MICROBIOLOGICAL RISKS OF MILK SHAKE SOLD IN ASSIUT CITY RESTAURANTS

MARWA M.N. EL-GENDI and MARIUM F. MANSY

Animal Health Research Institute (Assiut Provincial Lab.) Food Hygiene Department and Microbiology Department

ABSTRACT

An increase in the consumption of milk shakes has been observed in recent years all over the world. In this work, the hygienic quality of chocolate and fruity milk shake was examined. This study was carried out to evaluate presence of *E.coli*, *Enterococcus* spp. and *Staph. aureus* in 50 samples of milk shake which were collected from different restaurants in Assiut city. The obtained results revealed that *E.coli*, *Enterococcus spp*. and *S. aureus* were isolated and confirmed by chemical tests in percentages of 16%, 24%; 20%, 44% and 12% and 24% of examined samples of chocolate and fruity milk shake, respectively. Concerning the mean values of total psychrotrophic count of chocolate milk shake and fruity milk shake were $9.9x10^3$ and $1.2x10^4$ CFU/g, respectively. Whereas, it was indicated that total yeasts and molds counts of the examined samples of chocolate milk shake and fruity milk shake were $3.2x10^2$ and $2.1x10^3$, respectively. The obtained results revealed that the average counts of *Enterococci* were $4.9x10^3$ and $1.1x10^4$ CFU/g in the examined chocolate and fruity milk shake samples, respectively. In general the analysis indicates unsatisfactory quality of milk shakes and need to be microbiologically controlled by regulatory authorities and the public health significance of isolated strains as well as suggested control measures were discussed.

Key words: Microbiological Risks, Milk Shake, Restaurants

INTRODUCTION

Milk has played a major contribution in the human diet in many different countries across the world. Therefore, it is not surprising that considerable attention has been paid over many years to improve milk quality and in particular the hygienic quality. Bacterial contamination in milk can reduce the raw milk quality and create health hazards especially when the milk is contaminated with some certain species of bacteria with their associated enzymes and toxins that may survive pasteurization (*Oliver et al., 2005*). Microbiology to the dairy industry is an important issue, as recent outbreaks of food-borne illness were recorded as a result of consumption of milk and dairy products that had been contaminated with pathogenic organisms or their toxins. As a result, huge attention has been paid on the microbiological analysis of milk and dairy products to evaluate the quality and also to ensure that there are no public health hazards.

A milk shake is a sweet, cold beverage which is usually made from milk, ice cream, or iced milk, and flavorings or sweeteners such as butterscotch, caramel sauce, chocolate sauce, or fruit syrup.

Outside the United States, milkshakes using ice cream or iced milk are sometimes called a thick milkshake or thick shake; in New England, the term frappe may be used to differentiate it from thinner forms of flavored milk. Full-service restaurants, soda fountains, and diners usually prepare and mix the shake "by hand" from scoops of ice cream and milk in a blender or drink mixer using a stainless steel cup. Many fast food outlets do not make shakes by hand with ice cream. Instead, they make shakes in automatic milkshake machines which freeze and serve a premade milkshake mixture consisting of milk, a sweetened flavoring agent, and a thickening agent. However, some fast food outlets still follow the traditional method, and some serve milkshakes which are prepared by blending soft-serve ice cream (or ice milk) with flavoring or syrups. Milkshakes can also be made at home with a blender or automatic drink mixer (Bittman, 1998).

A milk shake can also be made by adding powder into fresh milk and stirring the powder into the milk. Milkshakes made in this way can come in a variety of flavors, including chocolate, caramel, strawberry, and banana. The manufacture of this product (milk shake) 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.

Among all microorganisms *E. coli* is frequently contaminating organism, and is reliable indicator of

Corresponding author: Dr. MARWA M.N. EL-GENDI
E-mail address: ahmednofel125@yahoo.com
Present address: Animal Health Research Institute (Assiut Provincial Lab.) Food Hygiene Department and Microbiology Department

fecal pollution generally in insanitary conditions of water, food, milk and other dairy products (Diliello. 1982). Two cases of hemolytic uraemic syndrome has been reported which provide evidence that raw milk may be a vehicle of transmission of E. coli O157: H7, both affected person consumed raw milk (Martin *et al.,* 1986). Recovery of *E. coli* from food is an indicative of possible presence of enteropathogenic and/or toxigenic micro-organism which could constitute a public health hazard. Enteropathogenic E coli (EEC) can cause severe diarrhea and vomiting in infants and young children (Anonymous 1975). In 1971 USA faced outbreak of food poisoning in which 387 persons were suffered with Enteropathogenic E.coli due to the consumption of imported French cheese. *E.coli* was isolated from milk products like Mawa/ Khoa, Cream Dahi, Cheese, Butter and Gulabjaman. *E. coli* is a normal inhabitant of the intestines of animals and humans but its recovery from food may be of public health concern due to the possible presence of enteropathogenic and/or toxigenic strains which lead to sever gastrointestinal disturbance (Soomro *et al.,* 2002). Its presence in processed foods results from recontamination, because this bacterium usually does not survive food preservation processes. The main reasons for the presence of E. coli in food products are nonobservance of relevant technological regimes, incompliance with recommended process standards, and the lack of personal hygiene (Law, 2000).

S. aureus is considered the third most important cause of disease in the world amongst the reported food-borne illnesses (Zhang *et al.,* 1998). This gram-positive bacterium has no particular nutritional and environmental requirement for its growth and it can grow at an pH above 4.8 and its minimum growth temperature is 18 - 45°C (Martin and Iandolo, 2000). Most strains are capable of producing one or more enterotoxins (Balaban and Rasooly, 2001) which are the cause of the gastrointestinal symptoms observed during intoxications (Tamarapu *et al.,* 2001). Even though food-borne *S. aureus* poisoning is a mild, generally self-limiting disease, with symptoms that include vomiting with or without diarrhea (Dinges *et al.,* 2000), hospitalization is required in approximately 10% of the cases (Holmberg and Blake, 1984).

Psychrotrophic bacteria are the most commonly isolated organisms which caused the spoilage of the heat treated milk and dairy products as the result of post-pasteurization contamination of the products (Santana *et al.,* 2004). Due to these properties of psychrotrophs, the spoilage and reduced quality of milk and dairy products can be both the consequence of the presence of live organisms and/or their thermos table enzymes (Koka and Weimer, 2001). Spoilage is occurred as the change of flavour, undesirable coagulation of milk proteins, and the increased concentration of free fatty and amino acids. In addition, depending on the type of dairy product, the atypical texture and proportion of certain undesirable organic compounds are occurred (Cempírková and Mikulová, 2009). With regard to other quality aspects, such as suitability of milk for the production of dairy products, psychrotophs have a significant negative effects on yields as well as on limiting shelf life of dairy products (Causin, 1982).

Some authors stated that the presence of enterococci in food matrices is not always to be attributed to direct fecal contamination (Birollo *et al.,* 2001 and Franz *et al.,* 2003). Although they do not represent major pathogens for animals, enterococci are known to be a leading cause of nosocomial infections in humans; they have been implicated in infective endocarditis and urinary tract infections in immunocompromised patients (Coque *et al.,* 1996).

Considering the above facts the present study was designed to isolate the *E. coli, S. aureus and Enterococcu*s from some milk shakes sold under restaurants conditions at Assiut city.

MATERIALS AND METHODS

A) Collection, preparation and serial dilutions of samples:

Total of 50 random samples of chocolate milk shakes and fruity milk shake (25 samples each) were purchased from different restaurants in Assiut city, Egypt. These samples were freshly prepared and transferred aseptically in clean sterile packages. Microbiological examination were done for samples where 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 tenfold serial dilutions were carried out according to (A.P.H.A., 1992).

B) Microbiological techniques:
1) Isolation and identification of *E. coli* according to ISO 7251 (2005):
Biochemical tests were performed to confirm *E. coli* using Gram staining, Catalase test, Indole, Methyl red, Voges- Proskauer test, Nitrate reduction, Urease production, Simon citrate agar, and various sugar fermentation tests.

2) Enumeration, isolation and identification of *Enterococci* was carried out according to *Deibel and Hartman (1982)* using KF streptococcal agar and Azide dextrose broth.

3) Isolation and identification of S. aureus according to ISO 6888-1:1999 A1 (2003).

4) Enumeration of total yeasts and molds counts according to ISO 6611 (2004)

5) Total Psychrotrophic count according to ISO 6730 (2005).

RESULTS

Table 1: Incidence of isolated microorganisms from the examined samples.

Microorganisms	Chocolate milk shake		Fruity milk shake	
Products	No./25	%	No./25	%
E. coli	4	16%	6	24%
Enterococcus	5	20%	11	44%
Staph. aureus	3	12%	6	24%

Table 2: Statistical analytical results of total yeasts and molds count of the examined samples.

Sample	Positive samples		Count/g		
	No./25	%	Min.	Max.	Average
Chocolate milk shake	4	16%	<100	4×10^3	3.2×10^2
Fruity milk shake	15	60%	<100	6×10^3	2.1×10^3

Table 3: Statistical analytical results of total psychrotrophic count of the examined samples.

Sample	Positive samples		Count/g		
	No./25	%	Min.	Max.	Average
Chocolate milk shake	6	24%	<100	2.23×10^5	9.9×10^3
Fruity milk shake	7	28%	<100	1.6×10^5	1.2×10^4

Table 4: Statistical analytical results of total Enterocci count of the examined samples.

Sample	Positive samples		Count/g		
	No./25	%	Min.	Max.	Average
Chocolate milk shake	5	20%	<100	6.4×10^4	4.9×10^3
Fruity milk shake	11	44%	<100	9.6×10^4	1.1×10^4

DISCUSSION

Food borne pathogens are virtually inescapable reaching every aspects of life (Elhaleem and Elkarim 2011). Furthermore microbial contaminants are extremely difficult to pinpoint precision of their presence and role in food system (Biswas *et al.,* 2011). The pattern of acute poisoning may be different even within the region or a country (Akhlaghi *et al.,* 2009). It is therefore essential to increase the number of studies carried out on the importance of food poisoning (Sahingoz and Sahin 2009).

Food-borne outbreaks due to consumption of dairy products constitute a chronic problem facing food hygienists, as milk and dairy products are subjected to different sources of contamination by many pathogens either from endogenous origin or directly and indirectly from exogenous origin. The origin of contamination by food-borne pathogens varies with the type of product and the mode of production and processing. Treatment and processing of milk inhibit or encourage the multiplication of such organisms as all the nutritional components that make milk and dairy products an important part of the human diet, also support the growth of these pathogenic organisms (Feganand Desmarchelier, 2010 and Basil *el al.,* 2012).

The results presented in Table 1, showed that 4 isolates of *E. coli* out of 25 examined chocolate milk

shake samples and 6 isolates out of 25 fruity milk shake samples were identified as *E. coli* with percentages of 16% and 24% respectively. Ahmed *et al.* (2009) found that different types of vended squeezed fruit juices samples have *E. coli* ranging from 43 to >2400/100 ml in Dhaka city, Bangladesh 17. Bagde and Tumane (2011) observed that fruit juices were found to be highly contaminated by *E. coli* in India. Higher results were also demonstrated by (Ahmad and Abdul Haq 2014).

Among all microorganisms *E. coli* is frequently contaminating organism, and is reliable indicator of fecal pollution generally in insanitary conditions of water, food, milk and other dairy products (Diliello. 1982). Recovery of *E. coli* from food is an indicative of possible presence of enteropathogenic and/or toxigenic micro-organism which could constitute a public health hazard (Georgi, 2011). The Findings of the present study revealed that the problem of *E. coli* contamination of milk shake products available in the chocolate and fruity milk shake exists. The presences of faecal indicator organisms not only indicate poor hygiene but also itself may be pathogenic.

Enterococcus spp. isolated from the examined chocolate milk shake samples and as recorded in Table 1, 5 (20%) out of 25 examined samples were confirmed by biochemical tests, also, 11 (44%) out of 25 examined samples of fruity milk shake were positive for *Enterococcus spp.* isolation.

An important clinical feature of *Enterococcus spp.* is the resistance to a wide range of antimicrobial agents as demonstrated in clinical, food and water isolates strains (Eaton and Gasson, 2001, Giraffa *et al.,* 1997 and Gold and Moellering Jr. 1996). *Enterococci* can be detected not only in raw materials but also in RTE (ready to eat) foods, of animal (e.g., milk, cheeses, meat and fish products) and plant origin (e.g., vegetable, fruits; Giard *et al.,* 2001; De Castro *et al.,* 2002 and Abriouel *et al.,* 2008). Despite the use of enterococci in the food industry, their effects on human biology vary widely. While some strains are used as probiotics, others are recognized as serious nosocomial pathogens causing bacteraemia, urinary tract infections, and endocarditis (*Kucerova et al., 2009*).

The results presented in *Table 1*, showed that *staph. aureus* was isolated within percentages of 12% and 24% from chocolate and fruity milk shake, respectively. Tambekar *et al.* (2009) reported Staphylococci prevalence in fruit juice samples in India. Ahmed *et al.* (2009) revealed the presence of staphylococci in squeezed fruit juice samples is also reported in Dhaka city. Rashed *et al.* (2013) found staphylococci in 30 out of 41 samples and total staphylococcal count for vended fruit juices samples was 6.95×10^5 cfu/ml in Dhaka city, Bangladesh.

Ahmad and Abdul Haq (2014) showed high prevalence of coagulase positive *Staphylococcal Spp*. where *Staphylococcus Spp* was found in 87 % of strawberry, 79% of banana and 90% of apple samples. High load of *Staphylococcal Spp* for strawberry, banana and apple samples was 6.1×10^7, 2.2×10^5 and 1.2×10^7, respectively.

The presence of *S. aureus* might indicate inadequate hygiene conditions during milkshake preparation. Foods requiring considerable handling during preparation and kept without refrigeration are usually involved in staphylococcal poisoning. This bacterium is able to grow in a wide temperature range (7-48 °C), with an optimal growth at 35-37 °C, a frequent value in warm climates (Baeza *et al.,* 2007). The presence of S. aureus may be resulted from either insufficient pasteurization of milk, or human exposure. In humans, the main reservoir of *S. aureus* is the nasal cavity. The organism finds their way to the skin and into wounds either directly or indirectly. The most common skin sources are arms, hands, and face. In addition to skin and nasal cavities, S. aureus may be found in the eyes, throat and intestinal tract. From these sources, the organism finds its way into air and dust, onto clothing, and in other places from which it may contaminate foods (Jay, 1996). It is obvious from the previous and the present data that milk shake samples are frequently subjected to Staphylococci spp. contamination which may indicate inadequate personel hygiene of workers or sales people.

Out of 25 samples of chocolate milk shake studied, 4 of them were contaminated by total yeasts and molds counts with percentage 16 % of samples with averages 3.2×10^2 varying between <100 to 4×10^3 UFC/ mL, as shown in Table 2. In addition, the average of total yeasts and molds counts from the fruity milk shake was 2.1×10^3 cfu/ml and varied between <100 to 6×10^3 cfu/mL, as shown in Table 2. The counts of yeast and molds in the samples suggest the need for the control of adequate heat treatment of milk shake and appropriate storage conditions in catering premises.

Total Psychrotrophic counts varied between <100 to 2.23×10^5 with an average count 9.9×10^3 cfu/mL in the examined samples of chocolate milk shake as shown in Table 3. While Psychrotrophic counts reached to 1.6×10^5 cfu/ml as maximum count and counted in 7 (28%) with average count 1.2×10^4 cfu/ml (Table 3). Psychrotrophic bacteria are generally able to form extracellular or intracellular termoresistant enzymes (proteases, lipases and phospholipases) which can cause spoilage of milk and dairy product. In addition, besides exhibiting spoilage features, some species belonging to the psychrotrophs are considered as opportunistic pathogenic bacteria that carry inherent resistance to antibiotics and/or to produce toxins. In sense of quality, psychrotrophic bacteria have become major problem for today's dairy industry as leading

causes of spoilage and significant economic losses (Samaržija *et al.,* 2012).

Only 20 % (5 samples) of the examined samples of chocolate milk shake in the current study where contaminated with *Enterococcus* spp. with minimum count <100cfu/ml and maximum count 6.4×10^4cfu/ml and average count 4.9×10^3cfu/ml. Comparing the results of chocolate milk shake with that obtained in fruity milk shake, it is found that 44% (11 samples) were positive for *Enterococcus spp.* with average count 1.1×10^4 and counts ranged from <100 to 9.6×10^4cfu/ml as recorded in Table 4. The count for *Enterococcus spp.* is being considered as one of the parameters used for food hygiene quality. Low hygienic quality of the samples regarding Enterococcus spp. occurrence in the previous and current work may have been resulted from insufficient heat treatment exposed to milk during the production or from post contamination.

The method of their manufacturing, handling, sale and transportation of these products are entirely based on the unhygienic measures. As observed during sample collection, the handling of products with bare hands, nonusage of aprons, absence of hair covering and handling of money during serving might also contribute to poor hygienic conditions. Such system could pose favorable environment for bacterial contamination. Poor personal hygiene, the use of unhygienic water, unhygienic surroundings act as source of contamination (Chukuezi 2010; Mensah *et al.,* 2002). Thus more hygienic preventive measures are required to reduce the bacterial contamination, so as to increase the wholesomeness of these products.

In developing countries, fruit and vegetable juices are considered as an affordable and readily available source of nutrition especially in summer. Such unpasteurized juices with attributes of fresh flavor are sold by road side shops and is prepared simply by mechanical extraction method. Final product in the form of juice is consumed directly by the consumer without any treatment. Pathogenic microorganisms find their way into fruits and vegetables through damage surfaces that occur during the process of growth and harvesting. In addition equipment contamination, raw material contamination, improper handling, unhygienic conditions also facilitate the entry of pathogens into juices of fruit and vegetables (Nicolas *et al.,* 2007 and Oliveira *et al.,* 2012).

Most of these milk shakes are prepare uses milk of open shops directly without any treatment which may act as an additional source of contamination. The personal hygiene of the manufacturer and the servant who prepare these milk shakes is also not satisfactory as most them are illiterate and does not know anything about microbiological aspect of juices preparation (Ahmad and Abdul Haq 2014).

CONCULUSION

The results of the present investigation revealed that samples were found contaminated with *E. coli, S. aureus* and *Enterococci.* The highest rate of contamination was recorded form the samples of fruity milkshake. These organisms are significant from public health point of view as they have been associated with the onset of food poisoning in human beings. However a large volume of these products are produced in unorganized sector, unbranded, with little precautions of food safety and quality. The counts of microorganisms above the recommended criteria and the presence of some groups of pathogenic bacteria may pose a risk for public health particularly for children and vulnerable elderly people. It is clear from the previous and current studies that there is a necessity for developing the hygienic status of locally produced milk shake in domestic or catering premises in Assiut. This investigation is a factual documentation of such a finding and suggestions as to the methods needed to improve the safety and quality. Findings of this study highlighted the need to improve and implement the hygienic practices related to dairy production and to apply the Egyptian standards of such products for effective monitoring throughout from production to consumption. Due to the importance of the research, more work on food hygiene is still ongoing.

RECOMENDIONS

1- Effective and continuous training accompanied with emphasize on the safety and health issues related to raw milk hazards, educational efforts to improve dairy farmers' awareness of milk borne zoonoses, risk factors associated with milk borne pathogens.
2- Efficient cleaning of all utensils and equipment and the consumers should take in consideration the cleanliness of sales persons.
3- The final retail containers used are preferred to be dispensable and efficiently closed or covered.
4- It is of outmost importance to examine the stool specimens of apparently healthy dairy handlers to clarify their role in shedding bacterial pathogenic agents. To protect public health, more stringent regulations and strategies are in demand.
5- Preventive measures rather than curative measures should be adopted.
6- Strict check and balance of the milk shakes quality in Assiut city is required.
7- Educating the manufacturers and servants of milk shakes shops about safety techniques and microbiological aspects of juice preparation can help in reducing these problems.
8- The use of pure water and pasteurized milk for shakes preparation must be ensured.
9- Personal hygiene and sanitization of the manufacturer and servants should be maintained.

10- Sterile equipments for milk shakes preparation should be used. The shops should be located in less crowded areas.

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المخاطر المكروبيولوجية لمخفوق اللبن المباع في مطاعم مدينة اسيوط

مروة محمد نبيل الجندي ، مريم فؤاد منسي

Email: ahmednofel125@yahoo.com   Assiut University web-site: www.aun.edu.eg

المِيلك شيك أو مخفوق الحليب شراب بارد حلو ويمكن تحضيره بنكهات عديدة كالفراولة أو الشوكولاتة ولكن يبقى اللبن من مكوّناته الأساسية. تم في هذه الدراسة تجميع ٥٠ عينة من مخفوق اللبن بالشوكولاتة و الفواكه من المطاعم الموجودة في مدينة اسيوط. لاستبيان تواجد بعض الميكروبات الممرضة مثل ميكروب الايشريشيا كولي و الميكروب الميكور العنقودي وقد اظهرت النتائج ان ميكروب الايشرشيا كولي تم عزله بنسبة ١٦% و ٢٤% من عينات مخفوق اللبن بالشيكولاتة والفواكه، علي التوالي. وتم عزل اجناس الانتيروكوكس من ٥ و ١١ عينة بنسبة ٢٠% و ٤٤% من عينات مخفوق اللبن بالشيكولاته والفواكه علي التوالي بينما تم عزل الميكور العنقوي من عينات مخفوق اللبن بالشيكولاته والفواكه بنسبة ١٢% و ٢٤ % علي التوالي. أظهرت نتائج فحص العينات ان متوسط العدد الكلي للبكتريا المحبة للبرودة $9.9x10^3$ و $1.2x10^4$ في عينات مخفوق اللبن بالشيكولاته والفواكه علي التوالي بينما كان متوسط العدد الكلي للخمائر والفطريات في عينات مخفوق اللبن بالشيكولاته والفواكه علي التوالي $3.2x10^2$ و$2.1x10^3$. وكانت متوسط العدد الكلي لأجناس للنتيروكوكي علي التوالي $4.9x10^3$و $1.1x10^4$. وقد تمت مناقشة تأثير الميكروبات محل الدراسة علي الصحة العامة و وضع التوصيات اللازمة للحد من انتشار هذه الميكروبات والحصول علي منتج صحي آمن للمستهلك.