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Assessment of Hygienic Quality of Beverage Cans Surfaces with and without **Protective Cover**

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ARTICLEINFO	ABSTRACT
Research Article	In this study, the microbial profiles of external top surfaces of beverage cans sold in Izmir markets, and the survival of <i>E. coli</i> on the top surfaces of cans were investigated through the storage period of 0, 10, 20 and 30 days at 4 and 25°C. Total aerobic mesophilic bacteria (TAMB),
Received: 10/06/2018 Accepted: 22/11/0000	coliforms and <i>E.coli</i> counts of 100 cans without protective cover and 20 cans with protective cover were examined. The numbers of TAMB on the cans without protective cover were in the range of 0.43-2.20×10 ³ cfu/cm ² . Lower bacteria counts in the range of <0.43-1.12×10 ¹ cfu/cm ² were observed on the cans with protective cover. <i>E. coli</i> was not detectable on the storage time of 20 days, whereas <i>E. coli</i> counts of higher than 2 log units were observed at the 10 days of storage.
Keywords: Beverage cans Hygienic status Escherichia coli Total aerobic mesophilic bacteria count Coliform	According to the results obtained from this study, the microbiological statuses of beverage cans are not the same quality, external top surfaces of beverage cans might be a potential risk for public health.

Türk Tarım - Gıda Bilim ve Teknoloji Dergisi 7(1): 61-67, 2019

Üzeri Koruyucu Ambalaj ile Kaplanmış ve Kaplanmamış Teneke Kutu İçecek Üst Yüzeylerinin Hijyenik Kalitesinin Değerlendirilmesi

MAKALE BİLGİSİ	ÖZ
Araştırma Makalesi Geliş : 10/06/2018 Kabul : 22/11/0000	Bu çalışmada, İzmir piyasasında satılmakta olan teneke kutu içeceklerin üst yüzeylerinin mikrobiyal profilleri belirlenmiş, 4 ve 25°C'de 0, 10, 20 ve 30 günlük periyotta teneke kutuların üst yüzeyinde <i>E.coli</i> ' nin canlılığı incelenmiştir. 100 adet üst yüzeyi ambalajsız ve 20 adet üst yüzeyi ambalajlı teneke içecek kutusunun toplam aerobik mezofilik bakteri (TAMB) sayısı, koliform ve <i>E.coli</i> sayıları belirlenmiştir. TAMB sayısı üst yüzeyi ambalajsız teneke kutularda (2.4.2.2.20) 103 kelekeri miki kutularda (2.4.2.2.2.20) 1
Anahtar Kelimeler: Teneke kutulu içecek Hijyenik durum Escherichia coli Toplam aerobik mezofilik bakteri Koliform	0,43-2,20×10³ kob/cm² aralığında saptanmıştır. Üst yüzeyi ambalajlı teneke kutulardaki bakteri sayısı <0,43-1,12×10¹ kob/cm²'dir. <i>E.coli</i> 10 günlük depolama süresinde 2 log'un üstünde olmasına rağmen, depolamanın 20. gününde <i>E.coli</i> saptanmamıştır. Çalışmada elde edilen sonuçlara göre, içecek kutularının mikrobiyolojik durumları aynı kalitede değildir, içecek kutularının dış üst yüzeyleri halk sağlığı için potansiyel risk olabilmektedir.

Introduction

Microbial attachment to surfaces is a potential way of transmission of pathogens in food processing industry, catering and the domestic environment (Kusumaningrum et al., 2003; Giauris and Nychas, 2006; Simoes et al., 2010; Kuda et al., 2015). Contaminations can be an intermediate step in transmission of pathogens from their original habitat in the environment to food contact surfaces (Reij et al., 2004; Silva and Martinis, 2013; Valero et al., 2017). Exposure of pathogens on surfaces may take place either by direct contact with contaminated objects or indirectly through airborne particles. Several studies indicated that various bacteria, including Escherichia coli, Staphylococcus aureus and Salmonella spp. survive on hands, sponges/cloths and utensils for hours or days after initial contact with the microorganisms (Scott and Bloomfield, 1990; Jiang and Doyle, 1999; Kusumaningrum et al., 2002; Kusumaningrum et al., 2003). Food stored under the sink or in locations where water, drain or heating pipes may pass; run an increased risk of pest invasion (Daniels, 1991; Michaels et al., 2003). Insects and rodents are common to these spaces, as they are difficult to seal and often contain moisture that is attractive to pests (Michaels et al., 2003). Cockroaches are known to carry Salmonella spp. and it is believed that they may represent reservoirs capable of spreading this organism to food products (Devi and Murray, 1991: Michaels et al., 2003). Dust on canned goods could also represent a potential infection hazard, as Salmonella spp. have been found viable in dust for up to 10 months (Michaels et al., 2003). Even though the cans are produced under hygienic conditions, they are exposed to some bacteria and germs during storage period, transportation and service. The risk of beverage cans to come in contact with rats, bugs, and other germs where they are stored is high (Ayçiçek and Küçükkaraarslan, 2003). Dirt accumulation of long-term storage, various abuse conditions described above, or even dripping of meat juices, is known to occur. Studies have reported that when food poisoning agents such as Salmonella Typhimurium and Staphylococcus aureus were dried and adhered onto stainless steel or glass surfaces in existence of nutrient rich food residue such as milk, meat and egg, they showed resistance to desiccation, surfactant disinfectant such as benzalkonium chloride, as well as 254-nm ultraviolet UV-C irradiation (Kuda et al., 2008; Kuda et al., 2011, Kuda et al., 2012; Li et al., 2014). This indicated that protein-, lipid-, and/or carbohydrate-rich food residue could preserve pathogens from stress conditions (Kuda et al., 2015). Because of these reasons, The Food and Drug Administration recommends washing all dirty canned goods with soap and water before opening. Even though the risks of having dirty beverage cans are quite different from food cans, there is a general abhorrence to drinking out of a can with a visibly dirty top, many people use a perfunctory to wipe of cans with paper products rather than rinsing or washing with soap and water (CFP 2000; US Navy, 2003; Michaels et al., 2003).

Chemical, physical and biological agents transmitted by food cause more than 200 recognized diseases in people (Kass and Riemann, 2006). Of these infectious biological agents are the most important causing the majority of foodborne diseases. Many of the infectious agents capable of causing foodborne diseases can be transmitted in ways other than via food or water. Agents transmitted by the fecal-oral route can cause infection through direct contact between hosts (Kass and Riemann, 2006). It was reported that, microorganisms present on the surface of beverage cans, which usually do not cause diseases directly, but might be opportunistic pathogens located in the tab area and contaminate the drink when the tab is opened, may cause serious diseases such as respiratory and urinary tract infections, and tuberculosis.

The aim of this study was to determine the contamination levels of external top surfaces of beverage cans with and without protective cover that are sold in the places such as supermarkets, grocery stores, restaurants and cafes; and also to investigate the survival of *Escherichia coli* inoculated to external top surfaces of beverage cans for 30 days.

Materials and Methods

Sample Collection

Commercially available beverage cans without any protective cover including ice tea (20), soda (20), beer (20), fruit juice (20), coke (20); and 20 cans (fruit juice and beer) with protective cover were randomly purchased, regardless of the brand and company names from supermarkets (41), grocery stores (38) and restaurants (21); 20 cans with protective cover in Izmir. Samples were taken the dates between 15.09.2015-15.02.2016 and analyzed within 24 hours after they were purchased.

Enumeration of Total Aerobic Mesophilic Bacteria, Coliforms and E.coli

Total aerobic mesophilic bacteria, coliform and E.coli counts were performed to determine the contamination levels of beverage cans. Swab samples were taken from the external top surfaces of beverage cans. Firstly, sterile swabs were wet with 0.1% sterile peptone water (PW, pH 7-7.4, Merck) in the test tube by immersion and excess fluid on the swab was leaked by repression of cotton swab on the inner surface of the tube. After getting the samples from the external top surface of can by using the sterile swab, it was immersed in 10 ml of sterile peptone water in the test tube and stirred about 30 seconds. Decimal dilutions were prepared and plated to Plate Count Agar (PCA, pH 6.8-7.2, Oxoid) plates. Inoculated petri dishes for total aerobic mesophilic bacteria count were incubated aerobically at 35±1°C for 48 hours (BAM, 2001). At the end of the incubation period, the colonies were counted and total viable counts were calculated as cfu/cm².

For total coliform bacteria count, 1 ml inoculum was taken from appropriate dilutions applied to 3 set tubes which contain Durham tubes and 10 ml Lauryl Sulphate Tryptose Broth (LSTB, pH 6.6-7.0, LabM). These tubes were incubated at 35±1°C for 24-48 hours. After the incubation period, gas positive tubes were determined and the possible coliform bacteria count was determined. In order to prove the results of presumptive test, inoculation was applied by using sterile loop from all gas positive

tubes to Brilliant Green Bile Broth (BGBB, pH 7.2-7.6, Oxoid) containing Durham tubes. At the end of the incubation period at 35±1°C for 24-48 hours, gas positive tubes were determined and confirmed coliform bacteria count was calculated by using MPN table (BAM, 2002). For the determination of fecal coliforms and E.coli, a loopful of inoculum from gas positive tubes of LSTB was transferred to a tube of EC broth (EC, pH 6.9±0.2, Oxoid). EC tubes were incubated at 44.5° C for 48 ± 2 h and examined for gas production to calculate fecal coliform MPN. To continue with E. coli analysis, gas producing tubes of EC broth was streaked on a Eosin Methylene Blue Agar (EMB, pH 6.8±0.2, Oxoid) plate and incubated for 18-24 h at 35°C. Plates were examined for suspicious E. coli colonies and used for further testing (BAM, 2002).

Survival of Escherichia coli on Beverage Cans

Escherichia coli DSM 1103 culture was used as test culture. Stock cultures were grown in Tryptone Soya Broth (TSB, pH 7.3, Oxoid) and incubated at 37°C for 24 h. Appropriate dilutions were prepared from this culture, the dilutions of about 6.0-6.5 log cfu/ml were prepared and the concentration applied was confirmed by using McFarland device and also by plating 0.1 ml of appropriately diluted culture on Plate Count Agar (PCA, pH 6.8-7.2, Oxoid).

Before the inoculation of *E. coli* to the external top surfaces of cans, 21 beverage cans were firstly washed with water and then top surfaces were disinfected with 70% and 96% alcohol for 15 minutes, respectively. Bacterial suspension of 0.1 ml (about 6.0-6.5 log cfu/ml) was spread on the disinfected surface of 21 beverage cans.

To determine the numbers of *E. coli* inoculated to the surface, 3 cans were analyzed just after the inoculation. The contaminated cans were incubated at 4°C and 25°C for 10, 20 and 30 days. *E. coli* inoculation was applied on the top surfaces of 3 beverage cans for each time and temperature, and numbers of *E. coli* were determined after the incubation periods as described above.

Statistical Analysis

Statistical analysis were carried out using IBM SPSS Statistics 20. Kruskal Wallis Test was used to assess differences among beverage cans purchased from supermarkets, grocery stores, restaurants while K-Independent Sample *t*-test was used to assess differences among beverage cans with and without protective cover. Differences were considered significant at P<0.05.

Results and Discussion

Total mesophilic aerobic bacteria count of external top surfaces of 100 beverage can samples without protective cover and total coliform bacteria count of 50 samples of these cans were analyzed to determine microbial profiles of external top surfaces of beverage cans sold in Izmir markets. The results of the analysis for the ice tea, soda, beer, fruit juice and coke samples without any protective cover taken from different places and different brands were given in Figures 1, 2, 3, 4 and 5, respectively. Considering the results of enumeration of total mesophilic aerobic bacteria of beverage cans without protective cover, it was observed that all the products were contaminated to varying degrees in the range of 0.43 cfu/cm² and 2.2×10³ cfu/cm².

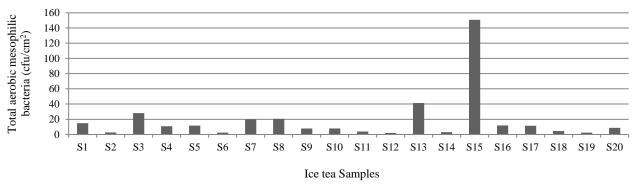


Figure 1 Total aerobic mesophilic bacteria counts of ice tea can top surfaces (cfu/cm²)

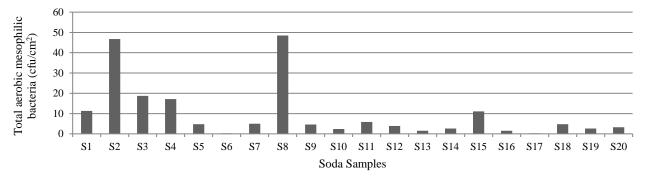


Figure 2 Total erobic mesophilic bacteria counts of soda can top surfaces (cfu/cm²)

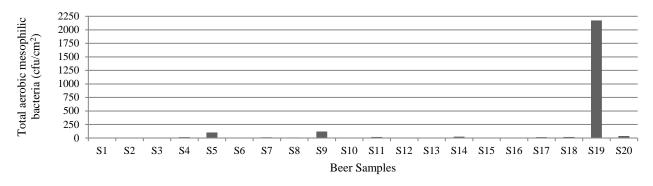


Figure 3 Total aerobic mesophilic bacteria counts of beer can top surfaces (cfu/cm²)

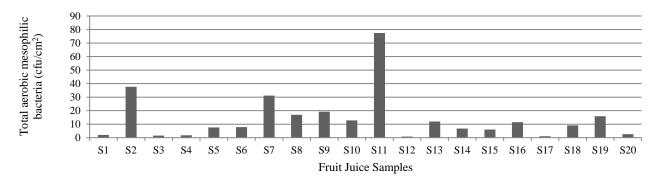


Figure 4 Total aerobic mesophilic bacteria counts of fruit juice can top surfaces (cfu/cm²)

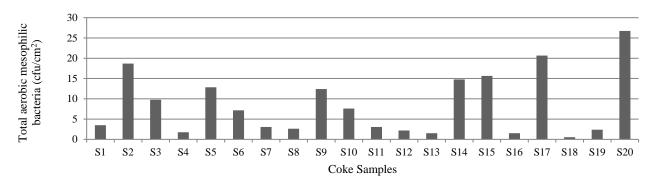


Figure 5 Total erobic mesophilic bacteria counts of coke can top surfaces (cfu/cm²)

There are no microbiological criteria for the external surfaces of beverage cans or the surfaces with direct contact with the mouth. Dantas et al. (2006), used an acceptable level of contamination lower than 50 cfu/cm² for total mesophilic aerobic microorganisms for hygienic cooking utensils. In another study, it is recommended that the satisfactory level of aerobic colony count is <10 cfu/cm², while 10 cfu/cm² or higher levels are considered as unsatisfactory level according to the US Public Health Service for swab from ready to eat food contact surfaces (Little and Sagoo, 2009). In the present study, microbiological results were compared with the criteria used by the US Public Health Service for swab from ready to eat food contact surfaces (Little and Sagoo, 2009) and also with the criteria suggested by Dantas et al. (2006). In our study, a total of 100 beverage cans without protective cover were analyzed and 56 out of 100 cans were in satisfactory hygienic condition, containing microorganisms below 10 cfu/cm² as specified in Little and Sagoo (2009). When the results are compared with the criteria suggested by Dantas et al. (2006), 95 out of 100 cans were in satisfactory hygienic condition. Average numbers of total mesophilic aerobic bacteria on the ice tea, soda, beer, fruit juice, coke samples and samples with protective cover as well as the percentages of samples with the satisfactory hygienic conditions were given in Table 1. Three of the beer and one of the ice tea samples were heavily contaminated with the numbers higher than 100 cfu/cm².

Aerobic mesophilic bacteria counts of beverage cans obtained from supermarkets, grocery stores and restaurants were not significantly different (P>0.05) and the percentage of samples containing unacceptable levels of TAMB according to Little and Sagoo (2009) were 49%, 40% and 43% among the samples tested, respectively (Table 2). Detecting a high percentage of microorganisms indicates that environmental contamination (water, earth, air etc.) is in significant

sizes. It was thought that the reason for microbial contamination on the top surfaces of beverage cans could be at storages, the transportation of beverage cans and intensity on enter and exit activities of goods and customers in market. Dantas et al. (2006) investigated the contamination levels of beverage cans and indicated that 83 out of 96 (86.5 %) of the cans analyzed presented total counts of mesophilic aerobic microorganisms lower than 50 cfu/cm². In a study by Michaels et al., (2003), aerobic and anaerobic bacteria, Bacillus spp., Clostridium perfringens, coagulase-negative Staphylococcus and mold counts were investigated. Escherichia coli, Salmonella spp., coagulase-positive Staphylococcus and yeast counts were recorded as non-detectable level (< 20 cfu/can). The average number of microorganisms listed for food and beverage cans revealed high contamination levels of 5.30 log cfu/can top surface area for bacterial counts and 5.84 cfu/ can top surface area mold spore counts. Major importance in these findings (on food and beverage cans) was the identification of Bacillus cereus and Clostridium perfringens, organisms very capable of causing foodborne illness was reported. Dantas et al., (2006) also observed that Clostridium perfringens were detected in 3 of 20 cans analyzed. Ayçiçek and Küçükkaraarslan, (2003) reported that they were obtained 127 isolates from beverage cans. The dominant microflora was *Bacillus* spp. (68 isolates); molds (21 isolates) and E. coli (10 isolates) were also isolated.

Total aerobic mesophilic bacteria count of beverage cans with protective cover are given in Figure 6. The numbers were between <0.43-1.12×10¹ cfu/cm². It was observed that 25 % of cans with protective cover have no growth and 95 % of the samples were in satisfactory hygienic condition according to Little and Sagoo (2009). Only one of them was slightly higher than the recommended limit which is 11 cfu/cm². In our study, the microbial load on all beverage cans without protective cover was detected in each of them. There is a statistically significant difference between microbiological profiles of beverage cans with and without protective cover was obtained (P<0.05). In the conducted analysis with beverage cans with protective cover, it was observed that microbial contamination is reduced to minimum levels. Effectiveness and necessity of using protective cover on the top surface beverage cans was proved. Different solutions are being developed by various domestic and foreign companies for the prevention of contaminations. Both producers and consumers who realize the dangers that would arise from the top surface of beverage cans offer solutions and demand. Beverages in cans are consumed directly from the cans and, in this case, the can surface enters into direct contact with the consumer's mouth. As the infectious dose can be as low as 10 microorganisms for some of the pathogens, this contact may give damage to health.

Table 1 Total aerobic mesophilic bacteria count of beverage cans with and without protective cover and percentages of cans with satisfactory hygienic levels

Beverage types	Total aerobic mesophilic bacteria count (cfu/cm²)		Percentages of cans with satisfactory levels of TAMB according to;	
	Range	Average	Little and Sagoo, (2009)	Dantas et al. (2006)
Icetea	1.96-150.87a	18.34 (32.72)	50	95
Soda	0.43-48.48 ^a	9.84 (13.92)	70	100
Beer	2.39-2173.91 ^a	130.14 (472.19)	50	85
Fruit Juice	0.87-77.39 ^a	14.08 (17.84)	55	95
Coke	0.52-26.74 ^a	8.42 (7.56)	65	100
Samples with protective cover	<0.43-11.18 ^b	2.22 (2.89)	95	100

Values on the same column followed by the same letter are not significantly different (P>0.05). Standart deviations are given in parenthesis.

Table 2 Total aerobic mesophilic bacteria counts of beverage cans obtained from three different places

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Beverage types	Average numbers of total aerobic mesophilic bacteria on beverage can surfaces (cfu/cm²)			
	Supermarket	Grocery stores	Restaurants	
Icetea	20.84 (36.17) ^a	8.68 (0) ^a	10.22 (10.22) ^a	
Soda	8.69 (6.84) ^a	2.91 (3.30) ^a	16.10 (21.66) ^a	
Beer	436.39 (946.51) ^a	25.25 (36.44) ^a	-	
Fruit Juice	6.52 (5.56) ^a	15.10 (20.48) ^a	16.23 (15.81) ^a	
Coke	7.45 (6.81) ^a	1.73 (7.08) ^a	11.29 (8.75) ^a	

Values on the same row followed by the same letter are not significantly different (p > 0.05). Standart deviations are given in parenthesis.

Table 3 Survival of *E.coli* on beverage can surfaces

Storage Time (day)	The numbers of <i>E.coli</i> on beverage can (log cfu/cm ²)		
	4°C	25°C	
0	3.91 (0.25)	3.91 (0.25)	
10	0.92 (0.27)	2.07 (0.60)	
20	_*	_*	
30	_*	_*	

^{*}Results are below the detection limit of 0.43 cfu/cm².

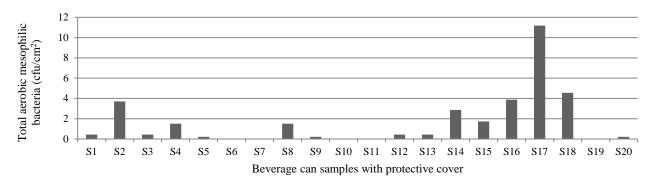


Figure 6 Total aerobic mesophilic bacteria counts of can top surfaces with protective cover (cfu/cm²)

After filling operation of beverage cans, the protection of packaging should be considered during transportation to points of sale to prevent contamination on the top surfaces of beverage cans. When beverage cans removed from their shrink packaging at the points of sale, the contact between outer surface of packaging with the top surfaces of beverage cans should not be allowed. Beverage cans should be placed on clean shelves with clean hands (Ayçiçek and Küçükkaraarslan, 2003). Top surfaces of beverage cans at shelves could be exposed to contamination through ambient air, consumers, dirty shelves, staff and insects. For this reason, at the places like market, grocery and restaurant, the important care measures must be taken on environment and personal hygiene and to struggle with pests and rodents (Ayçiçek and Küçükkaraarslan, 2003). In the present study, mold growth was observed on the external top surfaces of can at the refrigerator on the shelf during the collection of samples for two different places. Although, it is not very common to come across such visibly moldy cans, at rare circumstances, it is still possible to see them on shelves. Another dimension of the issue is, the staff could detect and rub the surface and make the moldy surface imperceptible to the customer, keeping it on the shelf for consumption and the customers could be at risk by consuming these products.

The presence of coliform was found just in one sample among 50 samples analyzed and total coliform bacteria count was determined as 0.17 MPN/cm² (4 MPN/can top surface area). Faecal coliform bacteria and E.coli was not detected on the analyzed samples. In the present study, the hygienic statuses of beverage cans were determined by using TAMB, coliform and E.coli count results as indicator microorganisms that widely used to assess the efficacy of sanitation programmes. Microbial indicators are a microorganism or group microorganisms that is indicative of the possible presence of pathogens and the detection and enumeration of indicator organisms and whose presence in given numbers points to inadequate processing for safety (Lues and Tonder, 2007).

Survival of *E. coli* inoculated on the top surface of cans at the 0th, 10th, 20th and 30th days of storage at 4°C and 25°C were given in the Table 3. *E. coli* counts were below the detection limit (0.43 log cfu/cm²) at 20 and 30 days of storage regardless of storage temperatures. It was detected that *E. coli* inoculated on the top surfaces of beverage cans survive at least 10 days, while the number

of *E. coli* was below the detection limit on 20th and 30th days of storage. Kusumaningrum et al. (2003) also suggested that pathogens remain viable on dry stainless steel surfaces and present a contamination hazard for considerable periods of time depending on the contamination levels and type of pathogen. If microorganisms remain on a given surface for a relatively long time, they can multiply and, eventually, form biofilms (Uhlich et al., 2006). Packaging materials supply a means to preserve, protect, market and distribute foods, on the other hand in this study demonstrated that the surface of beverage cans could be contaminated by the microorganisms.

Conclusions

For most of the people, microbial profile of top surfaces of beverage cans is very important because it is a product consumed by direct mouth contact with the top surface. As a result of analyses on the top surfaces of beverage cans sold as uncovered top surface, it was observed that there are serious contaminations. Since microorganisms can not seen with naked eyes while buying beverage cans, it is not possible to get an idea about their microbial profile. Even on top surfaces that appear very clean, extremely dangerous levels of contamination could be found. Considering all these situations and comparing the results of analysis of beverage cans sold as uncovered top surfaces and beverage cans which are covered with aluminium foil on the top surface, it was proved that covering top surfaces of beverage cans with a material is an applicable, useful and protective method.

According to the results obtained by the microbiological analysis of samples, it is concluded that the microbiological contamination levels of beverage cans are not the same and external surfaces of beverage cans might be potential risk for human health. As a result, the top surfaces of beverage cans pose a risk to health of consumers and some technologies used to protect the top surfaces of cans against external contamination is suggested to prevent contamination.

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