J. Agric. Sci. Mansoura Univ., 33 (12): 8843 - 8849, 2008

EVALUATION OF MICROBIOLOGICAL QUALITY OF SOME PROCESSED FRUIT JUICES IN EGYPTIAN MARKETS

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ABSTRACT

The present study was performed to evaluation the microbiological quality of processed packed juices like mango, guava, apple and cocktail purchased from five different local companies in Egypt. The microbiological analysis including total plate count (T.P.C), total coliforms (T.C), faecal coliforms (F.C.), lactic acid bacteria (L.A.B), yeasts, *Escherichia coli*, *Staphylococcus aureus* and *Clostridium perfingins*. The effect of *leuconostoc mesenteroids* on growth of *E. coli* and *Staph aureus* in vitro was evaluated. The results revealed that microbiological counts in the examined samples were ranged from 1x10 to $5.5x10^5$ cfu/ml for T.P.C., 3x10 to $2.5x10^5$ cfu/ml for T.C, 1x10 to $10x10^5$ cfu/ml for yeasts. All the examined samples were negative for *E. coli*, *Staph aureus*, *Colostridium perfringins* and faecal coliforms. The treatment with *lecuonostoc mesenteroids* induced complete eliminate of *E. coli* in mango, apple and cocktail juices and *Staph aureus* in mango and apple juices. The bacteria density of the same microorganisms (*E. coli and Staph aureus)* were decreased on enrichment broth.

Keywords: fruit, juices , *leuoconostoc* ,pathogens

INTRODUCTION

Fruits juices are recognized as an emerging cause of food borne illness (Parish, 1997). A major contributing factor in these raw agriculture commodities are contamination by animal or human waste and consumption without a processing step that will kill or remove associated bacterial pathogens. While a single piece of contaminated produce may infect a single person, contaminated produce that is co-mingled juices and served may infect many individuals. One potential source of entry of microorganisms into fruits is by environmental exposure with uptake occurring through either specific morphological structures in the plant and or through breaks in tissues that occur as a result of punctures wounds cuts and splits. These insults to the fruit can occur during growing or harvesting, additionally processing conditions and improper handling contribute substantially to the entry of bacterial pathogens into the product, especially in juices prepared from the fruits. Processed juices made from fruits have a very high consumer preference both in terms of tats and healthy effects through the word, however, in the current past such juices especially unpasteurized juices have been shown to be a potential source of bacterial pathogens notably, *E. Coli* O157:H7, (Ryu and Beuchat 1998, Uljas and Ingham, 1998, Zhuang *et al*. 1995). Bacteria, yeasts and molds are the microorganisms that can spoil the quality of soft drinks, yeast often colonize foods with a high sugar content and contribute to spoilage fruits and juices with a low PH (Elke. , 2007).

Lactic acid bacteria are a groupe of gram positive bacteria including species like *leuoconostoc* and *Lactobacills* which are useful in some food

production, but under low oxygne ,low temperature and acidic conditions these bacteria become the predominant spoilage organisms on a variety of foods .LAB may also produce large amounts of an exopolysaccharide that causes ropy spoilage in some beverages (Ellin, 2007). Soomro *et al*, 2004 reported that LAB have their ability to produce antimicrobial compounds called bacteriocins and in recent years these compounds has grown substantially due to their potential usefulness as natural substitute for chemical food preservation in the production of foods. The inhibitory effect of *lecuonostoc* in the gelatin system was caused by the production and activity of lecucocins (Hornback T. 2004).

The coliform population declines as the population of strain of lecuconostoc (John, L. 1998). Also bacteriocin produced by *leuconostoc sp*. previously been shown to inhibit the growth the wide range of pathogens (Ramnath, 2000).

MATERIALS AND METHODS

Thirty four of processed fruit juices including guava, mango, apple and cocktail samples were collected from different retail markets. Samples were collected in ice and transported immediately to the laboratory for the microbiological analysis. Determination of total bacterial count was carried out according to Berrang *et al*. (2001).

Total coliform and faecal coliform counts were carried out according to Mercuri and Cox, (1979). Lactic acid bacteria count was carried out according to Badis *et al*. (2004). Yeast count was carried out according to NMKL, (1999).

Determination of PH values were measured using laboratory PH meter with a glass electrode (Orion Research digital analysis)

Incidence of pathogenic bacteria in juices:

Isolation of *E.coli* was carried out according to Collins *et al*. (1998). *E.coli* colonies are green metallic sheen on Eosin Methylene Blue (E.M.B) agar medium.

Staphylococcus aureus was isolated based on carried out according to Gouda Hanan (2002). The isolation of *Staph. aureus* based on appears as black, convex, shiny colonies surrounded by a yellow zone on Vojel Johnson agar medium. Isolation of *Clostridium perpringins* was carried out according to FAO (1992). *Clostridium perpringins* appears as (black colonies) on cooked meat agar medium.

The bacterial cultures of *Staph. aureus* and *E. coli* were kindly obtained from Abdel Salam, (2005).

Preparation of bacterial inoculum:

Staph. aureus and *E. coli* was subcultured at least twice by loop inoculation of 100 ml volumes of 1% buffered peptone water (pH 7.2) for 24 h at 37ºC to achieved viable cell population $3.5x10^{10}$ cfu/ml of *E.coli* and $3.5x10^{11}$ cfu/ml of *Staph. aureus*. Leuconostoc was subcultured at least twice by loop inoculation of 100 ml volume of Mccleskey's broth medium according to Ebtsam ,1998 (sucrose 100 g/L, peptone 10g/L, yeast extract 59/L, and pH 6.7) for 24 h at 25ºC to achieved viable cell population of $5x10^{11}$ cfu/ml.

Effect of *leuconostoc mesenteroids* on growth of *staph aureus* and *E. coli* in vitro:

Erlenmeyer flasks (250 ml) contained 50 ml of 1% buffered peptone water (pH 7.2), beside another flasks contains different juices which inoculated with 0.5 ml of standard inoculum. The flasks were incubated at the 37 C^0 for 24h.

RESULTS

1- The microbial load of processed fruit juices :

Guava and Mango juices :

Table (1) describe the microbial load of Guava and Mango juices, from the obtained data it's obvious that the highest levels of total plate counts for Guava and Mango juices were $1.5x10^5$ and $5.5x10^5$ cfu/ml, while the highest values of total coliforms, lactic acid bacteria and yeasts in guava and mango juices were recorded as $4x10^4$ and $1.8x10^5$, $1.0x10^6$ and $5x10^5$ and $3x10^5$, $2x10^4$ cfu/ml respectively. All investigated samples were free for faecal coliform, *E. coli, Staph. aureus* and *Clostiridium perfringiens.*

Apple and cocktail Juices :

The data obtained in Table (2) showed that $2x10^5$ cfu/ml was recorded as the maximum level of total plate counts for cocktail juice. In the same table it's notable that only one apple juice sample was positive for total coliform counts ($2.5x10^5$ cfu/ml) and two samples from the same type were contain 1x10 and $6x10^2$ cfu/ml lactic acid bacteria. Fifty percent of cocktail juice were contain yeasts and the highest level was recorded as $1x10^3$ cfu/ml. No sample of cocktail juice was positive for faecal coliform, *E. coli, Staph. aureus* and *Clostiridium perfringins.* Concerning apple juice, the highest levels of total plate counts and total coliform counts were recorded as $3x10^5$ and $2x10^2$ cfu/ml, while the maximum counts of yeasts was observed as $1x10^5$ cfu/ml. All examined apple juice samples were negative for faecal coliforms, *E. coli, Staph.aureus* and *Clostiridium perfringins*.

2- Effect of *Leuconostoc mesenteroids* on growth of *Staph. aureus* and *E. coli:*

The obtained results in table (3) obviously showed that *Staph aureus* was completely elimination from Mango and apple juices samples while it decreased from $3.5x10^{11}$ cfu/ml to $1x10^7$, $7x10^5$ and $4.5x10^7$cfu/ml in guava, cocktail sample and enrichment broth respectively. Concerning *E. col* it was completely elimination from mango, apple and cocktail juices while it decreased from, cocktail and $3.5x10^{10}$ cfu/ml to $2x10^5$ and $1.5x10^8$ cfu/ml in guava juice and enrichment broth respectively.

Table (3): Effect of *leuconostoc mesenteroids* on growth of *Staph aureus* and *E. coli* (in vitro).

Microorganism	Count (cfu/ml)*					
	Initial inoculum cfu/ml	Guava	Mango	Apple	Cocktail	Enrichment broth
Staph aureus	3.5×10^{11}	1×10^{7}	-	-	7×10^{5}	4.5×10^{7}
E. coli	3.5×10^{10}	2×10^{5}	-	-	-	1.5×10^{8}

*** the use inoculation of *leuconostoc mesenteroids* in treatment was 5×10^{11} cfu/ml.**

DISCUSSION

According to Egyptian standards 2005 for processed fruit juices, all examined samples were acceptable for faecal coliforms, *E. coli, Staph. aureus and Cl. perfringines.* Concerning total plate counts the results were nearly agree with Afaf (2000) who reported that total plate counts of Mango and Guava juices were 3×10^{3} and 1.2×10^{4} cfu/ml, also Abdul Basar (2007), reported that total plate counts of mango juices was detect as 2.7×10^{3} cfu/ml. Concerning fruit juices which derived from different fruits which contain high acidity it's obvious that these juices contained large amounts of bacteria and yeasts and the values obtained within the range of 10^{2}-10^{5} cfu/ml for microbial populations (Hatcher *et al*., 1992), this is agree with the results study which obtained that PH values of all investigated samples were between 3.00 to 3.30.The study findings is agree with Peng *et al*., (2001) who reported that the presence of notable bacterial pathogens such as *E. coli*, *Staph. aureus* in fruit juices is considered a safety concern. Regarding to the samples which contain highest counts of bacterial contamination , Lateef *et al*, (2004) said that the processing units of the juices are likely primary causes of high bacterial load. Regarding to the effect of *Leuconostoc mesenteroids* on growth of *Staph.aureus* and *E.coli* , Savadoga *et al,*(2004) reported that the strains which identified to species *Lactobacillus fermentum* and *leuconostoc mesenteroids* are produced bacteriocins which exhibited activity against *Staph.aureus* and *E.coi*.

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تقييم الجودة الميكروبيولوجية لبعض العصائر المصنعة فى الأسواق المصرية

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أجريت هذه الدراسة من أجل تقييم الجودة الميكروبيولوجية لبعض العصائر المصنعة مثل المانجو والجوافة والتفاح والكوكتيل لعدد خمس شركات مختلفة فى الأسواق المصرية وكان الهدف من هذه الدراسة هو تقدير الكثافة الميكروبية مشتملة على أعداد البكتريا الكلية والعدد الكلى لبكتريا القولون و بكتريا القولون البرازية وبكتريا حامض اللاكتيك والخمائر وميكروبات الاشيرشياكولاى والاسيتافيلوكوكس اوريس والكلوستريديم بيرفيرنجس فى بعض العصائر مثل الجوافة، والتفاح والمانجو والكوكتيل التى تم شرائها من محلات البقالة المحلية.

كما تم دراسة تأثير ميكروب الليكونوستوك على نمو ميكروب الاشيرشيا كولاى والاستافيلوكوكس اوريس.

وأظهرت النتائج أن:

أعداد الميكروبات فى العينات المختبرة كانت تتراوح من 1×10 إلى 5.5× 10º خلية/مل بالنسبة لأعداد البكتريا الكلية و 3×10 إلى 2.5× 10º خلية/مل بالنسبة لبكتريا القولون و 1×10 إلى 10× 10º خلية/مل بالنسبة لبكتريا حامض اللاكتيك و1×10 إلى 3/ 10º خلية/مل بالنسبة للخمائر. اوضحت النتائج أيضا خلو جميع العينات تحت الدراسة من ميكروبات الأستافيلوكوكس أوريس و الكلوستريديم بيرفيرنجس و كذلك بكتيريا القولون البرازية.

كما أظهرت النتائج أن المعاملة بميكروب الليوكونوستوك أحدثت إزالة كاملة لميكروب الاشيرشيا كولاى فى عينات عصائر المانجو والتفاح والكوكتيل وميكروب الاستافيلوكوكس اوريس فى عينات عصائر المانجو والتفاح، كما أحدثت انخفاض فى الكثافة الميكروبية لنفس الميكروبات (الاشيرشيا كولاى، الاستافيلوكوكس اوريس) فى بيئات التنمية.

Table (1): The microbial load of guava and mango processed juices (cfu/ml).

Sample No.	Guava juice					Mango juice					
	Total plate counts	Total coliform	Faecal coliform	L.A.B	Yeasts	Sample No.	Total plate counts	Total coliform	Faecal coliform	L.A.B	Yeasts
1	$5.5x10^4$	$4x10^4$	0	$2.5x10^4$	$1x10^4$	1	$4x10^4$	0	0	$6x10^3$	$2x10^4$
2	$5.0x10^4$	0	0	$4x10^3$	$6x10^3$	2	$3x10^5$	$2.1x10^2$	0	$3.5x10^3$	$8.5x10^3$
3	$2x10^4$	0	0	$2x10^5$	$3x10^5$	3	$5.5x10^5$	$1x10^5$	0	$5x10^5$	$1.8x10^4$
4	$1.1x10^5$	$1.5x10^3$	0	$5x10^4$	$3x10^5$	4	$1.5x10^4$	0	0	$1x10^4$	$2x10^3$
5	$9x10^4$	0	0	$2x10^5$	0	5	$1x10^3$	0	0	0	$2x10^4$
6	$4x10^4$	0	0	$3x10^4$	2x10	6	$2x10^5$	$1.8x10^5$	0	0	$1x10^4$
7	$2x10^4$	0	0	$1x10^6$	-	7	$2x10^4$	0	0	$1x10^3$	0
8	$7x10^4$	0	0	$1x10^3$	$3x10^4$	8	$8x10^3$	0	0	0	0
9	$1.5x10^5$	$3x10^3$	0	$3x10^5$	$6x10^3$	9	$5.5x10^2$	0	0	0	$1x10^3$

L.A.B. : Lactic acid bacteria.
- ve : Negative.

Table (2): The microbial load of apple and cocktail processed juices (cfu/ml).

Sample No.	Apple juice					Cocktail juice					
	Total plate count	Total coliform	Faecal coliform	L.A.B	Yeasts	Sample No.	Total plate count	Total coliform	Faecal coliform	L.A.B	Yeasts
1	$3x10^5$	0	0	0	$1x10^3$	1	$2x10^5$	0	0	0	$1x10^2$
2	$2.7x10^2$	$1x10^2$	0	0	$1x10^5$	2	$1x10^2$	0	0	0	0
3	$5.5x10^4$	0	0	0	1x10	3	1x10	0	0	0	0
4	$1x10^4$	3x10	0	0	0	4	$2x10^3$	$2.5x10^5$	^{0}	$6x10^2$	1x10
5	$6x10^4$	0	0	0	0	5	$2x10^2$	0	0	0	$1x10^2$
6	$2x10^4$	5x10	0	0	$2x10^3$	6	1x10	0	0	0	$1x10^3$
7	$3x10^3$	0	0	0	$3x10^3$	7	1x10	0	0	0	0
8	$1x10^4$	$2x10^2$	0	0	0	8	1x10	0	0	0	0

L.A.B. : Lactic Acid Bacteria.
- ve : Negative.