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General Interest

Low–Water Activity Foods: Increased Concern as Vehicles of Foodborne Pathogens

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ABSTRACT

Foods and food ingredients with low water activity (a_w) have been implicated with increased frequency in recent years as vehicles for pathogens that have caused outbreaks of illnesses. Some of these foodborne pathogens can survive for several months, even years, in low- a_w foods and in dry food processing and preparation environments. Foodborne pathogens in low- a_w foods often exhibit an increased tolerance to heat and other treatments that are lethal to cells in high- a_w environments. It is virtually impossible to eliminate these pathogens in many dry foods or dry food ingredients without impairing organoleptic quality. Control measures should therefore focus on preventing contamination, which is often a much greater challenge than designing efficient control measures for high- a_w foods. The most efficient approaches to prevent contamination are based on hygienic design, zoning, and implementation of efficient cleaning and sanitation procedures in the food processing environment. Methodologies to improve the sensitivity and speed of assays to resuscitate desiccated cells of foodborne pathogens and to detect them when present in dry foods in very low numbers should be developed. The goal should be to advance our knowledge of the behavior of foodborne pathogens in low- a_w foods and food ingredients, with the ultimate aim of developing and implementing interventions that will reduce foodborne illness associated with this food category. Presented here are some observations on survival and persistence of foodborne pathogens in low- a_w foods, selected outbreaks of illnesses associated with consumption of these foods, and approaches to minimize safety risks.

Low-water activity (a_w) foods and food ingredients are either naturally low in moisture or they are produced from high-aw foods that are deliberately dried. The addition of large amounts of salt or sugar can also be regarded as a simulated drying process, as it results in a reduction of the amount of water available for microbial growth. The minimum aw at which microorganisms can grow (i.e., the aw below which physiological activities necessary for cell division are impaired) is 0.60. The minimum a_w for growth of most bacteria is approximately 0.87, although halophilic bacteria can grow at a_w as low as 0.75. Among the bacterial pathogens, Staphylococcus aureus is particularly well adapted to reduced-aw environments. According to the International Commission on Microbiological Specifications for Foods (76), under optimal conditions S. aureus can grow at a_w as low as 0.83 but in most foods the minimum is aw 0.85. A more recent predictive model shows that the lower a_w limit for growth of the pathogen is affected by the pH and type of solute used to achieve reduced a_w (157). The minimum a_w for growth of *S. aureus* in this model was reported to be 0.85 (pH 7.0), using sodium chloride as a humectant. Some species of xerophilic spoilage molds and osmophilic yeasts can grow at a_w 0.60 to 0.70, but the minimum a_w for mycotoxin production by molds is 0.80, with the majority not producing mycotoxins at $a_w < 0.85$ (45). In the context of this review, foods with $a_w < 0.85$ are referred to as low- a_w foods.

Foods with $a_w < 0.85$ include cereals, chocolate, cocoa powder, dried fruits and vegetables, egg powder, fermented dry sausage, flour, meal and grits, herbs, spices and condiments, honey, hydrolyzed vegetable protein powder, meat powders, dried meat, milk powder, pasta, peanut butter, peanuts and tree nuts, powdered infant formula (PIF), grains, and seeds (e.g., sesame, melon, pumpkin, linseed). Although low- a_w foods have clear advantages with respect to controlling growth of foodborne pathogens, there are nevertheless some major concerns. Microorganisms, including those capable of causing human diseases, are able to survive drying processes. Survival of only a few cells of some

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foodborne pathogens, e.g., Escherichia coli O157:H7 or Salmonella, may be sufficient to cause disease. Once in a desiccated state, metabolism is greatly reduced, i.e., growth does not occur, but vegetative cells and spores may remain viable for several months or even years. Pathogens can often persist longer in low-aw foods and dry food processing environments than in foods and environments with higher a_w, i.e., approaching the minimum for growth. Food processing environments in which dried foods are handled must be maintained at low humidity and kept dry, which gives rise to the need for specific cleaning and sanitizing procedures. Also, it is of concern that consumers sometimes wrongly believe that low-aw foods are sterile, which may lead to dangerous practices such as keeping reconstituted infant formula at ambient temperature for prolonged periods, thereby creating growth opportunities for pathogens.

It is difficult to eliminate pathogens from foods with low a_w by processes such as application of mild heat treatment, which works very well for high- a_w foods. The extent of enhanced heat resistance of pathogens in low- a_w foods is not easily predicted, and it does vary with the pathogen and type of solute present (19, 56, 65, 148). It is imperative that good manufacturing practices, good hygiene practices, and hazard analysis and critical control point (HACCP) systems (77), with special attention to preventing contamination and reducing persistence of foodborne pathogens in low- a_w foods, be implemented and effectively maintained on a continuous basis. Routine sampling for pathogens that may be present on surfaces where dust can accumulate provides valuable information concerning their potential presence in finished products and assessing safety risks.

Summarized here is information on the survival, persistence, and control of foodborne pathogens in low- a_w foods and in dry food processing environments. Pathogens that have been known to cause outbreaks of infections or intoxications associated with consumption of low- a_w foods are discussed. The content of this review was drawn and updated from a report published by the International Life Sciences Institute—Europe (13), with substantial modifications and permission to use.

PATHOGENS OF GREATEST CONCERN

Vegetative cells of foodborne pathogens, along with bacterial and fungal spores, may survive in foods and food ingredients with $a_w < 0.85$, as well as in dry processing environments, for long periods. Desiccation of microbial cells is, in fact, a process traditionally used in research laboratories to retain viability. Characteristics of bacterial pathogens that have been associated with and/or documented to have caused outbreaks of foodborne diseases as a result of consumption of low- a_w foods are summarized in Table 1.

Bacterial pathogens. *Cronobacter* species (formerly *Enterobacter sakazakii*) are opportunistic pathogens for vulnerable neonates, with infants becoming infected following the consumption of contaminated reconstituted PIF, a food that is most often based on dried milk powder (*122*). The pathogen has been isolated from a wide range of other dry foods and food ingredients, including milk powder,

grains, seeds, nuts, herbs, spices, flour and meal, adult and infant cereals, and cocoa powder (10, 83, 140). Cronobacter has been detected in surveys of several dry food processing facilities, on food contact surfaces, and in retail bakeries (93, 94, 150). There is evidence for strains persisting in some of these environments. Since Cronobacter can survive the spray-drying process (6, 62), control in the production environment must be achieved through a combination of several measures, including pasteurization prior to concentration and spray drying and control of the microbial ecology of the manufacturing facility. Guidelines for reconstituting, storing, and handling PIF have been issued by the World Health Organization, Food and Agriculture Organization (173).

Verotoxigenic E. coli (VTEC) has been shown to survive in moist farm environments, but survival is markedly reduced when the bacterium is exposed to dry conditions. Most cases of infection arise following the consumption of undercooked beef products or fresh produce. In an unusual finding, cookie dough served as a source in an outbreak of infections (121). Dough was intended for baking but was eaten raw, and 76 individuals were reported ill. It was suspected that flour contained the causative pathogen; but, in spite of extensive testing, E. coli O157:H7 was not isolated from this ingredient or from the finished product. Recent outbreaks of E. coli O157:H7 infections were attributed to consumption of hazelnuts (38, 115), and E. coli O104:H4 infections associated with sprouts produced from dry fenugreek seeds (17) have been described. E. coli O157:H7 infections also have been associated with consumption of reduced-aw meat products such as salami, semidried fermented sausage, and cured meat. In fermented, dry, vacuum-packaged sausage, VTEC can survive for at least 8 weeks (2-log reduction at 4° C) (67). Deng et al. (53) reported that E. coli O157:H7, at an initial population of 6.3 log CFU/g, survived in infant rice cereal (a_w 0.35 to 0.73) at 5 and 25°C for 16 and 22 weeks, respectively. At an initial population of 5.5 to 5.7 log CFU/g, the pathogen survived in apple powder (aw 0.16 to 0.23), buttermilk powder $(a_w 0.21 \text{ to } 0.38)$, Cheddar cheese seasoning $(a_w 0.21 \text{ to } 0.36)$, and powdered chicken (aw 0.34 to 0.38) stored at 5°C for 19 weeks and 21°C for 16 weeks.

Salmonella is readily destroyed by heat pasteurization of high-a_w foods. As the a_w is reduced by addition of solutes or by removal of water, heat resistance increases markedly. Temperatures in excess of 100°C for a few minutes may be necessary to reduce Salmonella by 1 log CFU/g of milk chocolate (9, 50, 68). Heating low-a_w wheat flour at 75 to 77° C for 2.5 min (5) and pecan nutmeats ($a_w 0.52$) at 120° C for 20 min reduces Salmonella by only about 1 log CFU/g (15). Heating peanut butter at 90° C for 50 min results in a reduction in Salmonella of 3.2 log CFU/g (149). Older cells are more heat resistant than young cells in peanut butter, and strains of the same serotype may have different levels of resistance (112). There is a high probability of infection at doses of $>10^5$ cells; but, in foods containing high levels of fat and/or protein, such as chocolate, salami, and Cheddar cheese, infection can result from ingesting as few as <10to 100 cells (162). Several outbreaks of salmonellosis following consumption of contaminated chocolate have

TABLE 1. Characteristics of	bacterial foodborne path	TABLE 1. Characteristics of bacterial foodborne pathogens associated with or documented to have caused outbreaks of illness associated with consumption of low-aw foods	nted to have caused outbreaks o	f illness associated with consun	$uption$ of $low-a_w$ foods	
Pathogen	Aerobic-anaerobic	Physiological features associated with more heat resistance	Relevance to dry foods	Minimum a _w for growth and toxin formation	Toxin formation or invasion of pathogen	Reference(s)
Bacillus cereus	Facultative anaerobe	Spores: $D_{95^{\circ}C}$ 1.2–36 min; z-value 7.9–9.9°C	Spores can survive in dry environments	Growth and emetic toxin formation: 0.92–0.93	Toxin formation or toxico-infection	147
Clostridium botulinum	Anaerobe	Psychrotrophic spores: $D_{100^{\circ}C}$ < 0.1 min; <i>z</i> -value 7–10°C Mesophilic spores: $D_{121^{\circ}C}$ 0.21 min: <i>z</i> -value 10°C	Spores can survive in dry environments	Psychrotrophic: 0.97 Mesophilic: 0.93	Toxin formation	153
Clostridium perfringens	Anaerobic	Spores: D _{95°C} 17.6-63 min	Spores can survive in dry environments	0.93 for growth	Toxico-infection: toxin produced during sporulation in GI tract	104
Cronobacter species (formerly Facultative anaerobe Enterobacter sakazakii)	Facultative anaerobe	D _{60°C} 2.5 min; <i>z</i> -value 5.8°C	Ability to survive in dry foods—up to 2 yr in powdered infant formula	Survival at 0.2; minimum for growth not known	Pathogen invasion; possible toxin formation	22, 57, 70
Escherichia coli 0157:H7	Facultative anaerobe	D _{63°C} 0.5 min; z-value 6°C	Ability to survive in dry foods, e.g., dry fermented meats	0.95 for growth	Toxico-infection	76, 113
Listeria monocytogenes	Facultative anaerobe	D _{60°C} 1.6–16.7 min in food substrates; 70°C for 2 min is the UK government– approved heat treatment for elimination of <i>Listeria</i>	Ability to survive in dry foods (a _w 0.83), e.g., dry fermented meats, and peanut butter (a _w 0.33)	0.90-0.93 for growth	Pathogen invasion	76, 98, 117
Salmonella	Facultative anaerobe	D _{60°C} 0.1–10 min; <i>z</i> -value 4–5°C; heat resistance is greatly increased in low-a _w and high-fat foods	Survives for weeks, months, or years in low-a _w foods (up to a _w 0.30)	0.94 for growth	Toxico-infection	12, 76
Staphylococcus aureus	Facultative anaerobe	D _{60°C} 1–2.5 min in phosphate buffer; z-value 8–10°C	Can survive for months in dry foods	0.83–0.85 for growth (0.85 in most foods); 0.87 for toxin formation	Toxin formation	76

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occurred, with low numbers of *Salmonella* being recovered from the incriminated chocolate many months after the outbreaks. For reviews focused on control of *Salmonella* in low- a_w foods and their processing environments, see Chen et al. (39, 40), Podolak et al. (131), and Scott et al. (148).

Survival and persistence of gram-positive foodborne pathogens in low-a_w foods is also a concern. Staphylococcal intoxications are of minor importance compared with the number of cases and severity of illnesses linked to *Salmonella* and VTEC, but they are particularly relevant to dried foods due to tolerance to low a_w. Salted and cured food products (defined as semidry), including ham, hard cheese, and salami, and especially foods where fermentation or drying (such as pasta) have been delayed or in "natural fermentations" where starter cultures are not used, are at risk of staphylococcal growth and toxin production.

Bacillus cereus, and very rarely *Bacillus subtilis* and *Bacillus licheniformis*, can produce one or two types of toxins. Heat-stable emetic toxin is produced by *B. cereus* in starchy foods such as cakes, pasta, and especially cooked rice. Diarrheagenic toxin is relevant only during growth in the gastrointestinal (GI) tract. Wet processing of dry food products, e.g., cereals, can present conditions suitable for growth and production of heat-stable toxins. *B. cereus* spores survive in dry foods such as rice cereal (*85*) and in dry food processing environments for long periods of time and can germinate and grow in reconstituted products that are not properly processed or stored.

Clostridium botulinum and Clostridium perfringens (and rare strains of Clostridium butyricum and Clostridium baratii) are associated with foodborne intoxications. Honey consumption by infants may give rise to infant botulism, a toxico-infection, whereby low numbers of spores germinate in the GI tract and produce toxin. Isolates of C. botulinum cultured from honey ($a_w < 0.60$) and linked to cases of infant botulism in the United States appear to reflect the same types found in the local soil (7). A case of infant botulism was associated with consumption of reconstituted infant formula milk powder (23). It was suggested in a later report, however, that the unopened brand of formula implicated in this case was not the source of transmission of spores to the infant (86). Intestinal toxemia botulism in adults has been linked to consumption of peanut butter (151). Peanut butter from the residence of one case patient yielded C. botulinum type A, which corresponded with type A spores found in the patient's feces. Food and clinical isolates were indistinguishable by pulsed-field gel electrophoresis (PFGE) analysis. Two of the case patients had Crohn disease, reinforcing the view that an underlying GI condition may be a risk factor in adult intestinal toxemia botulism. Spores of C. perfringens survive well in dust and on surfaces and are resistant to routine cooking temperatures. Sporulation of large numbers of vegetative cells of C. perfringens in the GI tract can result in the production of an enterotoxin. Spores of C. perfringens have been found in PIF and also in dried herbs and spices, including black pepper, which if added to cooked meat dishes, may give rise to an infective dose if the food is temperature abused during cooling or storage.

Listeriosis associated with consumption of low- a_w food containing *Listeria monocytogenes* has not been documented. However, the pathogen has been detected in several types of foods, including dried, smoked sausages (e.g., salami, chorizo, salpicao, and alheiras). Initial populations of 4.4 and 3.4 log CFU/g of peanut butter (a_w 0.33) and a chocolate-peanut spread (a_w 0.33), respectively, have been reported to decrease to 3.8 and 2.4 log CFU/g, respectively, within 24 weeks at 20°C (*98*).

Viral pathogens and mycotoxigenic molds. Outbreaks of illness attributed to foodborne viruses are being recognized with increased frequency. Norovirus and hepatitis A virus are the most commonly recognized viral agents linked to foodborne illness in humans. The infectious dose of norovirus is very low, but there are no documented infections implicating dried foods. Outbreaks of hepatitis A virus infection in Australia were associated with consuming semidried tomatoes (133). Poor hygiene in production plants was a likely reason for contamination. Related outbreaks also believed to be associated with consumption of semidried tomatoes have occurred in Europe (28, 63, 130). The extended incubation period, up to 2 months, makes detection, diagnosis, and identification of the original source of the virus difficult. Viruses can persist on dry hard surfaces for at least 42 days and be transferred to foods upon contact (58).

Molds capable of producing aflatoxin, ochratoxin, fumonisin, and other mycotoxins have been detected in a range of dry foods, including corn, rice, spices, coffee, cocoa, peanuts, tree nuts, seeds, and dried fruits (45, 155). Mold-infested legumes, grains, and spices stored in humid environments pose a serious risk to consumers. Carryover of mycotoxins from raw commodities to dry, processed products, e.g., coffee, cocoa, flour, and fermented beverages, is a public health concern.

PERSISTENCE OF BACTERIAL PATHOGENS IN DRY ENVIRONMENTS

Water is an important factor that contributes to microbial deterioration of foods and to the persistence of microorganisms in production environments. Pathogenic and spoilage microorganisms require nutrients and suitable a_w, pH, redox potential, and temperature in order to grow. During the production of dried foods, the control of moisture, and consequently the a_w, is key to controlling microbial growth. Dry cleaning, including the use of vacuum cleaners with integrated High Efficiency Particulate Air (HEPA) filters, is generally regarded as a useful approach.

While microorganisms cannot grow in the absence of water, nonetheless, vegetative cells and spores of some genera of bacteria can survive for several months, even years, in low- a_w foods and ingredients. Examples of survival characteristics of foodborne bacterial pathogens in low- a_w foods are listed in Table 2. Persistent strains with unique DNA fingerprint profiles, as determined by molecular subtyping methods such as PFGE, have been identified. In a study focused on investigating the survival of *Salmonella* in an oilmeal plant, Morita et al. (*118*) recovered

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TABLE 2. Evidence	Evidence of survival of foodborne pathogens in low-aw foods	ods and food ingredients	
Pathogen	Food	Survival	Reference(s)
Cronobacter species	Dried liquid infant formula	Inoculated (ca. 11 log CFU/g) with 6 capsulated and 4 noncapsulated strains, dried at 20–25°C, stored for up to 30 mo at 20–25°C; capsulated strains more resistant to drying and during storage; 2 strains	29
	Infant cereal	(capsulated) were recovered after 30 m0 Survival in infant cereals (rice, barley, oatmeal, and mixed grain; a _w 0.30–0.83) for 24 wk at 4, 21, and 30°C; increases in a or term accelerated the rate of death: survival was not affected by cereal commosition	011
	Powdered infant formula	Survived for 687 days at 20–22°C (a _w 0.14–0.27; initial population, ca. 6 log CFU/g); 2.4-log reduction in	57
		5 mo, 1.0-log reduction during subsequent 19 mo Survived in 4 milk-based and 2 soy-based formulas held at 4, 21, and 30°C for 12 mo; reductions were greater	70
		at a _w 0.43–0.50 than at 0.25–0.30; rate of inactivation was not markedly affected by composition of formula Reduction of 1–2 los CFIT/s during storage for 90 days at 30°C	48
		Reduction of <1 log CFU/g of formula (a. 0.21–0.31) after 1 mo and 4 log CFU/g after 12 mo at 25°C;	126
	Skim milk powder	thermal resistance decreased as storage time increased Three serotypes (initial population, 4.5-4.8 log CFU/g) survived for at least 14 days at 20-35°C; rate of reduction increased as the storage term increased	92
		Survived spray drying (inlet, 160°C; outlet, 90°C); recovered from dried milk (initially, 1.57–2.05 log CFU/g) stored for 12 wk at 18–20°C	6
Escherichia coli 0157:H7	Alfalfa seeds (for sprout production)	Initial population (2.9–3.2 log CFU/g) on seeds (a _w 0.15, 0.36, and 0.54) stored at 5°C for 52 wk was reduced by 0.6–0.9 log CFU/g; reductions on seeds stored at 25°C for 25 wk were 2.1, 3.0, and 2.9 log CFU/g, respectively.	16
		Detected on seeds (a _w 0.15 and 0.36) stored at 37°C for 8 wk but not on seeds stored for 14 wk Survived in seeds (5.1–6.2% moisture; initial population, 3.0 log CFU/g) stored at 5°C for at least 54 wk, and 25 and 37°C for 38 wk hut not 54 wk	161
		Initial population (2.0 log CFU/g) was reduced but detectable after 8 wk at 37°C; pathogen was not detected in 25-g samples after 13 wk at 37°C	174
	Almonds	Initial population (3.9 log CFU/g) on kernels did not decline at –19°C for 12 mo; slow decline at 24°C but detectable after 12 mo; linear rate of decline at 24°C was 0.60 log CFU/g/mo	001
	Apple powder, buttermilk powder, Cheddar cheese seasoning, powdered chicken, sour cream powder	Survived in products (aw 0.16-0.37, pH 4.07-6.49) stored for 19 wk at 5, 21, and 37°C; inactivation was enhanced by low pH and by increase in storage temp	53
	Beef powder	Rate of inactivation during storage for 8 wk was enhanced at a _w 0.28–0.41 compared with a _w 0.68, at 25°C compared with 5°C, and in powder containing 20% NaCl compared with 0.5 or 3% NaCl; acid adaptation or shock did not affect retention of viability	145
	Biscuit (cookie) cream	Rate of inactivation in biscuit cream ($a_w 0.75$) was dependent on storage temp. Initially at 2 log CFU/g, detected after 2 days at 38°C. 42 days at 22°C, and 58 days at 10°C	11
	Cereal (rice) Chocolate	Survived in infant rice cereal (a _w 0.35–0.75) at 5, 25, and 35°C for at least 24, 16, and 9 wk, respectively Rate of inactivation in milk chocalate (a _w 0.40) was dependent on storage temp. Initially at 4 log CFU/g,	53 11
	Infant cereal	but view for the days at 26 C, 90 days at 24 C, and -300 days at 10 C. Death was enhanced at reduced pH (4.0 vs 8.0) and a_w (0.35 vs 0.73) and as storage time (up to 24 wk) and	53
	Jerky (beef)	temp (5, 25, 35, and 45°C) increased Beef was treated with acidic marinades, dried at 60° C for 10 h to a _w 0.55–0.66, and stored for 60 days at	26
	Jerky (venison)	25°C; survival depended on acid adaptation of cells and marinade treatment Survived 24 days in environmental samples (dried gore and deer skin) and more than 13 mo in jerky stored at ca. 20°C	97

TABLE 2. Continued	4		
Pathogen	Food	Survival	Reference(s)
	Peanuts	Initial population (ca. 5 log CFU/g) remained stable on raw kernels for 181 days at -20 and 4° C; linear rate of decline at 23° C was 0.44 log CFU/g/mo	132
		Avg rates of reduction across 3 levels of inoculum (6, 4, and 2 log CFU/g of raw peanuts) were -0.63 , -0.16 , and -0.12 log CFU/mo for nuts stored at $23 + 3^{\circ}$ C. $4 + 2^{\circ}$ C. and $-20 + 2^{\circ}$ C. respectively	114
	Pecans	Initial population (ca. 5 log CFU/g) remained stable on kernels for 181 days at -20 and 4° C; linear rate of decline at 23°C was 0.45 log CFU/o/mo	132
	Pistachios	Initial population (4.6 log CFU/g) on in-shell nuts did not decline at -19 or 4°C for 12 mo; slow decline at 24°C but detectable after 12 mo; linear rate of decline at 24°C was 0.35 log CFU/g/mo	100
	Potato starch	Reduction of 2.5–3.8 log CFU/g of starch (a., 0.24–0.78) stored at 4°C for 33 wk; reductions were more ranid with increased storage temp (4, 20. and 37°C) and a., (0.24–0.26, 0.51–0.52, and 0.75–0.78)	128
	Walnuts	Reduction of 6.9 log CFU/g of walnut kernels stored at 23°C for 1,065 days (2.9 yr); calculated rate of decline was 0.21 log CFU/g/mo	20
Salmonella	Alfalfa seeds (for sprout production)	Initial population (4.8 log CFU/g) was reduced by 0.3 log/g of seeds (a. 0.21–0.60) stored at 5°C for 52 wk; reductions after storing seeds (a. 0.21, 0.40, and 0.60) for 52 wk at 25°C were 1.2, 1.2, and 4.5 log CFU/	16
	Almonds	g, respectively; reductions after 42 wk at 57 C were 2.4, 4.5, and 4.8 log CFU/g, respectively Kernels containing 7.1–8.0 log CFU/g were stored for 171 and 550 days at -20, 4, 23, and 35°C; no significant reductions in number after 550 days at -20 and 4°C; reductions of 0.18 and 0.30 log CFU/mo on kernels held at 23°C for 171 and 550 days, respectively	164
		Initial population (4.3 log CFU/g) on kernels did not decline at -19 or 4°C for 12 mo; slow decline at 24°C but detectable after 12 mo; linear rate of decline was 0.20 log CFU/g/mo	100
	Beef jerky	Beef was treated with acidic marinades and then dried for 10 h to $a_w 0.55-0.71$; exposure to marinades resulted in reduced tolerance to drying and subsequent storage for 60 days at 25° C	27
	Cake mix, skim milk powder, onion soup mix, gelatin-based dessert	Inactivation at 25°C for 25 days was minimal at a_w 0.00–0.22; survival decreased with increased a_w up to 0.53 (4–5-log CFU/g reduction in 25 days) and pH (cake mix 6.8 vs dessert 3.1); at a_w of foods as purchased, 2-log CFU/g reduction in cake mix (a_w 0.32), skim milk (a_w 0.22), onion soup mix (a_w 0.14), and dessert (a_w 0.42) within 10, 9, >27, and 2 wk. respectively	41
	Chocolate	Survived 19 mo; MPN values from composite samples were 4.3–24 cells per 100 g (initial no. not known) Initially at 100 CFU/g, decreased in milk chocolate to 14 MPN/100 g after storage for 15 mo at room temp	75 9
		Survived in milk chocolate and bitter chocolate for 15–18 mo at room temp Survival in milk chocolate and bitter chocolate for 6 mo at room temp Initially at 5.2 log MPN/100 g, decreased in milk chocolate (a., 0.38) to 0.89–1.11 log MPN/100 g in 9 mo	142 160 159
	Dried fish	Survived at 2°C in salted horse mackerel, salted dried mackerel, and dried mackerel for 60–65 days, 35–45 days, and 20 days, respectively; initial populations were 4.6–5.9 log CFU/g	116
	Dried milk products Egg powder	Survived in naturally contaminated products for 10 mo Reduction of 1.6–2.8 log CFU/g in 8 wk at 13°C (a _w 0.29–0.37); rate of inactivation more rapid at 37°C theorem 12°C and more induced by the transformation of any of any of the second s	136 90
	Fondant	Initial population (3.9–5.4 log CFU/g) in peanut butter-flavored fondant (a_w 0.65–0.69) decreased to <1 log CFU/g within 7 wk at room temp, but the pathogen was detected in samples stored for 1 vr	123
	Halva	Initial population of 3.87 log CFU/g (a., 0.18) decreased to 2.20–2.76 log CFU/g at 6°C and 2.15–2.70 log CFU/g at 18–20°C after storage for 8 mo; survival was better in vacuum-packaged halva compared with air-sealed halva	103

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TABLE 2. Continued	4		
Pathogen	Food	Survival	Reference(s)
	Paprika powder (on potato chips)	Populations of 0.04-0.45 CFU/g of powdered potato chips were detected; multiple serotypes survived for	107
	L	more than 8 mo	
	Fasta	Initial populations of 430–930 and 1.5–24 cells per 100 g (MLN) of pasta (12% moisture) decreased to 0.4– 23 and <0.3–1.5 cells per 100 g, respectively, during storage at room temp for 360 days	13/
	Peanuts	Initial population (ca. 5 log CFU/g) remained stable on raw kernels for 181 days at -20 and 4° C; linear rate	132
		of decline at 23°C was 0.32 log CFU/g/mo	
		Avg rates of reduction across 3 levels of inoculum (6, 4, and 2 log CFU/g of raw peanuts) were -0.72 , -0.00 and -0.10 log CFU/m for minor the second of $0.22 \pm 2^{\circ}$ $0.4 \pm 2^{\circ}$ $0.4 \pm 2^{\circ}$ $0.50 \pm 2^{\circ}$	114
	Peanut butter	Initial populations of 6–7 log CFU/g decreased by 0.15–0.65 and 0.34–1.29 log CFU/g in 5 brands of	129
		peanut butter stored at 4 and 22°C, respectively, for 14 days	
		Initial population (8.5 log CFU/g) in peanut butter (a., 0.55) decreased by 0.72 and 3.16 log CFU/g within 2	123
	Domit hitter nonit mand	and / Wk, respectively, at room temp Order of retention of violality in modules (2 - 0.20, 0.32) stored for 24 wh at 5 and 21°C was reason that ar	35
	r canut outtot, peanut spread	Other of referition of viability in products (a_w 0.20–0.33) stored for 24 we at 2 and 21 C was pealur other spreads > traditional (regular) and reduced-sugar. low-sodium nearly hutters > natural nearly hutter 6 of	07
		7 products initially containing 1.51 log CFU/g were positive after 24 wk at 5° C; at 21°C, 6 of 7 products	
		initially containing 5.68 CFU/g were positive after 24 wk at 5°C; 6 of 7 products at 21°C were positive	
	Pecans	In-shell pecans (5.8 log CFU/g) and nutmeats (6.2 log CFU/g) were stored for up to 78 and 52 wk, respectively,	14
		at -20 , 4, 21, and 37°C; no significant reduction on in-shell pecans and slight reduction on nutmeats at -20	
		and 4°C, 2.5–3.3-log CFU/g reduction on in-shell nuts and nutmeats stored at 21 and 37° C	
		Initial population (ca. $5 \log CFU/g$) remained stable on kernels for 181 days at -20 and $4^{\circ}C$; linear rate of	132
		decline at 23°C was 0.21 log CFU/g/mo	
	Pistachios	Initial population (4.8 log CFU/g) on in-shell nuts did not decline at -19 or 4° C for 12 mo; slow decline at	100
	:	24°C but detectable atter 12 mo; invertate of decline at 24°C was 0.15 log CFU/g/mo	
	Potato slices, carrot slices	Potato and carrot slices were dried at 60°C for 6 h; carrots were then heated at 80°C; reductions of 0.81 log	54, 55
		CFU/g of potatoes and 1./-2.6 log CFU/g of carrots during storage for 30 days at 25°C	10
	skim milk powder, cocoa powder	Kales of inactivation at a _w 0.43, 0.22, and 0.73 at 23°C for 14 wK were scrotype dependent; survival was markedly meater in milk nowder then in corres nowder and at a -0.43 and 0.53 command with a -0.75	16
	Walnuts	Interventy greated in titles poweed that in cocca poweed and at a worth and 0.25 bottparca with a worth Initial nonulations of 8.8 and 3.2 log CFU/o of kernels declined to 6.5 and 0.25 log CFU/o respectively during	20
		storage at 23°C for 3 yr; a population of 1.9 log CFU/g did not decline significantly on kernels stored at -20 or 4° C) I
		for 1 yr but declined by 1.4 CFU/g at 23°C; calculated rates of decline at 23°C were 0.05-0.1 log CFU/g/mo	
Bacillus cereus	Infant cereal	Survival of vegetative cells in infant rice cereal stored at 5, 25, 35, and 45°C for 36 wk was not affected by	85
		a_w (0.27–0.78) or pH (5.6 and 6.7); death of spores at 45°C for up to 48 wk was enhanced at a_w 0.78 but	
		unaffected by pH; loss of viability at 5, 25, and 35°C was unaffected by aw	
Listeria	Almonds	Initial population (4.4 log CFU/g) on kernels did not decline at -19 or 4° C for 12 mo; slow decline at 24° C	100
monocytogenes	ţ	but detectable after 12 mo; linear rate of decline at 24°C was 0.71 log CFU/g/mo	
	Peanuts	Initial population (ca. 5 log CFU/g) remained stable on raw kernels for 181 days at -20 and 4° C; linear rate	132
	Doonit hitten aboaclate maanit	01 decime at 25 U was 0.55 log UFU/g/m0 Initial monitation of 1.12 for CETU/s of account hutter at a 0.022 and 0.65 document to 0.67 for CETU/s in 24 md	00
	reanut outter, cnocotate-peanut hutter spread	initial population of 4.42 log CFO/g of peatin butter at a _w 0.55 and 0.65 decreased to 0.62 LFU/g III 24 WK and 0.48 log CFU/g in 8 wk respectively, at 20°C; initial nonulation of 3.37 log CFU/g of a chocolate and nearnit hutter	90
		spread at a _w 0.33 and 0.65 decreased to 0.90 log CFU/g in 16 wk and 0.95 log CFU/g in 4 wk, respectively, at 20°C	
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TABLE 2. Continued	pə		
Pathogen	Food	Survival	Reference(s)
	Pecans	Initial population (ca. 5 log CFU/g) remained stable on kernels for 181 days at -20 and 4° C; linear rate of decline at 23° C was 0.83 log CFU/g/mo	132
	Pistachios	Initial population (4.6 log CFU/g) on in-shell nuts did not decline at -19 or 4° C for 12 mo; slow decline at 24° C but detectable after 12 mo; linear rate of decline at 24° C was 0.86 log CFU/g/mo	001
	Walnuts	Calculated rate of decline was 1.1 log CFU/g/mo for kernels stored at 23°C for 105 days	20
Staphylococcus	Cake mix, skim milk powder, onion soup	Inactivation at 25°C for 27 days was minimal at a _w 0.00–0.22 but increased as a _w increased to 0.53; survival	41
aureus	mix, gelatin-based dessert	better in vacuum vs air and in food with higher pH (cake mix 6.8 vs dessert 3.1); at a_w of food as purchased, 2-log CFU/g reduction in cake mix (a_w 0.32), skim milk (a_w 0.22), onion soup mix (a_w 0.14), and dessert (a_w 0.42) within 27, 18, >27, and 1 wk, respectively	
	Pasta	Initial populations of approx 7 and 8 log CFU/g of pasta and egg pasta, respectively, decreased to approx $1-2$ and $3-4$ log CFU/g in 90 days at room temp; counts decreased to <100 cells per g after storage for 180 days	137

15 strains of Salmonella Anatum, 14 of which had the same DNA fingerprint. Environmental samples from processing floors, process conveyors, dust in the air, and rodents in the processing plant were analyzed for the presence of Salmonella over a 5-month period. Four serotypes of the pathogen were common to three distinct areas (receiving, manufacturing, and storage) of the plant. The rate of detection in rodents was 46.4%. Shoes and gloves of workers in the manufacturing area had prevalence rates of 100 and 90%, respectively. PFGE analysis showed that three serotypes isolated from the processing floor, work shoes, brooms, rodents, and dust were of the same origin, suggesting cross-contamination and persistence throughout the manufacturing area. Stocki et al. (158) studied colonization of Salmonella on egg conveyor belts to determine if the red dry and rough (rdar) morphotype, a conserved phenotype associated with aggregation and longterm survival, contributed to persistence. Higher numbers of Salmonella remained on a hemp-plastic belt than on a vinyl belt after washing and disinfection. The rdar morphotype was involved in colonizing belts but was not essential for persistence.

Mullane et al. (120) monitored PIF and its processing environment for Cronobacter species for a period of 1 year. The frequency of isolation in intermediate and final products was 2.5%, while frequencies up to 31% were found at specific locations in the processing environment. Nineteen PFGE types could be grouped into six clusters, each containing 5 to 32 isolates. Subtyping of 200 Cronobacter isolates recovered from a PIF factory revealed that the majority (70%) were clonally identical, demonstrating the persistence of a resident strain in the processing environment. The majority of isolates (72.5%) were of environmental origin, but no cluster was confined to a specific location. These findings further suggest that the manufacturing environment can serve as a key route for sporadic contamination of PIF with Cronobacter.

Baumgartner et al. (10) screened 268 ready-to-eat (RTE) foods from retail food shops for the presence of Cronobacter. Cronobacter was isolated from 7 (26.9%) of 25 samples of spices and dried herbs and 3 (7.1%) of 42 samples of confections. The public health implications of these findings are uncertain. Follow-up samples of each food found to be positive were analyzed to determine if Cronobacter persisted in particular products or at specific production sites. Isolates with indistinguishable PFGE profiles were recovered in five samples from two types of confectionery collected over an 11-month period from one bakery. It was concluded that this could be indicative of persistent contamination of the factory or retail premises. It is not known whether persistence in the baked foods was due to survival through the heating process or postprocess contamination. These observations, nevertheless, provide further evidence of the ubiquitous nature of Cronobacter. When compared with most other members of the Enterobacteriaceae, Cronobacter has a greater capacity to survive in dry foods and dry food environments for long periods of time (70, 125).

Pathogen	Environment	Remarks	Reference(s)
Cronobacter species (formerly	Dairy and dry-blending facilities	Detected in 4 (8%) of 50 samples	140
Enterobacter sakazakii)	Hospital, milk kitchen	Isolate from a blender noted to have a small crack at base; tested positive 5 mo after being used to prepare formula	8, 21
		Samples (256) from spoons, jars, bottles, blenders, sieves, and surfaces in infant formula preparation areas were analyzed; found in residue from nursing bottle and in cleaning sponge	127
	Powdered infant formula factory	Of 867 environmental samples, 35 (4.0%) were positive; 94.3% of the samples were powders	138
	Powdered milk factory	Detected in 88 (46%) of 192 environmental samples (includes floor sweepings, scrapings from processing areas, spilled dry products, contents of a vacuum cleaner bag) from 4 factories	69
		Frequency of isolation in product was 2.5%, while frequencies up to 31% were found at specific locations in the processing environment, suggesting that the environment serves as a key route for sporadic contamination	119, 120
		Detected in 18 (12%) of 152 environmental samples (scrapings from dust, vacuum cleaner bags, spilled product near equipment) taken from 3 factories	95
		Genotyping of 200 isolates over 25 mo showed 70% had same fingerprint, which indicates persistence; of the 156 isolates from the processing environment, most were from surfaces surrounding the dryer (floor, steps, walls, or cyclones), blenders, storage silo areas, silo vacuum, platform, and floors in packing areas and canning room	134
		Prevalence at 6 sampling sites during an 11-mo period was 14.3, 3.8, and 2.1%, respectively, in prefinal, prepackaged, and packaged final products; PFGE analysis showed long-term persistence in processing line; supply air was potential source of extrinsic contamination	73
		Isolated from 32% of 298 environmental samples in 5 factories; PFGE analysis showed that most clones were unique to each factory and 7 of 49 were isolated from both milk powder and other areas in the same factory, including tanker bays, evaporator rooms, an employee's shoes and external roofs	47
		Seven PFGE types were detected in the spray-drying area; textile fibers for exhaust air of the spray-drying towers were internal reservoirs; a 2nd transmission route was the roller dryer section of the factory	84
	Retail confectionery shop	Isolates with identical PFGE profiles recovered from 5 samples of 2 types of confections collected over an 11-mo period from 1 bakery; suggests persistent contamination of the factory or retail premises	10
	Various dry food facilities	Detected in 4 milk powder factories (14 [21%] of 68 samples) and a chocolate factory (2 [25%] of 8), cereal factory (4 [44%] of 9), potato factory (4 [27%] of 15), and pasta factory (6 [23%] of 26)	94
Salmonella	Oilmeal plant	Several serotypes were recovered from equipment in receiving, manufacturing, and storage areas, processing floors in manufacturing and storage areas, and rodents and dust in air in manufacturing area; processing floors represented the greatest risk of contamination	118
	Egg conveyor belt	Higher numbers remained on hemp-plastic belt than on vinyl belt after washing and disinfection; rdar morphotype, a conserved physiology associated with aggregation and long-term survival, was involved in colonization but not essential for persistence	158

TABLE 3. Presence and persistence of foodborne pathogens in dry food processing and preparation environments

Survival of resistant and dominant strains of *Cronobacter* and other foodborne pathogens in dry processing environments relies on their ability to adapt to high osmotic potentials and/or dry conditions (2, 126, 141). Lehner et al. (109) examined 56 strains of *Cronobacter* species for features important to persistence and survival. The ability of the pathogen to form biofilms with the production of cellulose as a component in the extracellular matrix, adherence to hydrophilic and hydrophobic surfaces, and production of extracellular polysaccharides along with cell-to-cell signaling molecules are thought to be factors

that enable *Cronobacter* species to adapt to physiologically stressful environments and that facilitate their persistence. Persistent strains within a biofilm and cells in the stationary phase of growth may use quorum sensing to modulate the collective activities of the bacterial population, thereby promoting enhanced resistance to adverse environments, e.g., cleaning agents, sanitizers, and dehydration (51, 75, 80, 105, 109). Examples of the presence and persistence of *Cronobacter* and *Salmonella* in dry food processing and preparation environments are listed in Table 3.

OUTBREAKS ASSOCIATED WITH LOW-AW FOODS

Shown in Table 4 is a partial list of documented outbreaks of foodborne illnesses that have been traced to consumption of dry foods and food ingredients. A large majority of these outbreaks has been caused by *Salmonella*. Of the 43 outbreaks listed, 33 (76.7%) were reported to be caused by *Salmonella*, 4 by *Cronobacter* species, 4 by *E. coli* O157:H7, 1 by *S. aureus*, and 1 by *C. botulinum*. Outbreaks of salmonellosis were caused by several different *Salmonella* serotypes and, while the more common Enteritidis and Typhimurium serotypes do appear, there were a number of other serotypes involved, potentially indicating the wide geographic origin of these foods and food ingredients.

Considering all food and ingredient types, powdered milk and dried infant food, formula, and cereal were implicated in the highest number (11 [25.6%] of 43) of outbreaks. This could be due to the nature of the consumer (infant) being more likely to become ill or to the nature of the product, which may be rehydrated and then stored before consumption, thus potentially allowing pathogens to grow, thereby increasing the risk of causing illness. Nuts, seeds, and their products caused 12 outbreaks, and chocolate caused 4. Although herbs and spices do not feature greatly, it is interesting that some issues linked to other foods may have originated with these items used in their manufacture. For example, the outbreak attributed to potato chips (crisps) was caused by contaminated paprika used as a seasoning (107). Some issues may not originate from the raw materials. At least one of the peanut butter-associated outbreaks and one chocolate outbreak were probably caused by contamination of equipment in the production area, which then cross-contaminated products that were being manufactured, with the dry nature of those products allowing the pathogen to survive for a long period of time. In an outbreak involving a corn snack flavoring, the Salmonella serotype responsible for illness, as well as two other serotypes, were isolated from an external water source used for cooling water (88).

MONITORING LOW-AW FOODS FOR PATHOGENS

Recalls and market withdrawals of low-aw foods due to potential contamination with Salmonella that occurred in the United States during the 8-year period of 2004 to 2011 (http://www.fda.gov/Safety/Recalls/ArchiveRecalls/default. htm) showed a predominance of contamination of nuts and seeds. Of the 55 types of low-aw products recalled or withdrawn, 24 (43.6%) were nuts (almonds, hazelnuts [filberts], peanuts, pecans, pine nuts, pistachios, and walnuts), seeds (celery, pumpkin, and sesame), or products (peanut butter, peanut paste, halva, and tahini) produced from or containing these foods. Following these, nine spices and herbs and eight mixes (cereal, batter, or soup) were among the types of products recalled. The latter category is a very heterogeneous mix, and the root cause of contamination may be from any one of the ingredients. Another interesting point to note is a large increase in number of low-a_w products recalled in 2009 to 2011, compared with earlier years. It is not known if this is indicative of a worsening safety problem in low- a_w foods or simply that more surveillance is ongoing and detection of contamination with foodborne pathogens is more likely.

The Rapid Alert System for Food and Feed (http://ec. europe.eu/food/food/rapidalert/index_en.htm) used by the European Union member states to alert each other of foods containing pathogens or undesired substances issued 71 warnings concerning pathogens in low-a_w foods during the period of 2008 to August 2011. Breakdown according to the country of origin yielded no consistent patterns. The occurrence of pathogen-food combinations varied considerably, with a notable peak of 10 warnings for *Salmonella* in nuts in 2009 but only 1 in 2010. *Salmonella* was found twice in infant formula, but *Cronobacter* species was found only once. *Salmonella* was clearly the pathogen most often detected, and sesame seeds, nuts and nut products, pepper, herbs, and spices (45 [72.6%] of 62) were the foods and ingredients in which it was found.

SOURCES OF PATHOGENS AND CONTROL OF ROUTES OF ENTRY INTO PRODUCTION AREAS

Producers of low-aw foods and food ingredients need to consider the ways consumers will use their products when assessing safety risks. For example, should the product be considered as RTE, i.e., not to be cooked before eating? The definition of RTE is given in European Commission Regulation 2073/2005 (59) as "food intended by the producer or manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level microorganisms of concern." However, producers must also consider what the consumer will actually do with the product and not just what they assume consumers will do. Processors intend some of their products to be cooked by the consumer before consumption, when in fact consumers may eat some or all of the product without cooking it. The outbreak of E. coli O157:H7 infections associated with consumption of uncooked ready-to-bake cookie dough (121) emphasizes the occasional aberrant behavior of consumers. An interview of patients revealed that several of them bought the dough with the intention of only eating it unbaked; they had no plans to actually bake cookies. When food producers design a cooking process to be used at the consumer level, they must consider the physiological condition of pathogens in dry environments. Because microbial cells often have a higher heat resistance in dry environments than in wet environments, the temperature and time a producer intends the consumer to cook a product need to be considered and validated to ensure effective inactivation of pathogens that may be found in the product.

Hazard identification and risk. A very wide range of raw materials and ingredients from primary agricultural origins are used to manufacture highly processed, low- a_w foods. It is therefore important that producers assess the risk that ingredients may contain pathogens of importance to

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Food	$\mathbf{Y}_{\mathbf{\Gamma}}$	Pathogen	Location	No. affected	Remarks	Reference(s)
Almonds	2000	S. Enteritidis PT30	USA, Canada	168	Outbreak strain was detected in raw almonds collected from orchards and distribution, warehouse, retail, and home locations, and from processing equipment 6–7 mo after being	82
	2003	S. Enteritidis PT9c	USA, Canada	29	used. Frequent and prolonged recovery suggests diffuse and persistent contamination. Raw almonds from an opened package, 1 environmental sample collected at the manufacturer, and 3 samples from 2 huller-shellers that supplied the manufacturer were nositive for <i>Colmonalia</i>	33
	2005	S. Enteritidis	Sweden	15	Phase type of isolates from patients was the same as the phase type of isolates from the 2000 almond-associated outheast	106
Cake mix Cereal (toasted)	2005 1998	S. Typhimurium S. Agona	USA USA	26 209	An opened box of cereal yielded a <i>S</i> . Agona isolate with a PFGE pattern indistinguishable from the predominant PFGE pattern among outbreak-associated	169, 175 31
Cereal (puffed)	2008	S. Agona	USA	28	Puffed rice and puffed wheat cereals implicated in the outbreak were manufactured at the same plant that manufactured toasted oat cereal implicated in a 1998 outbreak of S. Agona infections, S. Agona were consistent to a source of from the about and from how of muffed rice associations.	36
Chocolate	1973	S. Eastbourne	Canada, USA	95	Levels of 2–9 salmonellae per chocolate bar were estimated. Deficiencies in plant operations coupled with inadequate quality control contributed to spread of <i>Colmonality</i> to different means of the plant	49
	1982	S. Napoli	UK	245	Chocolate bars produced on at least 11 days over a 6-wk period contained <i>Salmonella</i> . Bars containing a low number of <i>Salmonella</i> caused illness at least 7 mo after	66
	1985	S. Nima	USA, Canada	33	Suspect chocolate contained 4.3–24.5. Nima per 100 g, suggesting that small numbers can cause clinical evontoms. Samules were nestive for S Nima at least 10 no after manufacture	74
	1987	S. Typhimurium	Norway, Finland	361	Levels of ≤ 10 S. Typhimurium per 100 g were detected in 91% of positive samples, suggesting that low numbers can cause infections. Other serotypes were isolated from dust collected from rooms in which cocoa beans were stored or rinsed.	96
	2001	S. Oranienburg	Germany, Denmark, Austria, Belgium, Finland, Netherlands, Sweden	439	Two brands of chocolate from 1 company were associated with illness in 7 countries; <i>Salmonella</i> -positive products reported in Canada, Croatia, and Czech Republic. Chocolate isolates had PFGE profiles that were indistinguishable from human isolates in the outbreak.	171
Cocoa	1970	S. Durham	Sweden	>109	Source of infection was traced to cocoa used to make confectionery items. Manufacturing process would not kill possible S Durham in cocoa	64
Coconut	1953	S. Typhi, S. Senftenberg, S. Potsdam, S. Orion	Australia	>50	Salmonellae were isolated from packets of desiccated coconut obtained from households and from unopened cartons at retail and wholesale.	172
	1999	S. Java PT Dundee	UK	18	One hundred twenty-eight (71%) of 181 samples of desiccated coconut obtained from retail nackets and sacks in warehouses vielded the outbreak strain.	168
Cookie dough	2009	E. coli 0157:H7	USA	76	Infections were associated with consumption of uncooked ready-to-bake cookie dough. Flour was the prime suspect ingredient for introducing $E. coli$ O157:H7 to the dough.	121
Fishmeal	1972	S. Agona	USA	17	Source of S. Agona was fishmeal fed to poultry; cross-contamination from poultry to other foods in a restaurant kitchen was hypothesized.	42

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TABLE 4. Continued	inued					
Food	Yr	Pathogen	Location N	No. affected	Remarks	Reference(s)
Halva (helva)	2001	S. Typhimurium DT104	Australia, Germany, Norway, Sweden, UK	>70	S. Typhimurium DT104 was isolated from jars of halva (plain, pistachio, and chocolate flavors) and other sesame seed-based products.	1, 24, 52, 111, 124
Hazelnuts (filberts)	2011	$E. \ coli$ O157:H7	USA	8	Inshell hazelnuts from bulk bins in retail stores were implicated; outbreak strain was isolated from mixed nuts containing in-shell hazelnuts	38, 115
Infant dried	1985	S. Ealing	UK	76	Source traced to defective factory spray drier. S. Ealing was isolated from scrapings	143
milk product	1996	S. Anatum	UK, France	>12	taken from a suo into which waste powder and dust (sweepings) were deposited. S. Anatum was isolated from unopened infant formula; linked to dried milk plant. Not	3
Infant food	1005	C Canffanhara	1112	v	found in routine surveillance of the production plant.	144
or cereal	C661		ND	n	Cleaning remains from minimg macminery were implicated as sources of contamination. The HACCP system was evaluated and highlighted this hazard.	144
Jerky (venison)	1995	E. coli 0157:H7	USA	9	E. coli O157:H7 was isolated from leftover jerky and uncooked, frozen venison (3 and 93 CFU/g of jerky; 150 CFU/g of uncooked venison); jerky stored at ca. 20°C for more than 13 mo was positive for the pathogen.	97
Peanuts	2001	S. Stanley, S. Newport	Australia, Canada, UK	109	Asian-style, dry-flavored or roasted peanuts in their shell were implicated as contaminated sources. Unopened packets were positive for <i>S</i> . Stanley, <i>S</i> . Newport, <i>S</i> . Lavinora, <i>C</i> . Kothue, and an universe contract	101
Deput butter	2000		Conodo	6	Contributed of Antonia and anticatoring (terms A and B) ware isolated from clinical	151
	0007		Callaua	r	A, which was indistinguishable from clinical isolates by PFGE analysis. Two of the case patients had Crohn disease.	101
	1996	S. Mbandaka	Australia	54	S. Mbandaka was isolated from opened and unopened jars. Roasted peanuts were implicated as the source of contamination. Positive samples of peanut butter contained <3-4 CFU/g. Outbreak highlighted the need for an effective HACCP movem throubout the moduction moress.	146
	2006	S. Tennessee	USA	628	S. Tennessee was isolated from opened and unopened jars. Peanut butter manufactured over at least a 6-mo period was positive for the pathogen.	34
	2008	S. Typhimurium	USA, Canada	684	S. Typhimurium was isolated from opened and unopened jars. S. Tennessee with a PFGE pattern indistinguishable from the peanut butter-associated outbreak strain in 2006–2007 outbreak was isolated from an unopened jar.	37
Pepper (black)	1981	S. Oranienburg	Norway	126	Consumption of minced meat and/or minced fish products containing ground black pepper was associated with infection. <i>S</i> . Oranienburg was isolated from an unopened package of pepper. Counts of 10 to >240/100 g were found in 12 samples positive for <i>S</i> . Oranienburg.	71
Pine nuts	2011	S. Entertitdis	USA	43	Two samples collected along the supply chain, 1 open retail sample, and multiple samples from consumers' homes tested positive for the same <i>S</i> . Entertidis strain found in clinical cases.	81
Potato chips	1993	S. Saintpaul, S. Rubislaw, Germany S. Javiana	Germany	1,000	Paprika powder and chips seasoned with powder contained salmonellae. Analysis of paprika powder yielded 2.5 salmonellae per g; a second count 8 mo later revealed 0.7 salmonellae per g; survived in spice mixtures for at least 1 yr. Infective dose was estimated at 4–45 CFU.	107

Powdered infant 1986 <i>C</i> formula 1988 <i>C</i> 1988 <i>C</i> 1993 <i>S</i> 1993 <i>S</i> 1993 <i>S</i> 1993 <i>C</i> 1993 <i>C</i> 1998 <i>C</i>	The second se		110. mm	Remarks	Reference(s)
1988 1993 1998	Cronobacier species	Iceland	3	Four strains of <i>Enterobacter sakazakii</i> (<i>Cronobacter</i> species) isolated from infected neonates were indistinouishable from 22 strains isolated from formula	18
	Cronobacter species	USA	4	Blender used to prepare formula was contaminated with <i>Cronobacter</i> . Pathogen was found in the powdered milk formula.	154
	S. Tennessee	Canada, USA	б	In addition to powdered infant formula, other spray-dried products (medical food supplement, protein supplement, medical meal replacement, powdered milk, diet beverage, and weaning formula) manufactured at the same plant were recalled.	30
	Cronobacter species	Belgium	12	<i>Cronobacter</i> was isolated from the implicated prepared milk formula as well as from unopened cans of powdered milk formula. Recommendations for preparing and handling infant milk formula were made. with the goal of enhancing safety.	166
2001 <i>C</i>	Cronobacter species	USA	11	<i>Cronobacter</i> was isolated from unopened and opened cans of powdered infant formula. PFGE patterns of these isolates were indistinguishable from a clinical isolate.	32
2008 S.	S. Give	France	8	All cases were associated with consumption of a single brand of powdered infant milk formula.	89
Powdered milk 1973 S.	S. Derby	Trinidad	3,000	Imported dried milk products were packaged in a single plant. Workers may have been vehicles of S. Derby.	170
2004 S.	S. aureus	China	150	Old milk powder was suspected to have been repackaged. Powder contained S. aureus toxin.	4
Salami 1994 E	E. coli O157:H7	USA	4	Isolates from intact packages of dry fermented salami collected from the plant warehouse, at retail, and from patients had identical PFGE patterns. Estimated infectious dose was 2–45 cells.	163
Snack (corn) 1989 S.	S. Manchester	UK	47	<i>S.</i> Manchester, <i>S.</i> Schwartzengrund, and <i>S.</i> Oranienburg were isolated from site producing yeast extract used in flavoring. These serotypes were also found in stream water used for cooling water in the factory.	88
Snack (rice- 2007 S. corn)	S. Wandsworth, S. Typhimurium	USA	75	<i>S</i> . Wandsworth and <i>S</i> . Typhimurium were isolated from sealed bags of a rice-corn vegetable-coated snack intended for children.	35
Snack (savory) 1994 S.	S. Agona PT15	UK, USA, Israel	>2,200	Snacks were manufactured on at least 7 separate dates over a 4-mo period. Levels were estimated to be 2–45 CFU/25-g packet of peanut-flavored snack.	99, 152
Tahini 2002 <i>S</i>	S. Montevideo	Australia, New Zealand	68	Three outbreaks occurred. Strains of <i>S</i> . Montevideo with closely related PFGE patterns were isolated from sesame-based products (tahini) imported from 2 countries. <i>S</i> . Tennessee and <i>S</i> . Orion were also isolated. Salmonellae were isolated from retail samples of tahini in the UK and Canada. Use of HACCP principles in the production of tahini was emphasized.	165
Tea (aniseed) 2003 S	S. Agona	Germany	42	<i>S.</i> Agona was isolated from 6 brands of tea containing aniseed. Various serotypes were isolated from 61 (11%) of 575 teas and other products containing aniseed. <i>S.</i> Agona survived when exposed to hot water during tea making.	102

human health. Once that risk is known, actions should be taken to control it during production of the final product. Assessment of risks associated with low- a_w foods and food ingredients can be achieved by asking a series of questions:

- Will the ingredient be included in a product that will receive an antimicrobial treatment before it leaves the production environment? Has that process been validated? This step on its own should produce a safe product.
- Will the ingredient be used in a way that it will not receive an antimicrobial treatment before it leaves the production site?
- Has the ingredient been processed in a manner effective to eliminate pathogens before it is used by the food processor? Has the process been validated to show this?
- Is the process used sufficient to reduce the pathogen risk in the end product to an acceptable level and has it been validated?
- Is the instruction given to the consumer on cooking before eating sufficient to reduce the pathogen risk to an acceptable level, and is the consumer likely to follow precisely the recommended cooking procedure? Have the instructions to consumers been validated for effective-ness at reducing pathogens? This point carries the greatest risk as there is little control that the producer can exert at this point over what the consumer actually does with the product. Resorting to this point should only be done after very careful consideration and assessment of risk.

Control of air flow. Food ingredients and final products may be exposed to air on many occasions in the production environment, and this will introduce a risk that they will become contaminated. Questions that should be asked concerning conditions potentially influencing the risk of contamination of low- a_w foods and food ingredients include the following:

- Is the production area effectively closed and are there any access points directly to an external environment, e.g., doors, windows, fans, skylights, ductways, and drains? Access to external environments introduces a risk that microorganisms from external sources may gain entry to the production area. These sources may also facilitate access to insects, birds, and rodents that could further increase the risk of product contamination.
- Is air used to convey product or material within the production area? Is the air filtered in any way and from where does it originate? Is the level of filtration effective for removing foreign bodies or microorganisms? Air can be a significant source of microbial contaminants and must be handled in ways to greatly minimize introduction of microorganisms in production areas.
- Is any part of production under positive pressure? How is this managed and where is the source of the air to maintain positive pressure? Is the incoming air filtered and is the level of filtration effective for removing foreign bodies or microorganisms? If the factory operates under a zoning system (see following section), then it

should be confirmed that air always flows from the highest hygiene zone to the lowest, with no chance of backflows that could introduce contamination into areas of highest care. The high hygiene zone must therefore always be under the highest positive pressure.

- Are there any negative pressures within the production area? These will tend to pull air into that area, thereby introducing contamination. Check for negative pressure areas and, unless required for any specific reason, try to eliminate them or ensure that the incoming air does not introduce significant numbers of microorganisms.
- Has adequate consideration been given to protecting exposed product from airborne contamination, e.g., covers over exposed parts of a production line? Use of covers can provide a degree of protection from airborne contamination and additionally from airborne foreign bodies dropping onto or into product. However, covers can also inhibit good cleaning by making access to product contact areas difficult.

Water is the enemy. In plants producing low- a_w products, it is usual to considerably reduce or eliminate water usage. Much of the microbiological control is centered on keeping production areas as dry as possible and thus preventing microbial growth. Allowing water to access such areas creates a potential for resuscitation and growth of dormant microorganisms. Growth can be rapid and high numbers can be reached if the area is warm. This can provide a source of contamination of the final product. Important points to consider include the following:

- Unless required for a specific use within production, limit the presence of water to an absolute minimum.
- If it is used, water should be contained within a specific area, e.g., hand washing should be well segregated from the processing area and waste water should always be drained away from production. Washing of production tools and utensils should be in well-segregated and dedicated areas with good drainage. Drainage water should not enter production areas and the tools should be dried before being taken back into these areas.
- Nonpotable water should never enter production areas. Nonpotable water includes water draining from washing systems and leaking rain water. Any indication that nonpotable water has entered production should result in a full risk assessment of the implications of this issue.
- Water used in contained systems that enter production (e.g., completely enclosed cooling systems) should be assessed for risk and the potential for leakage should be considered. Such systems are ideally operated with potable water.

Other potential sources of pathogens. Low- a_w food contact surfaces such as hoppers, conveying systems, pipework, elevators, and dispensing systems and tools such as scrapers should be pathogen free. There should be a regular cleaning schedule for such equipment to prevent residue buildup. Tools and items that can be removed from

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production areas may be wet cleaned as long as they are fully dried before bringing back into dry production areas.

Personnel can be a source of incoming pathogens into food production areas. Producers of $low-a_w$ foods and food ingredients should control the entry of staff into production areas, and suitable procedures to reduce the risk of product contamination should be adopted. Points to consider:

- Clothing and footwear worn in production areas should be designed to protect the product from contamination by the staff. If personal protective equipment is required, this too should not be a potential source of product contamination. Clothing should be regularly laundered before being worn by staff entering production areas. Consideration should be given to having footwear that is only worn within production areas and is regularly cleaned.
- Staff entering production areas must always wash their hands using warm water and soap, after which hands should be dried. Alcohol-based sanitizers may be considered as a useful feature to be used after hand washing, but not as a replacement.
- Health screening of staff for potentially pathogenic microorganisms must be considered.
- A notification system to enable staff to report suspect foodborne illness and visits to other countries must be considered.
- A policy to prevent staff that have contracted foodborne illness from entering food production areas, until the infection has cleared, should be in place.

Insects, birds, and rodents introduce a risk since they are likely carriers of pathogens that could contaminate food products. The ingress of pests of any sort into food warehouse, dry storage, and production areas introduces a risk that pathogens will also enter and contaminate finished products. Pest control programs that effectively minimize this risk involve placing screens over openings into production areas and the positioning of baits and traps around the production site. These programs are often operated by specialized pest elimination companies. Baits and traps should be regularly checked to ensure that pest problems are not developing. Traps should be regularly emptied to prevent the risk of buildup of sources of contamination.

CONTROL MEASURES IN LOW-A_W FOOD PRODUCTION ENVIRONMENTS

The level of safety of low- a_w foods can be increased through proper control of the production environment. The more sensitive the consumer is to the pathogen(s) that may be in the product, the stricter the end product specifications and the more critical the control measures during production should be to assure that products meet the specifications. Since PIF has very strict end product specifications, it is used here as an example of low- a_w foods to illustrate the most critical control measures. Control measures for other low- a_w foods with less stringent end product specifications can then be extrapolated.

Reduction of initial contamination. An important microbiological safety control measure for most low-a_w food

products is pasteurization in the wet stage to reduce contamination to an acceptable level. Not always considered in practice is that heat pasteurization conditions depend on the solids (dry matter) content. Cream containing 35% fat, for example, requires heating at 80°C for 15 s for pasteurization, i.e., to reduce Salmonella, Campylobacter, or L. monocytogenes by at least 5 log CFU/ml (43). This compares to heating milk with ca. 10% solids at 72°C for 15 s to achieve pasteurization. The solids content of liquids should always be taken into consideration when defining pasteurization conditions. Assuming that low-aw foods have been decontaminated by heat pasteurization or some other treatment (e.g., high pressure or irradiation) in a previous processing stage, the main route of entry of pathogens into these foods is by recontamination. Control measures discussed here are aimed at preventing contamination of dry foods in wet as well as in dry stages of production and storage.

Wet process line. After the critical heating step, wet process lines should be designed and constructed as high or ultra clean, like lines for ultrahigh-temperature products. Tubular sieves should not be cleaned in the wet processing environment but rather in a special high hygiene room, properly separated from the wet basic hygiene environment. An even better approach would be to clean tubular sieves in a special clean-in-place cycle. Sometimes, lecithin and/or mixtures of vegetable and other oils containing long-chain polyunsaturated fatty acids are added to obtain the desired composition and physical properties of the product. All additions defined as dry mix ingredients, even if added in the wet phase, should be free from pathogens.

Effective zoning in the dry process line. Proper zoning is crucial to prevent contamination in the dry stage. Zoning starts with defining wet and dry zones and, subsequently, high hygiene zones. In the wet part of the processing environment of dry foods, high hygiene zones usually do not occur, which is contrary to the processing environment of cooked meat products, for example. A separation between a basic hygiene level for the wet part of the line before the critical heat treatment and a medium hygiene zone for the wet part of the line after the critical heat treatment might be considered; this can be achieved by physical means such as a wall but is not as critical as the separation of high hygiene zones in the dry part.

To enable dry zones to be kept dry, they should be physically separated from wet zones by solid walls. Because the intermediate or final product is or can be exposed to the environment and hence a risk of contamination, the spray dryer building, dry mixing department, storage silos for dry mix ingredients, intermediate and finished product areas, and packaging department for primary packaging are high hygiene zones. Dry mix ingredients supplied in bags should be stripped in a special medium hygiene stripping zone. Dry mix ingredients in large bags are preferred over small bags, which need more handling, thereby introducing greater risk of contamination.

Personnel and materials that may pass from a basic hygiene zone into a high hygiene zone or vice versa should

always pass through a medium hygiene zone between these zones. Connections between the high hygiene zone and the basic hygiene zone should always function as an air lock, because high hygiene zones should be kept under a positive pressure of at least F7 as defined by filtration performance standards (60). The use of footbaths before entering zone areas creates a potential for introduction of water into the dry processing line area and should be considered in the overall approach to maintaining a high level of hygiene.

Dry parts of lines for spray-dried powdered products are seldom tightly closed. For this reason, it is necessary to introduce special precautions to prevent recontamination. Powder leaks can be observed frequently, highlighting the fact that the line is not tightly closed. If powder can come out of the line, air that may contain dust can easily enter the line, thereby increasing the risk of contamination. For this reason, and because some companies may operate with open spray dryers, high hygiene zones are always kept under filtered positive-pressure air of at least F7. High hygiene zones should never open directly to basic hygiene zones or to the outside. Air filters should be constructed such that, when they are changed, the filter cloths are removed at the lower hygiene side, preventing contamination of the higher hygiene zone. It could be considered useful if these filters were then checked microbiologically before being replaced and the microbiota detected compared with that observed as part of routine monitoring. If emergency doors for worker safety in the high hygiene zone open directly to the outside, they should be kept tightly closed and sealed to prevent use when not required. Emergency doors should never enable shortcuts; to prevent these shortcuts, emergency doors should be sealed and, if possible, electronically connected with the control room to alert operators if used unintentionally.

The practice of personnel changing their clothes and shoes before entering a high hygiene zone is becoming more common. Operators may introduce *Enterobacteriaceae* and other microorganisms with their clothes and shoes, especially when they operate on the wet evaporator side as well as on the drying side, which is usually the case if the process runs in line and is managed by the same personnel. Since dry processing is done under high hygiene conditions, entrance to the high hygiene zones should be limited as much as possible and controlled by the operators running the drying process. For this reason the control room should be the only entrance into the high hygiene processing area. In general, it is recommended not to have the control room in the high hygiene zone but rather in an area between wet processing and dry processing.

To help maintain dedicated dry zones, hand washing facilities should not be present in air locks between basic and high hygiene zones because they create an increase in humidity in the dry zone environment. Hand washing should have already been completed prior to entering the production zone, so no additional washing is necessary. Alternatively, dispensers with disinfecting gels (no sprays or liquids) can be placed in the air locks.

Engineers or maintenance staff may at times inadvertently use tools in a high hygiene zone after previous use in a lesser hygiene area. To prevent contaminated tools from contributing to the presence of pathogens in critical areas, it is recommended that dedicated tool kits and trolleys for tools remain within the dedicated zone.

Cleaning and sanitizing dry zone areas. Dry cleaning of spray dryers, dry mixers, storage silos, packaging machines, and adjacent equipment is essential. Vacuum cleaners can be used for dry cleaning the inside and outside of equipment and the environment. It is essential to dedicate vacuum cleaners and their tools, such as fittings, for different purposes, either because of hygiene reasons (inside of equipment versus outside) or because of sampling reasons. Although not yet suitable for all situations, more and more tools have been developed for use in dry cleaning areas. Examples include silo cleaning systems adapted for spray dryers and blasters that use lactose or calcium carbonate instead of sand and dry ice (CO₂); in the case of dry ice cleaning, care should be taken to prevent condensation.

Vacuum cleaning should never be done using a central vacuum cleaner system. Tubes in the system will collect dust, are prone to harbor insects, and cannot be easily cleaned internally. Alternatively, a central vacuum system can be installed with distinct small cyclones at locations where vacuum cleaning is necessary. These cyclones enable separation of dust from the air and are also equipped with filters. As with the filters, these cyclones should be microbiologically assessed.

If wet cleaning of certain parts of equipment, e.g., nozzles of the spray dryer and heads of the filling units in the filling machine, is necessary, it should be done in the high hygiene zone under controlled conditions in a special room within the dry high hygiene zone, properly separated from the dry zone. If the use of dry cleaning equipment is not possible but wet cleaning is necessary because of the need for elimination of allergens or because equipment cannot be dismantled for separate wet cleaning, it should be recognized that both the inside and outside of equipment as well as the immediate environment will become wet and that humidity is increased in the environment. For this reason, when wet cleaning is unavoidable, it should include all surfaces of the equipment and its environment. Sanitizing should follow wet cleaning.

The equipment as well as the surrounding environment should be dried after wet cleaning. Usually the equipment is dried by forced hot air. The environment does not become warm, however, if the spray dryer is insulated. To promote drying of the environment in the spray dryer building, elimination of all insulation from the spray dryer is recommended. An outbreak of salmonellosis associated with PIF has been linked with contaminated insulation in a spray dryer (143). Salmonella was present in the insulation and was dispersed from time to time into the spray dryer and the product flow through a crack in the spray dryer, resulting in its presence in the finished product.

Because dry zones must be kept dry, water should not be present, except for extinguishing fires. If dry cleaning is practiced, eliminate items such as water tubes, hoses, and drains that are not necessary. If the spray dryer needs to be wet cleaned, make sure that stagnant water is not present in the environment during production; drains with water locks should not be present in floors. Often, because of poor maintenance, air-handling units equipped with steam give rise to leakage and condensate formation. Proper maintenance of all equipment in high hygiene zones is critical. Preferably, auxiliaries are located outside the high hygiene zone of the spray dryer building.

If water has entered the dry zone, it can be removed using paper towels, followed by sanitizing. Sanitizing in dry environments can be done with chlorinated water or with alcohol-based sanitizers. The potential for residual water remaining after application of sanitizers is a concern, however, because high-moisture environments may promote the growth of microorganisms that may subsequently enter the dry zone area.

Dry mix ingredients. Ingredients necessary to formulate products such as PIF, for example, are not always combined in the wet phase and subjected to a heat treatment that would result in significant reduction of pathogens potentially present. The reason for this is often the heat sensitivity of the ingredient. Other reasons may be technological; e.g., some ingredients such as fat mixes are added during the wet phase just after the critical heat treatment, whereas others are added in the dry phase by dry mixing with a base powder. Dry mix ingredients are added and mixed without any further heat treatment that might result in a significant reduction of pathogens potentially present. Consequently, to ensure that products meet the very strict end product specifications, the base powder and dry mix ingredients should be produced under strictly controlled conditions.

VERIFICATION

There are several definitions of the term "verification" (44, 79), but in the context of food safety risk management they all refer to activities that aim at obtaining evidence that control measures have been correctly implemented and that the resulting product meets predefined safety criteria. Testing end products does not give sufficient guarantee that they will be safe for the consumer. Even the strictest specifications for testing, e.g., the specification used for PIF (absence of *Salmonella* in 60 samples of 25 g), have a certain percent chance of accepting contaminated lots (30% in case of a contamination level of 2% of the units). Less frequent sampling will only increase the chance of accepting contaminated lots.

Implementation of effective control measures for lowa_w foods can be verified at the production plant level by regular inspection of control charts, reviewing records to identify potential trends, and checking equipment for functionality and calibrations, as well as by internal and external plant audits. Verification of the safety of finished products is accomplished by microbiological analysis, in combination with monitoring consumer complaints. For low-a_w foods and dry food processing environments, there are some particular constraints that should be addressed in order to establish an efficient approach to verification. **Distribution of microorganisms in low-a**_w foods. Heterogeneous distribution of pathogens is of concern for many types of foods (72, 78, 87, 139); but it is exacerbated in low-a_w foods, rendering testing of finished products an even less reliable tool to verify safety because it may only serve to identify highly contaminated lots. Thorough mixing of samples before taking an aliquot for analysis or the application of continuous autosamplers will minimize but not eliminate the nonhomogeneous distribution of pathogens (44). To enhance the probability of identifying lots with low contamination levels, testing more samples and/or increasing the sample size should be considered. However, the associated increased analytical cost may be disproportional to the benefit.

Environmental monitoring. Verification of the effectiveness of zoning in plants that produce low-aw foods can be achieved by observations obtained by environmental monitoring. In order to make the program more effective, critical sampling sites and the pathogen(s) of concern must be identified. This requires detailed knowledge of the product and process, as well as detailed zoning in the factory, before establishing a meaningful sampling plan. Depending on the type of production, sampling can be focused on basic hygiene and medium hygiene zones where pathogens would most likely be detected when first entering the facility, thus enhancing proactive surveillance. Sampling in the high hygiene zone confirms the relevance of the monitoring program, but it is not the area to focus the investigation when the environmental pressure is limited to less critical areas. If a pathogen is found in multiple locations, molecular typing methods are recommended to determine the level of relatedness. This information may be crucial to elucidate the contamination route(s). Several studies (47, 108, 119, 120) have described how this approach has been successfully applied to trace Cronobacter in PIF plants.

Indicator microorganisms. It may be preferable to focus analysis on detecting indicators of the potential presence of pathogens rather than on the presence of pathogens, because it does not require specialized biosafety laboratories and methods for enumeration are usually more robust and less expensive. However, it is not always possible to accept the suitability of indicators for pathogens that are relevant for low-aw foods. It has been reported that testing for Enterobacteriaceae is not reliable to assess whether infant formula is likely to be free of Salmonella (61), for example, unless a sampling plan with the same stringency is applied and only if the method for detecting Enterobacteriaceae is equally or more sensitive than the method for detecting Salmonella. However, applying a sampling plan with the same stringency for Enterobacteriaceae as for Salmonella may not be a realistic option, because this could lead to the unjustified rejection of lots due to the presence of innocuous members of the Enterobacteriaceae. In addition, results of monitoring Enterobacteriaceae in wet and basic hygiene zones may not be properly interpreted (46). Nevertheless, using

Enterobacteriaceae as an indicator of good hygienic practices can be of value for $low-a_w$ food processing operations, including infant formula manufacturing.

Raw materials. Not all dry mixing operations are followed by a process that is aimed at reducing the presence of pathogens, which implies that the microbiological safety of such products depends directly on the quality of the ingredients. This is often reflected in very strict microbiological specifications for such ingredients. However, microbiological analysis of ingredients to verify compliance with specifications suffers from the same drawback as testing of finished products: it only serves to detect lots with relatively high levels of contamination. Therefore, it is recommended that resources be dedicated to supplier audits, in particular for ingredients that are considered to represent the highest safety risk.

Analytical methods. Because target microorganisms are likely to be stressed due to processing (e.g., drying and/ or heating), special measures such as the inclusion of a soaking step in the protocol to avoid abrupt rehydration are necessary for recovery of such cells (167). It may also be necessary to sample larger quantities of product in order to detect the target pathogen. For example, in an outbreak of salmonellosis associated with PIF, *Salmonella* Ealing was determined to be present at a level of 2 CFU/450-g packet of product (135). Due to the large variability in the lag phase of desiccation-stressed cells, an overnight incubation in preenrichment broth may not have been sufficient for resuscitation (156). Extending the incubation time, on the other hand, may lead to inactivation of the target microorganism, e.g., due to acid production by background microbiota.

Focusing verification activities on monitoring pathogens in the environment represents an analytical challenge, because environmental samples often contain large numbers of competitors that can mask the presence of the target microorganism. Nevertheless, standard methods such as International Organization for Standardization (ISO) 6579 (*Salmonella*) and ISO/TS 22964 (*Cronobacter*) for detection of pathogens specifically cover environmental samples. Likewise, the ISO procedure for validation of alternative analytical methods (ISO 16140) is an option if the source using the method claims that it can be used for this purpose.

CONCLUSIONS

Some foodborne pathogens can survive for long periods in low- a_w foods, food ingredients, and environments in which they are produced and stored, and in some cases at doses that can cause infections. Many microorganisms, including foodborne pathogens, can resist drying processes. If low- a_w foods are rehydrated before consumption, there is a possibility that bacterial pathogens will grow if products are subsequently stored under inappropriate conditions, potentially resulting in populations sufficient to cause infection or intoxication.

Characteristics unique to low- a_w foods can cause problems when testing for the presence of pathogens. Pathogens cannot be assumed to be homogeneously distributed in dry foods, and during detection and/or enumeration they may be outcompeted by nonpathogenic species. As a result, end-product testing is of limited value for verification of the microbiological safety of dry foods and should be complemented by environmental monitoring and audits, including supplier audits.

Possible ways consumers use low- a_w foods, which may include not cooking them or rehydration followed by prolonged storage at ambient temperature, should be taken into account when establishing microbiological criteria. The manufacturer cannot expect consumers to always use products in the intended way. Therefore, when setting microbiological criteria, foreseeable use, as well as abuse, should be considered.

Microorganisms, either pathogenic or spoilage, can enter products via raw materials or by contamination during or after the manufacturing process. These routes of entry must be effectively prevented. To be able to do so, detailed knowledge of the raw materials and their inherent safety risks and of possible sources and routes of contamination of low- a_w foods is necessary. This information can be used to identify critical control points. Control measures should be sufficiently robust to manage unusual and unexpected risks. The end product must be safe, even if the consumer does not completely follow the instructions on the label or generally accepted kitchen practices.

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