BC Centre for Disease Control
An agency of the Provincial Health Services Authority

Holding/Display Time of Potentially Hazardous Food: The Evidence

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Summary

Background

'Readily-consumed food' is consumed with no further processing; therefore, there is a microbiological risk associated with its consumption, especially with potentially hazardous foods. The *Canadian Food Retail and Food Service Code* authorizes a hold/display time of up to 2 hours for potentially hazardous food at room temperature, after which time items should be discarded.[1] Due to the impracticality of this code, the province of British Columbia is considering an extension of this time to 6 hours at 21°C, in line with the code predicated by the U.S. FDA.[2] Regarding this extension, the British Columbia Centre for Disease Control (BCCDC) is reviewing and evaluating the scientific basis/criteria for the U.S. FDA policies and attempting to locate additional resources to support these policies.

Intended audience

Policy-makers and public health officials

The issue

Examine the evidence to support policy change from a display time of 2 hours to 6 hours at 21°C for ready-to-eat food.

Search method used

Literature review

What is the evidence?

- The evidence used by the U.S. FDA is often dated; however, a search for more recent evidence did not bring new findings. In general, the evidence is limited and of variable quality, but valuable enough to help in the policy decision process.
- Evidence supports the use of time control as a strategy to prevent microorganism multiplication in ready-to-eat food.[3-8] The literature focuses on presence of spores and enterotoxin producing organisms in cooked food (*Bacillus* spp., *Clostridium* spp., and *Staphylococcus* spp.), although recent work done in the U.S. shows pathogens that caused the most fooborne illnesses are norovirus, nontyphoidal *Salmonella* spp., *C. perfringens*, and *Campylobacter* spp.[9]

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- In general, as far as spores producing *o*rganisms and toxins produced by *Staphylococcus aureus* are concerned, public health significance of extending display time from 2 hours to 6 hours, for cooked ready-to-eat food at 21°C, is minimal.
- Regarding ready-to-eat cut fruits and vegetables, due to the potential presence of Salmonellae in fresh herbs and the difficulty of washing ready-to-eat vegetables in general, some authors advise storage at temperatures below 8°C.[10,11] However, these results are based solely on the presence/absence of pathogens and not on their ability to grow and produce spores or toxins in this type of food. It is also expected that restaurants would keep this food refrigerated until consumption. Similarly, once fruits such as cantaloupes and watermelons are cut, they are considered PHF and must be stored below 4°C.

Implication for practice

- Generalizability of findings may be questionable, because results are specific to a certain type of bacterial strain and a certain type of environment; however, there is no strong evidence against the extension of hold/display time for ready-to-eat foods from 2 hours to 6 hours.
- Evidence shows that in certain cases the temperature margin at which food can be kept at room temperature for a 6-hour period is narrow.[3,4] An issue with respect to a time-temperature combination of 21°C/6 hours might arise when room temperature exceeds the safe zone.
- An attractive alternative strategy to temperature control, that covers the issue of generability and temperature buffer, is Time as a Public Health Control (TPHC).[8] This structured approach has been successfully used by Alberta Health Services and supports effective and consistent decision-making by both management and field staff.[8]

Gaps

Targeted studies addressing time control use at room temperature are limited; often one of the components in the time-temperature couple is missing. However more growth predictive models are becoming available and the USDA Agricultural Research Service created a Pathogen Predictive Modeling Program that can be used to predict the growth and inactivation of certain food-borne bacterial pathogens, under various environmental conditions.[12]

Introduction

Readily consumed food is becoming increasingly popular.[13] Since these products are consumed with no further processing, there is a microbiological risk associated with their consumption, especially with potentially hazardous foods (PHF). Lack of further processing opens opportunities for micro-organisms, present in innocuous levels, to multiply and reach infectious doses or produce enough toxins to impair food safety. As an alternative to temperature control, holding time prior to consumption is increasingly used to minimize the risk of food poisoning from PHF. This is reflected in the *Canadian Food Retail and Food Service Code*, an interpretative guide that advises a hold/display time of up to 2 hours for PHF at room temperature, after which time items should be discarded.[1] Yet, problems exist with the application of this code in the daily operation of food premises and the province of BC is considering a time extension to embrace the U.S. FDA code; permitting the storage of food for up to 6 hours under the condition that the holding temperature should not exceed 21°C[2]. In light

of this change, the National Collaborating Centre for Environmental Health (NCCEH) was charged by the British Columbia Centre for Disease Control (BCCDC) to review and evaluate the scientific basis/criteria for the U.S. FDA policies and attempt to locate additional resources to support these policies.

Literature Search Strategy

The first step was to analyse the 15 U.S. FDA code references to support section 3.501.19 *Time as a Pubic Health Control*.[2] These references included: peer-reviewed papers, books, conference proceedings, and a link to a modeling program.

A search for more recent evidence was conducted; the search strategy initially consisted of locating peer-reviewed journal articles with content relevant to ready-to-eat (RTE) foods. The following terms were used as keywords, either alone or in combination: ready-to-eat, street vendor cart, display case, market or baker, deli, school, cafeteria, food, egg, fish, seafood, tuna, salmon, vegetable, lettuce, broccoli salad, spinach or appetizer, bacteria, bacterial growth, contamination, microbiological risk, *Salmonella*, Norovirus, Listeria, Bacilli, temperature. A date restriction, January 2000 to December 2011, was imposed and English-only material was included. Literature searches did not tap grey literature; scientific literature was scoped using (i) the *Ebsco* database collection, available through the University of British Columbia Library; (ii) *Web of Science*, available through the University of British Columbia Library; and (iii) *Ingenta Connect*, available through a BCCDC subscription. This search generated 152 peer-reviewed papers. The reference list of each paper was consulted in an attempt to identify additional articles.

The quality of papers was assessed using the quality tools developed by the U.K. *Critical Appraisal Skills Programme* (CASP).[14-16] Depending on the study design, each article was rated on the 9 to 10 criteria as *strong*, *moderate* or *weak.* For a study to be rated as *strong*, none of the components could be rated as *weak.*[17] A rating of *moderate* was achieved only when one component was rated as *weak*. A rating of *weak* was given when two or more components were rated as *weak*.

Background

The U.S. FDA defines PHF in its 1999 food code as:

> ". . . food that is natural or synthetic and that requires temperature control for safety (TCS) because it is in a form capable of supporting:
>
> - the rapid and progressive growth of infectious or toxigenic micro-organisms;
> - the growth and toxin production of *Clostridium botulinum,*; or
> - in raw shell eggs, the growth of *Salmonella Enteritidis.*"[18]

The presence of pathogens on the foods, the characteristics of foods that support growth of pathogens, expected storage conditions, shelf life, and potential storage abuse are among the criteria used to include a food item as PHF.

Since 1999, this definition was updated and 'rapid and progressive growth' is defined as less than 1-log increase of a pathogen when food is stored at 24°C for a period of time that is 1.3

times the shelf life, as determined by the manufacturer.[19] This is based on the believe that the number of organisms in food is generally low (10 - 1,000 Colony Forming Unit (CFU)/g), therefore 1-log increase can be tolerated without putting health at risk.[8] For lower infectious doses, any introduction of organism into the food product will likely be sufficient to cause illness, irrespective of holding times and conditions.[8] Issues associated with food on hold or for display are similar to ready-to-eat foods (RET); the most common food-borne pathogenic bacteria found in refrigerated RET foods include: *Listeria monocytogenes*, *Salmonella enteritica*, *Escherichia coli* O157:H7, and *Clostridium perfringens*.[13] Improper cooling of prepared RTE foods is also a common cause of *C. perfringens* outbreak.[13]

According to the updated definition and common issues reported with RET, the literature search of this paper attempts to answer the following question: "Can food be displayed at room temperature for 6 hours without the bacterial population increasing by 1 log?"

If it takes more than 6 hours for the bacterial population to grow by 1 log, then the food is safe.

Results/Discussion

Evidence used by the U.S. FDA:

The U.S. FDA drew its evidence from 15 references dating from 1971 to 2000 (Table 1), including peer-reviewed papers, conference proceedings, and books related to:

- toxin production and/or spores germination of *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*;
- growth characteristics of *Listeria moncytogenes* and *Staphylococcus aureus.*

  Food investigated included: French fries, soy milk, rice, avian egg, sautéed onion, roast beef, chicken, and bottled chopped garlic.

Two references listed by the U.S. FDA but not analysed in this work include: *The Pathogen Modeling Program* by the USDA Agriculture Research Service[20] (because it refers to a modeling program) and the *Conference Proceeding* (2004), related to *time as a public health control* (only the summary, *Recommended Solution,* was available).[21]

Other references included challenge tests, surveys, and reviews:

- Mead et al. (1999) used multiple surveillance sources to examine food-borne illnesses and death in the U.S. from 1983 to 1997.[22] Authors concluded that among all illnesses attributable to food-borne transmission in the U.S., 67% are caused by viruses, 30% by bacteria, and 3% by parasite. The leading cause linking bacteria to food illnesses in the U.S. are: *Campylobacter* spp. with 47.1% of the total bacterial diseases, followed by *Salmonella* nontyphoidal (27.13%), *Shigella* spp. (8.61%), *Clostridium perfringens* (4.7%), and *Staphylococcus* spp. (3.55%). *Bacillus cereus* only represents 0.52% of the total food illnesses related to bacteria and *Listeria monocytogenes* only 00.5%. However, when comparing the death percentage to the total death attributed to food-borne illnesses, *Salmonella* non typhoidal (30.6%) and *Listeria* spp. (27.6%) are the leading bacterial organisms involved. For parasite, the leading cause of illnesses is *Giardia lamblia* (78.7%), while the leading cause of death is *Toxoplasma* (20.7%). With viruses, most deaths are linked to Norwalk-like viruses: 6.9%.

Therefore, although the majority of food-borne illnesses are due to viruses, deaths related to food intoxications are mainly caused by bacteria, in particular *Salmonella* spp. and *Listeria* spp.

- Bryan and Kilpatrick (1971)[23] conducted a time-temperature survey of the thawing, cooking, hot-holding, serving, chilling, and reheating processes during the preparation of roast beef sandwiches in a fast food service. Authors also investigated the presence of *Clostridium perfringens* in raw and cooked meat, as well as in workers and restaurant equipments, to pinpoint potential source of contamination.

  Results reveal that *Salmonella* spp. was only isolated from raw chicken while *C. perfringens* was omnipresent in the kitchen environment and in raw and cooked beef; 30% percent of swabs rubbed against raw beef and 19% of the 20 g raw beef sampled was positive for the presence of *C. perfringens.* This organism was also found in the environment, in stools, and on the hands of workers. Despite the lack of quantitative data regarding the concentration of *C. perfringens* in the samples analysed, the authors concluded that levels found will not cause any outbreaks unless the organism is given a chance to multiply, justifying the need for time control. In this regard, 2 steps in the sandwich-making process appeared to be particularly vulnerable, when the roast was in the warmer and during display on the slicer. During that time, the roast reached the optimal incubation range for *C. perfringens*; 46°C with an average generation time of 12 minutes. Although the authors emphasized the need for time control during that time, they did not provide any safe time range.

  This paper stresses the need for time control as a strategy to prevent micro-organisms growth on food that is on hold.

- The following two references were derived from textbook information on the growth and survival characteristics of *Clostridium perfringens* and *Bacillus cereus* in different types of food (ICMSF, 1996)[24,25]:
  - Chapter 2 is dedicated to *B. cereus* and relates to the growth characteristics of this organism in food. Perhaps the most interesting point of this section includes specific data, drawn from peer review papers, listing the toxin production pattern of *B.cereus* at different temperatures, in different types of food: with skim milk and pasteurised milk stored at 30°C, it takes between 7-8 hours before toxins can be detected; for skim milk stored at 15°C, the lag time is 68 to 92 hr; for rice meal, minced meat, and lasagne stored at 17°C, toxin can only be detected after 2 days.

  - Chapter 6 refers to *Clostridium perfringens* in food. Based on the literature review conducted by the authors, it appears that at 22°C the lag time for vegetative cells to grow and produce spores in raw minced chicken breast and leg is 6 hours. At 25 and 10°C, frankfurters and raw beef respectively will support growth but the generation time is 7 hours in frankfurters and 41 hours in raw beef.

  Judging from the above data, extending the hold/display time of PHF to 6 hours would not be a concern since, in the food tested (frankfurters, raw beef, raw minced chicken breast and leg), the earliest that *B. cereus* toxin can be detected is 7 hours while the lag time for *C. perfringens* vegetative cells to multiply and produce spores is at least 6 hours.

- Tatini (1973)[26] conducted a review of the environmental factors influencing the growth and toxin production of *S. aureus* in diverse type of food**.** Most of the review summarizes how nutrients, water activity, pH, oxygen availability, and associated growth of other organisms affects *S. aureus* toxin production. Lack of oxygen and associative growth of other organisms adversely affect enterotoxin production the most.

  This review also examines the influence of temperature on enterotoxin production. Results showed that production is less with a decrease of temperature within the range of 10°C to 40-45°C. The authors illustrate their result using the work of Donnely et al. (1968)[27]; in raw and pasteurized milk, a minimal incubation time of 18 and 36 hr was necessary at 25 and 20°C respectively to detect enterotoxin, after inoculating 10^6 CFU per ml of *S. aureus* cells.

  The data reveal that the production of enterotoxin by *S. aureus* decreases with a decrease in temperature within the range of 10°C to 40-45°C.

- Doan and Davidson's (1999a)[4] primary descriptive study examines the ability of *Bacillus cereus* spores to grow over time on oil blanched potato strips for home-style French fries at 21°C and 26.7°C. Low (3 log CFU/g) and high (5 log CFU/g) spore inoculums were seeded. Faster growth was achieved at 26.7°C, when compared to 21°C. *B cereus* increased by approximately 2 and 1.5 log CFU/g over 6 hours at 26.7°C for low and high inoculums respectively, while the bacterium did not reach the exponential growth phase at 21°C during the 9-hour time of the experiment. Viable *B.cereus* spores were reduced by 4.14 to 5.08 log CFU/g after 3.5 min at 185°C, depending on the fryer used.

  This study shows that oil blanched potato strips, to be used for French fries, can be stored at $\leq$ 21°C up to 9 hours, while this time should be restricted to 3-4 hours when stored at >21°C.

- Doan and Davidson (1999b)**[3]** did a similar experiment with *Staphylococcus aureus*, oil blanched potato strips, and French fries inoculated at about 3 or 5 log CFU/g. This time they investigated the ability of *S. aureus* to produce enterotoxin A at 21°C and 26.7°C. At 21°C the pathogen increased only slightly on oil blanched potato strips and no staphylococcal enterotoxins were detected on potato strips held during the 9-hour storage at 21°C. At 26.7°C, *S. aureus* grew and enterotoxins were detected after 5 hours of storage. *S. aureus* cells were not recovered once the contaminated oil blanched potato strips were fried; however, staphylococcal enterotoxins A were detected in both the native (fried) and renatured (refried) products. The authors estimated that a large order of French fries, produced under improper conditions, could contain approximately 31 to >124 ng of enterotoxins, which can be an issue knowing that the ingestion of as little as 100 to 200 ng can produce symptoms of staphylococcal intoxication.

  The authors concluded that oil blanched potato strips should be stored at 21°C or finish fried or discarded within 3 to 4 hours.

  This work indicates that oil blanched potato strips and French fries can be stored at 21°C for up to 9 hours.

- Melling and Capel (1978)[5] examined the emetic form of *Bacillus cereus* toxin, which has been almost entirely associated with consumption of cooked rice; intoxications due to the diarrhoeal form have been linked to other types of food. The goal of this work was to

investigate whether the association between the emetic form of the toxin and cooked rice can be explained by the physical properties of the toxin. Challenge tests were conducted to compare the resistance of the emetic and diarrhoeal toxins produced by *B. cereus* to various physical agents**.** Toxins were subjected to heat, pH, and enzymatic treatments. The emetic toxin appeared to be much more stable that the diarrhoeal toxin; emetic toxin remaining active after a heat treatment of 90 min at 126°C, while the diarrhoeal toxin is inactivated by a treatment at 56°C for 5 min. Similarly, the activity of the emetic toxin was unchanged after storage at 4°C for 7 days, while the activity of the diarrhoeal toxin was reduced.

This paper provides further insight into why the emetic form of *B. cereus* toxin is mainly associated with cooked rice; stressing the fact that once the toxin is present, it will remain active through cooking process. Therefore strategies to avoid food poisoning from cooked rice rely mainly on preventing the multiplication of *B. cereus*. The next paper will discuss how to prevent this multiplication.

- Johnson et al. (1983)[6] investigated the growth of *Bacillus cereus* in rice over a range of temperatures. The fastest growth occurred between 35 and 40°C with a generation time of 18-27 min. This work, however, does not provide information regarding the growth lag time or toxin production lag time at room temperature, although one reference mentioned by Parry and Gilbert (1980)[28] investigated the growth of different strains of this organism at 22°C in boiled rice. Boiled rice was inoculated with 3.2×10^2 and 2.7×10^4 spores/g of rice and incubated at 22°C. Results indicated that the 8 different strains tested grew well in boiled rice. After 6 hours of storage at 22°C, the population increased less that 10 times ($\leq$ log), with the exception of one strain which increased 1.58 log. Without any reference to toxin production, it is difficult to evaluate the health risks associated with the consumption of contaminated rice. Although, in its guidelines for the microbiology of ready-to-eat foods sampled at point sale, Gilbert et al (2000)[29] define as "acceptable" a concentration comprised between 10^3-10^4 CFU per g, while concentration $\geq 10^5$ qualified as potentially hazardous, probably because the minimum level required to cause illness has been estimated to be $>10^5$ g.[24]

  Knowing that *B.cereus* is found in food concentrations $<10^3$/g and mostly $<10^2$/,[24] a log increase after 6 hours of storage at 22°C would represent minimal health risks.

- Ferguson and Shelef (1990)[30] conducted a challenge test to examine the ability of *Listeria monocytogenes* to multiply in soymilk. Commercial milk from 2 different sources were inoculated with 10^2 and 10^4 CFU ml-1 and held at 5°C or 22°C. After a lag time of 4 hours at 22°C, the doubling time generation was 1.33 hours.

  Listeria monocytogenes is an opportunistic pathogen and not all strains will cause illnesses. However, when they do cause illnesses, the infectious dose is believed to be >100 viable cells.[31] Therefore, depending on the initial count of cells, storing contaminated soy milk at 22°C for more than 5 hours may represent a health risk. However, the generation time may be reduced at 20°C.

- Sionkowski and Shelef (1990)[32] investigated the ability of *Listeria monocytogenes* Brie-1 to grow in raw and heat treated whole eggs, albumen or yolk during storage at 5 or 20°C. Samples were inoculated with 10^5-10^6 cells/g. Results show an increase in cell numbers after 10 h of incubation at 20°C to reach $>10^9$ after 78 hours. Generation times were 2.6; 2.6, and 3.5 h in heat-treated whole eggs, yolks and albumen, respectively.

However, from the data presented in the paper, it is not clear whether the increase in cell number started at 10 hours or occurred earlier, as suggested by the authors' graph.

From the above two papers,[30,32] it is difficult to measure the health consequences of extending the display time from 2 hours to 6 hours.

- Solomon and Kautter (1986)[7] completed a study to follow up with an outbreak involving *Clostridium botulinum* type A and sautéed onion in margarine, used for sandwich making. The question was to determine whether *C. botulinum* spores could grow on sautéed onion and how long would it take to produce toxins. Sautéed onions were inoculated with various concentrations of spores produced by *C. botulinum* strain, ranging from 2 to 4.1 x 10^3 of spores/g of onions. The aim was to investigate their ability to convert into vegetative cells and produce toxins within 48 h at 35°C from an inocculum as low as 2 spores/g onions. No positive samples for the presence of toxin were found at 24 h of incubation for any of the strains tested. No data at 21°C is provided but, as a reference, earlier challenge tests performed at 20 and 22°C on potato reported time-to-toxin production in terms of days.[25]

- Solomon and Kauter (1988**)**[33] examined the implication of chopped garlic packed soybean oil in a 1985 outbreak in Vancouver. The challenge test studied the ability of *C. botulinum* type A and type B spores to grow and produce toxins in bottled chopped garlic stored at 35°C and at room temperature; however, room temperature is not defined. Results indicated that as few as 2 type A spores/g of chopped garlic produced high toxin titres within 5-10 days, while it took 10-15 days for type B strains to produce a similar amount of toxin. At room temperature, time-to-toxin production from an inoculum of 5 spores/g was extended to 30 days for type B spores. For type A spores, some strains started to produce toxin after 15 days.

  These data indicate that extending the display time to 6 hours for sautéed onion and chopped garlic packed soybean oil should not be an issue in terms of food poisoning due to *C. botulinum.*

NCCEH literature search

This search's aim was to answer the following question: If the food is slightly contaminated, does extending the storage to 6 hours allow "rapid and progressive growth", meaning 1-log bacterial growth as defined by the U.S. FDA? The search generated 153 articles. The reference list for each article was also consulted to find additional resources. However, few relevant articles were found (Table 2). Most articles included: challenge tests in ready-to-eat food, performed at temperatures other than 21°C; challenge tests at 21°C, but lacking a time component; ready-to-eat food contamination surveys; outbreak investigations; and effectiveness of the use of antimicrobial agents or treatment during food processing. Only nine papers were retained; they comprised a review on food-borne illnesses in the U.S., a review on biological hazards related to Clostridium spp. In food, a description of Time as a Public Health Control (TPHC), exposure assessment, and microbial survey of ready-to-eat food, including a component where food was tested for supporting bacterial growth. Studies were conducted in the U.S., Korea, Italy, Australia, and the U.K. Food being investigated comprised Asian food, fresh herbs, and Cicorino. Challenge tests were performed with *S.aureus*, *Salmonella enterica*, *Bacillus cereus,* and *E.coli.*

- Scallan et al. (2011)[9] used data from active and passive surveillance systems, risk factors studies, and current literature to estimate the proportion of pathogen specific illnesses caused by consumption of contaminated food in the U.S. This work updates the previous published results by Mead et al. (1999). Correcting for underdiagnosis and under-reporting, the authors estimated that 58% of total illnesses were caused by noroviruses, followed by non-thyphoidal *Salmonella* spp. (11%), *Clostridium perfringens* (10%), and *Campylobacter* spp. (15%). Authors established that the leading causes of hospitalizations and deaths were due to non-typhoidal *Salmonella* spp., 25% and 28% respectively.

- Hislop N. (2008)[8] describes an alternative to the use of temperature control for PHF, which is called Time as a Public Health Control (TPHC). This strategy, introduced by Alberta Health Services, has introduced TPHC principles, criteria, and processes across all zones of Alberta.[8] In the field, this principle translates into limiting the display of PHF at room temperature to less than four hours, time labelling the product on display, and discarding the food product at the end of that period; For those vendors who wish to display their products for longer than four hours, they need to go through an application, approval, and evaluation process to demonstrate that extending the time of display would not cause any microbial risk to consumer health.[8]

- The EFSA Scientific Panel on Biological Hazards (2005)[34] review summarizes biological hazards related to *Clostridium* spp. in food. The species of the genus *Clostridium* more involved in food-borne illness are *C. perfringens* and *C. botulinum.* Growth of *C. botulinum* has been observed in over-wrapped fresh mushrooms, in garlic oil, and in baked potatoes wrapped in aluminum foil when stored at ambient temperatures or when storage/time/temperature were not under control. Perhaps the most relevant information for our purpose is related to optimal growth and toxin production. The optimal growth of *C. botulinum* strains ranged from 28-40°C. The optimal temperature for growth of *C. perfringens* is 43-47°C and growth does not occur between 10-12°C. Almost all outbreaks result from conditions allowing multiplication of *C. perfringens* numbers reaching 10^6-10^7/g of food. Approximately 10^8 vegetative cells of enterotoxin-producing *C. perfringens* per serving are necessary to cause diarrhoea.

- Yoon et al. (2011)[35] used the Food Micromodel Predictive software to estimate *Staphylococcus aureus* growth and toxin production in Kimbab, a korean food where rice and different food items (e.g., ham, egg, seafood, vegetables) are rolled on a thin sheet of dried green (type of seaweed). Data used for the exposure assessment were derived from previous contamination studies data, surveillance results from Kimbab manufacturing companies, and prevalence of levels of *S. aureus*. Authors demonstrated that the growth of *S. aureus* accelerated proportionate to the holding temperatures investigated (10 to 30°C) and that the critical time for production of enterotoxin at 20°C was 22.6 hours.

  According to this article, displaying Kimbab for 6 hours would not cause health issues.

- McLean et al. (2010)[36] completed a pilot test including a microbial survey and challenge tests on traditional Asian ready-to-eat food available in Melbourne, Australia and normally stored at room temperature: nem chua, che dau trang, Kueh talam, banh tet nham man. Nem chua is a fermented sausage made with pork, salt and garlic, which is consumed without cooking. Che dau is a pudding-like sweet made with sticky rice, white

beans, and coconut milk. Kueh talam is made with rice flour, coconut milk, green pea flour, pandan leaf, and palm sugar. Banh tet nham man is sticky rice wrapped in banana leaves filled with either coconut milk or pork, onion, and pepper.

The overall microbiological quality of food was assessed upon purchase. In addition, microbial challenge experiments were performed at 25°C, by inoculating $1.5x10^6$ cfu/mL of a specific organism to 0.3 g of sample. The following organisms were tested: *S. aureus*, *Salmonella enterica*, *Bacillus cereus,* and *E.coli*.

Results of the microbial challenge tests showed that Kueh talam, che dau, and bahn tet nham man were able to support the growth of *S. aureus*, *Salmonella enterica*, *Bacillus cereus*, and *E.coli*., with 1-2 log increase over six hours at 25°C. In contrast, nem chua was unable to support the growth of any of these bacterial species.

- Elviss et al. (2009)[10] assessed a total of 3,750 ready-to-eat fresh herb samples from U.K. retail premises, for the presence/absence of *Salmonella* spp. and *E.coli* counts per gram of food including herbs that were loose or in bunches, pre-packed or growing in a pot. The authors found that 0.5% of the herbs were contaminated with *Salmonella* spp., the majority being *Salmonella senftenberg*, and 4% contained *E.coli* at $\geq 10^2$ cfu/g. Since effective washing/decontamination of ready-to-eat vegetables is difficult, the authors suggested storing fresh herbs at 8°C. However, the authors did not provide any levels of *Salmonella* spp. per gram of food nor did they give any indication related to the ability of fresh herbs to support the growth of *Salmonella* spp. and/or *E.coli.*

  Other than pointing out the possible health risk linked to storage of fresh herbs at room temperature, the lack of information renders interpretation of results difficult when evaluating the risk of extending the storage at room temperature from 2 to 6 hours.

- Riva et al. (2001)[37] assessed the microbiological quality of ready-to-eat fresh cut Cicorino during production steps and storage; assessment was for total bacterial count, total coliforms, and total lactic acid bacteria. Although this is an Italian study, the microbiological quality was assessed against French regulations, which set the acceptable limit to $5x10^7$ of total bacterial count per g. Cicorino is used to prepare salads alone or mixed with other vegetables or for decoration of other dishes. Two 200g packages of ready-to-use cicorino from four different Italian trademarks were investigated. Authors also performed shelf-life tests at 5, 10, and 20°C to determine the evolution of the microbial population during storage. Results show that cicorino was able to support the growth of all bacterial species investigated; starting from 10^5 to 10^6 CFU/g, all eventually reached 10^7 to 10^8 CFU/g. An increase of storage temperature reduced the lag phase and quickened the growth rate. This is reflected by the evolution of the total bacterial counts; results showed that at 5°C, 6.2 days were necessary to reach the total bacterial count legal limit whereas only 3.3 and 0.8 days were enough at 10°C and 20°C respectively.

  Ready-to-eat fresh cicorino would not represent any health risk when stored for 6 hours at 21°C; however, these results are based on the presence of indicators of quality and spoilage, which may not represent the growth pattern of all pathogens.

- Richardson and Stevens (2003)[38] investigated the microbiological quality of ready-to-eat stuffing used when roasting poultry or offered in sandwiches and rolls, in England. Food was examined for *E. coli*, S. aureus, *Bacillus* spp, *Campylobacter* spp., *Salmonella* spp,

and Enterobacteriaceae. Food was considered satisfactory, according to the guidelines published by Gilbert et al. (2000).[29] The authors also examined the microbiological quality according to the display temperature; however, no time component was released and these results are survey based with all the uncertainties associated with survey-based research. Results showed that display temperatures between 8 and 58°C significantly increase the likelihood of poor bacteriological results and authors concluded that if the product needed to be retained, it is essential that it be stored below 8°C.

This work highlights the possible health risk associated with consumption of stuffing held at room temperature; however, it is difficult to interpret the results since no display time was provided by the authors.

- Beuchat and Brackett (1991)[39] examined the fate of *Listeria monocytogenes* in tomato products, including whole and chopped raw tomatoes and commercially processed tomato products refrigerated and stored at 21°C. Tomato products were seeded with two different strains of *Listeria moncytogenes*. Results showed that growth of the pathogen occurred in raw whole tomatoes stored at 21°C with a maximum increase at 2 days of storage, but not in chopped tomatoes.

  Raw whole and chopped tomatoes can be stored at 21°C for 6 hours.

General consideration associated with the literature

- The quality of papers ranged from strong to weak. In general, weak scores were given to older papers, not because of scientific flaws but due to lack of information provided. The goal was to provide the reader with an idea of the ranking of quality of papers rather than an accurate measurement. It is expected that the use of different tools will provide different results, while preserving the raking order of papers.

- The literature focuses on bacterial toxin production and spore-forming bacteria in cooked food. Although viruses have been recognized as an important cause of food-borne disease,[40] they cannot grow in or on food and therefore are not an issue when time control is used as a preventive strategy for microbial growth. Similarly, cooked food is a predominant topic, despite increased growth of minimally processed refrigerated fruit and vegetables sold to consumers in a ready-to-eat or ready-to-use form. The increased sourcing of these products from overseas and concerns about the use of human and animal waste on agricultural land has led to new safety concerns in the food industry, because effective washing and decontamination of fruits and vegetables is difficult.[40] However, such products would likely be kept refrigerated until consumption, which may explain the paucity of papers related to unprocessed cut fruits or vegetables and the use of time control at room temperature.

- Papers pulled by the U.S. FDA focus mainly on spore producing organisms (*B. cereus* and *Clostridium perfringens*) and *Staphylococcus aureus* in cooked food (beef, rice, sautéed onion, French fries). Although evidence used by the U.S. FDA seemed dated, the search for more recent papers, while oriented towards less traditional food, did not bring any new findings. However, more growth predictive models are becoming available.[12]

- Due to lack of a time component incorporated into some results, it was sometimes difficult to evaluate the public health significance of these results. The literature is also very specific; growth of a certain microbial strain is tested under specific temperature and environment with specific food. It is a real challenge to transfer the specificity of these results to the broader level of ready-to-eat food where the strain type and chemical properties of the food might be totally different from the one tested. One microbial strain might not react the same way as another strain, under the same environmental conditions.
  Another issue relates to the application of policy in the field and temperature at which the display food should be kept during a 6 hour interval. The challenge, conducted with *B. cereus* and French fries,[3,4] demonstrates that oil blanched potato strips stored at 21°C can be held for up to 9 hours, but must be discarded within 4 hours if stored at 26.7°C. The temperature buffer margin is narrow and, in the field, an issue with respect to the time/temperature combination 21°C /6 hours might arise when temperatures exceed the temperature buffer margin.

Conclusion

In BC, it is advised to discard ready-to-eat after 2 hours of display at room temperature; however, it is not always practical for food premises to follow this code of practice. Extending the time length to 6 hours would be in line with the U.S. FDA code and more realistic in terms of daily operation of food premises.

From a microbiological point of view, ready-to-eat foods are regarded as more potentially hazardous than other foods because they are consumed with no further processing. The literature points to a possible risk linked to cooked food and the survival of bacterial spores and subsequent toxin production (e.g., *Bacillus cereus* or *Clostridium botulinum* spores) or the survival of enterotoxins produced by *Staphylococcus aureus*),[3-7,23-25,32,33,38] while non-typhoidal *Salmonella* spp., *C. perfringens,* and *Campylobacter* spp. represent the main cause of food-borne illnesses in the U.S.[9] In general, results support the use of time control as a key strategy to prevent micro-organism multiplication. According to the scientific evidence predicated by the U.S. FDA, although very specific and limited, no public health consequences should be expected when properly prepared, cooked, and cooled foods are kept at 21°C for 6 hours. A search for additional evidence highlighted the growing popularity of ready-to-eat fresh herbs and vegetables and the potential risk linked with their consumption, knowing that they are difficult to wash[10,11]; although some ready-to-eat fresh vegetables or cut fruits, such as fresh cicorino[37] or raw tomatoes, did not seem to represent an issue.[39] In terms of daily operation of a food premise, it is expected that ready-to-eat fresh vegetables and herbs will be kept refrigerated until consumption and should not be a concern in the view of the application of the new code.

Although the evidence supporting the extension of display time from 2 hours to 6 hours is specific, limited, and contains gaps, there is no strong evidence against the move forward of this policy; assuming that proper preparation, cooking, and cooling procedures are followed. Another consideration for practice is the limited buffer temperature zone associated with application of this rule. What is true at 21°C is not necessary valid at 26.7°C.[3,4] Strategies are needed to ensure the respect of the temperature/time combination. An attractive alternative strategy, that would cover limited evidence and issue associated with its generalization, is the use of TPHC in the field.[8]

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Table 1 Summary of evidence used by U.S. FDA

Authors	Study location/type/population/ method	Goal	Results	Conclusions related to time/ temperature control	Gaps/Quality
Bryan & Kilpatrick (1971)[23]	U.S. survey *C. perfringens* *Salmonell*a sp. Beef and chicken Fast-food service roast beef sandwich operation Temperature survey during roast beef sandwich making Test for the presence/absence of *C. perfringens* in meat, staff and kitchen environment	Identify critical steps in sandwich making Identify contaminatio n sources	*Salmonella* enteritis isolated from raw chicken only Omnipresence of *C.perfringens* in raw beef and the kitchen environment Vulnerable steps identified as follows: when roast is in the warmer and on the slicer	**Contamination sources**: kitchen staff and equipment **Control**: the only strategy is to prevent the organisms present from multiplying; therefore, need time-temperature control	Bacterial survey gives qualitative results only and not investigation of the presence of toxin Identification of critical steps in sandwich making based on temperature survey only, no microbial sampling was performed No time range provided for time control **Quality:** weak
Mead et al. (1999)[22]	U.S. study Review	Identify micro-organisms involved in food-borne illnesses in the U.S.	67% food-borne illnesses caused by viruses; 30% by bacteria 3% by parasites Leading causes of food-borne illnesses: **Bacteria**: *Campylobacte*r spp, *Salmonell*a non-typhoidal, *Shigella* spp, and *Stpahylococcus* spp **Parasites**: *Giardia lamblia* **Viruses**: Norwalk-like viruses Leading causes of food-borne death (compared to total death)**:** **Bacteria**: *Salmonella non typhoidal and Listeria spp.* **Parasites**: *Toxoplasma* spp **Viruses***:* Norwalk-like viruses Death related to food poisoning mainly		**Quality:** strong

			related to bacteria, namely: *Salmonella* spp.and *Listeria spp.*		
ICMFS (1996a,b)[24,25]	Textbook, literature review	Lag time for *B. cereus* to produce toxin: Skim milk and pasteurized milk stored at 30°C: 7-8 hours Skim milk stored at 15°C: 68-92 hours Rice meal, mince meat, and lasagne stored at 17°C: 2 days Lag time for *Clostridium perfringens* to grow and produce toxins**:** Minced chicken breast and leg stored at 22°C: 6 hours Frankfurters and raw beef stored at 10 and 25°C: 41 and 7 hours			**Quality:** NA
Tatini (1973)	U.S. Review *S. aureus*	Summarizes how nutrients, water activity, temperature, pH , oxygen, and associated organisms' growth affects *S. aureus* toxin production	Enterotoxin production decreases within the range of 10 to 40-45°C Illustrated by Donnely et al. (1968): with raw and pasteurised milk inoculated with 10^6 cells/mL, it takes 18 hrs at 25°C and 36 hrs at 20°C respectively to detect enterotoxin	The production of enterotoxin is less when temperature drops Donnely et al. (1968) showed that storing raw or pasteurised milk at 21°C for 6 hours would not be a threat as far as *S. aureus* is concerned	**Quality:** weak
Doan and Davidson (1999a,b)[3,4]	U.S. studies Challenge test *B. cereus* and *S.aureus* French fries	Ability of *B. cereus* spores and *S.aureus* to grow and produce toxins on oil blanched potato strips for home-style French fries at 21°C and 26.7°C Investigate toxin survival to the frying process	At 21°C, no *S.aureus* was detected on oil blanched potato held for 9 hours. Similarly, *B.cereus* did not reach exponential phase growth during that time At 26.7°C, *S. aureus* enterotoxin was detected after 5 hours on seeded oil blanched potato and enterotoxin survived the frying process. Similarly, *B. cereus* grew 1.5 log over 6 hours storage at 26.7°C. No toxin production was tested in this case.	Oil blanched potatoes can be stored at 21°C for 9 hrs	**Quality:** strong
Melling and Capel (1978)[5]	U.K. study Challenge test with the emetic and diarrhoeal form of the toxin produced by *B.*	Compare resistance to physical agents of	Emetic form of *B. cereus* toxin remains active after a heat treatment at	The emetic form of *B.cereus* toxin will remain active through	**Quality:** medium

	cereus	emetic versus diarrhoeal toxins	126°C for 90 min, while the diarrhoeal toxin is inactivated The activity of the emetic toxin was unchanged after storage at 4°C for 7 days while the activity of the diarrhoeal toxin was reduced.	cooking process; therefore, the only strategy to avoid food poisoning from rice is to prevent the multiplication of *B.cereus*	
Johnson et al. (1983)[6]	U.S. study Rice *B. cereus*	Investigate the growth of *B. cereus* in rice over a range of temperature	Critical temperature ranged between 35-40°C with a generation time of 18-27 min	Based on growth rate, the critical temperature range was comprised between 35-40°C, which is outside our temperature of interest. However, without any reference to time, it is difficult to evaluate the public health significance of these results Based on a reference listed by the authors, after 6 hours of storage at 22°C, *Bacillus cereus* population increased less than a log. Therefore 6 hours of storage of boiled rice at 22°C would represent a minimal health risk.	Does not provide any information regarding the growth lag time at 21°C or the pattern of toxin production **Quality:** strong
Sionkowski and Shelef (1990)[32]	U.S. study Avian egg *L. monocytogenes* strain Brie-1	Investigate growth at 5 and 20°C All samples were inoculated with 10^5-10^6 cells/g	At 20°C, increases in cell number occurred after 10h in whole egg, albumen, and yolk	These results are difficult to interpret due to the confusion around the growth lag time.	Not clear whether the growth started after 10 hours of storage at 20°C or before as suggested by the authors' graph (cont . . .) **Quality:** medium

Salomon and Kautter (1986)[7]	U.S. study *Clostridium botulinum* type A Sautéed onion in margarine	Investigate the growth of *C. botulinum* in sautéed onions and its ability to produce toxins at 35°C	No presence of toxin was found at 24 hr of incubation of inoculated sautéed onion at 35°C	If no toxin was present at 35°C, we can assume that the same lag time or shorter lag time will occur at a lesser temperature; therefore, storage at room temperature for 6 hours should not cause any health risks related to the presence of *C. botulinum*	**Quality:** strong

Table 2. Summary of relevant articles identified through the NCCEH literature review

Authors	Study location and type/population/ method	Goal	Results	Conclusion related to time/temperature control/quality/ gaps
Scallan et al. (2011)[9]	U.S. study Review	Identify micro-organisms involved in food-borne illnesses in the U.S.	58% of total illnesses were caused by noroviruses, followed by non-thyphoidal *Salmonella* spp. (11%), *Clostridium perfringens* (10%) and *Campylobacter* spp. (15%)	**Conclusion:** Viruses are not able to grow on food therefore whereas vegetative microbial cells may. Therefore, *Salmonella* spp., *Clostridium* spp. and *Campylobacter* spp. are the main concerns when it relates to the display of PHF. **Quality:** strong
EFSA Scientific Panel on Biological Hazards (2005)[34]	Europe Review	Identify biological hazards related to *Clostridium* spp. in foodstuffs	The species of the genus *Clostridium* more involved in food-borne illness are *Clostridium perfringens* and *C. botulinum* The optimal growth of *C. botulinum* strains ranged from 28-40°C Storage/time/temperature not under control: growth of *C.botulinum* has been observed in over-wrapped fresh mushrooms, in garlic	**Conclusion:** Prevent outbreaks related to *C.perfringens* by keeping the number of vegetative cells below 10^6-10^7/g of food. **Quality:** strong

			oil and in baked potatoes wrapped in aluminum foil when stored at ambient temperatures Almost all outbreaks result from conditions allowing multiplication of *C. perfringens* numbers reaching 10^6-10^7/g .Approximately 10^8 vegetative cells of enterotoxin-producing *C. perfringens* per serving are necessary to cause diarrhoea The optimal temperature for growth of C, perfringens is 43-47°C and growth does not occur between 10-12°C.	
Hislop (2008)[8]	Canadian study	Describes an alternative to the use of temperature control for PHF, which is called Time as a Public Health Control (TPHC)	In the field, this principle translates into limiting the display of PHF at room temperature to less than four hours, time labelling the product on display, and discarding the food product at the end of that period. For those vendors who wish to display their products for longer than four hours, they need to go through an application, approval, and evaluation process to demonstrate that extending the time of display would not cause any microbial risk to consumer health.	**Conclusion**: This structured approach has been successfully used by Alberta Health Services and supports effective and consistent decision-making by both management and field staff. **Quality**: Not applicable
Yoon et al.(2011)[35]	Korean study *Staphylococcus aureus* significance in Kimbab (korean fast food made with rice roll filled with different food materials) Exposure assessment using	Evaluate the risk of commercially available Kimbab	The growth of *S.aureus* accelerate with increase of the holding temperature (10 to 30°C) The critical times for the production of enterotoxin (cell numbers of 10^6 CFU/g) at 20°C was	**Conclusion**: The risk related to the presence of *S. aureus* in Kimbab stored at 20°C for 6 hours is minimal **Quality**: strong

	Food MicroModel (FFM)		determined at 22.6 hrs	
McLean et al. (2010)[36]	Australian study Microbial survey and challenge tests with *Staphylococcus aureus*, *Salmonella enterica* , *Bacillus cereus*, *E.coli* at 25°C Traditional Asian food consumed in Australia and normally stored at room temperature: nem chua,che dau trang, Kueh talam, banh tet nham man	Assess the levels of microbial contamination of samples upon purchase Assess their potential to support the growth of food-borne pathogens at 25°C	Keh talam, che dau and banh tet nham were able to support the growth of *B. cereus, E.coli*, *staphylococcus aureus,* and *Salmonella*: 1-2 log increases over 6 hours at 25°C	**Conclusion**: With no trial related to enterotoxin or spores production, it is difficult to evaluate the public health significance of these results. However, at least for *Staphylococcus aureus and B. cereus*, the growth at 21°C is expected to be slower than at 25°C. Therefore, depending on the initial concentration, these organisms may not be a concern. **Quality**: strong
Elviss et al. (2009)[10]	Survey of fresh herbs harvested in different countries for presence of Salmonellae and *E.coli* levels Levels of *E.coli* compared to EC regulations Ready-to-eat fresh herbs included those that were loose or in bunches, pre-packed or growing in a pot, that can be purchased in the U.K.	Gain insight into the frequency and types of *Salmonella* spp. in ready-to-eat fresh herbs	The majority of fresh herbs sampled were of satisfactory or acceptable microbial criteria 0.5% of samples were contaminated with *Salmonella* spp. (mostly *S. senftenberg*). Other studies found contamination levels of 1.7% up to 28% of fresh herbs 4% of the samples contained *E.coli* levels $\geq 10^2$ cfu/g	**Conclusion:** Because effective washing and decontamination of ready-to-eat vegetables is difficult, storing this type of product should be at or below 8°C. Without any reference to concentrations related to Salmonellae or any information regarding the ability of organisms to grow on fresh herbs, it is difficult to interpret the results for the purpose of our study **Quality**: strong
Riva et al. (2001)[37]	Italy Ready-to-eat fresh cut cicorino assessed for total bacterial counts, total coliforms, and total lactic acid bacteria Microbial quality assessed against	Microbial assessment during production steps and storage	Cicorino was able to support the growth of all bacterial species investigated Based on total bacterial counts, it took 0.8 days for cicorino to reach the legal limit, when stored at 20°C	**Conclusion** Ready-to-eat fresh cicorino would not cause health issues when stored for 6 hours at 20°C Results are based on the presence of indicators of quality and spoilage, which

	French regulations			may not represent the growth pattern of all pathogens **Quality**: strong
Richardson and Stevens (2003)[38]	England Ready-to-eat stuffing offered in sandwiches and rolls as with conventional poultry meals Microbial survey for *Salmonella* spp., *Campylobacter* spp., *Bacillus* spp., *S. aureus*, *E.coli, and* Enterobacteriaceae Results were analysed according to guidelines published by Gilbert et al. (2000)[29]	Microbial examination of ready-to-eat stuffing according to display temperature Total of 147 ready-to-eat samples were collected from 139 retail premises in England Investigate the effect on display temperature ranging from 0 to 94°C, but based on survey	Poor microbial results of ready-to-eat stuffing are related to post preparation factors Display temperatures between 8 and 58°C significantly increase the likelihood of poor bacteriological results	**Conclusion:** Display temperatures between 8 and 58°C are more likely associated with poor bacteriological results; however, no time component was provided The survey-based research regarding preparation mode and storage are subject to recall bias Authors recommended that prepared product be displayed at temperature <8°C or >58°C **Quality:** medium
Beuchat and Brackett (1991)[39]	U.S. Raw whole tomatoes, chopped tomatoes and other tomato products *Listeria monocytogenes*	Examine whether tomato products support the growth of *Listeria moncytogenes* at 10 and 21°C	Growth of the pathogen occurred in raw whole tomatoes stored at 21°C to reach approximately 1-log increase at 2 days, but not in chopped tomatoes	**Conclusion:** Storing raw whole or chopped tomatoes at room temperature for 6 hours does not cause a health risk. **Quality:** medium

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