**Bacterial Load, Incidence of *Escherichia Coli* and Proximate Analysis of Locally Produced Orange Juice in Gwagwalada**

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**Abstract:** The study on the bacterial load, incidence of *Escherichia coli* and proximate analysis of locally produced orange juice in Gwagwalada was conducted. The bacterial load, incidence of *Escherichia coli* was carried out using the Spread Plate Technique. The proximate values of the orange juice samples from five different locations which include Gwagwalada market, Phase I, Kaswandare, Phase 3 and Dagiri. Some of the juices contain impressive amounts of minerals, crude lipid, vitamin C, ash and moisture content. The moisture content ranged from 80.95±5.80 % to 94.16±6.18, with sample from Gwagwalada market having the highest (94. 16 %) while the least was recorded by sample from Kasuandare (80.95±5.80%). Vitamin C content ranges from 0.013±0.08 to 0.015±0.08 mg/100ml. The mineral content in the orange juice include calcium with an average concentration ranging from 20.2± 1.08 ppm to 26.2± 2.21, Magnesium (ranges from 12.4± 0.8 to 15.5± 0.9 ppm), low amounts of Fe (between 0.20± 0.07 to 0.50± 0.09 ppm) and Cu (0.10 ± 0.01 to 0.13± 0.01 ppm) were obtained in the juice. The juice was relatively acidic with pH values (at 25°C) ranging from 4.0 to 5.4, which was within the optimum pH range for fruit juices. Kasuwandere recorded the highest isolated *Escherichia coli* with 33.33 %, followed by Gwagwalada market and Phase III with 20 % each, Phase 1 and Dagiri with equal values have the least incidence of *Escherichia coli* in the orange juice with 13.33 % each. The result obtained from this study showed that the total bacteria count of the locally produced orange juice in Gwagwalada ranged from 1.2x105±0.40 to 6.6x107 ±0.13 CFU/ml for all the samples from the five different locations. The bacterial load, incidence of *Escherichia coli* were not significantly different at p < 0.05 level of significant.

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**Keywords:** Juice, *Escherichia coli*

**1. Introduction**

Most of the microorganisms present in fresh fruits are saprophytes, such as *coryniforms,* lactic acid bacteria, spore-formers, *coliforms, micrococci*, and pseudomonas, derived from the soil, air, and water (Doyle, 1991). Juice is a liquid naturally contained in fruits or vegetable tissues. Juice is prepared by mechanically squeezing or macerating fresh fruits or vegetables without the application of heat or solvent (Abdalla *et al*., 2009). Juice may be prepared in the home from fresh fruits and vegetables using variety of hand or electric juicers. Juice may be in concentrate form, sometime frozen, requiring the user to add water to constitute the liquid back to its “original state” (Abdullahi *et al*., 2005). However, concentrates generally have a noticeable different taste than their comparable “freshly squeezed” versions. Other juices are reconstituted before packaging for retail sale. Fruit juices consist of 100% pure juices and generally has no added ingredients (Aboloma, 2008). Although some minor exceptions exist like in cases where salt is added to Tomato juice to ensure the final product is of an acceptable taste. Also the juice may have been concentrated and later reconstituted with water suitable for the purpose of maintaining the essential composition and quality of the juice. Fruit juices account for more than 90% of the total fruit production in Nigeria (Adebayo-Tayo *et al*., 2012). The western part of Nigeria is the principal fruit juice producing region in the country (Adegoke *et al*., 1993). In fact, the concept of maintaining a fruit desired for juicing in its whole, intact form until the juicing is needed continues to be a sound principle (Aminu *et al*., 2006). It has been known that fruits constitute commercially and nutritionally important indispensable food commodity (Akintobi *et al*., 2002). Fruits play a vital role in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in human daily diet and that can help to maintain a good and normal body (Al-Hindi *et al*., 2011).

*Pseudomonas* and the group of *Klebsiella-Enterobacter-Serratia* from the enterobacteriaceae are the most frequent (Durgesh *et al*., 2008). Fungi, including *Aureobasidium, Fusarium*, and *Alternaria*, are often present but in relatively lower numbers than bacteria. Due to the acidity of raw fruits, the primary spoilage organisms are fungi, predominantly moulds and yeasts, such as *Sacharomyces cerevisiae*, *Aspergillus niger, Penicillum spp., Byssochlamys fulva*, *B. nivea*, *Clostridium pasteurianum*,and *Lactobacillus spp*., *Coletotrichum gloesporoides*, *Clostridium perfringes* (Droby, 2006; Durgesh *et al*., 2008).Psychrotrophic bacteria are able to grow in fruit and vegetable products; some of them are *Erwina carotovora*, *Pseudomonas fluorescens, P. auriginosa, P. luteola, Bacillus species, Cytophaga jhonsonae, Xantomonas campestri*, and *Vibrio fluvialis* (Durgesh *et al*., 2008). The existence of pathogenic bacteria in fruit juice products has been reported by Jawetz *et al*. (1998), which include *Listeria monocytogenes, Aeromonas hydrophila,* and *Escherichia coli* O157: H7. These bacteria are found in both fresh and minimally processed fruit and vegetable products. The aim of this study was to assess the bacterial load, incidence of *Escherichia coli* and proximate analysis of locally produced orange juice in Gwagwalada.

**2.0 Materials And Methods**

**2.1 Study area**

This research work was carried out at the Laboratory of the Department of Biology, School of Sciences, Federal Capital Territory College of Education, Zuba-Gwagwalada, Abuja, Nigeria.

**2.2 Collection of Samples**

A total of twenty (20) packs fresh locally produced orange juices were purchased randomly from five (5) different locations in Gwagwalada with four (4) samples each. Juice were purchased from Gwagwalada market, Phase I, Kasuandare, Dagiri as well as Phase III and transferred to the department of Microbiology laboratory of the University of Abuja for analyses.

**2.3 Media and Sterilizations**

Media used include: Nutrient agar (Made Micro), Mac Conkey agar (Made Micro) and Eosine Methylene Blue agar (Made Micro). The media were prepared according to the manufacturers’ instructions.

**2.4 Proximate Analysis**

The fruit juices were analysed for moisture content, vitamin C content, crude lipid and selected minerals such as calcium, copper, lead, magnesium as well as iron by Atomic Absorption Spectrophotometer (AAS). Similarly, the fruit juices were evaluated for fibre content and pH according to Amusa and Ashaye (2009).

**2.4.1 Moisture Content**

This test was carried out according to Amusa and Ashaye (2009). The sample was weighed into a pre-weighed crucible and placed in an oven at 105 0C for 3 hours. It was cooled in desiccators and weighed again. The moisture content was calculated thus:

Moisture Content (%) = W1-W2 X 100

 W1-W0

Where W1 = Weight of sample and crucible

W2 = Weight of sample and crucible after drying

W0 = Weight of crucible

**2.4.2 Crude Lipid**

About 100 ml of the orange juice samples were placed in a funnel and 20 ml of petroleum spirit was added and allowed to stand, the upper layer (petroleum spirit layer) was collected in pre-weighed beaker and the solvent evaporated, the beaker was reweighed and the weight recorded (Amusa and Ashaye, 2009). The crude lipid was calculated thus:

Crude Lipid = W1-W2 X 100

W0

Where W1 = Weight of sample

W2 = Weight of sample and beaker after drying the solvent

W0 = Weight of empty beaker

**2.4.3 Ash Content**

The samples were weighed into a pre-weighed crucible and then placed in a muffle furnace at 55 0C for 5 hours. They were cooled in desiccators and then weighed. Percentage ash content was calculated thus:

Ash Content (%) = W1-W2 X 100

W1-W0

Where W1 = Weight of sample and Petri dish

W2 = Weight of sample and crucible after drying

W0 = Weight of Petri dish

**2.4.4 Vitamin C**

Twenty-five milliliter (25 ml) of the juices samples were pipette into conical flasks, 1 ml of starch indicator solution was added to the sample and then titrated against iodine solution in the burette until a blue-black colouration was observed and the average titre value of three replicate values were determined (Amusa and Ashaye, 2009). The vitamin C content was calculated thus:

Vitamin C (g) = M x 0.25 g

21.50 cm3

Where, M = Average titre value of the juice

0.25 g of ascorbic acid was equivalent to 21.50 cm3.

**2.4.5 pH determination**

The sample was poured into a beaker and the pH meter was switched on, electrode was placed into a buffer which was then rinsed with distilled water and then placed in the sample. The pH of the samples were then read and recorded.

**2.5 Isolation of *E. coli* and bioloads**

Every juice pack was wiped with cotton wool soaked with 70 % alcohol and opened using a sterile surgical blade. The samples were subjected to microbiological analysis where plate count of *E. coli* was determined. One milliliter (1ml) of each fruit juice was mixed with 10 ml of sterile buffered peptone water and homogenized by manual shaking. Ten fold dilutions of solution were then made up to 10-8 and 0.1 ml was inoculated on the Nutrient agar and MacConkey agar using the spread plate method. The plates were incubated at 37o C for 24 hours and the number of colonies were counted using colony counter. The colonial density was calculated as the count multiplied by the dilution factor (Amusa and Ashaye, 2009).

**2.5.1 Preparation of Pure Cultures of Isolated *E. coli* and bioloads**

Representative of each colony type of the young colonies ofbacteria on MacConkey agar were aseptically transferred to freshly prepare sterile Eosin Methylene Blue agar to obtain pure cultures. The pure cultures were maintained in nutrient agar slants and stored at 4o C. Every isolate was resuscitated by sub-culturing for further studies (Cheesebrough, 2006).

**2.6 Identification of *E. coli***

Isolates obtained were identified on the basis of biochemical tests, Gram staining reactions which include the microscopic features and morphological assessment through macroscopic features. Among the characteristics used are: colonial characteristics such as size, surface appearance, texture and colour of the colonies (Cheesebrough, 2006).

**2.6.1 Cultural Characteristics**

Colonies were observed for size, texture, colour, shape, colony surface and edges/margin.

**2.6.2 Biochemical Tests**

The biochemical characteristics used include; oxidase test and IMViC test (citrate utilization test, indole test, methyl red and voges-proskauer test).

**2.6.2.1 Indole Test**

The test organism was inoculated in a bijou bottle containing 3 ml of sterile tryptone water and incubated at 37oC for 48 hours. To test for indole, 0.5ml of Kovac’s reagent was added and shaken gently to examine for a red colourring in the surface layer within 10 minutes. Red surface ring layer indicates positive indole test (Cheesbrough, 2006).

**2.6.2.2 Citrate Utilization Test**

This test was carried out by inoculating the slope and stabbing the butt of a 5 ml Simmon’s citrate agar with the test organism. An uninoculated control was setup in each case. These were incubated at 37oc for 48hours, growth indicated that the organism was able to use citrate as a sole carbon source and was usually accompanied by the medium turning from green to bright blue (Cheesebrough, 2006).

**2.6.2.3 Methyl Red Test and Voges-proskauer test**

The test organism was inoculated in peptone water and incubated for 24 hours. Prepared peptone water plus 0.5 g of D glucose plus 0.5g of potassium pallidium was sterilize and then allowed to cool and 2.5 ml of the test organism in peptone water was added and incubated for 24 hours. On addition the appearance of red color indicates positive reaction for methyl red. To the remaining 4ml broth in the stock bottle, 5 drops of 40% potassium hydroxide was added followed by 15 drops of 5% α-naphtha in ethanol and shake. The cap of the bottle was loosen and placed in a sleeping position within one hour of standing. No change in coloration indicates negative reaction for Voges-proskauer test.

**2.6.2.4 Oxidase Test**

A piece of filter paper was placed in a clean petri dish and three (3) drops of freshly prepared oxidase reagent was added using a glass rod, a colony of the test organism was removed and smeared on the filter paper. It was observed for the development of a blue-purple colour within a few seconds (Cheesebrough, 2006).

**2.7 Determination of Frequency of Occurrence of Bacterial Isolates**

The frequency of occurrence was determined by taking the sum of all the numbers of Cfu/ml of the organisms in each sample and the percentage was calculated as:

 Number of each Isolates × 100

|  |
| --- |
|  Total number of Isolates |

**2.7.1 Statistical Analysis**

Isolated bacteria were statistically analyzed using Analysis of Variance (ANOVA) from Ms Excel Statistics. Test applied was F-test statistic at p < 0.05.

**3.0 Results**

The results of the tests carried out in this research are reported below:

A total of 20 locally produced orange juice in Gwagwalada were collected from each of the five locations repeatedly and analysed for

i. Total bioload and

ii. Incidence of *Escherichia coli*

A total of fifteen (15) isolates were obtained

**Table 1: Total colony count of bacterial isolated from Nutrient agar**

|  |  |
| --- | --- |
| **Locations** |  **Sample(CFU/ml)** **A B C D** |
| **G M M****Phase 1****Kaswandare****Phase 3****Dagiri** | 5.2 x 105±0.02 4.2 x105±0.10 3.2 ×107 ± 1.50 1.2 ×106 ± 0.505.6 ×105 ± 0.12 6.6 x107±0.13 2.6 ×107 ± 3.17 2.1 ×107 ± 1.007.6 x 106±0.22 5.3 x106±0.02 1.8 ×107 ± 0.20 4.1 ×107 ±2.201.9 x 106±0.01 1.2 x107±0.11 3.3 ×105 ± 2.00 3.2 ×107 ±1.403.5 x 105±0.32 4.4 x105±0.31 1.1 ×106 ± 0.20 1.2 ×105 ±0.40 |

GMM=Gwagwalada main market. Each value represents Mean±Standard Deviation of three independent determinations

From the above Table 1, the total bacteria count of the locally produced orange juice ranged from 1.2x105±0.40 to 6.6x107 ±0.13 CFU/ml for all the samples from the five different locations.

**Table 2:** **Total coliform load of Isolated from MacConkey agar**

|  |  |
| --- | --- |
| **Locations** |  **Sample sites (CFU/ml)** **A B C D** |
| **G. M. M****Phase 1****Kaswandare****Phase 3****Dagiri** | 4.1 ×107 ± 3.40 2.8 ×106 ± 1.70 3.2 ×105 ± 1.50 1.2 ×106 ± 0.504.8 ×106 ± 3.17 1.7 ×106 ± 1.10 2.6 ×107 ± 3.17 2.1 ×105 ± 1.005.2 ×107 ± 2.23 4.0 ×105 ± 2.17 1.8 ×106 ± 0.20 4.1 ×106 ± 2.205.1 ×105 ±3.30 4.8 ×107 ± 3.10 3.3 ×107 ± 2.00 3.2 ×107 ±1.403.1 ×106 ±1.20 3.8 ×106 ±1.10 1.1 ×106 ± 0.20 1.2 ×105 ±0.40 |

Each value represents Mean±Standard Deviation of three independent determinations. GMM=Gwagwalada main market.

The above Table 2 showed that the coliform load obtained from the locally produced orange juice inoculated on MacConkey agar ranges from 5.2x107±2.23 to 1.2x105 ±0.40 CFU/ml.

**Table 3: Biochemical characteristics of isolates**

|  |
| --- |
| **Isolates Biochemical tests Probable organisms** **GR IN CI OX MR VP** |
| A1 - + - - + - *Escherichia coli*A2 + - - - - - Not *Escherichia coli*A3 - + - - + - *Escherichia coli*A4 - - - - - + Not *Escherichia coli*B1 + - - - - - Not *Escherichia coli*B2 - - + - - - Not *Escherichia coli*B3 - + - - + - *Escherichia coli*B4 - + - - + - *Escherichia coli*C1 - - - + - - Not *Escherichia coli*C2 - - - - - - Not *Escherichia coli*C3 - + - - + - *Escherichia coli*C4 - + - - + - *Escherichia coli*D1 - + - - + - *Escherichia coli*D2 - + - - + - *Escherichia coli*D3 + - - - - - Not *Escherichia coli*D4 - + - - + - *Escherichia coli*E1 - - - - - - Not *Escherichia coli*E2 - + - - + - *Escherichia coli*E3 - + - - + - *Escherichia coli*E4 - - - - - + Not *Escherichia coli* |

GR=Gram reaction, IN= Indole CI= Citrate OX= Oxidase, MR=Methyl red, VP=Voges-Proskauer

**Table 4: Morphological Characteristics of Microbial Isolates**

|  |
| --- |
| **Samples Morphological appearance Gram reaction** |
| **A G/lada Market**A 1 Circular, flat, pinkish and glistering surface colonies -veA 2 Small, circular pinkish shiny colonies -veA 3 Circular, flat, pinkish and glistering surface colonies -veA 4 Pale pink flat irregular shaped with mucoidal appearance -ve**B Phase I**B 1 Small, circular pinkish shiny colonies -veB 2 Pale pink flat irregular shaped with mucoidal appearance -veB 3 Circular, flat, pinkish and glistering surface colonies -veB 4 Circular, flat, pinkish and glistering surface colonies -ve**C Kasuwandere**C 1 Small, circular pinkish shiny colonies -veC 2 Circular, flat, pinkish and glistering surface colonies -veC 3 Circular, flat, pinkish and glistering surface colonies -veC 4 Circular, flat, pinkish and glistering surface colonies -ve**D Phase III**D 1 Circular, flat, pinkish and glistering surface colonies -veD 2 Circular, flat, pinkish and glistering surface colonies -veD 3 Pale pink flat irregular shaped with mucoidal appearance -veD 4 Circular, flat, pinkish and glistering surface colonies -ve**E Dagiri**E 1 Pale pink flat irregular shaped with mucoidal appearance -veE 2 Circular, flat, pinkish and glistering surface colonies -veE 3 Circular, flat, pinkish and glistering surface colonies -veE 4 Small, circular pinkish shiny colonies -ve |
| Key = G/lada market = Gwagwalada market, -ve = negative |

From the above Table 3 and 4, the isolates obtained were characterized and identified on the basis of their biochemical tests as well as Gram staining reaction and morphological assessment that is, macroscopic and microscopic features. Among the characteristics used includes: colonial characteristics such as size, surface appearance, texture and colour of the colonies.

**Table 5: Bacteria load and incidence of *Escherichia coli* in locally produced orange juice in Gwagwalada**

|  |
| --- |
| **Sample locations *Escherichia coli* Number of *Escherichia coli*** |
| **A Gwagwalada market**Sample 1 present 1Sample 2 Absent 0Sample 3 present 2Sample 4 Absent 0**Total 3****B Phase I**Sample 1 Absent 0Sample 2 Absent 0Sample 3 Present 1Sample 4 Present 1**Total 2****C Kasuwandere**Sample 1 Absent 0Sample 2 Present 1Sample 3 Present 2Sample 4 Present 2**Total 5****Sample locations *Escherichia coli* Number of *Escherichia coli*****D Phase III**Sample 1 Present 1Sample 2 Present 1Sample 3 Absent 0Sample 4 Present 1**Total 3****E Dagiri**Sample 1 Absent 0Sample 2 Present 1Sample 3 Present 1Sample 4 Absent 0 |
| **Overall Total 15** |

**Table 6: Frequency and percentage of *Escherichia coli* in locally produced orange juice in Gwagwalada**

|  |
| --- |
| **Sample locations Number of *E. coli* Percentages** |
| Gwagwalada market 3 20Phase I 2 13.33Kasuwandere 5 33.33Phase 3 3 20Dagiri 2 13.33 |
| **Total 15 100** |
|  |

From the above Table 5 and 6, Kasuwandere recorded the highest isolated *Escherichia coli* with 33.33 %, followed by Gwagwalada market and Phase III with 20 % each, Phase 1 and Dagiri with equal values have the least incidence of *Escherichia coli* in the orange juice with 13.33 % each.

**Table 7: Proximate analysis of locally produced orange juice in Gwagwalada**

|  |
| --- |
| **Nutrients L1 L2 L3 L4 L5** |
| Ash (%) 6.80 ± 0.8 7.60 ± 0.6 7.05 ± 0.4 6.99 ± 0.9 7.50± 0.8Moisture (%) 94.16±6.18 80.95±5.80 92.88±3.23 90.98±4.88 91. 89±3.38Crude lipid (%) 0.048± 0.01 0.037± 0.08 0.049± 0.02 0.044± 0.03 0.39± 0.04Crude fibre (%) N/A N/A N/A N/A N/AVitamin C 0.014±0.07 0.014±0.02 0.013±0.08 0.015±0.05 0.013±0.01(mg/100ml)pH 5.4 4.0 4.5 5.4 5.4**Minerals**Calcium (ppm) 21.3± 0.18 28.1± 0.28 20.2± 1.08 24.4± 1.12 26.2± 2.21Magnesium (ppm) 14.3± 0.8 12.4± 0.8 13.3± 0.7 15.5± 0.9 15.0± 0.8Iron (ppm) 0.24± 0.01 0.50± 0.09 0.20± 0.07 0.36± 0.01 0.45± 0.02Copper 0.12± 0.01 0.10± 0.01 0.11± 0.01 0.13± 0.02 0.12± 0.02 |

Each value represents mean ± standard deviation of two replicate determinations. Keys: L1=Gwagwalada market, L2= Kasuandare, L3=Phase I, L4= Phase III, L5= Dagiri, N/A=Not appreciable.

From the above Table 7 the proximate analysis of locally produced orange juice samples from five locations indicated that some of the juices contain impressive amounts of minerals, crude lipid, vitamin C, ash and moisture content.

**4. Discussions**

The proximate analysis of locally produced orange juice samples from five locations indicated that some of the juices contain impressive amounts of minerals, crude lipid, vitamin C, ash and moisture content. In terms of percentages, the moisture content in locally produced orange juice in Gwagwalada ranged from 80.95±5.80 % to 94.16±6.18, with sample from Gwagwalada market having the highest (94. 16 %) while the least was recorded by sample from Kasuandare (80.95±5.80 %). Vitamin C content ranges from 0.013±0.08 to 0.015±0.08 mg/100ml. Indigenous fruits are reported to have higher vitamin C content than the exotic ones. This trend could be attributed to the fact that ascorbic acid (Vitamin C) is easily destroyed by oxidation, a process which is greatly accelerated by heat (Droby, 2006). The availability of Vitamin C in the local orange juice is vitally important because vitamin C prevents major chronic diseases caused by free radicals. Lack of ascorbic acid in the diets causes a condition known as scurvy. Crude lipid in term of percentage also varies between juice samples and ranges from 0.037± 0.08 to 0.049± 0.02 % while the ash content of the locally produced juice collected from the five locations in Gwagwalada was between 6.80 ± 0.8 % and 7.60 ± 0.6 % while the percentage of the crude fibre was not appreciable (Table 6). The mineral contents include calcium with an average concentration ranging from 20.2± 1.08 ppm to 26.2± 2.21, Magnesium (ranges from 12.4± 0.8 to 15.5± 0.9 ppm), low amounts of Fe (between 0.20± 0.07 to 0.50± 0.09 ppm) and Cu (0.10 ± 0.01 to 0.13± 0.01 ppm) were obtained in the juice. The juice was relatively acidic with pH values (at 25°C) ranging from 4.0 to 5.4, which was within the optimum pH range for fruit juices (FEHD, 2005). The microbial load of the local juice are as follows: Kasuwandere recorded the highest isolated *Escherichia coli* with 33.33 %, followed by Gwagwalada market and Phase III with 20 % each, Phase 1 and Dagiri with equal values have the least incidence of *Escherichia coli* in the orange juice with 13.33 % each. The result obtained from this study showed that the total bacteria count of the locally produced orange juice ranged from 1.2x105±0.40 to 6.6x107 ±0.13 CFU/ml for all the samples from the five different locations. Improper washing of fruits adds these bacteria to juices leading to contamination (Durgesh *et al*., 2008). In addition lack of appreciation of basic safety issues by vendors contribute to augmentation of the microbial loads (Durgesh *et al*., 2008). These include use of crude stands and carts, unavailability of running water for dilution and washing, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies and airborne dust (Lateef *et al.*, 2006; Durgesh *et al*., 2008).

**5. Conclusion**

The degree of contamination of locally produced orange juice products largely depends on the initial load, source and kinds of microorganisms related to the fruits and care taken during collection, processing and product handling (Jolt *et al*., 1994). It is therefore concluded that the hygiene status of the locally produced juice in Gwagwalada can be fairly judged by the abundance of microorganisms associated with them and the incredence of *Escherichia coli* presence in the juice.

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