

## MICROBIOLOGICAL EVALUATION OF SOME DRIED DAIRY DRINKS SOLD IN ASSIUT CITY

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### ABSTRACT

This study was aimed to evaluate some dried dairy drinks as salep, hot chocolate and coffee milk powder. A total of 75 random samples (25 each) were purchased from different dairy shops and supermarkets in Assiut city, Egypt. Each sample was microbiologically examined in both forms; powdered form (as it was packed) and reconstituted form (as manufacturing instructions) as well as isolation of *Bacillus cereus* and detection of its toxigenic genes (*cytK*, and *hblC*) using PCR technique was performed. The obtained results showed that the average psychrotrophic counts in the powdered form were  $1.2 \times 10^3$ ,  $3.6 \times 10^2$  and 80 cfu/g, respectively; and the counts in the reconstituted form were  $1.25 \times 10^4$ ,  $1.69 \times 10^4$  and  $7.8 \times 10^3$  cfu/g, respectively. Moreover, the average thermophilic counts in the powdered form were  $2.68 \times 10^3$ , 40 and 40 cfu/g, respectively; and in the reconstituted form were  $6.8 \times 10^2$ , 40 and  $<10$  cfu/g, respectively. The average counts of total yeasts and molds in the powdered form of the examined samples were  $1.72 \times 10^3$ ,  $1.6 \times 10^2$  and  $1.01 \times 10^3$  cfu/g, respectively, and the counts in the reconstituted form were  $9.6 \times 10^2$ ,  $9.2 \times 10^2$  and  $2.6 \times 10^3$  cfu/g, respectively. Regarding the total *Bacillus* count, the average counts in the powdered form were  $4 \times 10^2$ ,  $3.2 \times 10^2$  and 40 cfu/g, respectively;  $1.8 \times 10^3$ ,  $1.96 \times 10^3$  and  $2 \times 10^2$  cfu/g were the counts in the reconstituted form, respectively. In addition, identification of the isolated *Bacillus species* was performed and recorded in the previous products. By using PCR, twenty eight strains of the isolated *B. cereus* were enterotoxigenic.

**Key words:** Salep, Hot chocolate, Coffee milk powder, Enterotoxigenic *B. cereus*

### INTRODUCTION

Nowadays, there are many dried dairy drinks that consumed as hot drinks in Egypt as salep, hot chocolate and coffee milk powder. The product salep is pronounced in Arabic dialect as Sahlab.

In the recent years, hot chocolate and coffee milk powder are generally prepared for immediate consumption and not stored in sealed bottles or cans. These drinks are consumed at home or through takeaway beverage shops, however, these drinks may have variable microbiological quality. Unhygienic handling of these ingredients may result in contamination and improper storage which allow microorganisms to multiply to a high level. Hot-served drinks are generally prepared with boiling hot water where microbiological hazards could be reduced by the high temperature; also, these drinks are more likely to be prepared upon ordering.

Salep was a popular beverage in the lands of the Ottoman Empire. Its consumption spread beyond

there to England and Germany before the rise of coffee and tea and it was later offered as an alternative beverage in coffee houses. In England, the drink was known as saloop, popular in the 17<sup>th</sup> and 18<sup>th</sup> centuries, its preparation required that the salep powder be added to water until thickened whereupon it would be sweetened, then flavored with orange flower or rose water. Substitution of British orchid roots, known as "dogstones", was acceptable in the 18<sup>th</sup> century for the original Turkish variants (Davidson, 1987).

Hot chocolate, also known as hot cocoa, is a heated beverage consisting of shaved chocolate, melted chocolate or cocoa powder, heated milk or water, and often sugar and considered a comfort food and is widely consumed in many parts of the world like USA (Sciscenti, 2015). Research has shown that the consumption of hot chocolate can be positive to one's health. A study conducted by Cornell University has shown that hot chocolate contains more antioxidants than wine and tea, therefore reducing the risk of heart disease (American Chemical Society, 2003).

Food safety control aims to safeguard public health and provide assurance on food safety. Microbiological analyses are useful ways to assess the safety and quality of food involved. Instant drinks

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are convenience products that require very little effort to reconstitute prior to consumption. Salep, ground cocoa based beverages (hot chocolate) and coffees milk powder are classified as instant foods that contain milk powder simply mixed with sugar or not. They are sources of high calorie intake and mineral nutrition (Shittu and Lawal, 2007).

Psychrotrophic bacteria are becoming increasingly dangerous to the dairy industry because they produce extracellular heat – resistant lipases and proteases (Sorhaug and Stepaniak, 1997). Moreover, the thermophilic bacilli could not only cause spoilage due to their production of acids and the most stable enzyme excretion, but also cause food borne illness (Burgess *et al.*, 2010).

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.. The presence of wild types of molds is undesirable as they may influence the organoleptic characteristics of the dairy products, they can produce mycotoxins and represent a potential health risk (Wouters *et al.*, 2002). Spoilage of dairy products by molds is frequent and a matter of concern for human health.

*Bacillus cereus* is a common contaminant frequently isolated from foods and dairy products and the fact that the bacterium has a remarkable ability to survive different environmental stresses makes it difficult to be removed from dairy products. The spores survive pasteurization and transfer from milk to pasteurized milk products. The psychrotrophic strains often limit the keeping quality of pasteurized milk and its products (Giffel *et al.*, 1996). Cases of *B. cereus* food poisoning in milk-based products have been reported (Meer *et al.*, 1991; Andersson *et al.*, 1995; Schoeni and Wang, 2005) and as many as 69-85% psychrotrophic *B. cereus* isolates from milk and milk products have been found to be enterotoxigenic (Griffiths, 1990). *B. cereus* produces two types of toxins – emetic (vomiting) and diarrhoeal – causing two types of illness. The emetic syndrome is caused by emetic toxin produced by the bacteria during the growth phase in the food. The diarrhoeal syndrome is caused by diarrhoeal toxins produced during growth of the bacteria in the small intestine (Ehling-Schulz *et al.*, 2006). *B. cereus* is responsible for several outbreaks of foodborne diseases due to its emetic toxin and enterotoxin. Enterotoxins, cytotoxin K (CytK), nonhemolytic enterotoxin (Nhe) and hemolysin BL (Hbl), have been recorded in several diarrheal cases due to food poisoning from *B. cereus* (Zhang *et al.*, 2016).

One of the objectives of this study was to use multiplex PCR to detect enterotoxin-producing *B. cereus* in some dried dairy drinks. The specificity of primers of two enterotoxins; *cytK* and *hblC* of *B.*

*cereus* was verified by inclusivity and exclusivity tests using single PCR.

The aim of the present study was to investigate the microbiological quality of some dried dairy drinks that consumed hot with special focus on the incidence of enterotoxigenic *B. cereus* in the examined products.

## MATERIAL AND METHODS

### A) Sampling:

A total of 75 random samples of salep, hot chocolate and coffee milk powder (25 each) were purchased from different dairy shops and supermarkets in Assiut city, Egypt; in the period from May to June 2015. All the samples were still valid for consumption. Cartons and packets of the samples were cleaned, thoroughly mixed and aseptically opened. Tenfold serial dilution was carried out according to A.P.H.A. (1992). Microbiological examination was done for the samples before preparation (powdered form) and after preparation (reconstituted form). Preparation of the samples was proceed according to the manufacturing methods by using sterile distilled water.

**B) Total psychrotrophic count:** was done according to Gilliland *et al.* (1976).

### C) Enumeration of total thermophiles:

One ml of the sample and its decimal dilutions was plated using milk plate count agar (MPCA) and incubated at 55° C for 48 h as described by Frank and Yousef (2004).

**D) Enumeration of total yeasts and molds:** was done according to Harrigan and MacCance (1976) by using malt extract agar (containing 500 mg each of chlortetracycline and HCL chloramphenicol).

### E) Total *Bacillus spp.* count:

One milliliter from each dilution was mixed with molten (45° C) mannitol–egg yolk–polymyxin (MYP) agar (FDA, 1998) and incubated at 37° C for 24 to 48 h. The colonies that appeared were counted and identified.

**F) Isolation of *B. cereus*:** was done according to Varadaraj (1993)

*B. cereus* was isolated using surface plating technique on polymyxin-pyruvate-egg yolk-mannitol-bromothymol blue agar (PEMBA) (Oxoid). *B. cereus* was appeared as peacock blue coloured colonies surrounded by a zone of precipitation of egg yolk. The isolates of typical colonies were picked for further identification.

### G) Identification of *B. cereus* isolates:

The isolated organisms were identified microscopically and biochemically according to Koneman *et al.* (1992):

**1. Microscopical identification:****1.1. Staining****1.2. Motility test****2. Biochemical identification:****2.1. Catalase test****2.2. Egg yolk lecithinase****2.3. Nitrate reduction test****2.4. Modified VogusProskauer test****2.5. Citrate utilization test****2.6. Gelatin hydrolysis****2.7. Starch hydrolysis****2.8. Indole production test****2.9. Sugar fermentation**

**3. Serological identification:** was done according to TeGiffel *et al.* (2000)

**H) Polymerase Chain Reaction (PCR) technique:**

1.1. Material used for PCR for detection of *B. cereus* enterotoxins genes:

Protocol applied for DNA Extraction using QIA amp kit was done according to Shah *et al.* (2009).

**1.2. Primer sequences of *B. cereus* used for PCR identification system:** Application of PCR for identification of heamolysin BL (*hblC*) and cytotoxic K (*cytK*) genes of *B. cereus* was performed essentially by using Primers (Pharmacia Biotech) as shown in the following table:

Target gene	Primer	Oligonucleotide sequence (5' → 3')	Product size (bp)	Reference
hblC	FHblC (F)	5' CCTATCAATACTCTCGCAA '3	695	Ngamwongsatit <i>et al.</i> (2008)
	RHblC (R)	5' TTTCTTTGTTATACGCTGC '3		
cytK	FCytK (F)	5' CGACGTCACAAGTTGTAACA '3	565	Ngamwongsatit <i>et al.</i> (2008)
	RCytK (R)	5' CGTGTGTAAATACCCAGTT '3		

**1.3. Amplification reaction of *B. cereus* (Ngamwongsatit *et al.*, 2008):**

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). The multiplex PCR amplification was performed in a final volume of 20 ul containing 5 µl of DNA templates with final concentration 1X PCR buffer (10mM Tris-HCl pH 8.3 and 50 mM KCl), 1.5mM MgCl<sub>2</sub>, 200 µM of each dNTP, 5U Taq DNA polymerase and 0.4µM hblC primer and 0.2 µM cytK primer. Reactions were carried out with the following cycling conditions: initial denaturation at 95°C for 5

min, followed by 30 cycles of 94°C for 45 sec, annealing at 54°C and 56°C for 1 min in case of hblC and at 58°C in case of cytK, elongation at 72°C for 2 min and final extension at 72°C for 5 min. Amplified products were analyzed by 1.5% of agarose gel electrophoresis (Applichem, Germany, GmbH) in 1x TBE buffer stained with ethidium bromide and captured as well as visualized on UV transilluminator. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes.

**RESULTS**

**Table 1:** Statistical analytical result of total psychrotrophic count of the examined samples (n=25 of each).

Positive no. of the examined samples	Salep		Hot chocolate				Coffee milk powder					
	powdered		reconstituted		powdered		reconstituted		powdered		reconstituted	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
	8	32	13	52	1	4	9	36	1	4	2	8
count/(cfu/g)	Max.	7x10 <sup>3</sup>	5.8x10 <sup>4</sup>		9x10 <sup>3</sup>		1.4x10 <sup>5</sup>		2x10 <sup>3</sup>		1.3x10 <sup>5</sup>	
	Min.	*<10	*<10		*<10		*<10		*<10		*<10	
	Average	1.2x10 <sup>3</sup>	1.25x10 <sup>4</sup>		3.6x10 <sup>2</sup>		1.69x10 <sup>4</sup>		80		7.8x10 <sup>3</sup>	

\*< 10 means no colonies were detected in the plates.

**Table 2:** Statistical analytical results of total the rmophilic count of the examined samples (n=25 of each).

Positive no. of the examined samples	Salep				Hot chocolate				Coffee milk powder				
	powdered		reconstituted		powdered		reconstituted		powdered		reconstituted		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
	11	44	4	16	1	4	1	4	1	4	0	0	
count/(cfu/g)	Max.	2.6x10 <sup>4</sup>		6x10 <sup>3</sup>		1x10 <sup>3</sup>		1x10 <sup>3</sup>		1x10 <sup>3</sup>		* <10	
	Min.	* <10		* <10		* <10		* <10		* <10		* <10	
	Average	2.68x10 <sup>3</sup>		6.8x10 <sup>2</sup>		40		40		40		* <10	

\* < 10 means no colonies were detected in the plates.

**Table 3:** Statistical analytical results of total yeasts and molds count of the examined samples (n=25 of each).

Positive no. of the examined samples	Salep				Hot chocolate				Coffee milk powder				
	powdered		reconstituted		Powdered		reconstituted		powdered		reconstituted		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
	7	28	6	24	3	12	8	32	8	32	7	28	
count/(cfu/g)	Max.	1X10 <sup>4</sup>		7X10 <sup>3</sup>		2X10 <sup>3</sup>		1X10 <sup>4</sup>		1.1X10 <sup>4</sup>		2.1X10 <sup>4</sup>	
	Min.	* <10		* <10		* <10		* <10		* <10		* <10	
	Average	1.72X10 <sup>3</sup>		9.6X10 <sup>2</sup>		1.6X10 <sup>2</sup>		9.2X10 <sup>2</sup>		1.01X10 <sup>3</sup>		2.6X10 <sup>3</sup>	

\* < 10 means no colonies were detected in the plates.

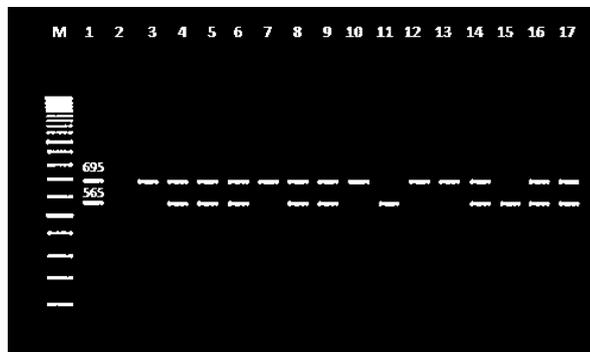
**Table 4:** Statistical analytical results of total Bacillus count of the examined samples: (n=25 of each).

Positive no. of the examined samples	Salep				Hot chocolate				Coffee milk powder				
	powdered		reconstituted		Powdered		reconstituted		powdered		reconstituted		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
	5	20	7	28	7	28	10	40	1	4	2	8	
Count/(cfu/g)	Max.	5x10 <sup>3</sup>		1.8x10 <sup>4</sup>		2x10 <sup>3</sup>		1.8x10 <sup>4</sup>		1x10 <sup>3</sup>		4x10 <sup>3</sup>	
	Min.	* <10		* <10		* <10		* <10		* <10		* <10	
	Average	4x10 <sup>2</sup>		1.8x10 <sup>3</sup>		3.2x10 <sup>2</sup>		1.96x10 <sup>3</sup>		40		2x10 <sup>2</sup>	

\* < 10 means no colonies were detected in the plates.

**Table 5:** Incidence of *Bacillus* spp. isolated from the examined samples: (n=25).

Products / <i>Bacillus</i> spp.	Salep				Hot chocolate				Coffee milk powder			
	powdered		reconstituted		powdered		reconstituted		powdered		reconstituted	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<i>B. megaterium</i>	2	8	0	0	2	8	2	8	0	0	1	4
<i>B. subtilis</i>	2	8	1	4	0	0	3	12	0	0	1	4
<i>B. circulans</i>	1	4	0	0	1	4	1	4	0	0	0	0
<i>B. licheniformis</i>	2	8	1	4	1	4	0	0	1	4	1	4
<i>B. macerans</i>	1	4	0	0	1	4	0	0	0	0	0	0
<i>B. sphaericus</i>	0	0	1	4	0	0	0	0	1	4	1	4
<i>B. coagulans</i>	0	0	2	8	0	0	1	4	1	4	0	0
<i>B. stearothermophilus</i>	0	0	1	4	1	4	1	4	0	0	1	4
<i>B. polymyxa</i>	0	0	1	4	0	0	0	0	0	0	1	4
<i>B. cereus</i>	6	24	9	36	4	16	4	16	2	8	3	12



**Figure 1a:** Agarose gel electrophoresis of multiplex PCR of hblC (695bp) and cytK (565 bp) virulent genes for characterization of *B. cereus*.

**Lane M:** 100 bp ladder as molecular size DNA marker.

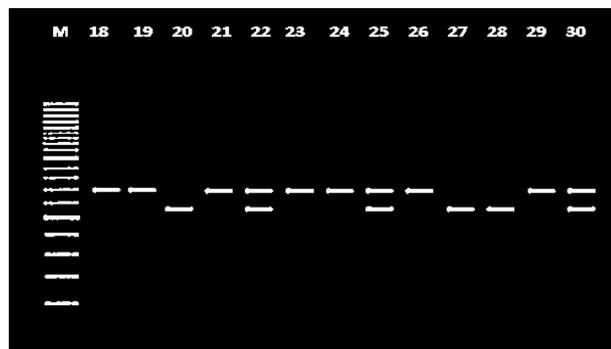
**Lane 1:** Control positive *B. cereus* for hblC and cytK genes.

**Lane 2:** Control negative.

**Lanes 3, 7, 10, 12 & 13:** Positive *B. cereus* strains for hblC gene.

**Lanes 11 & 15:** Positive *B. cereus* strains for cytK gene.

**Lanes 4, 5, 6, 8, 9, 14, 16 & 17:** Positive *B. cereus* strains for both hblC and cytK genes.



**Figure 2 b:** Agarose gel electrophoresis of multiplex PCR of hblC (695bp) and cytK (565 bp) virulent genes for characterization of *B. cereus*.

**Lane M:** 100 bp ladder as molecular size DNA marker.

**Lanes 18, 19, 21, 23, 24, 26 & 29:** Positive *B. cereus* strains for hblC gene.

**Lanes 20, 27 & 28:** Positive *B. cereus* strains for cytK gene.

**Lanes 22, 25 & 30:** Positive *B. cereus* strains for both hblC and cytK genes.

## DISCUSSION

The microbiological results of total psychrotrophic count of the tested dried dairy drinks are presented in Table 1. Out of 25 samples of salep, 8 samples in a percentage of 32% were positive for counting of total psychrotrophes in the powdered form. The maximum, minimum and average counts were  $7 \times 10^3$ ,  $<10$  and  $1.2 \times 10^3$  cfu/g, respectively. While,  $5.8 \times 10^4$ ,  $<10$  and  $1.25 \times 10^4$  were the maximum, minimum and average counts of the salep samples as the reconstituted form, respectively. The number of positive samples increased after reconstitution of the salep samples to become 13 samples in a percentage of 52%.  $9 \times 10^3$  and  $1.4 \times 10^5$  were the maximum counts of the hot chocolate examined samples of the powdered and reconstituted forms, respectively in percentages of 4 and 36% as shown in Table 1.4 and 8% of the examined samples of coffee milk powder were positive for total psychrotrophic count in the powdered and reconstituted forms, respectively with maximum numbers of  $2 \times 10^3$  and  $1.3 \times 10^5$  cfu/g.

The total the rmophilic counts ranged from  $2.6 \times 10^4$  to  $<10$  cfu/g with an average  $2.68 \times 10^3$  and ranged from  $6 \times 10^3$  to  $<10$  cfu/g with an average  $6.8 \times 10^2$  for the powdered and reconstituted forms of the salep samples, respectively (Table 2).

On other hand, only one positive sample of the hot chocolate has the rmophilic bacteria in a percentage of 4% for both powdered and reconstituted forms; 40 cfu/g was the average count of both powdered and reconstituted forms of the hot chocolate samples with a maximum count of  $1 \times 10^3$  cfu/g for both. The results of coffee milk powder samples for total the rmophilic count could be detected only in the powdered form in one sample (4%) with a count ranged from  $1 \times 10^3$  to  $<10$  cfu/g with an average count of 40 cfu/g.

Table 3 revealed that the average count of total yeasts and molds of the powdered form of the salep samples was  $1.72 \times 10^3$  cfu/g with counts ranged from  $1 \times 10^4$  to  $<10$  cfu/g, while in the reconstituted form, the average count was  $9.6 \times 10^2$  and the counts ranged from  $7 \times 10^3$  to  $<10$  cfu/g. 12 and 32% were the percentages of the positive examined samples of the powdered and reconstituted forms of the hot chocolate, respectively, with the maximum counts  $2 \times 10^3$  and  $1 \times 10^4$  cfu/g and the average counts  $1.6 \times 10^2$  and  $9.2 \times 10^2$  cfu/g. The maximum, minimum and average counts of the powdered and reconstituted forms of coffee milk powder samples were  $1.1 \times 10^4$  &  $2.1 \times 10^4$  and  $<10$  &  $<10$  and  $1.01 \times 10^3$  &  $2.6 \times 10^3$  cfu/g, respectively.

The results documented in Table 4 showed that the average counts of total *Bacillus* count of the powdered form of the examined samples of salep, hot chocolate and coffee milk powder were  $4 \times 10^2$ ,  $3.2 \times 10^2$  and 40 cfu/g, with maximum and minimum

counts ranged from  $5 \times 10^3$  to  $<10$ ,  $2 \times 10^3$  to  $<10$  and  $1 \times 10^3$  to  $<10$  cfu/g, respectively. The percentages of the positive samples of the reconstituted form of the same products respectively were 28, 40 and 8% where the maximum counts of the salep, hot chocolate and coffee milk powder samples recorded  $1.8 \times 10^4$ ,  $1.8 \times 10^4$  and  $4 \times 10^3$  cfu/g, respectively.

The manufacture of salep 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 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).

From Table 5, *B. megaterium*, *B. subtilis*, *B. circulans*, *B. licheniformis* and *B. macerans* were detected in percentages 8, 8, 4, 8 and 4% in the examined samples of salep in the powdered form while in the reconstituted form, *B. subtilis*, *B. licheniformis*, *B. sphaericus*, *B. coagulans*, *B. stearothermophilus* and *B. polymyxa* were isolated and identified in percentages of 4, 4, 4, 8, 4 and 4%, respectively. Other *Bacillus* spp. could be found in the examined samples of hot chocolate in the powdered form as *B. megaterium*, *B. circulans*, *B. licheniformis*, *B. macerans* and *B. stearothermophilus* in percentages of 8, 4, 4, 4 and 4%, respectively. After preparation of the hot chocolate samples according to manufacturing procedures, *B. megaterium*, *B. subtilis*, *B. circulans*, *B. coagulans* and *B. stearothermophilus* were detected in 2, 3, 1, 1 and 1 of the examined samples, respectively. *B. licheniformis*, *B. sphaericus* and *B. coagulans* were isolated from the powdered form of coffee milk powder samples in percentages of 4, 4 and 4%, respectively. *B. megaterium*, *B. subtilis*, *B. licheniformis*, *B. sphaericus*, *B. stearothermophilus* and *B. polymyxa* were present in the examined samples of coffee milk powder in the reconstituted form.

*B. stearothermophilus* are extremely heat resistant, up to 20 times more resistant than *Clostridium botulinum* spores (Iciek *et al.*, 2008). *B. stearothermophilus* spores typically survive canning and sterilization procedures of food products and may cause spoilage problems especially where foods must be stored at elevated temperatures for a long time. Growth of *B. stearothermophilus* spores results in flat sour spoilage because acid is produced but with little or no gas generated (Brackett, 2001).

Regarding, the incidence of *B. cereus* isolated from the powdered and reconstituted forms of salep

samples were 24 and 36%, respectively. Also, Table 5 showed that the incidence of *B. cereus* in both the powdered and reconstituted forms of hot chocolate samples was 16%. *B. cereus* was isolated from 8 and 12% of the powdered and reconstituted forms of coffee milk powder examined samples, respectively. A microbiological risk assessment defined the risk of *B. cereus* as gastroenteritis associated with consumption of vended hot chocolate. High level of *B. cereus* isolation does not indicate an immediate risk to public health; however, it may indicate a sub-optimal hygienic conditions and further improvement on the hygienic measures is required. The production of powdered hot chocolate drinks can involve the use of a variety of powdered products as; whey powder, powdered milk, powdered milk protein and cocoa powder which found to have the potentially contaminated *B. cereus* and also has been identified in hot drinks vended from automatic vending machines. Chung- Wang *et al.* (1993) could identify *B. cereus* in vended hot chocolate and cocoa, while Nelms *et al.* (1997) could isolate *B. cereus* within a vended hot chocolate drink, in and around the mixing bowl of the vending machine.

Figures 1a & b referred to the enterotoxigenic *B. cereus* that could be detected by multiplex PCR. Two types of virulent genes for characterization of *B. cereus* were used; hblC (695bp) and cytK (565 bp). All the 28 *B. cereus* isolates were tested for the presence of both enterotoxin genes. The figures cleared that out of 28 strains of *B. cereus*, 12 were harbored hblC gene. However, the cytotoxic (cytK) gene was found in 5 strains which lack hblC gene. Elsewhere, 11 strains carried both types of genes were noticed. So this study revealed that all the *B. cereus* isolates from the examined dried dairy products were enterotoxigenic.

Food manufacturer may need to observe good personal and hygienic practices on handling and storage of ingredients for preparation of salep; prevent packaging contamination and avoid prolonged storage under high temperatures.

Chung-Wang *et al.* (1993) examined 4 varieties of hot drinks obtained from automatic vending machines in Taiwan. A number of specific organisms were also isolated from the drinks and drinks powders by biochemical means. Coffee had a microbial load of 1.5 log cfu/ml. A powder of chocolate mix was free from *B. cereus*. The absence of *B. cereus* from cocoa powders may call into question the sensitivity of the test procedures used, as it is a common contaminant and was found within the vended drink. A variety of other Bacilli were identified within the powder including *B. subtilis* and *licheniformis*. *B. cereus* that found contaminating the drink was confirmed positive for emetic toxin production. Levels of *B. cereus* above  $10^5$  /g are thought to be required to produce sufficient toxin to cause illness (Granum, 1997).

Contamination of liquid milk with *B. cereus* and its spores can also lead to the presence of the organism within any powdered food product containing powdered milk. The contamination of powdered milk and other products derived from powdered milk with *B. cereus* has been widely reported (Wijnands *et al.*, 2006). *B. cereus* has also been found in other dried food products such as potato (Turner *et al.*, 2006), pepper (Chooet *et al.*, 2007) and importantly, cocoa and hot chocolate powder (Gabis *et al.*, 1970, TeGiffel *et al.*, 1996; Ardhana and Fleet, 2003).

The examined salep samples were found to have hygienic problems in the present study. Hygienic conditions of the ingredients used may be a contributing factor to the microbiological quality of this product.

Although *B. cereus* has been recognized as a cause of food poisoning for more than 30 years, it is still difficult to control, especially in the dairy industry. Morphological, biochemical, serological and toxigenic characteristics are commonly used to identify and classify *B. cereus* (Tallen and Kotewicz, 2012).

Further research should focus on the identification and characterization of the microorganisms found within the dried dairy drinks to provide information on the source of contamination and the population dynamic within the products.

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### التقييم الميكروبيولوجي لبعض المشروبات اللبنية المجففة والمباعة في مدينة أسيوط

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هدفت هذه الدراسة إلى تقييم بعض المشروبات اللبنية المجففة مثل السحلب والشيكولاته الساخنة ومسحوق القهوة باللبن ، حيث تم إحضار ٧٥ عينة عشوائية (٢٥ لكل منتج ) تحتوي كلها على اللبن المجفف المخلوط مع باقي المكونات من محلات الألبان المختلفة و السوبر ماركت بمدينة أسيوط ، مصر. وتم فحص كل عينة ميكروبيولوجيا في كل من الحالتين الحالة الجافة (الحالة المباعية) و الحالة السائلة ( بعد إحلالها بالماء المعقم طبقا لطريقة التحضير) و قد أظهرت نتائج فحص العينات أن متوسط العدد الكلي للبكتريا المحبة للبرودة في الحالة الجافة  $10^2$  و  $10^3$  ،  $10^3$  و  $3 \times 10^3$  ،  $10^4$  و  $80$  ميكروب / جم على التوالي ، و الأعداد في الحالة السائلة  $10^4$  ،  $10^6$  و  $10^7$  ميكروب / جم على التوالي و كان متوسط أعداد البكتريا المحبة للحرارة في الحالة الجافة  $10^2$  و  $10^3$  ،  $10^4$  ،  $10^5$  و  $10^6$  ميكروب / جم على التوالي وفي الحالة السائلة  $10^6$  و  $10^8$  ،  $10^9$  ،  $10^{10}$  ميكروب / جم على التوالي و نجد أن متوسط أعداد الخمائر و الفطريات في الحالة الجافة كان  $10^1$  و  $10^2$  ،  $10^3$  و  $10^4$  ،  $10^5$  ميكروب / جم على التوالي و متوسطها في الحالة السائلة كان  $10^6$  و  $10^9$  ،  $10^{10}$  ،  $10^{11}$  ميكروب / جم على التوالي و أما في الحالة السائلة كانت الأعداد  $10^8$  ،  $10^9$  و  $10^{10}$  ،  $10^{11}$  ميكروب / جم على التوالي و بالإضافة إلي هذا تم عزل و التعرف علي أنواع الباسيليس المعزولة وتسجيلها لكل منتج . من ناحية أخرى و باستخدام PCR تفاعل البلمرة المتسلسل تم الكشف عن تواجد الباسيليس سيريس المفرزة للسموم في ٢٨ من العينات السابق فحصها هذا و قد تم مناقشة النتائج و توضيح مدى خطورة الميكروبات المعزولة و أثرها على صحة المستهلك.

الكلمات الكاشفة: السحلب ، الشيكولاته الساخنة ، مسحوق القهوة باللبن ، الباسيليس سيريس المفرزة للسموم