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BIOLOGICAL HAZARDS OF MARKETABLE AND HOME MADE SOBIA IN ASSIUT CITY

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ABSTRACT

60 random samples of both sobia sold at shops and home made one (each 30 samples) were collected from juice shops and homes for microbiological examination to detect its hygienic quality. The mean values of total aerobic plate count were 1.01x 10^5 and 6.9x10^3 in the examined samples of marketable sobia and home made one, respectively. 7.79x10^4 and 5.87x10^2 cfu/g were the average count of total yeast and molds count of the examined samples of sold sobia and home made one. While, *Listeria monocytogenes* incidence were 33.3 and 0 % in the sold sobia and home made one samples, respectively. In addition, *Pseudomonas spp*. was detected in percentage of 93.3 and 56.7% in the previously mentioned samples, respectively. True fecal type of *E. coli O157:H7* could not be isolated in any of examined sample while other types of *E. coli* could be isolated (*O128:H2*, *O111:H4*, *O126*, *O124*, and *O1:H7* and *O119:H6* in percentage of 10, 6.7, 6.7, 3.3, 3.3 and 3.3 % respectively in the examined samples of sold sobia). *Enterobacter agglomerans*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Enterobacter hafniae*, *Klebsiella pneumoniae* and *Citrobacter diversus* could be isolated and identified from examined samples of sold sobia and home made one.

Key words: *Biological Hazards, Marketable, Home Made Sobia.*

INTRODUCTION

The safety of street foods is affected by several factors starting from the quality of the raw materials, to food handling and storage practices. Street foods are exposed to appalling environmental conditions, such as the presence of insects, rodents, domestic animals/other animals and air pollution (Hanashiro *et al.,* 2005). Besides, most food vendors do not observe good food handling practices, exposing foods to dangerous conditions such as cross contamination, unsafe storage and poor time-temperature conditions (Ekanem, 1998).

Ready-to-eat foods and beverages are prepared and/or sold by vendors or hawkers especially in the streets and other similar places. Provided that the consumer is informed and able to choose the proper combination of foods (WHO, 2006).

Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. Because of the specific production it is impossible to avoid contamination of milk with micro-organisms therefore the microbial content of milk is a major feature in determining its quality (Rogelj, 2003).

Yeasts themselves are not commonly the cause of defect in dairy products unless they ferment lactose. In this case, they can grow rapidly and produce a characteristic yeasty or fruity flavor and obvious gas (Davis and Wilbey, 1990). They also produce metabolites, e.g. short-chain fatty acids and other compounds, with known toxic effects against undesired micro-organisms in the intestinal tract (Jakobsen and Narvhus, 1996).

The presence of wild types of moulds is undesirable as they may influence the organoleptic characteristics of the dairy products, they can produce mycotoxins and represent a potential health risk (Jodral *et al.*, 1993; Wouters *et al.,* 2002). Spoilage of dairy products by moulds is frequent and a matter of concern for human health. Yeasts and moulds in milk might act as allergen and an irritant to human health (Karthikeyan and Dhanalakshmi, 2010).

Psychrotrophic microorganisms represent a substantial percentage of the bacteria in raw milk, with *Pseudomonads spp*. and related aerobic, Gram-negative, rod-shaped bacteria being the predominant groups. Typically, 65–70% of the psychrotrophs isolated from raw milk are *Pseudomonas* species (García *et al.,* 1989; Griffiths *et al.,* 1987). Important characteristics of *Pseudomonads* are their abilities to grow at low temperatures (37°C) and to hydrolyze and use large molecules of proteins and lipids for growth. Psychrotrophs can grow at refrigeration temperatures below 7°C, produce enzymes toxins and other metabolites (Jay, 1996) and contribute to high standard plate counts in both raw and pasteurised milk.

P. aeruginosa has been recognized as an infectious agent transmitted by food and water (Morais *et al.,* 1997). This organism is an opportunistic pathogen affecting primarily immunocompromised people and those suffering from cystic fibrosis. For this reason, current legislation in several countries demands that bottled water products test free of *P. aeruginosa* (Morais *et al.,* 1997). The lack of robust identification tools for these organisms can lead to the misidentification of nonpathogenic *Pseudomonas spp*. as pathogenic species, potentially forcing costly and unnecessary food product recalls (Morais *et al.,* 1997). As *P. aeruginosa* has been isolated from milk (Thomas and Druce, 1969), and as the dairy industry is likely to face increased domestic and international demand for products free of bacterial contaminants (Franck, 1997), development of reliable tools to identify and track spoilage strains and pathogens will help the industry meet future product quality and safety challenges.

In dairy industry, many problems associated with *L.monocytogenes* contamination are related to post-pasteurization contamination. *L.monocytogenes* can survive for longer period at low temperatures and on process equipment, and the ability of bacteria to survive on the equipment used in production is often cause of the outbreaks described in the literature (Conly and Johnston, 2008).

Pasteurization of milk destroys *L. monocytogenes*. However, to which extent the *L. monocytogenes* is destroyed in milk during the process of pasteurization depends on the resistance of individual strains within the same species. Pasteurization of milk which occurs at the temperature of 62,8°C for 30 minutes and 71,7°C for 15 seconds is enough to destroy listeria present in the population of 10^2 cfu/ml, but not in the population of 10^7 cfu/ml (Jayamanne and Samarajeewa, 2010). According to research by Pearson and Marth (1990), high pasteurization inactivates *L. monocytogenes*, but the minimum survival of the bacteria is still possible.

E. coli is a good indicator of fecal pollution and its presence in milk products indicates the presence of enteropathogenic microorganisms which constitute a serious public health hazard (Chye *et al.,* 2004). Several outbreaks of *E. coli* 0157 have been reported in developed countries ranging from mild diarrhea to potentially fatal hemolytic uremic syndrome (HUS), hemorrhagic colitis, and thrombotic thrombocytopenic purpura (Coia *et al.,* 2001). Enteropathogenic *E. coli* can also cause severe diarrhea and vomiting in infants, and young children.
The objective of this work was to evaluate the level of microbiological contamination of sobia sold in shops and other one made at home.

MATERIALS and METHODS

A. Collection, preparation and serial dilutions of samples:

A total of sixty random samples of sold sobia (30 samples) and home made sobia (30 samples) were collected from different juice shops and houses, respectively in Assiut city, Egypt. These samples were still valid for consumption and collected directly after preparation in clean, dry and sterile containers then thoroughly mixed, transported to laboratory and kept at 4 °C and examined microbiologically directly. Eleven grams of the prepared samples were mixed with 99 ml of sterile 0.1 % peptone water and thoroughly mixed to give a dilution of 1/10, and then ten fold serial dilutions were carried out according to A.P.H.A. (1992).

B. Experimental techniques:

1) Enumeration of total bacterial count according to A.P.H.A. (1992) by using standard plate count agar.

2) Enumeration of total yeasts and molds count according to Harrigan and MacCance (1976) by using malt extract agar (containing 500 mg each of chlortetracycline and HCL chloramphenicol).

3) Isolation and identification of *E. coli O157:H7*:
For detection of *E.coli O157:H7*, trypticase soy broth was supplemented with cefixime (0.05 mg/l), cefsulodin (10 mg/l) and vancomycin (8 mg/l) for pre enrichment (37ºC). After the addition of the samples into the modified trypticase soy broth, shake-incubation was performed at 37ºC the enriched samples were plated onto sorbitol Mac-Conkey agar (SMCA) supplemented with 0.05 mg/l cefixime and potassium tellurite (2.50 mg/l) after 4 and 24 hours. Presumptive *E.coli O157:H7* colonies (indole positive) were confirmed serologically using antibodies to the O157 antigen (*E.coli O157:H7* latex test, Oxoid DR 260). According to these results, agglutination and indole positive colonies were recognised as *E.coli O157* (AOAC, 1998).

a) Serodiagnosis of *E.coli*:
The isolates were serologically identified according to Kok *et al.* (1996) by using rapid diagnostic *E.coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types.

b) Identification of negative results of E. coli by using biochemical tests according to (USFDA., 2002)

5) Isolation and identification of L. monocytogenes:- A 25 g portion of each sample was weighed aseptically into a sterile stomacher bag containing 225 ml of sterilized 1% (w/v) peptone water and macerated in a laboratory blender stomacher for 3 min (Peng and Shelef, 2000). A

selective medium: Listeria Selective Agar (LSA) (Oxoid, Hampshire, UK) containing Listeria selective supplement (Oxford modified) (Oxoid, Hampshire, UK) was used for the isolation, enrichment and plating of Listeria (Gulmez and Guven, 2003). Bacteriological analyses were performed, by plating in duplicates (a volume of 0.1 ml of each dilution on agar plates containing appropriate selective media) (Gulmez and Guven, 2003). All analyses were conducted under aseptic conditions. Plated cultures were then incubated at 35°C for 48 h (Gulmez and Guven, 2003). Colonies that exhibited the *L. monocytogenes* morphology were preserved for further analyses. All bacteriological analyses were done according to the Compendium of Methods for the Microbiological Examination of Foods (Downes and Ito, 2001 and Horwitz, 2001).

Biochemical identification of the suspected L. monocytogenes isolates:
Colonies appearing on LSA were first selected based on their morphology then identified by biochemical tests. Black to brown colonies surrounded by black halos were chosen (Hitchins, 1995 and Aygun and Pehlivanlar, 2006). Those colonies were Gram stained. Only Gram-positive short rods were further tested for their ability to produce acids from the fermentation of D-xylose and L-rhamnose sugars, and were also subjected to the β-haemolysis test (Cocolin *et al.,* 2002 and Zhou and Jiao, 2005)

6) Isolation and identification of *Pseudomonas spp*.:- One ml from the previously prepared samples was mixed with 9 ml of pseudomonas enriched broth. All enriched samples were incubated for 24-48 h at 37°C. Loopfull from enriched broth was streaked onto pseudomonas selective media. The agar plates were incubated for 24-48 h at 37°C and samples of no growth were incubated for another 48h (Collins *et al.,* 1996). The typical colonies (creamy, light, nearly always tending to expand) were examined by microscopy for morphology and Gram reaction. One colony from morphological type was picked per plate and those showing bacteria morphologically similar to the genus pseudomonas underwent to diagnostic tests according to Mead *et al.* (1977) and Meyer *et al.* (2002).

RESULTS

Table 1: Statistical analytical results of aerobic plate count and total yeasts and molds count of the examined samples.

Sample	Aerobic plate count					Total yeasts and molds				
	Positive samples		Count/g			Positive samples		Count/g		
	No.	%	Min.	Max.	Average	No.	%	Min.	Max.	Average
Sold sobia (No. :30)	28	93.3%	>100	$5.4x10^5$	$1.01x10^5$	24	80%	>100	$8.5x10^5$	$7.79x10^4$
Home made Sobia (No. :30)	15	50%	>100	$9X10^4$	$6.9x10^3$	6	20%	>100	$9x10^3$	$5.87x10^2$

No. : Number of examined samples

Table 2: Frequency distribution of the positive samples based on their aerobic plate count and total yeasts and molds count:

Count/g	Aerobic plate count				Total yeasts and molds count			
	Sold sobia		Home made sobia		Sold sobia		Home made sobia	
	No./28	%	No./ 15	%	No./24	%	No./6	%
10^2 - < 10^3	2	7.1%	0	0	1	4.2%	2	33.3%
10^3 - < 10^4	8	28.6%	10	66.7%	8	33.3%	4	66.7%
10^4 - < 10^5	10	35.7%	5	33.3%	10	41.7%	0	0
10^5 - < 10^6	8	28.6%	0	0	5	20.8%	0	0
Total	28	%100	15	%100	24	100	6	%100

Table 3: Incidence of some microorganisms could be isolated from the examined samples:

Microorganisms / Products	Sold sobia		Home made sobia	
	No./30	%	No./30	%
E.coli O157:H7	0	0	0	0
Other E. coli type	10	33.3%	0	0%
L. monocytogenes	10	33.3%	0	0
Pseudomonas spp.	28	93.3%	17	56.7%
Enterobacter cloacae	3	10%	0	0
Enterobacter agglomerans	1	3.3%	0	0
Enterobacter aerogenes	0	0	1	3.3%
Enterobacter hafniae	1	3.3%	0	0
Citrobacter diversus	0	0	1	3.3%
Klebsiella pneumoniae	1	3.3%	0	0

Table 4: Incidence of *E. coli* could be isolated from the examined samples of sobia:

Identified bacterium	Products				Strain characteristic
	Sold sobia		Home made sobia		
	No.	%	No.	%	
O128:H2	3	%10	0	0	ETEC
O111:H4	2	6.7%	0	0	EHEC
O126	2	6.7%	0	0	ETEC
O124	1	3.3%	0	0	EIEC
O1:H7	1	3.3%	0	0	EPEC
O119:H6	1	3.3%	0	0	EPEC
Total	10	33.3%	0	0	

DISCUSSION

The manufacture of Sobia is based on traditional method without any regard to the quality of raw material used and/ or the hygienic quality of the products. Under such conditions, many microorganisms can find access to the milk products (Soomro *et al.,* 2002) leading to a low shelf life of the base products. Most of these products are sold in the market without proper packaging and unduly exposing them to atmospheric contamination (Khan, 2006) and has become an important public health issue and a great concern to everybody. In developing countries, fruit juices, drinks, meals and snacks sold by street-food vendors are widely consumed by millions of people (Tambekar *et al.,* 2011).

The result in Table 1 recorded that the aerobic plate count of examined samples of marketable sobia and home made sobia ranged from 0 to $5.4x10^5$ with an average count $1.01x10^5$ and from 0 to $9X10^4$ with an average count $6.9x10^3$ in examined samples, respectively. The majority of examined samples of marketable sobia (35.7%) occurred between 10^4 - < 10^5 while the majority of examined samples of home made sobia 66.7% occurred between 10^3 - < 10^4 (Table 2). The result of this work showed that the total viable count of sobia shop samples obtained from the different sources were higher than that obtained from home made ones, this may be due to poor quality of ingredients used by juice shop which indicates serious faults in raw material selection, production hygiene, unsatisfactory sanitation and unsuitable storage temperature.

Table 1 revealed that the results of total yeasts and molds count/g of examined samples. It ranged from >100 to $8.5x10^5$ with an average of $7.79x10^4$ in marketable sobia and from to $9x10^3$ with an average of $5.87x10^2$ in home made sobia. It is found that 41.7 and 66.7% of positive samples of marketable sobia and home made sobia occurred between 10^4 - < 10^5 and 10^3 - < 10^4, respectively (Table 2). Spoilage of dairy products by moulds is of frequent occurrence in Egypt due to the prevailing tropical climate and high humidity especially in coastal area like Assiut. Since the mould spores are transmitted through air, they are ubiquitous in nature. Gran, 2002 concluded that the hygienic aspects of dairy products are linked with transportation, preservation and handling.

Table 3 showed that *E. coli O157:H7* could not be detected in any of the examined samples of marketable sobia and home made sobia, while other types of *E. coli* could be detected in 33.3 and 0 % of examined samples of marketable and home made sobia, respectively. Similarly, other studies reported that the traditional product with high incidence of *E. coli* is indicative of unsanitary conditions (Riadh, 2005) and also *E. coli* may indicate evidence of contamination or pollution especially of fecal nature. *E. coli* bacteria could be due to inadequate hand washing by food workers and the absence of good manufacturing practices (Tambekar *et al.,* 2011).

E. coli O157:H7 is a high risk pathogen of considerable public health significance because of its involvement with serious human illnesses including Hemorrhagic Colitis (HC), Hemolytic Uremic Syn-drome (HUS) and Thrombotic Thrombocytopenic Pur-pura (TPP), which have been reported with increasing frequency ever since this organism was first reported in 1982 (Ferens and Hovde, 2011; Jay *et al.,* 2007 and Wells *et al.,* 1983). Transmission of this pathogen occurs primarily in ground beef (Griffin and Tauxe, 1991; Savoye *et al.,* 2011). However, other foods including raw and pasteurized milk, yoghurt and cheese have also been epidemiologically implicated (Chapman *et al.,* 1993).

Other strains of *E. coli* could be isolated and identified with serodiagnosis were documented in Table 4 where *O128:H2, O111:H4, O126, O124, O1:H7* and *O119:H6* in percentages of 10, 6.7, 6.7, 3.3, 3.3 and 3.3 % respectively in the examined samples of marketable sobia. Riley *et al.* (1983) stated that enterohaemorrhagic *E. coli* is a new emerging pathogen causing two principle types of illness in human, Hemorrhagic Colitis (HC) and Hemolytic Uremic Syn-drome (HUS). It was firstly identified as a cause of human illness in 1982 when it was associated with two food related outbreaks of HC in the states of Oregon (26 cases) and Michigan (21 cases). Varnam and Evans (1991) subdivided the pathogenic strains of *E. coli* on the basic of clinical symptoms, mechanisms of pathogenesis, biochemical and serological markers into five groups: enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative, and enterohaemorrhagic (EHEC). While, Piercefield *et al.* (2010) stated that one of the common non-*O157* VTEC in the USA is O111:H8 and one of the largest outbreaks was caused by an EHEC O111 in the USA in 2008 causing 341 illnesses.

Table 3 showed that *Enterobacter cloacae, Enterobacter agglomerans, Enterobacter hafniae* and *Klebsiella pneumoniae* could be isolated and identified in percentages of 10, 3.3, 3.3 and 3.3 % in the examined samples of marketable sobia while *Enterobacter aerogenes* and *Citrobacter diversus* could be detected in 3.3 and 3.3 % of examined samples of home made samples respectively. *Enterobacter* spp., particularly *E. aerogenes* and *E. cloacae,* have been associated with nosocomial outbreaks, and are considered opportunistic pathogens. *Enterobacter spp*. can cause numerous infections, including cerebral abscess, pneumonia, meningitis, septicemia, and wound, urinary tract (particularly catheter-related UTI), and abdominal cavity/intestinal infections. In addition, *Enterobacter spp*. has been noted in intravascular device-related infections, and surgical site infections (primarily postoperative or related to devices such as biliary stents). Many species can cause extra-intestinal infections (Pagotto *et al.,* 2003 and Farmer *et al.,* 2007).

Regarding the results in Table 3, the incidence of *L. monocytogenes* isolated from marketable sobia was 33.3% while, *L. monocytogenes* failed to be detected in any of examined samples of home made sobia. Human listeriosis is associated with consumption of contami nated milk, soft cheese, undercooked meat, and unwashed raw vegetables and cabbage (Oliver *et al.,* 2005; Aygun and Pelivanlar, 2006 and Colak *et al.,* 2007). It may range from mild flu-like sickness to severe manifestations. Groups at highest risk are pregnant women, neonates, adults with underlying disease, elderly and immunocompromised individuals (McLauchlin *et al.,* 2004).

Table 3 indicates the incidences of *Pseudomonas spp.* isolated from marketable and home made sobia samples in which 93.3% of sold sobia and 56.7% of home made sobia samples were contaminated with *Pseudomonas spp*. *Pseudomonas spp.* also plays an important role in milk spoilage. During the storage of raw milk they produce many thermo-tolerant lipolytic and proteolytic enzymes that reduce both the quality and shelf life of processed milk (Wiedmann *et al.,* 2000; Dogan and Boor, 2003). *Pseudomonas* spp. are important bacterial contributors to spoilage of conventionally pasteurized fluid milk products (Shah, 1994). These psychrotolerant organisms contribute to milk spoilage in two different ways. First, they produce the majority of lipolytic and proteolytic enzymes secreted into raw milk during preprocessing storage. Many of these enzymes can survive pasteurization (72°C for 15 s) and even ultra-high-temperature treatments (138°C for 2 s or 149°C for 10 s) and can thus reduce the sensory quality and shelf life of processed fluid milk products (Lopez-Fandino *et al.,* 1993 and Shah, 1994). Second, post pasteurization contamination contributes most of the microorganisms, primarily (Schroder, 1984). Although most *Pseudomonas spp*. are not considered to be human pathogens, several species of this group are associated with human and animal infections (Foght *et al.,* 1996). The occurrence of *P. aeruginosa* might be due to improper personal hygiene,

unhygienic surroundings, vehicular transmission, and sewage.

It was also revealed that microbial contamination of samples is strongly possible due to raw materials and poor production conditions in rural areas.

The higher microbial load may be due to contamination during post-preparation handling, transportation and storage of the finished product. The method of production, handling, transportation and marketing of these local vendors products are entirely depend upon traditional system. Such system could pose favorable environment for bacterial contamination. The unclean hands of workers, poor quality of milk, unhygienic conditions of manufacturing unit, inferior quality of material used and water supplied for washing the utensils could be the source of accelerating the bacterial contamination of milk products and post manufacturing contamination (Marrier, 1973; Kumar and Sinha, 1989; Grewal and Tiwari, 1990 and Kulshrestha, 1990).

In the present study, the bacteriological evaluation of marketable sobia found to be contaminated with different bacterial pathogens *like E. coli, Listeria, P. aeruginosa and yeast*. All these bacterial pathogens are responsible for the food borne and diarrheal diseases. The Local Government and the ministry should consider establishment of adequate facilities and utility services as well as provision of necessary information, education and training programmes for vendors and consumers. Our findings show the need for more respect of Good Manufacturing practices (GMP) and Good Hygiene Practices (GHP) to reduce street foods contamination (Titarmare *et al.*, 2009).

In conclusion, the present study is recommended to local vendors that strict hygienic measures should be practiced during pre and post-preparation handling, storage and marketing of the finished products to reduce its microbial load in the finished products.

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المخاطر الصحية لسوبيا الأسواق والمصنعة منزليا في مدينة أسيوط

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السوبيا واحدة من المشروبات الشعبية المصرية المشهورة التي تحتوي علي اللبن وتباع في محلات العصير كما يتم تحضيرها منزليا ويستخدام اللبن كمكون اساسي سواء كان لبن بودرة او لبن سائل مع بودرة الكريم الشانتيه والسكر ورائحة جوز الهند. شملت هذه الدراسة 60عينة من مشروب السوبيا المباع في المحلات وكذلك المعدة منزليا (بواقع 30 عينة لكل منتج) . فحصت العينات لتحديد مدي جودتها الصحية. أظهرت نتائج فحص العينات أن متوسط العدد الكلي للبكتريا الهوائية $1.01x\ 10^5$ في عينات السوبيا المباعة في محلات العصيرو $6.9x10^3$ في العينات المحضرة منزليا بينما كان متوسط العدد الكلي للخمائر والفطريات في عينات السوبيا المباعة والسوبيا المحضرة منزليا $7.79x\ 10^4$ و $5.87x\ 10^2$ علي التوالي. هذا وقد تم عزل الليستريا مونوسيتوجينز بنسبة 33.3 و 0 % في عينات السوبيا المباعة وتلك المحضرة منزليا علي التوالي. بالاضافة الي هذا تم عزل أجناس السيدوموناس بنسب مختلفة من كلا المنتجين. الميكروب القولوني النموذجي الايشريشيا كولاي (O157:H7) لم يتم عزله من كلا من العينات المفحوصة بينما وجدت أجناس أخري من الايشريشيا كولاي (O128:H2 ، O111:H4، O126 ، O124، O1:H7 و O119:H6 بنسب 10، 6.7، 6.7، 3.3، 3.3 و 3.3 % علي التوالي في عينات السوبيا المباعة. Enterobacter agglomerans ، Enterobacter cloacae ، Enterobacter aerogenes، Enterobacter hafniae ، Klebsiella pneumoniae ، Citrobacter diversus تم عزلها وتعريفها من كلا من عينات السوبيا المباعة والمحضر منزليا بنسب مختلفة. تؤكد هذه الدراسة أن مشروب السوبيا في محلات العصائر بالأسواق يحوي أنواعا كثيرة من الأحياء الدقيقة الضارة بالجسم. وعليه يحذر من عدم استخدام الطرائق الصحية في اعداد المشروب مما يعرضه للتلوث بكميات كبيرة من البكتريا خاصة بكتيريا القولون (كولاي) والتي تسبب المغص والإسهال والتسمم الغذائي، ويزداد الأمر سوءا بالإهمال في تداولها والذي نشاهده يمارس اليوم في الشارع. وللاستفادة من هذا المشروب ينبغى أن يغلى ثم يبرد قبل شربه، وتصبح عملية البسترة ضرورية إذا تم شراؤه من السوق.