

## Effect of Packaging Containers on the Bacteriological Profile of Soft Cheese

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**Abstract:** The effect of packaging containers (Plastic and cardboard) on the bacteriological profile of Egyptian soft cheese was studied at plant level. Samples were taken during the line of packaging from 3 stages (Cheese just before packaging, packaging containers and cheese after packaging). All bacterial groups (Aerobic plate count "APC", Aerobic sporeformers, Coliform, *Staphylococci* and *Enterococci* counts) were high after packaging either in plastic or cardboard laminated containers. The APC increased from  $3.26 \times 10^4 \pm 0.46 \times 10^4$  to  $1.91 \times 10^5 \pm 0.33 \times 10^5$  cfu/g in cheese after packaging in plastic containers and from  $1.62 \times 10^4 \pm 0.27 \times 10^4$  to  $7.09 \times 10^4 \pm 2.16 \times 10^4$  cfu/g after cardboard packaging. There were significant ( $p < 0.05$ ) positive relationship between the level of soft cheese contamination (Especially with APC, Aerobic sporeformers and *Staphylococci*) as a result of their packaging in plastic containers (0.67, 0.58 and 0.72, respectively) and cardboard laminated sheets (0.60, 0.53 and 0.51, respectively). *Citrobacter*, *Enterobacter*, *Kliebsilla*, *Bacillus*, *Staphylococci* and *Enterococci* species were detected in the examined soft cheese samples packaged in plastic and cardboard containers and also from the containers themselves with different percentages. In conclusion, plastic containers were found to be an obvious source for contamination of Egyptian soft cheese with different types of microorganisms as comparing with cardboard laminated packaging.

**Key words:** Soft cheese • Plastic containers • Cardboard laminated sheets • Bacteriological profiles

### INTRODUCTION

The main purposes of packaging are to contain and to protect the food product from deterioration in quality resulting from the activities of microorganisms or pests or from chemically or physically induced changes [1,2]. Traditionally, the primary roles of packaging for food safety have been to withstand thermal processing conditions and to act as barrier to contamination, but modern food packaging can also influence the nutritional and quality attributes of foods [3]. Certainly, when the packaging fails to perform its protective functions the result is an unsafe product as when package components migrate to a food or when there is a loss of its integrity (Seal rupture or leakers) resulting in contamination by pathogenic microorganism [4].

The types of packaging material used depends on the processing methods, properties of the product and the final storage conditions [1]. The most common package types in soft cheeses is the plastic container as it is more economic, protect the product from dirt, moisture and contamination [2]. Cardboard is the next choice of

packaging material as it is excellent for storage easily folding into a three- dimensional rigid box and usually be laminated to paper to allow printing. However, the package design should attract the consumer, define the product with clear printing and bar codes efficiently sealed with application of automation of packaging equipment during filling, sealing, labeling with computer- aided manufacture "CAM" may be also used for design of the food package [5].

Cheese was previously classified under "safe foods" but after 1980, many reports of infections and intoxications related to the consumption of contaminated cheese which poses a threat to public health and also quality defects in cheese leads to big economic losses [6].

*Coliforms*, *Enterococci* and *Staphylococci* are essential indicator organisms for detection the microbial food quality and their shelf life or their safety [7].

The aim of the current study was to investigate the bacteriological status of Egyptian soft cheese packages (Plastic and cardboard laminated containers) as well as the bacteriological profile of soft cheese before and after packaging.

## MATERIALS AND METHODS

**Collection of Samples:** Ninety random samples of soft cheese (60 samples) and their containers (30 samples) were collected from dairy plants to determine the influence of the packaging containers on the bacteriological profile of soft cheese. Accurately, 30 soft cheese samples were collected just before and after plastic container packaging (15 samples each) as well as another 30 soft cheese samples were collected just before and after cardboard laminated packaging (15 samples each) from the line of their manufacture. Also, 30 samples of the plastic containers and the cardboard laminated sheets (15 of each) were included. All samples were delivered to the laboratory to be examined bacteriologically after preparation of samples.

### Preparation of Samples

**Cheese Samples:** Ten grams of soft cheese sample were placed in a sterile plastic bag then 90 ml of 2% sodium citrate solution ( $45^{\circ}\text{C} \pm 1$ ) were added. The mixture was homogenized for 2 minutes which equal  $10^{-1}$  dilution, then tenth fold serial dilutions were prepared using sterile fourth strength ringers solution [8].

**Packaging Containers Samples:** Each plastic container (500 g capacity) was rinsed with 20 ml of sterile buffer solution as described by Sharma [9]. Swab method was applied for examination of cardboard laminated sheets according to Hickey *et al.* [10].

### Bacteriological Examination

**Determination of Aerobic Plate Count "APC":** The technique recommended by APHA [11] was carried out using Standard Plate Count agar and incubation at  $35^{\circ}\text{C}$  for 48 hours.

**Determination of Aerobic Sporeformers Count:** It was performed by the method outlined by Feng *et al.* [12] using Dextrose Tryptone agar medium with incubation at  $37^{\circ}\text{C}$  for 24 hours.

**Determination of Total Coliform Count:** The count was estimated as most probable number (MPN) using MacConkey broth with incubation at  $35^{\circ}\text{C}$  for 48 hours according to APHA [11].

**Determination of Total *Staphylococci* Count:** The Procedures recommended by ICMSF [13] was followed using Baird Parker agar medium with incubation at  $37^{\circ}\text{C}$  for 24 hours for determination of total *Staphylococci* count.

**Determination of Total *Enterococci* Count:** Enterococcus selective agar plates were used for enumeration and isolation of *Enterococci* after incubation at  $37^{\circ}\text{C}$  for 24 hours [11].

**Identification of Isolated Bacteria:** Identification of isolated bacteria was done by the identification schemes developed by Bailey and Scott and Koneman *et al.* [14,15].

**Statistical Analysis:** The obtained results were statistically evaluated according to Rosner [16].

## RESULTS

The average bacterial counts in the examined soft cheese samples were increased from  $3.26 \times 10^4 \pm 0.46 \times 10^4$  to  $1.91 \times 10^5 \pm 0.33 \times 10^5$  cfu/g for APC,  $9.58 \times 10^3 \pm 2.10 \times 10^3$  to  $4.07 \times 10^4 \pm 0.81 \times 10^4$  cfu/g for Aerobic sporeformers count,  $2.79 \times 10^2 \pm 0.36 \times 10^2$  to  $3.12 \times 10^2 \pm 0.57 \times 10^2$  MPN/g for *Coliforms*,  $1.66 \times 10^3 \pm 0.29 \times 10^3$  to  $7.47 \times 10^3 \pm 1.61 \times 10^3$  cfu/g for *Staphylococci* and  $5.30 \times 10^2 \pm 0.87 \times 10^2$  to  $8.13 \times 10^2 \pm 2.05 \times 10^2$  cfu/g for *Enterococci*, after packaging in the plastic containers (Table 1).

The mean values of the previous bacterial counts were  $1.62 \times 10^4 \pm 0.27 \times 10^4$ ,  $6.97 \times 10^3 \pm 1.45 \times 10^3$ ,  $4.01 \times 10^2 \pm 0.76 \times 10^2$ ,  $9.85 \times 10^2 \pm 2.39 \times 10^2$  and  $4.72 \times 10^2 \pm 0.73 \times 10^2$  cfu/g for soft cheese samples before cardboard laminated packaging and  $7.09 \times 10^4 \pm 2.16 \times 10^4$ ,  $2.28 \times 10^4 \pm 0.24 \times 10^4$ ,  $4.52 \times 10^2 \pm 0.85 \times 10^2$ ,  $3.17 \times 10^3 \pm 1.09 \times 10^3$  and  $5.64 \times 10^2 \pm 0.94 \times 10^2$  cfu/g for soft cheese samples after packaging in cardboard laminated containers, respectively (Table 3).

The mean values of the examined bacterial counts from plastic containers and cardboard laminated sheets were shown in Table (5).

Results recorded in Tables 2,4 and 6 showed the incidence of the isolated bacteria from the examined soft cheese samples before and after packaging in both plastic and cardboard laminated containers and also from the packaging containers themselves.

Table 1: Statistical analytical results of bacterial counts in the examined samples of soft cheese before and after packaging in plastic containers

Bacterial counts (cfu/g)	Before packaging (n=15)			After packaging (n=15)			Propagation	Correlation%coefficient (r <sup>2</sup> )
	Min	Max	Mean ± S.E	Min	Max	Mean ± S.E		
Aerobic plate count (APC)	3.1 X 10 <sup>3</sup>	1.8 X 10 <sup>6</sup>	3.26 X 10 <sup>4</sup> ± 0.46 X 10 <sup>4</sup>	7.4 X 10 <sup>3</sup>	5.7 X 10 <sup>4</sup>	1.91X 10 <sup>5</sup> ± 0.33 X 10 <sup>5</sup>	82.93	+ 0.67**
Aerobic sporeformer count	5.5 X 10 <sup>2</sup>	7.3 X 10 <sup>4</sup>	9.58 X 10 <sup>3</sup> ± 2.10 X 10 <sup>3</sup>	1.6 X 10 <sup>3</sup>	2.9 X 10 <sup>2</sup>	4.07 X 10 <sup>4</sup> ± 0.8X 10 <sup>4</sup>	76.46	+ 0.58*
Coliform count (MPN/g)	4.0 X 10	1.1 X 10 <sup>3</sup>	2.79 X 10 <sup>2</sup> ± 0.36 X 10 <sup>2</sup>	5.0 X 10	1.6 X 10 <sup>3</sup>	3.12 X 10 <sup>2</sup> ± 0.57X 10 <sup>2</sup>	10.58	+ 0.13 <sup>NS</sup>
Staphylococci count	1.0 X 10 <sup>2</sup>	2.0 X 10 <sup>4</sup>	1.66 X 10 <sup>3</sup> ± 0.29 X 10 <sup>3</sup>	3.0 X 10 <sup>2</sup>	8.0 X 10 <sup>4</sup>	7.47 X 10 <sup>3</sup> ± 1.61 X 10 <sup>3</sup>	77.78	+ 0.72**
Enterococci count	2.0 X 10	3.2 X 10 <sup>3</sup>	5.30 X 10 <sup>2</sup> ± 0.87 X 10 <sup>2</sup>	4.0 X 10	6.7 X 10 <sup>3</sup>	8.13X 10 <sup>2</sup> ± 2.05 X 10 <sup>2</sup>	34.81	+ 0.20 <sup>NS</sup>

\*\* Highly significantly

\* Significant

NS : Non Significant

Table 2: Incidence of different bacteria isolated from the examined samples of soft cheese before and after their Packaging in plastic containers

Isolated bacteria	Before packaging		After packaging	
	NO	%*	NO	%*
<b>Coliforms:</b>				
Citrobacter diversus	1	6.67	1	6.67
Citrobacter freundii	5	33.33	7	46.67
Enterobacter cloacae	3	20.00	3	20.00
Enterobacter hafniae	-	-	2	13.33
Kliebsilla ozaenae	-	-	3	20.00
Kliebsilla pneumoniae.	7	46.67	7	46.67
<b>Bacillus species:</b>				
Bacillus cereus	6	40.00	6	40.00
Bacillus coagulans	3	20.00	3	20.00
Bacillus licheniformis	3	20.00	5	33.33
Bacillus megaterium	-	-	1	6.67
Bacillus subtilis	4	26.67	4	26.67
<b>Staphylococci:</b>				
Staphylococci aureus	1	6.67	3	20.00
Staphylococci epidermidis	3	20.00	4	26.67
Micrococci	7	46.67	9	60.00
<b>Enterococci:</b>				
Enterococcus durans	-	-	2	13.33
Enterococcus faecalis	5	33.33	6	40.00
Enterococcus faecium	2	13.33	4	26.67
Enterococcus viridans	1	6.67	1	6.67

\* % calculated to No. of positive samples ( n=15)

Table 3: Statistical analytical results of bacterial count in the examined samples of soft cheese before and after packaging in cardboard laminated sheets

Bacterial counts (cfu/g)	Before packaging (n=15)			After packaging (n=15)			Propagation%	Correlation coefficient (r <sup>2</sup> )
	Min	Max	Mean ± S.E	Min	Max	Mean ± S.E		
Aerobic plate count (APC)	9.6 X 10 <sup>2</sup>	5.1 X 10 <sup>5</sup>	1.62 X 10 <sup>4</sup> ± 0.27 X 10 <sup>4</sup>	4.6 X 10 <sup>3</sup>	1.5 X 10 <sup>6</sup>	7.09 X 10 <sup>4</sup> ± 2.16 X 10 <sup>4</sup>	77.15	+0.60*
Aerobic sporeformer count	3.5 X 10 <sup>2</sup>	4.2 X 10 <sup>4</sup>	6.97 X 10 <sup>3</sup> ± 1.45 X 10 <sup>3</sup>	8.9 X 10 <sup>2</sup>	2.1 X 10 <sup>2</sup>	2.28 X 10 <sup>4</sup> ± 0.24 X 10 <sup>4</sup>	69.42	+0.53*
Coliform count (MPN/g)	6.0 X 10	2.7 X 10 <sup>3</sup>	4.01 X 10 <sup>2</sup> ± 0.76 X 10 <sup>2</sup>	6.0 X 10	3.3 X 10 <sup>3</sup>	4.52 X 10 <sup>2</sup> ± 0.85 X 10 <sup>2</sup>	11.28	+0.10 <sup>NS</sup>
Staphylococci count	1.0 X 10 <sup>2</sup>	7.0 X 10 <sup>3</sup>	9.85 X 10 <sup>2</sup> ± 2.39 X 10 <sup>2</sup>	2.0 X 10 <sup>2</sup>	1.0 X 10 <sup>4</sup>	3.17 X 10 <sup>3</sup> ± 1.09 X 10 <sup>3</sup>	68.93	+ 0.51*
Enterococci count	1.0 X 10	1.9 X 10 <sup>3</sup>	4.72 X 10 <sup>2</sup> ± 0.73 X 10 <sup>2</sup>	4.0 X 10	2.8 X 10 <sup>3</sup>	5.64 X 10 <sup>2</sup> ± 0.94 X 10 <sup>2</sup>	16.31	+ 0.11 <sup>NS</sup>

\*\* Highly significantly

\* Significant

NS : Non Significant

Table 4: Incidence of different bacteria isolated from the examined samples of soft cheese before and after their packaging in the cardboard laminated sheets

Isolated bacteria	Before packaging		After packaging	
	NO	%*	NO	%*
Coliforms:				
Citrobacter freundii	6	40.00	6	40.00
Enterobacter aerogenes	2	13.33	3	20.00
Enterobacter cloacae	-	-	1	6.67
Kliebsilla azaenae	1	6.67	2	13.33
Kliebsill apneumoniae.	4	26.67	4	26.67
Bacillus species:				
Bacillus cereus	5	33.33	5	33.33
Bacillus circulans	1	6.67	1	6.67
Bacillus coagulans	1	6.67	2	13.33
Bacillus licheniformis	3	20.00	3	20.00
Bacillus subtilis	2	13.33	3	20.00
Staphylococci:				
Staphylococcus aureus	1	6.67	1	6.67
Staphylococcus epidermidis	5	33.33	6	40.00
Micrococci	6	40.00	6	40.00
Enterococci:				
Enterococcus durans	3	20.00	3	20.00
Enterococcus faecalis	2	13.33	2	13.33
Enterococcus faecium	4	26.67	4	26.67
Enterococcus mutans	1	6.67	2	13.33

\* % calculated to No. of positive samples ( n=15)

Table 6: Incidence of different bacteria in plastic containers and cardboard laminated sheets used for packaging of the examined soft cheese samples

Isolated bacteria	Plastic containers		Cardboard laminated sheets	
	NO	% *	NO	% *
Coliforms:				
Citrobacter freundii	2	13.33	-	-
Enterobacter cloacae	-	-	1	6.67
Enterobacter hafniae	2	13.33	-	-
Kliebsiella azaenae	3	20.00	2	13.33
Bacillus species:				
Bacillus licheniformis	2	13.33	-	-
Bacillus megaterium	1	6.67	-	-
Bacillus subtilis	-	-	2	13.33
Staphylococci:				
Staphylococci aureus	3	20.00	-	-
Staphylococci epidermidis	1	6.67	1	6.67
Micrococci	4	26.67	1	6.67
Enterococi:				
Enterococcus durans	2	13.33	-	-
Enterococcus faecium	3	20.00	-	-
Enterococcus mutans	-	-	1	6.67

\* % calculated to No. of positive samples ( n=15)

Table 5: Statistical analytical results of bacterial counts in the examined plastic containers (cfu/package) and cardboard laminated sheets (cfu/ cm<sup>2</sup>) used for packaging of soft cheese samples

Bacterial counts	Plastic containers*(n=15)			Cardboard laminated packages*(n=15)		
	Min	Max	Mean ± S.E	Min	Max	Mean ± S.E
Aerobic plate count (APC)	5.1 X 10 <sup>2</sup>	1.6 X 10 <sup>4</sup>	4.49 X 10 <sup>3</sup> ± 0.91X 10 <sup>3</sup>	2.4 X10 <sup>2</sup>	3.6 X 10 <sup>3</sup>	1.17 X 10 <sup>3</sup> ± 0.20 X10 <sup>3</sup>
Aerobic sporeformer count	3.0 X 10	4.8 X 10 <sup>2</sup>	1.83 X 10 <sup>2</sup> ± 0.35 X 10 <sup>2</sup>	1.0 X 10	9.0 X 10	4.40 X 10 ± 0.79 X10
Coliform count (MPN)	2.0 X 10	1.1 X 10 <sup>2</sup>	6.14 X 10 ± 1.27 X10	1.0 X 10	4.0 X 10	2.33 X 10 ± 0.42 X 10
Staphylococci count	1.0 X 10 <sup>2</sup>	8.0 X 10 <sup>2</sup>	3.92 X 10 <sup>2</sup> ± 0.74 x 10 <sup>2</sup>	1.0 X10 <sup>2</sup>	5.0 X 10 <sup>2</sup>	2.94 X 10 <sup>2</sup> ± 0.61 X 10 <sup>2</sup>
Enterococci count	1.0 X 10 <sup>2</sup>	3.0 X 10 <sup>2</sup>	2.33 x 10 <sup>2</sup> ± 0.56 x 10 <sup>2</sup>	1.0 X 10 <sup>2</sup>	1.0 X 10 <sup>2</sup>	1.33 X 10 <sup>2</sup> ± 0.25 X 10 <sup>2</sup>

\*Significant differences (P<0.05)

## DISCUSSION

The ideal packaging materials should be odorless, hygienic and inert; not react with food article; relatively inexpensive, easily assembled into suitable packages, easily filled and sealed, withstand rough handling during transportation and storage [1].

In the present study it is obvious that there were significant positive relationship between the level of soft cheese contamination (Especially with APC, Aerobic sporeformers and *Staphylococci*) as a result of their packaging in plastic containers (0.67, 0.58 and 0.72 , respectively) and cardboard laminated containers (0.60, 0.53 and 0.51, respectively). In contrast, *Coliforms* and *Enterococci* counts had non significant correlation with package containers of cheese.

High levels of aerobic microbial counts (10<sup>8</sup>- 10<sup>9</sup>cfu/g) of packaged cheese samples were reported by O'Mahony *et al.* [17]. However, nearly similar results were recorded by Temelli *et al.* [18] for *Staphylococci* in soft cheese.

The presence of high APC in cheese before packaging may referred to environmental contamination of milk used for cheese manufacture [19]. While the high count after packaging may be due to presence of microorganisms on surface of packaging materials or indirect due to permeability of the materials to contaminating bacteria [20].

The presence of Aerobic sporeformers may related to main two reasons, the survival of pasteurization temperature as they are heat- resistant organisms or heating may give a shock to spores that stimulates their germination [21].

Regarding *Coliforms*, the count in the packaged soft cheese exceeded the permissible limit (10 MPN/g) suggested by Egyptian standards [22], which gives an indication either on inefficiently pasteurized milk used or poor plant sanitation. It was reported that *Coliform* count of 10<sup>7</sup>/g in soft cheese can result in swelling of plastic cheese packages due to early gas blowing of cheese with production of H<sub>2</sub> and Co<sub>2</sub> [23]. The contaminating

microbial sources of milk may be directly from faecal matter of human or animal origin or indirectly from contaminated water sources, animal coat, milking equipment or from bulk holding tanks [24].

The high incidence of *Staphylococci* isolates indicated the possible inefficient application of hygiene practices by the workers [25,18]. Personnel hands was contributed to greatly increase of *Staphylococci* counts in Turkish white cheese as mentioned by Temelli *et al.* [18]. Therefore, good sanitation among workers and at level of plant with use of active starters and good packaging are important factors in preventing *Staphylococci*, especially *Staphylococcus aureus* which related to food poisoning [26,27].

For *Enterococcal* isolates, previous studies showed that *Enterococcus faecalis* was the most abundant bacterium from this genus that isolated from cheese [28 - 30], while other investigators [24,31,32] reported that *Enterococcus faecium* and *Enterococcus durans* were the principle isolated species from cheeses. Gelsomino *et al.* [33] mentioned that humans involved in cheese manufacturing and the cheese making equipment are the main sources of *Enterococci* contamination of cheeses.

The high microbial load possibly reflects the differences in milk quality used, survival of heat resistant microorganisms during cheese- making and post-processing microbial contamination [34].

Presence of Sporeforming bacteria, *Coliforms* and *Staphylococcus aureus* pathogens in cheese may reflect the using of raw milk with poor microbial quality or contamination during cheese manufacture, handling or packaging [23].

Higher APC, Aerobic sporeformers and *Staphylococci* counts were detected in plastic containers examined by Saudi *et al.* [35]. While, higher *Coliform* counts in the examined containers was recorded by Kebede *et al.* [36]. Motsert and Jooste [20] suggested that the permissible limit for the microbial count should not exceed 50 cfu per package of more than 100 ml capacity and should not exceed 1cfu/cm<sup>2</sup> of product contact surface in case of laminated cardboard sheet as well as

they should be free from *Coliforms*. In this respect, the uncoated paper stock, prior to lamination, should meet a microbiological standard of not more than 250 cfu/cm<sup>2</sup> and should be free from *Coliforms* [10,37].

The high incidence of isolated *Coliforms* is suggestive of the unsanitary conditions or practices either during processing or storage of the dairy products [38]. In this respect, *Coliforms* detection in dairy products are intended to reflect overall dairy plant sanitation [39].

The presence of *Staphylococci* isolates in the examined soft cheese before cardboard laminated packaging may result in staphylococcal poisoning successfully in soft cheese as a result of *Staphylococcus aureus* heat-stable enterotoxin.

*Enterococcus faecalis* and *Enterococcus faecium* are the main isolated Enterococcal strains from cheese [28,29,40]. Their presence indicated faecal contamination as they are natural constituents of the intestinal flora of nearly all animals and human and they are primarily the two classic Enterococcal species of faecal origin. So *Coliforms* and the classic *Enterococci* are used as main indicators of food safety [7].

Packaging materials are significant source of cheese contamination as microorganisms can grow rapidly on food surface, especially the dairy products and also during sealing of the packages [1]. This clearly describe the higher microbial load of soft cheese after packaging (Especially in plastic containers).

The isolated bacteria from plastic containers surface were *Citrobacter freundii*, *Enterobacter hafniae*, *Kliebsilla ozaenae*, *Bacillus licheniformis*, *Bacillus megaterium*, *Staphylococcus auerus*, *Staphylococcus epidermidis*, *Micrococci*, *Enterococcus durans* and *Enterococcus facium* at percentages of 13.33, 13.33, 20, 13.33, 6.67, 20, 6.67, 26.67, 13.33% and 20%, respectively .While, *Enterobacter cloacae*(6.67%), *Kliebsilla ozaenae* (13.33%), *Bacillus subtilis*(13.33%) ,*Staphylococcus epidermis* (6.67%), *Micrococci* (6.67%) and *Enterococcus mutans* (6.67%) were the only isolated bacterial strains from cardboard laminated sheets.

The contamination of packaging materials may referred to the unefficient sanitation and sterilization of the main lines of packaging of the dairy products including; plastic packages surface, filling machine, equipment used for sealing and forming the cartons [41]. So the packaging material used for heat-treated milk should, first of all, be free from pathogenic bacteria and also from other microorganisms that able to multiply in the milk or product under the prevailing conditions [42]. Thus controlling bacterial cross- contamination of cheese during packaging is an important safety issue.

Finally, the best way to produce good bacterial quality soft cheese with desirable organoleptic characteristics and to prolong its shelf life is the application of strict sanitary practices through out the manufacturing steps starting from raw ingredients used till the packaging of the cheese.

In conclusion, as packaging materials, especially plastic containers are significant sources of Egyptian soft cheese contamination with different types of microorganisms, the application of hygienic requirements on manufacturing of packaging materials, containers and closures should be carried out.

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