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## EVALUATION OF MICROBIOLOGICAL PARAMETERS OF HACCP SYSTEM IN A JUICE PRODUCTION LINE IN LOCAL COMPANY

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M.S. Azab<sup>1</sup>, A.M. Fouda<sup>1</sup>, A.A. Radwan<sup>1</sup>, M.B. Emara<sup>2</sup>, and A.A Ebeid.<sup>3</sup>

<sup>1</sup> Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt.

<sup>2</sup> Food safety and hygiene advisor, Quality unit, Ministry of Tourism; <sup>3</sup> Lab and quality supervisor at food and juice industry

**\*\*Corresponding author:** [Ahmedradwan@azhar.edu.eg](mailto:Ahmedradwan@azhar.edu.eg)

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### ABSTRACT

This study was conducted to assess the Hazard Analysis and Critical Control Point (HACCP) system in a pasteurized juice production line through follow up prerequisite programs (PrP), Good Manufacturing Practices (GMP) and Critical Control Points (CCPs), the results were then compared to the standard specification. A total numbers of 361 samples were collected from the production line in local company including fruit concentrates, water, food contact surfaces, personnel swabs, environmental air and finished products samples. Standard methods were used to determine Total Plate Count (TPC), yeasts, moulds, coliform, heat resistant moulds and acid tolerant bacteria (*Alicyclobacillus*). Most of investigated samples were out of the standard acceptable ranges. The percentages of TPC, yeast and moulds in samples collected from fruit concentrates, food contact surfaces, water samples, personnel swabs, environmental air and finished products were significantly non-confirmed (22%, 85%, 41%, 44%, 14% and 4%), respectively. All these parameters counts lead to contamination in the products and cause high cost as a result of scraping these contaminated products. This was the first stage of problem evaluation and determining the CCP in order to overcome or reduce the phenomenon of microbial pollution in finished products.

**Key Words:** HACCP system, GMP, Juice microbiology and Evaluation

### INTRODUCTION

The consumption of contaminated food causes foodborne illness for the consumers and there are many sources for contaminants such as water, air, soil, plants, animals and human as well, food can be contaminated during the supply chain from the farm to the consumers and may probably cause disease outbreaks (CDC, 2010).

In food industries, food hazards should be identified including the potential hazards that associated with final products, raw materials, all ingredients, equipment with direct contact with the products and the environment of the production processes **Codex Alimentarius Commission (CAC), (2012)**. Foodborne pathogens and infections globally increased in the last twenty five years with a high level of risk due to pathogenic contamination (Oliver, et al., 2005).

The existence of microorganisms including bacteria, yeasts and molds in fruit juices are

responsible for fermentation, food spoilage and illness (Essien et al., 2011). Fruit juices, concentrates and fruit nectars may be fresh, unpasteurized and clean filled, or pasteurized, then cooled, preserved in aseptic, or clean filled in sterile pack (Stratford et al., 2000; Stratford and James, 2003).

Microorganisms responsible for fruit spoilage should be resistant to the acidic environment that is low in oxygen and nutrients with high CO<sub>2</sub> level. These microorganisms differ in their growth requirements (Back, 2005; Stratford 2006, Lawlor 2009 and Tribst et al., 2009). In 2005, data from World Health Organization (WHO) recorded that 1.8 million people died of gastroenteritis caused by contaminated food and water (WHO 2005).

In terms of the food industry, two factors should be considered: the need to ensure food safety and protection of consumer's health. The ensuring food safety is very important to preserve a company's image, reputation and to increase local and international market

distribution. The concern of food safety has become of worldwide concern making the public health agency and several countries looking for more efficient ways to monitor production chains (**Makiya and Rotondaro 2002**).

The systematic approach used in food industry to identify hazardous contamination of chemical, biological or physical agents and implements the proper controls to reduce or eliminate the hazards and risks factors at specific points related with the manufacture, production, storage and distribution of foods is known as Hazard Analysis and Critical Control Point (HACCP) (**Dillon *et al.*, 2003**).

The HACCP system is recognized as an important tool in the reduction of Food Borne Diseases (FBDs). It is recommended by the WHO, the International Commission on Microbiological Specifications for Foods (ICMSF), the Codex Alimentarius, and food regulatory agencies in various countries. More CCPs mean increased difficulty in the management of the HACCP plan, and affect the efficacy of food safety (**Roberto *et al.*, 2006**).

The application of HACCP system and its principles necessitates some prerequisite programs, such as GMP and cleaning procedures, which should be established to ensure the basic hygiene conditions in the processing areas. These prerequisite programs if correctly implemented will determine the principles for correct handling of foodstuffs, making HACCP more efficient and simple to manage (**Wallace and Williams, 2001**).

## 2. MATERIALS AND METHODS

### 2.1. Collection of Samples

A total of 361 samples were collected from all production steps along the pasteurized juice production process starting from raw materials and food contact surfaces until the final products as in table 1. Concentrate samples were collected in sterile bottles, treatment water samples in sterile syringes, food contact surfaces by sterile cotton swabs and personnel by sterile cotton swabs from hands, environmental air by plate settling method and end products from packet and all of these samples conducted for microbiological analysis.

### 1.2. Samples preparation

Samples of concentrates, personnel swabs, food contact surface swabs and water samples were mixed and diluted with 9 ml of buffered peptone water to dilute microbiological samples, while samples of environmental air were prepared by exposing petri plates containing 12-15 ml of DRBC and APC media and allowing the plates to be exposed to the surrounding air. For the finished products, one ml sample was inoculated directly onto petri plates with appropriate media such as DRBC, APC, VRBA, BAT and MEA.

### 1.3.2.3. Detection and enumeration of microorganisms in the different production steps.

#### 2.3.1. Detection and enumeration of TPC

The detection and enumeration of TPC was performed by direct inoculation of 1 ml direct from the samples (**ISO 6887, 2017**) in finished

**Table, 1: Samples collected from the different juice production steps**

Type of samples	Number	Percent (%) of total samples
Fruit concentrate	55	15.20%
Finished products	55	15.20%
Traditional swabs from food contact surfaces	78	21.60%
Water samples	63	17.40%
Traditional swabs from personnel	45	12.40%
Environmental air samples	65	18.00%
<b>Total</b>	<b>361</b>	<b>100.0%</b>

product samples or its dilution in concentrates, water, food contact surfaces and personnel swabs samples into petri dish and mixed with the plate count agar media (Merk) (ISO 11133, 2014) and incubated at  $30\pm 2^{\circ}\text{C}$  for 72 h. and the produced colonies were counted (ISO 4833-1, 2013).

### 2.3.2. Detection and enumeration of coliform

The detection and enumeration of coliform was assayed by direct inoculation of 1 ml of the sample or its dilution as mentioned in TPC test into petri dish and mixed with the violet red bile agar media (Merk) then incubated at  $35\pm 2^{\circ}\text{C}$  for 24 hr and the produced purple colonies were counted (ISO 4832, 2006).

### 2.3.3. Detection and enumeration of yeasts and moulds

The detection and enumeration of yeasts and moulds was determined by inoculating 1 ml of the sample or its dilution (ISO 7218, 2007) into petri dish and mixed with the Dichloran Rose Bengal Chloramphenicol media (DRBC) (Merk) and incubated at  $25\pm 2^{\circ}\text{C}$  for 2-5 days and the produced colonies were counted (ISO 21527-1, 2008).

### 2.3.4. Detection of *Alicyclobacillus*

The detection of *Alicyclobacillus* was determined by inoculating 10 ml of the pasteurized and cooled samples or its dilutions into petri dish and mixed with the *Bacillus Acidoterrestris* media (BAT) (Merk) and incubated at  $45 \pm 1^{\circ}\text{C}$  for 3-5 days and the produced colonies were counted (IFU standard Method, 2004).

### 2.3.5. Detection of heat resistant moulds (HRM)

The detection of heat resistant moulds was determined by inoculating 50 ml of the pasteurized juice samples in finished product and concentrate samples and exposed to heat shocked in a water bath at  $80^{\circ}\text{C}$  for 30 min. Then cooled samples or its dilutions were transferred to four petri dishes and mixed with the Malt Extract Agar (MEA) (Merk) and

incubated at  $30\pm 2^{\circ}\text{C}$  for 7-14 days, Most viable ascospores were germinated and formed visible colonies which were counted (Beuchat, *et al.*, 2001).

## 3. RESULTS AND DISCUSSION

### 3.1. Microbiological analysis of fruit concentrates (raw juice)

The obtained results of microbiological analysis for the fruit concentrate were summarized in Table 2 and illustrated in the Figure 1; the results indicated that there were 22% of samples didn't show conforming to the standard specifications. The average numbers of non-conforming samples were 3, 3, 4 and 1 recorded in black carrot, guava puree, frozen orange and peach, respectively. The other samples (78%) were free from any contamination including apples, carrot, cocktail, mango, orange high ratio, pineapple, pomegranate, red grapes and white grapes. Microbiological quality of fruit concentrates as raw material should be free from microorganisms to prevent the contamination and spoilage of finished product. In our study the non-conforming fruit concentrate samples result from uncontrolled storage temperature of fruit concentrate and the supplier evaluation system which not completely implemented.

### 3.2. Microbiological analysis of food contact surfaces

Food contact surface samples were collected by sterile cotton swabs from area of  $10\text{ cm}^2$  ( $2\times 5\text{ cm}$ ) from the detected surfaces. Cleaning and sanitation in general represent Sanitation of Standardization Operating Procedures (SSOPs) step which an important process in HACCP system implementation. Post contamination may arise from the food contact surfaces such as tanks, inside the pasteurization system, packaging materials, filling machines, conveyors, soap, lubrication systems and valve seals (Stratford, 2006). Swabs were tested by serial dilution for detection of total plate count and coliform. The results of the samples were summarized in

**Table, 2: Enumeration of different microbiological count in fruit concentrates (Mean  $\pm$  SD of colony count CFU/ml).**

Concentrate type	TPC	Coliform	Molds	Yeasts	Heat Resistant Moulds	<i>Alicyclo-Bacillus</i>
<b>Apple (5)*</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0	0
Non-conforming sample	0	0	0	0	0	0
<b>Black Carrot (3)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	161 $\pm$ 8	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	155-170	0	0
Non-conforming sample	0	0	0	3	0	0
<b>Carrot (3)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0	0
Non-conforming sample	0	0	0	0	0	0
<b>Cocktail (5)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0	0
Non-conforming sample	0	0	0	0	0	0
<b>Guava puree (5)</b>	260 $\pm$ 581.3	0 $\pm$ 0	60 $\pm$ 57.9	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0-1300	0	10-120	0	0	0
Non-conforming sample	1	0	2	0	0	0
<b>Mango (5)</b>	0 $\pm$ 0	0 $\pm$ 0	4 $\pm$ 8.95	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0-20	0	0	0
Non-conforming sample	0	0	0	0	0	0
<b>Frozen orange (5)</b>	920 $\pm$ 766.16	0 $\pm$ 0	20 $\pm$ 44.72	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	400-2000	0	0-100	0	0	0
Non-conforming sample	3	0	1	0	0	0
<b>Orange high ratio (5)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0	0
Non-conforming sample	0	0	0	0	0	0
<b>Peach (4)</b>	1250 $\pm$ 2500	0 $\pm$ 0	750 $\pm$ 1500	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0-5000	0	0-3000	0	0	0
Non-conforming sample	1	0	1	0	0	0
<b>Pineapple (3)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0	0
Non-conforming sample	0	0	0	0	0	0
<b>Pomegranate (5)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0	0
Non-conforming sample	0	0	0	0	0	0
<b>Red grapes (4)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0	0
Non-conforming sample	0	0	0	0	0	0
<b>White grapes (3)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0	0
Non-conforming sample	0	0	0	0	0	0

**Standard limits:** - TPC  $\leq$  100 CFU/ml, Coliform  $<$ 1 CFU/ml, Moulds  $\leq$  100 CFU/ml, Yeasts  $\leq$  10 CFU/ml, HRM  $<$ 1 CFU/10ml and Alicyclobacillus  $<$ 1 CFU/1ml according to **Egyptian standards 686-2(2005)**

\***Apple (5):** Apple ,type of sample and (5) mean number of samples

Table 3 and illustrated in the Figure 2. The results showed that there are 85% from samples not-conforming to the standard specifications. The mean and range results of TPC in non-conforming samples were ranged from 20 to  $5 \times 10^5$  CFU/cm<sup>2</sup> (the standard specification is up to 10 CFU/cm<sup>2</sup> from the detected surfaces). **Moyo and Baudi (2004)** proved that the first day of inspection of quality was 50% which was decreased to 48% on second day analysis on food contact surfaces in a canteen due to the proper sanitation and good hygiene practices. **Lambrechts et al. (2014)**, study showed that 60% from samples not conforming in total plate count. In our study the high percentage 85%

non-conforming indicates that the cleaning method is not effective. The tanks are probably unclean and may act as source for contamination for the products and lead to spoilage and increase the cost of products scrap.

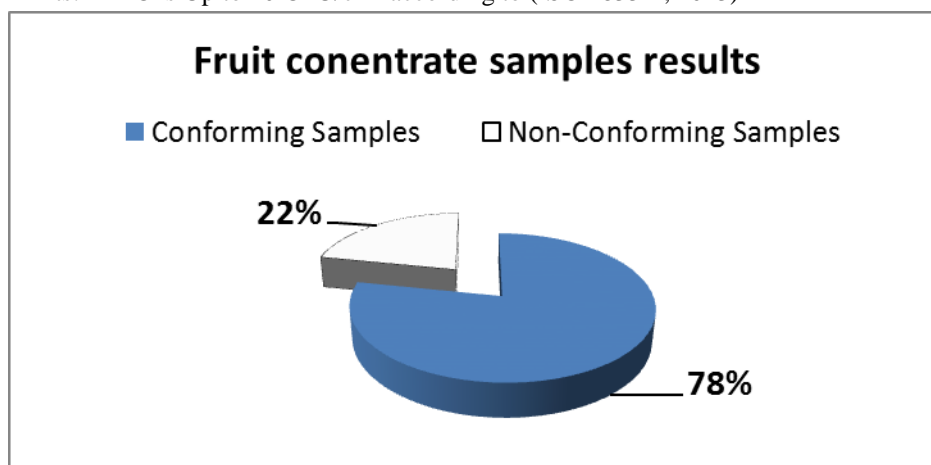
### 3.3. Microbiological analysis of personnel (food handlers)

Food handler surfaces swabs were collected by sterile cotton swabs from an area of 10 cm<sup>2</sup> (2×5 cm) from the detected hands and tested by serial dilution method for total bacterial count and coliform. Personnel hygiene represents an important pre-requisite program and good

**Table. 3: Enumeration of total plate count in food contact surfaces**

Tank No.	No. of Samples	Non-conforming samples	Range of results	T.P.C (CFU/cm <sup>2</sup> ) Mean ± SD
Concentrate Tank no.41	6	2	10-400	153 ± 191
Concentrate Tank no.42	5	2	0-5000	2006 ± 2733
Concentrate Tank no.43	4	1	0-150	82.5 ± 78.9
Concentrate Tank no.45	4	2	0-70	25 ± 31.1
Concentrate Tank no.46	4	0	20-70	42.5 ± 20.6
Concentrate Tank no.48	4	2	0-5000	1258 ± 2495
Rework tank no. 2	8	0	50-3000	856 ± 1324
Rework tank no. 3	5	0	20-5000	1062 ± 2203
Rework tank no. 4	9	0	170-5000	2232 ± 2184
Rework tank no. 5	9	0	100-5000	2013 ± 2264
Rework tank no. 9	12	2	10-5000	1834 ± 2163
Rework tank no. 11	4	1	0-40	25 ± 17.32
Rework tank no. 13	4	0	30-1310	380 ± 621

\*Standard Limits: - TPC is Up to 10 CFU/cm<sup>2</sup> according to (ISO 4833-1, 2013)



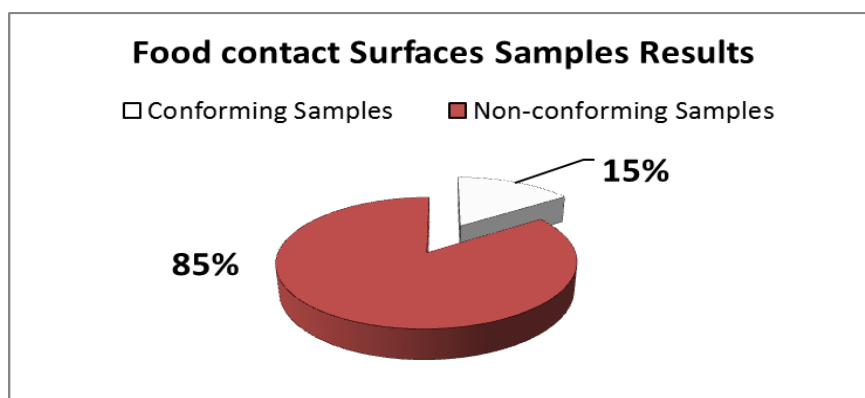
**Figure, 1:** The percentages of conforming and non-conforming results of fruit concentrate results

manufacturing practices in the application of HACCP system and food handlers should be clean and free from any pathogenic microorganisms. The samples were tested by serial dilution for total plate count and coliform. The results of the samples were summarized in Table 4 and illustrated in the Figure 3. The data recorded that 56% of samples were conformed to the standard specification and 44% of samples were non-conformed to the standard specifications. The mean and average results of total plate count in non-conforming samples were ranged from  $1.9 \times 10^2$  to  $5 \times 10^5$  CFU/cm<sup>2</sup> while the standard limit of total plate count is  $10^2$  CFU/cm<sup>2</sup>. **Rehman and Hayat (2016)** study showed that general bacterial growth was appeared on plate count agar (PCA) after serial dilution technique

for the surface swabs of worker hands and bacterial colonies were counted by colony counter and the percentage values of non-conforming samples were (20%) in total plate count while 0 % in *coliform*. In our study non-conforming samples results from the good hygiene practices (GHP) which was not implemented correctly. Also, these non-conformities results from the improper hands cleaning and disinfection and lack of awareness about the good hygiene practices before starting work.

### 3.4. Sampling and analyzing of water

Since juice production depends on water so, water safety is very important in juice production. Water supply plays a vital role in GMP step which is a prerequisite program for



**Figure, 2:** The percentages of conforming and non-conforming results of food contact surfaces swabs.

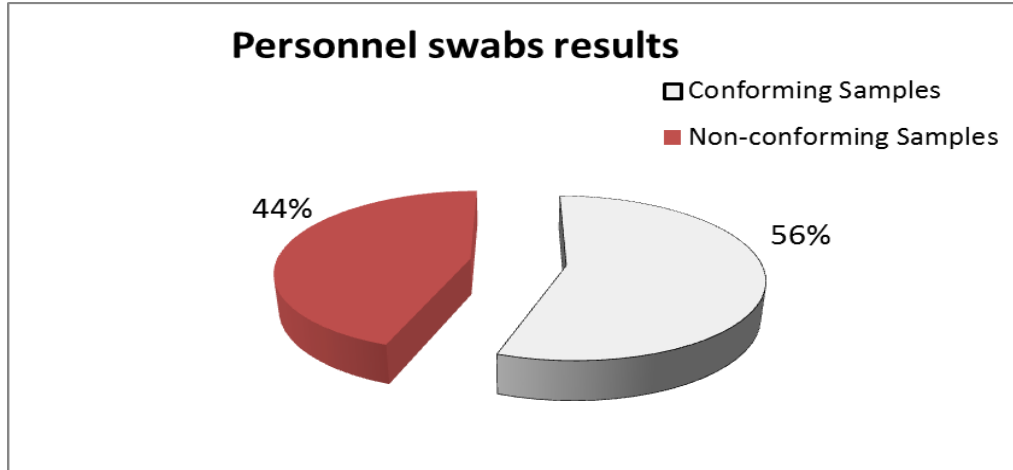
**Table, 4:** Enumeration of microbiological count in personnel hygiene swabs (Mean  $\pm$  SD of colony count CFU/10 cm<sup>2</sup>).

Name of Sample	TPC	Coliform
Daily worker (10)	2825 $\pm$ 2370	0 $\pm$ 0
Range	1070-5000	0
Non-conforming sample	7	0
Machine operator (20)	15.5 $\pm$ 28.37	0 $\pm$ 0
Range	0-90	0
Non-conforming sample	0	0
Processing worker (10)	2083 $\pm$ 2512	0 $\pm$ 0
Range	190-5000	0
Non-conforming sample	8	0
Rework daily worker (5)	1844 $\pm$ 2092	0 $\pm$ 0
Range	370-5000	0
Non-conforming sample	5	0

\*Standard Limits:- TPC up to 100 CFU/10 cm<sup>2</sup> and coliform negative

HACCP system. The microbiological quality of water should be free from pathogenic and spoilage bacteria (Lawlor *et al.*, 2009). Water samples were tested for TPC and coliform. The

results of the samples were summarized in table 5 and illustrated in the figure 4. The results show that 59% from samples were conforming to the standard specification and 41% from



**Figure, 3:** The percentages of conforming and non-conforming of personnel swab results

**Table, 5:** Enumeration of microbiological count in water (Mean  $\pm$  SD of colony count CFU/ml).

Name of Sample	TPC	Coliform
<b>Filtered ingredients (7)</b>	<b>1457<math>\pm</math>2421</b>	<b>0<math>\pm</math>0</b>
Range	<b>200-5000</b>	<b>0</b>
Non-conforming sample	<b>4</b>	<b>0</b>
<b>Production water tank (7)</b>	<b>2294<math>\pm</math>2150</b>	<b>0<math>\pm</math>0</b>
Range	<b>680-5000</b>	<b>0</b>
Non-conforming sample	<b>6</b>	<b>0</b>
<b>Raw water (7)</b>	<b>3571<math>\pm</math>2440</b>	<b>0<math>\pm</math>0</b>
Range	<b>0-5000</b>	<b>0</b>
Non-conforming sample	<b>6</b>	<b>0</b>
<b>Reverse osmosis water (7)</b>	<b>2294<math>\pm</math>2150</b>	<b>0<math>\pm</math>0</b>
Range	<b>140-5000</b>	<b>0</b>
Non-conforming sample	<b>7</b>	<b>0</b>
<b>Storage no.1 (7)</b>	<b>0<math>\pm</math>0</b>	<b>0<math>\pm</math>0</b>
Range	<b>0</b>	<b>0</b>
Non-conforming sample	<b>0</b>	<b>0</b>
<b>Storage no.2 (7)</b>	<b>714<math>\pm</math>1890</b>	<b>0<math>\pm</math>0</b>
Range	<b>0-5000</b>	<b>0</b>
Non-conforming sample	<b>1</b>	<b>0</b>
<b>Tank 1 (7)</b>	<b>0<math>\pm</math>0</b>	
Range	<b>0</b>	<b>0</b>
Non-conforming sample	<b>0</b>	<b>0</b>
<b>Tank 2 (7)</b>	<b>64.3<math>\pm</math>131.4</b>	<b>0<math>\pm</math>0</b>
Range	<b>100-350</b>	<b>0</b>
Non-conforming sample	<b>1</b>	<b>0</b>
<b>Tank 4 (7)</b>	<b>757<math>\pm</math>1872</b>	
Range	<b>200-5000</b>	<b>0</b>
Non-conforming sample	<b>3</b>	<b>0</b>

\***Standard Limits:** - TPC up to 100 CFU/1ml and coliform negative according to Egyptian standards 190-1/2007 and WHO 2006



samples were non-conforming to the standard specifications. The mean and average results of non-conforming samples were ranged from  $1.4 \times 10^2$  to  $5 \times 10^5$  CFU/1ml while the standard specification is up to  $10^2$  CFU/1ml. These non-conformities founded in processing water samples, raw water and storage water tanks. This means that the effectiveness of water treatment is not acceptable and preventive actions must be considered. Also, the cleaning and sanitation of water station and reverse osmosis (R.O) unit filters every four months and this lead to contamination in the treated and processing water.

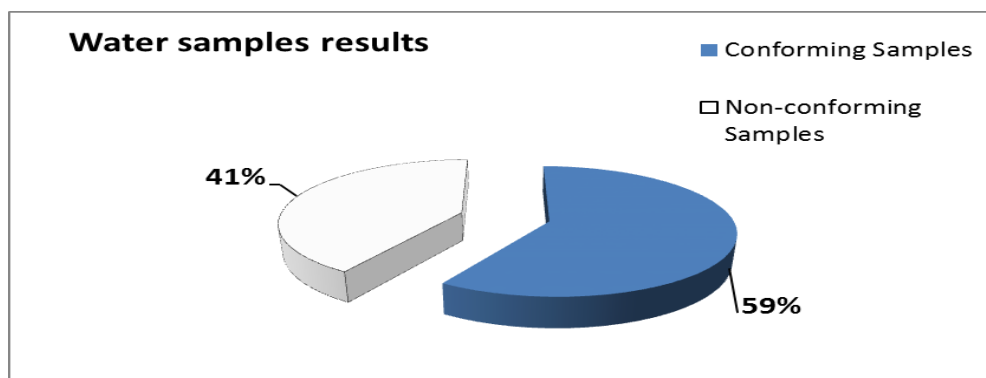
### 3.5. Isolation and analyzing of environmental air

Environmental air evaluation was detected for total plate count, yeast and moulds by settling plate method. Environmental air should be free from microbial contamination and dust particles. Microorganisms may contaminate the air through aerosols of personnel as well as with aerosols developed in the course of plant cleaning with high pressure hoses (Sperber, 2009). The results of the samples were summarized in table 6 and illustrated in the figure 5. The results showed that 78% from samples were conforming to the standard specification and 14% from samples were non-conforming to the standard specifications in yeast and moulds test while 94% from samples were conforming to the standard specifications and 6% from samples non-conforming to the

These high counts of TPC, yeast and moulds were founded in concentrate opening area, small ingredient and storage area. Fowoyo *et al* (2014) observed high percentage of total aerobic bacteria (31.6%), *coliform* (23%), as a result of polluted air emission from cement factory in the environment. These non-conformities in TPC, yeast and moulds indicate that self-closed system of doors is not correctly implemented and the persons not trained enough on the pre requisite programs to keep the inner environment clean and permanently closed.

### 3.5. Sampling and analyzing of finished products

Finished product is a pasteurized juice at  $95 \pm 3$  °C for 26 second and cooled at  $25 \pm 1$  °C then packed in sterile pack. Samples were taken from the pack for microbiological analysis to confirm the safety for the consumers, the standard specifications and effectiveness of HACCP system implementation mainly pasteurization process which is the only CCP in the production process. The results summarized in table 7 and illustrated in the figure 6 showing that 96% from samples were conforming to the standard specification and 4% from samples not-conforming to the standard specifications. Rehman and Hayat (2016) study show that the microbial load is lower in pasteurized fruit juices when compared to homogenized fruit juices before pasteurization. This is due to the



**Figure, 4:** The percentages of conforming and non-conforming of personnel swab results

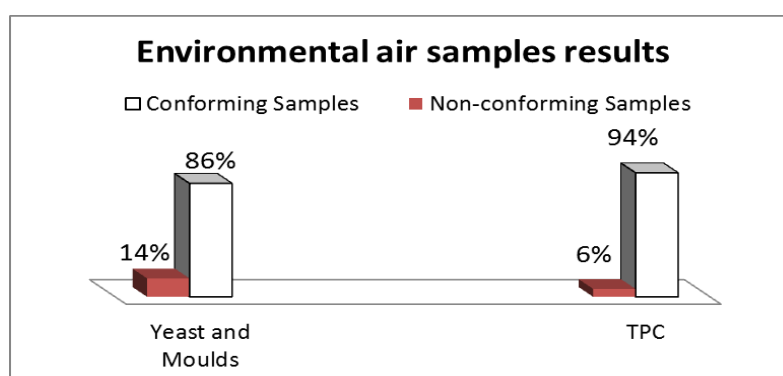
standard specifications in total plate count test.



**Table, 6: Enumeration of microbiological count in environmental air samples (Mean  $\pm$  SD of colony count CFU/cm<sup>2</sup>).**

Name of Sample	TPC	Yeats and Moulds
<b>Concentrate opening area (5)</b>	<b>2002<math>\pm</math>2737</b>	<b>12.6<math>\pm</math>9.1</b>
Range	<b>8-5000</b>	<b>17-21</b>
Non-conforming sample	<b>2</b>	<b>3</b>
<b>Filling machine (20)</b>	<b>0.9<math>\pm</math>2.8</b>	<b>0.65<math>\pm</math>0.8</b>
Range	<b>0-12</b>	<b>0-3</b>
Non-conforming sample	0	0
<b>Filling part 1 (5)</b>	1.4 $\pm$ 1.1	0.8 $\pm$ 0.83
Range	0-3	0-2
Non-conforming sample	0	0
<b>Filling part 2 (5)</b>	1.2 $\pm$ 1.1	2.6 $\pm$ 5.2
Range	0-3	1-12
Non-conforming sample	0	1
<b>Preparation tanks area (5)</b>	2.0 $\pm$ 2.5	1.4 $\pm$ 0.9
Range	0-6	0-2
Non-conforming sample	0	0
<b>Rework area (5)</b>	2.2 $\pm$ 2.1	6.4 $\pm$ 8.1
Range	0-5	12-18
Non-conforming sample	0	2
<b>Small ingredient (4)</b>	0 $\pm$ 0	0 $\pm$ 1
Range	0	0
Non-conforming sample	0	0
<b>Small tanks area (4)</b>	1250 $\pm$ 2500	5.7 $\pm$ 5.9
Range	0-5000	0-13
Non-conforming sample	1	1
<b>Storage area (4)</b>	1259 $\pm$ 2494	3.2 $\pm$ 2.5
Range	3-5000	2-7
Non-conforming sample	2	0
<b>Sugar area no. 1 (4)</b>	2.0 $\pm$ 1.4	6.2 $\pm$ 8.1
Range	0-3	0-18
Non-conforming sample	0	1
<b>Sugar area no. 2 (4)</b>	5.2 $\pm$ 5.5	4.7 $\pm$ 6.9
Range	0-10	0-15
Non-conforming sample	0	1

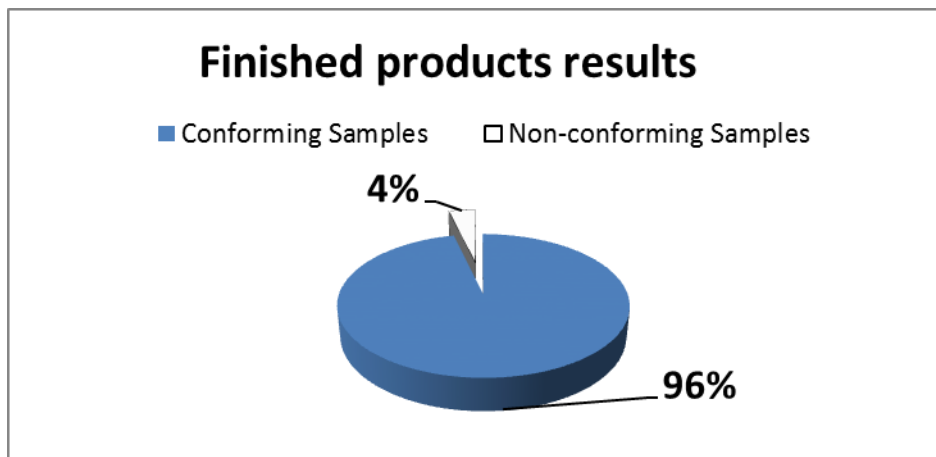
**\*Standard Limits:** - TPC up to 50 CFU/m<sup>2</sup>, yeast and moulds up to 10 CFU/m<sup>2</sup> according to Egyptian standards 190-1/2007 and WHO 2006.

**Figure, 5: The Percentages of conforming and non-conforming of environmental air results**

**Table, 7: Enumeration of different microbiological count in finished products samples (Mean  $\pm$  SD of colony count CFU/ml).**

Product Type	TPC	Coliform	Yeasts and Moulds	HRM	<i>Alicyclo-Bacillus</i>
<b>Classic apple (5)</b>	600 $\pm$ 1342	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0-3000	0	0	0	0
Non-conforming sample	1	0	0	0	0
<b>Classic cocktail (5)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0
Non-conforming sample	0	0	0	0	0
<b>Classic guava (5)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0
Non-conforming sample	0	0	0	0	0
<b>Classic mango (5)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0
Non-conforming sample	0	0	0	0	0
<b>Classic orange (5)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0
Non-conforming sample	0	0	0	0	0
<b>Classic pineapple (3)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0
Non-conforming sample	0	0	0	0	0
<b>Classic pomegranate (3)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0
Non-conforming sample	3	0	0	0	0
<b>Classic red grapes (4)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0
Non-conforming sample	0	0	0	0	0
<b>Pure apple (5)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0
Non-conforming sample	0	0	0	0	0
<b>Pure cocktail (3)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0
Non-conforming sample	0	0	0	0	0
<b>Pure guava (3)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0
Non-conforming sample	0	0	0	0	0
<b>Pure mango peach (3)</b>	0 $\pm$ 0	0 $\pm$ 0	0.67 $\pm$ 1.15	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0-2	0	0
Non-conforming sample	0	0	1	0	0
<b>Pure orange (3)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0
Non-conforming sample	0	0	0	0	0
<b>Pure red grapes (3)</b>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Range	0	0	0	0	0
Non-conforming sample	0	0	0	0	0

\*Standard limits :- TPC  $\leq$  100 CFU/ml, Coliform 0 CFU/ml, Moulds 0 CFU/ml, Yeasts 0 CFU/ml, HRM 0 CFU/10ml and *Alicyclobacillus* 0 CFU/1ml according to **Egyptian standards 1602-1/2017**.



Figure, 6: The Percentages of conforming and non-conforming of finished products results

pasteurization technique that was applied responsible for the reduction of the microbial load. They show that the pasteurization process prevents microbial contamination. Also **Gulhane et al. (2015)** stated that the combination of central process and pasteurization (heat treatment) inhibit the growth of *Pseudomonas* and *Coliform* at concentration of 0.06% applied for 15 minutes while *Klebsiella* and *Enterobacter* were inhibited at concentration of 0.09% for 10 and 5 minutes respectively. On the other hand, **Abisso et al. (2018)** recorded that the average aerobic mesophilic bacteria counts (CFU/ml) of fresh samples of avocado, mango and papaya were respectively  $2.2 \times 10^4$ ,  $1.3 \times 10^4$ , and  $7.4 \times 10^3$ . In our study these non-conformities results from the post contamination case after pasteurization process lead to spoilage of the finished product. The second case results from contaminated raw materials which the pasteurization process not completely prevents this contamination which matched directly with the results of **Kaddumukasa et al. (2017)**.

#### 4-CONCLUSION:-

In absence of good manufacturing processes; the richness of fruits and fruit juices nutrition makes the packed product as favorable medium for microbial contamination. High microbial count results from the use of poor processing techniques and lacks of good

hygiene which lowers quality and reduces stability in storage of juices. Storage temperature greatly reduces physicochemical parameters both at ambient and refrigeration temperatures. This implies that temperature control for unpasteurized juices is critical in order to inhibit microorganism metabolic activities which accelerate bio-deterioration leading to spoilage and short shelf life.

#### REFERENCES:-

- Abisso, T. G. ; Gugero, B. C. and Fissuh, Y. H. (2018):** Physical quality and microbiological safety of some fruit juices served in cafes/juice houses: The case of Hossana town, Southern Ethiopia, *Abisso et al., J Nutr Food Sci*, 8(3): 1-5.
- Back, W. (2005):** *Colour Atlas and Handbook of Beverage Biology*. W. Back (ed.). Verlag Hans Carl: Nürnberg, Germany, p. 317.
- Beuchat, L.R., Pit J.I. (2001):** Detection and enumeration of heat-resistant Molds. *Research gate*, p. 217-222.
- CDC (2010):** Preliminary Food Net data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2009. *Morb. Mortal. Wkly Rep.*, 59: 418–422.
- Codex Alimentarius Commission (CAC), (2012):** "Prevention and reduction of food and feed contamination." Rome, Italy. [ftp://ftp.fao.org/codex/Publications/Booklets/Contaminants/CCCF\\_2012\\_EN.pdf](ftp://ftp.fao.org/codex/Publications/Booklets/Contaminants/CCCF_2012_EN.pdf). Accessed June 2016.
- Dillon M, Griffith C., Zhao M. (2003):** How to HACCP. DN: M.D. Associates South

Humberside. FDA. 2001. NCIMS HACCP Pilot Program Phase II Expansion.

**Essien, E., Monago, C. and Edor, E.A., (2011).** Evaluation of the nutritional and microbiological quality of Kunun (A cereal based non-alcoholic beverage) in Rivers State, Nigeria. *Internet. J. Nutr. Wellness*, **10**. DOI:10.5580/8e7

**Fowoyo, Temitope P, Ogochukwu IE (2014):** Impact of air pollution on the microbiological quality of ready to eat hawked foods sold around a cement factory in Lokoja, Nigeria. *American Journal of Research Communication*. 2(11):138-157.

**Gulhane C, Gulhane D, Danga SK, Ashita Surve, Kalpana Dhuri (2015):** Scientific View towards Suvarnaprashana in Alternative Medicines, *Int J Ayu Pharm Chem*. 3(3).

**IFU STANDARDS (2004):** Method No. 12 on the detection of taint producing Alicyclobacillus in fruit juices. Revision march 2007.

**International Organization for Standardization (ISO) 4832, (2006):** Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique.

**International Organization for Standardization (ISO) 7218, (2007):** Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations.

**International Organization for Standardization (ISO) 21527-1, (2008):** Microbiology of food and animal feedings stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 2: Colony count technique in products with water activity less than or equal to 0, 95

**International Organization for Standardization (ISO) 4833-1, (2013):** consists of the following parts, under the general title Microbiology of the food chain — Horizontal method for the enumeration of microorganisms: Part 1: Colony count at 30 °C by the pour plate technique and Part 2: Colony count at 30 °C by the surface plating technique.

**International Organization for Standardization (ISO) 11133, (2014):** Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media.

**International Organization for Standardization (ISO) 6887, (2017):** Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.

**Kaddumukasa P.P., Imathiu S.M., Mathara J.M. and Nakavuma J.L. (2017):** Influence of physicochemical parameters on storage stability: Microbiological quality of fresh unpasteurized fruit juices. *Food Sci Nutr*. 5:1098–1105

**Lambrechts AA, Human IS, Doughari JH, Lues JFR (2014):** Bacterial contamination of the hands of food handlers as indicator of hand washing efficacy in some convenient food industries. *Pak J Med Sc*. 30(4):755-758.

**Lawlor, K., Schuman, J., Simpson, P. and Taormina, J. (2009):** In: Sperber, W.H. and Doyle, M.P. (eds.) Compendium of the Microbiological Spoilage of Foods and Beverages, *Food Microbiology and Safety*, pp. 245–283, Springer, New York.

**Makiya IK and Rotondaro RG. (2002):** System integration as GMP /HACCP /ISSO 9000 in the food industry. *Food Hygiene Journal*. 16 (99):46–50.

**Moyo DZ, Baudi I (2004):** A Bacteriological Assessment of the cleaning and Disinfection efficacy at the Midland State University Canteen, Zimbabwe, *Pak J Biol Sci*. 7(11):1996-2001.

**Oliver SP, Jayarao BM and Almeida RA (2005):** Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. *Foodborne Pathog. Dis.*, 2: 115–129.

**Rehman H U, Hyat A (2016):** Status of Hygienic Quality and HACCP Implementation in Food Industry. *Pak J Med Sc*. 4(4): 1136-1141.

**Roberto CD, Brandão SCC, da Silva CAB. (2006):** Cost and investments of implementing and maintaining HACCP in pasteurized milk plant. *Food Control*. 2006;17(8):599–603.

**Sperber, W.H. (2009):** Introduction to the Compendium of the Microbiological Spoilage of Foods and Beverages, *Food Microbiology and Safety*, p. 1–39, Springer, New York.

**Stratford, M.; Gökmen, Acar and James S.A. (2000):** Non-alcoholic beverages and yeasts. In: Boekhout, T. and Robert, V. (eds.) *Yeasts in Food*, Hamburg, Germany: B. Behr's Verlag GmbH & Co, Chapter 12, pp.309-345.

**Stratford, M. and James S.A. (2003):** Non-alcoholic beverages and yeasts. In: Boekhout, T.

and Robert, V. (eds.) Yeasts in Food, Hamburg, Germany: B. Behr's Verlag GmbH & Co, Chapter 12, pp.309-345.

**Stratford, M. (2006):** Food and Beverage Spoilage Yeasts, In: Querol, H. and Fleet, G. (eds.) Yeasts in Food and Beverages, Berlin, Germany: Springer-Verlag, Chapter 11, pp. 335–379.

**Tribst, A.A., Sant'Ana Ade, S. and de Massauer, P.R. (2009):** Review: Microbiological quality and safety of fruit juices--past, present and future perspectives. Critical Reviews in Microbiology, Vol. 35, pp. 310-339.

**Wallace C, Williams T. (2001):** Pre-requisites: a help or a hindrance to HACCP? Food Control; 12 (4):235–40.

**World Health Organization,(2006).** Guideline for drinking- Water quality, first addendum to third edition, annex 4 chemical summary table, volume 1- recommendation, 2006

**World Health Organization,(2007).** Food safety and foodborne illness. 2007. [http://www.who.int/foodsafety/foodborne\\_disease/in/](http://www.who.int/foodsafety/foodborne_disease/in/) . Accessed 1 May 2007.

## الملخص العربي

### التقييم الميكروبيولوجي لنظام تحليل المخاطر ونقاط التحكم الحرجة (HACCP) في أحد خطوط إنتاج العصائر في أحد الشركات المحلية

أ.د/ محمد صلاح عزب<sup>١</sup> ، د/ عمرو محمود فوده<sup>١</sup> ، د/ أحمد علي رضوان<sup>١</sup> ، د/ محمود بهاء عمارة<sup>٢</sup> ، أحمد عادل عبيد<sup>٣</sup>

<sup>١</sup> قسم النبات والميكروبيولوجي – كلية العلوم (بنين) – جامعة الأزهر بالقاهرة ،  
<sup>٢</sup> استشاري سلامة وصحة الأغذية – وحدة الجودة – وزارة السياحة – مصر ،  
<sup>٣</sup> مشرف معمل الجودة وسلامة الغذاء باحد مصانع الأغذية والعصائر

أجريت هذه الدراسة لتقييم العوامل الميكروبيولوجية المختلفة لنظام تحليل المخاطر ونقاط التحكم الحرجة (HACCP) في أحد خطوط إنتاج العصائر المبسترة من خلال متابعة برامج الإشتراطات المسبقة (PRP) ، وممارسات التصنيع الجيدة (GMP) ونقاط التحكم الحرجة (CCPs) ، ثم مقارنة النتائج بالموصفات القياسية.

تم جمع ٣٦١ عينة من خطوط الإنتاج المختلفة.

تم استخدام الطرق القياسية لتحديد العد الميكروبي الكلي (TPC) ، الخمائر والفطريات ، بكتريا القولون (الكوليفورم) ، الفطريات المقاومة للحرارة والبكتيريا المحتملة للأحماض (*Alicyclobacillus*) وكانت بعض العينات خارج النطاق القياسي المقبول . بنسبة ٢٠٪ ، ٨٥٪ ، ٣٨٪ ، ٤٤٪ ، ٦٪ و ٤٪ في نسبة العينات من TPC و الخميرة و الفطريات في العينات التي تم جمعها من مراكز الفاكهة ، الأسطح الملامسة للأغذية ، عينات الماء ، مسحات الموظفين ، الهواء البيئي والمنتجات النهائية. ، على التوالي.

ويرجع التلوث الميكروبي في بعض العينات وخاصة مراكز الفاكهة وبعض عينات المنتج النهائي الي حدوث تلوث ميكروبي نتيجة سوء التصنيع للمركبات وتلوث العصير بعد البسترة.

التلوث الناتج في العينات من الاسطح الملامسة للأغذية وفي المنتج النهائي ينتج عنه تكلفه عالية نتيجة لإعدام هذه المنتجات نتيجة تلوثها مما يعني ان التحكم في درجة الحرارة للعصائر المبسترة أمر حاسم من أجل منع الأنشطة الأيضية للكائنات الدقيقة التي تسرع من عملية التدهور الحيوي مما يؤدي إلى تلف وقصر فترة صلاحية المنتج.