# Effect of extracting methods on micro-organisms characterization in orange juice

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**Abstract:** The objective of this experiment was to evaluate the micro-organisms count in the raw and processed orange fruits using the Pour Plate Method and the Spread Plate Method. The study objective was to extract the juice from fresh orange juice using a Juice extractor, to determine the microbial count of orange juice and orange fruit, and to identify and characterize the different microbes present in the raw orange and processed fruit (juice) using different methods. In this study, different samples of orange fruits/juice were analyzed using Pour Plate Method *(PPM)* and Spread Plate Method *(SPM)*. Samples analyzed include Raw orange Fruit (*RF*) serving as control, Manually Extracted orange Juice (*MEJ*), Machine Extracted orange Juice (*MAEJ*), Pasteurized Machine Extracted orange Juice at 85$^{o}$C (*PMAEJ*, 85$^{o}$C) and Refrigerated Machine Extracted orange Juice at 4$^{o}$C (*RMAEJ*, 4$^{o}$C).The micro-organisms count considered include Total Heterotrophic Plate Count (*THPC*), Total Coliform Count (*TCC*), Total Faecal Coliform Count (*TFCC*), and Fungal Count (*FC*). A total of 15 micro-organisms were identified which were *Aeromonashydrophila*,*Bacillussubtilis*,*Micrococcussp*.,*Staphylococcus aureus*, *Staphylococcusepidermis*,*Listeriasp*.,*Lactobacillus fermenti*, *Providenciasp*., *Klebsiellapneumonia*, *Enterobacteraerogenes*, *Sacchoronyeescereoisioe*, *Penicillium sp*., *Aspergillusniger*, *Rhizopusstolonifer*,and *Nucorracemosus*. In all the samples, SPM was able to isolate more micro-organisms than PPM. Raw oranges showed high number of microbial load than any of the four samples while pasteurized orange juice showed the least number of microbial loads. Generally, for all the samples, the THPC ranges from 1.81 x $10^4$ to 0.16 x $10^4$cfumL$^{-1}$, TCC ranges from 0.23 x $10^3$ to 0.00 x $10^3$cfumL$^{-1}$, FCC ranges from 0.00 x $10^1$ to 0.00 x $10^1$cfumL$^{-1}$, and FC ranges from 0.90 x $10^4$ to 0.00 x $10^4$cfu mL$^{-1}$for Pour Plate method. While for spread plate method, THPC ranges from 1.83 x $10^4$ to 1.80 x $10^4$cfumL$^{-1}$, TCC ranges from 0.29 x $10^3$ to 0.00 x $10^3$cfumL$^{-1}$, FCC ranges from 0.00 x $10^1$ to 0.00 x $10^1$cfumL$^{-1}$, and FC ranges from 1.00 x $10^4$ to 0.00 x $10^4$cfumL$^{-1}$.From the two methods used in isolating micro-organisms, it can be recommended that the spread plate method is a more reliable method than the pour plate method in evaluating micro-organisms as it was able to isolate more micro-organisms in all the samples than pour plate method.

**Keywords:** orange, juice, coliform, plate, count

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## 1 Introduction

Fruit juices are liquid, non-alcoholic products with certain degree of clarity and viscosity obtained through pressing or breaking up of fruits with or without sugar or carbon dioxide addition(Akusu et al., 2016). Fruits and its juices constitute one of the most important foods for man. Their regular consumption maintains health and makes up for the losses in the human diet. Costescu et al. (2006)recommended the consumption of juices with pulp

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from foods and medicinal points of view. Nutritional, chemical composition and the effect of storage on various fruits (orange, pineapple and cashew apples) and their juices have been reported by Oguntona and Akinyele (1995) and Auta et al. (2011). Storage conditions on vitamin C and pH value of cashew apple juice was studied by Emelike and Ebere (2015). Many workers have described the changes that may contribute to the increase in diseases associated with the consumption of raw fruits and vegetables in industrialized countries and foods in general (Hedberg et al., 1994).

A healthy fruit surface harbors diverse range of microbes, which maybe the normal microflora, or the microbes inoculated during the processing of fresh produce (Hanklin and Lacy, 1992). However, themicroflora could be plant pathogens, opportunistic pathogens, or non-plant pathogenic species. According to Center for Disease Control and Prevention(CDCP, 1999), among the number of documented outbreaks of human infections associated with consumption of raw fruits, vegetables, and unpasteurized fruit juices, more than 50% of outbreaks occur with unidentified etiological agents. These new outbreaks of fresh-produce-related food poisoning include major outbreaks by tiny culprits as *Escherichia coli* O157:H7, *Salmonella*, *Shigella*, *Cyclospora*, Hepatitis A virus, Norwalk disease virus, on a variety of fruits as oranges, apples and other fruits (Kalia and Gupta, 2006). Orange fruit juice intended for the direct consumption is obtained from the edible portion of the fresh orange fruit by mechanical extraction process and can be preserved exclusive by chemical and physical means must have been concentrated, reconstituted with water suitable for the purpose of maintaining the essential composition and quality factor of the juice. Sugars or acids can be added to juices according to recommendations or personal preferences. Orange fruit juice contains antioxidant components that have health beneficial effects, such as reducing the risk of cancer and heart diseases.(Van Duyn and Pivonka, 2000)

Some food borne illnesses have been reportedly associated with the consumption of fruit juices in several places. Food borne diseases affect the gastrointestinal tract and can be transmitted through consumption of food or drink that has been contaminated with microbial pathogens. Reports suggest that some juices may be potential sources ofbacterial pathogens notably *E. coli* O157:H7, *Salmonella* sp, *Shigella*sp, and *Staphylococcus aureus*. (Aljoudi et al., 2010)

Fresh fruits have an external toughness, may be water proof, wax-coated protective covering, or skin that functions as a barrier for entry of most plant pathogenic microbes. The skin, however, harbors a variety of microbes and so the normal microflora of fruits is varied and includes both bacteria and fungi (Hanklin and Lacy, 1992). These microbes get associated with fruits, since a variety of sources such as the blowing air, composted soil, insects as *Drosophila melanogaster* or the fruit fly inoculate the skin/outer surface with a variety of Gramnegative bacteria (predominantly *Pseudomonas*, *Erwinia*, *Lactobacillus*) (Kalia and Gupta, 2006). Likewise, hand-picking the fresh produce inoculates the fruit surfaces with *Staphylococcus*. Contact with soil, especially partially processed compost or manure, adds diverse human pathogenic microbes generally of the fecal-oral type including the *Enterobacter*, *Shigella*, *Salmonella*, *E. coli* 0157:H7, *Bacillus cereus*, as well as certain viruses such as Hepatitis A Virus, Rotavirus, and Norwalk disease viruses that are transmitted by consumption of raw fruits(Kalia and Gupta, 2006). These microbes are restrained to remain outside on fruit surfaces as long as the skins are healthy and intact. Any cuts or bruises that appear during the postharvest processing operations allow their entry to the less protected internal soft tissue.

The routine culturing techniques require longer time to obtain results. To overcome this hurdle, nowadays, use of indicator organisms that provide rapid, simple, and reliable information without the requirement of isolation and identification of specific pathogens is performed. However, such tests could be used as the presumptive ones with the confirmation provided by a battery of biochemical tests, and may include specialized serological typing also (Swaminathan and Feng, 1994). The convectional microbiological techniques commonly

used include direct microscopic count, aerobic plate counts (APC) or total plate counts (TPC), *Howard Mold Count*,*Yeasts and Mold Counts*,*Thermophilic Spore Count* (Burnett et al., 2000; Kalia and Gupta, 2006;Wiley, 1994).However, modified conventional techniques include *Miniaturized Biochemical Assays*, *Modified Process/Specialized Media*, DNA-Based Assays, Antibody-Based Assays(Hartman et al., 1992; Curiale et al., 1991; Chen and Griffith, 2001; and Zhao et al., 2001).

In Nigeria, ready-to-eat snacks, meals and flavored drinks are sold by street food vendors and consumed by millions of people. This study was carried out to assess the microbial quality of commercially vended orange fruit and its juice with a view to assess their safety for human consumption and as possible sources of microbial pathogens. This research aimed at evaluating, identifying and characterizing the micro-organisms present in raw and processed oranges using different methods.

## 2 Material and methods

### 2.1 Sample collection

A total of 40 freshly harvested matured sweet orange fruits (Valencia) were collected from Ipata market in the Ilorin metropolis Ilorin on latitude 8º24′ N and 83º6′ N and longitude 4º10′ E and 4º 36′ E. Quality traits like uniformity in size, color, shape and abrasion-free were considered in choosing the orange fruits. The selected ones were sorted and washed thoroughly under running water.

### 2.2 Juicing orange fruits

The orange fruits were peeled (Figure 1)and split. 10 out of the 40 oranges were manually squeezed to extract the juice. The remaining 30 orange fruits were processed to orange juice using a fabricated juice extractor as shown in Figure 2. The juice extractor blended and also filtered the juice as shown in Figure 3. The juice was extracted and collected(Figures 4 and 5) and immediately packaged in the sterilized bottles prior to analysis as shown in Figure 6. The orange juice extracted using the machine was split into three parts. One part which was slightly pasteurized at 85ºC, the other part refrigerated at 4ºC and the last part left unpasteurized (raw). Altogether, Four samples were taken to the laboratory for microbial analysis: Manually extracted orange juice, pasteurized orange juice (85ºC), refrigerated orange juice (4ºC), and unpasteurized orange juice.

**Figure 1 Fabricated juice**

**Figure 2 Peeled oranges**

**Figure 3Peeled orangesextractorinside muslin clothduring extraction**

**Figure 4 Extraction process**

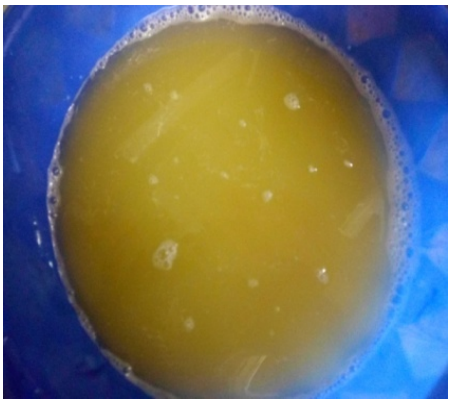

**Figure 5Extracted juice**

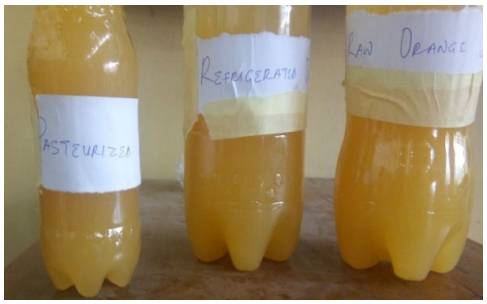



**Figure 6 Orange samples prior to analysis**

## 2.3 Microbial analysis methods

Two methods were used for the microbial analysis of the raw orange samples and extracted orange juice, and they were being evaluated for their relative effectiveness in the qualitative determination of the total number of mold species present in both raw orange samples and processed oranges: the pour plating method and the spread plating method. The micro-organisms count considered for this study include: Total Heterotrophic Plate Count (*THPC*), Total Coliform Count (*TCC*), Total Faecal Coliform Count (*TFCC*), and Fungal Count (*FC*).

### 2.3.1 Serial dilution

All two bacterial plate count methods that were performed in the laboratory required us to serially dilute the samples until we had 30-300 colony forming units (CFU) on the plate. The experimental procedures were performed according to procedures described by McLandsborough (2004) in a Food microbiology laboratory.

### 2.3.2 Pour plate method

The pour plate technique is used to determine the number of microbes/mL in a specimen. It has the advantage of not requiring previously prepared plates, and is often used to assay bacterial contamination of foodstuffs. The pour plate method and procedures below are followed according to Yousef and Ahmed (2003) in Food Microbiology.

### 2.3.3 Spread plate method

The spread plate technique involves using a sterilized spreader with a smooth surface made of metal or glass to apply a small number of bacteria suspended in a solution over a plate. The plate needs to be dried at room temperature so that the agar can absorb the bacteria more readily. A successful spread plate will have a countable number of isolated bacterial colonies evenly distributed on the plate (McLandsborough, 2004). The spread plate procedures were followed according to procedures described by Maheshwari (2002).

## 2.4 Colony forming unit

Procedure

1. Make a dilution series from a sample. (Serial dilution procedure)
2. Pipette out 0.1 ml from the appropriate desired dilution series onto the center of the surface of an agar plate.
3. Dip an L-shaped glass spreader into alcohol.
4. Flame the glass spreader over a Bunsen burner.
5. Spread the sample evenly over the surface of agar using the sterile glass spreader, carefully rotating the

Petri dish underneath at the same time.

6. Incubate the plate at 37°C for 24 hours.

7. Calculate the CFU value of the sample. Once you count the colonies, multiply by the appropriate dilution factor to determine the number of CFU/ml in the original sample.

8. Colony Forming Unit (CFU)/Amount Plated X Dilution Factor = Colony Forming Unit (CFU)/ml

### 2.5 Statistical analysis

The data generated from the experiment were analyzed using descriptive statistics are brief **descriptive** coefficients that summarize a given data set.

## 3 Results and discussion

The data obtained from the experiment were analyzed using descriptive statistics and the result was summarized inTable 1. The results showed that the different orange samples had different micro-organisms count as analyzed *via* the two methods (PPM and SPM). Irrespective of the method of analysis used, there are higher micro-organisms counts on raw orange fruits (RF) than other samples. This is accordance to the previous studies made by Reddi et al. (2015). This might suggest that the methods of analysis and /or orange samples used in this study affect the micro-organisms count. This was also observed by Sanders (2012) who used five different methods to evaluate micro-organisms and got different results. This means that micro-organisms count in the various orange samples used for this study is a function of *orange sample type* and *methods of analysis*. A cursory look at Table 1 shows that micro-organisms count seems to have higher count in SPM than PPM. The next section will use a more robust statistical analysis to draw a valid conclusion on this observed phenomenon.

**Table 1 Summary statistics of the data generated**

| Sample | Statistics | Pour plate method(CFUmL$^{-1}$) | | | | Spread plate method(CFU mL$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | THPC | TCC | TFCC | FC | THPC | TCC | TFCC | FC |
| Raw Fruit (*RF*) | Mean | 181 | 23 | 0 | 5 | 183 | 29 | 0 | 9 |
| | Std. Deviation | 2 | 1 | 0 | 1 | 2 | 2 | 0 | 1 |
| | Maximum | 183 | 24 | 0 | 6 | 185 | 30 | 0 | 10 |
| | Minimum | 179 | 22 | 0 | 4 | 181 | 27 | 0 | 8 |
| Manually Extracted Juice *(MEJ)* | Mean | 30 | 13 | 0 | 2 | 35 | 18 | 0 | 2 |
| | Std. Deviation | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 |
| | Maximum | 31 | 14 | 0 | 2 | 36 | 19 | 0 | 3 |
| | Minimum | 29 | 12 | 0 | 2 | 34 | 17 | 0 | 2 |
| Machine Extracted Juice (*MAEJ*) | Mean | 37 | 3 | 0 | 9 | 41 | 5 | 0 | 10 |
| | Std. Deviation | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 |
| | Maximum | 38 | 3 | 0 | 10 | 42 | 5 | 0 | 11 |
| | Minimum | 36 | 3 | 0 | 8 | 40 | 4 | 0 | 10 |
| Pasteurized(85°C) Machine Extracted Juice (*PMAEJ*, 85°C) | Mean | 16 | 0 | 0 | 0 | 18 | 0 | 0 | 0 |
| | Std. Deviation | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| | Maximum | 16 | 0 | 0 | 0 | 18 | 0 | 0 | 0 |
| | Minimum | 16 | 0 | 0 | 0 | 17 | 0 | 0 | 0 |
| Refrigerated(4°C) Machine Extracted Juice (*RMAEJ*, 4°C) | Mean | 20 | 0 | 0 | 5 | 23 | 0 | 0 | 5 |
| | Std. Deviation | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | Maximum | 20 | 0 | 0 | 5 | 24 | 0 | 0 | 5 |
| | Minimum | 20 | 0 | 0 | 5 | 22 | 0 | 0 | 4 |

Note: Total Heterotrophic Plate Count (THPC), Total Coliform Count (TCC), Total Faecal Coliform Count (TFCC), and Fungal Count (FC).

### 3.1 Effect of method of analysis (PPM and SPM) on micro-organisms count

This section investigates the effect of two methods (PPM and SPM) on micro-organisms count. The independent t-test which tests the significant differences between two independent samples was used and the result was as presented inTable 2. The following inferences can be drawn from Table 2.

***a. Raw fruits***: the average THPC count ($u_1$) observed in PPM was not significantly higher or lower than the average THPC count ($u_2$) in SPM. This is in accordance to previous studies made by Treuhaft (1982).However, the average TCC (23CFU mL$^{-1}$) and FC (5CFU mL$^{-1}$) count in PPM were significantly lower than the average TCC (29CFU mL$^{-1}$) and FC (9CFU mL$^{-1}$) count observed in SPM. This is also in accordance to Hoben (1982) who suggested that SPM produced more count than PPM.

***b. Manually extracted juice***; the THPC (35CFU mL$^{-1}$)

and TCC (18CFU $mL^{-1}$) counts in SPM were significantly higher than THPC (30CFU $mL^{-1}$) and TCC (13CFU $mL^{-1}$) observed in PPM. There was however no significant difference in FC counts of both methods.

***c. Machine extracted juice***; Similarly, THPC (41CFU $mL^{-1}$) and TCC (5CFU $mL^{-1}$) count were significantly higher in SPM compare to THPC (37CFU $mL^{-1}$) and TCC (3CFU $mL^{-1}$) observed in PPM. This implies that PPM significantly reduces the micro-organisms count (THPC and TCC) in machine extracted juice than SPM method.

***d. Pasteurized machine extracted juice at 85°C***; under this sample, only THPC count was observed for both methods because coliform, faecal coliform and fungi could not survive at that temperature (85°C). The test showed that THPC count was significantly lower in PPM than in SPM. Again, it could be concluded that PPM significantly reduced the THPC count in pasteurized machine extracted orange juice than SPM. Similar conclusion can also be drawn for refrigerated machine extracted juice at 4°C.

**Table 2Independent T-test on effect of micro-organisms count**

| Orange Sample | Sample | Test | T Statistics | Df | Sig. | MD | SED | 95% CI Lower | 95% CI Upper |
|---|---|---|---|---|---|---|---|---|---|
| RF | THPC | $u_1$-$u_2$ | -1.23 | 4 | 0.288 | -2 | 1.633 | -6.53 | 2.53 |
| | TCC | " | -5.38 | 3 | 0.009* | -6 | 1.054 | -8.79 | -2.55 |
| | FC | " | -4.90 | 4 | 0.008* | -4 | 0.816 | -6.27 | -1.73 |
| *MEJ* | THPC | " | -6.12 | 4 | 0.004* | -5 | 0.816 | -7.27 | -2.73 |
| | TCC | " | -6.12 | 4 | 0.004* | -5 | 0.816 | -7.27 | -2.73 |
| | FC | " | -1.00 | 2 | 0.423 | 0 | 0.333 | -1.77 | 1.10 |
| *MAEJ* | THPC | " | -4.90 | 4 | 0.008* | -4 | 0.816 | -6.27 | -1.73 |
| | TCC | " | -5.00 | 2 | 0.038* | -2 | 0.333 | -3.10 | -0.23 |
| | FC | " | -2.00 | 3 | 0.134 | -1 | 0.667 | -3.38 | 0.72 |
| *PMAEJ*, 85°C | THPC | " | -5.00 | 2 | 0.038* | -2 | 0.333 | -3.10 | -0.23 |
| | TCC | " | | | | | | | |
| | FC | " | | | | | | | |
| *RMAEJ*, 4°C | THPC | " | -5.00 | 2 | 0.038* | -3 | 0.667 | -6.20 | -0.47 |
| | TCC | " | | | | | | | |
| | FC | " | 1.00 | 2 | 0.423 | 0 | 0.333 | -1.10 | 1.77 |

Note: *significant at 5% level. CI=confidence interval, MD= mean difference, SED=standard error of mean difference

### 3.2 Effect of orange fruit/juice sample on micro-organisms count.

The analysis of variance test on Table 3 shows the effect of orange sample on micro-organisms count. The test shows that using either method, micro-organisms count differs significantly across the various samples used. This confirms the earlier assertion that different samples had significantly different micro-organisms counts with the raw sample having the significantly higher micro-organisms count in comparison to all other samples, irrespective of micro-organism under consideration. The high micro-organism counts in the raw samples are in accordance with previous studies made by Reddiet al. (2015). The next section will consider individual micro-organism count across sample.

**Table 3 Analysis of variance Test on effect of sample on micro-organism count**

| Method | Variable | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|---|
| | | Between Groups | 58664.400 | 4 | 14666.10 | 12220.000 | 0.000* |
| | THPC | Within Groups | 12.000 | 10 | 1.20 | | |
| | | Total | 58676.400 | 14 | | | |
| | | Between Groups | 1208.400 | 4 | 302.10 | 755.250 | 0.000* |
| Pour Plate | TCC | Within Groups | 4.000 | 10 | 0.40 | | |
| | | Total | 1212.400 | 14 | | | |
| | | Between Groups | 140.400 | 4 | 35.10 | 87.750 | 0.000* |
| | FC | Within Groups | 4.000 | 10 | 0.40 | | |
| | | Total | 144.400 | 14 | | | |
| Spread Plate | THPC | Between Groups | 57754.667 | 4 | 14438.67 | 9417.000 | 0.000* |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Within Groups | 15.333 | 10 | 1.53 | | |
| | Total | 57770.000 | 14 | | | |
| | Between Groups | 1921.600 | 4 | 480.40 | 655.091 | 0.000* |
| TCC | Within Groups | 7.333 | 10 | 0.73 | | |
| | Total | 1928.933 | 14 | | | |
| | Between Groups | 228.933 | 4 | 57.23 | 143.083 | 0.000* |
| FC | Within Groups | 4.000 | 10 | 0.40 | | |
| | Total | 232.933 | 14 | | | |

Note: *significant at 5% level

**THPC**

The new Duncan multiple range tests in Table 4 show the different mean values of micro-organism counts disaggregated by orange samples and methods of analysis employed for the experiment. THPC count was significantly higher in raw orange juice (181CFU $mL^{-1}$), followed by machine extracted juice (37CFU $mL^{-1}$), and then manually extracted juice (30CFU $mL^{-1}$). Pasteurized Machine Extracted Juice at 85ºChas statistically lowest THPC count at 5% level of significant after Refrigerated Machine Extracted Juice at 4ºC. This is in accordance to the work done by Batool et al.(2013) who also observed high THPC in raw juice compared to the pasteurized juice.

**TCC**

The average TCC count in raw orange juice was 23CFU $mL^{-1}$ which were significantly higher than TCC count of 13CFU $mL^{-1}$ observed in manually extracted orange juice. Only TCC count of about 3CFU $mL^{-1}$ was observed in machine extracted juice. No TCC count was observed in Pasteurized Machine Extracted Juice at 85ºCand Refrigerated Machine Extracted Juice at 4ºC. This is also in accordance to work done by Batool (2013) who also observed high TCC in raw juice compared to the pasteurized juice.

**FC**

FC count in machine extracted juice (FC=9CFU $mL^{-1}$) was significantly higher than that in all other samples. FC count in raw sample and Refrigerated Machine Extracted Juice at 4ºCwere same on the average (FC=5CFU $mL^{-1}$ respectively) which was significantly higher than FC count observed in manually extracted orange juice. No FC count was observed in Pasteurized Machine Extracted Juice at 85ºC.

The above inferences were based on the results of the experiment under Pour Plate Method (PPM), however, similar pattern was observed in Spread Plate Method (SPM) except that the magnitude of micro-organisms observed under the later method was higher. Therefore, same inferences drawn for the former method also applies to the later method. This is in accordance with Reasoner (2004) who concluded that SPM was a more accurate method than PPM in microbial evaluation.

**Table 4 Multiple comparison using the New Duncan Range Test**

| Method | Sample | THPC | TCC | FC |
|---|---|---|---|---|
| Pour Plate | Raw Fruit | 181a | 23a | 5a |
| | Manually Extracted Juice | 30b | 13b | 2b |
| | Machine Extracted Juice | 37c | 3c | 9c |
| | Pasteurized ($85^0$c) Machine Extracted Juice | 16d | 0d | 0d |
| | Refrigerated Machine Extracted Juice ($4^0$c) | 20e | 0d | 5a |
| Spread Plate | Raw Fruit | 183a | 29a | 9a |
| | Manually Extracted Juice | 35b | 18b | 2b |
| | Machine Extracted Juice | 41c | 5c | 10c |
| | Pasteurized($85^0$c) Machine Extracted Juice | 18d | 0d | 0d |
| | Refrigerated Machine Extracted Juice ($4^0$c) | 23e | 0d | 5e |

Note: Mean with same alphabet are not significantly different from each other.

**3.3Identification and characterization of bacterial isolates**

Table 5 and 6 show the occurrence of bacterial and fungal isolates respectively in the samples.

Bacterial identified in the raw fruits were Aeromonashydrophila, Bacillus subtilis, Micrococcus sp, Staphylococcus epidermis, Listeria sp., Providenciasp. and Enterobacteraerogenes. While bacterial observed in the manually extracted juice were Aeromonashydrophila, Bacillus subtilis, Staphylococcus epidermis, Staphylococcus aureus, Listeria sp., and Klebsiellapneumonia. This is in accordance to the work done by Aneja et al.(2014) who also identified Bacillus

subtilis and Listeria spin unpasteurized orange juice. Staphylococcus aureus and Klebsiella pneumonia may have found their way to the juice because of improper sanitation of the hands while manually extracting the juice. Bacterial identified in the machine extracted juice are Aeromonashydrophila, Bacillus subtilis, Micrococcus sp, Staphylococcus aureus, Lactobacillus fermenti, Providenciasp., Klebsiella pneumonia, and Enterobacteraerogenes. The presence of Aeromonashydrophila and Staphylococcus aureus in the raw fruits, machine extracted juice and the manually extracted juice is a primary concern because these bacteria have been associated with a number of outbreaks associated with fruit juice as reported by Mosqueda-Melgar et al.(2008).

**Table 5 Occurrence of bacterial isolates in the samples**

| Bacterial Isolates | Raw fruits | Manually extracted juice | Machine extracted juice | Pasteurized juice($85^0$c) | Refrigerated ($4^0$c) |
|---|---|---|---|---|---|
| *Aeromonas Hydrophila* | + | + | + | - | - |
| *Bacillus subtilis* | + | + | + | + | + |
| *Micrococcus sp* | + | - | + | + | + |
| *Staphylococcus aureus* | - | + | + | + | - |
| *Staphylococcus Epidermis* | + | + | - | - | - |
| *Listeria sp.* | + | + | - | - | + |
| *Lactobacillus fermenti* | - | - | + | + | + |
| *Providencia sp.* | + | - | + | - | - |
| *Klebsiella pneumonia* | - | + | + | - | - |
| *Enterobacter aerogenes* | + | - | + | - | - |

Bacillus subtilis, Micrococcussp, Staphylococcusaureus and Lactobacillus fermenti were the only bacterial identified in the pasteurized juice, while Bacillus subtilis, Micrococcus sp, Listeria sp., and Lactobacillus fermenti were the only bacterial identified in the refrigerated juice.

Fungi identified in the raw fruits and manually extracted juices are *Sacchoronyeescereoisioe*, *Penicilliumsp*. *Aspergillusniger*, *Rhizopusstolonifer*. This fungi isolate was also discovered in the work done by Aneja (2014). While fungi identified in the machine extracted juice were *Sacchoronyeescereoisioe*, *Penicilliumsp*. and *Rhizopusstolonifer*.

*Sacchoronyeescereoisioe* was the only fungus identified in the pasteurized juice. *Sacchoronyeescereoisioe* and *Nucorracemosus* were the only fungi identified in the refrigerated juice.

*Sacchoronyeescereoisioe*, *Penicilliumsp*. and *Aspergillusniger* are responsible for spoilage of fruits as reported by Aneja (2014).

**Table 6 Occurrence of fungal isolates in the samples**

| Fungal isolates | Raw fruits | Manually extracted juice | Machine extracted juice | Pasteurized juice($85^0$c) | Refrigerated ($4^0$c) |
|---|---|---|---|---|---|
| ***Sacchoronyeescereoisioe*** | + | + | + | + | + |
| ***Penicillium sp.*** | + | + | + | - | - |
| ***Aspergillusniger*** | + | + | - | - | - |
| ***Nucorracemosus*** | - | - | - | - | + |
| ***Rhizopusstolonifer*** | + | + | + | - | - |

## 4 Conclusions

From the results, juice squeezed from raw orange fruits contains microorganisms which are potentially hazardous to public health. Juices were spoiled with high level of bacteria and fungi. The presence of pathogenic microorganisms in juices is clearly indication of food borne outbreaks. Regardless of whether the juice was extracted manually or with a juice extractor, it still showed high level of microbial load and presence of bacteria and fungi. The microbial load reduced considerably after the juice was split and pasteurized at 85°C and also refrigerated at 4°C.From the two methods used in isolating micro-organisms, it can be concluded that the spread plate method is a more reliable method than the pour plate method in evaluating micro-organisms as it was able to isolate more micro-organisms in all the samples than pour plate method.

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