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 perfringins. 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 leuconostoc 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, leuconostoc, 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), Uijas 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 leuconostoc and Lactobacilis which are useful in some food
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production, but under low oxygen, 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 leucocins (Hornback T. 2004).

The coliform population declines as the population of strain of lecuonostoc (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 \textit{leuconostoc mesenteroids} on growth of \textit{staph aureus} and \textit{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° 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 $1.5 \times 10^5$ and $5.5 \times 10^5$ cfu/ml, while the highest values of total coliforms, lactic acid bacteria and yeasts in guava and mango juices were recorded as $4 \times 10^4$ and $1.8 \times 10^5$, $1.0 \times 10^6$ and $5 \times 10^5$ and $3 \times 10^5$, $2 \times 10^4$ cfu/ml respectively. All investigated samples were free for faecal coliform, \textit{E. coli}, \textit{Staph. aureus} and \textit{Clostiridium perfringiens}.


Apple and cocktail Juices :

The data obtained in Table (2) showed that $2 \times 10^5$ 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 ($2.5 \times 10^5$ cfu/ml) and two samples from the same type were contain $1 \times 10^6$ and $6 \times 10^2$ cfu/ml lactic acid bacteria. Fifty percent of cocktail juice were contain yeasts and the highest level was recorded as $1 \times 10^5$ cfu/ml. No sample of cocktail juice was positive for faecal coliform, \textit{E. coli}, \textit{Staph. aureus} and \textit{Clostiridium perfringiens}. Concerning apple juice, the highest levels of total plate counts and total coliform counts were recorded as $3 \times 10^5$ and $2 \times 10^2$ cfu/ml, while the maximum counts of yeasts was observed as $1 \times 10^0$ cfu/ml. All examined apple juice samples were negative for faecal coliforms, \textit{E. coli}, \textit{Staph.aureus} and \textit{Clostiridium perfringiens}.

2- Effect of \textit{Leuconostoc mesenteroids} on growth of \textit{Staph. aureus} and \textit{E. coli}:

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

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Count (cfu/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial inoculum</td>
</tr>
<tr>
<td>Staph aureus</td>
<td>3.5 x 10^11</td>
</tr>
<tr>
<td>E. coli</td>
<td>3.5 x 10^10</td>
</tr>
</tbody>
</table>

*the use inoculation of *leuconostoc mesenteroids* in treatment was 5x10^11 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 3x10^3 and 1.2x10^4 cfu/ml, also Abdul Basar (2007), reported that total plate counts of mango juices was detect as 2.7x10^3 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 mesenteroides* on growth of *Staph.aureus* and *E.coli*, Savadoga *et al.*, (2004) reported that the strains which identified to species *Lactobacillus fermentum* and *leuconostoc mesenteroides* are produced bacteriocins which exhibited activity against *Staph. aureus* and *E.coli*.

**REFERENCES**


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Tقييم الجودة الميكروبيولوجية لبعض العصائر المصنعة في الأسواق المصرية

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المركز الإقليمي للأغذية والأعلاف - مركز البحوث الزراعية

أجريت هذه الدراسة من أجل تقييم الجودة الميكروبيولوجية لبعض العصائر المصنعة مثل المانجو والجوافة والتفاح للتأكد منحتايات مختلفة في الأسواق المصرية وكان الهدف من هذه الدراسة هو تقديم الكثافة الميكروبية ممثلة على أعداد البكتيريا الكليلة والعدد الكلي للكولون البرازية وبكتيريا حامض اللاكتيك والخملييات ونيوكريبتات الأشيرة كولوكس ونيوكريبتات الأشيرة كولوكس أوريس في بعض العصائر مثل الجوافة والتفاح والمانجو والكمون التي تم شرائها من محلات البيعا المحلية.

كما تم دراسة تأثير ميكروب الليكونوستوك على نمو ميكروب الأشيرة كولوكس أوريس والنيوكريبتات الأشيرة كولوكس أوريس.

وأظهرت النتائج أن:

أعداد الميكروبات في العينات المختلفة كانت تتراوح من 1×10⁶ إلى 5.5×10⁹ خليلة/مل بالنسبة لأعداد البكتيريا الكليلة و 3×10⁵ إلى 10×10⁹ خليلة/مل بالنسبة للكولون البرازية و 1×10⁶ إلى 10×10⁹ خليلة/مل بالنسبة للكولون البرازية وبكتيريا حامض اللاكتيك و 1×10⁸ خليلة/مل بالنسبة للخمليبات. وسمعت النتائج أيضا أخذ جميع العينات تحت الدراسة من ميكروبات الأشيرة كولوكس أوريس والنيوكريبتات الأشيرة كولوكس أوريس وكذلك بكلا الکولون البرازية.

كما أن النتائج أن العلاقة بين ميكروب الليكونوستوك أحدثت إزالة كاملة لميكروب الأشيرة كولوكس أوريس في بعض العصائر والتفاح والكمون حيث أن ميكروب الأشيرة كولوكس أوريس في بعض العصائر والتفاح والكمون كما أحدثت اختفاء من الكثافة الميكروبية لنسف الميكروبات (الأشيرة كولوكس أوريس). في بيئات التماس.
Table (1): The microbial load of guava and mango processed juices (cfu/ml).

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Guava juice</th>
<th>Mango juice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total plate counts</td>
<td>Total coliform</td>
</tr>
<tr>
<td>1</td>
<td>5.5x10^4</td>
<td>4x10^4</td>
</tr>
<tr>
<td>2</td>
<td>5.0x10^4</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2x10^4</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1.1x10^5</td>
<td>1.5x10^3</td>
</tr>
<tr>
<td>5</td>
<td>9x10^4</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>4x10^4</td>
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</tr>
<tr>
<td>7</td>
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<td>0</td>
</tr>
<tr>
<td>8</td>
<td>7x10^4</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>1.5x10^3</td>
<td>3x10^3</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Apple juice</th>
<th>Cocktail juice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total plate count</td>
<td>Total coliform</td>
</tr>
<tr>
<td>1</td>
<td>3x10^5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2.7x10^2</td>
<td>1x10^2</td>
</tr>
<tr>
<td>3</td>
<td>5.5x10^4</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1x10^4</td>
<td>3x10</td>
</tr>
<tr>
<td>5</td>
<td>6x10^4</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>2x10^4</td>
<td>5x10</td>
</tr>
<tr>
<td>7</td>
<td>3x10^3</td>
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</tr>
<tr>
<td>8</td>
<td>1x10^4</td>
<td>2x10^2</td>
</tr>
</tbody>
</table>

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