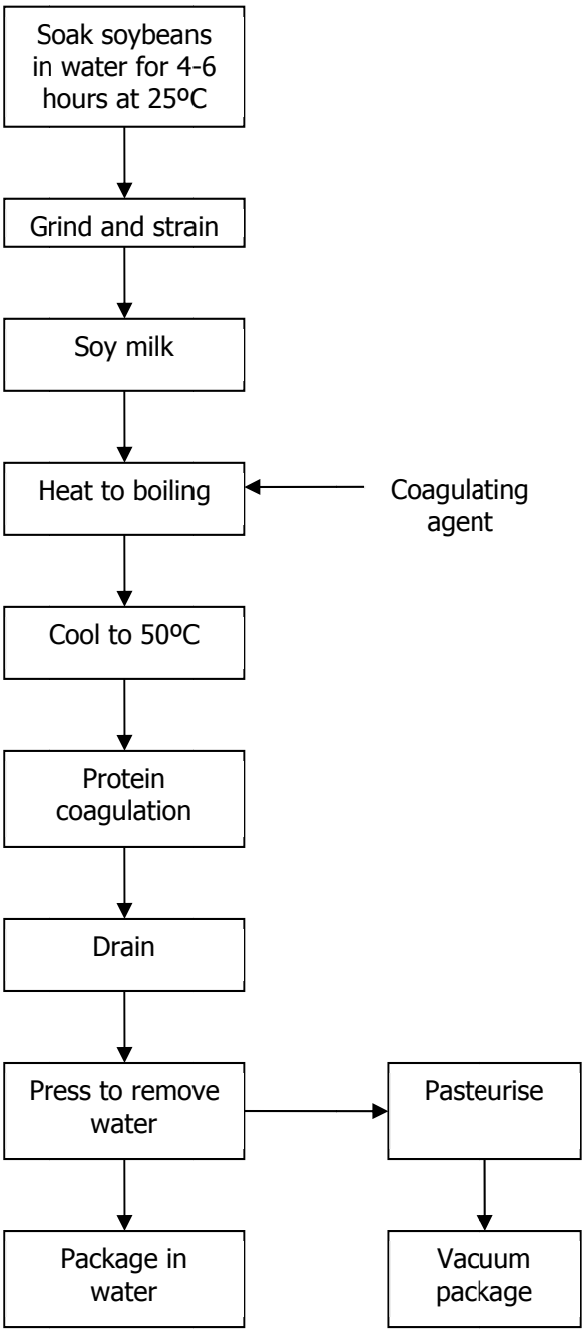
The mixture is stirred to ensure homogenous coagulation and put aside for 10 to 15 minutes to set. Tofu is set when the whey becomes clear yellow. The mixture is then poured into a tofu mould. A tofu mould is traditionally a box with holes that has been lined with cheesecloth or a similar filtering device. The tofu is then pressed to remove excess water and left to set.

Traditionally, tofu is prepared daily and packed with water in plastic containers and stored under refrigeration. Recent trends have been to pack in modified atmospheres to extend shelf life to several days under refrigeration. Tofu can either be pasteurised or not (Food Science Australia, 2000).

Critical Control Point (CCP)

The CCP in the tofu manufacture is cooking of the beans to eliminate vegetative microflora of the ingredients. Hygienic practices and proper handling are required throughout the manufacturing process to ensure that tofu remains safe.

Figure 1. Preparation of tofu (Food Science Australia, 2000)



Varieties

A wide variety of tofu styles are available. However, tofu products can be split into two main categories:

- fresh tofu, which is produced directly from soy milk
- processed tofu, which is produced from fresh tofu

Tofu production also creates important side products which are often used in various cuisines such as tofu skin and okara.

Fresh tofu

Depending on the amount of water that is extracted from the tofu curds, fresh tofu can be divided into three main varieties.

Soft/silken tofu

Silken tofu is made by using more viscous soy milk and there is no separation of the curds from the whey. The tofu sets to smooth custard like texture. Silken tofu may also be curdled straight into packaging.

Firm tofu

Firm tofu is a firmer version of soft tofu. It is manufactured using the same process as soft tofu but is pressed using a heavier weight to expel more whey from the curds, creating a firmer product.

Dried tofu

Dried tofu is firm tofu with very low moisture content. When sliced thinly, this tofu will crumble easily. Dried tofu may be reconstituted before cooking and has a tough chewy texture. Freeze dried tofu is popular in Japan.

Processed tofu

There are a number of forms of processed tofu. Some of these techniques likely originated from the need to preserve tofu before the days of refrigeration. Other production techniques are employed to create tofu with unique textures and flavours.

Stinky tofu

Stinky tofu is fermented tofu with strong odour. It originated from East and South East Asia. It is produced by marinating fresh tofu in a brine of fermented milk, vegetables and meat for several months. Other common brine ingredients include dried shrimp, amaranth greens, mustard greens, bamboo shoots, and Chinese herbs. Stinky tofu can be eaten cold, steamed, stewed or fried.

Pickled tofu (Sufu)

Pickled tofu is also known as tofu cheese, preserved tofu or fermented tofu. It originated from Vietnam and China. It is generally consumed as an appetiser or side dish with breakfast or steamed bread. Sufu is similar to tofu but has more spreadable consistency. The flavour and colour of Sufu is dependant on the processing method. Sufu can be red, white or grey depending on dressing mixture.

Most sufu products are produced by a similar principle, which involves four main steps: preparation of tofu, preparation of pehtze (fresh tofu overgrown with mould mycelia), salting and ripening.

Traditionally, the preparation of sufu involves natural fermentation. Cubes of tofu are placed in wooden trays, which then piled up and surrounded with straw for natural inoculation and fermentation. The temperature should be at 15 – 20ºC, which is favourable for Mucor spp only. This step might take five to fifteen days. At the end of this process, the pehtze must have white or light yellow – white mycelium. The pre-treated pehtze is transferred into a big earthen jar for salting, which takes six to twelve days. The salted pehtze is then removed from the jar, washed with water and transferred into another jar. Pehtze is mixed with dressing mixture which may consist of alcohol, salt, sugar, flour (or bean) paste, and spices. The jar is then sealed with clay and aged for 6 months for further maturation.

Nowadays, sufu is manufactured at an industrial scale, following the same four main steps as the traditional process. Pehtze is produced by inoculating tofu with pure culture moulds (Actinomucor, Mucor and Rhizopus). The inoculated tofu is placed in a wooden tray, incubated at 25ºC for a maximum of 48 hours. The pehtze can be salted in many ways, such as sprinkled with a layer of salt, transferred to vessels containing saturated salt solution, or immersed in an alcoholic saline solution (combining salting and ripening process together). Salted pehtze is then placed in 250ml to 1L jar containing a dressing mixture. Although nowadays the ripening time is shorter, the modern process still takes about 2 – 3 months (Han et al., 2001a).

Fried

Most tofu can be fried, although soft and silken tofu is generally not. Thin and soft tofu is deep-fried in oil until light and airy. Firm and dry tofu is deep fried until golden brown with a crispy skin. Fried tofu can be eaten on its own or with a light sauce.

Frozen tofu

By freezing tofu, large ice crystals develop within the tofu. This results in the formation of large cavities that give a 'layered' appearance to the tofu. The frozen tofu takes on a yellowish colour in the freezing process. 'Thousand layer' tofu is commonly made at home from soft tofu although it is also commercially sold as a specialty tofu. This tofu needs defrosting prior to use. Frozen tofu is usually soaked in warm water prior to use.

Flavoured

As tofu has little flavour, it is generally used as a base for other flavours and ingredients. Tofu can be flavoured sweet or savoury during manufacturing. Sweet tofu is served as a dessert and can be flavoured with peanut, almond, mango. Sweet tofu has sugar and flavours added to the soymilk prior to curdling. Most sweet flavoured tofu is silken or soft tofu. Egg is often used to flavour savoury tofu.

Tofu by-products

There are two main by products of the manufacture of tofu.

Okara

This is the left over soybean fibre after extracting the soymilk from the beans. Okara is generally used as animal feed but can be used as an ingredient in imitation meat products such as burgers. Okara can be sold as is, semi dried or dried.

Tofu skin (yuba)

This is the skin that can form on the soymilk during boiling. Yuba is also used to form imitation meat products. Often fried, it is can be used in a similar way to pastry and is sold as dried bean curd.

Standard and Regulation

Standard 1.3 of the Food Standards Code (FSC) specifies permitted additives and processing aid to be added to vegetable protein products, which include tofu.

The coagulants commonly used in the tofu manufacture (as specified above) are permitted food additives in vegetable protein products. Papain is also an approved processing aid.

Artificial colours are not permitted in these products.

Microbiological hazards

Cooking soybeans and soy milk eliminates the initial vegetative microflora of the ingredients. However further processing after cooking, such as pressing and packaging introduces the potential for contamination. Tofu has a neutral pH (6 to 7), high moisture content (70 to 80%) and high protein content (6 to 8.4%) which makes it a suitable medium for bacteria (Ashenafi, 1994; Han et al., 2001a). The competitive spoilage microflora is inactivated at the bean heating stage so there are no barriers to

the growth of pathogens. Therefore fresh tofu has a short shelf life of approximately 3 to 4 days, however, heat treatment or pasteurisation can increase this to much longer. Tofu is often only given a light heating before consumption although it can be eaten cold and cooking by the consumer cannot be relied upon to inactivate microbial pathogens (FSA, 2000).

Ashenafi (1994) conducted a study to identify the possible source of contamination during the production process of tofu and to evaluate the microbiological and keeping quality of the finished product. The study found that the slurry after filtration contained gram-negative organisms that imply that the cooking temperature of the beans was less than 80ºC. However, since the soy milk and precipitant had low bacterial count, the high microbial count of the slurry did not seem to affect the microbiological quality of the product. The high bacterial count in the finished products was probably caused by further contamination after pressing from the knife, cutting boards and hand contact. The isolation of Pseudomonas spp., *Bacillus* spp. and enterobacteria from the finished product imply that pathogens, if introduced, could survive and eventually proliferate in the product. Apart from the contamination during processing, major source of contamination for herb tofu was the highly contaminated herbs. Smoked tofu and fried tofu had a satisfactory microbiological quality due to either smoking or heat treatment at the end of the process.

Tuitemwong & Fung (1991) studied the microbiological quality of tofu juice and cake of seven different brands at 1 day and 30 days of storage in a refrigerator. Microbial load at day 1 was different from brand to brand, but cell counts in juice and cake were correlated. The level of organisms observed at day 30 was not related to initial cell count and it was affected mainly by the pH of the products. All brands spoiled after 30 days of storage at 7ºC.

Pathogens of concern that are capable of growth in tofu include, but are not limited to:

- *Bacillus cereus* – is known to be present on soybeans and its heat resistant spores can germinate in the products. Food Science Australia considered tofu as medium risk in relation to this organism because it can grow in the products during storage (FSA, 2000).
- *Staphylococcus aureus* – presents a risk in tofu due to its high protein content and the amount of handling subsequent to heating process. Prolonged storage at improper temperature can lead to *S. aureus* proliferation.
- *Listeria monocytogenes* – can be present in tofu due to its ability to survive mild heat treatment and the amount of handling subsequent to heating process. The capacity for *L. monocytogenes* to grow at refrigeration temperatures makes it a potential threat to the safety of food packaged under vacuum or modified atmosphere packaging such as tofu. A study conducted by Liu et al. (2010) found that *L. monocytogenes* grew and survived by competing with indigenous bacteria in tofu for 24 days at 4 to 7ºC and 48 hours at 22ºC.
- *Clostridium botulinum* – can be present in tofu since it can survive the heating process due to its heat resistant spores. The vacuum packaging creates a perfect environment for these anaerobic bacteria. Study conducted by Kovats et al (cited in Wang 1986) found that *C. botulinum* grew in water-packed tofu and produced toxin when stored at 15º and 25ºC but not at 5º and 10º during the six weeks of study.

For sufu, although pure culture is used in the pehtze fermentation, the process of sufu manufacture itself is sometimes carried out under non-sterile conditions, and microbial contamination may occur. Whereas most sufu contains considerable levels of salt (5 – 15%) and ethanol (1 – 7%), it is known that endospore-forming rods such as *Bacillus* spp. and *Clostridium* spp. may survive in these products (Han et al., 2001b)

Previous studies

Few surveys have been conducted globally on the natural microflora and microbiological quality of tofu (Table 3).

Table 3. Surveys on microbiological quality of tofu at retail

Year	Country	No of samples	Findings	Reference
Unknown	The Netherlands	23	- Samples: sufu (fermented tofu) - Three samples had *B. cereus* counts greater than 10^5 CFU/g - One sample contained *C. perfringens* at ~10^5CFU/g - *S. aureus* was not detected in any of the sample, but *S. aureus* enterotoxin A was detected in some of the white and grey sufu - *L. monocytogenes* was not detected in any of the samples	Han et al., 2001b
1999	Japan	29	- Samples include: fresh and fried tofu - SPC was higher in fried tofu compared to fresh tofu (3.6 log and 2.6 log respectively) - One fresh tofu sample contained *Listeria murrayi* (now *grayi*)	Kaneko et al., 1999
1999	USA	60	- Temperature of tofu at time of sale ranged from 1.7°C to 17°C - SPC ranged from $3.33x10^1$ to $7.73x10^7$ CFU/g - pH ranged from 4.68 to 6.36 - Two samples were bulging in the packet at time of purchase (stored at 16°C & 13.9°C)	Ashraf, 1999
1985	The Netherlands	154	- No *Salmonella* was detected in any of the samples - *B. cereus* was detected in one sample at the level of >10^5 CFU/g - *S. aureus* was detected in one sample at the level of >10^5 CFU/g - *E. coli* present in 36% of samples - Mould present in 14% of samples - Yeast present in 59% of samples at levels greater than 10^3 CFU/g - 95% had SPC >10^6 CFU/g - 35% of samples were sold at temperatures greater than 7°C - pH of samples ranged from <4.5 to ≥6.0, with the majority of samples at pH of 5.0 to 5.4.	Kooij & de Boer, 1985

Recurring issues identified in these surveys include:

- Manufacturing of products commonly occur under unsanitary conditions resulting in unsatisfactory microbiological results
- Shelf life of products appeared to be longer than expected (Ashraf, 1999; Van Kooij & de Boer, 1985)
- Tofu is often incorrectly stored at ambient temperature (Ashraf, 1999; Van Kooij & de Boer, 1985)

Outbreaks and recalls

There have been few outbreaks where contaminated tofu has been implicated (Table 4). The largest was an outbreak of shigellosis in USA in 1988 with case numbers estimated to be 3175.

Table 4. Outbreaks linked to tofu

Year	Country	No of cases	Pathogen	Vehicle	Reference
2012	USA (New York)	2	*C. botulinum*	Home fermented tofu	Chai, Choi, Guitierrez et al., 2013
2006	USA (California)	2	*C. botulinum*	Home fermented tofu	Meyers et al., 2007
2003	Australia (NSW)	20	*S.* Typhimurium 170	Restaurant tofu dish	OzFoodNet, 2004
1988	USA (Michigan)	3175	*Shigella sonnei*	Festival tofu salad	Lee et al., 1991
1981/82	USA	50	*Y. enterocolitica*	Tofu	Tacket et al., 1985

Several factors contributed to those outbreaks:

- Inadequate processing

  In the California outbreak, the fresh tofu was a commercially packaged product purchased at a retail market. At home, the tofu was boiled and cut into cubes and stored at room temperature for 10 to 15 days. The tofu was then transferred to glass jars with chilli powder, salt, cooking wine, vegetable oil and chicken stock and left for a further three days at room temperature. The fermented tofu was stored and eaten at room temperature.

  In this outbreak, the investigator stated that the growth of *C. botulinum* and production of toxin might have been facilitated by several factors such as:

  - the almost neutral pH of the product
  - boiling the tofu, followed by bottling, potentially creating an anaerobic environment
  - storage at room temperature for days during and after preparation

  Similar causes were believed to contribute to the latest outbreaks in New York. Two separate cases were observed. Fresh tofu was purchased from the same grocery store in Queens in the same month. A site visit to the grocery store revealed that bulk tofu was sold in unrefrigerated, uncovered, water-filled bins. After purchased, tofu was cubed, placed in plastic container and stored at room temperature for about 7 to 10 days. The tofu was then transferred to glass jars with addition of chilli pepper and salt. Fermented tofu was stored in refrigerator and was not heated before consumption. The laboratory detected botulinum toxin type B in leftover fermented tofu consumed by case 1. Toxin was not detected in tofu consumed by case 2. Previous

investigations reveal that botulinum toxin can be distributed unevenly in food, which might explain the negative test results for leftover tofu in case 2.

Homemade fermented bean products, including tofu, are the most common foods causing botulism in China. During 1958 to 1989, home fermented bean products were associated with 63% of approximately 2,000 cases of botulism in China (Meyers et al., 2007).

- Food handler & hygiene

  The Michigan outbreak was caused by infected food handlers and lack of hygiene during preparation of the salad. There was limited access to soap and running water at the festival (Lee *et al.*, 1991).

- Contaminated water

  The 1981/82 outbreak was caused by the use of untreated spring water in the commercial plant that manufactured the tofu (Tacket et al., 1985).

- Unknown

  In the NSW outbreak, twenty people were sick after consuming fried tofu, eggplant and prawn dish at yum cha. Further investigations revealed that the imported uncooked prawn meat was positive for *S.* Dublin. The exact cause of the outbreak is still unknown.

Overall, only one of these outbreaks can be traced back to poor processing of the product. The other four outbreaks are related to other factors.

From January 2004 to November 2013, there were five recalls of tofu products in Australia due to microbial contamination (Table 5). There was also a recall of a range of pre-packaged tofu products in USA in 2007 due to the presence of *L. monocytogenes* (FDA, 2007). In the EU, from 2003 to 2012, five alerts[1] were issued and one border rejection occurred in relation to imported tofu products due to elevated level of *B. cereus* or botulinum toxin (RAFFS).

Table 5. Recalls of tofu in Australia

Year	Products	Reason for recall
2012	Preserved bean curd	*B. cereus*
2009	Preserved bean curd (chilli)	*B. cereus*
2009	Preserved bean curd, fermented bean curd, preserved bean sauce	*B. cereus*
2006	Preserved bean curd	Microbial contamination
2005	Fried tofu, tofu cutlets	Microbial contamination

[1] Alert notifications are sent when a food presenting a serious health risk is on the market and when rapid action is required. The RASFF member that identifies the problem and takes the relevant actions (e.g. withdrawal of the product) triggers the alert. The goal of the notification is to give all RASFF members the information to confirm whether the product in question is on their market, so that they can also take the necessary measures.

Imported Food Inspection Scheme

AQIS undertakes testing of imported foods under its Imported Food Inspection Scheme for compliance with the Code. Foods which are considered to pose a low risk to public health and safety are classified as 'random surveillance foods' and are inspected at a rate of 5% of all consignments.

Tofu, soy bean curd and soy milk curd are classified as low risk and are analysed for *B. cereus* only. From January 2010 to October 2013, 36 bean curd and preserved bean curd products were rejected due to contamination with *B. cereus* at levels that ranged from 300 to >15,000 cfu/g. An additional three products were rejected because they were visually unsuitable for consumption, and two products were rejected due to the presence of glyceric acid which is not permitted.

In addition, one batch of instant bean curd was rejected for containing *B. cereus* at the level of 2,400 cfu/g and three batches of bean curd sheet were rejected due to elevated levels of *B. cereus* (ranged from <100 to 120,000 cfu/g).

2 Tempeh

Tempeh, originating in Indonesia, is made by fermenting dehulled and briefly cooked soybeans with *Rhizopus oligosporus* mould. Tempeh is used as a main dish and meat substitute in Indonesia. Vegetarians in the West have used tempeh as hamburger patties. Unlike most other fermented soybean foods which usually involve more than one organism, long brining, and an ageing process, tempeh fermentation is short, simple and requires only one mould (Wang, 1986).

Processing steps

Beans are first soaked at ambient temperature. During soaking uncontrolled growth of microorganisms (mainly bacteria) present naturally on the soybeans takes place. Depending on the temperature during soaking, bacteria may reach 10^8 to 10^{10} cfu/ml after 24 to 36 hours. This spontaneous fermentation results in a drop of pH from 6.5 to approximately 4.5. In addition to acidification, gas production causes foaming and the soak water becomes viscous (Nout et al., 1987; O'Toole, 2004)

The soaked beans are de-hulled and carefully cooked to avoid overcooking or undercooking. The beans are then drained, cooled to below 35ºC, and dusted with wheat flour to provide a good source of fermentable carbohydrate, and inoculated (O'Toole, 2004).

Traditionally, the dry beans are then placed in a container with small pieces of tempeh from the previous fermentation, which serve as a source of inoculum, and then left at room temperature for one to two days. However, a commercially available starter culture of *R. oligosporus* is more widely used in the West (Ashenafi, 1994; Wang, 1986).

During the fungal growth phase, oxygen must be controlled at a reduced level, otherwise the fungus will grow too quickly and form black spore masses that degrade the quality of the tempeh. The traditional way to control oxygen is to wrap the inoculated beans in banana leaves, but a modern innovation is the use of microperforated polyethylene plastic (O'Toole, 2004).

In the finished product the beans are 'knitted' together by a mat of white mycelia. The retention of the whole bean gives it a higher protein and fibre content compared to tofu. A mild acid, usually vinegar, may be added in order to lower the pH and create a selective environment that favours the growth of the starter culture over competitors (Ashenafi, 1994; Wang, 1986).

Figure 2 outlines the processing steps for tempeh production.

CCP

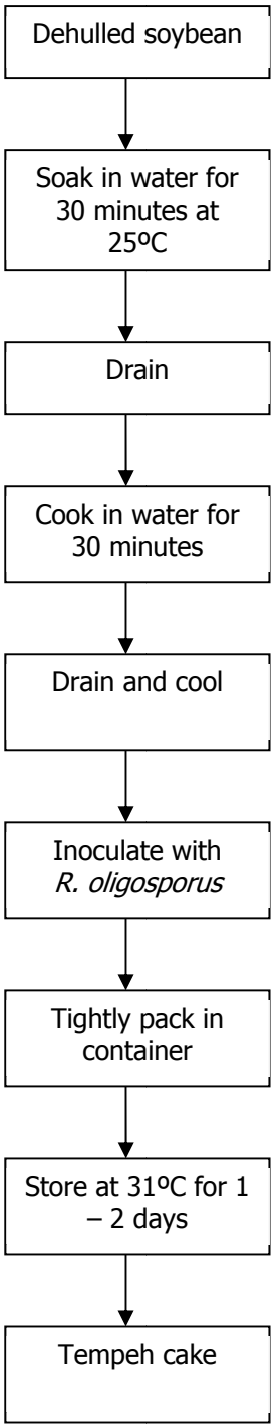
The most critical point in tempeh preparation is the fermentation process. *R. oligosporus* requires air to grow, but too much aeration will cause spore formation and also may dry the beans, resulting in poor mould growth. Therefore, both properly perforating the containers and packing the beans for fermentation are important.

Varieties

Traditionally tempeh is made from soybeans. However, copra (pressed coconut cake) and the by-product from making soybean milk have also been used in Indonesia to make tempeh known as tempeh bongkrek and tempeh gembus, respectively.

In the US, tempeh made from a mixture of wheat and soybeans has been available commercially since 1970 (Wang, 1986).

Figure 2. Flow diagram for tempeh fermentation (adapted from Wang, 1986)



Standard and Regulation

Standard 1.3.1 of the Code specifies permitted food additives that can be added to tempeh (vegetable protein products). Artificial colours are not allowed to be added to these products.

Microbiological hazards

Like tofu, tempeh should normally be relatively free of contaminated vegetative cells, but they may not be free of heat resistant spores. As the mould begins to grow rapidly, the temperature of fermenting beans rises a few degrees, and then falls as the growth of mould subsides. The pH increases steadily to above 7, presumably because of protein breakdown. Any contamination of the cooked beans will result in high bacterial population of the finished products (Ashenafi, 1994; Wang, 1986).

Failure of fermentation caused by bacterial contamination has been reported by tempeh producers. In order to assure successful fermentation, a starter with high viability and high level sanitary practice is very important (Wang, 1986). Steinkraus et al. (1965) stated that acidification during the soak was desirable in order to prevent growth of microbial contaminants during the fungal fermentation stage. They recommended that a sufficiently low pH of the cooked beans can be ensured by soaking them in dilute acids, e.g. lactic or acetic acid.

Tanaka et al. (1985) found that during manufacture of tempeh from unacidified soya beans, pathogens such as *C. botulinum*, *S. aureus*, *S.* Typhimurium, and *Y. enterocolitica* could grow very well and that clostridial and staphylococcal toxins could be produced. Nout et al. (1987) complemented the previous study by determining the effect of acidification on the growth of *B. cereus*. The study found that acidification of soya bean, either by biological or chemical means, is essential to inhibit *B. cereus* growth during the tempeh fermentation.

Previous studies

A study conducted by Samson et al. (cited in Nout et al., 1987) on microbiological quality of 110 retail samples of tempeh in the Netherlands revealed the general presence of lactic acid bacteria, Enterobacteriaceae and yeasts. In addition, 10% of the samples contained S. aureus and *B. cereus* in level that may cause illness.

Ashenafi (1994) conducted a study on the microbiological quality of tempeh during processing and storage. The study found that cooked beans for tempeh production, the water used for soybean cooking and the fresh tempeh product has plate counts of 8x103, 1.9x104 and $2.8x10^8$ CFU/g respectively. The psychrotrophic count of tempeh samples reached a level of $5x10^8$ CFU/g after two weeks of cold storage and the flora consisted of *Pseudomonas* spp., enterobacteria and enterococci. The study concluded that these steps should be followed to guarantee a wholesome tempeh:

- proper cooking of beans at temperatures that kill vegetative cells
- avoiding recontamination of cooked beans during further processing
- using uncontaminated inoculum

Outbreaks and recalls

To date, there is no reported outbreak or recall linked to tempeh in Australia.

There are hundreds of documented deaths in Indonesia linked to tempeh bongkrek (tempeh made from coconut after it has been pressed for oil). Tempeh bongkrek occasionally gets contaminated with the bacterium *Burkholderia cocovenenans*. The unwanted organism produces toxins namely bongkrek acid and toxoflavin, and kills the *Rhizopus* fungus due to the antibiotic activity of bongkrek acid.

In 2012, 89 cases of gastroenteritis related to infection with *S.* enterica ser Paratyphi B occurred in North Carolina (the United States). Eight of them required hospitalisation. Investigation revealed that the outbreak source was a *Rhizopus* spp. culture used in tempeh, that was contaminated with *Salmonella*. Unlike other commercial tempeh products, tempeh manufactured by this company is unpasteurised, and pathogens remained in the finished product (Griese et al., 2013).

Imported Food Inspection Scheme

No imported tempeh was identified in the NSW market.

3 Soymilk and milk products

Soymilk is a beverage made from soybeans. It is also the base for other products such as soy cheese and soy yoghurt.

Processing steps

Traditionally, soymilk production involves soaking the beans to rehydrate them, rinsing, resuspending in water, grinding of the beans, followed by filtration to separate soymilk and fibrous residue (okara). During soymilk production distinctive flavours developed that can be described as "beany", "painty", "rancid", or "bitter". The flavour is due to the action of a native soybean enzyme, lipoxygenase. Heating can prevent beany flavour development, but it may render some soy protein insoluble (O'Toole, 2004).

Modern continuous production systems try to balance those factors by minimise the incorporation of oxygen that reacts with lipoxygenase. A deodorisation step may also be included to remove volatile off-flavours and other undesirable matters (O'Toole, 2004).

Soy yoghurt is prepared using soy milk and yoghurt bacteria (*Lactobacillus delbrueckii* subs. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*). The process is similar to preparation of dairy-based yoghurt.

Varieties

Flavouring can be added to these products such as strawberry, chocolate, or vanilla.

Standard and Regulation

Standard 1.3.1 of the Code specifies permitted food additives that can be added to soy bean beverage (plain or flavoured), including steviol glycosides.

Microbiological hazards

FSA (2000) listed no microbiological hazards in relation to these products.

Previous studies

No information is available on this issue

Outbreaks and recalls

To date, there have been no outbreaks and recalls of soy milk and milk products in Australia in relation to microbiological contamination.

In late 2009, Bonsoy soy milk was recalled due to high level of iodine. The problem is not caused by the soymilk, but due to the use of kombu (seaweed) in the soymilk.

Imported Food Inspection Scheme

Tofu, soy bean curd and soy milk curd are classified as low risk and is analysed for *B. cereus* only. From January 2010 to October 2013, only one soymilk product was rejected at the border due to the presence of non-permitted additives.

Conclusion

The literature review found that there are a few microbiological hazards and outbreaks associated with tofu. There also seems to be an increase market share and availability of tofu in NSW. As a result, some tofu products will be tested to observe the microbiological quality and chemical properties of them. Tempeh and soy milk will not be included in the testing due to the lack of known hazards and outbreaks associated with them.

FERMENTED VEGETABLES

Fermentation is the "slow decomposition process of organic substances induced by micro-organisms, or by complex nitrogenous substances (enzymes) of plant or animal origin" (Walker, 1988). The changes caused by fermentation can be advantageous or disadvantageous. Fermentation, initiated by the action of microorganisms occurs naturally and is often part of the process of decay, especially in fruits and vegetables. However, fermentation can be controlled to give beneficial results. Fermentation is a relatively efficient, low energy preservation process, which increases the shelf life and decreases the need for refrigeration or other form of food preservation technology. It is therefore a highly appropriate technique for use in developing countries and remote areas where access to sophisticated equipment is limited (Battcock & Azar-Ali, 1998). The fermentation process also gives the raw vegetables a desirable taste, flavour or texture (Inatsu, 2007).

In terms of food processing, most vegetables are classified as 'low acid' foods. Low acid foods are more prone to deterioration by micro-organisms and can provide an ideal substrate for food poisoning organisms when in a moist environment. Low acid foods can be safely preserved by making them more acidic (through pickling), or by salting or drying (Anon, 1993).

Numerous fermented foods are consumed around the world. The most common organisms responsible for food fermentation are bacteria, yeasts and moulds. The most important bacteria in desirable food fermentations are the lactic acid bacteria (LAB) which have the ability to produce lactic acid from carbohydrates. Other important bacteria, especially in the fermentation of fruits and vegetables, are the acetic acid producing Acetobacter species. The most beneficial yeasts in terms of desirable food fermentation are from the Saccharomyces family, especially *S. cerevisiae* (Battcock & Azar-Ali, 1998).

Lactic acid fermentations are carried out under three basic types of condition: dry salted, brined and non-salted. Salting provides a suitable environment for lactic acid bacteria to grow which impart the acid flavour to the vegetable.

Kimchi

Kimchi is a unique traditional fermented vegetable product of Korea. Kimchi is made from Chinese cabbage pre-treated with brine and other ingredients such as garlic, ginger and red pepper. Kimchi has a sour/sweet and carbonated taste and is usually served cold. In Korea, production is estimated at over one million tons, mainly at household level. Daily consumption is estimated at 150 to 250 grams per person (Battcock & Azar-Ali, 1998; Cheigh & Park, 1994; Kim et al., 2008; Lee, 1997).

Processing steps

The principal steps in kimchi preparation are pre-treatment, brining, blending of ingredients, and fermentation.

Pre-treatments include grading, washing, and cutting. Appropriate cultivars of Chinese cabbage, with light-green coloured soft leaves and compact structures with no defects, are required for production of kimchi. After removing outer leaves and roots from the cabbage, it is cut into small pieces. Other ingredients are also graded, washed, and cut or chopped for the blending and fermentation steps.

Brining may be accomplished with either a brine solution or dry salt. Generally, in baechu kimchi, brining is carried out using a salt solution (8 to 15%) for two to seven hours in order to increase the salt content of the cabbage to between 2.0 to 4.0% (w/w). It is then rinsed several times with fresh water and drained to remove extra water by centrifugation or by allowing it to stand (Figure 3). For kaktugi, dry salt is added to radish cubes for a given time without rinsing.

Traditionally, kimchi is then packed in an earthen jar, buried in the ground, and pressed with a stone in order to immerse the ingredients in the juice. Nowadays, a fermentation vessel with lid on is commonly used. Fermentation may take one to three weeks at low temperature (2 to 10ºC) or one to three days at room temperature (20 to 25ºC) (Cheigh & Park, 1994; Lee, 1997).

Kimchi fermentation is carried out by various micro-organisms present in the raw materials and ingredients used in its preparation. After fermentation, the product can be left to mature for several weeks if refrigeration is available. If stored under warm conditions, kimchi deteriorates rapidly (Battcock & Azar-Ali, 1998).

CCP

The critical control point in kimchi production is the effective natural fermentation. It must proceed quickly and to completion (FSA, 2000). The fermentation of kimchi is carried out by various microorganisms, especially lactic acid bacteria (LAB) that are present naturally in the ingredients. Several factors, such as salt concentration, temperature, pH, population of other microorganisms and exposure to air, greatly influence the type of the principal LAB present (Cheigh & Park, 1994).

Brining is a very important step in the kimchi fermentation. Brining extracts the water from raw material by osmotic activity and suppresses the growth of some undesirable bacteria that can spoil the kimchi ingredients such as yeast and moulds. At the same time, it provides favourable conditions for LAB growth. The fermentation is normally carried out in anaerobic conditions and this minimises the growth of aerobic microorganisms (Cheigh & Park, 1994).

Varieties

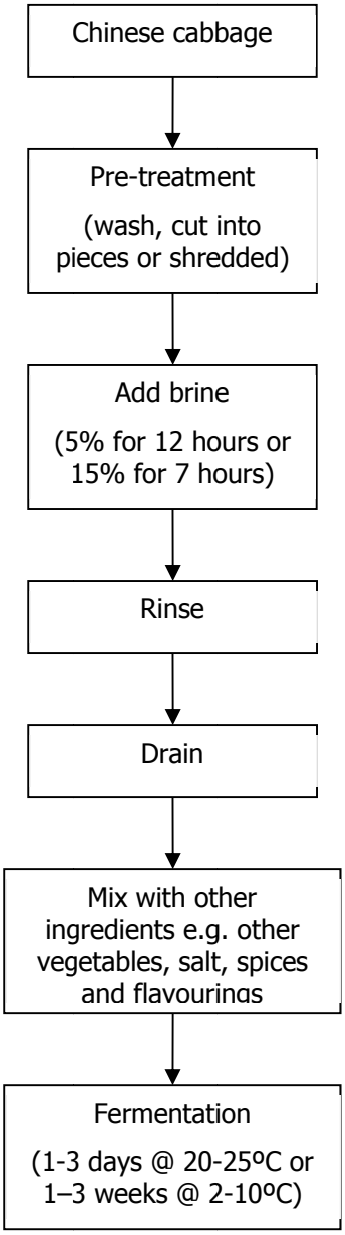
There are numerous (more than fifty) variations of kimchi depending on the ingredients and production technique. The main pickled cabbage kimchi are tongbaechu-kimchi (whole Chinese cabbage), baechu kimchi (cut Chinese cabbage), bossam-kimchi (wrapped-up Chinese cabbage), and kaktugi (diced radish kimchi) (Cheigh & Park, 1994).

Standard and Regulation

Standard 1.3.1 of the Code specifies additives that can be added to fermented vegetables. In addition, Standard 2.3.1 of The Code specifies that fruit and vegetables in brine, oil, vinegar or water, other than commercially canned fruit and vegetables, must not have a pH greater than 4.6.

CODEX has a standard for kimchi (CODEX STAN 223 – 2001) that outlines the product definition, composition, food additives and contaminants. In term of hygiene, kimchi production should adhere to the Code of Practice – General Principles of Food Hygiene (CAC/RCP 1 – 1969, Rev 4 – 2003). The product should also comply to the Principles for the Establishment and Application of Microbiological Criteria for Foods (CAC/GL 21 – 1997).

Figure 3. Flow diagram for kimchi production (Battcock & Park, 1998; Cheigh & Park, 1994; Lee, 1997)



Microflora

Taxonomically diverse groups of lactic acid bacteria (LAB) have been identified during the fermentation of kimchi. The best tasting kimchi is attained with an optimal product pH of 4.5 before overgrowth of *L. brevis* and *L. plantarum*. A number of studies have been carried out on the microbial composition of kimchi (Cheigh & Park, 1994; Choi et al., 2003; Kim et al., 2008; Lee, 1997).

One study found that fermentation of kimchi is initiated by LAB such as *Leuconostoc mesenteroides* under normal anaerobic conditions. As the pH drops to between 4.6 and 4.9 with organic acid accumulation, *L. mesenteroides* is relatively inhibited, but fermentation continues with other LAB such as *Streptococcus faecalis*, *Lactobacillus* brevis, *Pediococcus cerevisiae*, and *Lactobacillus plantarum*. The growth of each species depends on its initial number in the cabbage and other ingredients (Cheigh & Park, 1994).

Another study found that *L. citreum* appeared to be the predominant species in kimchi when fermented at 15º. This is not unexpected since the species is commonly found in both cabbage and garlic. However, as mentioned before, dominance during kimchi fermentation seems likely to be temperature dependent since *L. gelidium* was found to be dominant when fermentation was carried out at 8ºC (Choi et al., 2003). Low temperature is preferred in kimchi fermentation to prevent the production of strong acid and over-ripening and to extend the period of optimum taste (Lee, 1997).

However, according to Kim and Chun (2005), those studies have serious limitations including the availability of appropriate laboratory media. Also, the species were identified by phenotypic methods which can lead to misidentification and misinterpretation. In this study, a culture-independent molecular method involving direct DNA extraction, 16S rRNA gene amplification, amplified ribosomal DNA restriction analysis (ARDRA) and sequencing was used. The study found that the majority of LAB present in kimchi samples were species of the genus *Weissella* (46%), followed by *Leuconostoc* (39%) and *Lactobacillus* (15%).

In summary, those studies highlight the fact that kimchi has a unique and complicated bacterial community structure.

Microbiological hazards

In an inoculation test conducted by Ha (as cited in Lee, 1997), the antipathogenic activity of kimchi was demonstrated. *C. perfringens* disappeared after two days of kimchi fermentation, *S. aureus* and *Salmonella* Typhimurium after four days, and *L. monocytogenes*, V. parahaemolyticus and *E. coli* after five days.

The growth of pathogenic or spoilage microorganisms may be suppressed in this product, due to the following factors (Kim et al., 2008; Lee, 1997):

- The fermented kimchi has pH of 4.1 to 4.5 and salt concentration of 3 to 5%. The salt concentration will suppress the growth of undesirable bacteria without affecting that of LAB.
- Some of the kimchi ingredients such as garlic, green onion, red pepper, and ginger are known to contain sulphur containing compounds that have antimicrobial activity against pathogens.
- LAB are also known to produce a variety of antimicrobial agents such as organic acids, diacetyl, and hydrogen peroxide. Some LAB produce bacteriocins, which inhibit a variety of foodborne pathogens such as *B. cereus*.

However, outbreaks in Japan in 2001 raised concern over ability of some pathogens to survive in this product. Chinese cabbage are usually grown at 20 to 30ºC in soil and therefore can become contaminated from various sources such as polluted soil, animal manure, and contaminated water and equipment. Once contaminated, the bacterial population can increase during storage prior to fermentation above refrigeration temperature, even after the addition of salt (Inatsu et al., 2008).

Lee et al. (1995) conducted a study to determine the fate of *L. monocytogenes* during kimchi fermentation at 21 and 35ºC. The study found that *L. monocytogenes* was largely inactivated by kimchi ingredients and low pH, but viable cells still remained after ten days of fermentation.

Inatsu et al. (2004) conducted an inoculation study concerning the fate of four pathogenic bacteria in both commercial and laboratory prepared kimchi. It was found that *E. coli* O157:H7, *Salmonella* Enteritidis, *S. aureus*, and *L. monocytogenes* could survive in both commercial and laboratory-prepared kimchi when stored at 10ºC for seven days. In commercial kimchi, *S. aureus* level decreased rapidly from its initial inoculum level to a minimum detectable level within twelve days while *S.* Enteritidis and *L. monocytogenes* took sixteen days to reach a similar level. On the other hand, *E. coli* O157:H7 remained at high level throughout the incubation period. The result of the study suggested that contamination of kimchi with pathogenic bacteria at any stage of production or marketing could pose a potential risk.

Previous studies

A study was conducted in Taiwan on microbiological quality of kimchi and their histamine content. Thirty seven retail kimchi were tested for aerobic plate count (APC), total coliform and *E. coli*. The study found that all samples had pH between 3.6 and 5.1, with salt content ranging from 1.5 to 16%. Eighteen samples were unacceptable due to their APC content, fourteen samples were unacceptable in term of total coliform, and one sample contained *E. coli* at the level of 20 MPN/g.

Twenty three samples contained detectable level of histamine (LOD is 5mg/100g) and one sample contained histamine at the level of 535 mg/100g. Histamine is formed mainly through the decarboxylation of histadine due to the action of histadine decarboxylase which is produced by many bacterial species. LAB belonging to the species *Lactobacillus*, *Leuconostoc* and *Pediococcus* have been isolated from fermented food (Tsai et al., 2005).

Outbreaks and recalls

To date, there have been no outbreaks or recalls of kimchi in Australia.

However, there were two outbreaks associated with kimchi in Japan and one in Korea (Table 6) due to verotoxigenic *E. coli*. In the latest one, it was hypothesised that the outbreak was caused by insufficient ripening of products, hence there was increased opportunity for microbes to grow in the raw material such as cabbage and radish. The factors contributing to the other two outbreaks were unknown.

Table 6. Outbreaks linked to kimchi

Year	Country	No of cases	Pathogen	Vehicle	Reference
2012	Korea	1642	*E. coli* O169	Radish & cabbage kimchi	Hu, Seo & Choe, 2013
2001	Japan	Unknown	*E. coli* O157:H7	Locally made kimchi	Inatsu et al., 2004
1991	Japan	3	*E. coli* O169:H41	Kimchi purchased during a trip to Korea	Nishikawa et al., 1995

Imported Food Inspection Program

AQIS only tests imported kimchi (vegetables and fruits preserved in vinegar or acetic acid) for pesticides. No data is available on the results.

Conclusion

Kimchi is a very complex food due to the natural fermentation that takes place during processing. Kimchi supply has also grown as a consequence of Korean migration which has been running[2] at about 5000 people per annum over the last 5 years. Most Korean immigrants settle in NSW and their influence is obvious in Sydney's inner west. Thus, a number of kimchi products will be tested to observe their microbiological quality and chemical properties.

[2] Australian Government Department of Immigration and Citizenship

VEGETABLE-BASED DIPS & SAUCES

There are a number of vegetable-based dips and sauces available in the market. Dips are commonly consumed with finger foods, snack biscuits and other easily held foods. Some dips are hot filled and shelf stable, however, the majority of them require refrigeration.

Vegetable dips formulated with crushed cashews or grated parmesan cheese are now also in the marketplace. Vegetable dips generally have a pH below 4.6. However, vegetable dips containing cheese may have pH above 4.6.

Processing steps

The processing steps for these products vary depending on the type of dip. However, generally it involves grinding/mashing the main ingredient and mixing it with oil, spices, and various seasonings. Some of the ingredients may be cooked before mixing.

Varieties

Vegetable-based dips can be categorised into three types:

Sesame based dips

Tahini is made from roasted sesame seeds which are ground to form a thick paste suspended in oil. It forms a base for dips such as hommus and baba ghanoush. It can also be eaten mixed with molasses and honey and used to make confectionery (FSA, 2000). Due to its low water activity, it is normally sold in a plastic or glass jar on the shelf.

Hommus is a dip or spread made from cooked, pureed chickpeas, blended with tahini, olive oil, lemon juice, salt, garlic and other flavourings (FSA, 2000). Hommus has high water activity and pH of about 5.0, so it is commonly sold under refrigeration.

Baba ghanoush is a dish made from roasted, peeled, and mashed eggplant, blended with tahini, garlic, salt, white vinegar and lemon juice. Cumin and chilli powder can be added to mashed eggplant and mixed with various seasonings. It is often eaten as a dip or added to other dishes. It has high water activity, thus it is sold under refrigeration.

Salsa style dips

Salsa is a tomato-based sauce that is generally used in Mexican and South American foods. It can also be served as a dip. The common ingredients in salsa are tomatoes (cooked or raw), capsicum, garlic, onion, coriander, lime juice, other herbs and spices.

In Australia, salsa is commonly sold in glass jars or bottles (shelf stable) or in plastic tubs (require refrigeration). Shelf stable salsas have been cooked and some of them have also added vinegar. Tomatoes are acidic by nature, which along with the heat processing is enough to stabilise the product for grocery distribution.

Many grocery stores also sell "fresh" refrigerated salsa, usually in plastic containers. Fresh salsa has a shorter shelf life than jarred salsa. It may or may not contain added food acid.

Pesto style dips & tapenades

Pesto is a sauce originating in Italy. It is prepared by crushing basil leaves, garlic and coarse salt to a creamy consistency. Then pine nuts are then added and crushed together with the other ingredients. When the nuts are well-incorporated into the "cream", grated cheese and then olive oil are added and mixed. Commercial pesto is commonly available in either green (original) or red (with sun-dried tomatoes or red bell peppers) varieties. Cashew nuts or walnuts are often used instead of pine nuts, because they are less expensive and have a similar texture. Cheaper oils and other herbs, like parsley, may also be used. It is commonly used in pasta or other dishes.

Tapenade is an olive-based spread. The olives (most commonly black olives) and capers are finely chopped, crushed, or blended. Olive oil is then added until the mixture becomes a paste. Tapenade is often flavoured with other ingredients such as garlic, herbs, anchovies, lemon juice, or brandy. Some pastes based on sun dried tomato, capsicum or basil and parmesan are marketed as tapenades.

Standard and Regulation

Standard 1.3.1 of the Code specifies permitted food additives that can be added to tomato products with pH less than 4.5 eg salsa and fruit and vegetable spreads including jams, chutneys and related products.

Microbiological hazards

Since many of the products are not commercially sterile, their safety and quality is dependant on the combination of the following:

- the quality of the ingredients
- minimal heat treatment (if any)
- storage at a chilled temperature where relevant
- formulation
- restricted shelf life
- intrinsic properties of the food (eg pH, water activity, preservatives).

A range of pathogenic microorganisms can survive or even grow in non-sterile products during storage. Studies found that pathogens can survive longer in acidic food stored at refrigeration temperature than when stored at room temperature.

Sesame based dips

The survival of *Salmonella* in tahini is aided by the high oil and low moisture content of sesame seed. Products (such as hommus and baba ghanoush) made using contaminated tahini as an ingredient may in turn be contaminated. As these products are often consumed without cooking, any pathogens present may not be subjected to a heat kill step (FSANZ, 2005).

Hommus has high water activity, high nutrient content and relatively neutral pH, which support microbial growth. The presence of olive oil and citric acid in the products may have bactericidal effect (Al-Holy et al., 2006; Almualla et al., 2010), however, they have been linked to foodborne illness due to pathogenic organisms such as *Salmonella* and *C. perfringens*. There also have been a number of recalls of these products due to the presence of *L. monocytogenes* or *E. coli* or *Salmonella*.

Hommus and baba ghanoush can support the growth of *Salmonella* because they have higher water activity than tahini (FSANZ, 2005).

L. monocytogenes is widely distributed in the environment and can be easily introduced to these products from many sources in the processing environment such as raw materials, food handlers, equipment and the utensils. Hommus is rich in simple sugars, predominantly sucrose and raffinose, which can be utilised by *Listeria* spp. Storage under refrigeration temperature also allows the growth of *L. monocytogenes*, while inhibiting other pathogens (Al-Holy et al., 2006). A study by Gohil et al. (1996) reported that hommus stored at 4ºC had a slight increase in *L. monocytogenes* level over the 72 hours period of incubation.

Salsa style dips

Red salsa has a relatively low pH (less than 4.0). Historically, acidic foods have been regarded as safe, but in recent years several foodborne outbreaks have been associated with low pH foods, raising questions about their safety. In the US, from 1990 to 2006, salsa was linked to 70 foodborne outbreaks (2,280 illness cases). More than 70% of these outbreaks were associated with salsa consumed or purchased from restaurant. Some pathogens that have been associated with salsa outbreaks include *Salmonella*, norovirus, *Shigella* spp, *Clostridium* spp., *E. coli* O157:H7, and *S. aureus* (Franco et al., 2010). *Salmonella*, *S. aureus* and *L. monocytogenes* can survive in these products long enough to cause foodborne illness in consumers.

A study conducted by Franco et al. (2010) found that inoculated *Salmonella* survived in samples stored at room temperature (20 ± 2º) for 24 hours (sample had to be discarded after that period due to deterioration in appearance and smell) and during the first three days when stored at refrigeration temperature (4 ± 2º). Campbell et al. (2001) reported growth of *Salmonella* Thompson in freshly made salsa (pH 3.4) stored at room temperature, but when the samples were stored at refrigeration temperatures, the growth was not detected. Experimental details of the growth studies were not provided and growth of *Salmonella* at pH 3.4 is contrary to expectations. Subsequent studies did not confirm the observation. One study found that salsa containing fresh garlic in combination with lime juice (pH of 3.6) was consistently both inhibitory to growth and bactericidal to *Salmonella* regardless storage temperature (Ma et al., 2010)

Studies have also found that storage temperature and pH have a significant effect on the survival of *S. aureus* in acidic or acidified food products. Raghubeer et al. (2000) found that levels of *S. aureus* in fresh salsa stored at room temperature declined after a few days of storage, and the bacteria were not present after one week of storage. A similar result was obtained by Franco et al. (2010) who found *S. aureus* populations decreased over time, however remained relatively high after 24 hours and three days when stored at room temperature and refrigeration temperatures respectively.

Raghubeer et al. (2000) conducted a study to determine the survival of pathogens (*E. coli* O157:H7, *L. monocytogenes*, and enterotoxigenic *S. aureus*) in salsa. The study found that *L. monocytogenes* showed the longest survival in salsa at both refrigeration and room temperature. *L. monocytogenes* was recovered after 60 days and one week when stored at 4ºC and 21ºC respectively. It has shown remarkable resistance to acidic conditions and survives well under refrigeration conditions.

The same study found that there was a decrease in the level of EHEC at 4ºC after a week, and no EHEC was recovered during the same period when stored at room temperature (Raghubeer et al., 2000).

Previous studies

There have been two studies conducted in Australia on the microbiological quality of vegetable-based dips at retail (Table 7).

Table 7. Surveys conducted on the microbiological quality of vegetable-based dips

Year	Country	Food tested	No of samples	Findings	Reference
2004	Australia (National)	Sesame seeds, tahini, halva, hommus, baba ghanoush	40	1 sample was positive for *Salmonella* Richmond	FSANZ, 2005
1997	Australia (ACT)	Dairy and non-dairy dips (packaged & loose)	76 (35 non-dairy)	For non-dairy samples: - 1 sample had pH of 4.6 (unacceptable) - 8 samples contained SPC at the level of >1,000,000 CFU/g (3 packaged & 5 unpackaged) - No samples contained coagulase positive Staphylococcus 3 samples contained *E. coli* at low level (unpackaged) but there was no indication of the types of dip.	Christen et al., 1997

Outbreaks and recalls

There were a number of outbreaks related to vegetable-based dips, especially hommus and salsa in restaurant or takeaway settings. CDC stated that nearly one in 25 restaurant-associated foodborne outbreaks with identified food sources between 1998 and 2008 can be traced back to contaminated salsa or guacamole. Contributing factors to these outbreaks include the use of contaminated ingredients, preparation of salsa in big batches, inappropriate storage times or temperatures and/or cross contamination from food handlers. The same contributing factors may also be applicable to retail salsa or guacamole, if the products do not undergo heat treatment or contain preservatives.

However, only three outbreaks were recorded in relation to vegetable-based dips sold at retail (

Table 8). Contaminated produce was the contributing factor in the Australian outbreak. Sesame seeds used in the manufacturing of Egyptian tahini were contaminated with *Salmonella*, and the organism survived the heating process. In turn, the tahini was then used in the preparation of hommus in a few kebab outlets and restaurants in Australia and New Zealand (Unicomb et al., 2005).

Table 8. Outbreaks linked to vegetable-based dips and sauces

Year	Country	No of cases (hospitalised)	Pathogen	Vehicle	Reference
2013	Italy	(50)	*C. botulinum*	Pesto	Roberts, 2013
2002/03	Australia & NZ	68	*S.* Montevideo	Tahini	Unicomb et al., 2005
2000	USA	30	*Shigella sonnei*	5-layer party dip (bean, salsa, guacamole, nacho cheese, sour cream)	CDC, 2000

From 2004 to November 2013, nine recalls were carried out in Australia due to presence of pathogenic microorganisms in vegetable-based dips (Table 9).

Table 9. Recalls of pre-packaged vegetable-based dips and sauces in Australia

Year	Products	Pathogenic organism detected
2013	Mediterranean style spinach dip	*L. monocytogenes*
2013	Tahini dip	*Salmonella*
2010	Tahini	*Salmonella*
2008	Guacamole	Coagulase positive Staphylococcus
2007	Basil pesto dip	*L. monocytogenes*
2007	Pumpkin & pepita dip, macadamia & sundried tomato dip, cashew & coriander dip	*L. monocytogenes*
2007	Hommus	*L. monocytogenes*
2005	Hommus	*E. coli*
2004	Tahini	*Salmonella*

More than twenty recalls have been carried out in the US since 2004 in relation to these products due to pathogens such as *Salmonella*, *Clostridium botulinum*, and *L. monocytogenes* (FDA). In addition, since 2000, nine notifications were published by the EU due to contamination of sesame paste products with *Salmonella* (RASFF).

Imported Food Inspection Program

The association of salmonellosis with consumption of tahini has resulted in a policy change in Australia in 2004. All foods where sesame may be present are considered high risk and all imported products are tested for *Salmonella* at the border by AQIS.

From January 2010 to October 2013, nineteen tahini products failed due to contamination with *Salmonella*. In addition, eight hulled sesame seed, one sesame paste and eight halva products were also failed due to presence of *Salmonella*.

Conclusion

There are a number of vegetable-based dips and sauces available in the market. The literature review also found a number of outbreaks and recalls associated with these products. They are not sterile and their safety relies on a number of factors, including storage under refrigeration. A number of these products will be tested to observe the microbiological quality and chemical properties.

MIXED SALADS

Mixed salads include commercial salads that have mixed ingredients such as rice, pasta, potato as well as green vegetables. These types of salad normally use mayonnaise-based dressing. Apart from the mayonnaise and the major ingredients, sugar, spices or organic acids may also be present. Due to the vinegar in the mayonnaise or additional acids added, these salads normally have pH ranging from 4 to 5.5. Individual components may be cooked but the mixed salads do not normally undergo heating process (Michels & Koning, 2000).

Processing steps

Generally, these products are prepared by cooking some of the ingredients, such as rice, pasta or potato, chopping vegetables and herbs, and mixing the ingredients with the dressing and other seasoning.

Varieties

A number of mixed salads are available in the market such as pasta salad, rice salad, potato salad, coleslaw, and cous cous salad.

Standard and Regulation

There is no specific standard or regulation in relation to mixed salad.

Microbiological hazards

Vegetables carry a natural non-pathogenic microflora, which may be dispersed over the plant or appear as microcolonies embedded in the plant tissue. The majority of these are gram-negative bacteria which belong to either the Enterobacteriaceae or *Pseudomonas* group. The number of bacteria present will vary depending on seasonal and climatic variation and might range from 10^4 to 10^8 CFU/g (Lund, 1992; Szabo & Coventry, 2001).

Contamination of vegetables with foodborne pathogenic bacteria can result from bacteria present in soil including *B. cereus*, *C. perfringens*, *C. botulinum* and *L. monocytogenes*. Other sources of pathogens including *Salmonella*, *E. coli* and *L. monocytogenes* are manure, sewage sludge, polluted irrigation water, animals and birds (Lund, 1992). Pathogens can also be present due to unhygienic practices after harvest.

During harvest and transport, raw vegetables may be bruised resulting in the release of plant nutrients which support the growth of any microorganisms present on the surface of the vegetable. A sanitiser rinse is sometimes used to assist in reducing the number of microorganisms present. However, commercial shredding and packing of vegetables can provide a considerable increase in microbial contamination. Shredders used to prepare shredded lettuce and cabbage were reported to be major sources of contamination in the factory (Lund, 1992).

According to data from the Centers for Disease Control and Prevention in the US (CDC), foodborne illness due to contaminated vegetables is on the rise, with products implicated including baby spinach, lettuce, seed sprouts and green onions (FDA, 2004). Investigations into outbreaks have identified issues such as agricultural water quality, the use of manure as fertilisers, the presence of animals in fields or packing areas, and the health and hygiene of workers handling the fresh produce during production, packing, processing, transportation, distribution, or preparation. Many of these products are often consumed raw which can contribute to their potential as a source of foodborne illness.

Mayonnaise-based salads with a pH above 5.0 are likely to permit growth of pathogens when not kept under refrigerated conditions. Thus, it is important to ensure that good handling practices are followed to reduce contamination (Michels & Koning, 2000; Uyttendaele et al., 1999).

E. coli O157:H7 has also been implicated in outbreaks of these products. Studies showed that the survival of *E. coli* O157:H7 in coleslaw is due to its acid tolerance characteristics, and not temperature abuse (Beuchat, 2009).

L. monocytogenes is also an important pathogen to be considered in relation to these products. These products are generally stored under refrigeration temperature to extend the shelf life. Studies showed that the rate of *L. monocytogenes* growth decreased as the temperature decreased and the pH of the mayonnaise did not have a consistent effect on the rates of death (Beuchat, 2009).

Previous studies

There were five studies conducted overseas to determine the microbiological quality of these products at retail, pre-packaged or unpackaged (Table 10).

Table 10. Surveys on microbiological quality of mayonnaise-based salad at retail

Year	Country	Food tested (retail establishment)	No of samples tested	Organisms tested	Result Prevalence or mean	Reference
2005 - 2007	Belgium	Mayonnaise-based deli salads (retail)	1187	*L. monocytogenes*[1]	80 (6.7%)	Uyttendaele et al., 2009
2005 - 2006	South Africa	Assorted salads e.g. fruit & vegetables, mixed with mayonnaise (delicatessens)	35	APC (CFU/g) *S. aureus* (CFU/g) *B. cereus* (CFU/g) *L. monocytogenes*[1] *Salmonella*[1]	10^7 10^2 10^2 1 (3%) 4 (11%)	Christison et al., 2008
2000 - 2001	USA	Seafood salads Deli salads – potato, tuna, pasta & coleslaw	2446 8549	*L. monocytogenes*[1]	115 (4.7%) 202 (2.4%)	Gombas et al., 2003
1997 - 1998	Belgium	Mayonnaise based salad –ham, chicken, seafood, vegetables (retail)	874	*L. monocytogenes*[1]	186 (21.3%)	Uyttendaele et al., 1999b
1996	Japan	Cream based salad – potato, macaroni, coleslaw (packaged)	71	APC (>10^5cfu/g) *Listeria spp*[2]	13 (18.3%) 3 (4.2%)	Kaneko et al., 1999

Notes: [1] detected in 25g; [2] detected in 10g;

Outbreaks and recalls

Foodborne illness due to these products normally occurs when pathogens or toxin producing bacteria are present in the raw ingredients and allowed to grow. This may be due to a high level of handling, cross-contamination or temperature abuse. Pathogens that have been implicated in foodborne illness related to these products include *Salmonella*, pathogenic *E. coli*, *Shigella*, *L. monocytogenes*, *B. cereus*, and *S. aureus*. The most common vehicle for the outbreaks was potato salad. This might be due to the neutral pH of the potato with a low level of acetic acid which permits extended survival and growth of pathogens, particularly when temperature abuse takes place (Michels & Koning, 2000).

Little & Gillespie (2008) reviewed outbreaks related to prepared salads (both green leafy vegetables and mayonnaise-based salad) in England and Wales in the period of 1992 to 2006. The authors reported 82 outbreaks with 3434 people affected, 66 hospitalisations and one death. Most of the outbreaks took place in commercial food service premises, with functions at restaurants and hotels accounting for three quarters of outbreaks. Cross-contamination was the most common cause of outbreaks, followed by infected food handler and inappropriate storage.

The majority of outbreaks linked to these products in Australia also took place in commercial food service setting such as restaurants or commercial caterers (OzFoodNet).

To date, there were two outbreaks overseas due to pre-packaged coleslaw (

Table 11). Both outbreaks were caused by contaminated produce. In the 1981 outbreaks caused by coleslaw, cabbage was grown on a farm where two sheep had died of listeriosis. The farm used raw and composted sheep manure as fertiliser. The prolonged cold storage may have allowed *L. monocytogenes* to grow. In addition, two unopened packages of the implicated coleslaw, purchased at two different supermarkets and subjected to prolonged cold storage, tested positive for *L. monocytogenes* (Sewell & Farber, 2001).

A botulism outbreak in 1987 sent four circus performers to the hospital. Shredded cabbage, used in coleslaw and most likely packaged in a modified atmosphere, was the implicated vehicle of infection (Sewell & Farber, 2001).

Table 11. Outbreaks linked to pre-packaged mayonnaise-based salad

Year	Country	No of cases (hospitalised)	Pathogen	Vehicle	Reference
1987	USA	(4)	*C. botulinum*	Coleslaw	Sewell & Farber, 2001
1981	Canada	41 (17 deaths)	*L. monocytogenes*	Coleslaw	Sewell & Farber, 2001

Up until November 2013, there had been two mayonnaise-based pre-packaged salad recalls in Australia. Both of the recalls were related to undeclared allergens (milk solid in coleslaw and fish and egg in carbonara pasta salad). In addition, one chickpea and roast pumpkin salad was recalled due to contamination with *L. monocytogenes*.

Imported Food Inspection Program

Imports of pre-packaged mixed salad are not permitted.

Conclusion

Mixed salads are becoming more and more popular these days due to their convenience. Surveys carried out overseas found a number of pathogens in these products, especially *L. monocytogenes*. Thus, a number of these products sold in NSW will be tested to observe the microbiological quality and chemical properties.

FRESH CUT VEGETABLES EXCLUDED FROM THE FSS

1 Fresh herbs

According to Standard 1.4.2 Schedule 4 of the Code, herbs consist of leaves, flowers, stems and roots from a variety of herbaceous plants, used in relatively small amounts as condiments to flavour foods or beverages. They are used either in fresh or naturally dried form.

Herbs are widely used in food preparation all over the world. Cleanliness and flavour are considered as the two most important factors when evaluating the quality. In general, the microbiological quality of herbs reflects the hygienic situation of the region where they were produced (Garcia et al., 2001).

Australia's culinary herb industry can be described as a maturing industry. It began to accelerate in the early 1990's but growth escalated in the late 1990's/early 2000's due to innovative processing, changes in Australia's ethnic mix and lifestyle, and capture of overseas markets (Parker, 2004; RIRDC, 2006). In 2006/2007, it was recorded that Australia produced approximately 3.7 million kg parsley and 5.8 million kg of other herbs (e.g. coriander, basil etc), with a gross value of more than seventy million dollars. Of the total Australian production, NSW contributed to approximately 13% and 25% of parsley and other herbs respectively (ABS, 2008). In addition, the export industry was projected to grow at 100% per annum, giving an export farm-gate value of $100 million in 2009 (Parker, 2004; RIRDC, 2006).

Processing steps

Herb production is labour intensive. Hydroponics production, which accounts for about 5% of Australia's fresh herb production, is also very capital intensive. The post harvest handling of herbs is outlined in Figure 4.

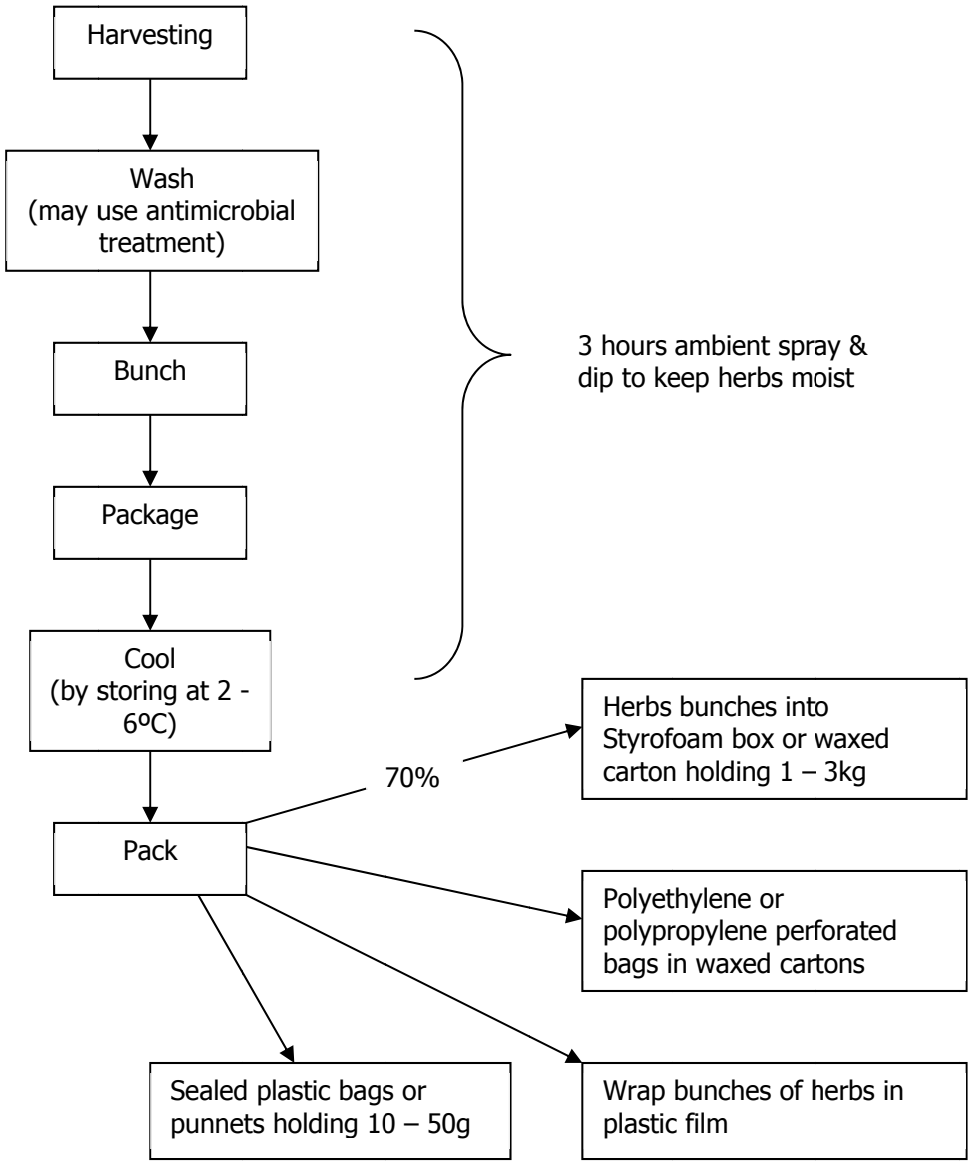
Varieties

The Australian market for culinary herbs falls into two major categories: fresh and dried herbs. This paper focuses on fresh herbs without further processing such as chopping. Fresh bunches of herbs are commonly used by consumers, restaurants and caterers. Due to import restrictions virtually all sales of fresh herbs are Australian grown product. Lemongrass is a possible exception. Because Australia has a wide diversity of climatic conditions, seasonality in general is not a major issue (Vinning & Hemphill, 2001). Table 12 outlines the ten most significant herbs traded in Australia.

Table 12. The ten most significant herbs traded in Australia (Parker, 2004; RIRDC, 2000)

Herbs	Estimated % of total production in 2000
Parsley – curled and continental	25
Coriander	20
Dill	20
Basil	15
Mint	6
Chives	6
Rosemary	2
Oregano	2
Thyme	2
Others e.g. lemon grass, garlic chives, marjoram, fennel and sage	2

Figure 4. Post harvest handling of herbs (Adapted from Lopresti & Tompkins, 1997)



Standard and Regulation

Standard 1.3.1 of the Code states that no additives can be added to fresh herbs (untreated fruits and vegetables).

Microbiological hazards

Hazards associated with herbs will be dependent on their use. Herbs used in processed foods would present little concern. However, they are often added to foods that undergo no further processing or are eaten raw. Herbs may be contaminated by pathogens occurring naturally in the soil and decaying vegetable matter or as a result of fertilisers or irrigation water used in the fields (Islam et al., 2004; Steele & Odumeru, 2004). A study conducted by Islam et al. (2004) found that parsley grown in contaminated soil (either from contaminated manure or irrigation water) contained detectable *Salmonella* for up to 231 days.

Contamination may also occur during harvesting and post harvest activities from contaminated processing water, equipment, transportation belts and during transport (FSA, 2000; Sewell & Farber, 2001). Johnston et al. (2005) found the level of total coliforms in parsley and coriander increased during the rinsing step. Even though chlorine is an effective disinfectant for drinking and recreational waters and an effective surface disinfectant, it is less effective for reducing microbial loads on fresh produce.

The risk analysis conducted by Food Science Australia stated that fresh cut herbs that may be consumed raw are categorised high risk for pathogenic *E. coli*, *Salmonella* and *L. monocytogenes* (FSA, 2000). The FAO & WHO also support that conclusion by stating that leafy fresh herbs present the greatest concern in terms of microbiological hazards associated with fresh produce. This assessment was based on the number of outbreaks reported around the world and the number of illnesses and deaths. Increase in consumption, complex production and distribution, and extensive international trade contribute to the problem (FAO/WHO, 2008). Pathogenic organisms of concern include Enterohaemorrhagic *E. coli*, *Salmonella*, *Campylobacter*, *Shigella* spp., *L. monocytogenes* and *Cyclospora cayetanensis*.

Fresh herbs may easily become contaminated with *E. coli*. This organism is an indicator of faecal contamination, and is a common environmental bacteria that is found in soil and water. The process of cutting may introduce additional contaminants, and the availability of moisture and nutrients on the cut surfaces creates conditions that generally favour microbial growth (Doyle & Erickson, 2008; Elviss et al., 2009).

Wu et al. (2000) conducted a study on the survival and growth of S. sonnei on whole and chopped parsley leaves held at 4 and 21ºC and found that the organism grew rapidly on chopped parsley held at 21ºC and remained viable for at least 14 days at 4ºC. The study also found that washing parsley with vinegar (containing ≥5.2% acetic acid) or chlorinated water (containing >150 ppm free chlorine) will greatly reduce, if not eliminate, S. sonnei.

Another study conducted by Hsu et al. (2006) investigated the survival and growth of *E. coli* O157:H7 and *Salmonella* on a number of fresh herbs (chives, coriander, parsley, basil, oregano and rosemary). It found that there was a significant reduction in the number of *E. coli* O157:H7 and *Salmonella* in the first five days of storage. After five days, decreases in populations varied with pathogen and storage time. However, both organisms were still detected at high concentration at day 24, even at 4ºC. The study also found that coriander had the highest populations of both pathogens, while rosemary had the lowest.

Phenolics and other compounds present in the essential oils of certain herbs are known to be inhibitory or lethal to microorganisms, including S. aureus and *Salmonella* (Karapinar & Aktug, 1984). A study carried out by Burnett and Beuchat (2001) found that the percentage recovery of *Salmonella* was substantially lower in herbs (25.9%) compared to fruits (41.7%) and vegetables (50.1%) using a direct plating method.

Previous studies

A number of surveys have been carried out in Australia and overseas on the microbiological quality of fresh herbs, especially coriander and parsley. Overall, there was a low prevalence level of pathogenic organisms such as *E. coli*, S. aureus, *Salmonella* and *Shigella* in these products (Table 13).

Table 13. Surveys conducted on the microbiological quality of fresh herbs

Year	Country	Food tested	No of samples	Findings	Reference
2008	Lebanon	Parsley	25	- Samples were collected from an area where irrigation water was drawn from a local river or ground water wells - Four samples (13.8%) were positive for *E. coli* and ten samples (38%) were positive for *S. aureus*	Halablab et al., 2011
2008	England	Fresh herbs	298	- Samples were tested for *Salmonella* only - Five samples (1.7%) were positive. They were coriander, curry leaves and holy basil	Surman-Lee et al., 2008
2007	UK	Various herbs e.g. basil, parsley, coriander, mint	3760	- Samples were RTE and include loose or in a bunch, pre-packed or grown in a pot - 18 samples (0.5%) were positive for *Salmonella* - 137 samples (3.6%) contained *E. coli* at a level greater than 100cfu/g	Elviss et al., 2009
2005-2007	Australia	Parsley Basil	15 2	- Samples were collected from field, farm gate, and retail - Samples were tested for *E. coli, E. coli* O157:H7 or VTEC, *Salmonella*, and *Listeria* spp - One parsley sample (6.7%) contained *E. coli* at the level of 3.6 MPN/g and one sample (6.7%) was positive for VTEC - One basil sample (50%) contained *E. coli* at the level of 23 MPN/g	FSANZ, 2009
2005 & 2007	Norway	Basil, mint, coriander	Unknown	- Samples were pre-cut and imported from SE Asia - 28% and 15% of samples were contaminated with *Salmonella*	Cited by Elviss et al., 2009

Year	Country	Food tested	No of samples	Findings	Reference
2002-2003	USA	Coriander Parsley Dill	141 72 9	- Samples included domestically produced and imported - Samples were collected from different step of processing - Samples were tested for APC, *E. coli*, *E. coli* O157:H7, *Salmonella*, *Shigella*, and *L. monocytogenes* - No pathogenic organisms were detected on the samples	Johnston et al., 2006
2000-2002	USA	Coriander Dill Parsley	94 12 78	- Samples were tested for *Salmonella*, *L. monocytogenes*, & *E. coli* O157:H7 - No pathogenic organisms were detected in any of the samples	Johnston et al., 2005
2000	USA	Coriander Parsley (domestically produced)	85 90	- Samples were collected from packinghouses, repackers, or wholesellers - Samples were tested for *E. coli* O157:H7, *Salmonella* & *Shigella* spp (parsley only) - No sample was positive for *E. coli* O157:H7 - One coriander sample (1.2%) was positive for *Salmonella* - One parsley sample (1.1%) was positive for *Shigella*	FDA, 2003
1999-2001	Norway	Various herbs Parsley & dill	130 100	- Three samples of various herbs (2.3%) and five samples of parsley/dill (5%) contained thermotolerant coliform - No sample contained *E. coli* O157:H7, *Salmonella*, *L. monocytogenes*, *Staphylococcus* spp or *Y. enterocolitica*	Johannessen et al., 2002
1999	USA	Coriander Culantro Parsley (imported)	177 12 84	- Samples were tested for *E. coli* O157:H7, *Salmonella* & *Shigella* spp (parsley only) - 16 coriander samples (9%) were positive for *Salmonella* - Six culantro samples (50%) were positive for *Salmonella* - One parsley sample (1.2%) was positive for *Salmonella* and one (1.2%) was positive for *Shigella*	FDA, 2001

Year	Country	Food tested	No of samples	Findings	Reference
1983	Spain	Parsley	23	- Samples were collected during four seasons and from different sources (field & retail) - 13 samples (56.5%) contained *E. coli* at a level greater than 1000cfu/100g - One sample (4.3%) contained *S.* Typhimurium	Ruiz et al., 1987

Outbreaks and recalls

The number of foodborne outbreaks traced back to fresh fruit and vegetables have increased in recent years, especially overseas, and few of them are associated with contaminated fresh culinary herbs (Table 14).

Table 14. Outbreaks linked to fresh herbs

Year	Country	No of cases (hospitalised)	Pathogen	Vehicle	Reference
2007	USA, North Europe, & Russia	74 (3)	*S.* Senftenberg	Fresh basil	Pezzoli et al., 2007
2006	Denmark	200	*S.* Anatum & enterotoxigenic *E. coli*	Basil used in pesto	Pakalniskiene et al., 2008
1999	USA	35	*S.* Thompson	Coriander	Campbell et al., 2001
1999	USA	62	*Cyclospora*	Basil	Lopez et al., 2001
1998	USA	300	*Shigella sonnei*	Coriander	Naimi et al., 2003
1998	USA & Canada	486 (163)	*Shigella sonnei*	Imported chopped uncooked parsley	Crowe et al., 1999
1998	USA	77 (12)	ETEC	Imported parsley	Naimi et al., 2003
1997	USA	48	*Cyclospora*	Basil used in pesto	Pritchett et al., 1997

In all of the outbreaks, contamination was traced back to the distribution chain or at some point all the way to the farm.

In the 1998 USA and Canada outbreak (Naimi et al., 2003), factors that contributed to the outbreaks, included:

- The use of recirculated, unchlorinated water to chill the parsley after harvest and to make ice used for cooling during transportation.
- Farm workers had limited knowledge of hygiene
- Facilities were not in sanitary condition
- Temperature abuse at restaurants; parsley was often chopped in the morning and left at room temperature for many hours before used

The 1999 *Cyclospora* outbreak investigation revealed 2 possibilities; one - that the basil was contaminated with sporulated oocyst through contact with water on the farm (such as when pesticides mixed in farm water were sprayed onto the plants); and the second is that the basil was contaminated with unsporulated oocysts when handled by an infected person, who could have been asymptomatic (Lopez et al., 2001).

The wide spread of contaminated basil with *S.* Senftenberg highlighted the extensive international trade with these products. The outbreak was first observed in the UK. An enquiry was then sent to Enter-net (the international surveillance network for the enteric infections organism *Salmonella*, VTEC O157 and *Campylobacter*). Response was received from Denmark, the Netherlands and the United States. Investigation revealed that the source of the outbreak was most likely to be contaminated basil that came from Israel (Pezzoli et al., 2007). Lessons learned from those outbreaks included:

- Growers, packers, and shippers should adopt good agricultural practices
- On the farm, efforts should be made to prevent contamination of water and produce by humans and animals
- At the packing shed, potable water (chlorinated) should be used for washing
- Workers should maintain good hygiene
- Diseased and decaying produce should be removed during harvesting, processing, packing, distribution and at retail sale
- Accurate records of the distribution of raw produce should be in place
- Chopped fresh herbs should be consumed promptly. Produce should be kept under refrigeration temperature and damaged herbs should not be consumed

To date, there has been only one recall in Australia for fresh herbs. In 2006, fresh curly parsley was recalled due to contamination with *L. monocytogenes* and *Salmonella*.

From the year 2000 to April 2013, there were 168 notifications in the EU in relation to detection of pathogenic microorganisms in fresh herbs. The majority of them are due to detection of *Salmonella* or elevated level of *E. coli*. Products included imported coriander, parsley, basil, mint and tarragon.

Imported Food Inspection Program

Most fresh herbs sold in Australia are grown in Australia.

2 Snow pea sprouts

There is no available literature on snow pea sprouts/shoots.

The industry often labels these products as 'microgreens'. They are normally grown in soil trays in a green house environment that are harvested by cutting the leaves or stem. The seed or root system is then discarded. There is anecdotal information from one of the biggest sprouts producers in Sydney that manure is not used in the soil where snow pea sprouts/shoots are grown and they have seven years 'pathogen free' microbiological data for snow peas in soil trays.

Conclusion

The risk analysis conducted by Food Science Australia stated that fresh cut herbs that may be consumed raw are categorised high risk for pathogenic *E. coli*, *Salmonella* and *L. monocytogenes*. The FAO & WHO also support that conclusion by stating that leafy fresh herbs present the greatest concern in terms of microbiological hazards associated with fresh produce. The microbiological quality of these products sold in NSW is unknown. Thus a number of fresh herbs without further processing will be tested to observe their microbiological quality.

EDIBLE SEAWEED

The Macquarie Dictionary (2010) defines seaweed as any plant or plants growing in the ocean, especially marine algae.

Processing steps

Between 1981 and 1994 world production of seaweed increased from 3.2 million tonnes (fresh weight) to nearly 7 million tonnes. Seaweeds that are most exploited are the brown algae with about 5.2 million tonnes (75%) followed by the red algae (1.73 million tonnes; 25%) and a small amount of green algae (about 0.5%).

Seaweed can be harvested from the wild or cultivated. As demand increases, natural populations frequently become inadequate and attempts are made to increase production by resource management techniques such as improving harvesting techniques, removing competing species, adding artificial habitats and seeding cleared areas. Such techniques are most highly developed in Japan, China and South-East Asia.

The seaweed that is most cultivated is the kelp Laminaria japonica, which accounts for most of Chinese production (about 3.8 million tonnes). The most valuable crop is the red alga Nori (*Porphyra* species, mainly *P. yezoensis*).

Large-scale edible seaweed mariculture is carried out only in Asia, where there is a very high demand for seaweed products and growing populations to create market growth. Cultivation of seaweeds is a relatively low-technology business with a high labour content in the operation.

For example, in Nori farming seeds are planted on nets, which are then placed in the sea attached to rafts. In the first month the fronds grow slowly but, once they reach about 1 cm long, growth to 10-15 cm can take place in 15 days. About 50 days after seeding the nets the fronds are 15-20 cm long and ready to be harvested. Nori is not sold in the fresh state but is immediately dried into sheet (Guiry, 2010).

Varieties

There are three common groups of edible seaweed, namely brown seaweed, green seaweed, and red seaweed (Guiry, 2010).

Brown seaweed

The brown colour of these algae results from the dominance of the xanthophyll pigment fucoxanthin. They are the most common seaweed consumed in the world, which includes species such as:

- *Laminaria* spp, *Laminaria japonica* (also known as Kelp, Kombu, Konbu, or Sea tangle). Plants are dried after harvesting and either cut into strips or powdered. In Japan, kombu is used in the preparation of fish, meat dishes, soups and also as a vegetable with rice. Powdered kombu is employed either in sauces and soups or is added to rice in the same way as curry.
- *Undaria* spp (also known as Wakame). The harvested algae are dried after washing in freshwater. After resoaking, the plant material is used as an additive to soups (wakame soup is served with virtually every meal in Japan); toasted (Yaki-wakame); used half resoaked, with boiled rice; and coated in sugar and tinned (Ito-wakame).
- *Sargassum fusiforme, Sargassum* spp (also known as Hiziki). This type of seaweed is known to contain a high level of inorganic arsenic, thus consumption of this type is limited.

Green seaweed

The green seaweed includes species such as *Ulva lactuca* and *Chlorella* spp. The green algae are more commonly used as dietary supplement.

Red seaweed

The red colour of these algae results from the pigments phycoerythrin and phycocyanin. The most common species consumed is *Porphyra* spp (commonly known as Nori or Laver). Nori is sold in sheets and they may be toasted to give a green colour and then flaked and added to sauces, soups and broths. Sometimes it is just soaked and eaten. Nori sheets are used in many sushi dishes, for rice balls and as a topping or condiment for various noodle and other dishes.

Palmaria palmata is commonly found in North Atlantic and North Pacific coastal waters. After harvest, product is packed loosely into small transparent plastic bags and commonly consumed in this raw state, without any further cooking or processing (Moore et al., 2002)

Standard and Regulation

Standard 1.4.1 of the Food Standards Code specifies the maximum level of inorganic arsenic in seaweed (edible kelp) of 1mg/kg.

Microbiological hazards

Seaweed is harvested from coastal regions. Microorganisms may be found in these products which originated from human sewage effluent and agricultural run-off which may contain faecal pathogens.

There is very limited data on the microbiological hazards on these products. A risk profile exercise conducted by Food Science Australia (FSA 2000) found that there was no microbiological hazard identified with these products. Furthermore, a study conducted by Gupta et al. (2010) found that seaweed (brown algae) in their raw state had almost 100% inhibition against *L. monocytogenes*. Heating the seaweed resulted in the reduction of antimicrobial activity, especially against Gram negative bacteria, including those associated with sewage effluent and agricultural run-off.

Chemical hazards

The hazards associated with seaweed are mainly chemical present in the marine environment. In the early 1980s there was a concern regarding the safety of imported seaweed products in Australia. This followed a reported case of arsenic poisoning on NSW where a patient had consumed large numbers of kelp tablets on a daily basis (FSA, 2000).

Arsenic

Arsenic can be present in food in many different forms which vary in toxicity, with inorganic arsenic forms, arsenite (As3+) and arsenate (As5+), being the most toxic. Most of the arsenic in the diet is present in the organic form. Inorganic arsenic species are typically present as one to three per cent of total arsenic found in food (FSA, 2004a).

The main adverse effects reported to be associated with long term ingestion of inorganic arsenic in humans are skin lesions, cancer, developmental toxicity, neurotoxicity, cardiovascular diseases, abnormal glucose metabolism, and diabetes. Neurotoxicity is mainly reported with acute exposure from deliberate poisoning, or at high concentrations in drinking water. There is emerging evidence of negative impacts on foetus and infant development, particularly reduced birth weight (EFSA, 2009).

Arsenic is bioaccumulated by many species of fish and shellfish and is present in poultry and livestock. Studies have demonstrated most arsenic found in finfish and shellfish occurs as methylated arsenic compounds, with only small amounts of inorganic arsenic present. The complex arsenic compounds are much less acutely toxic than soluble inorganic compounds, with arsenobetaine (the predominant form found in finfish) being virtually non-toxic (Schoof et al., 1999).

Less information is available on the type of arsenic present in seaweed. Since 2004, international food regulatory agencies issued warnings about the inorganic arsenic content of hijiki seaweed (CFIA, 2001; FSA, 2004c; FSA, 2010). Food Standards Australia New Zealand issued similar warnings in 2004 and border inspection regimes were tightened to target all shipments of hijiki seaweed. The warnings, however, do not apply to other edible seaweeds such as arame, nori, kombu and wakame (FSANZ, 2004).

From 2003 to 2009 the European Rapid Alert System lists 27 incidents of elevated arsenic levels in seaweed products: 26 edible seaweeds and 1 dietetic supplement. The species of seaweed was not specified (RASFF).

A survey conducted by the NSW Food Authority (the Authority) on dried seaweed product sold in NSW found one of 48 (1.7%) samples to contain inorganic arsenic at the level of 38mg/kg (NSW Food Authority, 2010).

Iodine

Iodine is a naturally occurring chemical element. Iodine is present in fairly constant amounts in seawater, but its distribution over land and fresh water is uneven. In Australia and New Zealand natural iodine levels are very low in the soils where we grow our vegetables, grains and graze livestock. Iodine is found in seafood, iodised salt and some vegetables. A number of types of seaweed, such as kelp, tends to be high in iodine, ranging from 0.03 – 0.45 dry weight per cent (The Australian Thyroid Foundation, n.d.).

In December 2009, FSANZ issued advice for consumer not to consume Bonsoy soy milk products. This advice was provided following a cluster of nine adults and one child who presented with thyroid problems due to the consumption of that particular product. Investigation found that the product was fortified with kombu which contained high level of iodine. As a result, the products were recalled and reformulated to not contain kombu.

Also, as a response to the incident, a national survey of iodine levels in seaweed and seaweed containing products was undertaken in 2010 (FSANZ, 2013). This survey found that:

- Iodine levels in seaweed varied between red and brown seaweed but were generally higher in brown seaweed
- Iodine concentrations in wakame and nori seaweed and seaweed containing products were generally low.
- Some other dried seaweed types had high iodine levels, considered to be unsafe for human consumption.

As a result of the survey, AQIS has now included brown algae/seaweed vegetables on the risk category food test and are monitoring at the border to ensure that only products with safe levels of iodine are imported (1000 mg iodine/kg dried weight).

Previous studies

Four studies have been conducted to determine the microbiological quality of seaweed (Table 15). Most of them found no pathogenic organisms were present in the sample.

Table 15. Surveys conducted on the microbiological quality of seaweed

Year	Country	Food tested	No of samples	Findings	Reference
2008	Ireland	*Laminaria digitata, L. saccharina, Himanthalia elongata* (brown algae)	-	Raw seaweeds showed complete absence of aerobic mesophiles, halophiles, yeast & moulds. Heating at 85ºC for 15 minutes resulted in spore germination, thus 10^7cfu of aerobic mesophiles and halophiles were observed. Heating above 95ºC for 15 minutes resulted in complete inactivation of surface microflora.	Gupta et al., 2010
1992-94	France	Eight types of dried edible seaweed (brown, red & green algae)	44	Samples were tested for faecal coliforms, *E. coli, C. perfringens, S. aureus, Salmonella* spp, yeast & moulds 66% failed to comply with the French official requirements	Delarras, 1997
Unknown	Ireland	*Palmaria palmata* (red algae)	100g	Samples were tested for *Salmonella, Campylobacter, S. aureus, Vibrio* spp., *L. monocytogenes, E. coli* O157:H7, yeast & moulds. No pathogens were detected.	Moore et al., 2002
Unknown	Argentina	*Monostroma undulatum* (green algae)	-	No pathogens and faecal indicators were detected	Gallardo et al., 2005

Outbreaks and recalls

Apart from the Bonsoy incident, there have been two other recorded illness associated with the consumption of seaweed, one was in Japan and another in Hawaii. However, no causative agents were found in those two incidents (Hanne et al., 1995; Noguchi et al., 1994).

In Australia, two products recalls have been conducted on dry seaweed. In 2005, products were recalled due to contamination with *Salmonella* and in 2010, products were recalled due to high level of iodine.

Imported Food Inspection Program

Iodine testing of seaweed as a risk category food was implemented in October 2010, which means that 100% of consignments are tested by AQIS before they enter the market.

There are two tariff codes in relation to seaweed and each type is tested for different chemical:

- seaweed – brown algae are tested for iodine with a limit of 1000mg/kg dry weight. An exclusion is applied to Nori, laver, Kim, Gim, Slake (*Porphyra species*), Dulse (*Palmaria palmata*), seafood snacks and sushi sheets
- Hijiki (*Sargassum fusiforme*) is tested for inorganic arsenic, with a limit of 1mg/kg (based on FSC Standard 1.4).

From November 2010 to December 2012[3], 138/1073 (13%) of dried seaweed samples have been rejected at border due to elevated level of iodine, ranged from 1400 to 9300 mg/kg. During the same period, one (2/13, 15%) hijiki sample was rejected because it contained inorganic arsenic above the limit.

Conclusion

A risk profile conducted by Food Science Australia found that no microbiological hazard identified with was associated with fresh seaweed. All seaweed products sold in NSW are in dried form and they are all imported. There was an issue with excessive iodine content with dried seaweed, so 100% of consignments are now tested for iodine at the border. No further work will be done with these products.

[3] Data retrieved from DAFF Biosecurity website on 3 December 2013.

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