Risk Assessment Studies

Report No.9

MICROBIOLOGICAL RISK ASSESSMENT ON SALADS IN HONG KONG

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Food and Environmental Hygiene Department

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Abstract

Recently, salads have become popular among consumers in Hong Kong. Many different styles of salads are commercially available in the market. In commercial settings, salads are either freshly prepared at restaurants or produced as pre-packaged food at food factories for supplies to retail outlets. Some restaurants have self-serve salad bars and food ingredients displayed there may be of additional risk of being contaminated by the patronizing customers. In reviewing the microbiological surveillance findings of 573 salad samples collected from 1999 through 2001 by the Food Surveillance Programme, 10 (1.7%) out of 573 samples exceeded the levels set out in the FEHD's Microbiological Guidelines for Ready-to-eat Food in terms of specific pathogens. *Listeria monocytogenes* was isolated in 6 (1.0%) of these samples and *Salmonella* spp. was isolated in the other 4 (0.7%) samples. With respect to hygienic quality, during the past 3 years, 63 (24.9%) out of 253 samples and 3 (1.3%) out of 239 samples were found unsatisfactory and exceeded the hygienic quality level of Aerobic Colony Count and *E.coli* (total) count respectively. Salad preparation often involves handling of pre-cooked or ready-to-eat ingredients with little or no further cooking steps to reduce the microbiological risk. Therefore, improper handling of ingredients and contamination after processing are the two main pathways to contaminate the final products. Both the trade and consumers are advised to take necessary precautions to enhance food safety in preparation and consumption of salads.

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Microbiological Risk Assessment on Salads in Hong Kong

OBJECTIVE

The aim of this paper is to evaluate the microbiological risk of salads in Hong Kong.

INTRODUCTION

2. Salads are mixtures of minimally processed ready-to-eat vegetables[a] with or without dressing[b]. Other common ingredients are fruits, poultry, meat, seafood, egg, pasta, herbs, nuts and cooked vegetables. Salads are usually served cold.

3. The ingredients for making salads usually impart fresh-like quality characteristics. In addition to such desirable quality, the consumption of fresh vegetables and fruits is increasing as consumers

[a] Minimally processed ready-to-eat vegetables consist of raw vegetables that have been washed, peeled, sliced, chopped or shredded.
[b] Typical salad dressings include mayonnaise, thousand island sauce, French dressing, Italian dressing, etc..

strive to eat healthy diets and benefit from the year-round availability of these products that up until recently were considered to be seasonal. Recently, salads have become popular among consumers in Hong Kong. Many different styles of salads are commercially available in the market. Where restaurants served various kinds of salads usually as appetizers, pre-packaged salads supplied by food factories are also commonly available in supermarkets and restaurants with take away service.

Production of Salads

4. The production of salads includes steps in purchasing and processing of raw materials, mixing of ingredients, packaging, transportation and sale. The general production of salads is illustrated in the flowchart at Annex I.

5. Major ingredients are minimally processed ready-to-eat vegetables that have been washed, peeled, sliced, chopped and shredded. Some of the raw materials, like poultry, meat, seafood, egg and pasta have to be cooked first. Others such as canned products, herbs, nuts and dressings are usually readily available for immediate use.

6. Salads may be served with or without dressing depending on consumers' preference. Some types of salads consist of ingredients mixed with dressings. Other types of salads, or in case of pre-packaged salads, may contain ingredients only and a separate package of dressing is available for consumers to mix with the ingredients by themselves.

Salad dressings usually impart characteristic flavours and may contribute some preservative effect to the product to a certain extent.

7. Large fast food chain stores usually purchase pre-prepared raw materials which only required a mixing procedure before serve. Other food premises may purchase and prepare the salad ingredients by themselves.

8. The finished products may be served on the premises. Otherwise, the fresh-made salads may be packaged and sold in restaurants with take-away service or packaged in food factory and delivered to retail outlets or supermarkets for sale.

9. Some restaurants have self-serve salad bars where customers can pick ingredients of their choice to make up their own dishes. Food ingredients displayed at self-serve salad bars may be of additional risk of being contaminated by the patronizing customers.

Potential Microbiological Hazards

10. Microbial pathogens associated with minimally processed ready-to-eat vegetables include those occur naturally in soil such as *Listeria monocytogenes, Clostridium perfringens, Bacillus cereus* and those from faeces of human or animal origin such as *Salmonella* spp..[1] The possible sources of pathogens in these products include the incoming raw vegetables themselves, the food handlers and the processing

environment. When vegetables are chopped and shredded, the release of plant cellular fluids provides a nutritive medium in which microorganisms can grow. The high moisture content of fresh vegetables, the lack of a lethal process to eliminate microbial pathogens and the potential for time and temperature abuse during preparation, distribution and handling further intensify the risk of foodborne illness.[2]

11. Raw egg is known to be a source of *Salmonella*. This pathogen may be introduced into the final product if raw and unpasteurized eggs or egg products are used to prepare salad dressings.

12. As salad preparation often involves handling of pre-cooked or ready-to-eat ingredients, good personal and environmental hygiene practices should be observed to minimize the chance of introduction of microbial hazards.

MICROBIOLOGICAL SURVEILLANCE ON SALADS

13. The Food and Environmental Hygiene Department (FEHD) has a Food Surveillance Programme in place for food surveillance and enforcement purposes. Food samples were collected at every stage of the supply process from the local market for chemical, microbiological and radiological testing. Microbiological tests were conducted by the Pathology Service of the Department of Health. Microbiological examination results of salads obtained in 1999 to 2001 were extracted from the food surveillance database and were reviewed and analyzed in

the present study. The microbiological tests included those for specific pathogens as well as for hygienic quality.

14. The results were evaluated based on the Microbiological Guidelines for Ready-to-eat Food recommended by this Department (Annex II). [3]

RESULTS

15. From 1999 to 2001, a total of 573 salad samples were taken for microbiological examination for specific pathogens and hygienic indicator microorganisms.

Specific Pathogens

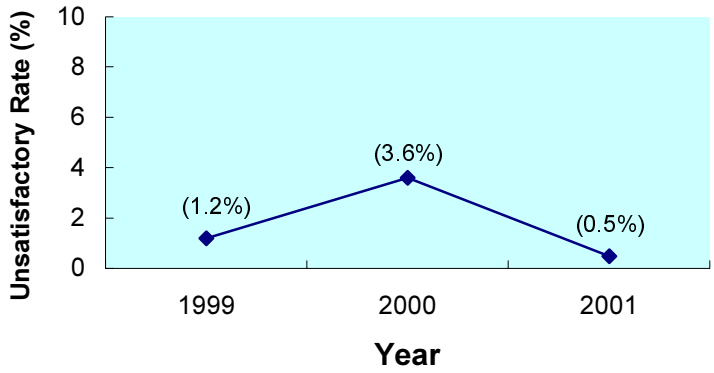
16. "Specific pathogens" refer to bacteria that may cause food poisoning or other illnesses. To safeguard public health, salads should not contain specific pathogens exceeding the levels as detailed in the FEHD's Microbiological Guidelines for Ready-to-eat Food (Annex II).[3]

17. Ten (1.7%) out of 573 samples were found unsatisfactory in terms of specific pathogens. The numbers of unsatisfactory samples were 2 (1.2%) out of 169 samples in 1999, 7 (3.6%) out of 194 samples in 2000 and 1 (0.5%) out of 210 samples in 2001 respectively (Table 1, Figure 1).

Table 1: Microbiological Surveillance Results of Salads – Specific Pathogens (1999 – 2001)

Year	Total Number of Sample	Number of Unsatisfactory Sample (%)
1999	169	2 (1.2%)
2000	194	7 (3.6%)
2001	210	1 (0.5%)
1999 – 2001	*573*	*10 (1.7%)*

Figure 1: Microbiological Surveillance Results of Salads – Specific Pathogens (1999 – 2001)



18. As regards the types of pathogens isolated, *Listeria monocytogenes* was detected in 6 of these samples, whereas *Salmonella* spp. was isolated in the other 4 samples (Table 2).

Table 2: Summary of Unsatisfactory Results – Specific Pathogens (1999 – 2001)

Year	Pathogens Isolated (Number of Samples)
1999	*Salmonella* spp. (1) *Listeria monocytogenes* (1)
2000	*Salmonella* spp. (2) *Listeria monocytogenes* (5)
2001	*Salmonella* spp. (1)

International Comparison

19. Several studies in relation to the microbiological status of salads and salad vegetables were reported in a World Health Organization (WHO) review document on fruits and vegetables eaten raw which was published in 1998.[4] The Public Health Laboratory Service (PHLS) of the United Kingdom had also conducted a study in 2001 on the microbiological quality of retail-prepared prepackaged ready-to-eat salad vegetables.[5] Table 3 summarized the results of these studies.

Table 3: Summary of Studies on Microbiological Status on Salad Conducted by Other Countries

Items	Country	Pathogen	Prevalence
Prepacked salads *	Northern Ireland	*Listeria monocytogenes*	3/21 (14.3%)
Salad vegetables *	Canada	*Listeria monocytogenes*	6/15 (40%)
Salad vegetables *	Germany	*Listeria monocytogenes*	6/163 (2.3%)
Salad vegetables *	Spain	*Listeria monocytogenes*	21/70 (30%)
Salad greens *	Egypt	*Salmonella* spp.	1/250 (0.4%)
Prepackaged ready-to-eat salad vegetables †	United Kingdom	*Listeria monocytogenes* *Salmonella* spp.	953/3851 (24.7%) 5/3851 (0.13%)

* Extracted from the WHO Review Report of Fruits and Vegetables Eaten Raw.

† Extracted from the PHLS study.

Hygienic Quality

20. Hygienic quality of food is reflected by the Aerobic Colony Count and *E. coli* (total) count.

Aerobic Colony Count (ACC)

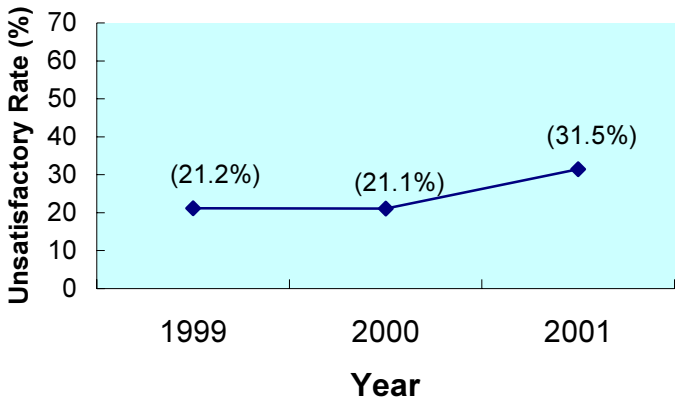
21. ACC is useful for indicating the overall microbiological quality of a food product. Generally, it does not relate to food safety hazards, but acts as an indicator for food quality, shelf-life and post-heat processing contamination. The quality level of ACC for salad recommended by this Department is not more than 10^6 colony-forming unit (cfu) per gram of food (Annex II). [3]

22. From 1999 to 2001, 253 salad samples were tested for ACC and 63 (24.9%) had unsatisfactory levels. The numbers of unsatisfactory samples were 18 (21.2%) out of 85 samples in 1999, 16 (21.1%) out of 76 samples in 2000 and 29 (31.5%) out of 92 samples in 2001 respectively (Table 4, Figure 2).

Table 4: Microbiological Surveillance Results of Salads – ACC (1999 – 2001)

Year	Total Number of Testing	Number of Unsatisfactory Result (%)
1999	85	18 (21.2%)
2000	76	16 (21.1%)
2001	92	29 (31.5%)
1999 – 2001	*253*	*63 (24.9%)*

Figure 2: Microbiological Surveillance Result of Salads – ACC (1999 – 2001)



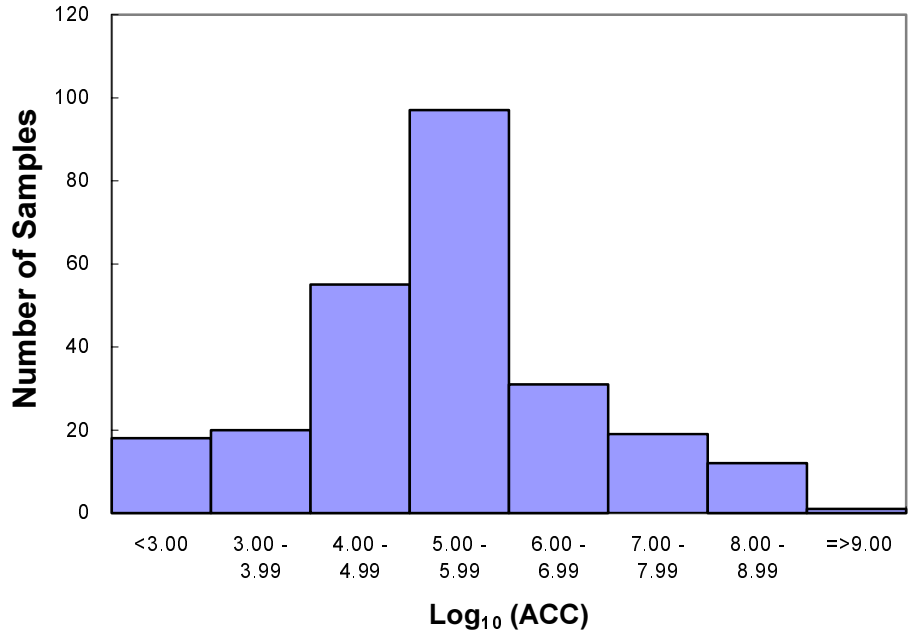
23. A breakdown of the unsatisfactory results of ACC was tabulated in Table 5.

Table 5: Breakdown of Unsatisfactory Results by Type of Premises – ACC (1999 – 2001)

Year / Type of Premises	1999		2000		2001	
	No. of samples taken	**No. of unsatisfactory sample (%)**	**No. of samples taken**	**No. of unsatisfactory sample (%)**	**No. of samples taken**	**No. of unsatisfactory sample (%)**
Restaurant	64	14 (21.9%)	60	10 (16.7%)	70	26 (37.1%)
Others	21	4 (19.0%)	16	6 (37.5%)	22	3 (13.6%)
Total	***85***	***18 (21.2%)***	***76***	***16 (21.1%)***	***92***	***29 (31.5%)***

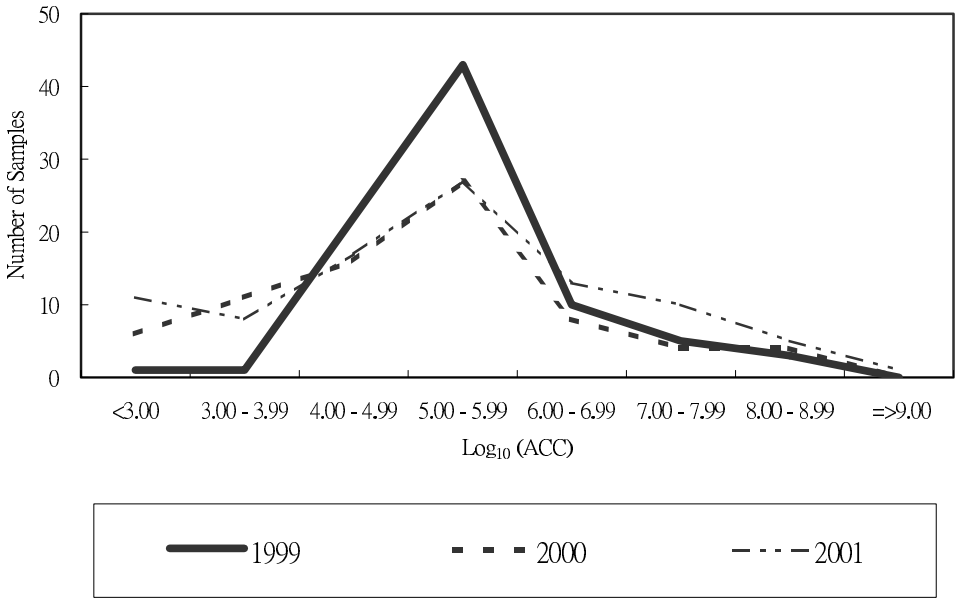
24. The distribution of ACC in salads was studied (Figure 3). It is shown that 97 (38.3%) out of 253 samples fall within values of $\log_{10}$ ACC range from 5.00 to 5.99, in which the upper range represents the margin for satisfactory level.

Figure 3: Aerobic Colony Count of Salads (1999-2001)



25. When the results are stratified by years, it can be seen that the distribution patterns for the three years are quite similar (Figure 4).

Figure 4: Distribution Curve of Aerobic Colony Count of Salads (1999-2001)



E. coli (total) count

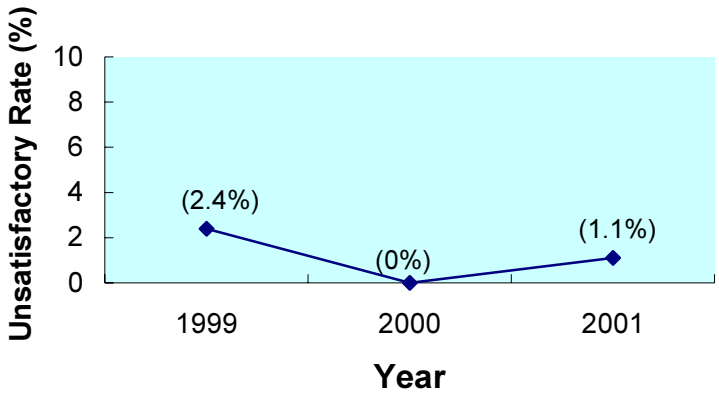
26. The presence of *E. coli* in foods generally indicates direct or indirect contamination from faecal origin. Substantial numbers of *E. coli* in food suggest a general lack of cleanliness in handling and improper storage. Its presence does not connote directly the presence of a pathogen, but implies a certain risk that may present. The quality level of *E. coli* recommended by this Department for salad is a total count of not more than 100 cfu per gram of food (Annex II). [3]

27. During 1999 to 2001, 239 salad samples were tested for *E. coli* and 3 (1.3%) were found unsatisfactory. The numbers of unsatisfactory samples were 2 (2.4%) out of 83 samples in 1999, 0 (0%) out of 66 samples in 2000 and 1 (1.1%) out of 90 samples in 2001 respectively (Table 6, Figure 5).

Table 6: Microbiological Surveillance Results of Salads – *E. coli* (total) count (1999 – 2001)

Year	Total Number of Testing	Number of Unsatisfactory Result (%)
1999	83	2 (2.4%)
2000	66	0 (0%)
2001	90	1 (1.1%)
1999 – 2001	*239*	*3 (1.3%)*

Figure 5: Microbiological Surveillance Results of Salads –*E. coli* (total) count (1999 – 2001)



DISCUSSION

28. *Listeria monocytogenes* and *Salmonella* spp. could be introduced by food handlers and cross-contamination between ready-to-eat and raw items.

29. *Listeria monocytogenes* was found in 6 of the 10 unsatisfactory samples for specific pathogens. *Listeria monocytogenes* is naturally found in soil and in decaying vegetables and can grow on salad vegetables such as lettuce at temperatures as low as 5°C.[6,7] As for *Salmonella*, the possible routes of contamination include direct contamination of the pre-harvested vegetables or fruits from faeces of birds and animals, the use of manure or sewage as fertilizer, or the use of contaminated waters for irrigation of the crops. Raw egg is also known to be a source of *Salmonella*. This pathogen may be introduced into the final product if raw unpasteurized eggs or egg products are used to prepare salad dressings. *Salmonella* may multiply in the finished

products if ambient conditions such as nutrient contents, pH value and water activity favour their growth. It is therefore of paramount importance to wash the salad vegetables thoroughly to reduce the microbiological hazards. Proper chilling of the salad items at 4°C or below is also essential to reduce microbial growth. The risk of *Salmonella* contamination by raw eggs can be reduced by using pasteurized eggs or egg products to prepare salad dressings.

30. Reports of several overseas studies regarding the microbiological quality of salads showed that the prevalence of pathogens was variable. It has been reported that the proportion of samples contaminated with *Listeria monocytogenes* ranged from 2.3% to 40%, and that contaminated with *Salmonella* spp. ranged from 0.13% to 0.4%. Our local surveillance results for *Listeria monocytogenes* compare favorably with those from overseas, with local prevalence of 1.0% (6 out of 573 samples) for *Listeria monocytogenes* in salads during 1999 to 2001. For *Salmonella* spp., the local prevalence was 0.7% (4 out of 573 samples), which is also comparable with overseas findings.

31. More than one-third (38.3%) of salad samples were tested to have marginal levels of ACC ($\geq 10^5$ to $< 10^6$ cfu) which are approaching the unsatisfactory level. As ACC is an indicator of overall hygienic quality of the product, good hygiene practices that are essential to improve the hygienic quality of salads should therefore be strengthened.

32. It is observed that there was an increase in unsatisfactory rate with respect to ACC in 2001. Analysis revealed that the increase was mainly contributed by samples taken from restaurants. As a great variety of foods were handled, processed and served by food handlers at restaurants, it is likely that cross-contamination between ready-to-eat and raw food items could contribute to the higher levels of ACC.

33. Samples found unsatisfactory with *E. coli* (total) count might be due to faecal contamination of salad crops. Such contamination might occur at the pre-harvested stage by way of using manure as fertilizers or using contaminated water for irrigation. Faecal contamination might also be introduced by food handlers if personal hygiene is not strictly observed.

34. Vegetables and fruits which are subject to processes such as peeling, slicing, chopping and shredding will remove the natural protective barriers of the intact plant and open the possibility for providing a suitable medium for the growth of microorganisms. These products are then eaten without a further heat processing step. It is therefore of paramount importance that these products should be washed thoroughly to minimize the microbiological hazards. Disinfectants may be applied to reduce the number of microorganisms on foods. However, when such chemical is used, the instructions from the suppliers of the disinfectants must be followed to prevent any chemical hazard being introduced into the final products.

35. Acid salad dressings such as mayonnaise may contribute some preservative effect to foods due to its relatively low pH. However, such preservative effect will depend on the initial contamination of the salad, the pH, presence of nutrients (sugars), etc.. The concentration of acetic acid (from vinegar) and the low pH of salad dressings are the major contributors to their anti-microbial properties. In case of mayonnaise, a total formula pH of less than or equal to 4.1 is essential to inactivate *Listeria monocytogenes*, *Salmonella* spp. in the dressing.[8,9,10] However, proper chilling of mayonnaise-based salad directly after production is important to provide protection against growth of microorganisms.[11] To be on the safe side, it is recommended to use only pasteurized eggs or egg products as an ingredient to produce salad dressings.

36. Food ingredients displayed at self-serve salad bars may be of additional risk of being contaminated by the patronizing customers. Storage temperature of the ingredients should be kept at 4°C or below. Sufficient numbers of tongs or ladles with suitably long handles should be provided and they should be replaced at a sufficient frequency (e.g. at 4 hours interval). Contaminated tongs and ladles (e.g. those dropped onto the floor) should be replaced with clean ones immediately. The salad bar area should be supervised by trained staff to prevent contamination of foods by customers.

CONCLUSION & RECOMMENDATIONS

37. To improve the hygienic quality of salads, good manufacturing

and handling practices should always be observed by the trade especially for restaurants providing self-serve salad bars. For consumers, they should observe hygiene rules to minimize the potential microbiological hazards. The followings are some recommendations.

Advice to Trade

(A) Handling of raw materials:

1. Purchase raw materials from reputable and reliable suppliers.
2. Wash all vegetables and fruits thoroughly.
3. Cook egg, poultry, meat and seafood thoroughly.
4. Use pasteurized eggs or egg products to prepare salad dressings.
5. Store salad dressings at 4°C or below once their packages are opened.
6. Avoid holding chilled ingredients (e.g. meat, seafood, cut fruits and vegetables) and finished products at above 4°C for more than 2 hours.

(B) Self-serve salad bars

1. Display salad ingredients in a proper manner and at 4°C or below.
2. Provide sufficient number of tongs or ladles with long handles and replace them with clean ones when appropriate (e.g. at 4 hour interval). Remove contaminated tongs and ladles (e.g. those dropped onto the floor) from the salad bar immediately.
3. Display ingredients in small portions and avoid topping up with fresh ones.

4. Discard leftovers.
5. Supervise the salad bar area by appropriately trained staff to protect the food against contamination by customers.

(C) Hygienic practices:

1. Observe good personal, food and environmental hygiene.
2. Prevent cross-contamination.

Advice to Consumers

(A) Purchase

1. Buy salads from reputable and reliable suppliers.
2. Check the "use-by date" and pay attention to the storage temperature of pre-packaged salads.

(B) Self-serve salad bars

1. Use appropriate utensils for picking up ingredients and avoid direct hand contacts.
2. Inform restaurant staff if mis-handling of food at salad bars is seen.

(C) Consumption

1. Consume the salads as soon as possible.
2. If salads are not consumed immediately, they (including home-made ones) should be:
   - packed and stored at 4°C or below.
   - separated from raw food.

- consumed within 1 to 2 days.

3. The elderly, children, pregnant women and persons with lowered immunity should be careful when choosing food especially high risk food, such as salads.

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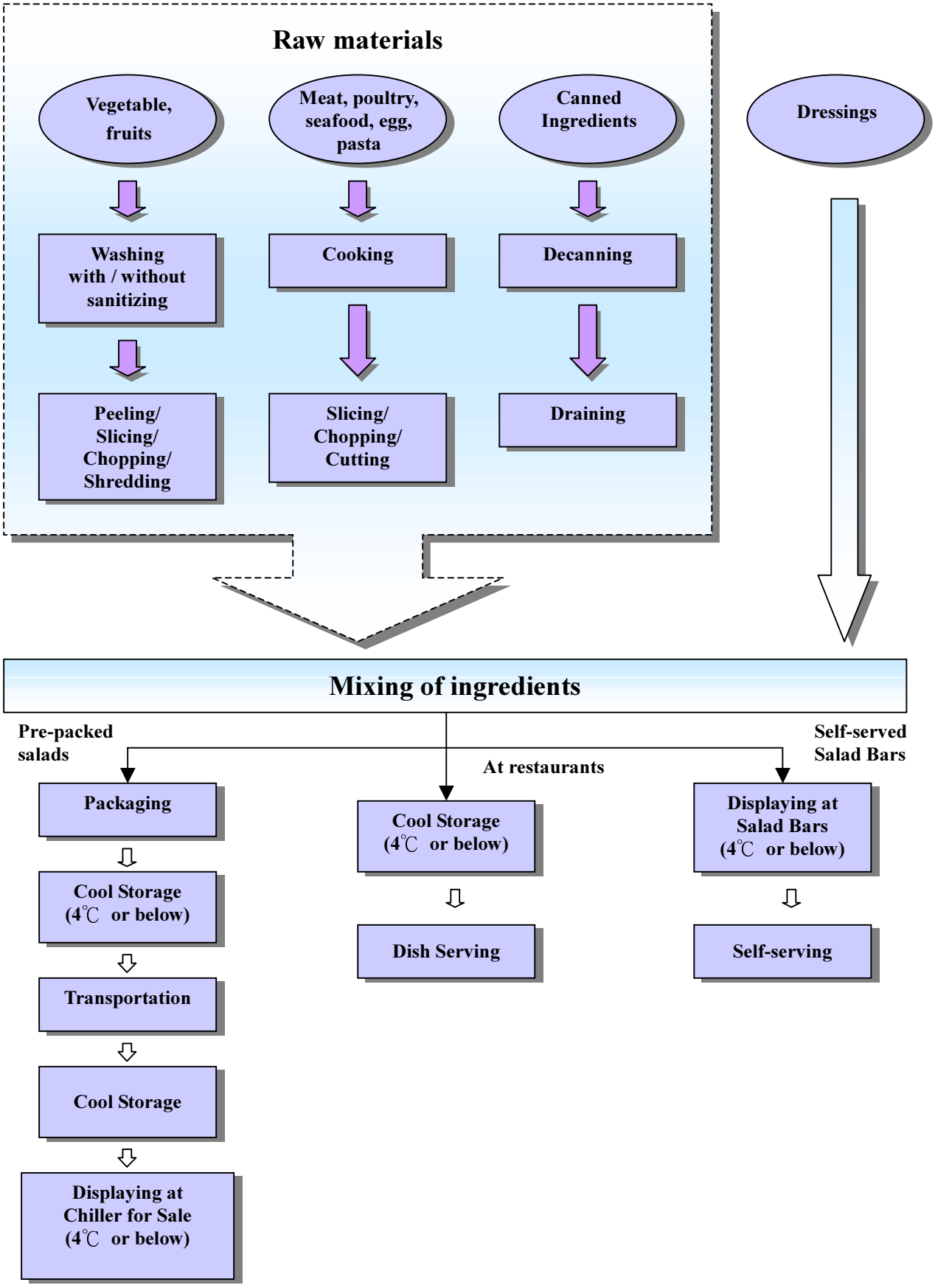
Annex I

Flow Chart of Salads Production



Annex II

Microbiological Guidelines for Salads

Hygienic quality	
Aerobic Colony Count (ACC)	≤ 10^6 cfu/g
E. coli (total)	≤ 100 cfu/g
Food safety	
Campylobacter spp.	Absent in 25g
E. coli O157	Absent in 25g
L. monocytogenes	Absent in 25g
Salmonella spp.	Absent in 25g
V. cholerae	Absent in 25g
V parahaemolyticus	< 10^3 cfu/g
S. aureus	< 10^4 cfu/g
C. perfringens	< 10^4 cfu/g
B. cereus	< 10^5 cfu/g